Applicability of biomarkers in the early diagnosis of prostate cancer

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Early diagnosis of prostate cancer can increase the curative success rate for this disease. Although serum prostate-specific antigen measurement is regarded as the best conventional tumor marker available, there is little doubt that it has great limitations. The threshold above which biopsies are indicated has now decreased to a serum prostate-specific antigen value of 3 ng/ml, which results in a negative biopsy rate of 70–80%. This can readily be explained by the fact that prostate-specific antigen is not specific for prostate cancer. Clinicians need more sensitive tools to help diagnose prostate cancer and monitor progression of the disease. Molecular oncology is playing an increasing role in the fields of diagnosis and therapy for prostate cancer and has already been instrumental in elucidating many of the basic mechanisms underlying the development and progression of this disease. The identification of new prostate cancer-specific genes, such as DD3PCA3, would represent a considerable advance in the improvement of diagnostic tests for prostate cancer. This could subsequently lead to a reduction of the number of unnecessary biopsies.


In the Western male population, prostate cancer has become a major public health problem. In many developed countries, it is not only the most commonly diagnosed malignancy but also the second leading cause of cancer-related deaths. Since the incidence of prostate cancer increases with age, the number of newly diagnosed cases continues to rise as the life expectancy of the general population increases. Approximately 230,110 men in the USA and 85,000 men in Europe are newly diagnosed with prostate cancer each year [1,2]. Epidemiology studies indicate that prostate cancer is an indolent disease and that more men die with prostate cancer than from it. However, 25–30% of the tumors behave aggressively and as a result, around 29,900 U.S. and 35,000 European men die from this disease annually. The high mortality rate is a consequence of the fact that there are no adequate therapeutic options for metastatic prostate cancer. Androgen ablation is the treatment of choice in men with metastatic disease. Initially, 70–80% of patients with advanced disease show a response to therapy. However, within 2–3 years, the majority of tumors become androgen independent and more aggressive. As a result, most patients will develop progressive disease. Currently, there is no effective treatment for this hormone-refractory stage of the disease. More than 70% of hormone-refractory patients suffer from painful bone metastases, which are the most important cause of morbidity.

Prostate cancer detection

Radical prostatectomy and radiotherapy are curative therapeutic options for prostate cancer but are limited to organ-confined disease. Early detection of prostate cancer, when the disease is confined to the prostate, is therefore pivotal. Since its discovery more than 20 years ago, prostate-specific antigen (PSA) has been the most valuable tool in the detection, staging and monitoring of prostate cancer. The European Randomised Study of Screening for Prostate Cancer (ERSPC), Rotterdam section, continuously evaluates screening procedures. Based on the knowledge of tumor characteristics and prevalence predictions of biopsy-detectable cancers per
PSA range, this ERSPC study group implemented changes in the screening protocol. Since 1997, the ERSPC study group accepted PSA values of more than or equal to 3 ng/ml as the standard biopsy indication. Digital rectal examination (DRE) and transrectal ultrasound (TRUS) have been entirely discarded as initial screening tests [3,4]. A large multicenter prostate cancer screening trial showed that approximately 50% of patients with serum PSA values greater than 10 ng/ml had advanced disease. Most patients with serum PSA values less than 10 ng/ml were diagnosed with early stage disease [5]. These findings have led to the conclusion that men with serum PSA values between 3 and 10 ng/ml most likely have clinically localized disease and would benefit from curative treatment. Due to the growing awareness of prostate cancer in the Western population and the introduction of the serum PSA test, the numbers of men newly diagnosed with localized prostate cancer has increased. However, concerns have arisen regarding the detection of clinically insignificant prostate cancers. The latter are tumors that do not pose a serious life threat and, as a result, do not require therapy. The tantalizing question for clinicians and researchers is how to distinguish a potentially dangerous from an indolent tumor.

Although widely accepted as a prostate tumor marker, PSA has turned out to be organ specific but not prostate cancer specific. PSA levels have been reported to be increased in men with benign prostatic hyperplasia (BPH) and prostatitis. This substantial overlap in serum PSA values between men with nonmalignant prostatic diseases and prostate cancer is the limitation of PSA as a prostate tumor marker. Moreover, PSA cannot be used to differentiate the aggressive from the indolent tumors. Upon detection of serum PSA values greater than 3 ng/ml, the conventional diagnostic approach is traditional sextant TRUS-guided prostate biopsies. However, the low specificity of serum PSA results in a negative biopsy rate of 70–80%. In some cases, biopsy specimens may not be representative, which also attributes to the failure to detect some cancers. Currently, most academic centers recommend extension of the diagnostic set to ten biopsies. In case of persistent rising serum PSA levels, repeated biopsies are indicated, which have at least 10% probability of finding cancer [6]. Moreover, if the combined use of serum PSA, DRE and TRUS biopsy do indicate clinically confined cancer, 40% of these men are found to have already extracapsular disease upon radical prostatectomy [7]. Therefore, noninvasive screening tests that can accurately identify men who have early stage, organ-confined prostate cancer and who would gain prolonged survival and improved quality of life from early radical intervention are urgently needed.

New prostate cancer screening tests

New prostate cancer screening tests have to meet four basic requirements to be an effective and practical approach for early detection [8]:

- Detection of prostate cancer should occur when the cancer is still confined to the prostate and radical treatment is potentially curative
- Distinguish the indolent from the aggressive tumors to avoid the problem of over diagnosis
- Be well accepted by the population targeted for screening and be inexpensive

As can be concluded from above, PSA as a screening marker for prostate cancer does not fulfill these requirements. The problem lies in the fact that PSA is not a prostate cancer-specific gene and does not discriminate between indolent and aggressive tumors. One approach to improve diagnostic accuracy of tests for prostate cancer and reduce the number of unnecessary biopsies is through the identification of prostate cancer-specific genes.

For the identification of new candidate markers for prostate cancer, it is necessary to study expression patterns in malignant as well as nonmalignant prostate tissues. Recent developments in molecular techniques have provided new tools that enable the comprehensive and rapid assessment of both genomic and proteomic alterations in samples. For instance, the identification of different chromosomal abnormalities such as changes in chromosome number, translocations, deletions, rearrangements and duplications in cells can be studied using fluorescence in situ hybridization (FISH) analysis. Comparative genomic hybridization (CGH) is able to screen the entire genome for large changes in DNA sequence copy number or deletions larger than 10 Mbp. Differential display analysis, serial analysis of gene expression (SAGE), oligonucleotide arrays and complementary DNA (cDNA) arrays characterize gene expression profiles. These techniques are often used in combination with tissue microarrays (TMAs) for the identification of genes that play an important role in specific biological processes [9].

Since genetic alterations often lead to mutated or altered proteins, the signaling pathways of a cell may become affected. Eventually, this may lead to a growth advantage or survival of a cancer cell. Proteomics is the term that refers to the identification of altered proteins in terms of structure, quantity and post-translational modifications. Disease-related proteins can be directly sequenced and identified in intact whole tissue sections using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). Additionally, surface-enhanced laser desorption/ionization (SELDI)-TOF MS can provide a rapid protein expression profile from tissue cells and body fluids such as serum or urine [10].

In the last 5 years, these molecular tools have led to the identification of hundreds of genes that are believed to be relevant in the development of prostate cancer. Not only have these findings led to a greater insight into the initiation and progression of prostate cancer, they have also shown that prostate cancer is a very heterogeneous disease. Several prostate tumors may occur in the prostate of a single patient due to the multifocal nature of the disease. Each of these tumors can show remarkable differences in gene expression and behavior that are associated...
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with varying prognoses. Therefore, in predicting the outcome of the disease, it is more likely that a set of different markers will become clinically important.

In 2003, an overview was given on prostate cancer biomarkers for cancer diagnosis, prognosis and prediction of disease survival [11]. This review will focus on the available data concerning the applicability of both old and new biomarkers in the early detection of prostate cancer in body fluids, such as urine and serum. These biomarkers are classified into four different prostate cancer-specific events: genomic alterations, prostate cancer-specific biological processes, epigenetic modifications and genes uniquely expressed in prostate cancer. Furthermore, this review will focus on whether current tests developed for several markers have the potential to reduce the number of unnecessary biopsies in men with total serum PSA values over 3 ng/ml.

Genomic alterations in prostate cancer
Prostate cancer-associated gene mutations
One of the strongest epidemiological risk factors for prostate cancer is a positive family history. A study of 44,788 pairs of twins in Denmark, Sweden and Finland has shown that 42% of prostate cancer cases were attributable to inheritance [12]. It has been observed that brothers of affected patients are consistently at higher risk for the disease compared with the sons of the same patients. This has led to the hypothesis that there is an X-linked or recessive genetic component involved in the risk for prostate cancer [13]. Genome-wide scans in affected families implicated at least seven prostate cancer-susceptibility loci: HPC1 (1q24), CAPB (1p36), PCAP (1q42), ELAC2 (17p11), HPC20 (20q13), 8p22–23 and HPCX (Xq27–28). Recently, three candidate hereditary prostate cancer genes have been mapped to these loci: HPC1/2′-5′-oligoadenylate dependent ribonuclease L (RNASEL) on chromosome 1q24–25, macrophage scavenger 1 gene (MSR1) located on chromosome 8p22–23 and HPC2/ELAC2 on chromosome 17p11 [14].

It has been estimated that prostate cancer susceptibility genes account for only 10% of prostate cancer cases. Familial prostate cancers are most likely associated with shared environmental factors or more common genetic variants of polymorphisms. Since such variants may occur at high frequencies in the affected population, their impact on prostate cancer risk can be substantial. Recently, polymorphisms in the genes coding for the androgen-receptor (AR), 5α-reductase Type II (SRD5A2), CYP17, CYP19, vitamin D receptor, PSA, GST-T1, GST-M1, GST-P1, insulin-like growth factor (IGF)-I and IGF-binding protein-3 have been studied. These studies were performed to establish whether these genes can predict the presence of prostate cancer in patients indicated for prostate biopsies due to PSA levels greater than 3 ng/ml. No associations were found between the AR, SRD5A2, CYP17, CYP19, vitamin D receptor, GST-M1, GST-P1 and IGF-binding protein-3 genotypes and prostate cancer risk. Only GST-T1 and IGF-I polymorphisms were found to be modestly associated with prostate cancer risk [15].

Unlike the adenomatous polyposis coli (APC) gene in familial colon cancer, none of the mentioned prostate cancer susceptibility genes and loci were by themselves responsible for the largest portion of prostate cancers. Epidemiology studies support the idea that most prostate cancers can be attributed to factors such as race, lifestyle and diet. The role of gene mutations in known oncogenes and tumor suppressor genes is probably very small in primary prostate cancer. For instance, the frequency of p53 mutations in primary prostate cancer is reported to be low but has been observed in almost 50% of advanced prostate cancers [16,17].

Screening men for the presence of cancer-specific gene mutations or polymorphisms is time consuming and costly. Moreover, it is very ineffective in the detection of primary prostate cancers in the general male population. Therefore, it cannot be applied as a prostate cancer screening test.

Mitochondrial DNA alterations
Mitochondrial DNA is present in approximately 1000 to 10,000 copies per cell [18]. Due to these quantities, mitochondrial DNA mutations have been used as a target for the analysis of plasma and serum DNA from prostate cancer patients. Recently, mitochondrial DNA mutations were detected in all three prostate cancer patients who had the same mitochondrial DNA mutations in their primary tumor [19]. Different urological tumor specimens must be studied and larger patient groups are needed to define the overall diagnostic sensitivity of this method.

Microsatellite alterations
Critical alterations in gene expression can lead to the progression of prostate cancer. Microsatellite alterations, which are polymorphic repetitive DNA sequences, often appear as loss of heterozygosity (LOH) or as microsatellite instability. Defined microsatellite alterations are known in prostate cancer. However, for the detection of microsatellite instability, the ratio of tumor to normal must be greater than 0.5%. For the detection of LOH, at least 20% of the analyzed genomic DNA must be obtained from tumor cells [20]. This might become a problem in blood or urine samples, in which a single prostate cancer cell must be detected in a huge background of normal cells. Furthermore, microsatellite analysis using small amounts of DNA is prone to artefacts. Due to the low amounts of genomic DNA, microsatellite analysis and LOH may fail in the detection of tumor DNA in body fluids.

Prostate cancer-specific biological processes
Since many proteins are shed into the circulation as a consequence of disease progression, screening the blood for overexpressed proteins appears to be an excellent way to search for new prostate cancer biomarkers. From recent studies, there is growing evidence that kallikreins and kallikrein-like genes are related to many types of malignancies. Recently, it has been suggested that there may be crosstalk between the kallikreins and that they participate in pathways that affect normal
physiological or pathological processes [22]. PSA is a member of the kallikrein family and since it has been successful in the diagnosis of prostate cancer, it is assumed that other kallikreins may also have diagnostic potential in prostate cancer.

Recently, 15 members of this gene family have been identified on chromosome 19q13.3–13.4. These 15 genes have a highly conserved structural organization and encode for putative secreted proteases. Their enzymatic activity may initiate or terminate biological events such as angiogenesis and growth factor release [22]. The release of these proteases into the blood circulation may reflect growth patterns of prostate cancer.

**PSA & serum subforms of PSA**

PSA, encoded by the KLK3 gene, is the most studied biomarker. Its drawings in the screening for prostate cancer have already been mentioned in this review. PSA is not overexpressed in prostate cancer cells. In fact, the PSA messenger RNA (mRNA) expression is approximately 1.5-fold lower in prostate tumor tissue compared with normal prostate tissue [23]. Therefore, it is assumed that the increase in serum PSA expression is a result of cancer progression. In the normal prostate, most of the produced PSA will be excreted into the semen where it acts as an androgen-regulated serine protease. Only a small amount of PSA will leak into the blood circulation. It is speculated that due to tumor development, the tissue architecture is altered by the disruption of the basal cell layer and basement membrane. It has been demonstrated that prostate cancer tissue releases 30-times more PSA into the circulation than normal prostate tissue [24]. Circulating PSA can occur in the serum either in an unbound free form or it can be bound to α1-antichymotrypsin or α2-macroglobulin.

There have been several attempts to improve the specificity of total PSA for the detection of prostate cancer in the range of 3 to 10 ng/ml, the so-called diagnostic PSA gray zone. These improvements include PSA density, transition zone PSA density, PSA velocity and age-specific PSA. These methods, all based on calculations with total PSA, have not been effective in the diagnostic gray zone to reduce the number of unnecessary biopsies [25,26].

Percent free PSA

Studies on percent free PSA (%fPSA), which is the ratio of free PSA to total PSA, appeared promising to increase the specificity in the diagnostic gray zone. Significantly higher %fPSA values have been observed in the serum of patients with BPH compared with those found in the serum of patients with prostate carcinomas. As a consequence, the probability of prostate cancer increased with a decrease of %fPSA values [27]. In patients with total serum PSA values of 4–10 ng/ml, %fPSA values at or below 25% resulted in the detection of 95% of prostate cancers upon biopsy and a reduction of 20% of unnecessary biopsies. Using a %fPSA cut-off value of 22% in the same group of patients, the sensitivity dropped to 90% but led to a 29% reduction of unnecessary biopsies [28].

On the other hand, patients with chronic prostatitis were demonstrated to have %fPSA values that are comparable with those of patients with prostate cancer [29]. This might lead to false-positive indications and thus to unnecessary biopsies. To date, controversy exists over using %fPSA values in addition to total PSA in screening for prostate cancer [30–32]. However, it has been reported that %fPSA appears to be more effective in the decision making of a repeat biopsy following an initial negative biopsy. In this way, a %fPSA cut-off value of 30% is optimal, leading to 90% sensitivity and a reduction of 50% of unnecessary biopsies [8].

**ProPSA**

Free PSA in serum is composed of distinct forms of inactive PSA, as reviewed by Mikolajczyk and coworkers [33,34]. ProPSA, which is one of the free PSA compounds in serum, is the name for native proPSA ([−7]proPSA) as well as truncated proPSA forms ([−2]proPSA, [−4]proPSA and [−5]proPSA). It has been demonstrated that in the diagnostic gray zone, total PSA had a specificity of 23%, %fPSA had a specificity of 33% and proPSA had a specificity of 13% at 90% sensitivity. A combination of these three variables resulted in a specificity of 44% in the diagnostic gray zone, which remarkably improved the specificity for early prostate cancer detection [35].

In a recent study, the development of highly sensitive and specific immunoassays for all intact and truncated forms of proPSA was described [36]. This study showed that the percentage of all proPSA forms (sum-proPSA/PSA; area under the curve [AUC] of 0.69) is able to improve the detection of prostate cancer compared with %fPSA (AUC of 0.63) and total PSA (AUC of 0.53) in the PSA gray zone used here of 4–10 ng/ml. Overall, [%−2]proPSA ([−2]proPSA/PSA) showed the same diagnostic potential as %sum-proPSA. However, in men with high (>25%) %fPSA values, [%−2]proPSA showed better diagnostic potential than %sum-proPSA. In these samples, [%−2]proPSA would have spared 36% of men from unnecessary biopsies, whereas %sum-proPSA would only have spared 29% of men. The additional forms of PSA mentioned here expand the possibilities for the use of PSA in the detection of prostate cancer.

**hK2**

There have been many studies on hK2, encoded by the KLK2 gene, which is another member of the kallikrein family. Like PSA, hK2 expression is regulated by androgens and has been detected in several other biological fluids, such as amniotic fluid, breast milk, breast cyst fluid and in malignant and non-malignant breast tissues. In vitro studies have shown that active hK2, prostate and hK15, which are also expressed at high levels in the prostate, convert latent proPSA into active PSA. The genes encoding hK2 and PSA show 80% homology and the two proteins show 78% amino acid sequence identity. hK2 is found at much lower concentrations (1–2% of PSA concentrations) in prostate tissue. The presence of hK2 in seminal plasma, albeit at only 0.1–1% of PSA concentrations,
sugests that hK2 is also involved in the cleavage of gel-forming proteins. The concentration of hK2 in serum is less than 3% of the PSA concentrations and, unlike PSA, it exists mainly in the free, unbound form. The development of specific hK2 immunoassays has been hampered by the high homology with PSA and the very low hK2 concentrations. This has resulted in highly sensitive assays for hK2 with very low detection limits (<0.1 ng/l) [37].

Like PSA, hK2 was expressed less in malignant prostate tissue compared with nonmalignant prostate tissue, albeit that the downregulation was less for hK2 than for PSA [38]. In a study where gene expression of PSA and KLK2 in Gleason grade (G) 4 or 5 tumors were compared with the gene expression of both genes in BPH specimens, both genes were found to be more than tenfold overexpressed compared with other genes [39]. However, there was no difference in gene expression between G4/5 tumors and the BPH samples. The clinical use of hK2 as a replacement for total PSA based on relative gene expression levels did not appear to be promising. Recently, it was shown that serum hK2 values alone were no more discriminatory than total PSA (TABLE 1) [40].

However, it has been demonstrated that the ratio of serum hK2 to free PSA could better distinguish prostate cancer from BPH than total PSA. In the diagnostic gray zone at 100% sensitivity, the ratio of hK2 to free PSA had a specificity of 48.2% [41]. This would have spared unnecessary biopsies in half of the men with elevated PSA levels.

Recently, it was shown that total PSA, free PSA or PSA-α1-ACT were not able to distinguish the poorly differentiated G3 tumors from the moderately differentiated G1 and G2 prostate tumors [42]. However, hK2 significantly improved the identification of G3 prostate tumors compared with %fPSA ratio. Furthermore, multivariate regression analysis revealed that combinations of hK2/free PSA and free PSA/(total PSA × hK2) were significant predictors of G3 tumors in the PSA range of 3 to 15 ng/ml. The free PSA/(total PSA × hK2) ratios were also helpful in prediction of organ-confined disease. Higher ratios showed a better chance for curative treatment and lower ratios showed a lower chance for successful surgery.

Furthermore, it has been determined that serum hK2 levels are remarkably higher in patients with advanced disease (median 116 ng/l) compared with patients with low Gleason scores (median 72 ng/l) or healthy men (median 26 ng/l) [43]. Moreover, serum hK2 levels alone or in combination with total PSA and free PSA have the power to distinguish pathologically organ-confined prostate cancer from locally advanced disease in patients with total PSA values less than 10 ng/ml [44].

These studies show that the ratio of hK2 to free PSA has diagnostic applicability in the diagnosis gray zone to distinguish prostate cancer patients from men with BPH (TABLES 1 & 2). As such, it can lead to a reduction of the number of unnecessary biopsies. Additionally, serum hK2 alone or in combination with total PSA and free PSA may improve the detection of extraprostatic or advanced disease. Multivariate regression analysis including hK2 may become more important for prostate cancer diagnosis since it allows the more accurate prediction of tumor grade, stage or organ-confined disease.

**KLK4**

KLK4, which encodes the hK4/prostate protein, is one of the more recently discovered members of the kallikrein gene family. Initial studies using northern blot analysis indicated that KLK4 expression was restricted to the prostate [45]. However, reverse transcription (RT)-PCR analysis demonstrated high KLK4 mRNA expression in prostate, testis, adrenals, uterus and thyroid. KLK4 expression was shown to be regulated either by androgens in the prostate cancer cell line LNCaP, or by androgens and progesterins in the breast cancer cell line BT-474 [46]. Using RT-PCR and immunohistochemistry (IHC) experiments, it has been shown that KLK4 is expressed both at the mRNA and protein level in normal human prostate tissues, primary prostate cancer tissues and metastatic prostate cancer tissues [47]. KLK4 mRNA expression was found to be higher in the majority of prostate cancer tissues compared with matched normal prostate tissues [48,49]. RNA in situ hybridization studies on normal and hyperplastic prostate tissue specimens in TMAs indicated that KLK4 is predominantly expressed in basal cells of the normal prostate [49].

Surprisingly, the hK4 protein concentrations were found to be frequently lower in the prostate cancer tissues compared with the matched normal prostate tissues. Furthermore, the hK4 protein concentration was found to be unexpectedly low in normal prostatic extracts (>104-fold lower than PSA) and seminal plasma (104–105-fold lower than PSA) [48]. Dotted mapping of the KLK4 mRNA 5’-end provided evidence that hK4 does not contain a signal peptide that would target the molecule for secretion. Furthermore, hK4 was found to be predominantly localized in the nucleus using immunofluorescence and cell fractionation experiments [49]. However, KLK4-specific antibodies have been detected in the sera of prostate cancer patients. These antibodies could have been generated by the immune system of prostate cancer patients after recognition of the hK4 protein. It is not known whether the protein enters the blood circulation in individuals with or without prostatic disease [47].

All these data suggest that KLK4 has a unique structure and function compared with the other members of the kallikrein family. In ovarian cancer, KLK4 expression appears to be associated with advanced ovarian carcinomas that have an unfavorable prognostic outcome of disease. Further studies are needed to assess the value of KLK4 mRNA or hK4 protein as biomarkers for prostate cancer.

**hK11**

The KLK11 gene encodes hK11, formerly known as hippo- 

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLK4</td>
<td>Prostate cancer</td>
<td>High</td>
</tr>
<tr>
<td>KLK11</td>
<td>Ovarian cancer</td>
<td>High</td>
</tr>
</tbody>
</table>

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serum hK11 values were significantly lower in prostate cancer patients compared with men with BPH. In this group of men with BPH, 45% would have avoided prostate biopsies based on %fPSA values of over 20%. When the ratio of hK11 to total PSA (>0.05) was applied, another 51.5% of these men with BPH would not have to undergo unnecessary biopsies. At 90% sensitivity, the ratio of hK11 to total PSA (0.05) had a specificity of 51.5% [51]. This indicates that in addition to PSA, %fPSA values combined with hK11 to total PSA ratios can lead to a higher reduction of unnecessary biopsies (TABLE 1). Future studies involving more patients are needed to confirm these preliminary data.

**KLK14**

KLK14 is another member of the kallikrein gene family. KLK14 expression has been found in the CNS and endocrine-related tissues, such as prostate, thyroid and testis [52]. In situ hybridization studies have shown KLK14 mRNA expression in secretory epithelial cells of the normal prostate, prostatic intraepithelial neoplasia (PIN) and malignant prostate cells [53]. This expression pattern is similar to that observed for PSA and hK2. In a recent study, a median 2.15-fold overexpression of KLK14 mRNA has been found in prostate cancer tissues compared with normal prostate tissues. Elevated expression levels of KLK14 appear to correlate with advanced and more aggressive tumors [54]. The potential of KLK14 in the diagnosis of prostate cancer is yet to be determined.

**KLK15**

The KLK15 gene has recently been identified. KLK15 is primarily expressed in the thyroid gland but expression has also been observed in the prostate, salivary gland, adrenal glands, colon, testis and kidney, albeit at much lower levels. Studies in the LNCaP prostate cancer cell line have shown that KLK15 expression was upregulated by steroid hormones. In prostate cancer tissues, KLK15 mRNA was found to be overexpressed compared with normal prostate tissues [55]. A recent study using quantitative real-time PCR has shown an association between the upregulation of KLK15 and advanced and more aggressive prostate tumors [56]. With KLK15 being a member of the family

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**Table 1. Current biomarker-based tests and their sensitivity and specificity in the diagnosis of prostate cancer in the diagnostic prostate-specific antigen gray zone.**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Application</th>
<th>Method</th>
<th>AUC</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>NPV (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA</td>
<td>Serum</td>
<td>Hybritech tandem PSA assays or dual ProStatus assay</td>
<td>0.50–0.70</td>
<td>90</td>
<td>10–31</td>
<td>[28,35,36,40]</td>
<td></td>
</tr>
<tr>
<td>%fPSA</td>
<td>Serum</td>
<td>Hybritech tandem free PSA assays or dual ProStatus assay</td>
<td>0.53–0.76</td>
<td>90</td>
<td>10–45</td>
<td>[28,35,36,40, 41,51]</td>
<td></td>
</tr>
<tr>
<td>%sum-proPSA</td>
<td>Serum</td>
<td>Research use dual monoclonal antibody immunoassay</td>
<td>0.69</td>
<td>90</td>
<td>31</td>
<td>[36]</td>
<td></td>
</tr>
<tr>
<td>Combination of total PSA, %fPSA and %sum-proPSA</td>
<td>Serum</td>
<td>Research use dual monoclonal antibody immunoassay</td>
<td>0.77</td>
<td>90</td>
<td>44</td>
<td>85</td>
<td>[35]</td>
</tr>
<tr>
<td>%[-2]proPSA</td>
<td>Serum</td>
<td>Research use dual monoclonal antibody immunoassay</td>
<td>0.64</td>
<td>90</td>
<td>21</td>
<td>[36]</td>
<td></td>
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<tr>
<td>hK2</td>
<td>Serum</td>
<td>In-house research immunofluorometric assay</td>
<td>0.68</td>
<td>90</td>
<td>20</td>
<td>[40]</td>
<td></td>
</tr>
<tr>
<td>fPSA/(tPSA×hK2)</td>
<td>Serum</td>
<td>Immunofluorometric assay for hK2</td>
<td>0.75</td>
<td>88</td>
<td>57</td>
<td>85</td>
<td>[42]</td>
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<tr>
<td>hK2/fPSA</td>
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<td>Immunofluorometric assay for hK2</td>
<td>0.86</td>
<td>100</td>
<td>48.2</td>
<td>[41]</td>
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<tr>
<td>hK11/tPSA</td>
<td>Serum</td>
<td>Immunofluorometric assay for hK11</td>
<td>0.77</td>
<td>90</td>
<td>51.5</td>
<td>[51]</td>
<td></td>
</tr>
<tr>
<td>GSTP1</td>
<td>Urine</td>
<td>MSP analysis</td>
<td>73</td>
<td>98</td>
<td>80</td>
<td>[68]</td>
<td></td>
</tr>
<tr>
<td>Telomerase</td>
<td>Urine</td>
<td>Telomeric repeat amplification assay</td>
<td>58</td>
<td>100</td>
<td>55</td>
<td>[79]</td>
<td></td>
</tr>
<tr>
<td>DD3PCA3</td>
<td>Urine</td>
<td>Quantitative RT-PCR assay</td>
<td>0.72</td>
<td>67</td>
<td>83</td>
<td>90</td>
<td>[85]</td>
</tr>
<tr>
<td>AMACR</td>
<td>Biopsy tissue</td>
<td>Immunohistochemistry</td>
<td>97</td>
<td>100</td>
<td></td>
<td>[74]</td>
<td></td>
</tr>
</tbody>
</table>

%fPSA: Percent free PSA; AMACR: α-methylacyl-CoA racemase; AUC: Area under curve; GSTP1: Glutathione S-transferase P1; MSP: Methylation-specific PCR; NPV: Negative predictive value; PSA: Prostate-specific antigen; RT: Reverse transcription.
of serine proteases, these high mRNA levels may indicate the presence of a protein. When shed into the serum as a result of cancer development, serum KLK15 may become important as a diagnostic and prognostic marker for prostate cancer.

**50.8-kDa protein**

Using peptide mass fingerprinting, a 50.8-kD protein (previously known as NMP48) was shown to be related to a vitamin D-binding protein. The protein has been detected in sera of 96% of prostate cancer-affected men and in 53% of men with high-grade PIN. No expression has been found in the sera of men with benign prostates (75%), BPH (70%), status after radical prostatectomy (80%) or healthy controls (96%). This small preliminary study indicates a role for this protein as a biomarker for the early detection of prostate cancer (TABLE 2) [57].

These preliminary studies indicate that a combination of these serum markers can remarkably improve the specificity for the detection of prostate cancer (TABLE 1). In particular, the combination of serum hK2 or hK11 with different forms of serum PSA has the potential to reduce the number of unnecessary biopsies in the diagnostic gray zone. Moreover, serum hK2 may improve the detection of extraprostatic disease and KLK15 may be used to discriminate between the more aggressive and indolent tumors.

Support for using a panel of proteins came from a recent study in which a novel technique, similar to SELDI-TOF MS, was combined with bioinformatics. It showed that complex serum protein profiles have the potential to identify prostate cancer and that the combination of two array types with different surface chemistries increased the number of more clinically significant discriminators. This new tool had an 85% sensitivity and specificity for the detection of prostate cancer [58]. In addition, such a serum-based proteomic pattern analysis has already proven to be successful in the early detection of ovarian cancer [59]. Using this method, the diagnosis of ovarian cancer was based on a panel of individual proteins, each of which was not independently discriminatory for the disease.

**Epigenetic modifications**

Alterations in DNA, without changing the order of bases in the sequence, often leads to changes in gene expression. These epigenetic modifications include changes such as DNA methylation and histone acetylation/deacetylation. Many gene promoters contain GC-rich regions known as CpG islands. Abnormal methylation of CpG islands results in decreased transcription of the gene into mRNA. Recently, it has been suggested that the DNA methylation status may be influenced in early life by environmental exposures, such as nutritional factors or stress, and that this leads to an increased risk for cancer in adults [60]. Changes in DNA methylation patterns have been observed in many human tumors [61]. A technique known as methylation-specific PCR (MSP) is used for the detection of promoter hypermethylation. In contrast to microsatellite or LOH analysis, this technique requires a tumor-to-normal ratio of only 0.1–0.001%. This means that by using this technique, hypermethylated alleles from tumor DNA can be detected in the presence of $10^6$–$10^5$ excess amounts of normal alleles [62]. Therefore, DNA methylation

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**Table 2. Biomarkers and their potential application in the detection of prostate cancer.**

<table>
<thead>
<tr>
<th>Goal</th>
<th>Biomarker</th>
<th>Application</th>
<th>Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discrimination of organ-confined disease from locally advanced disease</td>
<td>hK2, fPSA/(tPSAxhK2)</td>
<td>Serum</td>
<td>Immunofluorometric assays</td>
<td>[42–44]</td>
</tr>
<tr>
<td>Prediction of grade 3 tumors</td>
<td>hK2/fPSA, fPSA/(tPSAxhK2)</td>
<td>Serum</td>
<td>Immunofluorometric assays</td>
<td>[42]</td>
</tr>
<tr>
<td>Distinguish the more aggressive tumors from the indolent ones</td>
<td>Hepsin, RASSF1A</td>
<td>Serum</td>
<td>Immunofluorometric assays</td>
<td>[77]</td>
</tr>
<tr>
<td>Urine</td>
<td>MSP analysis</td>
<td>[63, 64]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction of the number of unnecessary biopsies</td>
<td>50.3-kDa protein, PSMA, hK11/tPSA, hK2/fPSA, combination of tPSA, %fPSA and %sum-pro-PSA</td>
<td>Serum</td>
<td>Immunofluorometric assays</td>
<td>[57, 83], [41, 51]</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Urine</td>
<td>MSP analysis</td>
<td>[68]</td>
<td></td>
</tr>
<tr>
<td>DD3&lt;sup&gt;PCA3&lt;/sup&gt;</td>
<td>Urine</td>
<td>Quantitative RT-PCR</td>
<td>[85]</td>
<td></td>
</tr>
<tr>
<td>Candidate for molecular probe in imaging modalities</td>
<td>AMACR</td>
<td>Imaging modalities</td>
<td>[73]</td>
<td></td>
</tr>
<tr>
<td>Prediction of disease progression (circulating tumor cells)</td>
<td>PSMA/PSMA, DD3&lt;sup&gt;PCA3&lt;/sup&gt;</td>
<td>Tissue, blood</td>
<td>Quantitative RT-PCR</td>
<td>[81]</td>
</tr>
</tbody>
</table>

%fPSA: Percent free PSA; AMACR: α-methylacyl-CoA racemase; GSTP1: Glutathione S-transferase P1; MSP: Methylation-specific PCR; PSA: Prostate-specific antigen; PSMA: Prostate-specific membrane antigen; RASSF1A: Ras-association domain family protein isoform A; RT: Reverse transcription.
can serve as a useful marker in cancer detection. Recently, there have been many reports on hypermethylated genes in human prostate cancer. Two of these genes are Ras-association domain family protein isoform A (RASSF1A) and glutathione S-transferase P1 (GSTP1).

**RASSF1A**

Hypermethylation of RASSF1A is a common phenomenon in breast, kidney, liver, lung and prostate cancer. RASSF1A hypermethylation has been observed in 60-70% of prostate tumors, demonstrating a clear association with aggressive prostate tumors. No RASSF1A hypermethylation has been detected in normal prostate tissue [63,64]. These findings suggest that RASSF1A hypermethylation may distinguish between the more aggressive and indolent tumors (TABLE 2). Further studies are needed to assess its diagnostic value.

**GSTP1**

The most thoroughly described epigenetic alteration in prostate cancer is the hypermethylation of the GSTP1 promoter. GSTP1 belongs to the cellular protection system against toxic effects and as such, is involved in the detoxification of many xenobiotics. GSTP1 hypermethylation has been reported in approximately 6% of proliferative inflammatory atrophy (PIA) lesions and in 70% of PIN lesions [65]. It has been shown that some PIA lesions merge directly with PIN and early carcinoma lesions, although additional studies are necessary to confirm these findings. Hypermethylation of GSTP1 has been detected in more than 90% of prostate tumors, whereas no hypermethylation has been observed in BPH and normal prostate tissues [66]. In another study, hypermethylation of the GSTP1 gene has been detected in 50% of ejaculates from prostate cancer patients but not in men with BPH. Due to the fact that ejaculates are not always easily obtained from prostate cancer patients, hypermethylation of GSTP1 was determined in urinary sediments obtained from prostate cancer patients after prostate massage. Cancer could be detected in 77% of these sediments [67]. Moreover, hypermethylation of GSTP1 has been found in urinary sediments after prostate massage in 68% of patients with early confined disease, 78% of patients with locally advanced disease, 29% of patients with PIN and 2% of patients with BPH. These findings resulted in a specificity of 98% and a sensitivity of 73% [68]. The negative predictive value of this test was 80%, which shows that this assay bears great potential to reduce the number of unnecessary biopsies.

Recently, GSTP1 hypermethylation has been detected in 40-50% of urinary sediments that were obtained from patients who recently underwent prostate biopsies. GSTP1 hypermethylation was detected in urinary sediments of patients with negative biopsies (33%) and patients with atypia or high-grade PIN (67%). Since hypermethylation of GSTP1 has a high specificity for prostate cancer, it suggests that these patients may have occult prostate cancer [69]. This indicates that the test could also be used as an indicator for a second biopsy.

**Genes uniquely expressed in prostate cancer**

Microarray studies have been very useful and informative in identifying genes that are consistently up- or downregulated in prostate cancer compared with benign prostate tissue [11]. These genes can provide prostate cancer-specific biomarkers and provide a greater insight into the etiology of the disease. For the molecular diagnosis of prostate cancer, genes that are highly upregulated in prostate cancer compared with low or normal expression in normal prostate tissue are of special interest. Such genes could enable the detection of one tumor cell in a huge background of normal cells and thus be applied as a diagnostic marker in prostate cancer detection.

**TMPRSS2**

cDNA microarray analysis in the prostate cancer cell line LNCaP has led to the discovery of the serine protease TMPRSS2, which was found to be upregulated by androgens. In situ hybridization studies have shown that TMPRSS2 was highly expressed in the basal cells of normal human prostate tissue and in adenocarcinoma cells. Low expression of TMPRSS2 has been found in colon, lung, kidney and pancreas. A 492-amino acid protein has been predicted for TMPRSS2. This predicted protein is a Type II integral membrane protein, most similar to the hepsin family of proteins. These proteins are important for cell growth and maintenance of cell morphology. There are speculations that TMPRSS2 could be an activator of the precursor forms of PSA and hK2 and that TMPRSS2, like other serine proteases, may play a role in prostate carcinogenesis [70]. Since TMPRSS2 has a low prostate cancer specificity, it cannot be applied in the detection of prostate cancer cells in urinary sediments.

**AMACR**

The gene coding for α-methylacyl-CoA racemase (AMACR) on chromosome 5p13 has been found to be consistently upregulated in prostate cancer. This enzyme plays a critical role in peroxisomal β-oxidation of branched chain fatty acid molecules obtained from dairy and beef [71]. Interestingly, the consumption of dairy and beef has been associated with an increased risk for prostate cancer [72].

In clinical prostate cancer tissue, a ninefold overexpression of AMACR mRNA has been found compared with normal prostate tissue. IHC studies and western blot analyses have confirmed an upregulation of AMACR at the protein level. Furthermore, it has been shown that 88% of prostate cancer cases and both untreated metastases and hormone-refractory prostate cancers were strongly positive for AMACR [73]. AMACR expression has not been detected in atrophic glands, basal cell hyperplasia and urothelial epithelium or metaplasia. IHC studies also showed that AMACR expression in needle biopsies had a 97% sensitivity and a 100% specificity for prostate cancer detection [74]. Combined with a staining for p63, a basal cell marker absent in prostate cancer, AMACR greatly facilitated the identification of malignant prostate cells. Its high expression and cancer cell specificity implies that AMACR may also
be a candidate for the development of molecular probes that may facilitate the identification of prostate cancer using noninvasive imaging modalities (TABLE 2) [73].

**Hepsin**

Using cDNA microarray analysis, it has been shown that hepsin, a Type II transmembrane serine protease, is one of the most differentially overexpressed genes in prostate cancer compared with normal prostate and BPH tissues [39,75,76]. Using quantitative real-time PCR analysis, it has been shown that hepsin is overexpressed in 90% of prostate cancer tissues. In 59% of prostate cancers, this overexpression was more than tenfold. There has also been a significant correlation between the upregulation of hepsin and tumor grade. Further studies must determine the tissue specificity of hepsin and the diagnostic value of this serine protease as a new serum marker. Since hepsin is upregulated in advanced and more aggressive tumors, a role as a prognostic tissue marker is suggested in determining the aggressiveness of a tumor (TABLE 2) [77].

**Telomerase**

Telomerase, a ribonucleoprotein, is involved in the synthesis and repair of telomeres that cap and protect the ends of eukaryotic chromosomes. The human telomeres consist of tandem repeats of the TTAGGG sequence as well as several different binding proteins. During cell division, telomeres cannot be fully replicated and become shorter. Telomerase can lengthen the telomeres and thus prevents the shortening of these structures. Cell division in the absence of telomerase activity will lead to shortening of the telomeres. As a result, the lifespan of the cell becomes limited and this will lead to senescence and cell death [78].

In tumor cells, including prostate cancer cells, telomeres are significantly shorter than in normal cells. In cancer cells with short telomeres, telomerase activity is required to escape senescence and to allow immortal growth. High telomerase activity has been found in 90% of prostate cancers and was shown to be absent in normal prostate tissue. In a small study on 36 specimens, telomerase activity has been used to detect prostate cancer cells in voided urine or urethral washing after prostate massage. This test had a sensitivity of 58% and a specificity of 100% [79]. The negative predictive value of the test was 55%. Although it was a small and preliminary study, the low negative predictive value indicates that telomerase activity measured in urine samples is not very effective in reducing the number of unnecessary biopsies.

The quantification of the catalytic subunit of telomerase, hTERT, showed a median overexpression of hTERT mRNA of sixfold in prostate cancer tissues compared with normal prostate tissues. A significant relationship was found between hTERT expression and tumor stage but not with Gleason score. The quantification of hTERT using real-time PCR showed that hTERT could accurately discriminate between prostate cancer and nonmalignant prostate tissue. However, hTERT mRNA is expressed in leukocytes, which are regularly present in body fluids such as blood and urine [80]. This may cause false positivity. As such, quantitative measurement of hTERT in body fluids is not very promising as a diagnostic tool for prostate cancer.

**PSMA**

Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein that is expressed on the surface of prostate epithelial cells. The expression of PSMA appears to be restricted to the prostate. It has been shown that PSMA is upregulated in prostate cancer tissue compared with benign prostate tissues. No overlap in PSMA expression has been found between BPH and prostate cancer, indicating that PSMA is a very promising diagnostic marker (TABLE 2) [81]. Recently, it has been shown that high PSMA expression in prostate cancer cases correlated with tumor grade, pathological stage, aneuploidy and biochemical recurrence. Furthermore, increased PSMA mRNA expression in primary prostate cancers and metastasis correlated with PSMA protein overexpression [82]. Its clinical utility as a diagnostic or prognostic marker for prostate cancer has been hindered by the lack of a sensitive immunoassay for this protein. However, a combination of ProteinChip® (Ciphergen Biosystems) arrays and SELDI-TOF MS has led to the introduction of a protein biochip immunoassay for the quantification of serum PSMA. It was shown that the average serum PSMA levels for prostate cancer patients were significantly higher compared with those of men with BPH and healthy controls [83]. These findings implicate a role for serum PSMA to distinguish men with BPH from prostate cancer patients. However, further studies are needed to assess its diagnostic value.

RT-PCR studies have shown that PSMA in combination with its splice variant PSMA´ could be used as a prognostic marker for prostate cancer. In the normal prostate, PSMA´ expression is higher than PSMA expression. In prostate cancer tissues, the PSMA expression is more dominant. Therefore, the ratio of PSMA to PSMA´ is highly indicative of disease progression. Designing a quantitative PCR analysis that discriminates between the two PSMA forms could yield another application for PSMA in diagnosis and prognosis of prostate cancer (TABLE 2) [81].

δ-catenin

δ-catenin (p120/CAS), an adhesive junction-associated protein, has been shown to be highly discriminative between BPH and prostate cancer. In situ hybridization studies showed the highest expression of δ-catenin transcripts in adenocarcinoma of the prostate and low to no expression in BPH tissue. The average overexpression of δ-catenin in prostate cancer compared with BPH is 15.7-fold. Neither quantitative PCR nor in situ hybridization analysis could find a correlation between δ-catenin expression and Gleason score [81]. Further studies are needed to assess the tissue specificity and diagnostic value of δ-catenin. However, it is clear that it has limitations when used as a prognostic marker for prostate cancer.
**DD3PCA3**

DD3PCA3 has been identified using differential display analysis. DD3PCA3 was found to be highly overexpressed in prostate tumors compared with normal prostate tissue of the same patient using northern blot analysis [84]. Moreover, DD3PCA3 was found to be strongly overexpressed in more than 95% of primary prostate cancer specimens and prostate cancer metastases. Furthermore, expression of DD3PCA3 is restricted to prostatic tissue, that is, no expression has been found in other normal human tissues [80,85]. The gene encoding DD3PCA3 is located on chromosome 9q21.2. The DD3PCA3 mRNA contains a high density of stop codons. Therefore, it lacks an open reading frame, resulting in noncoding RNA. Recently, a time-resolved quantitative RT-PCR assay (using an internal standard and an external calibration curve) has been developed. The accurate quantification power of this assay showed a median 66-fold upregulation of DD3PCA3 in prostate cancer tissue compared with normal prostate tissue. Moreover, a median upregulation of 11-fold was found in prostate tissues containing less than 10% of prostate cancer cells. This indicated that DD3PCA3 was capable of detecting a small number of tumor cells in a huge background of normal cells.

This hypothesis has been tested using the quantitative RT-PCR analysis on voided urine samples. PSA mRNA expression was shown to be relatively constant in normal prostate cells and only a weak downregulation (~1.5-fold) of PSA expression has been reported in prostate cancer cells. Therefore, PSA mRNA has been used as a housekeeping gene to correct for the number of prostate cells present in urinary sediments. These urine samples were obtained following extensive prostate massage from a group of 108 men who were indicated for prostate biopsies based on total serum PSA value of more than 3 ng/ml. This test had 67% sensitivity and 83% specificity using prostatic biopsies as a gold standard for the presence of a tumor. Furthermore, this test had a negative predictive value of 90%, which indicates that the qualitative determination of DD3PCA3 transcripts in urinary sediments obtained after extensive prostate massage bears great potential in the reduction of the number of invasive TRUS-guided biopsies in this population of men [85].

The tissue specificity and the high overexpression in prostate tumors indicate that DD3PCA3 is the most prostate cancer-specific gene described so far. Therefore, validated DD3PCA3 assays could become valuable in the detection of disseminated prostate cancer cells in serum or plasma [86]. Multicenter studies using the validated DD3PCA3 assay can provide the basis for molecular diagnostics in clinical urological practice (TABLES 1 & 2).

**RT-PCR-based detection of circulating tumor cells**

With the introduction of highly sensitive PCR technology, the detection of a single tumor cell in the huge background of predominantly normal cells became feasible to improve cancer diagnosis in blood samples. It is assumed that transcripts of epithelial cells do not normally occur in the blood circulation. Therefore, the detection of these transcripts in the serum or plasma indicates the presence of disseminated prostate cancer cells. In the last 12 years, many reports have been written on the RT-PCR-based detection of disseminated prostate cancer cells using PSA mRNA as the target. However, remarkable differences were observed in the sensitivity of the RT-PCR-based assays since these assays were qualitative, not standardized and difficult to reproduce in various laboratories [87]. To enhance the sensitivity of these assays, researchers used nested PCR. This led to the amplification of illegitimate transcripts [88]. These are transcripts that have been produced and secreted in small amounts by any normal cell in the body, such as normal blood or epithelial cells. As a result, PSA mRNA transcripts were found in the serum of women and healthy controls [89]. As such, these RT-PCR-based methods were of limited value. New sensitive, quantitative and more reproducible assays using exogenous internal standards for the detection of PSA and hK2 mRNA transcripts overcome this problem [90]. However, another problem arose when using organ-specific and not cancer-specific transcripts, such as PSA or hK2 mRNA. PSA mRNA transcripts were detected in the serum or plasma of men with and without prostate cancer after prostate biopsies. This led to a false-positive indication for the presence of a disseminated cancer cell [91,92]. However, the identification of highly overexpressed prostate cancer-specific genes combined with the validated quantitative RT-PCR assays could become valuable in the detection of disseminated cancer cells in serum or plasma.

**Conclusions**

Prostate cancer is a very sneaky disease since it develops within and even outside the prostate before an initial diagnosis is made. Since there are no adequate therapeutic options for advanced prostate cancer, it is imperative that it is detected at an early stage when it is potentially curable. The screening tests for PSA show high sensitivity for prostate cancer detection. However, in the diagnostic gray zone, the specificity of PSA is only 20%, resulting in a negative biopsy rate of 70–80%. It has become clear that new markers are urgently needed to improve the specificity of PSA in the diagnostic gray zone.

Innovations in the field of molecular technology are rapidly growing. Recent technologies such as microarrays and proteomics have already provided new markers. In this review, several markers have been discussed that might have clinical utility in the early diagnosis of prostate cancer. The number of serum markers is rapidly growing due to the extensive human kalrein family. By themselves they will not be able to improve the specificity for the early detection of prostate cancer. However, combined as a panel of markers, the specificity can be remarkably improved. Since prostate cancer is a heterogeneous disease, it becomes clear that a defined set of markers will become important in early prostate cancer diagnosis. Novel tests based on GSTP1 hypermethylation and the DD3PCA3 gene, which is highly overexpressed in prostate cancer, enabled the noninvasive detection of prostate cancer in body fluids such as urine or ejaculates. It becomes clear that through evaluation and clinical testing of the markers described herein, a greater insight into their true diagnostic potential emerges.
**Expert opinion**

The application of new technologies has shown that a large number of genes are upregulated in prostate cancer. For non-invasive screening tests, only those genes that are over-expressed in more than 95% of prostate cancer tissues compared with normal prostate or BPH will be important. Moreover, the upregulation of these genes in cancer should be more than tenfold in prostate cancer compared with normal prostate to enable the detection of a single prostate cancer cell in a huge background of normal cells in body fluids. The close collaboration and communication between clinicians and researchers is essential in clinical testing of these markers to assess their true diagnostic potential and to evaluate the impact of these tests on the reduction of unnecessary biopsies and disease mortality.

**Five-year view**

Despite the success of free PSA in cancer detection, several limitations remain. In the coming years, novel assays will be developed for the measurement of the distinct forms of serum free PSA, particularly proPSA. Only evaluation of these parameters in clinical trials will demonstrate their applicability in prostate cancer detection and lead to a reduction in the number of unnecessary biopsies.

The combination of hK2 with several forms of PSA offers promising approaches for the discrimination of prostate cancer patients from men with BPH, and may be used in the prediction of organ-confined disease. Clinical studies using large patient groups will demonstrate the applicability of hK11 in the reduction of the number of unnecessary biopsies. The combination of %fPSA values with hK11/tPSA ratios appears especially promising. The application of artificial neural networks and various logistic regression models may aid in the improvement of the positive predictive value of these serum markers. Large multicenter studies will show the real diagnostic application of these kallikreins and may lead to promising new serum-based tests. Another challenge of the coming years is to unravel the biological and physiological functions of hK4, hK11, KLK14 and KLK15.

In the next few years, the number of potential biomarkers will grow substantially, as will our understanding of the etiology of prostate cancer. For diagnostic purposes, it is very important that the potential biomarkers are tested in terms of tissue specificity and discrimination potential between prostate cancer, normal prostate and BPH. Until now, only telomerase, GSTP1 and DD3PCA3 have been studied for their potential to reduce the number of biopsies. Ironically, TRUS-guided prostate biopsies are used as the gold standard in these studies. Therefore, many of the patients who are currently regarded as being negative for prostate cancer may become cancer patients in the near future. Consequently, follow-up data of these clinical studies will become very important to achieve improved outcomes and management of prostate cancer.

To date, the most prostate cancer-specific gene is DD3PCA3. Its potential to reduce the number of biopsies in men with PSA values between 3 and 10 ng/ml is currently being validated in a large multicenter trial. A clinically applicable assay that will be reproducible within and between laboratories, which can be used as a reflex test following total PSA testing, is also under development. This urinary DD3PCA3 test will soon become available to urologists.

Innovations in new technologies will enable the development of more sensitive and accurate marker-based tests. Proteomic analysis and TMA will become increasingly important in discovering a pattern of proteins or genes which can discriminate between prostate cancer patients and men with BPH. These analyses may also indicate men at risk for progression of disease. Validated quantitative RT-PCR assays that are based on the detection of truly prostate cancer-specific genes may become important for the identification of patients with malignant disease.

Noninvasive marker-based assays used as screening tests in the early detection of prostate cancer are rapidly becoming reality.

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**Key issues**

- Early detection of prostate cancer when it is still organ confined can increase the cure rate and prevent mortality from this disease.
- Currently, the low specificity of prostate-specific antigen (PSA) leads to 70–80% of unnecessary biopsies in the diagnostic PSA gray zone of 3–10 ng/ml.
- Recent developments in the field of molecular techniques have provided new tools that have led to the discovery of many new promising biomarkers for prostate cancer. These biomarkers may be instrumental in the development of new screening tests that have a high specificity in the diagnostic gray zone and as such are able to reduce the number of unnecessary biopsies.
- For diagnostic purposes, is it important that the potential biomarkers are tested in terms of tissue specificity and discrimination potential between prostate cancer, normal prostate and benign prostatic hyperplasia.
- The results of multiple marker-based assays may enhance the specificity for cancer detection in the diagnostic gray zone and may discriminate between more aggressive and indolent tumors.

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Recently developed molecular techniques

Biomarkers in the early diagnosis of prostate cancer


38 Extensive overview on the molecular forms of prostate-specific antigen (PSA) and hK2 and their applicability in the early diagnosis of prostate cancer.


49 Xi Z, Klokki T, Korkmaz K et al. Kallikrein 4 is a predominantly nuclear protein and is overexpressed in prostate cancer. Cancer Res. 64, 2365–2370 (2004).


• Demonstrates the diagnostic potential of the combination of hK11, percent free PSA and total PSA in the reduction of prostate biopsies.


• Demonstrates that complex serum protein profiles have the potential to identify prostate cancer and could be applied as a new tool for prostate cancer diagnosis.


Demonstrates that the detection of glutathione S-transferase P1 methylation can be used as a urine-based diagnostic tool for the detection of prostate cancer.


Introduces the D D 3 (PСА3)-bаsed аssау fоr thе dеtесtioн of prostate cancer cеlls in уrіnе sеdiхents.


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