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Review

PSA and other tissue kallikreins for prostate cancer detection

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ABSTRACT

Prostate cancer is the most common neoplasia of middle-aged men. Prostate specific antigen (PSA) is the first FDA-approved tumour marker for early detection of cancer and it is now in widespread clinical use. The discovery of different PSA molecular forms in serum (free PSA, PSA complexed with various protease inhibitors) in the early 1990s renewed clinical research to enhance the specificity of PSA. Also, the use of a homologous prostate-localised antigen, human glandular kallikrein 2 (KLK2) may further reduce the number of unnecessary prostate biopsies. More recently, promising data is emerging regarding molecular forms of free PSA (proPSA, BPSA, 'intact' PSA) and other members of the expanded human kallikrein family. These new findings may add substantial clinical information for early detection of prostate cancer.

1. Prostate specific antigen and prostate cancer detection

Prostate cancer (PCa) is the most common malignancy in the western world. In 2006, the number of expected new cases is ~235,000 in the USA alone.[1] It is controversial whether screenings for this common and often indolent disease may reduce mortality, despite other positive effects such as stage migration and reduction of primarily metastatic PCa (from 20% down to 5%). For example, in Tyrol, a reduction of PCa mortality may be attributed to the use of prostate specific antigen (PSA) test, free of charge, for more than 5 years.[2] On the other hand, a recent case-control study showed no effect on PCa mortality with the use of PSA, in comparison to an equivalent patient group without PSA measurements.[3] In the years 2008/2009 results from two large, randomised studies in Europe and USA will hopefully provide sufficient information as to whether PSA can reduce PCa mortality. Despite this controversial discussion, there is no question it has been that PSA revolutionised the management of PCa, especially for early detection, with increased chances of curative treatment (reviewed in Ref. 4). The serine protease PSA was characterised and named in 1979[5] and detected in serum in 1980.[6] PSA is secreted into the seminal plasma and is responsible for semen liquefaction. The retrograde release of PSA into the bloodstream is a rare event in healthy men. This occurs with a frequency of less than one PSA molecule per million secreted PSA molecules, leading to a concentration of <4 μg/L in serum, which is a million-fold lower than the PSA concentration in seminal plasma (0.5–5 g/L). The prostate volume influences

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the PSA level in serum and increased levels are observed in some patients with benign prostate hyperplasia (BPH). However, destruction of the basement membrane of prostate epithelial cells may result in excessive escape of PSA into the circulation. Thus, PCa, benign prostate diseases, as well as physical trauma of the prostate, can result in significant increases of serum PSA. Therefore, an elevated serum PSA indicates pathologies of the prostate gland, including PCa, but PSA is not a cancer-specific marker. The non-cancerous alterations have challenged the usefulness of PSA as a tumour marker for the early detection of PCa. One of the main drawbacks of PSA is its low specificity and low positive predictive value. Consequently, about 60–80% of prostate biopsies are due to false-positive PSA values and they are unnecessary and invasive procedures. Several calculated parameters, such as PSA density (PSA divided by the prostate volume), PSA transition zone density, PSA velocity (PSA change over time) or age- and race-specific PSA ranges, were only partially successful in enhancing the specificity of PSA.[7,8] Due to large biological variations in the measured PSA concentrations (up to 20–30%)[9] a repeat measurement of PSA can avoid significant numbers of unnecessary prostate biopsies.[10]

The discovery of different PSA forms, like free PSA and PSA bound to alpha$_1$-antichymotrypsin (PSA-ACT) in the early 1990s renewed the clinical research on this biomarker.[11,12]

2. Molecular forms of PSA

PSA circulates in serum in free (unbound) and complexed (bound to protease inhibitors) forms.[11–13] Approximately 65–95% of the PSA is bound to ACT (PSA-ACT) whereas free PSA represents, on an average, only 5–35% of the total PSA (tPSA). The relative amount of free PSA tends to be increased in benign disease compared to PCa. The ratio of free to total PSA is being used routinely to increase specificity for PCa and to reduce unnecessary biopsies. PSA complexes with alpha$_2$-macroglobulin (PSA-A2M) and with alpha$_1$-protease inhibitor (PSA-API) are now measurable in serum as well.[14–16] Complexed PSA is being evaluated in numerous studies to determine its clinical utility. The Bayer complexed PSA assay measures both PSA-ACT and PSA-API and has been proposed as a single assay, alternative to the free and total PSA assays.[17–19] Other existing PSA complexes are so far not measurable in serum.[20] Free PSA has recently been shown to exist in at least three molecular forms; proPSA,[21] BPSA[22] and inactive 'intact' PSA (iPSA).[23]

The reason for the differences between BPH and PCa regarding the molecular forms of PSA, and especially the higher PSA-ACT in serum of cancer patients, is not completely understood. It is assumed that due to the loss of tissue architecture in PCa, the active PSA gains quicker access to the circulation and the protease inhibitors (like ACT and A2M) can complex with it more easily.[24] If PSA reaches the circulation from normal or BPH cells, it first has to leak backwards into the extracellular space, where it is susceptible to proteolytic degradation.[24] After degradation, the inactive PSA can still form complexes with A2M, but only to a very small degree with ACT.[25] This may explain the decreased capability of PSA to form complexes with ACT and the higher amounts of PSA-A2M in BPH patients.[26] An earlier hypothesis that higher intracellular ACT production in the prostatic epithelium leads to higher serum PSA-ACT concentrations in PCa patients compared to BPH patients could not be confirmed, as prostatic tissue PSA is present almost exclusively as uncomplexed PSA.[22,27,28]

3. Clinical use of percent free PSA

The use of percent free PSA (%fPSA), which is the ratio of free to total PSA, has already been established as a routine clinical parameter since the mid 1990s.[29–32] Various retrospective (reviewed in Refs. 4,20) and prospective studies have demonstrated a significant improvement in specificity for the 4–10 μg/L PSA range and for lower PSA values <4 μg/L.[33,34] Generally, with %fPSA cutoffs at 90–95% sensitivity, the number of unnecessary biopsies could be reduced by approximately 15–20%. This has also been shown for the indication to repeat biopsies.[35] However, for a better interpretation of %fPSA values, possible confounding factors such as prostate volume, tPSA, stage and grade, prostatic intraepithelial neoplasia (PIN) and race as well as sample stability, prostate manipulations or drug treatment history should be considered.[4,20] A brief summary of the most important influencing factors of %fPSA will be given below.

In two studies with more than 1500 patients[36,37] the authors confirmed the earlier findings of a positive correlation between %fPSA and prostate volume.[29,32,38]

The %fPSA tends to be inversely correlated to tPSA. A significant downward trend of %fPSA for the tPSA ranges <4 μg/L, 4–10 μg/L and >10 μg/L could be shown.[36,39] However, another study with patients with non-malignant prostate diseases within the tPSA range 2.6–9.9 μg/L revealed no influence of total PSA on %fPSA.[40]

The use of %fPSA for predicting the pathological stage of PCa is controversial. Some authors found an inverse relationship of %fPSA and pathological stage[41–43] while others did not find such an effect.[44–46] Regarding the histological grade and Gleason grade, it appears that low %fPSA values are more associated with higher grades.[43,47,48] A prospective multicentre trial demonstrated that %fPSA, followed by the Gleason sum, was the strongest predictor of pathological outcome.[49] Moreover, aggressive cancer may be detected much earlier using %fPSA instead of tPSA.[50,51]

Several new investigations have focused on the impact of isolated PIN as precursor of PCa on %fPSA.[52–54] The release of PSA into the bloodstream may be different due to the integrity of the basal cell layer in PIN tissue, in comparison to cancerous tissue. Significantly higher mean %fPSA levels in patients with exclusively PIN lesions compared to PCa patients were found.[52–54] Therefore, a decrease of %fPSA values in PIN patients should be considered as a possible concomitant evidence of PCa.

It has been proposed to use %fPSA as a priority decision tool for first time biopsy in men with unsuspicious digital rectal examination within the tPSA range of 4–10 μg/L, as well as for lower PSA values.[55,56] This will further enhance the number of detected cancers per biopsy. However, there are only limited data on %fPSA cutoff recommendations for tPSA <4 μg/L.[34,55] With specificity cutoffs of 90–95% the number of unnecessary biopsies can be reduced using %fPSA at low tPSA concentrations but many cancers will pass undetected.[55] Data on men with PSA concentrations ranging from 2.6 to 4.0 μg/L

argue for a high %fPSA sensitivity or even a general biopsy within this low tPSA range.[57] The need for high sensitivities at low tPSA concentrations is further supported by recent data from 2950 biopsied men with tPSA values <4 μg/L.[58] PCa was diagnosed in 15.2% of all men and the prevalence of PCa increased from 6.6% amongst men with a tPSA of 0–0.5 μg/L to 23.9% and 26.9% amongst those with tPSA values of 2.1–3 and 3.1–4 μg/L.[58] Also, the prevalence of high-grade PCa increased from 12.5% at tPSA of 0–0.5 μg/L to 25% at tPSA of 3.1–4 μg/L.[58] Thus, these data at tPSA concentrations between 2 and 4 μg/L are comparable to the 4–10 μg/L tPSA range. Expanding the range of additional free PSA measurements from 4–10 μg/L to 2 or 2.5–10 μg/L could be beneficial for detecting significant PCa at such low PSA values.

4. Use of other molecular forms of PSA

4.1. PSA-ACT and complexed PSA

For the measurement of different molecular PSA forms, only assays for PSA-ACT and complexed PSA have been available. It is known that PSA-ACT is the predominant form of PSA in serum.[11,12] Early analytical problems of overestimation due to the non-specific binding of ACT-cathepsin G-complex, loss of immunoreactivity or complex dissociation have now been solved.[59] To date, no study has shown an advantage of the PSA-ACT or the PSA-ACT/tPSA ratio, compared with %fPSA, to enhance the specificity of PCa detection.[60–64] However, for methodological reasons, determination of the ratio of PSA-ACT to tPSA is more demanding than that of fPSA to tPSA but this assay is not commercially available.[65] A research version of this assay (Roche) was not brought to the market.

The complexed PSA (cPSA) assay (Bayer Immuno 1) utilises a blocking antibody against free PSA and detects the PSA-ACT and PSA-API, but not the PSA-A2M complex.[66] Proposals to use the cPSA test alone have been debated since 1998 but, in general, the use of the cPSA/total PSA ratio has resulted in similar sensitivity and specificity compared with %fPSA.[62,67–69] While one multicentre study indicated an advantage by using cPSA alone compared to tPSA[70] another one could not prove an advantage by using cPSA compared to tPSA or %fPSA.[71] Despite the fact that cPSA has theoretically a small advantage compared with tPSA as a first line parameter, only the ratio of cPSA to tPSA could reach specificity levels comparable to %fPSA.[20,72–74] The conclusion in a meta analysis that cPSA performs equal to %fPSA in the tPSA range 2–10 μg/L[75] is therefore highly questionable and was debated.[74]

4.2. PSA-A2M and PSA-API

The measurement of the PSA-A2M complex in serum has been demonstrated using PSA immunoadsorption followed by pH manipulation to release the encapsulated PSA from the 25-fold larger molecule A2M.[14] PSA-A2M represents a considerable proportion of tPSA in serum and the ratio of PSA-A2M to PSA is higher in BPH patients (12%) compared to PCa patients (8%).[76] It was also shown that the sum of %fPSA and PSA-A2M could further enhance the specificity of tPSA and %fPSA.[76]

A method to analyse PSA-API has also been reported by the same investigators.[15] In a study the amount of PSA-API was 1.6% of tPSA in BPH patients and 0.9% of tPSA in PCa patients.[77]

4.3. BPSA, iPSA and proPSA

The BPSA is a specifically clipped subform of free PSA, which is highly associated with the transition zone, containing BPH nodules in prostate tissue.[22] A dual monoclonal assay with a detection limit of 0.06 μg/L showed that BPSA represents 0–60% of fPSA but this measure cannot distinguish between BPH and PCa.[78] However, BPSA can be a marker for BPH and may enhance specificity of %fPSA in combination with proforms of fPSA, or may be used for therapeutic control of BPH treatment.[79]

Based on the development of novel anti-PSA antibodies that do not recognise internally cleaved PSA at Lys145-Lys146, and thus are specific for intact, unclipped PSA[80] the same group developed an assay with a detection limit of 0.035 μg/L for the non-clipped free PSA called 'intact' PSA (iPSA).[81] The iPSA assay detects both proPSA and other inactive, non-clipped free PSA and has been shown to be useful for discriminating BPH and PCa.[23]

Recently, proPSA forms were isolated in serum and tissue from PCa patients.[21,82] The complete natural proPSA protein contains 244 amino acids (−7) compared to free PSA (237 amino acids). The proPSA in serum and prostate tissue exists as a mixture of different designated forms including the (−7), (−5), (−4) forms and partially the (−2) and (−1) forms.[83] Mikolajczyk and co-workers[84] found an overrepresentation of the (−2) form in serum samples from PCa patients compared with BPH samples. The same group showed that the percentage of proPSA (%pPSA) affords better cancer detection in the 4–10 μg/L range than did %fPSA and complexed PSA.[85] Furthermore, (−2)proPSA significantly discriminated PCa from BPH in men whose serum had >25% free PSA.[85] In a study on 1,091 patients, the %pPSA significantly improved specificity for cancer detection and decreased the number of unnecessary biopsies in the PSA range of 2–10 μg/L.[86] Sub-analysis of the same patient group revealed that %pPSA was superior to %fPSA and calculated cPSA for the detection of more aggressive PCa, as indicated by Gleason score 7 or greater and/or extracapsular tumour extension.[87]

The other assay for (−5, −7) proPSA was recently used in two studies to assess the validity of these proPSA forms to detect early PCa and to distinguish pathological grade.[88,89] Contrary to the above mentioned studies,[85,87] data from the (−5, −7) proPSA studies showed limited ability of proPSA, compared with %fPSA, to improve PCa detection.[88,89] A recent review summarises available proPSA studies and their potential implications.[90] The commercial availability of the (−2)proPSA assay in 2007 will facilitate more studies. Combined measurements of proPSA and BPSA could further enhance the sensitivity and specificity of %fPSA and PSA.

5. The kallikrein gene family

Until recently, only three human kallikrein genes were identified: the pancreatic/renal kallikrein *KLK1*, the human glandular kallikrein 2 (*KLK2*) and *KLK3*, which is widely known as PSA.[91,92] Recently, 12 new members of the human kallikrein

family have been characterised.[93] This family of proteases now consists of 15 members, identified by new nomenclature introduced in the year 2000.[94] In 2006, a new nomenclature for human, mouse and rat kallikreins has been proposed, in order to further simplify the classification of all kallikreins.[95]

The human kallikrein genes (named *KLK1 to KLK15*; encoding for the proteins KLK1 to KLK15) share significant homologies, genomic motifs and other similarities and cluster within a 300-kb region on human chromosome 19q13.4. All genes have 5 coding exons and are highly homologous at the DNA and amino acid levels (40–80%). Most kallikreins are regulated by steroid hormones.[93] Besides PSA, KLK2 has shown to add significant information for detecting PCa, especially at low PSA values.[96–98] Additionally, it has been shown that KLK2 can convert proPSA to active PSA and that these two kallikreins may act in concert in extraprostatic locations.[99–102] Studies with KLK6 and KLK10 indicated possible roles as serum biomarkers for non-prostate diseases, especially ovarian cancer.[103–106] It is possible that several kallikreins, in addition to PSA, may add clinical information for various cancers including PCa.

5.1. *KLK2 and PCa*

PSA and KLK2 share the highest homology amongst kallikreins with 78% and 80% identity at the amino acid and DNA level, respectively.[93] Both kallikreins have also been detected in relatively small quantities in non-prostatic tissues and biological fluids.[20,93] The *KLK2* mRNA amounts to 10–50% of the PSA mRNA in the prostate tissue but in serum and seminal plasma, KLK2 concentration is only 1–3% of that of PSA. The low levels in serum pose analytical challenges for KLK2 measurements but reliable assays are available in several research laboratories.[107–109]

Kwiatkowski et al.[96] reported first that the ratio of KLK2 to free PSA enhances discrimination of PCa and BPH patients. In a larger study with 937 serum samples, enhanced PCa detection rates were described using both %fPSA and KLK2/fPSA within the PSA ranges of 2–4 μg/L and 4–10 μg/L.[98] Other studies confirmed the advantage of using KLK2 and its ratios to fPSA and %fPSA, especially at low PSA.[97,110,111] KLK2 was found to discriminate between high and low grade tumours and between stage 2 and stage 3 tumours.[112,113] Furthermore, it has been shown, that KLK2 in PCa more closely correlates with total PCa volume and high grade PCa volume compared with tPSA or fPSA.[114] These data may be of importance because until now, the Gleason grade 4/5-cancer volume is the only independent predictor of biochemical failure after radical prostatectomy and therefore a serum marker is critically needed.[115]

It has been proposed that KLK2 is a powerful predictor of organ-confined disease and pathologic stage of clinically localised PCa.[116] In a further study, KLK2 and the KLK2 density could independently predict pT2a/b PCa.[117] However, improvement in predictive accuracy was marginal when nomograms based on traditional variables were also used.[117] Stephan et al.[118] could not find an advantage for KLK2 in distinguishing between pT2 and pT3 PCa patients, whereas %fPSA and the ratios KLK2/fPSA and KLK2/%fPSA were all significantly different. However, neither KLK2 and fPSA ratios nor KLK2 or %fPSA alone could distinguish between G2 and G3 or between Gleason score >7 and ⩽6.[118] Inclusion of KLK2 into an artificial neural network provided only marginal benefits at lower tPSA values (<4 μg/L).[119]

Analogous to PSA, KLK2 was also found in different molecular forms in serum but contrary to PSA, free KLK2 is the predominant form and KLK2-ACT represents only 4–19% of total KLK2.[107] A recently developed free KLK2 and total KLK2 assay yielded a mean free to total KLK2 ratio of 85% (17% to 131%).[120] The wide variation in the free-to-total KLK2 ratio suggests that KLK2 in plasma is not consistently in the free, non-complexed form in patients with PCa.[120] The proform of KLK2 is also present in serum and is increased in prostate diseases.[121] The measurement of complexed KLK2 may be used for improving PCa specificity.[122] A novel complex of hK2, hK2-PI6, has been shown to be highly associated with prostate tumour tissue relative to transition zone and peripheral zone normal cells.[123]

5.2. *Other kallikreins as PCa markers*

The differential regulation of kallikrein genes or other genes in cancerous versus non-cancerous prostatic tissues might lead to the development of potential new serum-markers. Amongst all kallikreins, at least 8 (*KLK2-4, KLK10-13 and KLK15*) are expressed in relatively high amounts in prostatic tissue.[93] Despite high expression of *KLK4* in prostate tissue[124] no commercial serum immunoassay exists so far.[125–128]

With the first reported immunological assay for KLK11, elevated serum levels were found in 70% of women with ovarian cancer and in 60% of men with PCa.[129] The KLK11 to tPSA ratio and %fPSA in combination were good predictors of PCa.[130] Further analysis will help confirm this hypothesis.[131]

KLK12 and *KLK13* are also highly expressed in prostate tissue but so far there are no further studies available.[93,132–135] *KLK14*, like PSA and *KLK2*, was downregulated in PCa tissue compared to non-cancerous tissue.[136]

The regulation of *KLK15* was initially analysed in matched prostate tissue samples.[137] On quantitative analysis, *KLK15* expression was significantly higher in cancerous than in non-cancerous prostate tissue.[138] Using a newly developed immunoassay, KLK15 was detected in prostate, colon and thyroid tissues, as well as in breast milk and seminal plasma.[139] Further studies with serum will reveal if KLK15 is a biomarker for PCa and other cancers.

All these newly cloned genes and associated proteins, which are prostate localised and/or members of the kallikrein family, are worth studying in the future as potential new markers for PCa detection.

6. Conclusions

The %fPSA has shown effectiveness in improving specificity over tPSA alone. There is a need for evaluating new and promising molecular forms of PSA, such as proPSA, BPSA, 'intact' PSA, PSA-A2M and PSA-API as well as KLK2 and its molecular forms. The development of antibodies for various other kallikreins will be of great interest. In the future, one of the important goals for PCa serum marker development, is to search for a

marker, which can predict the Gleason grade 4/5, which is so far the only independent prognostic factor for biochemical failure after radical prostatectomy. None of the hitherto existing serum markers fulfills this important clinical need. The molecular forms of free PSA show potential to clarify this issue, while other kallikreins remain potential candidates.

Conflict of interest statement

None declared.

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