Review – Prostate Cancer

Screening for Prostate Cancer in 2008 II: The Importance of Molecular Subforms of Prostate-Specific Antigen and Tissue Kallikreins

Flip H. Jansen*, Monique Roobol, Guido Jenster, Fritz H. Schröder, Chris H. Bangma

Department of Urology, Erasmus MC, Rotterdam, The Netherlands

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Abstract

Context: Over the past decades, prostate-specific antigen (PSA), its isoforms, and other members of the tissue kallikrein family have been of continuous interest with regard to early detection and screening for prostate cancer (PCa).

Objective: This review strives to give an overview of the possible clinical utilities of these markers, focused on early diagnostics and PCa screening.

Evidence acquisition: Using the Medline database, a literature search was performed on the role of molecular subforms of PSA and other members of the tissue kallikrein family in PCa detection.

Evidence synthesis: With respect to PSA isoforms, only the combination of the various truncated forms (pPSA) shows additional value over total PSA (tPSA) and free PSA (fPSA) in PCa detection within the range of 2–10 ng/ml tPSA. At a high sensitivity for PCa, the specificity of the ratio of pPSA to fPSA (%pPSA) is, in general, better than that of the ratio of fPSA to tPSA (%fPSA), with a gain of 5–11%. The (−2)pPSA, (−4)pPSA, (−5)pPSA, (−7)pPSA, and benign PSA (BPSA) isoforms generally show no additional value over either pPSA or the existing parameters of tPSA and fPSA. Of the other members of the tissue kallikrein family, most studies on human kallikrein 2 (hK2) show an additional value of the ratio of hK2 to fPSA (%hK2) over %fPSA alone in PCa prediction. Other tissue kallikreins cannot be recommended for diagnosing PCa, due to the lack of additional value over tPSA or fPSA or to insufficient research. Regarding a prognostic role, the value of PSA subforms as well as of other members of the tissue kallikrein family is limited with regard to existing parameters.

Conclusions: pPSA and hK2 are able to improve PCa diagnosis in the range of 4–10 ng/ml tPSA over the existing variables tPSA and fPSA.

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1. Introduction

Prostate-specific antigen (PSA; also known as human kallikrein 3 [hK3]) is a member of the tissue kallikrein family. It is synthesized in prostate tissue, and after being released into the seminal fluid, it lyses the gel proteins, resulting in the liquefaction of the semen [1]. Normally, PSA is confined within the prostate and only a minute amount leaks into the circulation. The increased serum concentrations in prostate cancer (PCa) patients are not the result of increased expression of PSA but rather of an increased release of PSA into the bloodstream, likely the result of disruption of the prostate architecture in PCa (Fig. 1) [2,3]. A substantial fraction of PSA that enters the circulation is intact and forms a complex with the protease inhibitor α1-antichymotrypsin or with other inhibitors. PSA that is catalytically inactive does not form complexes and circulates as free PSA (fPSA). The major part of fPSA consists of three distinct forms: inactive PSA (iPSA), similar to active native PSA; a variety of precursor isoforms of PSA (pPSA); and a form designated as benign PSA (BPSA), as it was initially found in patients with benign prostatic hyperplasia (BPH) (Fig. 2) [4].

Sequencing of the human genome resulted in the identification of a total of 15 tissue kallikrein genes located on chromosome 19q13.4 [5]. They are expressed in multiple tissues, and almost all are steroid hormone regulated (Fig. 3) [6]. Of these, human kallikrein 2 (hK2; kallikrein-related peptidase 2 [KLK2]) and human kallikrein 4 (hK4; kallikrein-related peptidase 4 [KLK4]) are primarily expressed in prostate tissue and are androgen regulated [7,8].

The diagnostic, predictive, and prognostic characteristics of PSA, its isoforms, and other members of the tissue kallikrein family have been of continuous interest over the past decades, especially within the lower PSA ranges. This review strives to give an overview of the possible clinical utilities of these markers, focused on early diagnostics and PCa screening.
2. Evidence acquisition

Relevant publications were collected by searching the Medline database using the search terms pPSA, proPSA, PSA isoform, BPSA, benign PSA, (−2)proPSA, (−4)proPSA, (−5)proPSA, (−7)proPSA, tissue kallikreins, hK1–hK15 alone or in combination with prostatic neoplasms and benign prostatic hyperplasia, with no temporal limitations. This search resulted in 504 hits, of which 471 were written in the English language. Of these manuscripts, titles and abstracts were reviewed, focusing on the diagnostic, predictive, and prognostic characteristics of PSA isoforms and other members of the tissue kallikrein family. Reference lists of the retrieved articles were scrutinized for additional relevant publications. This resulted in a final selection of 54 manuscripts.

3. Evidence synthesis

3.1. Prostate-specific antigen isoforms

PSA is produced as a preproprotein, containing 261 amino acids. After removal of the 17-amino acid

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**Fig. 3** – Expression of the 15 tissue kallikrein genes in various tissues. Human kallikrein 2 (hK2), prostate-specific antigen (human kallikrein 3 [hK3]), and human kallikrein 4 (hK4) are primarily expressed in prostate cancer. Red reflects overexpression of the given member of the tissue kallikrein family, whereas white or blue represents underexpression. Data extracted from the exon 1.0 ST array sample data set on Affymetrix.com (http://www.affymetrix.com/support/technical/sample_data/exon_array_data.affx). KLK = kallikrein-related peptidase.

**Fig. 4** – Prostate-specific antigen (PSA) is initially produced as a 261-amino acid preproprotein. After co-translational removal of the amino-terminal leader sequence, the noncatalytic zymogen (−7) precursor isoform of PSA ((−7)pPSA) results. After cleavage by human kallikrein 2 (hK2), the seven-amino acid propeptide is removed and catalytically active mature PSA (237 amino acids) is formed. Alternatively, truncated forms of (−7)pPSA are formed due to cleavage within the propeptide. As a result of internal cleavage within active PSA between residues 145-146 and 182-183, the inactive benign PSA (BPSA) can be produced. Figure adapted from Balk et al [3].
leader sequence, an inactive 244–amino acid pre-
cursor protein termed proPSA (pPSA) results (Fig. 4) [9].
After cleavage by hK2, pPSA is converted to mature,
active PSA (237 amino acids) [10,11]. Originally, pPSA
was defined as the only precursor form of PSA,
consisting of 244 amino acids including a seven–
amino acid propeptide leader, and was therefore
also termed (−7)proPSA or (−7)pPSA. However, later
reports presented several other truncated forms of
pPSA, such as (−1)pPSA, (−2)pPSA, (−4)pPSA, and
(−5)pPSA, containing one, two, four, or five amino
acids, respectively, in the propeptide leader instead of
the native seven amino acids (Fig. 4) [12,13]. In this
review, pPSA is regarded as the combination of the
various truncated forms of pPSA. The molecular basis
for the increased serum levels of truncated pPSA
forms in PCa is unknown but most likely reflects
decreased cleavage by hK2 in PCa cells. Overall,
roughly one-third of the fPSA fraction in cancer
serum is composed of pPSA forms (Fig. 2).

One of the first studies to show evidence for the
presence of nicked forms of PSA was by Noldus et al,
who isolated fPSA from a pool of PCa sera [14].
Subsequent studies by others produced and identi-
fied various pPSA forms [11,15]. pPSA was shown to
be differentially elevated in peripheral zone cancer
and to be undetectable in most specimens of the
transition zone (TZ), leading to the conclusion that
pPSA represented a more cancer-specific form of
PSA [16]. Initial attempts to confirm the presence of
pPSA in serum were unsuccessful, but others later
unequivocally confirmed the presence of several
pPSA forms in serum of PCa patients [12–14,17]. In
2000, Mikolajczyk et al published a report in which
prostate cancer tissue was examined to understand
the origin of pPSA [16]. Sequencing showed that the
pPSA in peripheral zone cancer consisted mainly of
(−2)pPSA, containing a proleader peptide of two amino
acids. A subsequent study confirmed the presence of (−2)pPSA in serum of men with PCa, in
which (−2)pPSA ranged from 25–95% of the fPSA
fraction, in contrast to 6–19% in biopsy-negative
men [12]. As a result of the presence of only a two–
amino acid propeptide, hK2 was shown to be unable
to activate (−2)pPSA to mature PSA, stabilizing
(−2)pPSA as an inactive isoform of PSA in serum.

3.2. Combined precursor isoforms of prostate-specific
antigen

Several studies have investigated the use of pPSA in
PCa detection. Naya et al compared 43 men with or
without PCa having small or large prostates and
found no significant difference in pPSA levels or in
the ratio of pPSA to fPSA (%pPSA) between men with
and without PCa independent of prostate volume
(PV) or fPSA [18]. These findings contrasted with
several other studies that found differences in
%pPSA between PCa and non-PCa subjects [19–22].
The %pPSA improved the specificity for a positive
biopsy from 10% to 15% in the tPSA range of 2–4 ng/
ml. Within the tPSA range of 4–10 ng/ml, the
reported improvements in specificity varied from
20% to 31% or 10% to 19%, respectively.

With regard to PCa aggressiveness, it was shown
that within the 4–10-ng/ml tPSA range, %pPSA is
associated with Gleason grades ≥7 and with the
existence of extracapsular extension [20,23].
Although different selection criteria were used to
define unfavourable PCa, this finding was confirmed
in a study by de Vries et al [23]. However, both
studies showed that the benefit of pPSA over
(−2)pPSA, the ratio of fPSA to tPSA (%fPSA), and
tPSA to detect aggressive PCa is limited.

In conclusion, the studies incorporating larger
study populations show that %pPSA is able, to some
extent, to increase cancer detection within the 2–10-
ng/ml PSA range and usually performs slightly better
than %PSA (Table 1). Also, at a high sensitivity for
PCa, the specificity of %pPSA generally is better than

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients</th>
<th>PSA range (ng/ml)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>[18,21]</td>
<td>43</td>
<td>4.4–8.5</td>
<td>No difference in pPSA and %pPSA levels between PCa and non-PCa patients</td>
</tr>
<tr>
<td>[19]</td>
<td>1091</td>
<td>2–10</td>
<td>%pPSA levels increased in PCa; higher PCa specificity and prediction than %PSA</td>
</tr>
<tr>
<td>[22]</td>
<td>380</td>
<td>4–10</td>
<td>Higher predictive value and specificity for PCa of %pPSA compared with %PSA</td>
</tr>
<tr>
<td>[23]</td>
<td>61</td>
<td>&lt;15</td>
<td>%pPSA not related to PCa prognosis; pPSA related to poor prognosis in 4–10-ng/ml PSA range</td>
</tr>
<tr>
<td>[20]</td>
<td>1091</td>
<td>2–10</td>
<td>No significant relationship between %pPSA and pathologic stage; limited relationship with biopsy or pathologic Gleason grade</td>
</tr>
</tbody>
</table>

PCa = prostate cancer; PSA = prostate-specific antigen; %pPSA = ratio of pPSA to free PSA; %fPSA = ratio of free PSA to total PSA.
that of %pPSA, but again, the gain is limited to 5–11%. In addition, the ability of pPSA to predict tumour stage or grade seems restricted.

3.3. (−2) precursor isoform of prostate-specific antigen

Catalona et al measured (−2)pPSA values in a cohort of 1091 serum samples from men with and without PCAs within the PSA ranges of 2–4 ng/ml and 4–10 ng/ml [19]. This two-centre study showed that (−2)pPSA levels were higher in PCa patients compared with non-PCa patients, but only one of the two centres reached statistical significance within the 2–4-ng/ml PSA range. The predictive power of the ratio of (−2)pPSA to fPSA (%−2)pPSA) was smaller compared to %pPSA. Mikolajczyk et al later confirmed these results for the PSA range of 4–10 ng/ml in 380 serum samples [22]. In contrast, Naya et al found no significant difference in (−2)pPSA or %−2)pPSA levels in men with and without PCa [18,21].

A second study by Catalona et al analysed pPSA forms in relation to PCa aggressiveness [20]. The (−2)pPSA isoform could not discriminate between cancers with pathologic stages less than or greater than pT3a and did not perform significantly better than %pPSA and %pPSA in the detection of cancers with a Gleason score >7. These results were later confirmed by de Vries et al in a smaller study [23].

In summary, (−2)pPSA and %−2)pPSA levels are generally increased in the circulation of men with PCa but perform worse compared with pPSA or %pPSA on a predictive as well as a prognostic level (Table 2).

3.4. (−4) precursor isoform of prostate-specific antigen

Most studies incorporate the (−4)pPSA isoform within the measurement of pPSA, so data regarding the individual performance of (−4)pPSA in PCa detection are sparse. Mikolajczyk et al showed that the ratio of (−4)pPSA to fPSA was significantly higher in PCa serum samples compared to benign serum samples. However, (−4)pPSA performed less than (−2)pPSA and pPSA and was therefore not incorporated in the backward logistic regression analysis [22]. Other studies by Naya et al also concluded that (−4)pPSA performed less than (−2)pPSA and did not discriminate men with PCa from men without PCa [18,21]. Furthermore, no relationship between (−4)pPSA levels and PV was found.

3.5. (−5, −7) precursor isoforms of prostate-specific antigen

One of the first reports on the possible clinical use of the (−5)pPSA and (−7)pPSA forms was published by Bangma et al [24]. Because the (−5)pPSA and (−7)pPSA forms were recognized simultaneously with one antibody, they were designated (−5, −7)pPSA. This pilot study of 143 men with a negative biopsy, 142 men with BPH, and 146 men with biopsy-proven PCa showed that (−5, −7)pPSA had an additional value in detecting PCa over variables such as age, tPSA, and %pPSA, but (−5, −7)pPSA did not improve the specificity of pPSA for discriminating BPH and PCa. Also, no correlation was found between (−5, −7)pPSA levels and pathologic tumour grades or confined versus unconfined disease. A later report on the performance of (−5, −7)pPSA in an artificial neural network (ANN) analysis, based upon measurements in 898 men with or without PCa, failed to show an improved specificity of (−5, −7)pPSA over %PSA at a 95% sensitivity [25]. Within all PSA ranges, the area under the curve (AUC) of (−5, −7)pPSA was smaller than that of either tPSA or %pPSA. Also, others showed that the addition of (−5, −7)pPSA did not increase the predictive value for PCa over tPSA, fPSA, or pPSA [18,22,26].

In short, several studies have shown that (−5, −7)pPSA has no additional value over fPSA or tPSA in diagnosing or assessing the prognosis of PCa (Table 3).

3.6. Benign prostate-specific antigen

Characterization of fPSA in normal, hyperplastic, and cancerous prostatic tissue led to the identifica-

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Table 2 – Summary of the results of studies focusing on (−2) precursor isoform of prostate-specific antigen ((−2)pPSA).

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients</th>
<th>PSA range (ng/ml)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>[19]</td>
<td>1091</td>
<td>2–10</td>
<td>Value of %−2)pPSA in predicting PCa smaller than %pPSA.</td>
</tr>
<tr>
<td>[22]</td>
<td>380</td>
<td>4–10</td>
<td>PCa specificity and predictive value of %pPSA greater than %−2)pPSA.</td>
</tr>
<tr>
<td>[20]</td>
<td>1091</td>
<td>2–10</td>
<td>%−2)pPSA related to PCa aggressiveness, although generally not</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>performing better than %pPSA.</td>
</tr>
<tr>
<td>[23]</td>
<td>61</td>
<td>&lt;15</td>
<td>(−2)pPSA related to poor prognosis but weaker than pPSA.</td>
</tr>
<tr>
<td>[18,21]</td>
<td>43</td>
<td>4.4–8.5</td>
<td>No significant difference in (−2)pPSA or %−2)pPSA levels in men with</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>and without PCa</td>
</tr>
</tbody>
</table>

PCa = prostate cancer; PSA = prostate-specific antigen; %pPSA = ratio of pPSA to free PSA; %−2)pPSA = ratio of (−2)pPSA to free PSA.
tion of a specific molecular form of clipped fPSA, called BPSA, most likely the consequence of posttranslational proteolytic processes within the prostate [27]. Further characterization showed that BPSA contained 237 amino acids, like PSA, but was clipped at amino acid residues lysine 145-146 and lysine 182-183 (Fig. 4). The ratio of BPSA to tPSA (%BPSA) was significantly increased within the TZ of patients with BPH compared with patients without BPH. Initial measurements of BPSA concentrations in serum showed that BPSA represented 25% of the fPSA in biopsy-negative men [28].

Canto et al showed BPSA and fPSA to be strongly correlated with both age and TZ volume in 91 biopsy-negative men [29]. Furthermore, BPSA outperformed fPSA as well as tPSA in the prediction of TZ enlargement. A study from Naya et al demonstrated that BPSA, %BPSA, and %fPSA performed similarly in the prediction of PV [18]. BPSA could not predict the presence of PCa and was not related to tumour volume. A recent report by de Vries et al in 61 men diagnosed with PCa showed that BPSA failed to discriminate between favourable and poor prognostic PCa [23].

Briefly, studies investigating BPSA in relation to PCa and BPH have shown that BPSA is related to PV and, more precisely, to TZ enlargement. Its role in discriminating men with PCa from men without PCa, however, seems limited (Table 4).

### 3.7. Human kallikrein 2

hK2 shares 80% homology with PSA and is responsible for the cleavage of pPSA to active mature PSA, as described previously [10,11]. In the circulation, hK2 is present at levels of 1–2% compared with PSA [30,31]. However, because the covariance of hK2 and PSA is <60% and because different expression patterns are seen on an immunohistochemistry level, hK2 may be a marker independent of PSA [32,33]. Of all of the members of the tissue kallikrein family, hK2 is the most intensively studied in relation to PCa. One of the first reports showed that hK2 concentrations did not significantly differ between PCa and BPH patients, but the ratio of hK2 to fPSA (%hK2) enhanced the discrimination of PCa from BPH within the 4–10-ng/ml PSA range over %fPSA [34]. Several others have reported a non-significant relationship of hK2 concentrations and PCa [35–37], although the majority of later studies found significantly higher levels of hK2 in PCa versus BPH or non-PCa samples [24,38–44]. Almost all reported an enhanced discrimination between PCa and non-PCa patients attributed to %hK2 [35–38,40–42]. For the 2.5–10-ng/ml PSA range, the predictive value of %hK2 was greater than %fPSA in most studies, although Partin et al found a higher predictive value for %fPSA in a large study [35,36,41,42]. For PSA levels <4 ng/ml, the predictive value of %hK2 performed worse than %fPSA [36,42].

### Table 3 – Summary of the results of studies focusing on (−5) and (−7) precursor isoforms of prostate-specific antigen ((−5, −7)pPSA).

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients</th>
<th>PSA range (ng/ml)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>[24]</td>
<td>431</td>
<td>0.9–10.2</td>
<td>No improvement over fPSA in discriminating PCa from BPH; not related to tumour stage or grade</td>
</tr>
<tr>
<td>[25]</td>
<td>898</td>
<td>1–10</td>
<td>No improved specificity for PCa over %fPSA</td>
</tr>
<tr>
<td>[26]</td>
<td>2055</td>
<td>0.28–81</td>
<td>Predictive value of (−5, −7)pPSA for PCa smaller than %fPSA</td>
</tr>
<tr>
<td>[22]</td>
<td>380</td>
<td>4–10</td>
<td>Higher levels of (−5, −7)pPSA in men with PCa compared to men without PCa; predictive value not reported</td>
</tr>
<tr>
<td>[18]</td>
<td>43</td>
<td>4.4–8.5</td>
<td>(−7)pPSA not discriminative in PCa vs non-PCa samples</td>
</tr>
</tbody>
</table>

PCa = prostate cancer; PSA = prostate-specific antigen; fPSA = free PSA; BPH = benign prostatic hyperplasia; %fPSA = ratio of free PSA to total PSA.

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients</th>
<th>PSA range (ng/ml)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>[28]</td>
<td>170</td>
<td>1.5–17</td>
<td>Ratio of BPSA to tPSA higher in BPH vs PCa serum</td>
</tr>
<tr>
<td>[29]</td>
<td>91</td>
<td>0.9–20.9</td>
<td>BPSA more accurate than fPSA in prediction of transition zone enlargement</td>
</tr>
<tr>
<td>[18]</td>
<td>43</td>
<td>4.4–8.5</td>
<td>BPSA similarly related to PV as %fPSA</td>
</tr>
<tr>
<td>[23]</td>
<td>61</td>
<td>&lt;15</td>
<td>BPSA has no additional value in discriminating favourable- from poor-prognosis PCa</td>
</tr>
</tbody>
</table>

PCa = prostate cancer; PSA = prostate-specific antigen; tPSA = total PSA; BPH = benign prostatic hyperplasia; %fPSA = ratio of free PSA to total PSA.

<table>
<thead>
<tr>
<th>Table 4 – Summary of the results of studies focusing on benign prostate-specific antigen (BPSA).</th>
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</thead>
<tbody>
<tr>
<td>Reference</td>
<td>No. of patients</td>
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<tr>
<td>-----------</td>
<td>-----------------</td>
</tr>
<tr>
<td>[28]</td>
<td>170</td>
</tr>
<tr>
<td>[29]</td>
<td>91</td>
</tr>
<tr>
<td>[18]</td>
<td>43</td>
</tr>
<tr>
<td>[23]</td>
<td>61</td>
</tr>
</tbody>
</table>

PCa = prostate cancer; PSA = prostate-specific antigen; tPSA = total PSA; BPH = benign prostatic hyperplasia; PV = prostate volume; %fPSA = ratio of free PSA to total PSA.
Regarding PCa stage and grade, several studies demonstrated that hK2 is able to discriminate between high- and low-grade tumours and between organ- and non–organ-confined disease [40,45–49]. Haese et al showed in three subsequent studies that hK2 levels were significantly higher in organ-confined versus non–organ-confined disease, although improvements were marginal when traditional variables such as Gleason grade and clinical stage were added to a multivariate logistic regression model [45–47]. These results were recently confirmed by Steuber et al [49]. In contrast with these results, others showed that hK2 was unable to discriminate organ-confined from non–organ-confined PCa or failed to show additional value over existing parameters [38,50,51].

In summary, most studies show significantly elevated levels of hK2 in men with PCa compared to men without PCa, although several larger studies did not (Table 5). An increased predictive value for %hK2 over %fPSA alone is supported by most studies. Most studies investigating the possible prognostic role of hK2 show a limited increase in predicting stage and tumour grade after addition of hK2 or hK2 incorporated in ratios with fPSA or tPSA.

### 3.8. Human kallikrein 11

Cloning of the human kallikrein 11 (hK11) gene showed that it is highly expressed in prostate tissue as well as in stomach, trachea, skin, and colon epithelial cells [52–54]. Diamandis et al developed an assay to measure serum hK11 levels and demonstrated 60% higher concentrations in men with PCa [52]. In a larger study, hK11 levels and the ratio of hK11 to tPSA were shown to be significantly lower in men with PCa compared to BPH [55]. The predictive power of the hK11-to-tPSA ratio was equal to that of %fPSA. Stephan et al also found significantly lower levels of hK11 in men with PCa; however, the predictive value of the hK11-to-tPSA ratio was smaller than that for %fPSA, and hK11

### Table 5 – Summary of the results of studies focusing on human kallikrein 2 (hK2).

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients</th>
<th>PSA range (ng/ml)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>[24]</td>
<td>431</td>
<td>0.9–10.2</td>
<td>Discrimination between PCa and PCa not enhanced by hK2; hK2 not related to tumour grade</td>
</tr>
<tr>
<td>[34]</td>
<td>90</td>
<td>4–10</td>
<td>hK2 levels not different between PCa and BPH samples; higher specificity of hK2-to-fPSA ratio compared to %fPSA</td>
</tr>
<tr>
<td>[35]</td>
<td>206</td>
<td>2.5–10</td>
<td>hK2 levels not different between PCa and BPH samples; higher predictive value of hK2-to-fPSA ratio compared to %fPSA</td>
</tr>
<tr>
<td>[36]</td>
<td>937</td>
<td>&gt;2</td>
<td>hK2 levels not different between PCa and BPH samples; specificity increased by addition of hK2-to-fPSA ratio over %fPSA</td>
</tr>
<tr>
<td>[37]</td>
<td>475</td>
<td>1–20</td>
<td>hK2 levels not different between PCa and BPH samples; PCa detection improved after addition of hK2 in 1–4-ng/ml PSA range</td>
</tr>
<tr>
<td>[38]</td>
<td>247</td>
<td>1.3–172</td>
<td>hK2 levels higher in PCa vs BPH; predictive value of hK2 smaller than tPSA; predictive value of (hK2 × tPSA)/PSA higher than %fPSA.</td>
</tr>
<tr>
<td>[39]</td>
<td>604</td>
<td>3–220</td>
<td>hK2 levels higher in PCa vs BPH; predictive value of hK2 smaller than tPSA; predictive value of (hK2 × tPSA)/PSA higher than tPSA.</td>
</tr>
<tr>
<td>[40]</td>
<td>324</td>
<td>3–144</td>
<td>hK2 levels higher in PCa vs non-PCa; high hK2 levels 5–8-fold increase in risk of PCa detection.</td>
</tr>
<tr>
<td>[41]</td>
<td>162</td>
<td>≤2–≥100</td>
<td>Higher specificity of hK2-to-fPSA ratio vs %fPSA within 4–10-ng/ml PSA range</td>
</tr>
<tr>
<td>[42]</td>
<td>345</td>
<td>0.26–393</td>
<td>Predictive value of hK2-to-fPSA ratio greater than %fPSA in 4–10-ng/ml PSA range; hK2-to-fPSA ratio independent predictor of PCa</td>
</tr>
<tr>
<td>[43]</td>
<td>355</td>
<td>≥4</td>
<td>No difference in hK2 levels between PCa and non-PCa samples within 2–10-ng/ml PSA range; hK2 not a significant predictor for PCa</td>
</tr>
<tr>
<td>[44]</td>
<td>1793</td>
<td>Not reported</td>
<td>Additional value of hK2 over fPSA and tPSA in PCa detection in older men</td>
</tr>
<tr>
<td>[45]</td>
<td>68</td>
<td>Not reported</td>
<td>Higher hK2 and fPSA levels in non-organ vs organ-confined disease; hK2 highest value in predicting non–organ-confined PCa</td>
</tr>
<tr>
<td>[46]</td>
<td>245</td>
<td>0.27–87.1</td>
<td>Limited improvement in predicting organ-confined disease by hK2</td>
</tr>
<tr>
<td>[47]</td>
<td>161</td>
<td>&lt;10</td>
<td>hK2 correlated to PCa pathologic stage; predictive value for non–organ-confined PCa higher for (hK2 × tPSA)/PSA than tPSA</td>
</tr>
<tr>
<td>[48]</td>
<td>122</td>
<td>Not reported</td>
<td>Additional value of hK2 in predicting organ-confined tumour stage and grade</td>
</tr>
<tr>
<td>[49]</td>
<td>867</td>
<td>Not reported</td>
<td>hK2 best predictor of locally advanced PCa; best predictor of biochemical recurrence for PSA levels &lt;10ng/ml</td>
</tr>
<tr>
<td>[50]</td>
<td>222</td>
<td>0.5–48</td>
<td>No value of hK2 in predicting PCa tumour grade; limited value in predicting organ-confined disease</td>
</tr>
<tr>
<td>[51]</td>
<td>188</td>
<td>0.90–44.81</td>
<td>No additional value in predicting PCa tumour stage and grade</td>
</tr>
</tbody>
</table>

PCa = prostate cancer; PSA = prostate-specific antigen; BPH = benign prostatic hyperplasia; fPSA = free PSA; tPSA = total PSA; %fPSA = ratio of fPSA to tPSA.
individually performed worse than tPSA and %fPSA [56]. In an ANN analysis, models including hK11 showed an increase in predictive value for PCa [57]. In contrast, Ochiai et al found no diagnostic advantage in using either hK11 or the hK11-to-tPSA ratio in discriminating between PCa and non-PCa within the PSA range of 2.5–10 ng/ml [58]. This difference with previous studies, especially by Nakamura et al [55], most likely results from better matching of tPSA levels between men with and without PCa. Also, in the latter study, hK11 levels could not be quantified in a substantial proportion of men.

In short, the majority of studies on hK11 show that hK11 cannot be recommended for diagnosing PCa because it possesses no additional value next to fPSA and/or tPSA (Table 6). A possible prognostic role for hK11 remains to be investigated.

3.9. Other tissue kallikreins

The other members of the tissue kallikrein family are intensively studied for their value in PCa detection and prognosis, as they are also expressed in prostate tissue because they are closely related to PSA and hK2. Human kallikrein 1 (hK1; kallikrein-related peptidase 1 [KLK1]) has been shown to be expressed in prostate tissue at low levels, but data regarding the clinical use of KLK1 are lacking [59]. hK4 (KLK4) has been shown to be overexpressed in primary and metastatic PCa compared with normal prostate tissue [60,61]. An immunoassay showed its presence in prostatic tissue extracts and seminal plasma [62,63]. Future research will have to elucidate its potential as a serum marker for PCa. Kallikrein-related peptidase 5 (KLK5) is overexpressed in normal versus cancerous prostatic tissue [64,65]. A preliminary analysis demonstrated an inverse relationship between KLK5 levels and pathologic tumour stage and Gleason grade [65]. An immunohistochemical study on the expression of kallikrein-related peptidase 6 (KLK6) showed a decreased expression in PCa compared with normal prostate tissue. The value of KLK6 for PCa prognosis seems limited, as KLK6 did not correlate with tumour grade, pathologic stage, and recurrence of PCa [66]. Although an immunoassay to quantify KLK6 levels in serum has been developed, no results have been reported yet. Kallikrein-related peptidase 7 (KLK7) has been shown to be expressed in seminal plasma as well as in prostate tissue [67]. Clinical potential has not been evaluated yet. Kallikrein-related peptidase 8 (KLK8) has not been shown to be expressed in prostate tissue. Kallikrein-related peptidase 9 (KLK9) is mainly expressed in the testis and seminal vesicles but is also expressed in normal prostate tissue [59,68]. Its presence in serum or expression in benign versus malignant prostate tissue has not been evaluated yet. Kallikrein-related peptidase 10 (KLK10) is expressed in relatively high levels in prostatic tissue and is downregulated in PCa [66]. Furthermore, a tumour-suppressor role for KLK10 in PCa has been suggested [69]. Kallikrein-related peptidase 12 (KLK12) and kallikrein-related peptidase 13 (KLK13) have been demonstrated to be expressed in prostatic tissue, but so far, clinical studies are lacking [70,71]. As with KLK6 and KLK10, the expression of KLK13 is decreased in PCa tissue compared with normal prostate [66]. Recently, kallikrein-related peptidase 14 (KLK14) has been shown to play a major role in seminal clot liquefaction [72]. Inconclusive evidence exists on the expression of KLK14 in PCa [73–76]. Rabien et al found evidence for a prognostic role, as KLK14 expression was increased in men with shorter progression-free survival [74]. Also, a higher KLK14 expression was seen in non–organ-confined PCa but was not related to Gleason grade, in contrast with Yousef et al [76]. Most interestingly, Borgono et al showed significantly increased KLK14 serum levels in a small series of men with PCa compared with healthy men [73]. Kallikrein-related peptidase 15 (KLK15) has been demonstrated to be upregulated in PCa versus normal prostate tissue and was also found to be related with higher Gleason scores or tumour stage, although not significantly [77,78]. An immunoassay for the measurement of KLK15 levels in body fluids has been developed, although results on serum levels in men with and without PCa have not been published yet [79].

**Table 6 – Summary of the results of studies focusing on human kallikrein 11 (hK11).**

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of patients</th>
<th>PSA range (ng/ml)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>[55]</td>
<td>150</td>
<td>0.17–48</td>
<td>Predictive value of hK11 in discriminating PCa from BPH equal to fPSA</td>
</tr>
<tr>
<td>[56]</td>
<td>371</td>
<td>0.5–20</td>
<td>Discriminatory power in distinguishing PCa from BPH lower for hK11 than tPSA</td>
</tr>
<tr>
<td>[57]</td>
<td>357</td>
<td>0.58–19.90</td>
<td>Predictive value of hK11 in discriminating PCa from BPH lower than tPSA; predictive value of hK11-to-tPSA ratio higher than tPSA alone</td>
</tr>
<tr>
<td>[58]</td>
<td>114</td>
<td>2.5–10</td>
<td>No additional value of hK11 in discriminating PCa from non-PCa</td>
</tr>
</tbody>
</table>

PCa = prostate cancer; PSA = prostate-specific antigen; BPH = benign prostatic hyperplasia; fPSA = free PSA; tPSA = total PSA.
4. Conclusions

Although PSA is one of the best tumour markers currently available for medical practice, the major drawback of PSA is its relative lack of specificity for PCa, especially within the lower PSA range of 4–10 ng/ml. Within this range, it has been shown that about 60% of all men receive unnecessary biopsies [80–82]. Moreover, there is no PSA concentration that rules out the presence of PCa, illustrated by the fact that 15% of men with a PSA serum level <4.0 ng/ml have PCa, of which 15% have a Gleason score >7 [83]. Also, PSA is unable to differentiate aggressive from indolent disease. Commonly used predictors to assess tumour aggressiveness such as biopsy Gleason score and clinical tumour stage are subject to intra- and interobserver biases. Consequently, major efforts are being put into the discovery of new markers for PCa. This effort has not yet resulted in any additional markers that are suitable for clinical use and implementation, such as in PCa guidelines by the American Urological Association (AUA), the European Association of Urology (EAU), and the National Comprehensive Cancer Network (NCCN). This review focused on the clinical potential of the various isoforms of PSA as well as on the other members of the tissue kallikrein family and their potential value in PCa detection and prognosis.

A drawback regarding most reviewed publications is that the studied populations are retrospectively collected based on earlier PSA measurements, instead of relying on prospectively collected fresh serum samples. Also, the assay systems and antibodies utilized to measure the concentrations of PSA subforms and other tissue kallikreins differ widely between the reviewed studies, complicating outcome analyses and possibilities regarding PCa detection. Lastly, most of the study results are based on relatively small populations.

The studies with a larger population evaluating the potential of pPSA showed that %pPSA is able to improve PCa detection over %fPSA within the tPSA range of 2–10 ng/ml. Also, specificity for PCa is slightly increased by %pPSA in comparison with %fPSA. Taken together, this implies that %pPSA could be useful as an additional marker in early diagnosis and screening for PCa in the 2–10-ng/ml PSA range. Data regarding the performance of %pPSA for tPSA levels of <2 ng/ml are currently insufficient to draw conclusions. Available studies hint at a limited relationship of pPSA with tumour stage and grade. Generally, (−2)pPSA performs worse than pPSA regarding PCa detection as well as PCa prognosis. Regarding (−5, −7)pPSA and BPSA, no additional value over tPSA and fPSA in the detection and prognosis of PCa has been shown.

Of the other members of the tissue kallikrein family, hK2 holds particular promise. Generally, hK2 levels are significantly higher in men with PCa compared with men without PCa. In the PSA range of 4–10 ng/ml, hK2 or %hK2 performed better than fPSA alone in the detection of PCa. In contrast, hK2 tends to perform worse than fPSA in men with PSA levels <4 ng/ml. Most studies investigating the possible prognostic role of hK2 show a limited increase in predicting stage and tumour grade after addition of hK2 or hK2 incorporated in ratios with fPSA or tPSA. Data on hK11 show that it cannot be recommended for diagnosing PCa, as it possesses no additional value over fPSA and/or tPSA. Regarding the remaining members of the tissue kallikrein family, future research should clarify whether there is a possible role in PCa diagnosis or prognosis.

In conclusion, it has been shown that the early diagnosis of PCa within the 4–10-ng/ml tPSA range can be significantly improved by the addition of pPSA and hK2. For tPSA levels <4 ng/ml, the role for hK2 is limited, whereas the possibilities for pPSA have been insufficiently studied. The prognostic value of PSA subforms as well as of other members of the tissue kallikrein family is limited with regard to existing parameters, such as the biopsy Gleason score. Before pPSA or hK2 can be implemented in existing PCa guidelines, future research should clarify whether the promises of pPSA and hK2 with respect to an improved diagnosis of PCa hold in a prospective setting and are suitable for routine application. Aside from pPSA and hK2, other promising novel markers should be thoroughly investigated to create a comprehensive set of markers for the entire spectrum of PCa within the male population. Key factors are the use of appropriate study designs and data analyses to obtain unbiased and reproducible results. Importantly, investigation from an economic point of view should assess whether the increase in PCa predictive value of these additional markers over existing markers is large enough to be commendable.

Author contributions: Flip H. Jansen had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Schröder, Bangma.
Acquisition of data: Jansen, Jenster.
Analysis and interpretation of data: Jansen, Bangma.
Drafting of the manuscript: Jansen.
Critical revision of the manuscript for important intellectual content: Schröder, Bangma, Roobol.
Statistical analysis: None.
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Supervision: None.
Other (specify): None.

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