

Review

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Emerging biomarkers in the detection and prognosis of prostate cancer

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Abstract: The prostate-specific antigen (PSA) is currently the most used tumor marker in the early detection of the prostate cancer (PCa), despite its low specificity and low negative predictive value. New biomarkers, including urine prostate cancer gene 3 (*PCA3*) score, Prostate Health Index (PHI), and the four-kallikrein panel, have been investigated during recent years especially with the aim of detecting aggressive PCa. Results suggest the ability of these biomarkers to improve the specificity of PSA in the detection of PCa, although there are not enough results directly comparing these biomarkers to know their complementarity. The relationship with PCa aggressiveness seems to be confirmed for PHI and for the four-kallikrein panel, but not for *PCA3* score. However, available results suggest that emerging biomarkers may be useful as part of a multivariable approach for the screening and prognosis of PCa. Nevertheless, larger prospective studies comparing these biomarkers are necessary to evaluate definitely their value in the management of early PCa.

Keywords: four-kallikrein panel; proPSA; prostate cancer; prostate cancer gene 3 (*PCA3*); Prostate Health Index; prostate-specific antigen (PSA).

Introduction

The prostate-specific antigen (PSA) is currently the most used tumor marker in the early detection of the prostate cancer (PCa), despite the disadvantages of its use [1]. First,

several benign prostate pathologies (benign prostatic hyperplasia, acute prostatitis) and the manipulation of the prostate can cause elevation of PSA serum levels. There is certainly a relationship between the elevation of PSA and the probability of PCa in the biopsy. However, the biopsy is positive in only 25% of patients with PSA in the range between 2 and 10 µg/L [2]. A second problem is related to the increasing detection of no clinically significant tumors in relation to the extensive use of PSA for the last years. This fact, together with the decision to do a biopsy every time with lower and lower PSA concentrations, has led to the detection of very low aggressive tumors, so overdiagnosis and overtreatment of PCa affects between 27% and 56% of new diagnoses [3, 4].

New biomarkers of PCa have been described during recent years with the aim of increasing the diagnostic specificity and differentiating between aggressive cancers and clinically insignificant cancers [5, 6]. This review covers the recent developments in this area and remarks the most promising biomarkers in the management of patients with PCa. Some of the new biomarkers are related to PSA, such as Prostate Health Index (PHI), which is calculated with serum concentrations of [–2] proPSA (p2PSA), total PSA, and free PSA, and four-kallikrein panel, which includes total PSA, free PSA, intact PSA, and human kallikrein 2 (hK2). A completely different way is the study of genes associated with PCa, notably the *PCA3* assay (prostate cancer gene 3), which involves the measurement of the messenger RNA (mRNA) of this gene in urine obtained after a prostate massage.

PCA3 score

Cumulative data suggest the role of non-coding RNAs (ncRNAs) in the initiation and development of PCa. Some ncRNAs are detectable in several body fluids, including plasma and urine, opening a promising frame for the management of early PCa. MicroRNAs (miR) (e.g., miR 141, miR 375) and long ncRNAs (e.g., *PCA3*) are the most studied of those biomarkers [7].

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PCA3 gene, initially called *DD3*, was discovered in 1999 by Bussemakers et al. [8] as a widely overexpressed gene in PCa. *PCA3* is composed of four exons and three introns and is located on chromosome 9q21–22 in antisense orientation for within intron 6 of the Prune Homolog 2 gene (*PRUNE2* or *BMCC1*) [9]. The most common transcript contains exons 1, 3, 4a, and 4b (Figure 1) [10], although exon 2 has been described as specifically amplified in samples with PCa, but not in hypertrophic tissue [9]. Further characterization of the *PCA3* transcript sequence revealed alternative splicing at exon 2 and alternative polyadenylation at exon 4, and due to a very short open reading frame, it was designated as an ncRNA. Ferreira et al. [11] found that *PCA3* silencing with siRNA decreases cell growth and induces apoptotic cell death, suggesting the relationship between *PCA3* and tumor aggressiveness.

The *PCA3* test is the measurement of mRNA of the gene *PCA3* in urine obtained after a prostate massage to release prostate cells into the urinary tract. Measurement of mRNA of *PCA3* must be performed simultaneously with the mRNA of *PSA* gene because the last one is not overexpressed in PCa, and this fact allows the normalization of the amount of mRNA of *PCA3* in the studied samples.

The first published works to assess the performance of *PCA3* in the detection of PCa used a quantitative reverse transcriptase real-time polymerase chain reaction technique (RT-qPCR) [12–14]. Groskopf et al. [15], in 2006, introduced a new test that included isolation, amplification, and quantification of mRNA from *PCA3* and *PSA* using the Gen-Probe GTS400 system. This test obtained the Conformité européenne (CE) in November 2006 and was approved by the Food and Drug Administration (FDA) in 2012 to decide the repetition of the prostate biopsy in men older than 50 years old who have one or more previous negative biopsies. The company Gen-Probe, now merged with Hologic, has promoted the automation and standardization of this test; therefore, the number of

studies published on *PCA3* has increased progressively, and now the PubMed database includes 266 items (of which 47 cases are reviews) when a search for keywords “*PCA3*” and “PCa” is done. This documentation includes only three meta-analyses and a comparative review of the effectiveness of *PCA3* score that were published between 2010 and 2014. The meta-analysis published in 2010 by Ruiz-Aragón and Márquez-Peláez [16], based in 14 moderate to high-quality studies, concluded that the measurement of *PCA3* score in urine is useful in the detection of PCa. According to this meta-analysis, sensitivity ranged from 46.9% to 82.3%, specificity ranged 56.3%–89%, positive predictive value from 39% to 75.8%, and negative predictive value from 61.4% to 89.7%.

The FDA has recommended using a cutoff value of 25 for the *PCA3* test of Progenisa to indicate the repetition of biopsy, despite the most widely used discriminant value is 35, as Ruiz-Aragón and Márquez-Peláez explained in their meta-analysis.

The comparative review published by Bradley et al. [17] identifies 34 observational studies and concludes that the diagnostic accuracy of *PCA3* score is higher than the diagnostic accuracy of tPSA, but also notes that the evidence is not enough to conclude that the measurement of the *PCA3* score allows obtaining improved global results. The sensitivity of *PCA3* score in this study was from 94.3% for the cut-off value of 10%–61.1% for the cutoff value of 35%, with a percentage of missed cancers of 6% and 39%, respectively. The percentage of saved biopsies was 22% and 70%, respectively. The results of two recent meta-analyses published by Luo et al. [18, 19] are consistent with previously published data to emphasize that *PCA3* can be used for early diagnosis of PCa and to avoid unnecessary biopsies, although they also highlight the heterogeneity of published studies. Luo et al. [18] reported that the sensitivity ranged between 46.9% and 82.3%; specificity was from 55% to 92%; positive

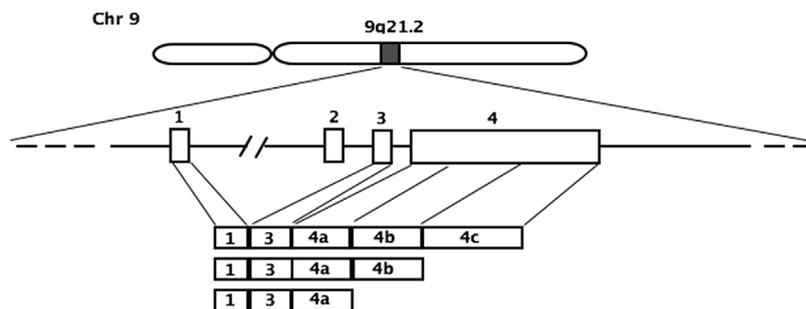


Figure 1 Structure of the *PCA3* gene.

PCA3 has four exons. The most frequent transcript contains exons 1, 3, 4a, and 4b. Exon 2 is only present in 5% of transcripts, but it has been described as specifically amplified in samples with PCa.

predictive value had a range of 39%–86%; the negative predictive value was 61%–89.7%.

To sum up, the available results suggest that PCA3 score is useful in the detection of early PCa, especially for patients with previous negative biopsy (Table 1). However, one of the most controversial issues about PCA3 score concerns the definition of the cutoff used to detect PCa with an appropriate sensitivity and specificity. Haese et al. [20] indicated that a PCA3 score of 35 provided the optimal balance between sensitivity (47%) and specificity (72%),

even when 21% of high-grade tumors would have been missed. The number of missed cancers may be reduced using lower cutoffs. Crawford et al. [21] showed a reduction of missed cancers from 21.6% to 5.6% when the cutoff is changed from 35 to 10. Meanwhile, a high PCA3 score, even higher than 100, does not ensure the existence of a tumor. According to Haese et al. [20], the PCa detection rate was only of 47% in patients with PCA3 score >100. This is an unclear point due to tissue specificity of PCA3 and the differential expression of this biomarker in normal

Table 1 Performance of PCA3 score in the management of early PCa.

Author	Cohort	AUC	Relation with aggressiveness
Hessels et al. [12]	108 Consecutive patients admitted for prostatic biopsies based on serum PSA levels above 3 ng/mL	0.72	Not reported
Fradet et al. [13]	517 Men undergoing transrectal ultrasound-guided prostate biopsy at five medical centers	0.86	Not reported
Tinzl et al. [14]	Prospective study including 201 patients with an elevated serum tPSA level and/or abnormal DRE referred for prostate biopsy	0.87	Not reported
Groskopf et al. [15]	Three groups: men scheduled for prostate biopsy (n=70), healthy men (<45 years with no known PCa risk factors; n=52), men who had undergone RP (n=21)	0.746	Not reported
Ruiz-Aragón and Márquez-Peláez [16]	Meta-analysis	0.63–0.87	Not reported
Luo et al. [18]	Meta-analysis	0.63–0.87	Not reported
Luo et al. [19]	Meta-analysis	0.577–0.730	Not reported
Haese et al. [20]	Prospective cohort study of 463 patients with one or two negative biopsies	0.658	Yes, with Gleason score and clinical stage
Crawford et al. [21]	Prospective and multicenter study of 1962 men with PSA >2.5 ng/mL and/or abnormal DRE	0.706	Yes, with Gleason score
Capoluongo et al. [22]	734 Patients who underwent first prostate biopsy for suspected PCa	0.775	No correlation with Gleason score
van Poppel et al. [23]	Pooled analysis (n=1009; 173 treated with RP) of data from two multicenter, European, prospective trials evaluating patients at initial or repeat biopsy	Not reported	Yes, with biopsy and pathological Gleason score
Durand et al. [24]	Prospective, multicenter study including 160 patients with localized PCa	Not reported	Yes, with biopsy and pathological Gleason score, and with pathological stage
Hessels et al. [25]	351 Men admitted for prostate biopsies based on serum PSA >3 ng/mL, and abnormal DRE	Not reported	No correlation with clinical and pathological stage or with biopsy and pathological Gleason score
Ploussard et al. [26]	106 Consecutive prospective low-risk PCa; patients treated with RP	Not reported	Useful to select patients for AS No correlation with overall unfavorable disease (pT3 and/or primary Gleason pattern 4)
van Gils et al. [27]	62 Patients with PCa treated with RP	Not reported	No correlation with pathological Gleason score or pathological stage
Chevli et al. [28]	Retrospective study; 3073 men who underwent initial prostate biopsy	0.697	No correlation with Gleason score
Foj et al. [29]	122 Patients who underwent prostate biopsy for PSA >4 µg/L	0.804	No correlation with Gleason score or clinical stage

PCa, prostate cancer; AUC, area under curve; DRE, digital rectal examination; RP, radical prostatectomy; AS, active surveillance.

and cancerous prostate cells. High PCA3 score levels in patients without PCa could be explained by the existence of tumors that are not diagnosed on biopsy. In this way, Roobol et al. [30] indicated that after a monitoring period of 19 months, the percentage of PCa detected was higher in patients with PCA3 score ≥ 100 (30% vs. 18.8%). However, the authors concluded that their findings do not offer a direct explanation of why PCA3 scores can be excessively high despite the absence of biopsy-detectable PCa [30, 31].

The relationship between PCA3 score and the aggressiveness of PCa is also controversial, with studies suggesting this relation [22–24], whereas others concluding no relationship of PCA3 score with Gleason score or with clinical and pathological stages [25–27]. A recent study performed in a cohort of 3073 men, including 1341 men with PCa, concluded that PCA3 score was statistically significantly associated with biopsy Gleason score [28], even when ROC analysis showed that PCA3 did not significantly outperform PSA in the prediction of high-grade PCa (AUC 0.682 vs. 0.679, respectively, $p=0.702$). Also, in a recent evaluation concerning PCA3 score, we did not find correlation with Gleason score or with clinical and pathological stages [29]. No explanation has been proposed by any author for this disagreement.

p2PSA and PHI

tPSA circulates together with several protease inhibitors, such as α -1-antichymotrypsin, whereas a small fraction that has been previously inactivated circulates as free PSA. There are several commercial tests for measuring free PSA, whereas the complexed PSA test, which is

commercialized by Siemens, measures the PSA bound to the α -1-antichymotrypsin. The utility of the percentage of free PSA in the detection of PCa has been described in several studies [32, 33], although they also highlight its limitations. In this way, a meta-analysis published in 2006 confirms that %fPSA only provides additional information when levels reach extreme values [34]. The description of several isoforms of fPSA (BPSA, iPSA, and proPSA) has introduced an improvement in the detection of PCa [35, 36]. Of the three isoforms, only proPSA is associated with the presence of PCa. The native form of proPSA is [-7] proPSA, which contains a 7-amino acid N-terminal pro-leader peptide. Through the proteolytic cleavage of this peptide, promoted by the kallikreins hK2 and hK4, the other fractions of proPSA known as [-2] [α 4] and [α 5] proPSA are formed (Figure 2).

New strategies to increase the accuracy of tPSA have been described since the description of a new assay for the measurement of p2PSA, the most stable proPSA isoform. Several studies have suggested the value of two tests derived from p2PSA (Table 2). On the one hand, the percentage of p2PSA related to fPSA (%p2PSA), and on the other hand, PHI, which combines the concentration of p2PSA, fPSA, and tPSA according to the formula $(p2PSA/fPSA) \cdot \sqrt{tPSA}$. This index, which in 2012 received approval by the FDA, would be indicated in men older than 50 years, tPSA between 4 and 10 $\mu\text{g/L}$ and negative digital rectal examination (DRE), in which group of patients would help to reduce the number of negative biopsies. Despite the high cost of the test for determining p2PSA, the use of PHI in the detection of PCa decreases global costs. The additional blood test costs are compensated by the savings on the costs of physician office visits and the avoidance of unnecessary biopsies [47, 48].

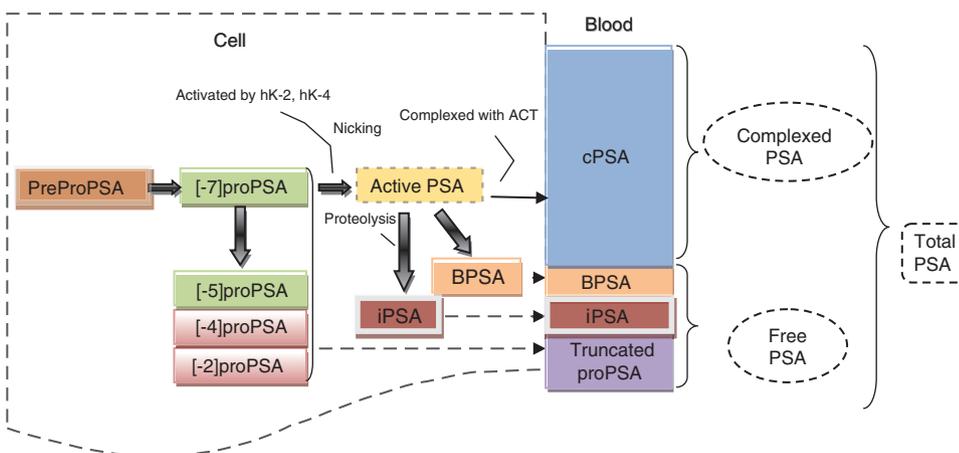


Figure 2 Molecular forms of PSA.

Table 2 Performance of PHI and %p2PSA in the management of early PCa.

Authors	Cohort	Biomarker or multivariate model	AUC	Relation with aggressiveness
Filella et al. [37]	Meta-analysis	%p2PSA	0.635–0.78	Not available
		PHI	0.703–0.77	
		tPSA	0.50–0.585	
		%fPSA	0.58–0.77	
Wang et al. [38]	Meta-analysis	%p2PSA	0.638–0.78	Not available
		PHI	0.69–0.781	
		tPSA	0.50–0.60	
		%fPSA	0.52–0.77	
Stephan et al. [39]	1362 Patients from 4 different study sites with tPSA 1.6–8.0 µg/L	%p2PSA	0.72	Yes, with Gleason score
		PHI	0.74	
Lazzeri et al. [40]	Prospective cohort study of 646 patients from five European urologic centers with tPSA 2–10 ng/mL	%p2PSA	0.67	Yes, with Gleason score
		PHI	0.67	
Filella et al. [41]	354 Patients with positive or negative prostatic biopsy	%p2PSA	0.723	Yes, with Gleason score and clinical stage
		PHI	0.732	
Sokoll et al. [42]	Prospective multicenter study including 566 patients with prostate biopsy	Multivariate model (age, race, DRE, and PCa family history+PSA+%fPSA+%p2PSA). tPSA 2–10 µg/L	0.76	Yes, with Gleason score
		Multivariate model (age, prostate volume, DRE, tPSA, %fPSA+PHI or %p2PSA)	0.75	
Stephan et al. [43]	586 Patients with positive or negative prostatic biopsy	Multivariate model (age, prostate volume, DRE, tPSA, %fPSA+PHI or %p2PSA)	0.75	Yes, with pathological Gleason score and pathological stage
		Multivariate model (age, prostate volume, tPSA, %fPSA+%p2PSA)	0.82	
Guazzoni [44]	Prospective cohort study of 268 patients with tPSA 2.0–10 ng/mL and negative DRE	Multivariate model (age, prostate volume, tPSA, %fPSA+%p2PSA)	0.83	Yes, with Gleason score
		Multivariate model (age, prostate volume, tPSA, %fPSA+PHI)	0.80	
Lughezzani et al. [45]	883 Patients who were scheduled for a prostate biopsy from five different European tertiary care centers	Multivariate model (age, prostate volume, DRE biopsy history+PHI)	0.80	Not available
Filella et al. [46]	220 Patients with PSA <10 µg/L	Logistic regression model (tPSA, %fPSA, age, prostate volume+PHI+%p2PSA)	0.802	Yes, with Gleason score
		Artificial neural network model (tPSA, %fPSA, age, prostate volume+PHI+%p2PSA)	0.815	
		Logistic regression model (tPSA, %fPSA, age+PHI+%p2PSA) without prostate volume	0.762	
		Artificial neural network model (tPSA, %fPSA, age+PHI+%p2PSA) without prostate volume	0.775	

PCa, prostate cancer; AUC, area under curve; DRE, digital rectal examination.

According to data collected in a meta-analysis that we published in 2013 [37] concerning 12 relevant articles evaluating these tests, the AUCs were between 0.635 and 0.78 for %p2PSA and between 0.703 and 0.77 for PHI. This accuracy was higher than that observed for tPSA, with AUCs between 0.50 and 0.585, or for %fPSA, with AUCs between 0.58 and 0.77. A recent meta-analysis published in 2014 obtained similar conclusions [38], showing that the pooled sensitivity, specificity, and AUC were 0.86, 0.40, and 0.72, respectively, for %p2PSA and were 0.85, 0.45, and 0.70, respectively, for PHI.

Among the articles currently published, we highlight two multicenter studies that have assessed the utility of PHI in patients with tPSA between 2 and 10 µg/L. Stephan

et al. [39], in a study including 1362 patients, showed AUCs of 0.72 and 0.74 for %p2PSA and PHI, respectively. Furthermore, Lazzeri et al. [40] published a prospective European study including 646 patients that showed AUCs of 0.67 for both biomarkers. Our group obtained similar results in a study including patients with tPSA between 1.92 and 36.90 µg/L, showing AUCs of 0.723 and 0.732 for %p2PSA and PHI, respectively [41].

Published studies showed that %p2PSA as well as PHI are related to PCa aggressiveness, with higher levels of these tests in patients with Gleason score higher than 6 and in patients with locally advanced tumors [42–44, 49]. In our experience, %p2PSA and PHI are significantly higher in patients with biopsy Gleason score ≥ 7 , whereas

no differences among these groups of patients were found in relation to %fPSA. Also, we found a significant association between elevated levels of %p2PSA and PHI and clinical stage T2–3 [41]. The availability of aggressiveness biomarkers is a relevant point because a substantial proportion of new diagnosed tumors are candidates to active surveillance, due to their features of clinically insignificant PCa. Significantly, Tosoian et al. [50] suggested that PHI appears to provide improved prediction of biopsy reclassification during follow-up of patients on active surveillance.

Mikolajczyk et al. [51] have suggested the utility of combining several biomarkers in a multivariate model for the detection of PCa, considering that this fact would reflect the multidimensional nature of prostate disease, which ranges from metastatic cancer to benign hyperplasia and inflammation. The inclusion of %p2PSA in a multivariate model was suggested firstly by Sokoll [42], obtaining an AUC of 0.76 for patients with tPSA from 2 to 10 $\mu\text{g/L}$. That model included tPSA, %fPSA, and %p2PSA, together with several clinical and demographic factors. Afterward, several studies have assessed the inclusion of %p2PSA and PHI in a multivariate model to detect PCa. Stephan et al. [39] improved accuracy in the detection of PCa by adding %p2PSA or PHI (AUC 0.75, in both cases) to a multivariate model based on patient age, prostate volume, DRE, tPSA, and %fPSA, which had an AUC of 0.69. Also, a multivariate model described by Guazzoni et al. [44] showing a gain in predictive accuracy of 0.10 or 0.11, respectively, when %p2PSA (AUC 0.82) or PHI (AUC 0.83) were added to a base model including patient age, prostate volume, tPSA, and %fPSA (AUC 0.72).

Otherwise, Lughezzani et al. [52] developed a PHI-based nomogram to assist clinicians in the decision of performing a biopsy. Including PHI in a multivariable logistic regression model based on patient age, prostate volume, DRE, and biopsy history significantly increased predictive accuracy from 0.73 to 0.80. This nomogram was externally validated by a multicenter European study based on 833 patients, obtaining an AUC of 0.752 [45].

Our group, in a recent study, showed that accuracy increased from 0.762 to 0.802 (logistic regression model) or 0.815 (artificial neural network) when PHI and %p2PSA were included in a multivariable analysis based on patient age, prostate volume, tPSA, and %fPSA [46]. Furthermore, our results showed the relationship between prostate volume and PHI values. PHI performance was better in patients with small prostate volume (AUC 0.818) compared with those with medium (AUC 0.716) or large prostate volume (0.654). In addition, excluding prostate

volume of the multivariate models to detect PCa make the accuracy to decrease substantially (AUC was 0.762 using a logistic regression model and 0.775 using an artificial neural network), showing that prostate volume is a key factor in the interpretation of the values of PHI.

Four-kallikrein panel

The kallikrein family is composed of 15 substances characterized by its protease activity [53]. The human kallikrein type 2 (hk2) is the one that has a greater interest in the detection of PCa, regardless of kallikrein 3, better known as PSA. Although hk2 and PSA are widely expressed in prostate tissue, both kallikreins differ in their enzymatic activity and show independent expression patterns [54]. Thus, hk2, which shows 80% homology with the sequence of PSA, is responsible, together with hk4, for post-translational proteolytic cleavage of proPSA into the active form of PSA [53]. hK2 also differs substantially from PSA in relation of its concentration because it is only about 2% of the concentration of tPSA.

The initial studies that assessed the utility of hk2 showed that the percentage of hk2 compared with fPSA could be used to increase the specificity in the detection of PCa in patients with PSA between 2 and 10 $\mu\text{g/L}$ [55, 56]. More recently, Stephan et al. [57] observed that a neural network that included hk2 improved the outcome of %fPSA but not the outcome of hK2/(f/tPSA) or hk2/fPSA in almost all analyzed subgroups. Despite discrepant results [58], there are data that suggest the relation of hK2 with a high Gleason score and with extracapsular extension of the tumor [59, 60].

In addition, the group led by Lilja and Vickers included hk2 in a multivariate model to predict the presence of PCa [61–67]. This model, which incorporates hk2, tPSA, fPSA, and iPSA, together with DRE, the age of the patient, and previous prostate biopsy, improves the accuracy obtained with other models. Table 3 illustrates the performance of four-kallikrein panel according to several studies published by this group, showing the gain in accuracy in relation to the basic laboratory model or the basic clinical model. Results are shown considering the usefulness of the four-kallikrein panel in the detection of any cancer and in the detection of a high-grade cancer (Gleason score ≥ 7). Accuracy was higher in the prediction of cancer in unscreened patients (0.764–0.832 for four-kallikrein panel; 0.776–0.836 for four-kallikrein panel+DRE) and in the detection of high-grade cancer (AUC 0.793–0.870 for four-kallikrein panel; 0.798–0.903 for four-kallikrein panel+DRE). Also, according to

Table 3 Performance of four-kallikrein panel in the management of early PCa.

Authors	Cohort	Increase in AUC: base laboratory model vs. four-kallikrein panel	Increase in AUC: base clinical model vs. four-kallikrein panel+DRE
Vickers et al. [61]	740 Unscreened men, Goteborg, ERSPC	Any cancer: 0.680 vs. 0.832 High-grade cancer: 0.816 vs. 0.870	Any cancer: 0.724 vs. 0.836 High-grade cancer: 0.868 vs. 0.903
Vickers et al. [62]	2914 Unscreened men, Rotterdam, ERSPC	Any cancer: 0.637 vs. 0.764 High-grade cancer: 0.776 vs. 0.825	Any cancer: 0.695 vs. 0.776 High-grade cancer: 0.806 vs. 0.837
Vickers et al. [63]	1501 Previously screened men, Rotterdam, ERSPC	Any cancer: 0.557 vs. 0.713 High-grade cancer: 0.669 vs. 0.793	Any cancer: 0.585 vs. 0.711 High-grade cancer: 0.709 vs. 0.798
Vickers et al. [64]	1241 Men who underwent biopsy for elevated PSA during their second or later visit, Gothenburg, ERSPC	Any cancer: 0.564 vs. 0.674 High-grade cancer: 0.658 vs. 0.819	Any cancer: 0.622 vs. 0.697 High-grade cancer: 0.717 vs. 0.828
Gupta et al. [65]	925 Men with a previous negative prostate biopsy and PSA ≥ 3 $\mu\text{g/L}$, Rotterdam, ERSPC	Not evaluated	Any cancer: 0.584 vs. 0.681 High-grade cancer: 0.764 vs. 0.873
Benchikh et al. [66]	262 Men, biopsy was based on clinical judgment following additional workup such as DRE or additional PSA test, Tarn, ERSPC	Not evaluated	Any cancer: 0.628 vs. 0.782 High-grade cancer: 0.767 vs. 0.870
Vickers et al. [67]	792 Men with PSA ≥ 3 $\mu\text{g/L}$, Malmö Diet and Cancer cohort	Any cancer: 0.654 vs. 0.751 Palpable cancer: 0.708 vs. 0.803	Not evaluated

Base laboratory model: patient age, tPSA; base clinical model: patient age, tPSA, and DRE.

Carlsson et al. [68], the four-kallikrein panel distinguishes between pathologically insignificant and aggressive disease on pathological examination of the radical prostatectomy specimens in a series of 392 patients. The addition of four-kallikrein panel enhanced the base model, increasing AUC from 0.81 to 0.84 ($p < 0.005$), distinguishing between pathologically insignificant and aggressive disease after radical prostatectomy with good accuracy. Besides, according to data related to a study including 12,561 men followed up for more than 15 years, the four-kallikrein panel increased accuracy in predicting from 15 to 20 years the risk of metastasis among men with PSA ≥ 2 $\mu\text{g/L}$ at age 50 or 60 years [69].

A recent meta-analysis summarizes the published results from published studies concluding that the four-kallikrein panel has the potential to improve patient outcomes and to reduce costs [70]. According to this meta-analysis, this improvement ranged between 10% and 13% for identification of any PCa and between 8% and 10% for identification of high-grade prostate tumors. Also, the authors underline that the detection of high-grade tumors was delayed in only 0.63%–0.70% of patients compared with biopsying all patients with PSA ≥ 3 $\mu\text{g/L}$, using, respectively, the four-kallikrein panel or the four-kallikrein panel+DRE. More recently, a multicenter and prospective evaluation of the four-kallikrein panel in the

detection of high-grade PCa has been published [71]. The study included 1012 men scheduled for prostate biopsy, obtaining an AUC of 0.82. The authors reported a reduction of 30%–58% in the number of biopsies, missing only 1.3%–4.7% of high-grade PCa patients, depending on the threshold (between 6% and 15%, for young and old patients, respectively) used for biopsy.

Conclusions

tPSA remains the most used biomarker in the management of early PCa, despite the controversies around its use as a screening tool. Actually, findings show that screening based only on PSA results in a small or no reduction of PCa-specific mortality and is associated with harms related to overdiagnosis and overtreatment [72, 73]. Meanwhile, tPSA is a strong predictor of PCa [74] and is related to PCa aggressiveness [75]. Emerging biomarkers should improve the performance of tPSA in these points. Available results showed that PHI and four-kallikrein panel outperform the specificity of tPSA and %fPSA, maintaining the relationship with aggressiveness. Furthermore, PCA3 score, even when the most appropriate cutoff has to be established, performs better than tPSA and %fPSA,

although its relationship with the aggressiveness of the tumor is controversial.

A comparison between these biomarkers is necessary to evaluate accurately their complementary usefulness in the detection of PCa and in the prediction of its aggressiveness. At the moment, the widest comparison between PCA3 score and PHI has been published by Stephan et al. [76], showing no significant differences in accuracy between PCA3 score and PHI (AUC 0.74 vs. 0.68), even when only PHI correlated with biopsy Gleason score. However, significant differences between both biomarkers has been reported by Scattoni et al. [77], showing that PHI was more accurate than PCA3 score for predicting PCa (AUC 0.70 vs. 0.59). Meanwhile, Tallon et al. [78] showed that PCA3 score and PHI are predictors of pathological characteristics of PCa at radical prostatectomy. PCA3 score was related to tumor volume ≥ 0.5 mL and multifocality, whereas PHI was related to tumor volume ≥ 0.5 mL, Gleason score ≥ 7 , and extracapsular extension. More recently, two new studies compared the four-kallikrein panel with PHI and PCA3 score in unscreened and previously screened cohorts, respectively. Nordström et al. [79] evaluated the four-kallikrein panel and PHI in a series of 531 men with PSA levels between 3 and 15 $\mu\text{g/L}$. Both tests performed similarly in the detection of any-grade PCa and high-grade PCa (AUCs: 0.69 and 0.718 for the four-kallikrein panel and 0.704 and 0.711 for PHI). Finally, Vedder et al. [80] compared PCA3 and the four-kallikrein panel in a prescreened cohort of 708 patients in which biopsy was performed if PSA was ≥ 3 $\mu\text{g/L}$ or PCA3 score ≥ 10 , interestingly avoiding bias caused by the selection of patients according to PSA serum levels, even if a previous biopsy was performed in 29% of all participants according to PSA serum levels and DRE. In 202 men with an elevated PSA, the four-kallikrein panel discriminated better than PCA3 score (AUC 0.78 vs. 0.62), but PCA3 score ran better in the global population (AUC 0.63 vs. 0.56). Additionally, the authors showed that both tests added value to a multivariate model (0.73 for PCA3 score and 0.71 for the four-kallikrein panel), even if significant differences in relation to multivariate model (AUC 0.70) were found only for PCA3 score ($p=0.02$).

To summarize, current results suggest that emerging biomarkers may be useful as part of a multivariable approach to screening and prognosis of PCa. However, larger prospective studies, including the four-kallikrein panel and avoiding bias due to pre-selection of patients according to tPSA serum levels, are necessary to evaluate definitely their value in the management of early PCa.

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