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Review Article

Clinical Use of [-2]proPSA (p2PSA) and Its Derivatives (%p2PSA and Prostate Health Index) for the Detection of Prostate Cancer: A Review of the Literature

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Prostate-specific antigen (PSA) is recognized as an organ-specific marker with low specificity and sensitivity in discriminating prostate cancer (PCa) from other benign conditions, such as prostatic hyperplasia or chronic prostatitis. Thus, in the case of clinical suspicion, a PCa diagnosis cannot be made without a prostate biopsy. [-2]proPSA (p2PSA), a precursor of PSA, has been investigated as a new marker to accurately detect PCa. The aim of this systematic review was to discuss the available literature regarding the clinical validity and utility of p2PSA and its derivatives, p2PSA/fPSA (%p2PSA) and the Prostate Health Index (PHI). A systematic search of the PubMed and Scopus electronic databases was performed in accordance with the PRISMA statement (http://www.prisma-statement.org), considering the time period from January 1990 to January 2014 and using the following search terms: proprostate specific antigen, proenzyme PSA, proPSA, [-2]proPSA, p2PSA, Prostate Health Index, and PHI. To date, 115 studies have been published, but only 35 were considered for the qualitative analysis. These studies suggested that p2PSA is the most cancer-specific form of PSA, being preferentially expressed in PCa tissue and being significantly elevated in the serum of men with PCa. It is now evident that p2PSA, %p2PSA, and PHI measurements improve the specificity of the available tests (PSA and derivatives) in detecting PCa. Moreover, increasing PHI values seem to correlate with more aggressive disease. Some studies have compared p2PSA and its derivatives with other new biomarkers and found p2PSA to be significantly more accurate. Indeed, the implementation of these tests in clinical practice has the potential to significantly increase the physician's ability to detect PCa and avoid unnecessary biopsies, while also having an effective impact on costs. Further studies in large, multicenter, prospective trials are required to confirm these encouraging results on the clinical utility of these new biomarkers.

Keywords: *[-2]proPSA; Diagnosis; Prostate cancer; Prostate health index*

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INTRODUCTION

Prostate-specific antigen (PSA) is widely known as a serum biomarker for the early detection of prostate cancer (PCa). Its introduction in clinical practice in the early 1990s changed PCa diagnosis and management. Currently in Western countries, PSA-based opportunistic or systematic screening has resulted in a stage migration to more organ-confined tumors at the time of diagnosis [1], with a consequently consistent reduction in PCa-related mortality observed over time [2]. However, PSA is recognized as an organ-specific marker but not a perfect PCa marker.

Indeed, it has low specificity and sensitivity, especially in the total PSA (tPSA) range of 4 to 10 ng/mL (the so-called diagnostic gray zone) [3]. PSA levels may increase as a result of benign conditions such as benign prostatic hyperplasia (BPH) [4] and chronic prostatitis [5]. Moreover, PSA levels are also affected by biological variability, which may be related to differences in androgen levels, prostate manipulation, or ejaculation [6]. Finally, sample handling, laboratory processing, and assay standardization can all alter PSA measurements [7]. Owing to these factors, it is difficult to find an appropriate PSA cutoff for PCa diagnosis (which for many years was considered to be 4 ng/mL).

Thus, definitive diagnosis is still based on prostate biopsy. According to the European Association of Urology guidelines, the need for prostate biopsy should be determined by PSA level, suspicious digital rectal examination (DRE) result, patient's biological age, potential comorbidities, and therapeutic consequences. However, biopsies are positive in only approximately 30% of patients [8]. Consequently, prostate biopsy needs to be repeated if PSA rises or is persistently elevated, if the DRE result remains suspicious, or if there is a pathological diagnosis of atypical small acinar proliferation or extensive (multiple biopsy sites) prostatic intraepithelial neoplasia from a previous biopsy [9]. Finally, PCa is not rare among men with PSA levels of less than 4 ng/mL, with a risk ranging from 6.6% in men with PSA $\leq$0.5 ng/mL to 26.9% in men with PSA of 3.1 to 4.0 ng/mL [10]. It is also important to remember that clinically significant PCa (Gleason Score [GS] $\geq$7) may be diagnosed in 15% of patients with PSA levels of less than 4 ng/mL [11].

Considerable efforts have been made to find new markers to accurately detect but also discriminate between clinically significant and insignificant PCa. Accordingly, the introduction of several PSA derivatives (free PSA [fPSA], percentage of free PSA [%fPSA], PSA density, and PSA velocity) has improved the accuracy of tPSA in detecting PCa in clinical practice. Recently, fPSA was found to include several subforms, such as a precursor form of PSA (proPSA). PSA is an androgen-regulated chymotrypsin-like serine protease that is produced in high levels within the prostatic ductal and acinar epithelium. PSA has a 17–amino acid leader sequence (preproPSA) that is cleaved co-translationally to generate an inactive precursor protein (proPSA) with seven additional amino acids compared with mature PSA [12-14]. The partial removal of the leader sequence of the preproPSA leads to other truncated forms of proPSA. Thus, theoretically, seven isoforms of proPSA should exist, although only [-1], [-2], [-4], [-5], and [-7]proPSA have been found. There is still no evidence of [-3] or [-6]proPSA [15,16]. However, all forms of proPSA are enzymatically inactive [17]. It is possible to detect three truncated forms of proPSA in serum ([-2], [-4], and [-5/-7]proPSA), of which [-2]proPSA (p2PSA) is the most stable form [15,18].

Notably, p2PSA was found to be elevated in peripheral gland cancer tissue and to be specifically higher in serum from patients with PCa [18,19]. Thus, in the past decade, it has been under investigation as a potentially more accurate test for PCa detection in clinical practice. This systematic review focused on recently published studies investigating the clinical validity and utility of p2PSA and its derivatives, p2PSA/fPSA (%p2PSA) and the Prostate Health Index (PHI).

MATERIALS AND METHODS

1. Search strategy

A systematic search of the PubMed and Scopus electronic databases was performed by three investigators (A.A., G.L., M.L.) in accordance with the PRISMA statement (http://www.prisma-statement.org). Title, abstract, or keyword lists were searched, from January 1990 to January 2014, for combinations of the following free search terms: "proprostate specific antigen," "proenzyme PSA," "proPSA," "[-2]proPSA," "p2PSA," "prostate health index," and "PHI." The search was performed for each term alone or in combination with "prostate cancer" and "prostate biopsy."

2. Eligibility criteria

Titles and abstracts of each available study were reviewed, with a focus on the diagnostic and predictive characteristics of p2PSA, %p2PSA, and PHI compared with PSA and other available PCa biomarkers. Only scientific articles in English that reported original data were included. Priority was given to the most complete studies when the same population was reported and similar results were shown. Studies that failed to report a specific and detailed outcome or those not adding any novelty were excluded.

RESULTS

Of the more than 115 published papers, 35 were considered in this review (Fig. 1).

1. Clinical validity of proPSA isoforms in improving PSA specificity

Four important studies investigated the clinical validity of proPSA, which was defined as the sum of the [-2], [-4], and [-5/-7] forms. The first study by Sokoll et al. [20] involved archival serum obtained before biopsy from 119 men (31 PCa, 88 noncancer) with PSA of 2.5 to 4.0 ng/mL. The serum levels of tPSA, fPSA, and proPSA and the proPSA/fPSA ratio (%proPSA) were analyzed: PSA and %fPSA values were similar between the noncancer and PCa groups, whereas %proPSA was relatively higher in PCa patients (50.1% ± 4.4%) than in the noncancer group (35.5% ± 6.7%, p=0.07). The areas under the curve (AUCs) for %proPSA and %fPSA were 0.688 and 0.567, respectively. At fixed sensitivity (75%), the specificity was significantly greater for %proPSA (59%) than for %fPSA (33%, $p<0.0001$). These results were then confirmed in a follow-up study of the same group [21]. In multivariate logistic regression analyses, at fixed sensitivity (90%), the combination of proPSA with tPSA and %fPSA showed significantly higher specific-

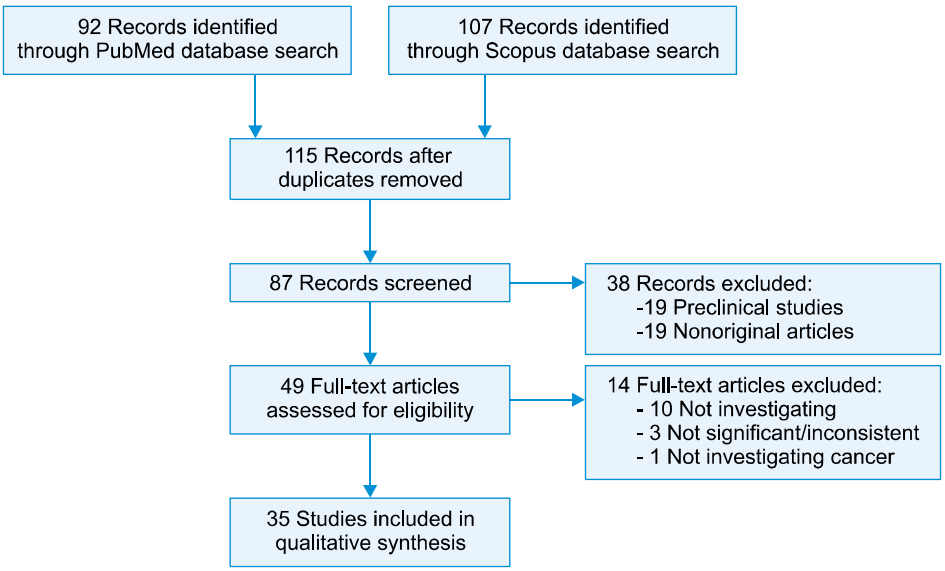

FIG. 1. Flow of information through the different phases of the systematic review. PSA, prostate-specific antigen; p2PSA, [-2]proPSA.

TABLE 1. Studies investigating the accuracy of p2PSA and %p2PSA in detecting PCa

Study	Year	No. of patients	PSA range (ng/mL)	Results
Sokoll et al. [24]	2008	123	0.48–33.18	%p2PSA had the greatest area under the curve (AUC, 0.69) followed by p2PSA (AUC, 0.63) and %fPSA (AUC, 0.61)
Sokoll et al. [25]	2010	566	0.29–310.6	Including %p2PSA in a multivariate prediction model incorporating PSA and %fPSA improved the performance ($p<0.01$); in the 2–4 ng/mL PSA range, %p2PSA outperformed %fPSA (AUC, 0.73 vs. 0.61, p=0.01)
Stephan et al. [26]	2009	586	0.26–28.4	Multivariable model utilizing %p2PSA, %fPSA, tPSA and age, had the highest AUC (0.84) and best specificities (53.1%) compared to tPSA (22.7%) and %fPSA (45.5%) at 90% sensitivity; the %p2PSA furthermore distinguished better than tPSA and %fPSA between pT2 and pT3, and GS<7 and ≥7 PCa
Rhodes et al. [27]	2012	443	0.7–1.8[a]	The annual increase rate in p2PSA was significantly greater for men who developed enlarged prostates (median, 3.5%) or PCa (median, 8.1%) compared to those who did not develop enlarged prostates (median, 1.9%) or PCa (median, 3.5%)
Rhodes et al. [28]	2012	748	0.5–1.8[a]	Baseline p2PSA was slightly higher in black men (median, 6.3 pg/mL) than in white men (median, 5.6 pg/mL; p=0.01); it was also highly predictive of biopsy confirmed PCa

PSA, prostate-specific antigen; p2PSA, [-2]proPSA; %p2PSA, [-2]proPSA/fPSA; PCa, prostate cancer; tPSA, total PSA; fPSA, free PSA; %fPSA, fPSA/tPSA; GS, Gleason score; AUC, area under the curve.
[a]:25th–75th Percentiles.

ity (44%) for early PCa detection than did the individual variables (13%, 23%, and 33%, respectively).

Catalona et al. [22] confirmed these results in a later study in which they analyzed serum specimens from 1,091 patients (635 noncancer, 456 PCa) who underwent prostate biopsies. In men with PSA of 2 to 4 ng/mL, %proPSA (at a threshold of 1.8) detected 90% of cancers, including all (16/16) extracapsular tumors and 96.6% (28/29) of cancers with a GS≥7.

In 2004, Mikolajczyk et al. [23] retrospectively evaluated the serum samples of 380 men (238 PCa, 142 noncancer) with tPSA of 4 to 10 ng/mL. Accordingly, %proPSA had a higher AUC than %p2PSA, fPSA, and complexed PSA (AUC: 0.69, 0.64, 0.63, and 0.57, respectively). In men with %fPSA>25, %p2PSA had the highest accuracy (AUC, 0.77). At a threshold of 2.5, %p2PSA had a sensitivity of 90% and would have resulted in 36% of prostate biopsies being avoided. However, in patients with %fPSA<15, at 90% sensitivity, %proPSA had a higher accuracy (AUC, 0.703; specificity, 36%) than did %p2PSA (AUC, 0.669; specificity, 21%).

2. Clinical validity of p2PSA and %p2PSA

As shown above, p2PSA is a more cancer-specific PSA isoform. Table 1 shows studies investigating p2PSA validity and their main results. Sokoll et al. [24] evaluated the relationship between p2PSA and PCa by using serum samples of 123 men (51% PCa, 49% noncancer) enrolled in the Early Detection Research Network study. Overall, the %fPSA was significantly lower, whereas p2PSA and %p2PSA were higher, in PCa patients. Additionally, in the PSA range of 2 to 10 ng/mL, p2PSA and %p2PSA continued to be significantly associated with PCa: the AUC for

%p2PSA was 0.73 compared with 0.53 for %fPSA. Sokoll et al. [25] investigated the potential correlation between p2PSA and PCa aggressiveness and found that %p2PSA performed significantly better than %fPSA at lower (2–4 ng/mL) PSA levels.

However, multivariate regression models incorporating clinical information and p2PSA were shown to perform better than PSA forms individually. Stephan et al. [26] included 475 patients (264 PCa, 211 noncancer) with tPSA of 2 to 10 ng/mL and showed that the multivariable model including %p2PSA, %fPSA, tPSA, and age (but not prostate volume) reached the highest AUC (0.84) and specificity (53.1%) compared with tPSA (22.7%), %fPSA (45.5%), and %p2PSA (41.7%) alone at fixed sensitivity (90%).

More recently, p2PSA level changes over time were suggested as a potential predictor of PCa development. In 2012, Rhodes et al. [27] reported that p2PSA increased with advancing age and prostate volume. However, the greatest p2PSA level changes were seen in men who subsequently developed PCa (+8.1%/y) compared with those who did not (+3.5%/y) after a median follow-up of 7 years.

In a subsequent study [28], the same group reported that the baseline p2PSA levels in black men were slightly higher than those in white men (median, 6.3 pg/mL vs. 5.6 pg/mL, respectively). Furthermore, more interestingly, white men (from the Olmsted County Study of Urinary Symptoms and Health Status among Men cohort) with higher baseline p2PSA had an almost eight-fold higher risk of subsequent PCa diagnosis (hazard ratio, 7.8; 95% confidence interval, 2.2–27.8). Thus, baseline p2PSA and p2PSA changes over time might be useful predictors of PCa development, and this warrants further investigation.

These clinical studies investigating p2PSA showed very promising results. However, they were all retrospective, involving serum specimens collected in different preanalytical settings and stored for up to 16 years [28].

3. PHI: clinical validity and utility

Because p2PSA appears to have the highest predictive ability when associated with other variables, Beckman Coulter Inc. developed the PHI, a mathematical algorithm that is defined as follows: $(p2PSA/fPSA) \cdot \sqrt{tPSA}$. Fifteen studies have investigated the utility of p2PSA and PHI, and the main results of these studies are reported in Table 2.

Le et al. [29] were the first to evaluate the predictive ability of p2PSA and PHI in a prospective PCa screening setting. Their study involved 2,034 men undergoing PCa screening: 322 patients were advised to undergo prostate biopsy for an elevated PSA level (>2.5 ng/mL) and/or suspicious DRE. Eventually, only 74 patients underwent prostate biopsy; 63 of them had a tPSA level of 4 to 10 ng/mL and a normal DRE result. ROC analysis showed that PHI had the highest predictive ability (AUC, 0.77), followed by %p2PSA (AUC, 0.76) and %fPSA (AUC, 0.68). tPSA alone lacked sensitivity and specificity in the range of 2.5 to 10 ng/mL (AUC, 0.50). At a sensitivity of 88.5%, PHI and %p2PSA outperformed %fPSA or tPSA (specificity: 64.9% and 48.6% vs. 40.5% and 24.3%, respectively).

In 2010 Jansen et al. [30] retrospectively evaluated serum samples of 405 patients enrolled in the Rotterdam arm of the ERSPC study and 351 samples from Innbruck Medical University to investigate the use of p2PSA, PHI, and benign prostatic hyperplasia–associated PSA. The authors found significantly higher PCa predictive value and specificity for PHI and %p2PSA. However, p2PSA had limited additional value in identifying aggressive PCa (GS≥7). At 90% sensitivity, PHI and %p2PSA had the highest specificity (31%–35%) compared with tPSA (only 10%–16%).

Afterwards, Catalona et al. [31] conducted a multicenter, double-blind, case-control clinical trial to validate PHI in the PSA range of 2.0 to 10.0 ng/mL. Of 1,372 men enrolled in eight medical centers from October 2003 to June 2009, 892 patients met the eligibility criteria: age≥50 years, normal DRE result, and PSA of 1.5 to 11 ng/mL. PHI was found to have the greatest PCa predictive accuracy (AUC, 0.703) compared with %fPSA (AUC, 0.648), fPSA (AUC, 0.615), p2PSA (AUC, 0.557), and tPSA (AUC, 0.525), directly correlating with GS (p=0.013), with an AUC of 0.724 for GS≥4+3 disease. Moreover, men with a PHI>55.0 had a 52% likelihood of being diagnosed with PCa at biopsy compared with 26% of men with a PHI<25.0. In particular, compared with a PHI<25.0, the relative risk of PCa detection was 1.6-, 3.0-, and 4.7-fold higher at PHI values of 25.0–34.9, 35.0–54.9, and ≥55.0, respectively. At a PHI cutoff of 21.3, GS was ≥7 in 25% of missed cancers, resulting in the authors suggesting careful surveillance.

The same group recently published a prospective, multicenter study involving 892 men undergoing prostate biopsy [32]. The AUC for PHI (0.704) was significantly higher than for %fPSA (0.649, p=0.005) and tPSA (0.527, p<0.001) in men with a PSA of 1.6 to 7.8 ng/mL (World Health Organization [WHO] calibration [corresponding to 2–10 ng/mL Hybritech calibration]). Moreover, higher PHI values were associated with higher PCa risk and GS. The authors concluded that PHI had comparable performance characteristics by use of Hybritech and WHO standardization.

In 2011, Guazzoni et al. [33] conducted a prospective observational study of 268 consecutive men with PSA between 2 and 10 ng/mL and normal DRE results who underwent prostate biopsy. In this cohort, %p2PSA and PHI were the strongest predictors of positive prostatic biopsy outcome. PHI and %p2PSA improved the accuracy of a base multivariate model (including tPSA, fPSA, prostate volume, and age) by 11% and 10%, respectively (p<0.001). Similarly, in patients with tPSA of 4 to 10 ng/mL, the inclusion of PHI and %p2PSA significantly increased the multivariate predictive accuracy from 72% to 83% (+11%) in both models (p<0.001).

Furthermore, Lazzeri et al. [34] prospectively evaluated a clinical cohort of men with previous negative biopsies but persistent suspicion of PCa. Again, %p2PSA and PHI were the most accurate predictors of disease. In multivariable

TABLE 2. Studies investigating the accuracy of the PHI in detecting PCa

Study	Year	No. of patients	PSA range (ng/mL)	Results
Le et al. [29]	2010	2034	2.5–10	%p2PSA (AUC, 0.76) outperformed PSA (AUC, 0.50) and %fPSA (AUC, 0.68) in differentiating between PCa and benign disease; the Beckman Coulter PHI (AUC, 0.77) had the best overall performance characteristics
Jansen et al. [30]	2010	756	2–10	The highest PCa predictive value was achieved by PHI (AUC, 0.750), compared to tPSA (AUC, 0.585) and %fPSA (AUC, 0.675); also, %p2PSA showed significantly higher accuracy compared to tPSA and %fPSA
Catalona et al. [31]	2011	892	1.5–11	In the 2–10 ng/mL PSA range, PHI AUC exceeded those of PSA and %fPSA; an increasing PHI was associated with a 4.7-fold increased risk of PCa and a 1.61-fold increased risk of GS≥7 (4+3) disease on biopsy
Loeb et al. [32]	2012	892	2–10	PHI had comparable performance characteristics using Hybritech and WHO standardization
Guazzoni et al. [33]	2011	268	2–10	PHI and %p2PSA were the most accurate predictors of PCa (AUC, 0.756 and 0.757, respectively), followed by PSAD (61%), %fPSA (58%), and tPSA (53%); in multivariate accuracy analyses, both PHI (+11%) and %p2PSA (+10%) significantly improved the accuracy of established predictors in determining the presence of PCa at biopsy ($p<0.001$)
Lazzeri et al. [34]	2012	222	0.3–46.4	%p2PSA and PHI were the most accurate PCa predictors; they significantly increased the accuracy of multivariable models including PSA and prostate volume with or without %fPSA and PSAD by 8% to 11% ($p\leq0.034$); at a %p2PSA cutoff of 1.23, 153 biopsies (68.9%) could have been avoided, missing PCa in 6 patients; at a PHI cutoff of 28.8, 116 biopsies (52.25%) could have been avoided, missing PCa in 6 patients
Lazzeri et al. [35]	2013	646	2–10	In multivariable logistic regression models, p2PSA, %p2PSA, and PHI significantly increased the accuracy of the base multivariable model by 6.4%, 5.6%, and 6.4%, respectively ($p<0.001$); at a PHI cutoff of 27.6, a total of 100 (15.5%) biopsies could have been avoided
Lazzeri et al. [36]	2013	158	1.1–57.5	Univariable accuracy analysis showed %p2PSA (AUC, 0.733) and PHI (AUC, 0.733) to be the most accurate predictors of PCa at biopsy in patients with positive family history, significantly outperforming tPSA (AUC, 0.549), fPSA (AUC, 0.489) and %fPSA (AUC, 0.600) ($p\leq0.001$); in multivariable logistic regression models, %p2PSA and PHI achieved independent predictor status and significantly increased the accuracy of multivariable models including PSA and prostate volume by 8.7 and 10%, respectively ($p\leq0.001$)
Stephan et al. [37]	2013	1362	1.6–8.0	Significantly higher PHI median values were observed for patients with a GS≥7 (PHI, 60) compared with a GS<7 (PHI, 53; p=0.0018). The proportion of aggressive PCa (GS≥7) increased with the PHI score
Ito et al. [38]	2013	239	2–10	When sensitivity was fixed at 95%, unnecessary biopsies could be avoided in 28% of men when PHI was used as a biopsy indication
Ng et al. [39]	2013	230	3.18–9.98	The AUC for tPSA, PSAD, %fPSA, %p2PSA, and PHI were 0.547, 0.634, 0.654, 0.768, and 0.781, respectively; PHI was the best predictor of the prostate biopsy results. At a sensitivity of 90%, the use of PHI could have avoided unnecessary biopsies in 104 patients (45.2 %)
Lazzeri et al. [40]	2013	267	4–10	Considering chronic histologic prostatic inflammation (CHPI) in prostate biopsy samples, univariable accuracy analysis revealed %p2PSA (AUC, 0.73) and PHI (AUC, 0.73; lower %p2PSA and PHI result in higher probability of CHPI) to accurate discriminate PCa from CHPI at biopsy
Lughezzani et al. [41]	2012	729	0.5–19.9	On accuracy analyses PHI emerged as the most informative predictor of PCa (AUC, 0.70) compared to established predictors, such as total PSA (AUC, 0.51) and %fPSA (AUC, 0.62); including PHI in a multivariable logistic regression model (based on patient age, prostate volume, DRE and biopsy history) significantly increased predictive accuracy by 7% from 0.73 to 0.80 ($p<0.001$); decision curve analysis showed that using the PHI based nomogram resulted in the highest net benefit
Lughezzani et al. [42]	2013	833	0.5–19.9	In accuracy analyses, PHI was the most informative predictor of PCa (AUC, 0.68), outperforming tPSA (AUC, 0.51) and %fPSA (AUC, 0.64). The predictive accuracy of the previously developed nomogram was 75.2%; calibration of the nomogram was good in patients at a low-intermediate predicted probability of PCa, while calibration was suboptimal in high-risk patients, with a tendency to overestimate the presence of PCa

PSA, prostate-specific antigen; tPSA, total PSA; fPSA, free PSA; %fPSA, fPSA/tPSA; PSAD, PSA density; p2PSA, [-2]proPSA; %p2PSA, [-2]proPSA/fPSA; PHI, Prostate Health Index; PCa, prostate cancer; GS, Gleason score; WHO, World Health Organization; DRE, digital rectal examination; AUC, area under the receiver-operating characteristic curve.

logistic regression models, %p2PSA and PHI achieved independent predictor status and significantly increased the accuracy of multivariable models by 8% to 11% ($p \leq 0.034$). At a PHI cutoff of 28.8, 116 biopsies (52.25%) could have been avoided and PCa would have been overlooked in 6 patients, but none with a GS ≥ 7, demonstrating a real clinical utility.

Recently, Lazzeri et al. [35] confirmed previous results in an observational, prospective, multicenter European cohort (PROMEtheuS Project). This study involved 646 patients from five European urology centers with tPSA of 2 to 10 ng/mL who were subjected to initial prostate biopsy for suspected PCa. p2PSA, %p2PSA, and PHI significantly increased the accuracy of the base multivariable model by 6.4%, 5.6%, and 6.4%, respectively ($p < 0.001$). At 90% sensitivity, the PHI cutoff of 27.6 could result in the avoidance of 100 biopsies (15.5%), with 26 cancers (9.8%) being overlooked (23 with GS 6, 3 with GS 3+4).

Interestingly, the same group [36] published a nested case-control study from the same PROMEtheuS database, evaluating 158 patients with a positive family history for PCa (at least one first-degree relative with PCa), in a PSA range of 1.1 to 57.5 ng/mL. %p2PSA and PHI were directly associated with GS and were more accurate than tPSA, fPSA, and %fPSA in predicting PCa. At 90% sensitivity, the thresholds for %p2PSA and PHI were 1.20 and 25.5, sparing a total of 39 (24.8%) and 27 biopsies (17.2%), respectively, and missing 2 cases (3.8%) of PCa, each with a GS of 7. Again %p2PSA and PHI significantly increased the accuracy of multivariable models by 8.7% and 10%, respectively ($p \leq 0.001$). Although the PROMEtheuS study had a well-planned observational design, the main limitation was that patients were included for their PSA and DRE-related risk of PCa and not through a p2PSA screening protocol.

Another European multicenter study was published by Stephan et al. [37]. This study involved 1,362 patients with tPSA between 1.6 and 8.0 g/L (668 PCa, 694 noncancer). Serum concentrations of tPSA and fPSA were both calibrated against a WHO reference material. %p2PSA and PHI were significantly higher in all PCa subcohorts (positive initial or repeat biopsy results or negative DRE result) compared with patients without PCa ($p < 0.0001$). PHI had the largest AUC (0.74) and provided significantly better clinical performance for predicting PCa compared with %p2PSA (AUC, 0.72; p=0.018), p2PSA (AUC, 0.63; $p < 0.0001$), %fPSA (AUC, 0.61), or tPSA (AUC, 0.56). Significantly higher PHI was observed for patients with GS ≥ 7 (PHI 60) compared with GS < 7 (PHI 53, p=0.0018). The proportion of aggressive PCa (GS ≥ 7) increased with PHI.

Two recent studies from Asia confirm previous results in another population setting. Ito et al. [38] reported data on 239 consecutive men with tPSA between 2.0 and 10.0 ng/mL who underwent prostate biopsy. When PHI was used as a biopsy indicator and sensitivity was fixed at 95%, unnecessary biopsies could be avoided in 28% of men. Accordingly, Ng et al. [39] retrospectively analyzed archived serum samples from 230 patients over 50 years of age who had undergone their first prostate biopsy with a PSA of 4 to 10 ng/mL and a negative DRE result. PHI was found to be the best predictor of the prostate biopsy results. At a sensitivity of 90%, the use of PHI could have resulted in the avoidance of unnecessary biopsies in 104 patients (45.2%).

Interestingly, Lazzeri et al. [40] showed that p2PSA, %p2PSA, and PHI values might specifically discriminate PCa from chronic histologic prostatic inflammation (CHPI) or BPH, but not CHPI from BPH, in men with tPSA of 4 to 10 ng/mL and normal DRE. Univariable accuracy analysis revealed %p2PSA (AUC, 0.73) and PHI (AUC, 0.73) to be the most accurate predictors of CHPI at biopsy (lower %p2PSA and PHI result in higher probability of CHPI), outperforming the other biomarkers. Again, multivariable models including p2PSA, %p2PSA, and PHI showed the highest net benefit in discriminating between patients with and without PCa in a probability of pathologic outcome range (threshold probability) between 25% and 90%.

Finally, Lughezzani et al. [41] developed and validated, on over 729 patients, a PHI-based nomogram to predict PCa at extended prostate biopsy. Including PHI in a multivariable logistic regression model based on patient age, prostate volume, DRE, and biopsy history significantly increased predictive accuracy by 7% from 0.73 to 0.80 ($p < 0.001$). Decision curve analysis showed that using the PHI-based nomogram resulted in the highest net benefit. This nomogram was also externally validated in a recent multicenter European study [42].

Overall, studies to date suggest that %p2PSA and PHI are more accurate than standard reference tests in predicting prostate biopsy outcome and could result in the avoidance of unnecessary biopsies.

4. p2PSA and PHI as predictors of final histology in radical prostatectomy specimens

Further studies are necessary before definitively proving that PHI and p2PSA can predict PCa aggressiveness on prostate biopsies, as well as on final histology after radical prostatectomy (RP), potentially limiting overtreatment.

Accordingly, Guazzoni et al. [43] conducted an observational, prospective study of 350 consecutive men diagnosed with clinically localized PCa who underwent RP. Preoperative %p2PSA and PHI were significantly higher in patients with pT3 disease, pathological GS ≥ 7 and those with GS upgrading ($p < 0.001$). These measures might therefore be useful in the preoperative counseling of patients with newly diagnosed, clinically localized PCa.

Interestingly, in 2013 Heidegger et al. [44] found that p2PSA values were highly differentiated ($p < 0.001$) between GS ≥ 8 and GS ≤ 7 as early as 3 years before diagnosis and that preoperative p2PSA values were significantly higher in men with pT3a or higher compared with pT2c or lower PCa up to 4 years before diagnosis ($p < 0.01$). p2PSA was shown to have a high positive predictive value concern-

ing GS≥8 and GS≤7 and also extraprostatic extension.

Although these two studies were well designed and conducted, and the results were strong, they do not completely resemble the general population and need to be confirmed in larger multicenter studies.

5. p2PSA and PHI in active surveillance regimens

PSA screening has resulted in an increasing number of patients being diagnosed with potentially low-risk, clinically insignificant cancers. To reduce overtreatment, active surveillance (AS) has been proposed as an alternative strategy for these patients [9]. An effective program should include regular periodic DREs, PSA testing, and repeated prostate biopsies.

Makarov et al. [45] assessed the association of proPSA with outcomes among men with PCa in AS. The authors found that the p2PSA/%fPSA ratio in serum was significantly higher at diagnosis in men with unfavorable biopsy results (0.87±0.44) than in those with favorable biopsy results (0.65±0.36, p=0.02). Moreover, p2PSA/%fPSA (hazard ratio, 2.53; p=0.02) was significantly associated with an unfavorable biopsy result in Kaplan-Meier and Cox analyses.

In 2011, the same group analyzed the role of the PHI in this same cohort of patients [46]. The PHI was significantly greater in men who ultimately had unfavorable biopsy findings (37.23±15.76 vs. 30.60±12.28, p=0.03). Moreover, PHI (p=0.003) and p2PSA/%fPSA (p=0.004) were significant predictors of unfavorable biopsy conversion in a Cox regression analysis.

Tosoian et al. [47] reported data from 167 men scheduled in a single-institution AS program. Risk of biopsy reclassification was significantly associated with lower %fPSA (p=0.002) and higher %p2PSA (p<0.0001) and PHI (p<0.0001) at baseline.

Recently, Hirama et al. [48] evaluated the predictive impact of baseline p2PSA and related indexes on the pathological reclassification at 1 year in 67 patients enrolled over 134 candidates for AS. %p2PSA and PHI at baseline were significantly different between the reclassification and nonreclassification groups (2.44 vs. 1.88 [p=0.003] and 60.3 vs. 47.8 [p=0.01], respectively). Multivariate logistic regression analysis revealed baseline %p2PSA and PHI (both p=0.008) to be the only independent predictive factors for pathological upgrade at the 1-year mark during AS.

Therefore, baseline p2PSA and derivative values seem to help to identify those men at risk of future unfavorable reclassification during AS, but further studies are needed to define the role of these variables in selecting men who would most benefit from AS.

6. p2PSA and other molecular markers

Recently, four studies compared the accuracy of p2PSA in detecting PCa with that of other interesting biomarkers, in particular, urinary prostate cancer antigen 3 (PCA3). Ferro et al. [49] were the first to report that %p2PSA, PHI, and PCA3 are comparably good indicators of malignancy (AUC: 0.73, 0.77, and 0.71, respectively). PHI had the highest AUC, but it was not statistically different from PCA3 (p=0.368).

In a subsequent study by the same group [50], PHI (AUC, 0.82; p<0.001), PCA3 (AUC, 0.77; p=0.015), and their combination (AUC, 0.83; p<0.001) improved the diagnostic accuracy (AUC, 0.72) of the base multivariable model (including age, PSA, %fPSA, DRE, and prostate volume). However, the AUC of the multivariable model did not improve over both PHI and PCA3 alone (p>0.05).

Scattoni et al. [51] showed that PHI accuracy was higher than that of PCA3 at both the initial prostate biopsy (AUC: 0.69 vs. 0.57) and the repeat biopsy (AUC: 0.72 vs. 0.63), although accuracy in these two settings (initial or repeat biopsy) was not statistically different. Including PCA3 in the base multivariable model (PSA, %fPSA, prostate volume) did not increase predictive accuracy in either setting (AUC: 0.79 vs. 0.80 and 0.75 vs. 0.76, respectively). Conversely, PHI improved the predictive accuracy of the base model by 5% (AUC: 0.79 to 0.84) and 6% (AUC: 0.75 to 0.81) in initial and repeat settings, respectively.

Moreover, Stephan et al. [52] compared PHI, PCA3, and the transmembrane protease, serine 2 (TMPRSS2):v-ets erythroblastosis virus E26 oncogene homolog (avian) (*ERG*) gene fusion (TMPRSS2:ERG). These markers showed the highest accuracy (AUC: 0.68, 0.74, 0.63, respectively). PCA3 had the largest AUC, although it was not statistically different from that of PHI. The combination of both markers modestly enhanced diagnostic power (AUC gain: 0.01–0.04). Although PCA3 had the highest AUC also in the repeat-biopsy cohort, the highest AUC for PHI was observed in DRE-negative patients with PSA of 2 to 10 ng/mL.

If these results are confirmed in larger multicenter studies, PHI seems to be the best compromise between diagnostic accuracy and ease of sampling and analysis.

7. Cost-effectiveness of p2PSA and PHI

Owing to its high accuracy in predicting PCa, PHI could result in the avoidance of a considerable number of negative prostate biopsies, thus reducing direct costs. In two subsequent studies, Nichol et al. [53,54] evaluated the cost-effectiveness of PHI.

In the first study [53], the authors constructed two budget impact models by using PSA cutoff values of ≥2 ng/mL (model #1) and ≥4 ng/mL (model #2) for recommending a prostate biopsy in a hypothetical health plan with 100,000 male members aged 50 to 75 years old. The budgetary impact on the 1-year expected total costs for PCa detection was calculated. The addition of PHI to the current PSA screening strategies (using tPSA and %fPSA) increased the total cost of blood tests by $51,524 (model #1) and $13,611 (model #2), but produced higher cost savings in model #1 ($356,647) than in model #2 ($94,219) with a small short-term reduction in the number of positive tests.

In the second study [54], the same group evaluated the cost-effectiveness of early PCa detection with PHI asso-

ciated with a PSA test compared with the PSA test alone from a United States of America societal perspective. Over 25 annual screening cycles, the strategy of PSA plus PHI was estimated to save $1,199 or $443 with an expected gain of 0.08 or 0.03 quality-adjusted life years per person for PSA thresholds of ≥2 and ≥4 ng/mL, respectively. Because the strategy of PSA plus PHI is expected to increase the number of true-positive tests while reducing false-positives in men aged 50 to 75 years, the authors suggested that the increased total costs of the laboratory assay (PSA+fPSA+p2PSA) could be offset by reducing unnecessary prostate biopsies.

DISCUSSION

This review has summarized current knowledge about the early diagnosis of PCa with p2PSA, %p2PSA, and PHI and has presented indications that %p2PSA and PHI may discriminate men with or without PCa with higher accuracy than the reference standard tests. Furthermore, the results of observational prospective international studies support the association between these new biomarkers and cancer aggressiveness [35,37]. Several authors have shown that PHI correlates with the GS and might result in the avoidance of unnecessary biopsies without missing significant PCa [31,34,35,37]. PHI was also shown to be a useful clinical marker in patients with a positive family history of PCa [36]. Thus, the results reported above suggest that the new diagnostic tests may be particularly useful in patients with a tPSA range of 2/4 to 10 ng/ml. Furthermore, a strong correlation between %p2PSA and PHI and the pathological characteristics in whole gland samples was found after RP. Finally, we also reported the results of studies comparing p2PSA and derivatives with other available biomarkers, in particular PCA3. These studies showed a slightly higher accuracy for PHI than for PCA3 but an improvement in accuracy with their combination. Moreover, it is notable that other biomarkers (TMPRSS2:ERG, 4Ks, miRNAs, circulating tumor cells) are emerging and these will need to be taken into account in future studies. To our knowledge, only one study recently compared TMPRSS2:ERG to p2PSA [52], and this study was reported in this review.

The present systematic review had a number of limitations that must be taken into account. Most of the studies were retrospective and different biopsy protocols were used. Even though the gold standard for biopsy was used, some groups limited the number of biopsy cores to 12, whereas others extended the core number to 18 to 24, possibly causing significant heterogeneity. Further heterogeneity was found regarding study design (retrospective, prospective, screening), race (most of the studies included Caucasian men), and preanalytic and analytic phases. Another limitation was the potential duplication of results in related publications that could bias the conclusions. In fact, there may have been some overlap between subsequent studies, but being that this article was not a meta-analysis, we decided to include all the studies because of their different aims. Finally, the issue of costs remains unsolved.

CONCLUSIONS

p2PSA and PHI are more accurate than the currently used tests (PSA and derivatives) in predicting the presence of PCa at biopsy. Their implementation in clinical practice has the potential to significantly increase physicians' ability to detect PCa and avoid unnecessary biopsies. Further work is needed to confirm and generalize these conclusions to wider populations.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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