Review

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Clinical utility of the (-2)proPSA and evaluation of the evidence: a systematic review

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Abstract

Background: Diagnostic studies usually provide important information about the analytical and diagnostic performances. We investigated the clinical utility of (-2)proPSA in identifying patients with prostate cancer (PCa).
Methods: We performed electronic searches in five databases as well as a list of reference literature. Studies were included if they evaluated the diagnostic accuracy of (-2)proPSA in men with PSA value ranged from 2.0 to 10 μg/L. We also analyzed data about total PSA (tPSA), %(-2)proPSa, freePSA (fPSA), its percentage (%fPSA) and the prostate health index (phi). The selection of the studies, the screening of the full texts and the data extraction, as well as the assessment of risk of bias using the QUADAS-2 tool were conducted independently by two authors. Grading the quality of the evidence was carried out according to the GRADE method. The random effects model was used for the meta-analyses.
Results: We included 17 studies, including 6912 patients. The pooled sensitivity of (-2)proPSA was 90% and the summary specificity was 13%. The tPSA sensitivity and specificity were 89% and 25%, respectively. Considering (-2)proPSA, 225 men out of 1000 have been identified having PCa true positives (TP). However, 652 persons have been incorrectly identified and undergo biopsy. The majority of studies were judged to carry a moderate risk of bias. Therefore, the overall quality of evidences was deemed to be low.
Conclusions: The (-2)proPSA could be useful to identify men at risk of PCa, but its accuracy still remains uncertain and the level of evidence does not support an improved clinical utility.

Keywords: GRADE; proPSA; systematic review.

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Introduction

Prostate cancer (PCa) is the main cause of worldwide death in men and its great prevalence is due to inappropriate life custom and environmental risk factors, as well as genetic factors [1]. Prostate specific antigen (PSA) is the marker used for screening the male population at risk of PCa, although being unspecific for this cancer. PSA levels increase in patients with PCa, independently of the level of PCa aggressiveness. This is especially limiting when the PSA values fall in the diagnostic gray zone, ranging between 2 and 10 μg/L. The clinical significance of the test feels the effect of these limitations, recording a high number of false positives (FP) and therefore an increment in unnecessary prostate biopsies [2]. Considering the limitations unsatisfactory diagnostic accuracy of this molecule, new biomarkers should be developed. Serum contains two forms of PSA, the complex and uncomplex form (free PSA), the combination of which constitute the total PSA (tPSA). The free PSA (fPSA) is enzymatically inactive and is composed of three different types of PSA: benign PSA usually associated with benign prostatic hyperplasia, intact PSA which is similar to the active form, and proPSA usually associated with cancer [3]. Different truncated forms of proPSA have been identified: (-4) and (-2) proPSA are resistant to activation and are biochemical features differentiating from (-5) and (-7)proPSA which are rapidly activated by human kallikrein 2 [4, 5]. Recently, researchers have focused their interest on the (-2)proPSA, because its serum levels seem to be higher in men with

PCa compared to men without cancer. Therefore, this molecule appears to be more cancer specific than PSA alone.

To investigate the diagnostic significance of the (-2)proPSA, we performed a methodological assessment, applying the GRADE method [6]. The GRADE approach facilitates the description of the grade of evidence available, based on the quantity of information produced in a study and considering four domains: risk of bias, consistency, directness and precision.

Aim of this paper is the evaluation of the diagnostic accuracy and clinical utility of (-2)proPSA in patients with the PSA value in the gray zone (from 2 to 10 μg/L). We analyzed, also, the diagnostic accuracy of the other available PCa biomarkers.

Materials and methods

We performed a systematic review and meta-analysis according to the recommendations indicated in the Cochrane Handbook for Diagnostic Test Accuracy (DTA) Reviews (http://srdta.cochrane.org/handbook-dta-reviews) and we reported data adapting the preferred reporting items from systematic reviews and meta-analysis (PRISMA) checklist [7] to diagnostic accuracy studies.

Search strategy, eligibility criteria and study selection

A systematic research was performed in five databases: Medline, Embase, Web of Science (WOS), Scopus and The Cochrane Register of Diagnostic Test Accuracy Studies (CRDTAS), to identify all possible eligible studies. The search strategy was carried out using the terms “prostatic neoplasm”, “prostate-specific antigen”, “(-2)pro-prostate-specific antigen”, “proPSA”, “p2PSA”, “sensitivity and specificity” and it was adapted to all databases. Furthermore, we checked the reference list of all selected studies.

We included studies which respected the following eligibility criteria: i) included male patients with suspicious PCa; ii) included male patients with PSA between 2.0 and 10 μg/L; iii) data about the diagnostic accuracy of (-2)proPSA, tPSA and its derivate [%p2PSA, fPSA, %fPSA, prostate health index (phi)] were reported; iv) published in English, Italian, Spanish, or French. The search strategy was performed by one author (VP). Two independent authors (VP, LR) screened the titles and abstracts, and subsequently reviewed all potentially eligible studies after the removal of duplicates. Disagreements between authors were resolved by consensus.

Data collection

One author (VP) used a standardized data extraction form to collect relevant publication details, regarding, the study methods, and the results, and the second author (LR) checked the data. The authors collected data about: study characteristics (i.e. authors, year of publication, title, reference, study design and inclusion criteria); ii) patient characteristics (i.e. age, number of enrolled patients and number of patients with PCa); iii) detailed information about the index test (i.e. method of assay and cut-off) and reference standard; iv) diagnostic study data (i.e. TP, TN, FP, FN). Disagreements were resolved by discussion. We defined reference standard the test whose results are compared with the index test as reported in the QUADAS-2 tool.

Outcomes

The primary outcome was the diagnostic accuracy of (-2)proPSA defined as the number of TP, FP, false negatives (FN) and true negatives (TN) reported in each study. When these data were not available, they were calculated from sensitivity and specificity data. We evaluated the number of unnecessary prostate biopsies and the number of missed PCa diagnoses. We also considered the changes of tPSA, (-2)proPSA and tPSA concentration in patients with and without PCa.

Assessment of risk of bias

The methodological quality of each selected paper was assessed independently by two reviewers (VP and LR) according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist [8] which considers four domains (patients selection, index test, reference standard and flow and timing), each rated in terms of their risk of bias and applicability to the research question. Risk of bias was judged as “low”, “high”, or “unclear”. If all signaling questions for a domain were answered “yes” then risk of bias was judged “low”. Concerning applicability, the authors recorded the information for which the study did not match the review question. Concerns regarding applicability were rated as “low”, “high” or “unclear”. Any disagreements were resolved through discussion.

Evidence profile using the GRADE approach

We evaluated the evidence using the GRADE approach. Prospective studies were initially considered at high quality but were downgraded according to: their risk of bias, directness of evidence, consistency or imprecision. Directness refers to the link between test of interest and disease or populations evaluated. Consistency concerns the degree of homogeneity (direction and magnitude) of results across the different studies. Precision describes the grade of uncertainty across the effects estimate, in other words the width of confidence intervals for diagnostic accuracy measurement [6, 9].

Statistical analysis

For each study, we constructed two-by-two tables and pooled TP, FP, TN and FN, calculating sensitivity and specificity with 95% confidence intervals. We used the random effects bivariate models to create separate forrest plots. We explored heterogeneity first through visual examination of the forrest plot and then through the analysis of the receiver operating characteristics (ROC) plot. Statistically heterogeneity was measured using I^2 tests. All analyses were performed using the Stata 11 and Meta-Disc softwares.

Results

Studies selection

The literature search from Medline, Embase, WOS, Scopus and CRDTAS, after the exclusion of duplicates and irrelevant records, identified 4017 references. Of these, 3987 were discarded because they did not meet the inclusion criteria. Thirty were considered eligible for inclusion and their full texts were evaluated for details. Of these, 13 were excluded because: i) diagnostic accuracy data was not reported (n=7); ii) definition of reference range was not reported (n=2); male patients suspected of PCa were not included (n=3); iii) (-2)pPSA was not considered (n=1). A total of 17 studies [1, 4, 10–24] were included in the current analysis (Supplemental Material, Figure 1).

Characteristic of studies

We included 17 cohort studies; 11 of which utilized a prospective design, four retrospective studies and two were defined as prospective and retrospective. Table 1 reports details of the studies selected, including information about the biopsy Gleason score. Overall, 6912 men were enrolled, of these 2993 (43.3%) had PCa. The number of participants ranged from 63 to 1091 and the age ranged from 62 to 68 years old. All trials were conducted between 2003 and 2014 and used immunoassays to quantify the serum markers. All studies used the prostate biopsy to diagnose PCa, and only four studies specified the tPSA as reference standard whose results were compared with the (-2)proPSA. In 15 studies, all patients underwent first serum biomarkers determination and then prostate biopsy [1, 4, 10–16, 18–24]. One study extended their research through an active surveillance program, of patients with a negative biopsy and with increasing PSA values [17]. All studies reported the performance of (-2)pPSA at 90% sensibility, but not all reported the cut-off values that, when recorded, ranged from 7 to 9 μg/L.

(-2)pPSA, fPSA and phi levels were slightly higher in PCa patients than in men without PCa in all studies. Results for values of serum biomarkers in patients with or without PCa are shown in Supplemental Table 1.

The evidence profile

The results of the methodological quality of the included studies were shown in Supplemental Table 2. Four studies were retrospective, nine studies (53%) enrolled consecutive patients presenting to the urology department and in three studies the enrolment was unclear. The authors were blinded about the results of index test and reference standards in seven studies and the markers cut-off value were defined in 10 studies only. The majority of studies (n=11, 65%) reported that the blood samples were collected before any prostate manipulation. In all studies all patients received the same reference standard.

Using the GRADE approach, we assessed the overall quality of the evidence about the levels of (-2)proPSA in patients with PSA levels between 2.0 and 10.0 μg/L. Because some studies were retrospective, the GRADE scores were downgraded for risk of bias for all outcomes. Our meta-analyses did not suffer from serious imprecision. We subtracted one point for inconsistency in meta-analysis showing substantial heterogeneity, and an additional point for indirectness. The quality of evidences was very low for true negative, false positive and false negative patients. Thus, the available evidences suggest that the measurement of (-2)proPSA does not procure substantial clinical utility in identifying patients with PCa. However, it seems more informative than tPSA alone (Table 2).

Table 1: Characteristics of included studies.

Authors	Study design	Inclusion criteria	Method assay	Age of patients[a], years	Patients, n	Patients with cancer, n	Gleason score			Reference standard
							6≤	7	≥8	
Catalona et al. [10]	Retrospective	Incl: patients undergo prostate biopsy, PSA range 4–10 ng/mL	Immunoassay	63	1091	456	n.a.	n.a.	n.a.	Not define
Catalona et al. [11]	Prospective and retrospective	Incl: patients >50 years of age, no PCa history, non suspicious DRE, pre study PSA 1.5 to 11 ng/mL, 6-core or greater biopsy, histological diagnosis from prostate biopsy Excl: previous prostate surgery, urinary tract infection, no blood or biopsy draw at the appropriate time, previous androgen therapy	Beckman Coulter Immunoassay	62.8±7	892	430	n.a.	n.a.	n.a.	Not define
Filella et al. [12]	Prospective and retrospective	Incl: patients selected for biopsy because of an elevated serum PSA level and/or abnormal DRE, as well as patients diagnosed of Pca Excl: medical therapy to affects PSA serum levels, prostatitis, urinary tract infection, invasive treatment	Beckman Coulter Immunoassay	68 (38–88)	354	175	82	59	21	Not define
Guazzoni et al. [13]	Prospective	Incl: PSa range 2–10 ng/mL, negative DRE, patients with suspected PSA Excl: prostatitis, previous prostate surgery, use of drugs may alter PSA levels	Beckman Coulter Immunoassay	63.3±8.2	268	107	55	52	–	tPSA fPSA %fPSA
Ito et al. [14]	Prospective	Incl: PSA range 2–10 ng/mL	Beckman Coulter Immunoassay	66 (37–82)	239	53	12	33	4	Not define
Jansen et al. [15] (site 1)	Prospective	Incl: patients >50 years of age, tPSA range 2–10 ng/mL, TRUSPB, histologically confirmed diagnosis	Beckman Coulter Immunoassay	66 (55–75)	405	226	168	122	–	Not define
Jansen (site 2)		Excl: history of PCa, prostatitis, urinary tract infection, prostate surgery, use of drugs may akter PSA levels	Beckman Coulter Immunoassay	60 (50–77)	351	174	55	48	–	Not define
Khan et al. [16]	Prospective	Incl: tPSa range 4–10 ng/mL Patients with suspected PCa	Beckman Coulter Immunoassay	n.a.	93	41	n.a.	n.a.	n.a.	Not define
Lazzeri et al. [17]	Prospective	Incl: negative first biopsy, elevated PSA, suspicious DRE, atypical small acinar proliferation Excl: man received dutasterine or finasteride, previous invasive treatment, urinary tract infection	Beckman Coulter Immunoassay	63.9±7.1	222	71	n.a.	n.a.	n.a.	tPSA fPSA %fPSA

Table 1 (continued)

Authors	Study design	Inclusion criteria	Method assay	Age of patients[a], years	Patients, n	Patients with cancer, n	Gleason score			Reference standard
							6≤	7	≥8	
Lazzeri et al. [18]	Prospective	Incl: patients >45 years of age, PSA range 2–10 ng/mL Excl: prostatitis, previous prostate surgery, renail failor or other condition may alter PSA values	Beckman Coulter Immunoassay	64.2±7.5	646	264	125	139	–	tPSA, fPSA, %fPSA
Le et al. [19]	Prospective	Incl: PSA range 2.5–10 ng/mL, nonsospicious DRE	Beckman Coulter Immunoassay	65	63	26	19	11	–	Not define
Mearini et al. [20]	Prospective	Incl: tPSA 2.0–10 ng/mL Excl: prostatitis, precious prostate surgery, use of drug may alter PSA levels	Beckman Coulter Immunoassay	65.4±6.8	275	86	60	26	–	tPSA %fPSA PSAD
Mikolajczyk et al. [4]	Retrospective	Incl: PSA range 4–10 μg/L patients undergo prostate biopsy	Beckman Coulter Immunoassay	66	380	238	n.a.	n.a.	n.a.	Not define
Miyakubo et al. [1]	Prospective	Incl: PSa range 2–10 ng/mL	Beckman Coulter Immunoassay	n.a.	239	53	n.a.	n.a.	n.a.	Not define
Ng et al. [21]	Retrospective	Incl: tPSA 4–10 ng/mL, negative DRE Excl: history of TRUSPB,	Beckman Coulter Immunoassay	65.7 (50–84)	230	21	n.a.	n.a.	n.a.	Not define
Sokoll et al. [23]	Retrospective	Incl: man with an indication for prostate biopsy, No prior prostate cancer history	Beckman Coulter Immunoassay	62.2±8.2	123	63	n.a.	n.a.	n.a.	Not define
Sokoll et al. [22]	Prospective	Incl: Patients >40 years of age, No prior prostate surgery, biopsy or history pf PCa, No use of 5-α reductase inhibitors	Beckman Coulter Immunoassay	61.7±8.6	566	245	107	96	42	Not define
Stephan et al. [24]	Prospective	Incl: patients referred to the department of urology for suspected PCa	Beckman Coulter Immunoassay	n.a.	475	264				Not define

n, number; n.a., not available; Incl, inclusion; Excl, exclusion; DRE, digital rectal examination; TRUSPB, transrectal ultrasonography prostatic biopsy; [a]age reported as mean±SD or median (range).

Table 2: Evidence profile of the (-2)proPSA accuracy to identify patients with PCa using GRADE approach.

Outcomes	Studies, n	Patients[a], n	Factors that may decrease quality of evidence[b]				Overall quality[f]
			Risk of bias	Indirectness	Inconsistency	Imprecision	
True positive	6	760	Serious[c]	None	Serious[e]	None	Low
True negative	6	171	Serious[c]	Serious[d]	Serious[e]	None	Very low
False positive	6	1116	Serious[c]	Serious[d]	Serious[e]	None	Very low
False negative	6	82	Serious[c]	Serious[d]	Serious[e]	None	Very low

[a]n Patients reported by included studies; [b]downgrade quality of evidence: none, serious (–1), very serious (–2); [c]six prospective studies and only one retrospective study; [d]there is uncertainty about the consequences for these patients; [e]the studies included use different cut-off and the heterogeneity is very high; [f]quality range: high, moderate, low, very low.

Valuation of the diagnostic accuracy

The diagnostic accuracy of (-2)proPSA was evaluated in six studies with 2129 patients. The pooled sensitivity was 0.90 (95% CI 0.88–0.92, I^2=0%), the polled specificity was 0.13 (95% CI 0.11–0.15, I^2=93.1%) and the area under the curve (AUC) was 0.95 (SE=0.03) (Figures 1–3). Even if the heterogeneity related to specificity was very high, we estimated that 877 of 1000 patients had a positive test results, 225 of these had PCa (TP), while 652 of these people were FP and they were undergo to prostate biopsy. One hundred and twenty three of 1000 patients had no evidence of cancer,

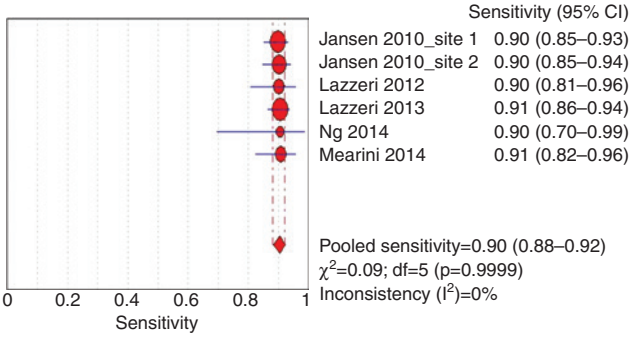

Figure 1: The pooled sensitivity of (-2)proPSA.

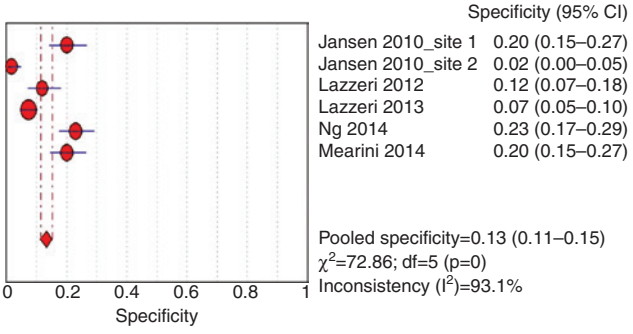

Figure 2: The pooled specificity of (-2)proPSA.

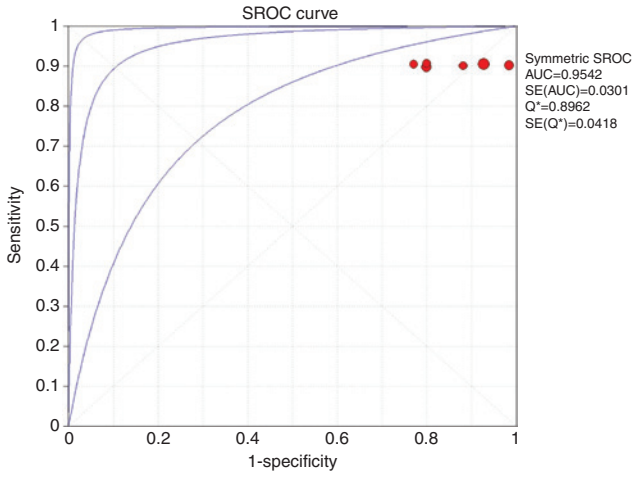

Figure 3: SROC of (-2)proPSA.

98 of these could have avoided a biopsy, while 25 patients with PCa would have been missed (Table 3). The pooled positive and negative likelihood ratios (LR) were 1.04 and 0.87, respectively.

To obtain clinically relevant estimates of the performance of other available PSA serum markers, we conducted a separate meta-analysis for each of them. Twelve studies evaluated tPSA for 4077 patients. The pooled sensitivity and AUC were inferior to those reported for (-2)proPSA: 89%, 0.94 (SE=0.035), respectively. For all other biomarkers (%(-2)pPSA, fPSA,%fPSA, phi) at fixed sensibility of 90% reported in all studies, the connected specificity was very low and the heterogeneity was very high, probably due to different cut-offs used. The results from these meta-analyses are presented in Table 3.

Discussion

PCa is the most common male cancer affecting middle age men older than 50 years. The serum biomarker

Table 3: Summary of meta-analyses table.

Test	Studies, n		Sensitivity		Specificity				per 1000[a]		AUC (SE)	LR+	LR−
	Patients, n		(95% CI)	I^2	(95% CI)	I^2	TP rate	FP rate	TN rate	FN rate		(95% CI)	(95% CI)
(-2)proPSA	5 (2129)		0.90 (0.88–0.92)	0%	0.13 (0.11–0.15)	93.1%	225	652	98	25	0.95 (0.03)	1.04 (0.96–1.13)	0.87 (0.47–1.61)
tPSA	12 (4077)		0.89 (0.87–0.9)	27.8%	0.25 (0.23–0.27)	96.9%	223	562	188	27	0.94 (0.03)	1.22 (1.10–1.35)	0.47 (0.34–0.65)
%p2PSA	14 (4566)		0.89 (0.88–0.9)	11.7%	0.32 (0.3–0.34)	86.8%	223	510	240	27	0.89 (0.06)	1.35 (1.24–1.46)	0.34 (0.27–0.43)
fPSA	4 (2516)		0.92 (0.9–0.93)	55.5%	0.08 (0.07–0.10)	92.9%	230	690	60	20	0.82 (0.09)	1.01 (0.96–1.06)	0.98 (0.60–1.60)
%fPSA	15 (4758)		0.89 (0.88–0.9)	6.8%	0.22 (0.20–0.24)	89.3%	223	585	165	27	0.84 (0.05)	1.15 (1.09–1.22)	0.49 (0.39–0.60)
PHI	11 (4184)		0.90 (0.89–0.91)	0%	0.31 (0.29–0.33)	89.8%	225	518	232	25	0.95 (0.03)	1.35 (1.25–1.46)	0.34 (0.29–0.41)

AUC, area under the curve; TP, true positives; FP, false positives; TN, true negatives; FN, false negatives; [a]assuming a pre-test probability of 25%.

recommended in most guidelines in the screening phase for patients at risk of PCa is PSA, but its clinical value is questionable due to a lack of specificity. Despite its widespread use, due to the reduced specificity and sensitivity in patients within the PSA grey zone, this test is unable to discriminate between patients with and without PCa, and between aggressive or indolent cancer types. PSA levels can also be effected by benign prostate conditions or prostatitis, prostate manipulations, some inflammatory processes or infections, specific drugs (NSAIDs, statins, thiazide diureticsor finestaride and five alpha reductase inhibitors) and androgen therapy [25], commonly administered to, or present in, middle aged men. All these factors should be taken into consideration when evaluating PSA values.

Recently, to enhance the selection of patients for prostate biopsy, some authors have proposed numerous serum biomarkers with improved PCa specificity, particularly PSA derivatives such as fPSA,%fPSA, PSA density and PSA velocity. However, the precursor of PSA (proPSA) seems to be the most promising among the candidate molecules. There are four types of proPSA in serum which can be differentiated according to the number of amino acids forming the pro-leader peptide sequence [2, 3, 5]. Of these, the (-2)proPSA is localized in the peripheral zone cancer and its serum levels are higher in patients with PCa than patients without cancer [26].

Despite the unsatisfactory accuracy of tPSA and fPSA, we evaluated the diagnostic accuracy of (-2)proPSA and its derivatives, in discerning men at risk of cancer when PSA was within the diagnostic grey zone. The GRADE approach was again applied to assess clinical utility of the serum biomarker determination.

Our meta-analyses showed that at sensibility 90%, the specificity of (-2)proPSA was 13% and its prognostic value was consistent (AUC 0.95). We also report a good specificity of %(-2)proPSA (32%) and phi (31%). We show that (-2)proPSA was equivalent in term of diagnostic accuracy (sensitivity and specificity) compared to other PSA derivatives, but the number of unnecessary biopsies was reduced unsatisfactory, as well as the number of the false negative. These results suggest a limited clinical utility of (-2)proPSA, due to a high rate of FP and low level of evidence.

It is interesting to note that the accuracy of (-2)proPSA is limited by lack of specificity. Furthermore, for (-2) proPSA the authors reported AUC ranging from 0.51 [18] to 0.62 [15], highlighting a better performance for %(-2) proPSA (AUC from 0.63 [4] to 0.78 [24]) and phi (AUC from 0.67 [18] to 0.78 [21]). For these biomarkers, whereby, we report a significantly high accuracy for detecting PCa (AUC 0.89 and 0.95, respectively) and they seem to be of greater clinical interest.

Our results suggest, also that the %(-2)proPSA and phi improve the detection of PCa in patients with PSA in the grey zone, according to previous systematic reviews. A published systematic review including 12 studies [27] reported similar %(-2)proPSA specificity (32.5 % vs. 32%) and phi specificity (31.6% vs. 31%). However, Wang [28] reported, a very high %p2PSA specificity (40% vs. 32%), phi specificity (45% vs. 31%). The authors suggest that %(-2)proPSA and phi have higher diagnostic accuracy rates than other PSA derivatives and seem to be more useful in PCa diagnosis. Additionally, other authors have suggested that these biomarkers may be potential candidates in predicting aggressive PCa in association with the Gleason score, and may help clinicians in guiding the decision to opt for a biopsy and in choosing appropriate treatment [27, 28].

To assessment of clinical utility, the levels of evidence of the included studies were validated by the GRADE approach [6]. From the level of evidence, to the current study sought to determine whether the (-2)proPSA should be introduced as a preferred biomarker in routine laboratory examinations for the identification of patients with PCa. The level of evidence was evaluated as low quality because the studies included in the current analysis have important methodological shortcomings: some studies have a retrospective design, and in others the outcome assessor was not blinded with respect to the reference or standard test. Despite the encouraging results, the clinical significance of serum PSA remain controversial due to different levels of evidence provided by included studies, and as they are enabling which cannot suggest a clear benefit. Nevertheless the majority of the included studies claimed that the (-2)proPSA has a higher accuracy than traditional marker as the tPSA, the valuation of the evidence through the GRADE method shown a questionable clinical application. This approach could be a valid method also for the laboratory professionals to evaluate the evidence, but its implementation is still far.

Our findings are in line with current recommendations in many international guidelines [29–32], which do not support the widespread use of (-2)proPSA in identifying patients at risk of PCa. The main American and European urologist associations recommend the consideration of digital rectal examination (DRE) findings, prostate size, ethnicity, age, comorbidity, family history, previous biopsy results, as well as tPSA values before to perform a biopsy. Moreover, at the moment, tPSA is the only biomarker recommended to monitor patients after radical therapy. Other serum biomarkers still require further investigation to establish their clinical usefulness.

As a result of excess detection of non-aggressive PCa from tPSA levels, active surveillance, identifying patients with quick disease progression during the observation time has been proposed as a strategy to reduce the number of negative prostate biopsies [33]. In patients with previous biopsy and who show increasing PSA values throughout follow-up, (-2)proPSA monitoring could assist in any further prostate biopsy decision making processes [3]. The success of this strategy depends on the ability to identify appropriate patients. Many features were investigated to more precisely characterize candidate patients, and only age, race and family history seem to be associated with active surveillance outcomes [34]. Some authors suggest that (-2)proPSA may have a role in stratifying the risk of progression during the active surveillance program and to improve the treatment decisions, identifying patients requiring a second prostatic biopsy, but further studies are needed to confirm the performance of this biomarker throughout the follow-up.

Although this systematic review provides useful information, some limitations could be considered. First, many studies did not report the cut-offs utilized, making difficult the generalizability of the results. Second, although the study's results were accurate, the meta-analyses were affected by high heterogeneity, probably due to the variety of cut-offs used, the difference in the enrolment methods and the number of core obtained during the biopsy. In this, the concept of harmonization should be highlighted, as the differences among decision limits used in the primary studies compromise the result interpretations and the possibly derived recommendations. Third, studies with retrospective design were included.

Furthermore, we conducted a methodological exercise trying to apply the GRADE method also for the evaluation of a diagnostic test. This approach could be a valid method also for the laboratory professionals to evaluate the evidence, but its implementation is still far due to some levels of criticality. The first obstacle is the lack of explicit diagnostic key question and a clear definition of the diagnostic accuracy outcome. The applicability of the GRADE criteria is laborious often due to the poor reporting of the evaluated studies. So, develop a profile defining the sensibility and specificity in term of number of patients that could benefit or not from the evaluated test, helps readers to judge the test validity. Moreover, the further limitation of our work was the difficulty to engage a structured multidisciplinary and multiprofessional panel with GRADE experienced, ensuring the methodological validity and the clinical relevance of the (-2)proPSA.

In conclusion, the current evidences do not support the clinical utility of (-2)proPSA in the diagnostic process. Transparent and explicit method as GRADE could lead the way for the interpretation and implementation of a new test.

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