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The Percentage of [-2]Pro–Prostate-Specific Antigen and the Prostate Health Index Outperform Prostate-Specific Antigen and the Percentage of Free Prostate-Specific Antigen in the Detection of Clinically Significant Prostate Cancer and Can Be Used as Reflex Tests

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- Context.—There is a need to avoid the overdiagnosis of prostate cancer (PCa) and to find more specific biomarkers.

- Objective.—To evaluate the clinical utility of [-2]pro–prostate-specific antigen ([-2]proPSA) derivatives in detecting clinically significant PCa (csPCa) and to compare it with prostate-specific antigen (PSA) and with the percentage of free PSA (%fPSA).

- Design.—Two hundred thirty-seven men (PSA: 2–10 ng/mL) scheduled for a prostate biopsy were enrolled. Parametric and nonparametric tests, receiver operating characteristic (ROC) curves, and logistic regression analysis were applied. Outcomes were csPCa and overall PCa.

- Results.—Both [-2]proPSA derivatives were significantly higher in csPCa and overall PCa (P < .001). The areas under the curves for the prediction of csPCa were higher for the percentage of [-2]proPSA (%[-2]proPSA) (0.781) and the prostate health index (PHI) (0.814) than for PSA (0.651) and %fPSA (0.724). There was a gain of 11% in diagnostic accuracy when %[-2]proPSA or PHI were added to a base model with PSA and %fPSA. Twenty-five percent to 29% of biopsies could have been spared with %[-2]proPSA (cutoff: ≥1.25%) and PHI (cutoff: ≥27), missing 10% of csPCa’s. The same results could have been achieved by using [-2]proPSA as a reflex test, when %PSA was 25% or less (cutoffs: ≥1.12% and ≥24 for %[-2]proPSA and PHI, respectively).

- Conclusions.—The [-2]proPSA derivatives improve the diagnostic accuracy of csPCa, when the PSA value is between 2 and 10 ng/mL, allowing to spare unnecessary biopsies and to select patients for active surveillance. [-2]proPSA can be used as a reflex test when %PSA is 25% or less, without reducing the diagnostic accuracy for csPCa and the number of spared biopsies.

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Prostate cancer (PCa) is estimated to be the malignancy with the highest incidence in men in 2020, both in the United States and in Europe,1,2 being the second leading cause of cancer death in men, after lung cancer.3 The importance of this neoplasia has led to increasing efforts to improve its detection, while keeping in mind the potential harms of overtreatment and overdiagnosis. Although the European Randomized study of Screening for Prostate Cancer (ERSPC),3 after a 16-year follow-up, showed that prostate-specific antigen (PSA) screening significantly reduces PCa mortality, overtreatment and overdiagnosis still remain a concern. Thus, there is a need to evaluate new diagnostic tests, which could assist in predicting the aggressiveness of PCa, in order to avoid overdiagnosis of nonsignificant cancers.4

In this respect, several free PSA (fPSA) subforms have been studied, such as benign PSA, intact PSA, and proPSA.5 Among these subforms, proPSA was shown to have higher values in PCa, while the other two are related to benign tissue.6 Several circulating forms of proPSA were identified in serum: [-2], [-4], [-5], and [-7].7 Of these proPSA forms, [-2]proPSA has a greater stability in serum, and Beckman
Coulter developed an automated immunoassay for its detection.\(^6\) Mostly since the launch of this assay, several observational studies have consistently reported that both \([-2]\text{proPSA}\) derivatives—the percentage of \([-2]\text{proPSA}(%\text{-}[2]\text{proPSA})\) and the prostate health index (PHI)—could improve PCa detection when compared with PSA and with the percentage of fPSA (%fPSA), allowing a reduction in the number of unnecessary biopsies.\(^7,8,9\)

Despite these studies, there is yet no agreement about the best cutoff for both PHI and \(%\text{-}[2]\text{proPSA}\).\(^10-12\) Moreover, the European Association of Urology states that there is too limited evidence to implement these tests into routine screening programs.\(^13\) The same applies to the American Urological Association, which still underlines that more research is needed to confirm \([-2]\text{proPSA}\) benefits in the early detection of PCa, and its ability to reduce unnecessary biopsies, while keeping the capacity to detect clinically significant prostate cancer (csPCa).\(^14\) Based on these requirements, and since most of the previous studies about \([-2]\text{proPSA}\) focused on the overall detection of PCa, in this study we wanted to address the need to avoid the overdiagnosis of nonsignificant PCa. Hence, our main objective was to evaluate the clinical utility of \([-2]\text{proPSA}\) derivatives in the detection of csPCa, and to find suitable cutoffs for this purpose. We also aimed to make a comparison between the diagnostic accuracy of these new parameters and the already fully implemented tests—PSA and %fPSA—in order to optimize the combined use of all these tests in men with a PSA value between 2 and 10 ng/mL.

**MATERIALS AND METHODS**

**Study Subjects**

We enrolled all consecutive patients with a prostate biopsy scheduled for suspicion of PCa, who met the inclusion criteria and signed the informed consent, at the Urology Department of the Central Lisbon University Hospital Center (Lisbon, Portugal), between December 2017 and October 2019. The study was approved by the Institution’s Research Ethics Committee and complies with the Declaration of Helsinki. In total, we enrolled 237 patients, who underwent a first or repeated prostate biopsy with at least 12 fragments: 203 patients underwent a transrectal ultrasound (TRUS)–guided needle core biopsy, and the remaining 34 patients underwent a multiparametric magnetic resonance imaging/ultrasound (MRI/US) fusion-targeted transperineal biopsy.

Patients were eligible for inclusion if they had a PSA level between 2 and 10 ng/mL and no previous history of PCa, irrespective of the digital rectal examination findings. Patients were excluded if any of the following situations occurred: previous transurethral resection of the prostate, therapy with drugs that may affect PSA concentration (5-\(\alpha\)-reductase inhibitors and androgens), urinary infection contemporary to blood collection, acute bacterial prostatitis in the 3 months before biopsy, hemophilia or history of multiple blood transfusions, serum total protein concentration above 8 g/dL, chronic renal failure, or heavily hemolyzed serum samples.

All biopsy and radical prostatectomy specimens had a pathologic assessment by the same expert genitourinary pathologist, to avoid any possible assessment biases. All histologic diagnoses were given with the updated Gleason grading, according to the definitions of the 2014 consensus conference of the International Society of Urological Pathology (ISUP).\(^15\) Patients with atypical small acinar proliferation or high-grade prostatic intraepithelial neoplasia were considered as not having PCa. All patients with PCa were prospectively followed up until staging was completed (January 2020). For patients who underwent a radical prostatectomy, the final Gleason score (GS) used for the purpose of this study was the one obtained from the prostatectomy histologic examination, instead of the GS from the biopsy.

**Blood Sample Processing and Laboratory Assays**

Blood collection took place on the same day of the biopsy, but before the procedure. We followed the criteria described by Semjonow et al\(^16\) for the preanalytic in vitro stability of \([-2]\text{proPSA}\). Blood samples were collected in tubes without any anticoagulant and containing a separator gel, centrifuged at 2876 g for 10 minutes, and refrigerated (2°C–8°C) within 3 hours of the blood draw. From each sample, 500 μL of serum was separated into secondary tubes, which were frozen at −80°C, within 8 hours after the blood draw. Samples were thawed only once and processed with the Beckman Coulter Access 2 immunoassay analyzer (Beckman Coulter, Brea, California), which was used to measure the serum concentrations of PSA, fPSA, and \([-2]\text{proPSA}\) (Hybritech calibration).

The %fPSA and the \[%\text{-}[2]\text{proPSA}\] were defined as proportions: \(\text{fPSA}/\text{PSA} \times 100\) and \((\text{-}[2]\text{proPSA} [\text{ng/mL}]/\text{fPSA} [\text{ng/mL}] \times 1000)\) × 100, respectively. The PHI was calculated through the formula \([-\text{[2]proPSA}/\text{fPSA}]\) × \(\sqrt{\text{PSA}}\).

**Primary and Secondary Outcomes**

The primary outcome was csPCa on biopsy, which we defined according to the criteria of the Prostate Cancer Research International Active Surveillance (PRIAS) study, for patients with a PSA value below 10 ng/mL.\(^17\) Therefore, we classified as csPCa any cancer with at least one of the following characteristics: regional lymph node metastasis (N1), distant metastasis (M1), extracapsular disease (T3), PSA density of 0.2 ng/mL or greater, GS of 7 or higher, number of positive biopsy cores greater than 2 or, whenever saturation biopsies were made (≥20 cores), more than 15% of positive cores (or more than 4 positive cores, if 15% of positive cores exceeded this number).\(^18\) All the other cancers were classified as nonsignificant. This definition is also consistent with the National Comprehensive Cancer Network criteria for low-risk PCa.\(^19\)

Besides evaluating the diagnostic accuracy of the \([-2]\text{proPSA}\) derivatives in detecting this primary outcome (csPCa), we also looked at their ability to detect overall PCa (secondary outcome).

**Statistical Analysis**

Continuous variables were reported as mean and standard deviation (SD), if normally distributed, or as median and interquartile range (IQR) if nonnormally distributed. Student \(t\) test or ANOVA were used to compare normally distributed continuous variables, and the Mann-Whitney \(U\) test or Kruskal-Wallis test was used to compare nonnormally distributed ones. Categorical variables were described by their frequencies, and comparisons were done with \(\chi^2\) test or Fisher exact test. Univariate and multivariate logistic regression analysis were applied for the prediction of the primary and secondary outcomes. Odds ratios (ORs) with 95% CIs were also calculated. The predictive accuracy of the biomarkers was quantified as the area under the ROC curve (AUC). A 2-sided \(P\) value <.05 was considered to indicate statistical significance. Statistical analysis was performed with IBM SPSS Statistics 26.0 (2019; IBM Corp, Armonk, New York). Positive (PPV) and negative (NPV) predictive values, as well as Youden indices, were calculated with the 2020 MedCalc Software version 19.6.1 (MedCalc Software Ltd, Ostend, Belgium).

**RESULTS**

The main demographic and clinical characteristics of the study participants, including the biomarkers results, are depicted in Table 1. Patients with and without PCa had similar age distributions and most were white (234 of 237 [98.7%]).

Of the 237 patients enrolled, 118 (49.8%) had PCa, including 100 (42.2%) who had csPCa. The percentage of positive biopsies was around 50% both in the patients...
Table 1. Summary of Characteristics of the Study Patients

|                                | All Cases | Without PCa | Overall PCa | P       | Without PCa or With Nonsignificant PCa | Clinically Significant PCa | P     |
|--------------------------------|-----------|-------------|-------------|---------|----------------------------------------|---------------------------|-------|
| Patients, n (%)                | 237 (100) | 119 (50.2)  | 118 (49.8)  | -       | 137 (57.8)                            | 100 (42.2)                | -     |
| Age, median (IQR) or mean (±SD), y | 68.0 (61.5–73.0) | 65.8 (±7.8) | 69.0 (62.8–73.0) | .03⁶⁷  | 66.0 (61.0–71.5) | 67.9 (±7.8) | .07⁶⁷ |
| Ethnic group                   |           |             |             | .58⁸⁸  | .58⁸⁸                                 | .58⁸⁸                     |       |
| White, n (%)                   | 234 (98.7)| 118 (99.2)  | 116 (98.3)  | -       | 136 (99.3)                            | 98 (98.0)                 | -     |
| Black, n (%)                   | 3 (1.3)   | 1 (0.8)     | 2 (1.7)     | -       | 1 (0.7)                               | 2 (2.0)                   | -     |
| Family history (first-degree relatives) |         |             |             | .49⁸⁸  | .49⁸⁸                                 | .49⁸⁸                     |       |
| Yes, n (%)                     | 30 (12.7) | 11 (9.2)    | 19 (16.1)   | -       | 15 (10.9)                             | 15 (15.0)                 | -     |
| No, n (%)                      | 196 (82.7)| 102 (85.7)  | 94 (79.7)   | -       | 115 (83.9)                            | 81 (81.0)                 | -     |
| Missing, n (%)                 | 11 (4.6)  | 6 (5.0)     | 5 (4.2)     | -       | 7 (5.1)                               | 4 (4.0)                   | -     |
| DRE                            |           |             |             | .10⁸⁸  | .10⁸⁸                                 | .10⁸⁸                     |       |
| Suspicious, n (%)              | 61 (25.7) | 27 (22.7)   | 34 (28.8)   | -       | 29 (21.2)                             | 32 (32.0)                 | -     |
| Nonsuspicious, n (%)           | 166 (70.0)| 88 (73.9)   | 78 (66.1)   | -       | 101 (73.7)                            | 65 (65.0)                 | -     |
| Missing, n (%)                 | 10 (4.2)  | 4 (3.4)     | 6 (5.1)     | -       | 7 (5.1)                               | 3 (3.0)                   | -     |
| Biopsy type                    |           |             |             |        |                                        |                           |       |
| TRUS-guided needle core biopsy, n (%) | 203 (85.7) | 101 (84.9)  | 102 (86.4)  | -       | 114 (83.2)                            | 89 (89.0)                 | -     |
| MRI/US fusion-targeted transperineal biopsy, n (%) | 34 (14.3) | 18 (15.1)   | 16 (13.6)   | -       | 23 (16.8)                             | 11 (11.0)                 | -     |
| Tumor markers                  |           |             |             |        |                                        |                           |       |
| PSA, mean (±SD), ng/mL         | 6.24 (±2.00) | 5.89 (±1.98) | 6.60 (±1.97) | .006⁷ | 5.81 (±1.97)                        | 6.84 (±1.90)              | <.001⁷ |
| fPSA, median (IQR), ng/mL      | 0.93 (0.64–1.26) | 0.99 (0.69–1.28) | 0.81 (0.61–1.17) | .02⁶ | 0.98 (0.66–1.28) | 0.80 (0.62–1.17) | .04⁶ |
| %fPSA, median (IQR) or mean (±SD) | 16.29 (±6.32) | 18.29 (±6.43) | 14.27 (±5.55) | <.001⁶ | 17.81 (14.11–21.71) | 13.04 (10.14–16.96) | <.001⁶ |
| [−2]proPSA, median (IQR), pg/mL | 12.99 (9.25–18.76) | 11.92 (8.39–17.00) | 14.60 (10.49–21.19) | .002⁷ | 11.92 (8.35–17.79) | 14.72 (10.94–21.26) | .001⁷ |
| %[−2]proPSA, median (IQR)      | 1.56 (1.16–1.95) | 1.29 (0.98–1.61) | 1.86 (1.44–2.24) | <.001⁷ | 1.31 (1.04–1.62) | 1.93 (1.57–2.35) | <.001⁷ |
| PHI, median (IQR)              | 36.57 (26.93–49.87) | 29.79 (23.91–37.48) | 45.59 (34.48–60.75) | <.001⁷ | 30.10 (23.92–37.50) | 49.66 (38.90–62.60) | <.001⁷ |

Abbreviations: DRE, digital rectal examination; fPSA, free prostate-specific antigen; IQR, interquartile range; MRI/US, magnetic resonance imaging/ultrasound; PCa, prostate cancer; PHI, prostate health index; PSA, prostate-specific antigen; TRUS, transrectal ultrasound; %fPSA, percentage of free prostate-specific antigen; %[−2]proPSA, percentage of [−2]proPSA.

⁷ Student t test.
⁸ Mann-Whitney U test.
⁹ Fisher exact test.
¹⁰ χ² test with continuity correction.
undergoing a TRUS-guided needle core biopsy (50.25%; n = 102 of 203) and a MRI/US fusion-targeted transperineal biopsy (47.06%; n = 16 of 34). Extracapsular extension (T3) was present in 18 of the 237 patients (7.6%), regional lymph node metastasis (N1) was identified in 3 patients (1.3%), and 9 (3.8%) had distant metastasis (M1). Among the 118 patients with PCa, 29 (24.6%) had a GS of 6; 48 (40.7%) a GS of 7 (3+4); 25 (21.2%) a GS of 7 (4+3); 7 (5.9%) a GS of 8; and 9 (7.6%) had a GS of 9 or 10.

When looking at the primary outcome, both %-2proPSA and PHI (Figure 1, A and B) had significantly higher values (P < .001) for the patients with csPCa (median values, 1.93% and 49.66, respectively), than for patients with negative biopsies or with nonsignificant PCa (median values, 1.31% and 30.10).

When considering the secondary outcome, we also found significant differences between the patients with and without overall PCA for %-2proPSA (median values, 1.86% versus 1.29%; P < .001) and for PHI (median values, 45.59 versus 29.79; P < .001), respectively.

The levels of both %-2proPSA and PHI increased from the lower to the higher Gleason grade groups (Figure 2, A and B). However, only PHI showed a significant difference when all grade groups were compared (P = .006). When comparing the values of the two %-2proPSA derivatives between patients within ISUP grade groups 2 (GS = 3+4) and 3 (GS = 4+3), there were no significant differences (P = .52 for %-2proPSA; P = .33 for PHI), although the values of PHI were higher for patients within grade group 3 (GS = 4+3). When comparing grade group 1 (GS = 6) with all the other groups (GS ≥ 7), there is a significant difference between the values of both %-2proPSA (P = .02) and PHI (P = .001).

The ROC curve analysis (Figure 3) showed a greater diagnostic accuracy, considering csPCA as the outcome, for %-2proPSA (AUC = 0.781) and PHI (AUC = 0.814), when compared to PSA (AUC = 0.651) and %fPSA (AUC = 0.724). The difference between the AUC was significant between %-2proPSA and PSA (P = .008), PHI and PSA (P < .001), PHI and %fPSA (P < .001), but not significant between %-2proPSA and %fPSA (P = .17). Univariate logistic regression analysis is consistent with these findings, revealing that %-2proPSA and PHI are predictors of csPCa (P < .001) (Table 2).

In multivariate logistic regression analysis, %-2proPSA and PHI, when added to a base model that included PSA and %fPSA (AUC = 0.753), increased the diagnostic accuracy for csPCA by 11% (AUC = 0.836 with %-2proPSA; AUC = 0.837 with PHI). Multivariate analysis

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also showed that both tests are independent predictors of csPCa when added to the base model (Table 2).

For the secondary outcome, the diagnostic accuracy was also greater for \(\%[\text{[1–2]proPSA}]\) and PHI (AUC = 0.781) than for PSA and \%PSA (AUCs of 0.601 and 0.691, respectively) and the difference was significant between the new tests and the older markers, except between \%[1–2]proPSA and \%PSA \((P = .09)\). In addition, univariate logistic regression analysis revealed that both \%[1–2]proPSA and PHI are predictors of overall PCa \((P < .001)\). Multivariate analysis showed an increase in diagnostic accuracy of 14\% (AUC = 0.799) and 15\% (AUC = 0.804) when \%[1–2]proPSA and PHI, respectively, were added to the base model (AUC = 0.699). Both tests are independent predictors of overall PCa, when added to the base model \((P < .001)\).

We also performed the same analysis for the subset of patients with a PSA level between 4 and 6 ng/mL \((n = 84)\), since this is a very challenging range regarding biopsy decisions. Even in this range, there was a significant difference between patients without and with csPCa for \%[1–2]proPSA (median values, 1.28\% versus 1.94\%; \(P < .001)\) and for PHI (median values, 29.45 versus 45.18; \(P < .001)\). On univariate analysis both parameters maintained a predictor status for the detection of csPCa \((OR = 8.208; P < .001)\) and for PHI \((OR = 1.107; P < .001)\). The AUCs were greater for \%[1–2]proPSA \((AUC = 0.819; 95\% CI, 0.728–0.910)\) and PHI \((AUC = 0.842; 95\% CI, 0.757–0.927)\) than for PSA \((AUC = 0.635; 95\% CI, 0.509–0.762)\) and \%PSA \((AUC = 0.750; 95\% CI, 0.639–0.862)\). On multivariate analysis, there was a gain of 13\% in diagnostic accuracy when \%[1–2]proPSA \((AUC = 0.846)\) or PHI \((AUC = 0.847)\) was added to the base model \((AUC = 0.747)\). ROC curve analysis provided the values of the diagnostic sensitivity and specificity for csPCa (Table 3) and overall PCa (Table 4), for several \%[1–2]proPSA and PHI cutoffs, as well

Figure 3. Receiver operating characteristic (ROC) curves for the prostate-specific antigen (PSA), the percentage of free prostate-specific antigen (%fPSA), the percentage of \%[1–2]proPSA \%(1–2]proPSA) and the prostate health index (PHI), regarding clinically significant prostate cancer detection.

Table 2. Univariate and Multivariate Analyses of Tumor Markers for Predicting Clinically Significant Prostate Cancer

| Predictor       | Univariate Analysis | Multivariate Analysis | Base Model and PHI |
|-----------------|---------------------|-----------------------|-------------------|
| PSA             | AUC (95\% CI)       | OR (95\% CI)          | P                 |
|                 | 0.753 (0.689–0.817) | 1.233 (1.061–1.433)   | < .001            |
| %fPSA           | 0.724 (0.659–0.790) | 0.859 (0.812–0.908)   | < .001            |
| %\%[1–2]proPSA  | 0.814 (0.756–0.873) | 0.814 (0.756–0.873)   | < .001            |

Abbreviations: AUC, area under the curve; OR, odds ratio; PHI, prostate health index; PSA, prostate-specific antigen; %fPSA, percentage of free prostate-specific antigen; %\%[1–2]proPSA, percentage of \%[1–2]proPSA.
Table 3. Diagnostic Sensitivities, Specificities, and Predictive Values for Clinically Significant Prostate Cancer

| PSA, ng/ml | Sensitivity, % (95% CI) | Specificity, % (95% CI) | PPV, % (95% CI) | NPV, % (95% CI) | Spared Biopsies, n (%) of 237 | Missed Clinically Significant PCa, n (%) of 100 |
|------------|-------------------------|-------------------------|----------------|----------------|-------------------------------|-----------------------------------------------|
| ≥ 4.00     | 92.00 (84.84–96.48)     | 19.71 (13.41–27.36)    | 45.54 (43.05–48.06) | 77.14 (61.56–87.68) | 35 (14.77)                   | 8 (8)                                       |
| ≥ 4.30     | 90.00 (82.38–95.10)     | 21.90 (15.29–29.76)    | 45.69 (42.97–48.43) | 75.00 (60.61–85.40) | 40 (16.88)                   | 10 (10)                                     |
| ≥ 5.73 (YI = 0.2674) | 72.00 (62.10–80.50) | 54.74 (46.00–63.30) | 53.73 (48.22–59.16) | 72.81 (65.38–79.15) | 102 (43.04)                  | 28 (28)                                     |
| ≥ 8.60     | 21.00 (13.49–30.29)     | 90.51 (84.32–94.85)    | 61.76 (45.95–75.43) | 61.08 (58.33–63.77) | 203 (85.65)                  | 79 (79)                                     |
| %fPSA      | ≤ 25.00                 | 96.00 (90.07–98.90)    | 13.14 (7.98–19.97) | 44.65 (42.77–46.55) | 81.82 (61.11–92.80)          | 22 (9.28)                                   |
|           | ≤ 20.00                 | 90.00 (82.38–95.10)    | 31.39 (23.73–39.87) | 48.91 (45.66–52.18) | 81.13 (69.4–89.06)           | 53 (22.36)                                  |
|           | ≤ 14.01 (YI = 0.4037)   | 63.00 (52.80–72.40)    | 77.37 (69.40–84.10) | 67.02 (59.03–74.14) | 74.12 (68.59–78.98)          | 142 (59.92)                                 |
|           | ≤ 11.00                 | 32.00 (23.02–42.08)    | 90.51 (84.32–94.85) | 71.11 (57.69–81.63) | 64.58 (61.20–67.83)          | 192 (81.01)                                 |
| %[−2]proPSA | ≥ 1.25                 | 90.00 (82.38–95.10)    | 43.07 (34.64–51.80) | 53.57 (49.59–57.51) | 85.51 (76.06–91.63)          | 69 (29.11)                                  |
|           | ≥ 1.66 (YI = 0.4810)    | 70.00 (60.0–78.80)     | 78.10 (70.20–84.70) | 70.00 (63.29–76.65) | 78.10 (72.29–82.97)          | 138 (58.23)                                 |
|           | ≥ 2.00                  | 41.00 (31.26–51.29)    | 90.51 (84.32–94.85) | 75.93 (64.12–84.77) | 67.76 (63.89–71.40)          | 183 (77.22)                                 |
| PHI        | ≥ 27.00                 | 90.00 (82.38–95.10)    | 36.50 (28.44–45.15) | 50.85 (47.28–54.41) | 83.33 (72.74–90.36)          | 60 (25.32)                                  |
|           | ≥ 37.96 (YI = 0.5610)   | 78.00 (68.60–85.70)    | 78.10 (70.20–84.70) | 72.23 (65.08–78.39) | 82.94 (76.89–87.67)          | 128 (54.01)                                 |
|           | ≥ 45.70                 | 57.00 (46.71–66.86)    | 90.51 (84.32–94.85) | 81.43 (71.78–88.31) | 74.25 (69.57–78.43)          | 167 (70.46)                                 |

Abbreviations: NPV, negative predictive value; PCa, prostate cancer; PHI, prostate health index; PPV, positive predictive value; PSA, prostate-specific antigen; YI, Youden index; %fPSA, percentage of free prostate-specific antigen; %[−2]proPSA, percentage of [−2]proPSA.
| PSA, ng/mL | Sensitivity, % (95% CI) | Specificity, % (95% CI) | PPV, % (95% CI) | NPV, % (95% CI) | Spared Biopsies, n (%) of 237 | Missed Overall PCa, n (%) of 118 | Missed csPCa, n (%) of 100 |
|-----------|---------------------|------------------------|-----------------|-----------------|-------------------------------|-------------------------------|-------------------------------|
| ≥ 4.00    | 87.29 (79.90–92.71) | 16.81 (10.58–24.76)   | 50.99 (48.34–53.64) | 57.14 (41.79–71.24) | 35 (14.76)                   | 15 (12.71)                    | 8 (8)                         |
| ≥ 3.80    | 90.68 (83.93–95.25) | 13.45 (7.88–20.91)    | 50.95 (48.67–53.23) | 59.26 (41.35–75.01) | 27 (11.39)                   | 11 (9.32)                     | 6 (6)                         |
| ≥ 5.64 (YI = 0.1821) | 66.95 (57.70–75.30) | 51.26 (41.90–60.50)   | 57.68 (52.14–63.02) | 60.99 (53.40–68.09) | 97 (40.93)                   | 39 (33.05)                    | 27 (27)                       |
| ≥ 8.60    | 19.49 (12.78–27.80) | 90.76 (84.06–95.29)   | 67.65 (51.64–80.37) | 53.20 (50.56–55.82) | 203 (85.65)                  | 95 (80.51)                    | 79 (79)                       |
| %fPSA     | ≤ 25.00             | 94.92 (89.26–98.11)   | 13.45 (7.88–20.91) | 52.09 (50.04–54.14) | 72.73 (51.94–86.81)          | 22 (9.28)                     | 6 (5.08)                      | 4 (4)                         |
|           | ≤ 21.40             | 90.68 (83.93–95.25)   | 26.89 (19.18–35.79) | 55.15 (52.09–58.18) | 74.42 (60.63–84.60)          | 43 (18.14)                    | 11 (9.32)                     | 7 (7)                         |
|           | ≤ 14.01 (YI = 0.3409) | 56.78 (47.30–65.90)   | 77.31 (68.70–84.50) | 71.28 (63.23–78.18) | 64.33 (58.93–69.38)          | 142 (59.92)                   | 51 (43.22)                    | 37 (37)                       |
|           | ≤ 10.80             | 25.42 (17.86–34.26)   | 90.76 (84.06–95.29) | 73.17 (58.93–83.83) | 55.10 (52.12–58.05)          | 196 (82.70)                   | 88 (74.58)                    | 70 (70)                       |
| %[-2]proPSA | ≥ 1.12             | 90.68 (83.93–95.25)   | 35.29 (26.76–44.58) | 58.15 (54.59–61.63) | 79.25 (67.41–87.58)          | 53 (22.36)                    | 11 (9.32)                     | 8 (8)                         |
|           | ≥ 1.57 (YI = 0.4345) | 70.34 (61.20–78.40)   | 73.11 (64.20–80.80) | 72.18 (65.36–78.11) | 71.30 (64.83–77.01)          | 121 (51.05)                   | 35 (31.36)                    | 25 (25)                       |
|           | ≥ 1.95              | 40.66 (31.73–50.11)   | 90.76 (84.06–95.29) | 81.36 (70.47–88.86) | 60.67 (56.80–64.42)          | 178 (75.11)                   | 70 (59.32)                    | 54 (54)                       |
| PHI       | ≥ 24.00             | 90.68 (83.93–95.25)   | 26.05 (18.44–34.89) | 54.87 (51.85–57.85) | 73.81 (59.80–84.23)          | 42 (17.72)                    | 11 (9.32)                     | 7 (7)                         |
|           | ≥ 37.96 (YI = 0.4933) | 70.34 (61.20–78.40)   | 78.99 (70.60–85.90) | 76.86 (69.69–82.75) | 72.86 (66.70–78.25)          | 128 (54.01)                   | 35 (29.66)                    | 22 (22)                       |
|           | ≥ 45.70             | 50.00 (40.66–59.34)   | 90.76 (84.06–95.29) | 84.29 (74.81–90.64) | 64.67 (60.24–68.87)          | 167 (70.46)                   | 59 (50)                      | 43 (43)                       |

Abbreviations: csPCa, clinically significant prostate cancer; NPV, negative predictive value; PCa, prostate cancer; PHI, prostate health index; PPV, positive predictive value; PSA, prostate-specific antigen; YI, Youden index; %fPSA, percentage of free prostate-specific antigen; %[-2]proPSA, percentage of [-2]proPSA.
as for PSA and %fPSA. For these two already implemented tests, we have included in the analysis the usual cutoffs of 4 ng/mL and 25%, respectively. Moreover, we calculated the cutoffs that provided 90% sensitivity, 90% specificity, and the best balance between both, given by the maximum Youden index value. NPVs and PPVs for all parameters were also calculated, considering all the aforementioned cutoffs. For %[-2]proPSA, a 90% (95% CI, 82.38–95.10) diagnostic sensitivity for csPCa was achieved at a cutoff of 1.25%, allowing to spare 69 of the 237 biopsies (29%), although missing 10 of the 100 csPCa’s (10%). On the other hand, a lower cutoff of 1.12% allows a 90.68% (95% CI, 83.93–95.25) sensitivity for overall PCa, which could have avoided 53 of the 237 biopsies (22%), but 11 of the 118 cancers (9%) would have been missed (including 8 csPCa’s). Regarding PHI, a 90% (95% CI, 82.38–95.10) diagnostic sensitivity is reached with a cutoff of 27.00. This threshold for PHI would have spared a total of 60 of 237 biopsies (25%), missing 10 of the 100 csPCa’s (10%). When considering overall PCa, a cutoff of 24.00 for PHI allows a diagnostic sensitivity of 90.68% (95% CI, 83.93–95.25), and a total of 42 of 237 biopsies (18%) could have been avoided, missing 11 cases of 118 PCa’s (9%) (of which 7 were csPCa’s).

We also calculated how many biopsies we could have spared, considering the sequential use of %fPSA and the %[-2]proPSA derivatives for detecting csPCa (Table 5). Using a cutoff of 20% for %fPSA (90.00% of diagnostic sensitivity for csPCa) and testing %[-2]proPSA only in patients with %fPSA of 20% or less, we determined that with a cutoff of 1.25% for %[-2]proPSA, 102 of the 237 biopsies (43.04%) would have been spared, but 17 csPCa’s would have been missed. On the other hand, applying a cutoff of 27.00 to PHI, a total of 93 of 237 biopsies (39.24%) would have been avoided, but missing 15 cases of csPCa. The same algorithm of reflex testing was evaluated for overall PCa, using a cutoff of 21.40% (90.68 of sensitivity) for the %fPSA and also the commonly used cutoff of 25% (94.92% of sensitivity). When a threshold of 21.40% is used for %fPSA, applying %[-2]proPSA as a reflex test (cutoff: 1.12%), we would avoid 80 of the 237 biopsies (33.76%), missing 18 cancers of 118 (15.25%), of which 12 were csPCa’s. Applying PHI as a reflex test (cutoff: 24.00) when %fPSA was 21.40% or lower, 72 of the 237 biopsies (30.38%) could have been spared, but 19 cancers of the 118 (16.10%) would have been missed, 12 of which were csPCa’s. If the most commonly used cutoff for %fPSA is applied, the %[-2]proPSA with a cutoff of 1.12% would spare 69 of the 237 biopsies (29.11%), missing 15 cancers, of which 10 are csPCa’s. With the use of PHI, 59 of the 237 biopsies (24.89%) would have been avoided, missing also 15 cancers, 10 of these being csPCa’s.

### Table 5. Benefits of Using the Percentage of %[-2]proPSA and the Prostate Health Index as Reflex Tests, Applied to Different Cutoffs for the Percentage of Free Prostate-Specific Antigen

| %fPSA | %[-2]proPSA | PHI | Spared Biopsies, n (%) of 237 | Missed Overall PCa, n (%) of 118 | Missed Clinically Significant PCa, n (%) of 100 |
|-------|-------------|-----|-------------------------------|-----------------------------|---------------------------------|
| ≤ 20.00 | ≥ 1.25 | - | 102 (43.04) | 26 (22.03) | 17 (17) |
| | - | ≥ 27.00 | 93 (39.24) | 24 (20.34) | 15 (15) |
| ≤ 21.40 | ≥ 1.12 | - | 80 (33.76) | 18 (15.25) | 12 (12) |
| | - | ≥ 24.00 | 72 (30.38) | 19 (16.10) | 12 (12) |
| ≤ 25.00 | ≥ 1.12 | - | 69 (29.11) | 15 (12.71) | 10 (10) |
| | - | ≥ 24.00 | 59 (24.89) | 15 (12.71) | 10 (10) |

Abbreviations: PCa, prostate cancer; PHI, prostate health index; %fPSA, percentage of free prostate-specific antigen; %[-2]proPSA, percentage of %[-2]proPSA.

### DISCUSSION

In this study, we found that both %[-2]proPSA and PHI were more accurate predictors of csPCa than PSA and %fPSA. In our population, they had higher PPVs and NPVs than did PSA and %fPSA, and could lead to a reduction in the number of unnecessary biopsies. Both %[-2]proPSA and PHI had significantly higher levels in patients with csPCa (P < .001), being independent and significant predictors of csPCa, when added to the base model, as shown by logistic regression analysis.

The better diagnostic accuracy of these newer tests was revealed by higher AUCs for %[-2]proPSA and PHI. Moreover, multivariate logistic regression analysis showed that the addition of %[-2]proPSA and PHI to a base model that included the commonly used markers (PSA and %fPSA) could provide a gain in diagnostic accuracy for csPCa of 11%. Both %[-2]proPSA derivatives maintain their diagnostic accuracy and advantages when compared to PSA and %fPSA, even when the PSA value is between 4 and 6 ng/mL, which can be important, considering that this PSA range can be very challenging when considering biopsy decisions.

The ROC curve analysis, as well as the logistic regression analysis, also revealed a better diagnostic accuracy for these newer tests, considering the detection of overall PCa.

The ability of %[-2]proPSA derivatives in predicting csPCa is also revealed by increasing values of %[-2]proPSA and PHI with increasing grade groups. When comparing values between the patients with GS = 6 and GS of 7 or higher, the differences were statistically significant for both tests. This fact can make them valuable tools in evaluating the risk of aggressiveness of PCa, since in many centers the GS is perhaps the most important factor for risk stratification. However, none of the parameters could differentiate well between the two GS = 7 subgroups, that is, 3+4 or 4+3. These findings are consistent with previous studies that report higher levels of these newer tests in patients with a GS of 7 or higher.2–4 In particular, a study performed by Guazzoni et al21 showed that both %[-2]proPSA and PHI are accurate predictors of several pathologic characteristics of PCa, namely the presence of pT3 disease, GS of 7 or higher, and tumor volume below 0.5 mL, which are criteria quite similar to those used in the PRIAS study for patients with a PSA value below 10 ng/mL, which we adopted for defining csPCa in our study.

Our results show that the measurement of %[-2]proPSA and PHI can increase the specificity for csPCa. Actually, for equivalent diagnostic sensitivities of 90.00%, %[-2]proPSA and PHI had diagnostic specificities of 43.07% and 36.50%, respectively.
respectively, whereas the specificities of PSA and %fPSA were lower (21.90% and 31.39%). The higher specificities give these newer tests better predictive values, especially higher NPVs when compared to the formerly implemented tests, which can help exclude the likelihood of csPCa in more patients, hence allowing a reduction in the number of unnecessary biopsies.

We believe that the best cutoffs for the [−2]proPSA derivatives to be used in clinical practice should be those that can allow a sensitivity of around 90%, in order to maximize PCa detection. In most of the studies already performed, the cutoffs that allow a 90% sensitivity have been chosen as the better ones to use. With a 90% diagnostic sensitivity for csPCa, biopsies could have been avoided in 29.11% (n = 69 of 237) of the cohort, or in 43% (n = 59) of the 137 men with no PCa or with clinically insignificant PCa, if only patients with %[−2]proPSA of 1.25% or lower had undergone biopsy. By doing this, a similar number, 10, of the 100 csPCa’s (10%), would have been missed. A similar benefit is verified with PHI: if only men with PHI of 27.00 or greater had undergone biopsy, we could have spared 25.32% (n = 60) of the 237 biopsies (missing also 10% of the 100 csPCa’s), or 36.50% (n = 50) of unnecessary biopsies, that is, in the 137 men without PCa or with insignificant PCa. These results are similar to those obtained by Loeb et al who also investigated the role of PHI in selectively identifying csPCa, with a cutoff of 28.60. However, these authors used a cohort of patients only with PSA values between 4 and 10 ng/mL, while we used a broader range from 2 to 10 ng/mL.

When considering the detection of overall PCa, lowering the cutoff for PHI (from 27.00 to 24.00) and for %[−2]proPSA (from 1.25% to 1.012%) can provide a 90.68% sensitivity (with 26.05% specificity for PHI and 35.29% for %[−2]proPSA), allowing to spare between 42 and 53 of the 237 biopsies (17.72%–22.36%), and missing 11 of the 188 cancers (9.32%), of which between 7 and 8 were csPCa’s (5.93%–6.78%). This means that, for similar sensitivities (around 90%), both [−2]proPSA derivatives can improve specificity for PHI, compared to PSA (13.45%). The %[−2]proPSA has also a higher specificity than %fPSA (26.89%), although PHI has a similar specificity to that of %fPSA for overall PCa. These values of specificity at 90% sensitivity are similar to those found in other studies, for %fPSA for overall PCa. These values of specificity at 90% sensitivity are similar to those found in other studies, for %fPSA for overall PCa. These values of specificity at 90% sensitivity are similar to those found in other studies, for %fPSA for overall PCa.

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In summary, the [−2]proPSA derivatives can improve the diagnostic accuracy for csPCa when the PSA value is between 2 and 10 ng/mL, allowing to reduce the number of unnecessary biopsies and to select patients for active surveillance. It is also possible to use them as reflex tests, based on the %fPSA results. This strategy allows a cost-effective approach, without reducing the diagnostic accuracy for csPCa or the number of spared biopsies.

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