# Serum Index Test %[-2]proPSA and Prostate Health Index are More Accurate than Prostate Specific Antigen and %fPSA in Predicting a Positive Repeat Prostate Biopsy

Massimo Lazzeri,* Alberto Briganti, Vincenzo Scattoni, Giovanni Lughezzani, Alessandro Larcher, Giulio Maria Gadda, Giuliana Lista, Andrea Cestari, Nicolòmaria Buffi, Vittorio Bini, Massimo Freschi, Patrizio Rigatti, Francesco Montorsi and Giorgio Guazzoni

*From the Department of Urology, San Raffaele Turro, Vita-Salute San Raffaele University (ML, GL, AL, GMG, GL, AC, NB, GG), Department of Urology (AB, VS, PR, FM) and Department of Pathology (MF), Vita-Salute San Raffaele University, Milan, and Department of Internal Medicine, University of Perugia, Perugia (VB), Italy*

**Purpose:** We tested the hypothesis that serum isoform [-2]proPSA derivatives %p2PSA and Prostate Health Index are accurate predictors of prostate cancer in men scheduled for repeat biopsy.

**Materials and Methods:** The study was an observational prospective evaluation of a clinical cohort of men with 1 or 2 previous negative prostate biopsies, with persistent suspicion of prostate cancer. They were enrolled in the study to determine the diagnostic accuracy of %p2PSA using the formula, (p2PSA pg/ml)/(free prostate specific antigen ng/ml $\times$ 1,000)]$\times$ 100, and Beckman-Coulter Prostate Health Index using the formula, (p2PSA/free prostate specific antigen) $\times$ $\sqrt{}$total prostate specific antigen), and to compare it with the accuracy of established prostate cancer serum tests (total prostate specific antigen, free prostate specific antigen and percent free prostate specific antigen). Multivariable logistic regression models were complemented by predictive accuracy analysis and decision curve analysis.

**Results:** Prostate cancer was found in 71 of 222 (31.9%) subjects. %p2PSA and Prostate Health Index were the most accurate predictors of disease. %p2PSA significantly outperformed total prostate specific antigen, free prostate specific antigen, percent free prostate specific antigen and p2PSA in the prediction of prostate cancer ($p \leq 0.01$), but not Prostate Health Index ($p = 0.094$). Prostate Health Index significantly outperformed total prostate specific antigen and p2PSA ($p \leq 0.001$) but not free prostate specific antigen ($p = 0.109$) and free/total prostate specific antigen ($p = 0.136$). In multivariable logistic regression models %p2PSA and Prostate Health Index achieved independent predictor status, and significantly increased the accuracy of multivariable models including prostate specific antigen and prostate volume with or without percent free prostate specific antigen and prostate specific antigen density by 8% to 11% ($p \leq 0.034$). At a %p2PSA cutoff of 1.23, 153 (68.9%) biopsies could have been avoided, missing prostate cancer in 6 patients. At a Prostate Health Index cutoff of 28.8, 116 (52.25%) biopsies could have been avoided, missing prostate cancer in 6 patients.

**Conclusions:** Serum %p2PSA and Prostate Health Index are more accurate than standard reference tests in predicting repeat prostate biopsy outcome, and could avoid unnecessary repeat biopsies.

**Key Words:** prostate-specific antigen, prostatic neoplasms, biopsy, early detection of cancer, diagnosis

**Abbreviations and Acronyms**

DCA = decision curve analysis
DRE = digital rectal examination
fPSA = free prostate specific antigen
%fPSA = free/total prostate specific antigen
PCa = prostate cancer
PCA3 = prostate cancer gene 3
phi = Prostate Health Index
PSA = prostate specific antigen
p2PSA = PSA isoform [-2]proPSA
tPSA = total prostate specific antigen

Submitted for publication January 23, 2012.
Study received ethics committee approval.

* Correspondence: Department of Urology, San Raffaele Turro, Vita-Salute San Raffaele University, Via Stamira D'Ancona 20, 20127 Milan, Italy (telephone: +39.022643.3357; FAX: +39.0226433442; e-mail: lazzeri.maximus@gmail.com).

**See Editorial on page ●●●.**

0022-5347/12/1884-1137/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2012.06.017
Vol. 188, 1137-1143, October 2012
Printed in U.S.A.

CURRENTLY the early detection of PCa primarily relies on an abnormal DRE and/or an increased PSA. Unfortunately, the positive predictive values of tPSA and %fPSA remain low, especially in men with tPSA in the 2.5 to 10 ng/ml range and the outcome of 75% of initial biopsies in a screening setting may be negative.[1] However, up to 10% to 35% of these men have PCa detected on repeat (second or more) biopsy.[2] In men with a negative first biopsy but persistent suspicion of PCa, the European Association of Urology guidelines recommend a repeat biopsy.[3] However, in approximately 80% of men these repeat biopsies are negative. Economic aspects as well as anxiety, discomfort and sometimes severe complications are associated with repeat biopsies.[2] In this respect the PCA3 assay, a new PCa gene based marker, has shown promising results.[4,5] Nevertheless, the PCA3 test has not yet been incorporated in the clinical practice. In addition, it requires future studies as an appropriate cutoff with acceptable performance characteristics has not yet been defined.[6,7]

Recent findings have shown that the serum PSA isoform [-2]proPSA (p2PSA) and its derivatives, namely percentage of p2PSA to free PSA (%p2PSA) and phi, improve the accuracy of tPSA and %fPSA in predicting the presence of PCa at initial prostate biopsy, and may predict PCa aggressiveness.[8–13] There is currently no evidence to support the use of these new biomarkers in men undergoing repeat prostate biopsy for persistent suspected PCa. Thus, in this study we determined the performance characteristics and clinical usefulness of the isoform [-2]proPSA and its derivatives in detecting PCa in men scheduled for repeat biopsy.

## MATERIALS AND METHODS

This study was an observational prospective evaluation of a clinical cohort of men with a negative first biopsy but persistent suspicion of PCa who were scheduled for repeat biopsy according to the European Association of Urology guidelines of increasing and/or persistently elevated PSA, suspicious DRE, atypical small acinar proliferation and high grade prostate intraepithelial neoplasia. Men receiving medical therapy known to affect serum PSA (dutasteride and finasteride) were excluded from analysis. Patients who previously had invasive treatment for benign prostatic hyperplasia (such as transurethral resection of prostate), had clinical signs and symptoms of a current urinary tract infection or had acute prostatitis were also excluded from our study. Furthermore, subjects with marked blood protein alterations (plasma normal range 6 to 8 gm/100 ml), hemophiliacs or those previously polytransfused were not included in the study as these conditions may alter the p2PSA concentration. The study was approved by the hospital ethics committee (Protocol N. 2PROPSA/13.03.2010) and reported according to STARD (STAndards for the Reporting of Diagnostic Accuracy) guidelines (http://www.stard-statement.org).

The primary end point of the study was to determine the diagnostic accuracy of p2PSA, %p2PSA using the formula, (p2PSA pg/ml)/(fPSA ng/ml $\times$ 1,000) $\times$ 100, and Beckman-Coulter phi using the formula, (p2PSA/fPSA) $\times$ $\sqrt{}$tPSA (index tests), and to compare it with the accuracy of established PCa predictors (tPSA, fPSA and %fPSA) (reference standard tests). The secondary end point was to translate the statistical findings into clinical practice cutoffs and determine the percentage of biopsies that could be avoided without missing aggressive PCa (Gleason sum 7 or greater).

A blood sample was drawn at the time of repeat biopsy just before any prostatic manipulations to avoid any transient increase of biomarkers. The blood samples were processed with the UniCel® DxI 800 Immunoassay System analyzer and were managed according to the methods of Semjonow et al.[14] Analysis of the serum samples were performed using Hybritech calibrated Access® tPSA and fPSA assays. Transrectal ultrasonography was used to determine prostate and transition zone volume. Patients underwent ambulatory transrectal ultrasonography guided prostate biopsies according to a standardized institutional scheme to obtain the highest detection rate.[15] Specimens were processed and evaluated by a single experienced genitourinary pathologist blinded to index test results. PCa was identified and graded according to the 2005 consensus conference of the International Society of Urological Pathology definitions.[16] Patients diagnosed with high grade prostate intraepithelial neoplasia and/or atypical small acinar proliferation were not considered as having developed the outcome of interest (PCa).

In terms of statistical analysis the Kolmogorov-Smirnov test was used to assess the normal distribution of variables. Student's t test and the Mann-Whitney U test were used for comparisons of normally and not normally distributed continuous variables, respectively. Multivariate logistic regression models were fitted for the prediction of the presence of PCa at biopsy, incorporating as explanatory variables all variables that showed a $p \leq 0.25$ on bivariate analysis.[17] To avoid multicollinearity problems, predictors that were in strong correlation with other explanatory variables were dropped from the models. Goodness of fit of logistic regression models was checked using the Hosmer and Lemeshow test, and ORs with 95% confidence intervals were also calculated. Qualitative data were analyzed with the chi-square test with Yates continuity correction.

Multivariate logistic regression models were complemented by predictive accuracy tests. Predictive accuracy was quantified as the area under the receiver operating characteristics curve. To test the ability of %p2PSA and phi in determining the presence of PCa at biopsy, these variables were added to the base multivariate model including PSA, prostate volume, PSA density and DRE with or without %fPSA and PSA density. The increase in predictive accuracy was quantified and AUCs were compared using the DeLong method.[18] To reduce overfitting bias, multivariate predictive accuracy tests were subjected to 200 bootstrap re-samples.

To evaluate whether incorporating %p2PSA and phi levels into the statistical models improved the accuracy of the prognostic model and the consequent clinical manage-

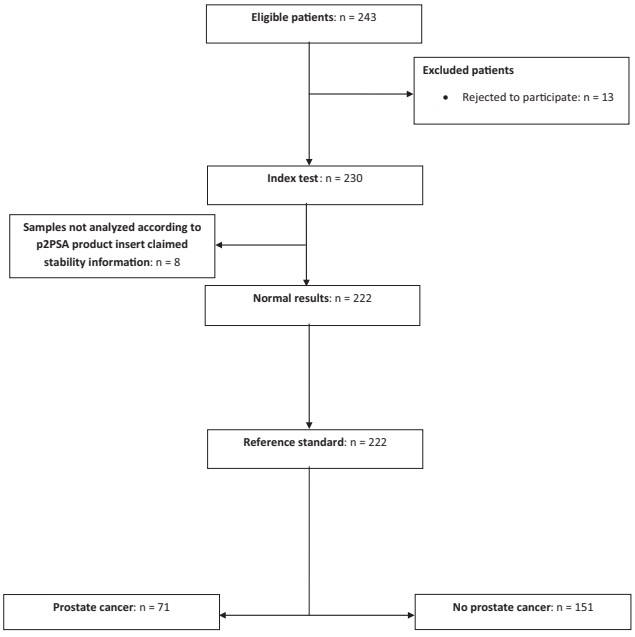


**Figure 1.** STARD flowchart diagram

ment of the patient, we used the DCA.[19] DCA is constructed by plotting net benefit against threshold probability. In our study this analysis estimates the magnitude of benefit resulting from altering clinical management in patients with different threshold probabilities of prostate cancer. The threshold probability is the minimum probability of prostate cancer at which a patient (or clinician) would opt for intervention. As the threshold probabilities can vary from patient to patient, the net benefit is calculated across a range of probabilities.

Finally, the relationship between p2PSA (and its derivatives) and Gleason score at biopsy was tested using the Spearman's rho coefficient analysis. All calculations were performed with PASW® release 17.0.2 with a 2-sided $p < 0.05$ considered significant.

## RESULTS

From June 2010 to June 2011, 243 men underwent repeat biopsy at our single high volume center and 222 patients were included in the final analysis (fig. 1). Descriptive characteristics of the study population are summarized in table 1. Prostate cancer was found in 71 of 222 (31.9%) subjects. When comparing patients with and without PCa at repeat biopsy, no difference was found in age (mean age 64.3 vs 63.7 years, $p = 0.554$) and body mass index (median 25.8 vs 25.7 kg/m$^2$, $p = 0.922$). Median tPSA (7.2 vs 7.8 ng/ml, $p = 0.660$) and p2PSA (17.8 vs 15.1 pg/ml, $p = 0.149$) did not differ significantly between men with and those without PCa. Conversely, %p2PSA (1.9 vs 1.4) and phi (51.6 vs 37.0) values were significantly higher (both $p < 0.0001$), while fPSA (0.9 vs 1.2 ng/ml) and %fPSA (0.14 vs 0.16) were lower ($p = 0.025$ and $p = 0.014$, respectively). In addition, prostate volume (54 vs 68 ml, $p = 0.004$) and transition zone volume (31 vs 38 ml, $p = 0.002$) were statistically significantly lower in patients with PCa.

On univariate accuracy analysis %p2PSA (AUC 72.5%) and phi (AUC 67.2%) were the most accurate predictors (fig. 2). %p2PSA significantly outperformed tPSA (AUC 51.8%), fPSA (AUC 59.3%), %fPSA (AUC 60.2%) and p2PSA (AUC 56%) in the prediction of prostate cancer ($p \leq 0.01$), but not phi ($p = 0.094$). phi significantly outperformed tPSA and p2PSA ($p \leq 0.001$) but not fPSA ($p = 0.109$) and %fPSA ($p = 0.136$) in the prediction of prostate

**Table 1.** *Descriptive characteristics of the study population*

| | Overall | Absence of PCa | Presence of PCa | p Value |
|---|---|---|---|---|
| No. pts (%) | 222 | 151 (68.1) | 71 (31.9) | — |
| Mean ± SD pt age | 63.9 ± 7.1 | 63.7 ± 7.1 | 64.3 ± 6.9 | 0.554* |
| Median kg/m$^2$ body mass index (range) | 25.7 (16.9–34.9) | 25.7 (18.8–32.6) | 25.8 (16.9–34.9) | 0.922† |
| No. Gleason score (%): | | | | |
| Less than 7 | Not available | Not available | 21 (29.5) | — |
| 7 or Greater | Not available | Not available | 50 (70.5) | — |
| No. pos DRE (%) | 31 (14.0) | 16 (10.6) | 15 (21.1) | 0.057‡ |
| Median ml prostate vol (range) | 64 (19–230) | 68 (19–230) | 54 (19–140) | 0.004† |
| Median ml transition zone vol (range) | 35 (4–178) | 38 (5–178) | 31 (4–103) | 0.002† |
| Median cores (range) | 20 (12–26) | 20 (12–26) | 20 (12–24) | 0.974† |
| Median ng/ml total PSA (range) | 7.6 (0.3–46.4) | 7.8 (0.3–30.4) | 7.2 (1–46.4) | 0.660† |
| Median ng/ml free PSA (range) | 1.1 (0.1–5.6) | 1.2 (0.1–4.1) | 0.9 (0.2–5.6) | 0.025† |
| %fPSA (range) | 0.15 (0.05–0.65) | 0.16 (0.07–0.65) | 0.14 (0.05–0.41) | 0.014† |
| Median pg/ml p2PSA (range) | 16.1 (0.1–143.5) | 15.1 (0.1–72.7) | 17.8 (4.6–143.5) | 0.149† |
| Median PSA density (range) | 0.11 (0.02–0.91) | 0.10 (0.02–0.76) | 0.12 (0.03–0.91) | 0.094† |
| Median %p2PSA (range) | 1.6 (0.1–6.8) | 1.4 (0.1–6.8) | 1.9 (0.5–4.4) | <0.0001† |
| Median phi (range) | 40 (11–284) | 37 (11–122) | 51.6 (16–284) | <0.0001† |

* Student's t test.
† Mann-Whitney test.
‡ Chi-square test with Yates continuity correction.

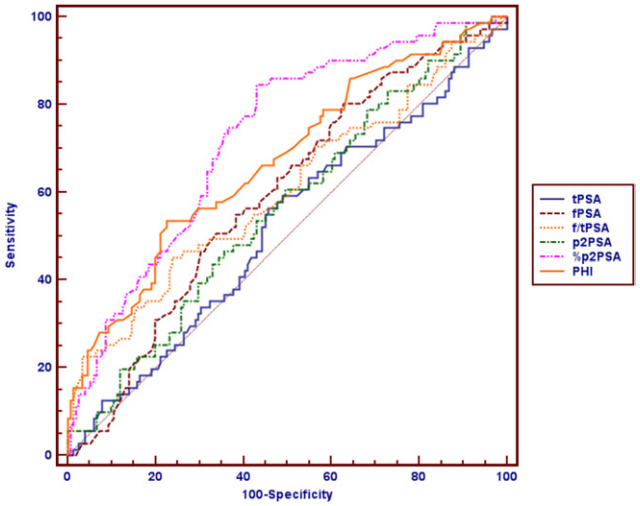


**Figure 2.** Predictive accuracy, quantified as area under receiving operating characteristics curve, for single biomarkers.

cancer. Table 2 shows the cutoffs of predictive variables at different levels of sensitivity and specificity as well as negative and positive predictive values. In particular, for %p2PSA a cutoff of 1.68 showed the best balance between sensitivity and specificity (67.6%, 95% CI 55.5–78.2, and 66.9%, 95% CI 58.8–74.3, respectively). For phi a cutoff of 40.4 showed the best balance between sensitivity and specificity (62%, 95% CI 49.7–73.2, and 59.6%, 95% CI 51.3–67.5, respectively). At 90% sensitivity the cutoff of %p2PSA and phi were 1.23 and 28.8 with a specificity of 40.4% and 25.2%, respectively. At a %p2PSA cutoff of 1.23, a total of 153 (68.9%) biopsies could have been avoided, while an overall of 6 patients with PCa would have been missed in addition to 1 (5%) with a Gleason score of 7 or greater. At a phi cutoff of 28.8 a total of 116 (52.25%) biopsies could have been avoided, while an overall of 6 patients with PCa would have been missed but no patient with a Gleason score of 7 or greater would have been missed.

In multivariable logistic regression models testing the predictors of PCa at biopsy, %p2PSA and phi achieved independent predictor status and significantly increased the accuracy of a multivariate model including PSA, prostate volume, PSA density, %fPSA and DRE by 9% ($p < 0.05$) and 10% ($p < 0.01$), respectively (table 3).

Figure 3 presents the DCA for the models shown in table 3. Models 3 (base plus %fPSA and p2PSA), 4 (base plus %fPSA and %p2PSA) and 5 (base plus %fPSA and phi) result in greater net benefit in the PCa probability threshold range of 25% to 40% compared with model 1 (base) and model 2 (base plus %fPSA), which are perfectly superimposed. Finally, the Spearman's rho coefficient analysis demonstrated a significant relationship between Gleason score and phi level (rho 0.299, $p = 0.013$), but not between Gleason score and %p2PSA (rho 0.175, $p = 0.155$).

**Table 2.** *Sensitivity, specificity, PPV and NPV at 3 levels of predictive variables (high sensitivity, best combination, high specificity) for prediction of cancer*

| Cutoff | % Sensitivity (95% CI) | % Specificity (95% CI) | Pos Predictive Value (95% CI) | Neg Predictive Value (95% CI) |
|---|---|---|---|---|
| Total PSA (ng/ml): | | | | |
| 17.2 | 93.0 (84.3–97.6) | 8.6 (4.7–14.3) | 32.4 (26.1–39.3) | 72.3 (0.49–0.87) |
| 7.45 | 56.3 (44.0–68.1) | 54.3 (46.0–62.4) | 36.7 (27.6–46.5) | 72.5 (63.3–80.5) |
| 3.23 | 12.7 (6.0–22.7) | 92.0 (86.5–95.8) | 42.8 (21.7–65.9) | 69.1 (62.2–74.5) |
| Free PSA (ng/ml): | | | | |
| 2.04 | 90.1 (80.7–95.9) | 19.9 (13.8–27.1) | 34.6 (27.8–42.0) | 81.0 (64.8–92.0) |
| 1.08 | 57.7 (45.4–69.4) | 56.3 (48.0–64.3) | 38.3 (29.1–48.3) | 73.9 (64.9–81.6) |
| 0.45 | 7.0 (2.4–15.7) | 90.7 (84.9–94.8) | 26.2 (9.1–51.0) | 67.5 (60.5–73.9) |
| %fPSA: | | | | |
| 0.24 | 91.6 (82.5–96.8) | 13.9 (8.8–20.5) | 33.4 (26.8–40.4) | 77.9 (57.4–91.6) |
| 0.15 | 54.9 (42.7–66.8) | 56.3 (48.0–64.3) | 37.1 (27.9–47.2) | 72.6 (63.6–80.5) |
| 0.09 | 23.9 (14.6–35.5) | 91.4 (85.7–95.3) | 56.7 (37.1–74.8) | 71.8 (64.9–78.1) |
| [−2]proPSA (pg/ml): | | | | |
| 7.7 | 90.1 (80.7–95.9) | 11.9 (7.2–18.2) | 32.5 (26.0–39.5) | 71.9 (50.0–88.1) |
| 16.4 | 54.9 (42.7–66.8) | 55.0 (46.7–63.1) | 36.5 (27.3–46.4) | 72.2 (63.0–80.1) |
| 35.7 | 11.3 (5.0–21.0) | 90.1 (84.1–94.3) | 34.9 (16.1–58.0) | 68.3 (61.4–74.7) |
| %[−2]proPSA: | | | | |
| 1.23 | 90.1 (80.7–95.9) | 40.4 (32.5–48.7) | 41.6 (33.7–49.8) | 89.7 (79.9–95.7) |
| 1.68 | 67.6 (55.5–78.2) | 66.9 (58.8–74.3) | 49.0 (38.7–59.4) | 81.4 (73.5–87.8) |
| 2.31 | 31.0 (20.5–43.1) | 91.4 (85.7–95.3) | 62.9 (45.0–78.6) | 73.8 (66.8–79.9) |
| phi: | | | | |
| 28.8 | 90.1 (80.7–95.9) | 25.2 (18.5–32.9) | 36.2 (29.1–43.8) | 84.4 (70.5–93.5) |
| 40.4 | 62.0 (49.7–73.2) | 59.6 (51.3–67.5) | 41.9 (32.4–52.0) | 76.9 (68.2–84.2) |
| 62.0 | 29.6 (19.3–41.6) | 90.7 (84.9–94.8) | 60.0 (42.1–76.1) | 73.2 (66.3–79.5) |

**Table 3.** *Bivariate and multivariate analyses predicting the probability of PCa at biopsy in the rebiopsy population*

| | | | Multivariate Analysis OR (95% CI)/p Value | | | | |
|---|---|---|---|---|---|---|---|
| | AUC of Individual Predictor Variables (95% CI) | Bivariate Analysis OR (95% CI)/p Value | Base Model | Base Model + %fPSA | Base Model + %fPSA + p2PSA | Base Model + %fPSA + %p2PSA | Base Model + %fPSA + PHI |
| Age* | 0.51 (0.43–0.59) | 1.013 (0.973–1.055)/0.519 | — | | | — | — |
| Prostate vol | 0.63 (0.55–0.72) | 0.984 (0.972–0.996)/0.011 | 1.004 (0.984–1.025)/0.671 | 1.005 (0.984–1.026)/0.655 | 1.006 (0.983–1.030)/0.596 | 1.006 (0.985–1.028)/0.563 | 1.005 (0.983–1.029)/0.642 |
| Adenoma vol† | 0.65 (0.56–0.73) | 0.977 (0.961–0.993)/0.005 | — | — | — | — | — |
| PSA density‡ | 0.58 (0.49–0.67) | 1.003 (1.001–1.005)/0.042 | 1.005 (0.998–1.012)/0.160 | 1.005 (0.998–1.012)/0.162 | 1.007 (0.997–1.017)/0.159 | 1.005 (0.997–1.013)/0.192 | 1.006 (0.9961–1.016)/0.230 |
| DRE | 0.55 (0.47–0.64) | 2.260 (1.046–4.882)/0.038 | 1.816 (0.757–4.356)/0.181 | 1.821 (0.759–4.369)/0.180 | 2.053 (0.805–5.235)/0.132 | 1.957 (0.779–4.919)/0.153 | 2.030 (0.789–5.225)/0.142 |
| tPSA | 0.52 (0.45–0.59) | 1.010 (0.479–1.016)/0.061 | 1.015 (0.881–1.168)/0.841 | 1.018 (0.878–1.180)/0.810 | 0.891 (0.692–1.147)/0.369 | 0.947 (0.800–1.121)/0.528 | 0.842 (0.651–1.089)/0.190 |
| fPSA | 0.59 (0.53–0.67) | 0.698 (0.339–0.928)/0.024 | 0.353 (0.145–0.860)/0.022 | 0.338 (0.118–0.967)/0.043 | 0.202 (0.045–0.912)/0.038 | 0.662 (0.208–2.113)/0.487 | 0.609 (0.168–2.210)/0.451 |
| %fPSA§ | 0.60 (0.53–0.67) | 0.995 (0.991–0.999)/0.036 | — | 1.000 (0.995–1.006)/0.874 | 0.998 (0.991–1.006)/0.605 | 0.998 (0.991–1.005)/0.598 | 1.000 (0.993–1.007)/0.960 |
| p2PSA | 0.56 (0.49–0.63) | 1.017 (1.001–1.034)/0.044 | — | — | 1.089 (1.037–1.147)/0.001 | — | — |
| %p2PSA | 0.72 (0.66–0.78) | 2.544 (1.663–3.890)/<0.001 | — | — | — | 2.593 (1.280–5.257)/<0.001 | — |
| phi | 0.67 (0.61–0.73) | 1.029 (1.015–1.044)/<0.001 | — | — | — | — | 1.045 (1.021–1.069)/<0.001 |
| AUC of multivariate models (95% CI) | | | 0.68 (0.60–0.74) | 0.68 (0.60–0.74) | 0.77 (0.70–0.83) | 0.77 (0.70–0.83) | 0.78 (0.71–0.84) |
| Increase in predictive accuracy (95% CI) | | | — | 0.001 (0.0–0.06) | 0.09 (0.02–0.17)‖ | 0.09 (0.02–0.16)‖ | 0.10 (0.03–0.18)¶ |

The area under the curve reflects the predictive value of individual variables (columns) as well as of the multivariate models in predicting the probability of having PCa.

* Not included in multivariate base model because p >0.25 in bivariate analysis.

† Not included in multivariate base model because the strong correlation with prostate volume (rho = 0.933).

‡ Expressed as tPSA (pg/ml)/prostate volume (ml) to scaling OR in a more intelligible range.

§ Expressed as fPSA (pg/ml)/PSA (ng/ml) to scaling OR in a more intelligible range.

‖ p <0.05 (relative to the multivariate base model with %fPSA, DeLong method).

¶ p <0.01 (relative to the multivariate base model with %fPSA, DeLong method).

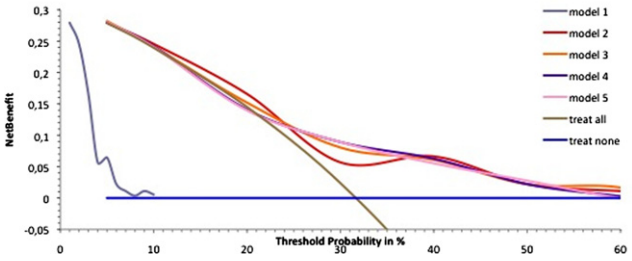


**Figure 3.** DCA for models shown in table 3. Models 3 (base plus %fPSA and p2PSA), 4 (base plus %fPSA and %p2PSA) and 5 (base plus %fPSA and phi) resulted in greater net benefit in PCa probability threshold range from 25% to 40% compared with model 1 (base) and model 2 (base plus %fPSA), which are perfectly superimposed.

## DISCUSSION

Our study demonstrates for the first time to our knowledge that %p2PSA and phi were significantly higher in men with PCa at repeat biopsy, and are the most accurate predictors of outcome compared with reference standard tests. They could significantly spare a relevant number of unnecessary biopsies, missing a negligible number of aggressive (Gleason sum 7 or greater) cancers.

It has already been established that %p2PSA and phi can significantly predict the presence of PCa at initial biopsy better than tPSA.[8–13] In the current study %p2PSA and phi showed similar characteristics when applied to a group of patients who underwent repeat prostate biopsy. Furthermore, we also confirmed a direct relationship between phi and PCa aggressiveness at repeat biopsy, as Sokoll[9] and Guazzoni[12] et al found at initial biopsy.

In the last 5 years 2 separate studies in America and Europe investigated the gene based urine biomarker PCA3 as a diagnostic tool to aid in the decision to perform repeat biopsy in men with 1 or more previous negative biopsies for suspected PCa.[4,5] In the former study data analysis was performed in 233 American men (mean age 64 years) who had a PSA of 2.5 ng/ml or greater (mean 7.4) and 1 or more negative biopsies.[4] Repeat biopsies were positive for 60 men (27%) and correlated with the PCA3 score in that the higher the PCA3 score, the greater the probability of a positive repeat biopsy. In the European study 470 men with 1 to 2 previous negative biopsies scheduled for repeat biopsy were enrolled (mean age 64.4 years),[5] with a positive result in 128 (28%).

To assess the ability of a biomarker to predict biopsy outcome, ROC curve analysis is generally performed using the biopsy result as the reference method. Previous studies showed that serum tPSA had little diagnostic value for a population who underwent repeat biopsy.[4,5] We confirm such data as the serum tPSA test yielded an area under the ROC curve (AUC) of 0.518 (95% CI 0.450 to 0.59). Marks et al reported an AUC of 0.678 (95% CI 0.597–0.759) for the PCA3 score and the difference between the PCA3 and serum tPSA AUCs was statistically significant ($p = 0.008$).[4] Haese et al constructed a ROC curve for the %fPSA test which was considered more accurate than tPSA for predicting positive outcome at repeat biopsy.[5] %fPSA was chosen as a comparator because the majority of patients had a serum PSA 4 to 10 ng/ml, in which range the use of %fPSA improves the specificity of tPSA for PCa detection.[20,21] The %fPSA test yielded an area under the ROC curve (AUC) of 0.578 indicating it had little diagnostic value for this subject population. In our population, which was not stratified according to a pre-biopsy serum tPSA of 4 to 10 ng/ml, we found that %fPSA had an AUC of 0.602 and %p2PSA but not phi significantly outperformed %fPSA. These results are in line with our findings in patients who underwent initial biopsy with a tPSA range of 4 to 10 ng/ml.[12]

Previous studies investigated the relationship between prostate volume at repeat biopsy and the PCA3 score. Haese et al found that men with a higher prostate volume had a higher mean serum PSA, with 5.9, 8.6 and 9.5 ng/ml for a prostate volume of less than 30, 30 to 50 and greater than 50 ml, respectively, but the mean PCA3 score was independent of prostate volume at 42.3, 45.0 and 41.5 in the 3 groups, respectively.[5] The Pearson correlation coefficient confirmed the lack of relationship between PCA3 score and prostate volume with $r = 0.03547$ and $p = 0.4549$. Guazzoni et al did not find any correlation between prostate volume and phi in men who underwent initial biopsy.[12] Similar data were found in men who underwent radical prostatectomy.[22] We also confirmed that %p2PSA and phi values are not correlated with prostate volume in men undergoing repeat biopsy.

In clinical practice the aim of a new biomarker for patients scheduled to undergo a repeat biopsy should be to avoid unnecessary biopsies and minimize the risk of missing significant cancers. We found that at a sensitivity of 90%, %p2PSA and phi had the highest utility regarding the decision of performing biopsy and whether the cancer found on repeat biopsy was clinically insignificant.

One of the main strengths of this study is that phi was shown to be an accurate independent predictor in multivariable logistic regression models with and without %fPSA. In contrast to previously published studies on p2PSA which were based on a retrospective analysis and were more susceptible to selection bias, our study was a prospective observational study in a contemporary cohort of candidates for repeat prostate biopsy. Finally, blood samples were handled according to the guidelines of Semjonow et al[14] and archived serum was not used.

Despite its strengths, our study is not without limitations. Because few patients had undergone 2 or more biopsies, no stratification according to the number of previous biopsies was performed. In addition, not all data from the initial biopsy screening were available and, therefore, information regarding the original biopsy scheme and average time between biopsies was not available. Although a nomogram including age, PSA, %fPSA, DRE, prostate volume and PCA3 score was developed to predict the probability of PCa at repeat biopsy, we did not integrate our findings in a new nomogram. Finally, a single pathologist reviewed all the biopsy samples and no blind analysis was available on pathological samples.

## CONCLUSIONS

Serum index tests %p2PSA and phi were shown to be more accurate than standard reference tests (tPSA, fPSA and %fPSA) in predicting repeat prostate biopsy outcome in a tPSA range of 0.3 to 46.4 ng/ml, and may be indicative of cancer aggressiveness. International multicenter studies are required to confirm our findings and translate the results into clinical practice cutoffs. Thus, we can improve the selection of candidates for repeat biopsy and avoid unnecessary biopsies without missing aggressive prostate cancer.

## ACKNOWLEDGMENTS

UniCel DxI 800 Immunoassay System analyzer p2PSA ([-2]proPSA) reagents were provided by Beckman Coulter Inc. and Beckman Coulter Italy. The UniCel DxI 800 Immunoassay System analyzer was provided by Beckman Coulter Italy.

Amy Sussman provided linguistic assistance.

## REFERENCES

1. Heijnsdijk J, der Kinderen A, Wever EM et al: Overdetection, overtreatment and costs in prostate-specific antigen screening for prostate cancer. BJC 2009; **101:** 1833.
2. Raja J, Ramachandran N, Munneke G et al: Current status of transrectal ultrasound-guided prostate biopsy in the diagnosis of prostate cancer. Clin Radiol 2006; **61:** 142.
3. EAU Guidelines on Prostate Cancer, 2011 Edition. Available at www.uroweb.org. Accessed December 2011.
4. Marks LS, Fradet Y, Deras IL et al: PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. Urology 2007; **69:** 532.
5. Haese A, de la Taille A, van Poppel H et al: Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. Eur Urol 2008; **54:** 1081.
6. Roobol MJ: Contemporary role of prostate cancer gene 3 in the management of prostate cancer. Curr Opin Urol 2011; **21:** 225.
7. Roobol MJ, Schröder FH, van Leenders GL et al: Performance of prostate cancer antigen 3 (PCA3) and prostate-specific antigen in prescreened men: reproducibility and detection characteristics for prostate cancer patients with high PCA3 scores (≥ 100). Eur Urol 2010; **58:** 893.
8. Rhodes T, Jacobson DJ, McGree ME et al: Distribution and associations of [-2]proenzyme-prostate specific antigen in community dwelling black and white men. J Urol 2012; **187:** 92.
9. Sokoll LJ, Sanda MG, Feng Z et al: A prospective, multicenter, National Cancer Institute Early Detection Research Network Study of [-2]proPSA: improving prostate cancer detection and correlating with cancer aggressiveness. Cancer Epidemiol Biomarkers Prev 2010; **19:** 1193.
10. Le BV, Griffin CR, Loeb S et al: [-2]Proenzyme prostate specific antigen is more accurate than total and free prostate specific antigen in differentiating prostate cancer from benign disease in a prospective prostate cancer screening study. J Urol 2010; **183:** 1355.
11. Jansen FH, Van Schaik RH, Kurstjens J et al: Prostate-specific antigen (PSA) isoform p2PSA in combination with total PSA and free PSA improves the diagnostic accuracy in prostate cancer detection. Eur Urol 2010; **57:** 921.
12. Guazzoni G, Nava L, Lazzeri M et al: Prostate-specific antigen (PSA) isoform p2PSA significantly improves the prediction of prostate cancer at initial extended prostate biopsies in patients with total PSA between 2.0 and 10 ng/ml: results of a prospective study in a clinical setting. Eur Urol 2011; **60:** 214.
13. Catalona WJ, Partin AW, Sanda MG et al: A multicenter study of [-2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. J Urol 2011; **185:** 1650.
14. Semjonow A, Köpke T, Eltze E et al: Pre-analytical in-vitro stability of [-2]proPSA in blood and serum. Clin Biochem 2010; **47:** 926.
15. Scattoni V, Raber M, Abdollah F et al: Biopsy schemes with the fewest cores for detecting 95% of the prostate cancers detected by a 24-core biopsy. Eur Urol 2010; **57:** 1.
16. Epstein JI, Allsbrook WC Jr, Amin MB et al: The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol 2005; **29:** 1228.
17. Hosmer DW and Lemeshow S: Applied Logistic Regression. New York: John Wiley & Sons 2000.
18. DeLong ER, DeLong DM and Clarke-Pearson DL: Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988; **44:** 837.
19. Vickers AJ and Elkin EB: Decision curve analysis: a novel method for evaluating prediction models. Med Decis Making 2006; **26:** 565.
20. Catalona WJ, Partin AW, Slawin KM et al: Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: a prospective multicenter clinical trial. JAMA 1998; **279:** 1542.
21. Djavan B, Zlotta A, Remzi M et al: Optimal predictors of prostate cancer on repeat prostate biopsy: a prospective study of 1,051 men. J Urol 2000; **163:** 1144.
22. Guazzoni G, Lazzeri M, Lughezzani G et al: Preoperative prostate-specific antigen isoform p2PSA and its derivatives, %p2PSA and prostate health index, predict pathologic outcomes in patients undergoing radical prostatectomy for prostate cancer. Eur Urol 2012; **61:** 455.