Clinical performance of serum prostate-specific antigen isoform [-2]proPSA (p2PSA) and its derivatives, %p2PSA and the prostate health index (PHI), in men with a family history of prostate cancer: results from a multicentre European study, the PROMEtheuS project

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Objectives

- To test the sensitivity, specificity and accuracy of serum prostate-specific antigen isoform [-2]proPSA (p2PSA), %p2PSA and the prostate health index (PHI), in men with a family history of prostate cancer (PCa) undergoing prostate biopsy for suspected PCa.
- To evaluate the potential reduction in unnecessary biopsies and the characteristics of potentially missed cases of PCa that would result from using serum p2PSA, %p2PSA and PHI.

Patients and Methods

- The analysis consisted of a nested case–control study from the PRO-PSA Multicentric European Study, the PROMEtheuS project.
- All patients had a first-degree relative (father, brother, son) with PCa.
- Multivariable logistic regression models were complemented by predictive accuracy analysis and decision-curve analysis.

Results

- Of the 1026 patients included in the PROMEtheuS cohort, 158 (15.4%) had a first-degree relative with PCa. p2PSA, %p2PSA and PHI values were significantly higher ($P < 0.001$), and free/total PSA (%fPSA) values significantly lower ($P < 0.001$) in the 71 patients with PCa (44.9%) than in patients without PCa.
- Univariable accuracy analysis showed %p2PSA (area under the receiver-operating characteristic curve [AUC]: 0.733) and PHI (AUC: 0.733) to be the most accurate predictors of PCa at biopsy, significantly outperforming total PSA ([tPSA] AUC: 0.549), free PSA ([fPSA] AUC: 0.489) and %fPSA (AUC: 0.600) ($P \leq 0.001$).
- For %p2PSA a threshold of 1.66 was found to have the best balance between sensitivity and specificity (70.4 and 70.1%; 95% confidence interval [CI]: 58.4–80.7 and 59.4–79.5 respectively). A PHI threshold of 40 was found to have the best balance between sensitivity and specificity (64.8 and 71.3%, respectively; 95% CI 52.5–75.8 and 60.6–80.5).
- At 90% sensitivity, the thresholds for %p2PSA and PHI were 1.20 and 25.5, with a specificity of 37.9 and 25.5%, respectively. At a %p2PSA threshold of 1.20, a total of 39 (24.8%) biopsies could have been avoided, but two cancers with a Gleason score (GS) of 7 would have been missed. At a PHI threshold of 25.5 a total of 27 (17.2%) biopsies could have been avoided and two (3.8%) cancers with a GS of 7 would have been missed.
- In multivariable logistic regression models, %p2PSA and PHI achieved independent predictor status and significantly increased the accuracy of multivariable

models including PSA and prostate volume by 8.7 and 10%, respectively ($P \leq 0.001$).
- p2PSA, %p2PSA and PHI were directly correlated with Gleason score (ρ: 0.247, $P = 0.038$; ρ: 0.366, $P = 0.002$; ρ: 0.464, $P < 0.001$, respectively).

Conclusions

- %p2PSA and PHI are more accurate than tPSA, fPSA and %fPSA in predicting PCa in men with a family history of PCa.
- Consideration of %p2PSA and PHI results in the avoidance of several unnecessary biopsies.
- p2PSA, %p2PSA and PHI correlate with cancer aggressiveness.

Keywords

prostate-specific antigen, p2PSA, prostate health index, familial prostate cancer, positive biopsy, predictive models

Introduction

Prostate cancer (PCa) is currently the most frequent neoplasm in men and the second most common cause of death from cancer in the USA [1]. The aetiology of PCa remains multifactorial. Family history, however, does seem to be a very important factor in PCa occurrence. It has been estimated that 5–10% of PCa cases are caused by a dominantly inherited susceptibility to the disease [2–5].

Interestingly, relative risk for PCa increases according to the number of affected family members, their degree of relatedness, and the age at which they were diagnosed with PCa [5]. Early onset PCa (≤55 years), in particular, has a significant genetic component [6].

The risk of PCa is about 2.5 times higher in men with a first-degree relative (father, brother, son) diagnosed with PCa, and higher still with more than one first-degree relative having PCa, a relative diagnosed with PCa at age <60 years, or men <65 years with a first-degree relative diagnosed with PCa [7].

The quality, quantity and limitations of the current literature make definitive recommendations regarding the role of PSA screening for early detection of PCa in the overall male population difficult [8] and controversial [9]. The American Cancer Society recommends that screening of men at high risk (i.e. those with a first-degree relative with PCa) should be initiated at an earlier age than in the general population. Among patients with a positive family history of PCa, it is usually recommended to begin routine PSA screening and DREs at 45 years of age [10].

Because a positive family history of PCa improves the positive predictive value (PPV) of PSA testing [11], men with a PSA >3 ng/mL are advised to undergo a prostate biopsy [5]; however, a reported PCa detection rate of 35–40% in these patients [12] implies a high rate (60–65%) of negative biopsies. Patients with a negative biopsy are advised to undergo regular urological evaluations, eventually repeating the biopsy if necessary [6]. Given the possible relatively younger age of men with a family history of PCa who currently undergo biopsy, it is important to find a more reliable way to differentiate probable PCa-positive from PCa-negative cases before biopsy, to spare as many men as possible from an unnecessary biopsy.

According to preliminary investigations and observational studies, [-2]proPSA (p2PSA), a serum isoform of PSA, and its derivatives, namely %p2PSA (p2PSA as a proportion of free PSA [fPSA]) and the Beckman Coulter (Brea, CA, USA) prostate health index (PHI), may improve both discrimination between men with and without PCa and between cases of aggressive and less aggressive disease, as indicated by the Gleason score (GS) [13–16]. So far, the clinical performance of p2PSA and its derivates in men with a family history of PCa has not been investigated.

In the present study, we tested the hypothesis that p2PSA and its derivatives were more accurate than total PSA (tPSA), fPSA and free/total PSA (%fPSA) in detecting cancer and could result in the avoidance of unnecessary biopsies, within a prospectively collected, multicentre European, large and contemporary cohort of men with a family history of PCa.

Patients and Methods

Patients

The analysis consisted of a nested case–control study from the PRO-PSA Multicentric European Study (PROMEtheuS) project (detailed descriptions of study design, setting, ethics, centres and patients are available at http://www.controlled-trials.com/ISRCTN04707454).

The overall study population included patients undergoing prostate biopsy for suspected PCa according to indications from their referring physicians. Demographic characteristics and medical history of patients were recorded on a specific requisition form [13,15,16]. Inclusion was limited to patients enrolled in the PROMEtheuS project who had a first-degree relative (father, brother, son) with PCa. Exclusion criteria were the following: patients with bacterial acute prostatitis in the 3

months before biopsy and patients subjected to previous endoscopic surgery of the prostate. Patients subjected to previous prostate biopsy or patients being treated with dutasteride or finasteride were excluded. Furthermore, subjects with chronic renal failure, marked blood protein alterations (plasma normal range 6–8 g/100 mL), patients with haemophilia or those who had previously undergone multiple transfusions were not included in the study as these conditions may have altered the concentration of p2PSA [17].

Methods

Before prostate biopsy, blood was drawn to measure the pre-biopsy tPSA, fPSA and p2PSA levels. The samples were centrifuged within 3 h of the blood draw [18]. The samples were then frozen at −80 °C and centrally processed using an Access 2 Immunoassay System, an automated random-access analyser that performs immunoassays on body fluid samples (Beckman Coulter, Brea, CA, USA). tPSA and fPSA were determined using the Hybritech calibration. Subsequently, the clinician performed a DRE. TRUS was used to measure the prostate and adenoma volume (using the ellipsoid volume formula, length × height × width × π/6), and to determine the presence of abnormalities within the prostate gland. TRUS-guided prostate biopsies were performed according to a standardized extended scheme: at least 10 biopsy cores were taken from the peripheral portion of the prostate gland (apex, mid-gland and base), with additional cores taken when necessary according to patients' age, prostate volume, ultrasonography-visible abnormalities or institutional protocols. Prostate biopsy specimens were placed in specific single-core specimen containers filled with 10% buffered formalin. The specimens were processed and evaluated at each centre by an experienced genitourinary pathologist who was able to review and exchange data (histological results) between centres if doubts were encountered. PCa was identified and graded according to the 2005 consensus conference of the International Society of Urological Pathology definitions [19]. Patients diagnosed with high grade intraepithelial neoplasia or suspicious lesions but not adenocarcinoma (atypical small acinar proliferation of prostate), according to the contemporary diagnostic criteria, were not considered positive for the outcome of interest (PCa).

Outcomes

The primary outcome was a test of the sensitivity, specificity and accuracy of serum p2PSA, %p2PSA ([(p2PSA pg/mL)/(fPSA ng/mL × 1000)] × 100) and Beckman Coulter PHI $\left((\text{p2PSA}/\text{fPSA}) \times \sqrt{\text{PSA}}\right)$, and a comparison of their performance with the established PCa serum tests (tPSA, fPSA and %fPSA) for the identification of PCa in men with a family history of PCa (first-degree relative), according to the Standards for the Reporting of Diagnostic Accuracy studies methodology (http://www.stard-statement.org).

The potential reduction in unnecessary biopsies and the characteristics of the potentially missed PCa cases were reported as a secondary outcome.

Statistics

The multicentric nature of this study made for a complex sample in which the subjects were enrolled not by simple random sampling but rather with an unequal probability of selection. It was therefore necessary to adjust the data using inverse probability weighting according to the data from the 2012 EUROSTAT Census to arrive at an estimate of the multinational population (http://epp.eurostat.ec.europa.eu).

The Shapiro–Wilk 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 non-normally distributed continuous variables, respectively. Spearman's ρ coefficient analysis was used to test correlations between variables. Bivariable and multivariable logistic regression models were fit for the prediction of the presence of PCa and, in particular, GS ≥7 PCa at biopsy. To avoid multicollinearity problems, predictors that strongly correlated with other explanatory variables were dropped from the models. Goodness-of-fit of logistic regression models (internal calibration) were checked using the Hosmer and Lemeshow test. Odds ratios with 95% CIs were also calculated.

Multivariable logistic regression models were complemented by predictive accuracy analysis and decision-curve analysis. Predictive accuracy was quantified as the area under the receiver-operating characteristic curve (AUC). To test the ability of p2PSA, %p2PSA and PHI to determine the presence of PCa at biopsy, these variables were added to the base multivariable model. The gain in predictive accuracy was quantified and AUCs were compared using the DeLong method [20]. To reduce overfit bias, multivariable predictive accuracy tests were subjected to 200 bootstrap resampling.

To evaluate whether incorporating p2PSA, %p2PSA and PHI into the statistical models improved the accuracy of the prognostic model and the consequent clinical management of the patient, we used decision-curve analysis [21]. Decision curves are constructed by plotting 'net benefit' against threshold probability. In the present study, this analysis estimated the magnitude of benefit resulting from altering clinical management in patients with different threshold probabilities of PCa. The threshold probability

was the minimum probability of PCa at which a patient (or clinician) would opt for intervention. As the threshold probabilities can vary from patient to patient, the net benefit was calculated across a range of probabilities.

Statistical analyses, including Complex Sample Analysis, were performed using IBM-SPSS® version 20.0 (IBM Corp., Armonk, NY, USA, 2011). A two-sided *P* value <0.05 was considered to indicate statistical significance. Receiver-operating characteristic curves were plotted using MedCalc release 9.3.7.0, (MedCalc Software, Mariakerke, Belgium, 2007). Decision curves were plotted using a macro developed in Excel (Microsoft, Redmond, WA, USA) by one of the authors (V.B.).

Ethics

This study was part of a larger multicentre study which obtained the approval of the local ethical committee of each institution involved.

Results

Of the 1026 patients enrolled in the PROMEtheuS cohort, 158 (15.4%) had a first-degree relative with PCa. Demographic and clinical characteristics of the study population are listed in Table 1. PCa was found in 71 subjects (44.9%), of whom 24 had a GS of 6 (33.8%), 34 a GS of 7 (47.8%), and three and five subjects (4.2 and 7%) with GSs of 8 and 9, respectively. p2PSA, %p2PSA and PHI values were significantly higher (median values: 15.9 pg/mL, 2.0 and 46.6, respectively), and %fPSA values significantly lower (median 0.15) in patients with PCa than in patients with a negative biopsy (median values: 13.2 pg/mL, 1.4, 33.2 and 0.17; $P = 0.011$, $P < 0.001$, $P < 0.001$ and $P = 0.030$, respectively). Conversely, no significant differences were found for tPSA or fPSA between patients with and without PCa.

In bivariate logistic regression models, prostate volume ($P = 0.031$), tPSA ($P = 0.004$), p2PSA ($P < 0.001$), %p2PSA ($P = 0.001$) and PHI ($P = 0.001$) were significant predictors of PCa at biopsy. By contrast, patient age ($P = 0.640$), transition zone volume ($P = 0.173$), fPSA ($P = 0.137$) and %fPSA ($P = 0.233$) were not significantly associated with the presence of PCa at biopsy (Table 2).

Univariable accuracy analysis showed %p2PSA (AUC: 0.73) and PHI (AUC: 0.73) to be the most accurate predictors of PCa at biopsy, significantly ($P \leq 0.001$) outperforming tPSA (AUC: 0.55), fPSA (AUC: 0.49) and %fPSA (AUC: 0.60 [Fig. 1]).

Information regarding sensitivity and specificity is found in Table 3. For %p2PSA a threshold of 1.66 showed the best balance between sensitivity and specificity (70.4 and 70.1%; 95% CI: 58.4–80.7 and 59.4–79.5, respectively). The best balance for PHI was found using a threshold of 40.3 (sensitivity = 64.8 and specificity = 71.3%; 95% CI: 52.5–75.8 and 60.6–80.5, respectively). At a %p2PSA threshold of 1.66, a total of 82 (51.9%) biopsies could have been avoided but 21 (13.3%) cancers would have been missed: 12 with GS 6 (3+3) and nine with a GS of 7 (seven with GS 3+4, and two with GS 4+3). At a PHI threshold of 40.3, a total of 88 (55.7%) biopsies could have been avoided but 25 (15.8%) cancers would have been missed: 13 with GS 6 (3+3) and 12 cancers with a GS of 7 (10 with GS 3+4, and two with GS 4+3).

Table 1 Descriptive characteristics of the study population.

	Overall	Absence of PCa	Presence of PCa	*P*
Patients, *n* (%)	158	87 (55.1)	71 (44.9)	–
Mean (SD) age, years	63.6 (7.5)	63.1.(8.1)	64.2 (6.7)	0.538*
GS categories, *n* (%)				
<7	NA	NA	24 (33.8)	–
≥7	NA	NA	47 (66.2)	–
Round of biopsy				
1	NA	NA	20	–
2	NA	NA	27	–
3	NA	NA	24	–
Median (min, max) no. of cores	14 (10–36)	12 (10–21)	14 (10–36)	0.021
Median (min, max) prostate volume, mL	47.5 (13–222)	50 (26–222)	45 (13–155)	0.030†
Median (min, max) adenoma volume, mL	23 (0–169)	27 (0–169)	21.5 (0–140)	0.190†
Median (min, max) tPSA, ng/mL	5.6 (1.1–57.5)	5.5 (1.1–29.8)	5.7 (1.4–57.5)	0.292†
Median (min, max) fPSA, ng/mL	0.9 (0.2–6.3)	1.0 (0.2–6.3)	0.9 (0.2–6.3)	0.811†
Median (min, max) %fPSA	0.17 (0.02–0.48)	0.17 (0.06–0.48)	0.15 (0.02–0.38)	0.030†
Median (min, max) p2PSA, pg/mL	13.7 (2.7–395.7)	13.2 (2.7–111.5)	15.9 (3.7–395.7)	0.011†
Median (min, max) %p2PSA	1.6 (0.6–7.7)	1.4 (0.6–5.3)	2.0 (0.6–7.7)	<0.001†
Median (min, max) PHI	38.6 (10.9–403.6)	33.2 (12.9–197.6)	46.6 (10.9–403.6)	<0.001†

**Student's t-test.*
†Mann–Whitney test.
NA, not available; PHI, Prostate Health Index.

Table 2 Bivariate and multivariate analyses predicting the probability of PCa. The AUC reflects the predictive value of individual variables (columns), as well as of the multivariable models in predicting the probability of having PCa.

Predictors	AUC of individual predictor variables (95% CI)	Bivariate analysis		Multivariate analysis							
				Base model		Base model plus p2PSA		Base model plus %p2PSA		Base model plus PHI	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age	**0.53 (0.45–0.61)**	1.018 (0.924–1.121)	0.640	1.049 (0.975–1.129)	0.143	1.057 (0.960–1.164)	0.184	1.057 (0.947–1.180)	0.236	1.054 (0.953–1.166)	0.220
Prostate volume	**0.61 (0.52–0.69)**	0.984 (0.970–0.998)	0.031	0.973 (0.962–0.984)	0.002	0.972 (0.956–0.989)	0.010	0.975 (0.959–0.990)	0.011	0.972 (0.955–0.990)	0.011
Adenoma volume†	**0.59 (0.47–0.71)**	0.990 (0.974–1.007)	0.173	–		–		–		–	
tPSA	**0.55 (0.47–0.63)**	1.059 (1.031–1.088)	0.004	1.060 (0.990–1.134)	0.077	1.059 (0.945–1.187)	0.233	1.073 (1.021–1.127)	0.017	1.024 (0.967–1.086)	0.313
fPSA‡	**0.49 (0.41–0.57)**	1.227 (0.903–1.665)	0.137	1.140 (0.691–1.879)	0.508	0.414 (0.045–3.797)	0.331	0.819 (0.368–1.823)	0.527	0.730 (0.255–2.087)	0.452
%fPSA‡	**0.60 (0.52–0.68)**	0.996 (0.987–1.004)	0.233	1.001 (0.989–1.012)	0.848	1.003 (0.987–1.020)	0.610	1.004 (0.991–1.018)	0.441	1.004 (0.989–1.019)	0.478
p2PSA	**0.62 (0.54–0.69)**	1.024 (1.018–1.030)	0.0004	–		1.037 (0.991–1.085)	0.088	–		–	
%p2PSA	**0.73 (0.66–0.80)**	2.358 (1.798–3.093)	0.001	–		–		2.081 (1.291–3.353)	0.013	–	
PHI	**0.73 (0.66–0.80)**	1.022 (1.015–1.028)	0.001	–		–		–		1.020 (1.002–1.039)	0.039
AUC of multivariate models (95% CI)				**0.673 (0.588–0.753)**		**0.760 (0.679–0.830)**		**0.773 (0.693–0.841)**		**0.760 (0.679–0.830)**	
Gain in predictive accuracy (95% CI)				–		0.087 (0.021–0.151)*		0.100 (0.025–0.173)*		0.087 (0.021–0.151)*	

*P < 0.001 (relative to the multivariate base model; DeLong method).
†Not included in multivariate base model because the strong correlation with prostate volume ($\rho = 0.893$)
‡Expressed as fPSA (pg/mL)/PSA (ng/mL) to scaling OR in a more intelligible range.
OR, odds ratio.

Fig. 1 Receiver-operating characteristic curves depicting the accuracy of individual predictors of PCa at initial extended biopsies.

At 90% sensitivity, the threshold of %p2PSA and PHI were, respectively, 1.20 and 25.5 with a specificity of 37.9 and 23.0%. At a %p2PSA threshold of 1.20, a total of 39 (24.7%) biopsies could have been avoided, but seven cancers would have been missed: four with GS 6 (3+3) and three with a GS of 7 (3+4). At a PHI threshold of 25.5, a total of 26 (16.5%) biopsies could have been avoided but six cancers would have been missed: four with GS 6 (3+3) and two with a GS of 7 (3+4).

In multivariable logistic regression models, %p2PSA and PHI achieved independent predictor status, significantly increasing the accuracy, by 8.7 and 10%, respectively ($P < 0.001$), of the multivariable base model consisting of patient age, prostate volume, tPSA, fPSA and %fPSA (Table 2). Figure 2 shows the decision-curve analysis for the models shown in Table 2. The models that include p2PSA, %p2PSA and PHI (models 2, 3 and 4) resulted in a greater net benefit in a probability of pathological outcome range (threshold probability) of 35–65%. A threshold probability indicates the risk probability of PCa at which one would choose to perform a biopsy. Table 4 shows examples of how many biopsies could be avoided and how many cases of cancer would go undetected at various threshold probabilities. For example, if biopsy is recommended when the probability of PCa is ≥30%, using Model 1 we could avoid 19 of 73 (26%) unnecessary biopsies; 27 of 73 (37%) with Model 3, and with Model 4 (including PHI) we would avoid 24 of 73 (32.9%) biopsies in patients without PCa (Table 4A).

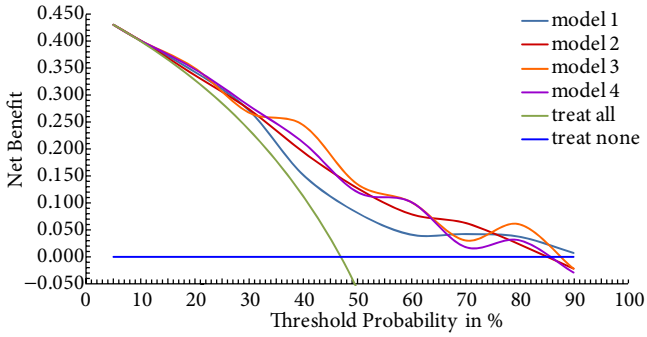
Within the subgroup of patients with PCa, only age ($P = 0.027$) was a significant predictor of GS ≥7 PCa at biopsy in bivariable and multivariable logistic regression models; however, in patients with PCa, cancer aggressiveness was

Table 3 Sensitivities and specificities in predicting the presence of PCa at initial biopsy.

Criterion	Sensitivity (%)	95% CI	Specificity (%)	95% CI	PPV	95% CI	NPV	95% CI
Total PSA (ng/mL)								
≥3.18	**90.1**	80.7–95.9	**6.9**	2.6–14.4	**44.1**	36.1–52.2	**46.2**	19.1–73.3
≥5.65	**53.5**	41.3–65.5	**54.0**	43.0–64.8	**48.7**	37.6–59.8	**58.8**	48.0–69.5
≥11.6	**19.7**	11.2–30.9	**91.9**	84.1–96.7	**66.7**	46.5–86.8	**58.4**	50.1–66.6
Free PSA (ng/mL)								
≥0.35	**90.1**	80.7–95.9	**5.7**	1.9–12.9	**43.8**	35.8–51.9	**41.7**	13.8–69.6
≥0.91	**50.7**	38.6–62.8	**47.1**	36.3–58.1	**43.9**	33.2–54.6	**53.9**	42.7–65.2
≥1.95	**15.5**	8.0–26.0	**91.9**	84.1–96.7	**61.1**	38.6–83.6	**57.1**	48.9–65.3
%fPSA								
≤0.229	**90.1**	80.7–92.8	**19.5**	11.8–29.4	**47.8**	39.3–56.2	**70.8**	52.6–89.0
≤0.164	**56.3**	44.0–68.1	**57.5**	46.4–68.0	**51.9**	40.8–63.1	**61.7**	51.1–72.3
≤0.093	**12.7**	6.0–22.7	**90.8**	82.7–95.9	**52.9**	29.2–76.7	**56.0**	47.8–64.2
p2PSA (pg/mL)								
≥6.9	**90.1**	80.7–95.9	**11.5**	5.7–20.1	**45.4**	37.2–53.6	**58.8**	35.4–82.2
≥14.0	**54.9**	42.7–66.8	**56.3**	45.3–66.9	**50.6**	39.5–61.8	**60.5**	49.8–71.1
≥25.6	**29.6**	19.3–41.6	**90.0**	81.3–95.1	**70.0**	53.6–86.4	**60.9**	52.5–69.4
%p2PSA								
≥1.20	**90.1**	80.7–95.9	**37.9**	27.7–49.0	**54.2**	45.2–63.2	**82.5**	70.7–94.3
≥1.66	**70.4**	58.4–80.7	**70.1**	59.4–79.5	**65.8**	55.1–76.5	**74.4**	64.9–83.8
≥2.35	**31.0**	20.5–43.1	**90.0**	81.3–95.1	**71.0**	55.0–86.9	**61.4**	53.0–69.9
PHI								
≥25.5	**90.1**	80.7–95.9	**23.0**	14.6–33.2	**48.9**	40.3–57.4	**74.1**	57.5–90.6
≥40.3	**64.8**	52.5–75.8	**71.3**	60.6–80.5	**64.8**	53.7–75.9	**71.3**	61.8–80.8
≥50.9	**45.1**	33.2–57.3	**90.8**	82.7–95.9	**80.0**	67.6–92.4	**66.9**	58.5–75.4

For each test, three different criterion are provided which represent the threshold values based on different levels of sensitivity and specificity (90% sensitivity, best balance between sensitivity and specificity, and 90% specificity). PPVs and NPVs are shown for each criterion.

Fig. 2 Decision-curve analysis of the effect of prediction models on the detection of PCa. Net benefit is plotted against various threshold probabilities. A threshold probability indicates the risk probability of PCa at which one would choose to perform a biopsy. Model 1 is a basic model including age, prostate volume, tPSA, fPSA, and %fPSA. Model 2 is a basic model including all factors in model 1 plus p2PSA. Model 3 is a basic model including all factors in model 1 plus % p2PSA to fPSA. Model 4 is a basic model including all factors in model 1 plus the PHI.



correlated with p2PSA, %p2PSA and PHI index. All these variables increased with increasing GS (ρ: 0.247, $P = 0.038$; ρ: 0.366, $P = 0.002$; ρ: 0.464, $P < 0.001$, respectively).

Discussion

In the current multicentre European study, we found that %p2PSA and PHI were the most accurate predictors of PCa presence at biopsy in men with a family history of PCa, had the best PPV and negative predictive value (NPV), and would also result in the avoidance of several unnecessary biopsies.

It is well known that, of the risk factors related to PCa, such as age and race, one of the strongest is a positive family history [22]. The aggregation in a family of PCa cases that do not fulfill the criteria for hereditary PCa (i.e. a definitive genetic basis) is called 'familial PCa', reflecting not only shared genes but also shared environment and common behaviours [3].

The proportion of hereditary PCa cases is estimated to be ~5–10% [2–4]. The foremost evidence of the role of family history was demonstrated in two meta-analyses, reporting a relative risk of ~2–3.5 [4,7]. The lifetime absolute risk of clinical PCa has been estimated to range from 12% for a man with a father who is diagnosed with PCa after age 60 years to 35–45% for a man with three or more relatives diagnosed with PCa, compared with an 8% absolute risk in men without a family history of the disease. In particular, family history seems to be a very important factor in patients affected by PCa at a young age: ~18% of men diagnosed with PCa aged <65 years have a positive familiar history. Men diagnosed at age ≤55 years show a stronger underlying genetic aetiology [6].

Among men who have a family history of PCa, the rate of PSA test positivity (based on a threshold of 4 ng/mL) varies

Table 4 (A) The number of potential avoided biopsies and cases of undetected PCa among patients in our sample with available data regarding age and prostate volume based on the decision-curve analysis. (B) Estimates of potential avoided biopsies and cases of undetected cancer among all patients in our sample.

(A) Models built on 134 patients with available prostate volume and age data (73 no PCa, 61 PCa)																
Cancer probability threshold (%)	Model 1				Model 2				Model 3				Model 4			
	Avoided biopsies	%	Undetected cancers	%	Avoided biopsies	%	Undetected cancers	%	Avoided biopsies	%	Undetected cancers	%	Avoided biopsies	%	Undetected cancers	%
25	14	19.2	1	1.6	15	20.5	2	3.3	17	23.3	2	3.3	16	21.9	2	3.3
30	19	26.0	2	3.3	22	30.1	3	4.9	27	37.0	6	9.8	24	32.9	3	4.9
35	29	39.7	9	14.8	33	45.2	7	11.5	36	49.3	9	14.8	35	47.9	8	13.1
40	35	47.9	16	26.2	36	49.3	15	24.6	38	52.1	13	21.3	37	50.7	14	23.0
(B) Estimates based on all 158 patients (87 no PCa, 71 PCa)																
Cancer probability threshold (%)	Model 1				Model 2				Model 3				Model 4			
	Avoided biopsies	%	Undetected cancers	%	Avoided biopsies	%	Undetected cancers	%	Avoided biopsies	%	Undetected cancers	%	Avoided biopsies	%	Undetected cancers	%
25	17	19.5	1	1.4	18	20.7	2	2.8	20	23.0	2	2.8	19	21.8	2	2.8
30	23	26.4	2	2.8	26	29.9	3	4.2	32	36.8	7	9.9	29	33.3	3	4.2
35	35	40.2	11	15.5	39	44.8	8	11.3	43	49.4	11	15.5	42	48.3	9	12.7
40	42	48.3	19	26.8	43	49.4	17	23.9	45	51.7	15	21.1	44	50.6	16	22.5

Model 1 is a basic model including age, prostate volume, tPSA, fPSA, and %fPSA. Model 2 is a basic model including all factors in model 1 plus p2PSA. Model 3 is a basic model including all factors in model 1 plus % p2PSA to fPSA. Model 4 is a basic model including all factors in model 1 plus the PHI.
Percentages are calculated on the total number of patients without cancer for avoided biopsies, and on the total number of patients with cancer for missed cancer.

between 9 and 11%, with a cancer detection rate of ~3%, a relatively poor PPV (28–32%), and high specificity (92–94%) [23,24]. PSA thresholds of 2.0 ng/mL or 2.5 ng/mL have been shown to have higher PPVs among men with a positive family history (38–43%) than in men at average risk of PCa, and variable specificity (64–93%) [23,25].

In line with reports from the literature, in the current study we found that a tPSA threshold of 3.18 ng/mL showed a sensitivity of 90.1% with a PPV of 44.1%; however, with the same criteria (sensitivity of 90.1%), a threshold of 1.20 for %p2PSA and 25.5 for PHI (respective specificity of 37.9% and 23.0%) showed the best PPV (54.2 and 48.9%, respectively [Table 3]). Interestingly %p2PSA and PHI also showed the best NPV (82.5 and 74.1%, respectively). These data indicate that %p2PSA and PHI could have important clinical implications as an effective tool for PCa screening in the population considered in the present study.

Recently it was demonstrated that serum isoform p2PSA and its derivative PHI could be valid tools for discriminating between men with or without PCa, and would aid in the avoidance of overdiagnosis and over-treatment in patients with a tPSA between 2.0 and 10 ng/mL. Indeed, %p2PSA and PHI were shown to be the strongest predictors of PCa at initial and repeat extended biopsy, showing significantly greater accuracy than the currently used tests (tPSA, %fPSA and PSA density) in determining the presence of PCa [13,16]. Even when patients are stratified according their risk categories (as in the case of the family history), PHI maintains high accuracy and therefore potential clinical use.

The strength of the present study lies in its multicentre European design in which a large population from several different geographic areas is represented. The diagnostic procedures of the study adhered strictly to protocol. Moreover, the study appears to be the first in which p2PSA and its derivatives were prospectively evaluated in a group of patients with a positive family history of PCa.

Nevertheless, the study also has some limitations. Although it was a European multicentre analysis, it was conducted on a highly selected subset sample of the PROMEtheuS project and therefore, our findings may not generalize fully to a broader population. It is true that we found a relatively high proportion of moderately to poorly differentiated disease, which is not an accurate reflection of the norm. We believe that this large proportion of high grade diagnosed PCa may have resulted from the strategy of our departments to discriminate dichotomously between PCas that could be safely ignored, or better, not diagnosed at all and those that, if left undiagnosed and untreated, would compromise either quality or quantity of life. Furthermore, our cohort was already slated for biopsy, and one could consider the population with family history to be inherently at higher risk as they undergo more intense and earlier prostate exams. Owing to the relatively small sample size, there may be unclear generalizability to other at-risk populations, such as African-Americans. Patients were included for their PCa risk and not primarily for their family history risk. Different PCa risk categories, based on the number of relatives with PCa and their degree of relatedness, were not taken into consideration. Another limitation which merits attention is the fact that the men in whom cancer was detected had, on average, undergone more biopsies and had a higher number of cores sampled, than men who were found to be cancer-free. Both of these factors could have resulted in a bias to find more cancer in these men. Finally we did not evaluate the possibility of dividing our population in two different subgroups: PSA <4 ng/mL and 4–10 ng/mL, or stratifying them according previous negative biopsies.

In conclusion, our findings support the hypothesis that %p2PSA and PHI are more accurate than the reference standard tests (tPSA, fPSA and %fPSA) in predicting PCa in men with a positive family history of PCa within a contemporary cohort of European men who underwent prostate biopsy.

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Conflict of Interest

None declared.

References

1 **Siegel R, Naishadham D, Jemal A.** Cancer statistics, 2012. *CA Cancer J Clin* 2012; 62: 10–29

2 **Bratt O, Kristoffersson U, Lundgren R, Olsson H.** Familial and hereditary prostate cancer in southern Sweden. A population-based case-control study. *Eur J Cancer* 1999; 35: 272–7

3 **Carter BS, Bova GS, Beaty TH et al.** Hereditary prostate cancer: epidemiologic and clinical features. *J Urol* 1993; 150: 797–802

4 **Bruner DW, Moore D, Parlanti A, Dorgan J, Engstrom P.** Relative risk of prostate cancer for men with affected relatives: systematic review and meta-analysis. *Int J Cancer* 2003; 107: 797–803

5 **Bratt O.** Hereditary prostate cancer: clinical aspects. *J Urol* 2002; 168: 906–13

6 **Lange EM, Salinas CA, Zuhlke KA et al.** Early onset prostate cancer has a significant genetic component. *Prostate* 2012; 72: 147–56

7 **Johns LE, Houlston RS.** A systematic review and meta-analysis of familial prostate cancer risk. *BJU Int* 2003; 91: 789–94

8 **Basch E, Oliver TK, Vickers A et al.** Screening for prostate cancer with prostate-specific antigen testing: American Society of Clinical Oncology Provisional Clinical Opinion. *J Clin Oncol* 2012; 30: 3020–5

9 **Moyer VA.** Screening for prostate cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2012; 157: 120–34

10 **Wolf AM, Wender RC, Etzioni RB et al.** American Cancer Society guideline for the early detection of prostate cancer: update 2010. *CA Cancer J Clin* 2010; 60: 70–98

11 **Narod SA, Dupont A, Cusan L et al.** The impact of family history on early detection of prostate cancer. *Nat Med* 1995; 1: 99–101

12 **Aprikian AG, Bazinet M, Plante M et al.** Family history and the risk of prostatic carcinoma in a high risk group of urological patients. *J Urol* 1995; 154: 404–6

13 **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: 214–22

14 **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: 1650–5

15 **Guazzoni G, Lazzeri M, Nava L 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: 455–66

16 **Lazzeri M, Briganti A, Scattoni V et al.** 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. *J Urol* 2012; 188: 1137–43

17 **Lazzeri M, Lughezzani G, Larcher A et al.** Reply to Sergey Tadtayev, Thomas A. McNicholas, and Gregory B. Boustead's Letter to the Editor re: Giorgio Guazzoni, Massimo Lazzeri, Luciano Nava, **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: 455–66. *Eur Urol* 2012; 62: e16–7

18 **Semjonow A, Kopke T, Eltze E, Pepping-Schefers B, Burgel H, Darte C.** Pre-analytical in-vitro stability of [-2]proPSA in blood and serum. *Clin Biochem* 2010; 43: 926–8

19 **Epstein JI, Allsbrook WC Jr, Amin MB, Egevad LL.** The 2005 International Society of Urological Pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma. *Am J Surg Pathol* 2005; 29: 1228–42

20 **DeLong ER, DeLong DM, Clarke-Pearson DL.** Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; 44: 837–45

21 **Vickers AJ, Elkin EB.** Decision curve analysis: a novel method for evaluating prediction models. *Med Decis Making* 2006; 26: 565–74

22 **Bostwick DG, Burke HB, Djakiew D et al.** Human prostate cancer risk factors. *Cancer* 2004; 101: 2371–490

23 **Makinen T, Tammela TL, Stenman UH et al.** Family history and prostate cancer screening with prostate-specific antigen. *J Clin Oncol* 2002; 20: 2658–63

24 **Giri VN, Beebe-Dimmer J, Buyyounouski M et al.** Prostate cancer risk assessment program: a 10-year update of cancer detection. *J Urol* 2007; 178: 1920–4; discussion 1924

25 **Catalona WJ, Antenor JA, Roehl KA, Moul JW.** Screening for prostate cancer in high risk populations. *J Urol* 2002; 168: 1980–3; discussion 1983–4

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Abbreviations: p2PSA, isoform [-2]proPSA; PHI, prostate health index; PCa, prostate cancer; AUC, area under the receiver-operating characteristic curve; tPSA, total PSA; fPSA, free PSA; %fPSA, free/total PSA; PPV, positive predictive value; GS, Gleason score; NPV, negative predictive value.