The Added Value of Percentage of Free to Total Prostate-specific Antigen, PCA3, and a Kallikrein Panel to the ERSPC Risk Calculator for Prostate Cancer in Prescreened Men.

Vedder, Moniek M; de Bekker-Grob, Esther W; Lilja, Hans; Vickers, Andrew J; van Leenders, Geert J L H; Steyerberg, Ewout W; Roobol, Monique J

Published in: European Urology

DOI: 10.1016/j.eururo.2014.08.011

Published: 2014-01-01

Document Version
Peer reviewed version (aka post-print)

Citation for published version (APA):

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal
The added value of %freePSA, the prostate cancer antigen gene (PCA3) and a kallikrein panel to the ERSPC risk calculator for prostate cancer in pre-screened men

Authors: M.M. Vedder¹, E.W. de Bekker-Grob¹, H.G. Lilja², A.J. Vickers³, G.J.L.H. van Leenders⁴, E.W. Steyerberg¹, M.J. Roobol⁵

¹ Department of Public Health, Erasmus Medical Center, Rotterdam, the Netherlands
² Departments of Surgery (Urology), Laboratory Medicine, and Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA; Nuffield Department of Surgical Sciences, University of Oxford, Oxford, UK; Department of Laboratory Medicine and Clinical Sciences in Malmö, Lund University, Skåne University Hospital, Malmö, Sweden; and Institute of Biomedical Technology, University of Tampere, Tampere, Finland.
³ Department of Epidemiology & Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA
⁴ Department of Pathology, Erasmus Medical Center, Rotterdam, the Netherlands
⁵ Department of Urology, Erasmus Medical Center, Rotterdam, the Netherlands

Address for correspondence:
Moniek Vedder, MSc, Department of Public Health, Erasmus MC, PO Box 2040, 3000 CA Rotterdam, The Netherlands. Tel: 31 10 704 3732; fax: 31 10 703 8475; e-mail: m.vedder@erasmusmc.nl
Keywords: %freePSA, Kallikrein panel (4k-panel), Prostate biopsy, Prostate cancer, Prostate Cancer Antigen 3 (PCA3), Prostate cancer risk calculator, Validation

Word count total: 2,905
ABSTRACT

BACKGROUND: PSA testing has limited accuracy for early detection of prostate cancer (PCa).

OBJECTIVE: To assess the added value of %freePSA, Prostate Cancer Antigen 3 (PCA3), and a kallikrein panel (4k-panel) to the European Randomized study of Screening for Prostate Cancer (ERSPC) multivariable prediction models: risk calculators (RCs) 4, including trans rectal ultrasound, and 4+DRE, for pre-screened men.

DESIGN, SETTING, AND PARTICIPANTS: Participants were invited for rescreening between October 2007 and February 2009 within the Dutch part of the ERSPC study. Biopsies were taken in men with PSA level ≥3.0ng/ml or PCA3 score ≥10. Additional analyses of 4k-panel were done on serum samples.

OUTCOME MEASUREMENTS AND STATISTICAL ANALYSIS: Outcome was defined as sextant biopsy detectable PCa. ROC curve and decision curve analyses were performed to compare the predictive capabilities of %freePSA, PCA3, 4k-panel, the ERSPC RCs, and their combinations in logistic regression models.

RESULTS AND LIMITATIONS: PCa was detected in 119 out of 708 men. %freePSA did not perform better univariately or added to the RCs compared to the RCs alone. In 202 men with elevated PSA, the 4k-panel discriminated better than PCA3 when modelled univariately (AUC 0.78 vs. 0.62; p=0.01). The multivariable models with PCA3 or 4k-panel were equivalent (AUC of 0.80 for RC 4+DRE). In the total population, PCA3 discriminated better than 4k-panel (univariate AUC 0.63 vs. 0.56, p=0.05). There was no statistically significant difference between the multivariable model with PCA3 (AUC=0.73) vs. the model with 4k-panel (AUC=0.71, p=0.18). The multivariable model with PCA3 performed better than the reference model (0.73 vs. 0.70, p=0.02). Decision curves confirmed these patterns, although numbers were small.
CONCLUSION: Both PCA3 and, to a lesser extent, a 4k-panel have added value to the DRE based ERSPC RC in detecting PCa in pre-screened men.

PATIENT SUMMARY: In this paper, we studied the added value of novel biomarkers to previously developed risk prediction models for prostate cancer. We found that inclusion of these biomarkers resulted in an increase in predictive ability.
INTRODUCTION

PSA testing is the mainstay of early detection of prostate cancer (PCa) (1). However, PSA has limited specificity and sensitivity in determining the presence of prostate cancer, which leads to unnecessary biopsies and diagnosis of potentially indolent PCa (2, 3). PSA-based multivariable prediction tools have been developed to improve the prediction of having a biopsy detectable PCa. Well known externally validated models are the European Randomized Study of Prostate Cancer (ERSPC) risk calculators (http://www.prostatecancer-riskcalculator.com/) (4), the Prostate Cancer Prevention Trial (PCPT) calculator (http://deb.uthscsa.edu/URORiskCalc/Pages/calcs.jsp) (5) and the Montreal Model (6).

The addition of new biomarkers to an existing prediction tool may increase the accuracy. Novel and promising markers in the field of PCa include the Prostate Cancer Specific Antigen 3 (PCA3), a non-coding mRNA, highly over-expressed in PCa tissue (7, 8) which can be assessed using urine obtained after digital rectal exam (DRE). A promising serum-based biomarker is the kallikrein panel (4k-panel), which consists of total-PSA, free-PSA, intact-PSA, and human-kallikrein-related peptidase-2 (hK2) (9, 10). The 4k-panel has been shown to increase predictive capability as compared to PSA and DRE alone.

In this study, we aimed to assess the added value of %freePSA, PCA3, and 4k-panel to the ERSPC risk calculators (RCs) for pre-screened men.
METHODS

Participants

Participants were recruited from the Dutch part of the ERSPC study (11, 12). We included 965 men who were invited for rescreening (3rd, 4th or 5th time) between October 2007 and February 2009. The serum based PSA level and PCA3 were measured in all men. The PCA3 score is the ratio of PCA3:PSA mRNAs multiplied by 1,000 (8). Men with a PSA level ≥3.0ng/ml and/or a PCA3 score ≥10 were invited to undergo a DRE, trans rectal ultrasound (TRUS) and a lateral sextant biopsy. We set the cut-off for PCA3 on ≥10 to evaluate performance characteristics of the PCA3 in comparison to a biopsy indication driven by PSA values of ≥3.0 ng/ml (13). Assessed prostate volume was categorised with cut-points of <30 cc, 30-50 cc, and ≥50 cc (14). In case of a hypoechogetic lesion, a seventh biopsy was taken. Permission for the present study (ISBN 978-90-5549-653-2) was granted by the Medical Ethics Committee, University Medical Center Rotterdam and the Dutch Ministry of Health.

Tests to predict PCa

The PSA test (Hybritech, Beckman Coulter Inc., Fullerton, CA, USA) was carried out in a standard fashion at the clinical laboratory of the Erasmus University Medical Center, the Netherlands. The PCA3 test (Progensa™, Gen-Probe Inc., San Diego, CA, USA) was done at the laboratory of experimental urology at Radboud University Nijmegen Medical Center. Measurements of the 4k-panel, consisting of four markers (total-PSA, free-PSA, intact-PSA, and human-kallikrein-related peptidase-2 (hK2)), were performed in the Department of Laboratory Medicine (Lund University, Malmo, Sweden) on stored serum samples (15). Separate marker values as well as an overall 4k-panel predictor were derived using a pre-specified formula, i.e.
the study is an independent validation of a previously specified model (9). The formula was a mix of linear terms and non-linear spline transformations of the four markers. A specialised pathologist (GvL) handled the histologic examinations of the biopsy specimens.

Reference model

Two models from the ERSPC Rotterdam RCs (http://www.prostatecancer-riskcalculator.com/, RC4+DRE and RC4, including TRUS) were used as reference models:

1. RC 4+DRE: A model including total PSA (ng/ml), DRE (normal/abnormal), DRE assessed volume of the prostate (<30 cc, 30-50 cc, and ≥50 cc), and whether or not there was a previous (negative) biopsy;

2. RC4: A model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), TRUS assessed prostate volume (ml) and a whether or not there was a previous (negative) biopsy.

Both models are used for men who have previously had PSA screening and a previous biopsy, if indicated according to the ERSPC Rotterdam screening algorithm (16). It predicts the chance of a positive sextant biopsy and its degree of aggressiveness; the RC4+DRE model including information on prostate volume without the necessity of a TRUS (17).

Statistical analyses

The primary outcome measure was any form of PCa vs. no cancer, detected by a sextant biopsy, in men with elevated PSA levels (≥3.0ng/ml). Secondary, we assessed the predictive value of %freePSA, PCA3, and the 4k-panel in the total population and in the population with PSA<3.0ng/ml.
We assessed the predictive value of %freePSA, PCA3, and the 4k-panel, using univariate and multivariable regression models. We refitted the original RCs: RC4 and RC4+DRE to use as the reference. We subsequently refitted the models including %freePSA, PCA3 and/or the 4k-panel. We used the area under the ROC curve (AUC) to quantify the predictive accuracy of five models: (i) the first reference model (RC 4+DRE), (ii) the reference model + PCA3, (iii) the reference model + 4k-panel, (iv) the reference model + PCA3 and the 4k-panel, and (v) the reference model + %freePSA. We used the original RC4 (i.e. including information from TRUS) as the second reference model and used the likelihood ratio test for differences between models.

We applied decision curve analysis (DCA) (18, 19) to evaluate the potential clinical usefulness of making decisions based on the models including the markers. We estimated net benefit (NB) for prediction models by summing the benefits (true positive biopsies) and subtracting the harms (false positive biopsies). The harms were weighted by a factor related to the relative harm of a missed cancer versus an unnecessary biopsy. This weighting was derived from the threshold probability (p_t) of PCa at which a patient would opt for a biopsy. This threshold can vary between men; we used a p_t between 0% and 40% (20). The interpretation of a decision curve is straightforward; a model with the highest net benefit at a particular threshold should be chosen over alternative models. The net benefit was used to calculate for the reduction in numbers of biopsies per 100 men with a PSA level of ≥3.0ng/ml (9) and/or a PCA3 score ≥10.

We used the following formula: reduction in biopsy per 100 men = (∆NB/(p_t/(1-p_t)))*100.

Standard statistical software was used (SPSS v 18.0, SPSS Inc., Chicago, Ill; R version 2.15.2, R Foundation for Statistical Computing, Vienna, Austria; Stata v 12.0, StataCorp. 2011. College Station, TX: StataCorp LP).
RESULTS

Of 965 invited men, 721 (75\%) underwent a biopsy. 163 (17\%) men did not meet the PSA or PCA3 inclusion criteria, 39 (4\%) could not have a biopsy because of contraindications, and 42 (4\%) men refused biopsy. Records of 708 out of 721 (98\%) biopsied participants were complete, including PCA3 and 4k-panel results.

These 708 men were invited for rescreening: 339 originated from the 3rd, 357 originated from the 4th and 12 originated from the 5th screening round. Participants were aged 64-75 years at time of the visit. A previous biopsy was taken from 206 (29\%) of all men. PCa was found in 119 (17\%) of the 708 biopsied men, of which 40 in the 202 men with elevated PSA levels (Table 1).

Few men had an abnormal TRUS or DRE. Of 708 men, 503 had a PCA3 score ≥10 and a PSA score <3.0 ng/ml. Total PSA and PCA3 levels differed significantly between men with and without PCa (Table 1).

In men with PSA levels ≥3.0ng/ml the 4k-panel had a higher AUC value as compared to PCA3 when studied univariately (AUC 0.78 vs. 0.62, p=0.01; Table 2; Supplementary figures.). The multivariable models with PCA3 or 4k-panel were equivalent (AUC 0.80 for RC 4+DRE, 0.78 vs. 0.79 for RC 4 with PCA3 and the 4k-panel respectively).

In the total population, PCA3 discriminated better than the 4k-panel (univariate AUC 0.63 vs. 0.56, p=0.05, Table 3). There was no statistically significant difference between the multivariable model with PCA3 (AUC=0.73) vs. the model with 4k-panel (AUC=0.71, p=0.18). The multivariable model with PCA3 performed better than the reference model (0.73 vs. 0.70, p=0.02). A multivariable model with both markers did not perform better than the multivariable model with PCA3 alone (AUC 0.73 vs. 0.73) in the total dataset. %freePSA did not perform
better univariately or added to the RCs compared to the RCs alone in the total population (Table 3).

Analyses in men with PSA levels <3.0 ng/ml showed no value for the 4k-panel, but some added value of PCA3 (univariate AUC 0.64 (0.58-0.70), AUC 0.70 vs. 0.66 when added to the reference models, p=0.01 for RC4 and p<0.01 for RC4+DRE) (see appendix Table A1).

In men with elevated PSA levels, the net benefits of all models were higher than in the total dataset (Figure 1). In this subgroup the use of a model was clinically useful from a threshold of 5%. The reduction in biopsies per 100 men differed between a threshold of 10 to 30% in the total dataset, in favour of the multivariable model with PCA3 and PCA4 + 4k-panel. In the subgroup of men with elevated PSA, different models were in favour depending on the specific threshold, which also reflected the low number of PCa cases at these thresholds (Figure 2).

The prediction models had added value over biopsy in all men if the threshold for performing a biopsy exceeded 9% (Figure 1-2). Between thresholds of 9 and 40% the multivariable model with PCA3 or PCA3 + 4k-panel had the highest net benefit and performed better than the reference model at all thresholds. With a cut-point of PSA ≥3.0 ng/m and PCA3>10, reduction in the number of biopsies per 1000 men at a threshold probability of 12.5% was 89 when PCA3 was added, 50 when the 4k-panel was added, and 124 when both the PCA3 and the 4k-panel marker were added to the original RC. At a threshold probability of 20%, there was a reduction of 11 biopsies per 1000 men when PCA3 was added to the original RC, and 7 per 1000 men when both PCA3 and the 4k-panel were added. In contrast, no reduction in the number of biopsies was noted in men with PSA level ≥3.0 ng/ml.

Results were similar for each of the considered reference models (RC4 with DRE or RC4 with TRUS, data not shown).
DISCUSSION

In the current study, adding the 4k-panel to a previously developed PCa risk prediction model increased the predictive value in participants with PSA ≥3.0 ng/ml. Adding PCA3 to the previously developed PCa risk prediction model increased the AUC in pre-screened men regardless of total PSA level at time of biopsy. This was equally seen in reference models with and without the inclusion of TRUS and TRUS assessed volume. Therefore, we advise for the model with DRE to estimate prostate volume.

In the past, %freePSA has been shown to significantly increase the accuracy of DRE and total PSA (21). Its limited cost and wide availability in labs that run total PSA values are attractive attributes for clinical use. We found very limited predictive value of %freePSA alone or combined with the RCs.

The usefulness of PCA3 testing for the detection of PCa and possible reduction of unnecessary biopsies has been shown before (22, 23). These studies assessed the added value of PCA3 after selecting men for biopsy solely on the basis of a PSA cut-off level. This implies that PCa in men with PSA values below the threshold will be missed. In addition, assessing the added value of PCA3 in men with a previous negative biopsy, initially selected on the basis of an elevated PSA level, is by definition biased. The benefit from PCA3 as compared to PSA is then overoptimistic. To overcome this attribution bias in the current study, men with a PCA3 score ≥10 were biopsied, even if their PSA level was <3.0 ng/ml (13, 24).

Predictions based on the 4k-panel did not differ significantly between cancer and non-cancer cases in the total study group, while some markers such as intact-PSA and Hk2 did differ. In the subgroup analyses of men with PSA level ≥3.0, the PCA3 and 4k-panel scores differed significantly between men with and without PCa, whereas intact-PSA and hK2 did not (Table 1).
Free-PSA differed significantly among those in the subgroup men with PSA level ≥3.0. Free PSA may hence be the most relevant element in the 4k-panel for rescreened men with elevated PSA levels.

The 4k-panel is developed in men with elevated PSA levels and has up to now only been tested in that particular but clinically most relevant setting. Previous studies showed that predictions based on levels of four kallikrein markers in blood distinguish between pathologically insignificant and aggressive PCa with good accuracy (15, 25). We confirmed these results with an increase in predictive capability in addition to a risk prediction model that already had an AUC ≥0.7, albeit in a relatively low number of patients.

With respect to cost-effectiveness, data suitable for a direct comparison with our study are scarce. While data on the cost effectiveness of PCA3 are weak (26), another comparable but cheaper combination of serum-based sub forms of PSA, the Prostate Health Index (PHI) has been found to be cost-effective for screening purposes (27). For the current study, we assessed cost-effectiveness with arbitrarily assumed costs for the PCA3 test and for prostate biopsy (€300 and €249, (28)). The 4k-panel is not commonly available, and may be cheaper than a PCA3 test (9). When adding PCA3 and/or the 4k-panel to previously developed PCa risk prediction model, less biopsies are needed to find the same amount of cancers (increased net benefit, Fig. 1 and Fig. 2). However, this did not result into a substantial reduction in prostate biopsies as compared to the original RCs alone for ps between 0 and 40%, making it very unlikely that the extended risk model will be cost-effective.

One limitation of this study was the pre-screened nature of our study cohort. Therefore we compared the performance of models with PCA3 or the 4k-panel to reference models developed for pre-screened men, allowing for a fair comparison. This, and the fact that all men
were from the Netherlands, may affect external validity. However, elevated PCA3 scores have
particularly been demonstrated to increase the probability of a positive repeat biopsy in men with
a prior negative biopsy result, independent of PSA (29, 30).

Another limitation of this study is the small number of men included, specifically men
with PSA ≥ 3.0ng/ml. The relative utility of PCA3 and the 4k-panel need to be confirmed. The
number of serious cancers was low (N=22, of which 9 in men with PSA levels ≥3.0ng/ml),
limiting separate analyses for this group of patients. In men with PSA ≥3.0ng/ml (N=202, of
whom 40 had cancer), we used the original RC consisting of 4 variables and extended this with 1
or 2 variables – giving an events per variable (EPV) ratio of 8 or 6.7 – which could lead to
overfitting of the model. Ideally the EPV would be higher, but EPV values from 5 have been
shown to be valid in the context of statistical adjustment for baseline risk factors (31).

We used sextant biopsying in a repeat screening setting and found a 17% cancer detection
rate (N=119), and it is likely that we missed some cases. Even using sextant biopsy for repeat
screening, deaths due to PC occurred at a rate of only 0.03%, compared to 0.35% overall (32).

CONCLUSION

Both the PCA3 and, to a lesser extent, a 4k-panel have added value in detecting PCa to the DRE
based ERSPC Rotterdam RC for pre-screened men. Further validation is however needed, and
should focus on biomarkers capable of identifying men at elevated risk for potentially aggressive
PCa. This is most relevant for men with a previous negative biopsy, where such markers may
especially be useful.
CONFLICTS OF INTEREST

Hans Lilja holds patents for free PSA, hK2, and intact PSA assays, and is named, along with Andrew Vickers, on a patent application for a statistical method to detect prostate cancer.

FINANCIAL DISCLOSURE

Supported in part by funds from National Cancer Institute (NCI) [R01CA160816 and P50-CA92629], the Sidney Kimmel Center for Prostate and Urologic Cancers, David H. Koch through the Prostate Cancer Foundation, the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre Program, Swedish Cancer Society (project no. 11-0624), and Fundacion Federico SA. MV and ES received funding from the Center for Translational Molecular Medicine (CTMM) [The Prostate Cancer Molecular Medicine (PCMM) project grant]. MR received funding from the Dutch Cancer Society (KWF94-869, 98-1657, 2002-277, 2006-3518, 2010-4800); The Netherlands Organisation for Health Research and Development (ZonMW-002822820, 22000106, 50-50110-98-311, 62300035), The Dutch Cancer Research Foundation (SWOP), and an unconditional grant from Beckman-Coulter-Hybritech Inc. Performing the PSA test (Hybritech, Beckman Coulter Inc., Fullerton, CA, USA), the PCA3 test (Progensa, Gen-Probe Inc., San Diego, CA, USA) and the 4k-panel measurements (performed in the Department of Laboratory Medicine (Lund University, Malmo Sweden)) were sponsored. The funding source did not have any role in the design or conduct of the study; the collection, management, analysis, or interpretation of the data; or the preparation, review, or approval of the manuscript.


### Table 1. Characteristics of men rescreened in the ERSPC trial

<table>
<thead>
<tr>
<th></th>
<th>PSA ≥3.0ng/ml (N=202)</th>
<th>Total set (N=708)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Cancer N=162 (80%)</td>
<td>Cancer N=40 (20%)</td>
<td></td>
</tr>
<tr>
<td>Age1</td>
<td>70.3 (68.1;72.7)</td>
<td>70.2 (68.6;72.4)</td>
<td>0.98</td>
</tr>
<tr>
<td>Previous Biopsy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>41 (25%)</td>
<td>26 (65%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>121 (75%)</td>
<td>14 (35%)</td>
<td></td>
</tr>
<tr>
<td>Total PSA (ng/ml)</td>
<td>4.6 (3.7;6.4)</td>
<td>4.4 (3.6;6.9)</td>
<td>0.95</td>
</tr>
<tr>
<td>DRE3</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Normal</td>
<td>133 (82%)</td>
<td>31 (77.5%)</td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>29 (18%)</td>
<td>9 (22.5%)</td>
<td></td>
</tr>
<tr>
<td>Volume classes DRE</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>&lt;30 cc</td>
<td>9 (6%)</td>
<td>6 (15%)</td>
<td></td>
</tr>
<tr>
<td>30-50 cc</td>
<td>51 (31%)</td>
<td>17 (42.5%)</td>
<td></td>
</tr>
<tr>
<td>≥50 cc</td>
<td>102 (63%)</td>
<td>17 (42.5%)</td>
<td></td>
</tr>
<tr>
<td>TRUS4</td>
<td></td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>Normal</td>
<td>155 (96%)</td>
<td>38 (95%)</td>
<td></td>
</tr>
<tr>
<td>Abnormal</td>
<td>7 (4%)</td>
<td>2 (5%)</td>
<td></td>
</tr>
<tr>
<td>4k-panel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free PSA</td>
<td>1.14 (0.86;1.62)</td>
<td>0.93 (0.68;1.39)</td>
<td>0.02</td>
</tr>
<tr>
<td>Intact PSA</td>
<td>0.42 (0.32;0.60)</td>
<td>0.40 (0.25;0.58)</td>
<td>0.40</td>
</tr>
<tr>
<td>hK25</td>
<td>0.05 (0.04;0.07)</td>
<td>0.05 (0.04;0.07)</td>
<td>1.00</td>
</tr>
<tr>
<td>4k-panel score</td>
<td>-2.81 (-3.37;-2.18)</td>
<td>-1.69 (-2.45;-1.09)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Probability 4k-panel</td>
<td>0.06 (0.03;0.10)</td>
<td>0.16 (0.08;0.25)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PCA3 score5</td>
<td>29.5 (14.0;57.5)</td>
<td>44.0 (20.0;118.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1C</td>
<td>31</td>
<td>78%</td>
<td>87</td>
</tr>
<tr>
<td>T2A</td>
<td>8</td>
<td>20%</td>
<td>28</td>
</tr>
<tr>
<td>T2B</td>
<td>1</td>
<td>3%</td>
<td>2</td>
</tr>
<tr>
<td>T2C</td>
<td>0</td>
<td>0%</td>
<td>1</td>
</tr>
<tr>
<td>T3A</td>
<td>0</td>
<td>0%</td>
<td>1</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gleason</td>
<td>Count</td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>78%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>3%</td>
<td></td>
</tr>
</tbody>
</table>

**Serious cancer**

22 18%

---

1. Continuous variables are noted as median (interquartile range)
2. Nominal variables are noted as number and percentage
3. DRE = digital rectal exam
4. TRUS = Trans rectal ultrasound
5. hK2 = kallikrein protein 2
6. PCA3 score = the ratio of PCA3: PSA mRNAs multiplied by 1,000
Table 2. Incremental enhancement in discrimination for the subgroup of 202 men rescreened in the ERSPC trial with PSA ≥3.0 ng/ml

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Added to original risk calculator 4&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Added to original risk calculator 4+DRE&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sup&gt;3&lt;/sup&gt; (95% CI)</td>
<td>C (95% CI)</td>
<td>C (95% CI)</td>
</tr>
<tr>
<td>Reference value&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.53 (0.44-0.64)</td>
<td>0.78 (0.69-0.86)</td>
<td>0.76 (0.68-0.83)</td>
</tr>
<tr>
<td>Kallikrein panel</td>
<td>0.78 (0.69-0.85)</td>
<td>0.80 (0.71-0.87)</td>
<td>0.79 (0.71-0.86)</td>
</tr>
<tr>
<td>PCA3</td>
<td>0.62 (0.52-0.73)</td>
<td>0.80 (0.71-0.87)</td>
<td>0.78 (0.70-0.85)</td>
</tr>
<tr>
<td>Kallikrein panel AND PCA3</td>
<td>0.75 (0.65-0.84)</td>
<td>0.81 (0.72-0.88)</td>
<td>0.80 (0.72-0.87)</td>
</tr>
<tr>
<td>% freePSA</td>
<td>0.65 (0.55-0.75)</td>
<td>0.80 (0.71-0.88)</td>
<td>0.79 (0.71-0.85)</td>
</tr>
</tbody>
</table>

<sup>1</sup> A model including total PSA (ng/ml), DRE (normal/abnormal), assessed DRE volume of the prostate (<30 cc, 30-50 cc, and ≥50 cc)
<sup>2</sup> A model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), and TRUS assessed prostate volume (ml)
<sup>3</sup> Area under the receiver operator curve
<sup>4</sup> The reference value for the univariate analysis is total PSA (ng/ml) and DRE (normal/abnormal), for the multivariate analyses it is the original risk calculator
Table 3. Incremental enhancement in discrimination in 708 men rescreened in the ERSPC trial

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Added to original risk calculator 4</th>
<th>Added to original risk calculator 4+DRE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C (95% CI)</td>
<td>C (95% CI)</td>
<td>C (95% CI)</td>
</tr>
<tr>
<td>Reference value^4</td>
<td>0.61 (0.56-0.67)</td>
<td>0.70 (0.64-0.75)</td>
<td>0.70 (0.64-0.75)</td>
</tr>
<tr>
<td>Kallikrein panel</td>
<td>0.56 (0.50-0.61)</td>
<td>0.71 (0.65-0.76)</td>
<td>0.71 (0.65-0.76)</td>
</tr>
<tr>
<td>PCA3</td>
<td>0.63 (0.58-0.69)</td>
<td>0.73 (0.67-0.78)</td>
<td>0.73 (0.67-0.77)</td>
</tr>
<tr>
<td>Kallikrein panel AND PCA3</td>
<td>0.66 (0.61-0.70)</td>
<td>0.73 (0.68-0.78)</td>
<td>0.73 (0.68-0.78)</td>
</tr>
<tr>
<td>%freePSA</td>
<td>0.57 (0.51-0.63)</td>
<td>0.70 (0.65-0.76)</td>
<td>0.70 (0.64-0.75)</td>
</tr>
</tbody>
</table>

1 A model including total PSA (ng/ml), DRE (normal/abnormal), assessed DRE volume of the prostate (<30 cc, 30-50 cc, and ≥50 cc)
2 A model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), and TRUS assessed prostate volume (ml)
3 Area under the receiver operator curve
4 The reference value for the univariate analysis is total PSA (ng/ml) and DRE (normal/abnormal), for the multivariate analyses it is the original risk calculator
Figure 1. Net benefit of prediction models with PCA3 and/or the 4k-panel in the subgroup of men with PSA ≥3.0ng/ml (N=202)
Figure 2. Net benefit of prediction models with PCA3 and/or the 4k-panel in all men (N=708)
Table A1. Incremental enhancement in discrimination in 506 men rescreened in the ERSPC trial with PSA <3.0ng/ml

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Added to original risk calculator 4¹</th>
<th>Added to original risk calculator 4+DRE²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C² (95% CI)</td>
<td>C (95% CI)</td>
<td>C (95% CI)</td>
</tr>
<tr>
<td>Reference value⁴</td>
<td>0.63 (0.56-0.69)</td>
<td>0.66 (0.59-0.73)</td>
<td>0.66 (0.58-0.73)</td>
</tr>
<tr>
<td>Kallikrein panel</td>
<td>0.50 (0.43-0.56)</td>
<td>0.66 (0.59-0.73)</td>
<td>0.66 (0.59-0.73)</td>
</tr>
<tr>
<td>PCA3</td>
<td>0.64 (0.58-0.70)</td>
<td>0.70 (0.62-0.76)</td>
<td>0.70 (0.63-0.77)</td>
</tr>
<tr>
<td>Kallikrein panel AND PCA3</td>
<td>0.63 (0.57-0.69)</td>
<td>0.70 (0.63-0.76)</td>
<td>0.70 (0.64-0.77)</td>
</tr>
</tbody>
</table>

¹ A model including total PSA (ng/ml), DRE (normal/abnormal), assessed DRE volume of the prostate (<30 cc, 30-50 cc, and ≥50 cc)
² A model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), and TRUS assessed prostate volume (ml)
³ Area under the receiver operator curve
⁴ The reference value for the univariate analysis is total PSA (ng/ml) and DRE (normal/abnormal), for the multivariate analyses it is the original risk calculator
Figure A1. ROC curves for the subgroup of 202 men rescreened in the ERSPC trial with PSA ≥3.0ng/ml (Table 2).

A. Univariate analysis, with PSA (ng/ml) and DRE (normal/abnormal) as a reference.
B. Multivariate analysis, with risk calculator 4, a model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), and TRUS assessed prostate volume (ml), as a reference.
C. Multivariate analysis, with risk calculator 4+DRE, a model including total PSA (ng/ml), DRE (normal/abnormal), assessed DRE volume of the prostate ((<30 cc, 30-50 cc, and ≥50 cc), as a reference.
Figure A2. ROC curves for the subgroup of 708 men rescreened in the ERSPC trial (Table 3).

A. Univariate analysis, with PSA (ng/ml) and DRE (normal/abnormal) as a reference
B. Multivariate analysis, with risk calculator 4, a model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), and TRUS assessed prostate volume (ml), as a reference.
C. Multivariate analysis, with risk calculator 4+DRE, a model including total PSA (ng/ml), DRE (normal/abnormal), assessed DRE volume of the prostate ((<30 cc, 30-50 cc, and ≥50 cc), as a reference.
Figure A3. ROC curves for the subgroup of 506 men rescreened in the ERSPC trial with PSA <3.0ng/ml (Table A1).

A. Univariate analysis, with PSA (ng/ml) and DRE (normal/abnormal) as a reference.
B. Multivariate analysis, with risk calculator 4, a model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), and TRUS assessed prostate volume (ml), as a reference.
C. Multivariate analysis, with risk calculator 4+DRE, a model including total PSA (ng/ml), DRE (normal/abnormal), assessed DRE volume of the prostate ((<30 cc, 30-50 cc, and ≥50 cc), as a reference.