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.

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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

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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 hypoechogenic 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., Fulleron, 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 = $(\Delta NB/(p_t/(1-p_t)))*100$.

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

168 better univariately or added to the RCs compared to the RCs alone in the total population (Table
169 3).

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

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

179 The prediction models had added value over biopsy in all men if the threshold for
180 performing a biopsy exceeded 9% (Figure 1-2). Between thresholds of 9 and 40% the
181 multivariable model with PCA3 orPCA3 + 4k-panel had the highest net benefit and performed
182 better than the reference model at all thresholds. With a cut-point of PSA ≥3.0 ng/m and
183 PCA3>10, reduction in the number of biopsies per 1000 men at a threshold probability of 12.5%
184 was 89 when PCA3 was added, 50 when the 4k-panel was added, and 124 when both the PCA3
185 and the 4k-panel marker were added to the original RC. At a threshold probability of 20%, there
186 was a reduction of 11 biopsies per 1000 men when PCA3 was added to the original RC, and 7
187 per 1000 men when both PCA3 and the 4k-panel were added. In contrast, no reduction in the
188 number of biopsies was noted in men with PSA level ≥3.0 ng/ml.

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

191

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 ≥3.0ng/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 p_ts 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

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REFERENCES

1. Heidenreich A, Bellmunt J, Bolla M, Joniau S, Mason M, Matveev V, et al. EAU guidelines on prostate cancer. Part 1: screening, diagnosis, and treatment of clinically localised disease. Eur Urol. 2011 Jan;59(1):61-71.
2. Draisma G, Boer R, Otto SJ, van der Cruijsen IW, Damhuis RA, Schroder FH, et al. Lead times and overdetection due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer. J Natl Cancer Inst. 2003 Jun 18;95(12):868-78.
3. Heijnsdijk EA, der Kinderen A, Wever EM, Draisma G, Roobol MJ, de Koning HJ. Overdetection, overtreatment and costs in prostate-specific antigen screening for prostate cancer. Br J Cancer. 2009 Dec 1;101(11):1833-8.
4. Steyerberg EW, Roobol MJ, Kattan MW, van der Kwast TH, de Koning HJ, Schroder FH. Prediction of indolent prostate cancer: validation and updating of a prognostic nomogram. J Urol. 2007 Jan;177(1):107-12; discussion 12.
5. Thompson IM, Ankerst DP, Chi C, Goodman PJ, Tangen CM, Lucia MS, et al. Assessing prostate cancer risk: results from the Prostate Cancer Prevention Trial. J Natl Cancer Inst. 2006 Apr 19;98(8):529-34.
6. Karakiewicz PI, Benayoun S, Kattan MW, Perrotte P, Valiquette L, Scardino PT, et al. Development and validation of a nomogram predicting the outcome of prostate biopsy based on patient age, digital rectal examination and serum prostate specific antigen. J Urol. 2005 Jun;173(6):1930-4.
7. Bussemakers MJ, van Bokhoven A, Verhaegh GW, Smit FP, Karthaus HF, Schalken JA, et al. DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Res. 1999 Dec 1;59(23):5975-9.
8. Hessels D, Schalken JA. The use of PCA3 in the diagnosis of prostate cancer. Nat Rev Urol. 2009 May;6(5):255-61.
9. Vickers AJ, Cronin AM, Aus G, Pihl CG, Becker C, Pettersson K, et al. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Goteborg, Sweden. BMC Med. 2008;6:19.
10. Gupta A, Roobol MJ, Savage CJ, Peltola M, Pettersson K, Scardino PT, et al. A four-kallikrein panel for the prediction of repeat prostate biopsy: data from the European Randomized Study of Prostate Cancer screening in Rotterdam, Netherlands. Br J Cancer. 2010 Aug 24;103(5):708-14.
11. Schroder FH, Denis LJ, Roobol M, Nelen V, Auvinen A, Tammela T, et al. The story of the European Randomized Study of Screening for Prostate Cancer. BJU Int. 2003 Dec;92 Suppl 2:1-13.
12. Schroder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med. 2009 Mar 26;360(13):1320-8.
13. Roobol MJ, Schroder FH, van Leeuwen P, Wolters T, van den Bergh RC, van Leenders GJ, et al. Performance of the prostate cancer antigen 3 (PCA3) gene and prostate-specific antigen in prescreened men: exploring the value of PCA3 for a first-line diagnostic test. Eur Urol. 2010 Oct;58(4):475-81.

14. Roobol MJ, van Vugt HA, Loeb S, Zhu X, Bul M, Bangma CH, et al. Prediction of prostate cancer risk: the role of prostate volume and digital rectal examination in the ERSPC risk calculators. Eur Urol. 2012 Mar;61(3):577-83.
15. Vickers A, Cronin A, Roobol M, Savage C, Peltola M, Pettersson K, et al. Reducing unnecessary biopsy during prostate cancer screening using a four-kallikrein panel: an independent replication. J Clin Oncol. 2010 May 20;28(15):2493-8.
16. Roobol MJ, Zhu X, Schroder FH, van Leenders GJ, van Schaik RH, Bangma CH, et al. A Calculator for Prostate Cancer Risk 4 Years After an Initially Negative Screen: Findings from ERSPC Rotterdam. Eur Urol. 2013 Apr;63(4):627-33.
17. Roobol MJ, Schroder FH, Kranse R, Erspc R. A comparison of first and repeat (four years later) prostate cancer screening in a randomized cohort of a symptomatic men aged 55-75 years using a biopsy indication of 3.0 ng/ml (results of ERSPC, Rotterdam). Prostate. 2006 May 1;66(6):604-12.
18. Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. Med Decis Making. 2006 Nov-Dec;26(6):565-74.
19. Steyerberg EW, Vickers AJ, Cook NR, Gerds T, Gonen M, Obuchowski N, et al. Assessing the performance of prediction models: a framework for traditional and novel measures. Epidemiology. 2010 Jan;21(1):128-38.
20. Vickers AJ, Cronin AM, Elkin EB, Gonen M. Extensions to decision curve analysis, a novel method for evaluating diagnostic tests, prediction models and molecular markers. BMC Med Inform Decis Mak. 2008;8:53.
21. Steuber T, Vickers A, Haese A, Kattan MW, Eastham JA, Scardino PT, et al. Free PSA isoforms and intact and cleaved forms of urokinase plasminogen activator receptor in serum improve selection of patients for prostate cancer biopsy. Int J Cancer. 2007 Apr 1;120(7):1499-504.
22. Auprich M, Haese A, Walz J, Pummer K, de la Taille A, Graefen M, et al. External validation of urinary PCA3-based nomograms to individually predict prostate biopsy outcome. Eur Urol. 2010 Nov;58(5):727-32.
23. Auprich M, Chun FK, Ward JF, Pummer K, Babaian R, Augustin H, et al. Critical assessment of preoperative urinary prostate cancer antigen 3 on the accuracy of prostate cancer staging. Eur Urol. 2011 Jan;59(1):96-105.
24. Roobol MJ, Schroder FH, van Leenders GL, Hessels D, van den Bergh RC, Wolters T, 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 Dec;58(6):893-9.
25. Carlsson S, Maschino A, Schroder F, Bangma C, Steyerberg EW, van der Kwast T, et al. Predictive Value of Four Kallikrein Markers for Pathologically Insignificant Compared With Aggressive Prostate Cancer in Radical Prostatectomy Specimens: Results From the European Randomized Study of Screening for Prostate Cancer Section Rotterdam. Eur Urol. 2013 May 2.
26. Malavaud B, Cussenot O, Mottet N, Rozet F, Ruffion A, Smets L, et al. Impact of adoption of a decision algorithm including PCA3 for repeat biopsy on the costs for prostate cancer diagnosis in France. J Med Econ. 2013;16(3):358-63.
27. Nichol MB, Wu J, Huang J, Denham D, Frencher SK, Jacobsen SJ. Cost-effectiveness of Prostate Health Index for prostate cancer detection. BJU Int. 2012 Aug;110(3):353-62.
28. Fandella A. Analysis of costs of transrectal prostate biopsy. Urologia. 2011 Oct-Dec;78(4):288-92.

29. Haese A, de la Taille A, van Poppel H, Marberger M, Stenzl A, Mulders PF, et al. Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. Eur Urol. 2008 Nov;54(5):1081-8.
30. Gittelman M, Hertzman B, Bailen J, Williams T, Koziol I, Henderson RJ, et al. PROGENSA(R)PCA3 molecular urine test as a predictor of repeat prostate biopsy outcome in men with previous negative biopsies: A prospective multicenter clinical study. J Urol. 2013 Feb 14.
31. Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. Am J Epidemiol. 2007 Mar 15;165(6):710-8.
32. Schroder FH, van den Bergh RC, Wolters T, van Leeuwen PJ, Bangma CH, van der Kwast TH, et al. Eleven-year outcome of patients with prostate cancers diagnosed during screening after initial negative sextant biopsies. Eur Urol. 2010 Feb;57(2):256-66.

385 TABLES AND FIGURES

386 Table 1. Characteristics of men rescreened in the ERSPC trial
387

	PSA ≥3.0ng/ml (N=202)					Total set (N=708)				
	No Cancer N=162 (80%)		Cancer N=40 (20%)		P-value	No Cancer N=589 (83%)		Cancer N=119 (17%)		P-value
Age[1]	70.3	(68.1;72.7)	70.2	(68.6;72.4)	0.98	70.3	(68.1;72.5)	70.3	(68.4;72.3)	0.97
Previous Biopsy					<0.01					<0.01
No	41	25%	26	65%		403	68%	99	83%	
Yes	121	75%	14	35%		186	32%	20	17%	
Total PSA (ng/ml)	4.6	(3.7;6.4)	4.4	(3.6;6.9)	0.95	1.7	(0.9;3.2)	2.1	(1.4;3.7)	<0.01
DRE[3]					0.51					<0.01
Normal	133	82%	31	77.5%		504	86%	88	74%	
Abnormal	29	18%	9	22.5%		85	14%	31	26%	
Volume classes DRE					0.03					0.53
<30 cc	9	6%	6	15%		115	20%	23	19%	
30-50 cc	51	31%	17	42.5%		263	45%	60	50%	
≥50 cc	102	63%	17	42.5%		204	35%	36	30%	
TRUS[4]					0.85					0.38
Normal	155	96%	38	95%		573	97%	114	96%	
Abnormal	7	4%	2	5%		16	3%	5	4%	
4k-panel										
Free PSA	1.14	(0.86;1.62)	0.93	(0.68;1.39)	0.02	0.47	(0.28;0.84)	0.56	(0.39;0.86)	0.06
Intact PSA	0.42	(0.32;0.60)	0.40	(0.25;0.58)	0.40	0.20	(0.12;0.34)	0.23	(0.16;0.39)	0.04
hK2[5]	0.05	(0.04;0.07)	0.05	(0.04;0.07)	1.00	0.03	(0.02;0.05)	0.04	(0.03;0.05)	<0.01
4k-panel score	-2.81	(-3.37;-2.18)	-1.69	(-2.45;-1.09)	<0.01	-1.33	(-2.27;-0.98)	-1.28	(-1.76;-0.97)	0.04
Probability 4k-panel	0.06	(0.03;0.10)	0.16	(0.08;0.25)	<0.01	0.21	(0.09;0.27)	0.22	(0.15;0.28)	0.04
PCA3 score[6]	29.5	(14.0;57.5)	44.0	(20.0;118.3)	0.01	31.0	(18.0;58.5)	46.0	(28.0;97.0)	<0.01
Stage										
T1C			31	78%				87	73%	
T2A			8	20%				28	24%	
T2B			1	3%				2	2%	
T2C			0	0%				1	1%	
T3A			0	0%				1	1%	
Grade										

Gleason 6	31	78%	99	83%
Gleason 7	5	13%	13	11%
Gleason 8	3	8%	5	4%
Gleason 9	1	3%	2	2%
Serious cancer[2]	9	23%	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 ≥3.0ng/ml

	Univariate		Added to original risk calculator 4[1]		Added to original risk calculator 4+DRE[2]	
	C[3]	(95% CI)	C	(95% CI)	C	(95% CI)
Reference value[4]	0.53	(0.44-0.64)	0.78	(0.69-0.86)	0.76	(0.68-0.83)
Kallikrein panel	0.78	(0.69-0.85)	0.80	(0.71-0.87)	0.79	(0.71-0.86)
PCA3	0.62	(0.52-0.73)	0.80	(0.71-0.87)	0.78	(0.70-0.85)
Kallikrein panel AND PCA3	0.75	(0.65-0.84)	0.81	(0.72-0.88)	0.80	(0.72-0.87)
%freePSA	0.65	(0.55-0.75)	0.80	(0.71-0.88)	0.79	(0.71-0.85)

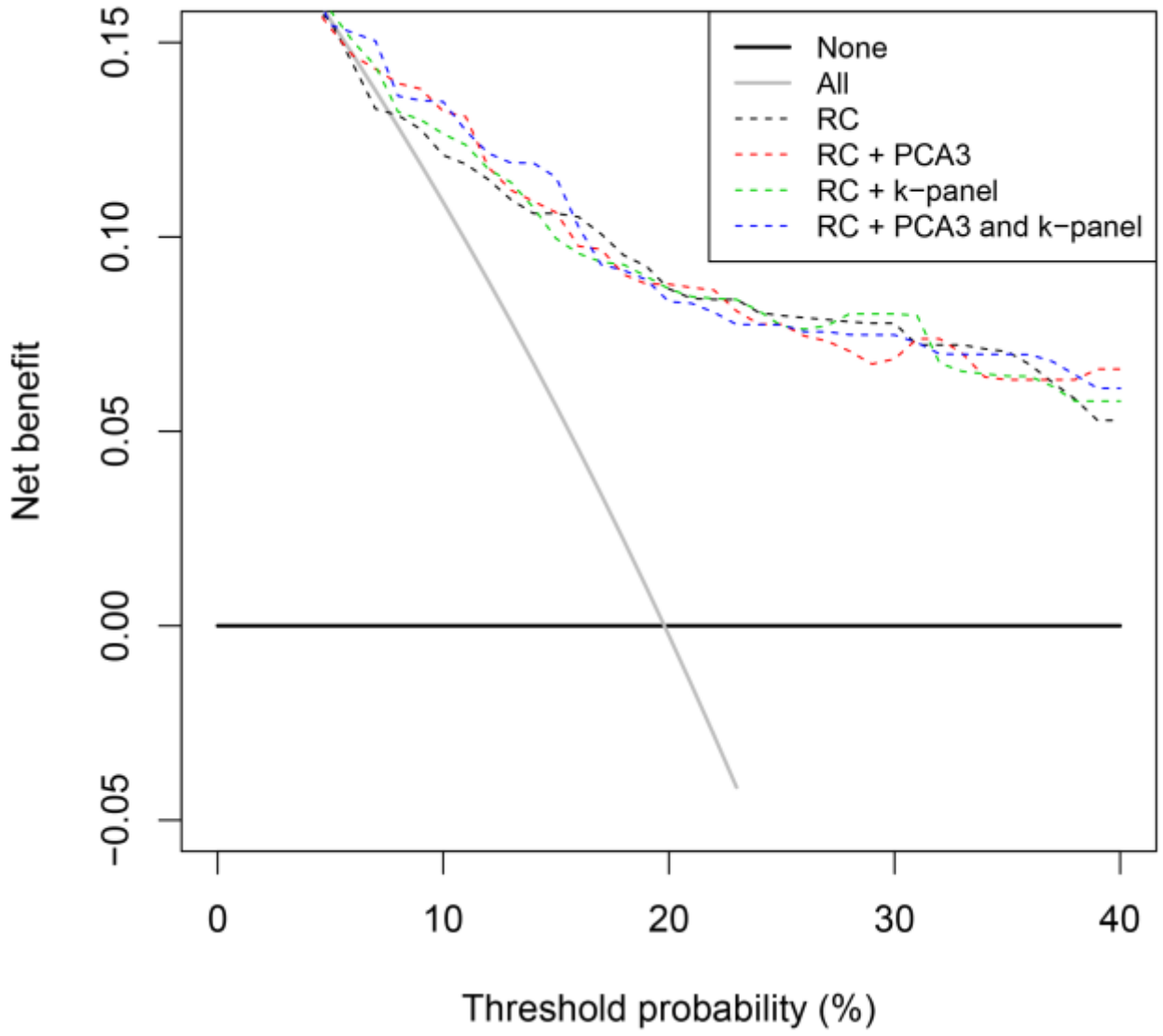
[1] A model including total PSA (ng/ml), DRE (normal/abnormal), assessed DRE volume of the prostate (<30 cc, 30-50 cc, and ≥50 cc)
[2] A model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), and TRUS assessed prostate volume (ml)
[3] Area under the receiver operator curve
[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

Table 3. Incremental enhancement in discrimination in 708 men rescreened in the ERSPC trial

	Univariate	Added to original risk calculator 4[1]	Added to original risk calculator 4+DRE[2]
	C[3] (95% CI)	C (95% CI)	C (95% CI)
Reference value[4]	0.61 (0.56-0.67)	0.70 (0.64-0.75)	0.70 (0.64-0.75)
Kallikrein panel	0.56 (0.50-0.61)	0.71 (0.65-0.76)	0.71 (0.65-0.76)
PCA3	0.63 (0.58-0.69)	0.73 (0.67-0.78)	0.73 (0.67-0.77)
Kallikrein panel AND PCA3	0.66 (0.61-0.70)	0.73 (0.68-0.78)	0.73 (0.68-0.78)
%freePSA	0.57 (0.51-0.63)	0.70 (0.65-0.76)	0.70 (0.64-0.75)

[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

408 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)



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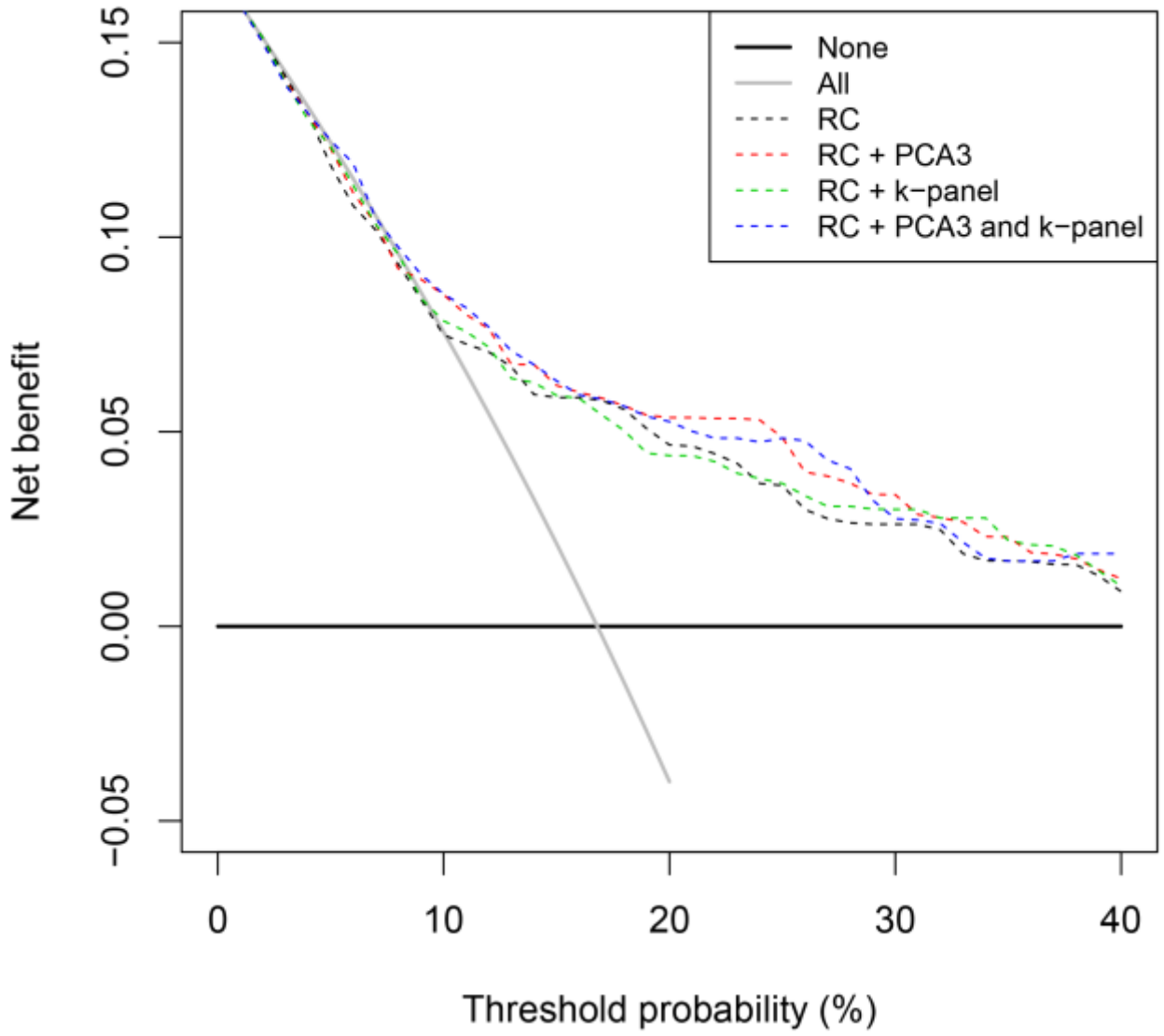
410 Figure 2. Net benefit of prediction models with PCA3 and/or the 4k-panel in all men (N=708)



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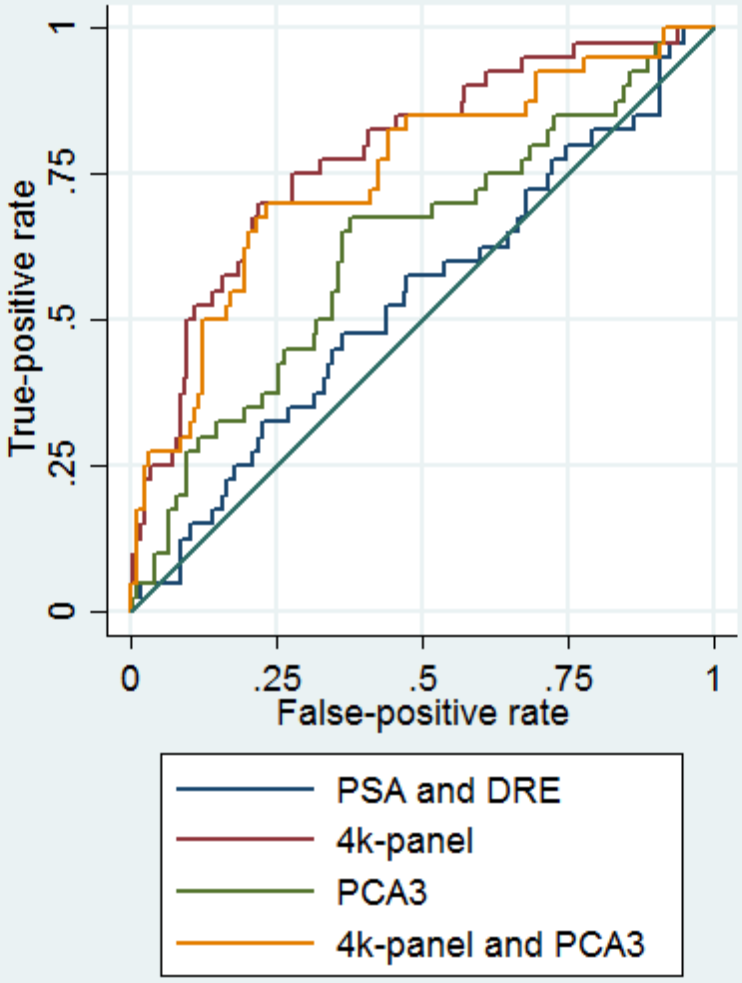
APPENDIX

Table A1. Incremental enhancement in discrimination in 506 men rescreened in the ERSPC trial with PSA <3.0ng/ml

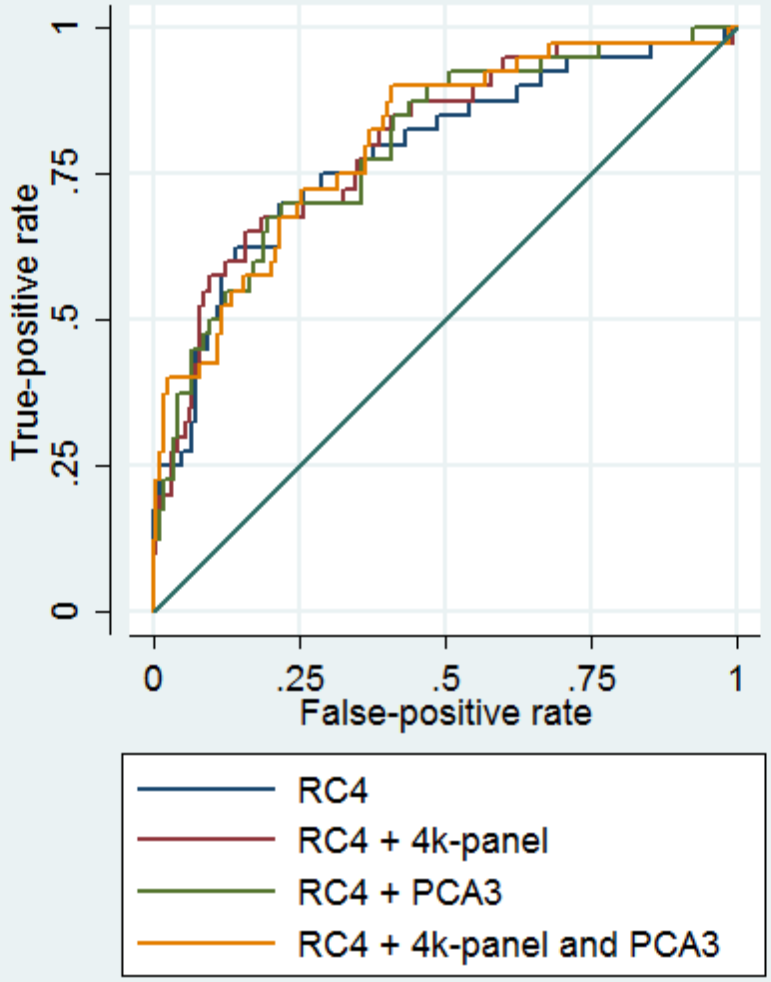
	Univariate		Added to original risk calculator 4[1]		Added to original risk calculator 4+DRE[2]	
	C[3]	(95% CI)	C	(95% CI)	C	(95% CI)
Reference value[4]	0.63	(0.56-0.69)	0.66	(0.59-0.73)	0.66	(0.58-0.73)
Kallikrein panel	0.50	(0.43-0.56)	0.66	(0.59-0.73)	0.66	(0.59-0.73)
PCA3	0.64	(0.58-0.70)	0.70	(0.62-0.76)	0.70	(0.63-0.77)
Kallikrein panel AND PCA3	0.63	(0.57-0.69)	0.70	(0.63-0.76)	0.70	(0.64-0.77)

[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

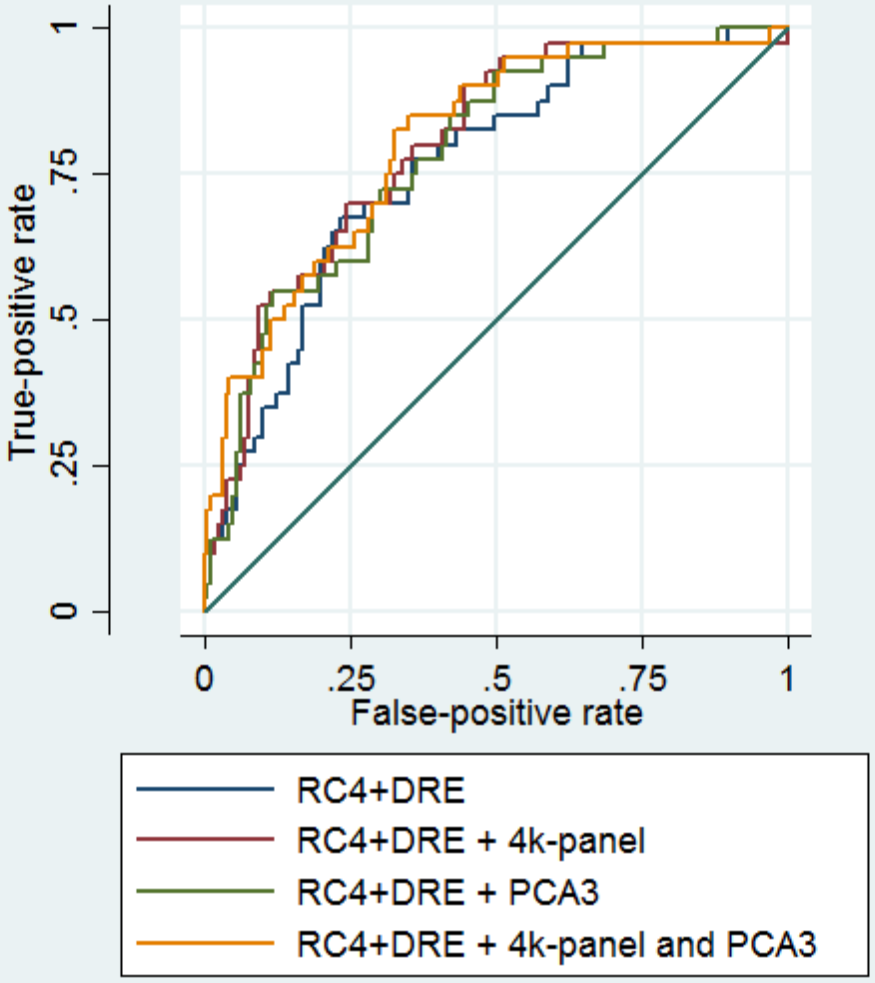
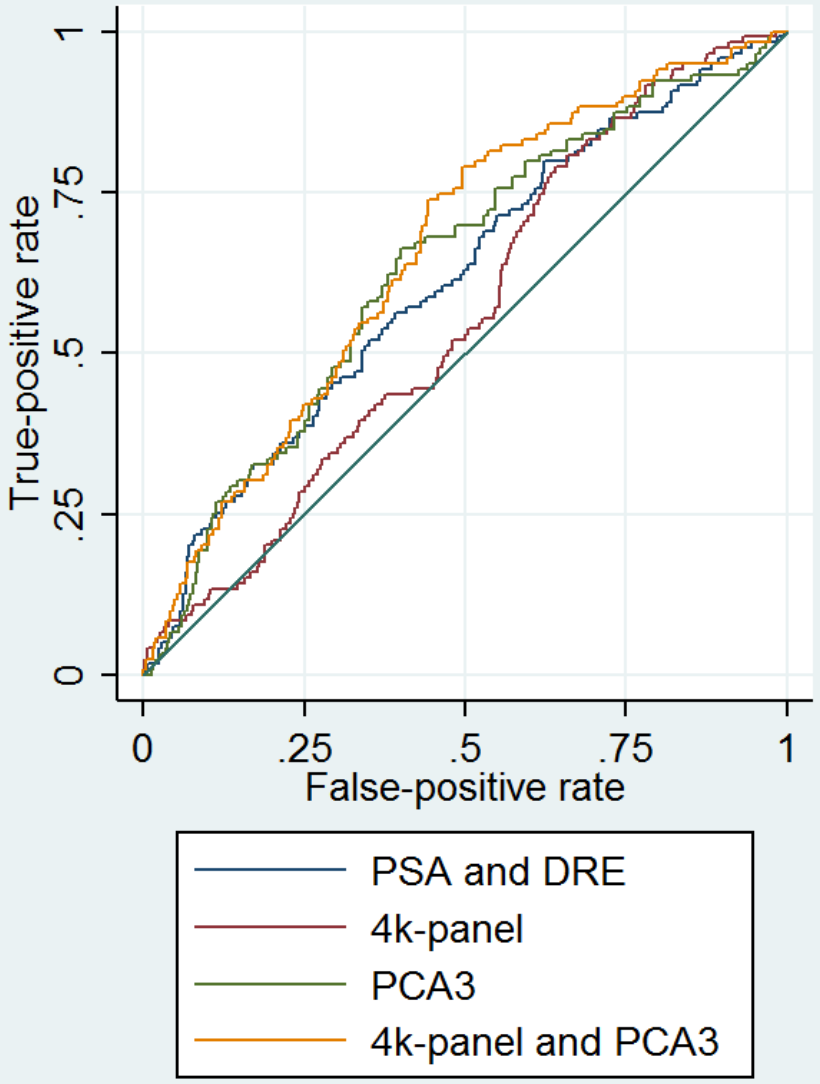
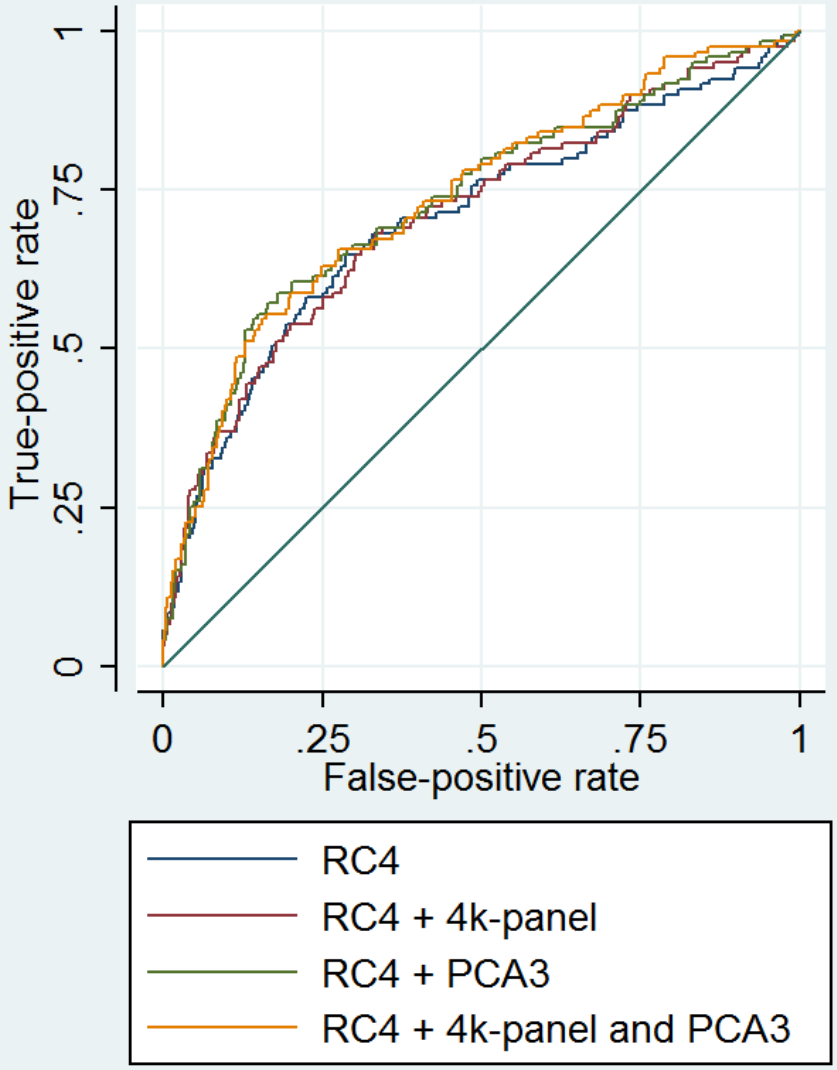


Figure A2. ROC curves for the subgroup of 708 men rescreened in the ERSPC trial (Table 3). A. Univariate analysis, with PSA (ng/ml) and DRE (normal/abnormal) as a reference



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

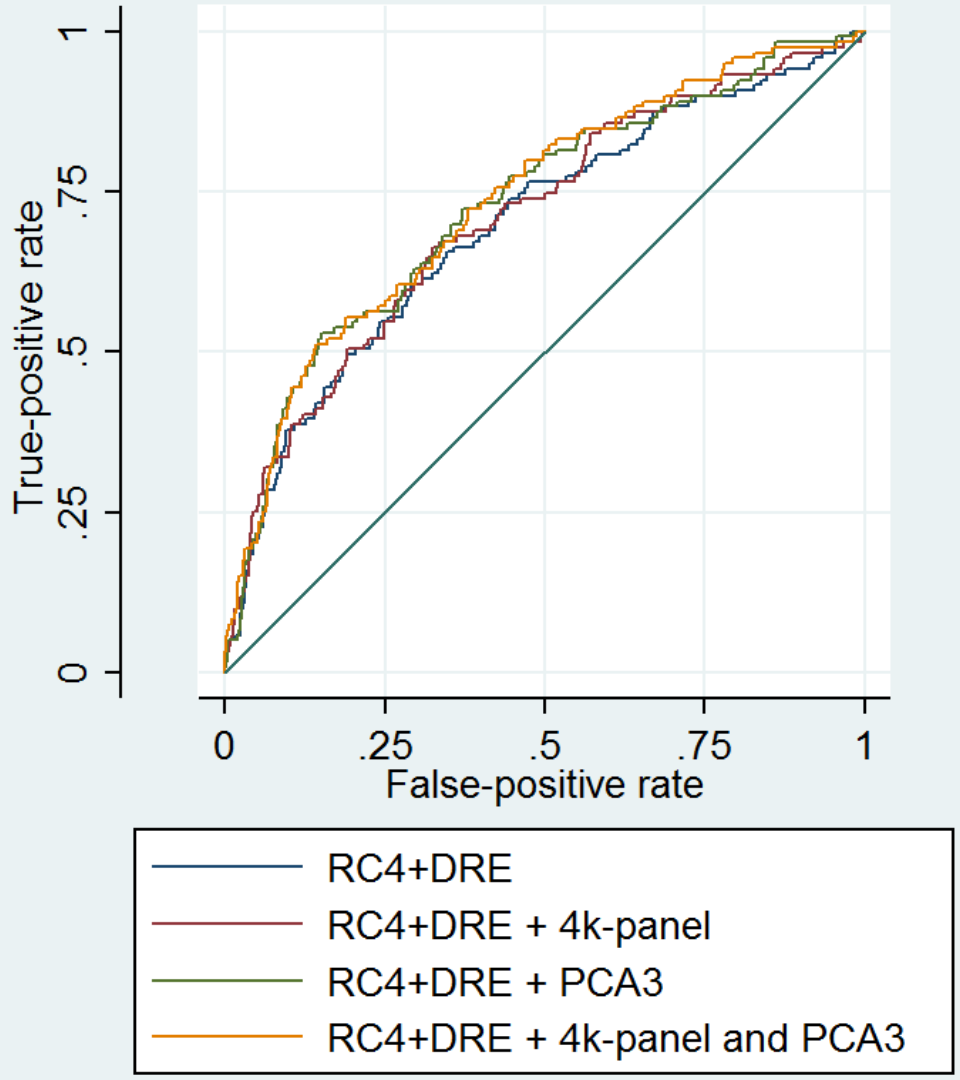
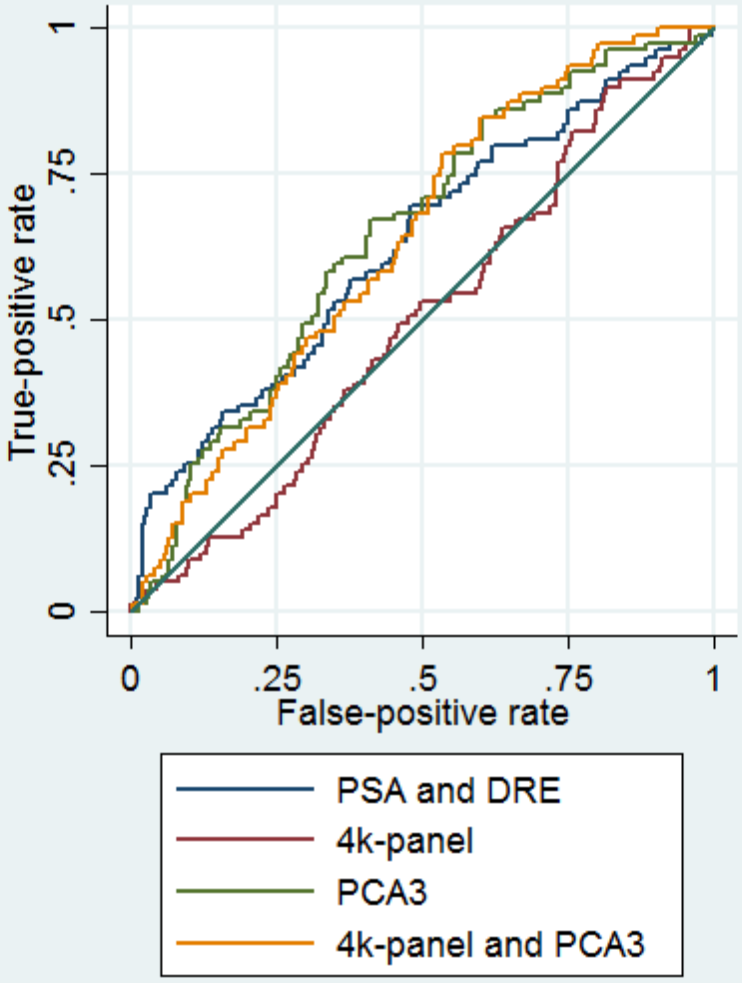
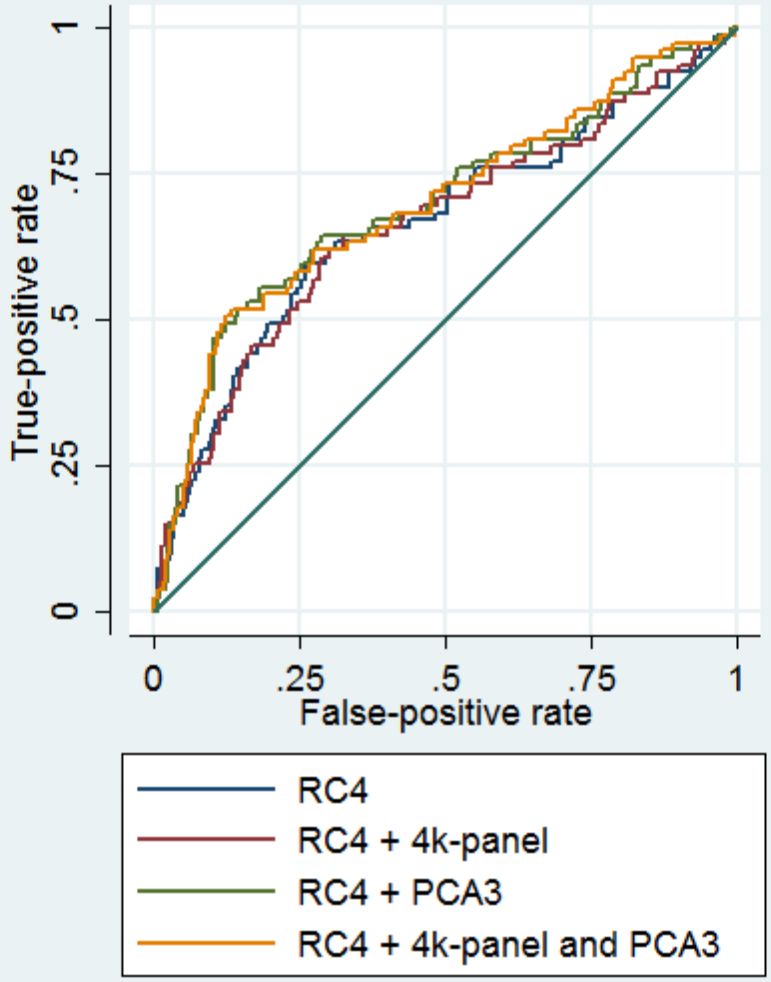


Figure A3. ROC curves for the subgroup of 506 men rescreened in the ERSPC trial with PSA <3.0ng/ml (Table A1). A. Univariate analysis, with PSA (ng/ml) and DRE (normal/abnormal) as a reference



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