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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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1 The added value of %freePSA, the prostate cancer antigen gene (PCA3) and a kallikrein panel to
2 the ERSPC risk calculator for prostate cancer in pre-screened men

3

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22

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25

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27

28 ABSTRACT

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

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

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

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

42 RESULTS AND LIMITATIONS: PCa was detected in 119 out of 708 men. %freePSA did not
43 perform better univariately or added to the RCs compared to the RCs alone. In 202 men with
44 elevated PSA, the 4k-panel discriminated better than PCA3 when modelled univariately (AUC
45 0.78 vs. 0.62; $p=0.01$). The multivariable models with PCA3 or 4k-panel were equivalent (AUC
46 of 0.80 for RC 4+DRE). In the total population, PCA3 discriminated better than 4k-panel
47 (univariate AUC 0.63 vs. 0.56, $p=0.05$). There was no statistically significant difference between
48 the multivariable model with PCA3 (AUC=0.73) vs. the model with 4k-panel (AUC=0.71,
49 $p=0.18$). The multivariable model with PCA3 performed better than the reference model (0.73
50 vs. 0.70, $p=0.02$). Decision curves confirmed these patterns, although numbers were small.

51 CONCLUSION: Both PCA3 and, to a lesser extent, a 4k-panel have added value to the DRE
52 based ERSPC RC in detecting PCa in pre-screened men.

53

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

57

58 INTRODUCTION

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

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

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

76

77 METHODS

78 *Participants*

79 Participants were recruited from the Dutch part of the ERSPC study (11, 12). We included 965
80 men who were invited for rescreening (3rd, 4th or 5th time) between October 2007 and February
81 2009. The serum based PSA level and PCA3 were measured in all men. The PCA3 score is the
82 ratio of PCA3:PSA mRNAs multiplied by 1,000 (8). Men with a PSA level ≥ 3.0 ng/ml and/or a
83 PCA3 score ≥ 10 were invited to undergo a DRE, trans rectal ultrasound (TRUS) and a lateral
84 sextant biopsy. We set the cut-off for PCA3 on ≥ 10 to evaluate performance characteristics of
85 the PCA3 in comparison to a biopsy indication driven by PSA values of ≥ 3.0 ng/ml (13).
86 Assessed prostate volume was categorised with cut-points of < 30 cc, 30-50 cc, and ≥ 50 cc (14).
87 In case of a hypoechoic lesion, a seventh biopsy was taken. Permission for the present study
88 (ISBN 978-90-5549-653-2) was granted by the Medical Ethics Committee, University Medical
89 Center Rotterdam and the Dutch Ministry of Health.

90

91 *Tests to predict PCa*

92 The PSA test (Hybritech, Beckman Coulter Inc., Fullerton, CA, USA) was carried out in a
93 standard fashion at the clinical laboratory of the Erasmus University Medical Center, the
94 Netherlands. The PCA3 test (ProgenSA™, Gen-Probe Inc., San Diego, CA, USA) was done at the
95 laboratory of experimental urology at Radboud University Nijmegen Medical Center.
96 Measurements of the 4k-panel, consisting of four markers (total-PSA, free-PSA, intact-PSA, and
97 human-kallikrein-related peptidase-2 (hK2)), were performed in the Department of Laboratory
98 Medicine (Lund University, Malmo, Sweden) on stored serum samples (15). Separate marker
99 values as well as an overall 4k-panel predictor were derived using a pre-specified formula, i.e.

100 the study is an independent validation of a previously specified model (9). The formula was a
101 mix of linear terms and non-linear spline transformations of the four markers. A specialised
102 pathologist (GvL) handled the histologic examinations of the biopsy specimens.

103

104 *Reference model*

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

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

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

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

117

118 *Statistical analyses*

119 The primary outcome measure was any form of PCa vs. no cancer, detected by a sextant biopsy,
120 in men with elevated PSA levels (\geq 3.0ng/ml). Secondary, we assessed the predictive value of
121 %freePSA, PCA3, and the 4k-panel in the total population and in the population with
122 PSA<3.0ng/ml.

123 We assessed the predictive value of %freePSA, PCA3, and the 4k-panel, using univariate
124 and multivariable regression models. We refitted the original RCs: RC4 and RC4+DRE to use as
125 the reference. We subsequently refitted the models including %freePSA, PCA3 and/or the 4k-
126 panel. We used the area under the ROC curve (AUC) to quantify the predictive accuracy of five
127 models: (i) the first reference model (RC 4+DRE), (ii) the reference model + PCA3, (iii) the
128 reference model + 4k-panel, (iv) the reference model + PCA3 and the 4k-panel, and (v) the
129 reference model + %freePSA. We used the original RC4 (i.e. including information from TRUS)
130 as the second reference model and used the likelihood ratio test for differences between models.

131 We applied decision curve analysis (DCA) (18, 19) to evaluate the potential clinical
132 usefulness of making decisions based on the models including the markers. We estimated net
133 benefit (NB) for prediction models by summing the benefits (true positive biopsies) and
134 subtracting the harms (false positive biopsies). The harms were weighted by a factor related to
135 the relative harm of a missed cancer versus an unnecessary biopsy. This weighting was derived
136 from the threshold probability (p_t) of PCa at which a patient would opt for a biopsy. This
137 threshold can vary between men; we used a p_t between 0% and 40% (20). The interpretation of a
138 decision curve is straightforward; a model with the highest net benefit at a particular threshold
139 should be chosen over alternative models. The net benefit was used to calculate for the reduction
140 in numbers of biopsies per 100 men with a PSA level of ≥ 3.0 ng/ml (9) and/or a PCA3 score ≥ 10 .
141 We used the following formula: reduction in biopsy per 100 men = $(\Delta NB / (p_t / (1 - p_t))) * 100$.

142 Standard statistical software was used (SPSS v 18.0, SPSS Inc., Chicago, Ill; R version
143 2.15.2, R Foundation for Statistical Computing, Vienna, Austria; Stata v 12.0, StataCorp. 2011.
144 College Station, TX: StataCorp LP).

145

146 RESULTS

147 Of 965 invited men, 721 (75%) underwent a biopsy. 163 (17%) men did not meet the PSA or
148 PCA3 inclusion criteria, 39 (4%) could not have a biopsy because of contraindications, and 42
149 (4%) men refused biopsy. Records of 708 out of 721 (98%) biopsied participants were complete,
150 including PCA3 and 4k-panel results.

151 These 708 men were invited for rescreening: 339 originated from the 3rd, 357 originated
152 from the 4th and 12 originated from the 5th screening round. Participants were aged 64-75 years at
153 time of the visit. A previous biopsy was taken from 206 (29%) of all men. PCa was found in 119
154 (17%) of the 708 biopsied men, of which 40 in the 202 men with elevated PSA levels (Table 1).
155 Few men had an abnormal TRUS or DRE. Of 708 men, 503 had a PCA3 score ≥ 10 and a PSA
156 score < 3.0 ng/ml. Total PSA and PCA3 levels differed significantly between men with and
157 without PCa (Table 1).

158 In men with PSA levels ≥ 3.0 ng/ml the 4k-panel had a higher AUC value as compared to
159 PCA3 when studied univariately (AUC 0.78 vs. 0.62, $p=0.01$; Table 2; Supplementary figures.).
160 The multivariable models with PCA3 or 4k-panel were equivalent (AUC 0.80 for RC 4+DRE,
161 0.78 vs. 0.79 for RC 4 with PCA3 and the 4k-panel respectively).

162 In the total population, PCA3 discriminated better than the 4k-panel (univariate AUC
163 0.63 vs. 0.56, $p=0.05$, Table 3). There was no statistically significant difference between the
164 multivariable model with PCA3 (AUC=0.73) vs. the model with 4k-panel (AUC=0.71, $p=0.18$).
165 The multivariable model with PCA3 performed better than the reference model (0.73 vs. 0.70,
166 $p=0.02$). A multivariable model with both markers did not perform better than the multivariable
167 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 or PCA3 + 4k-panel had the highest net benefit and performed
182 better than the reference model at all thresholds. With a cut-point of $PSA \geq 3.0$ ng/ml 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

192 DISCUSSION

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

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

203 The usefulness of PCA3 testing for the detection of PCa and possible reduction of
204 unnecessary biopsies has been shown before (22, 23). These studies assessed the added value of
205 PCA3 after selecting men for biopsy solely on the basis of a PSA cut-off level. This implies that
206 PCa in men with PSA values below the threshold will be missed. In addition, assessing the added
207 value of PCA3 in men with a previous negative biopsy, initially selected on the basis of an
208 elevated PSA level, is by definition biased. The benefit from PCA3 as compared to PSA is then
209 overoptimistic. To overcome this attribution bias in the current study, men with a PCA3 score
210 ≥ 10 were biopsied, even if their PSA level was < 3.0 ng/ml (13, 24).

211 Predictions based on the 4k-panel did not differ significantly between cancer and non-
212 cancer cases in the total study group, while some markers such as intact-PSA and Hk2 did differ.
213 In the subgroup analyses of men with PSA level ≥ 3.0 , the PCA3 and 4k-panel scores differed
214 significantly between men with and without PCa, whereas intact-PSA and hK2 did not (Table 1).

215 Free-PSA differed significantly among those in the subgroup men with PSA level ≥ 3.0 . Free
216 PSA may hence be the most relevant element in the 4k-panel for rescreened men with elevated
217 PSA levels.

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

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

235 One limitation of this study was the pre-screened nature of our study cohort. Therefore
236 we compared the performance of models with PCA3 or the 4k-panel to reference models
237 developed for pre-screened men, allowing for a fair comparison. This, and the fact that all men

238 were from the Netherlands, may affect external validity. However, elevated PCA3 scores have
239 particularly been demonstrated to increase the probability of a positive repeat biopsy in men with
240 a prior negative biopsy result, independent of PSA (29, 30).

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

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

252

253 CONCLUSION

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

259

260 CONFLICTS OF INTEREST

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

263

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281

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383
384

385 TABLES AND FIGURES

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

387

	PSA \geq 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
Age¹	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
DRE⁵					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%	
\geq 50 cc	102	63%	17	42.5%		204	35%	36	30%	
TRUS⁴					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 score⁶	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 cancer²	9	23%	22	18%

388 ¹ Continuous variables are noted as median (interquartile range)

389 ² Nominal variables are noted as number and percentage

390 ³ DRE = digital rectal exam

391 ⁴ TRUS = Trans rectal ultrasound

392 ⁵ hK2 = kallikrein protein 2

393 ⁶ PCA3 score = the ratio of PCA3: PSA mRNAs multiplied by 1,000

394

395 Table 2. Incremental enhancement in discrimination for the subgroup of 202 men rescreened in the ERSPC trial with PSA ≥ 3.0 ng/ml
 396

	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)

397 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)

398 ² A model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), and TRUS assessed prostate volume (ml)

399 ³ Area under the receiver operator curve

400 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

401

402 Table 3. Incremental enhancement in discrimination in 708 men rescreened in the ERSPC trial
 403

	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)

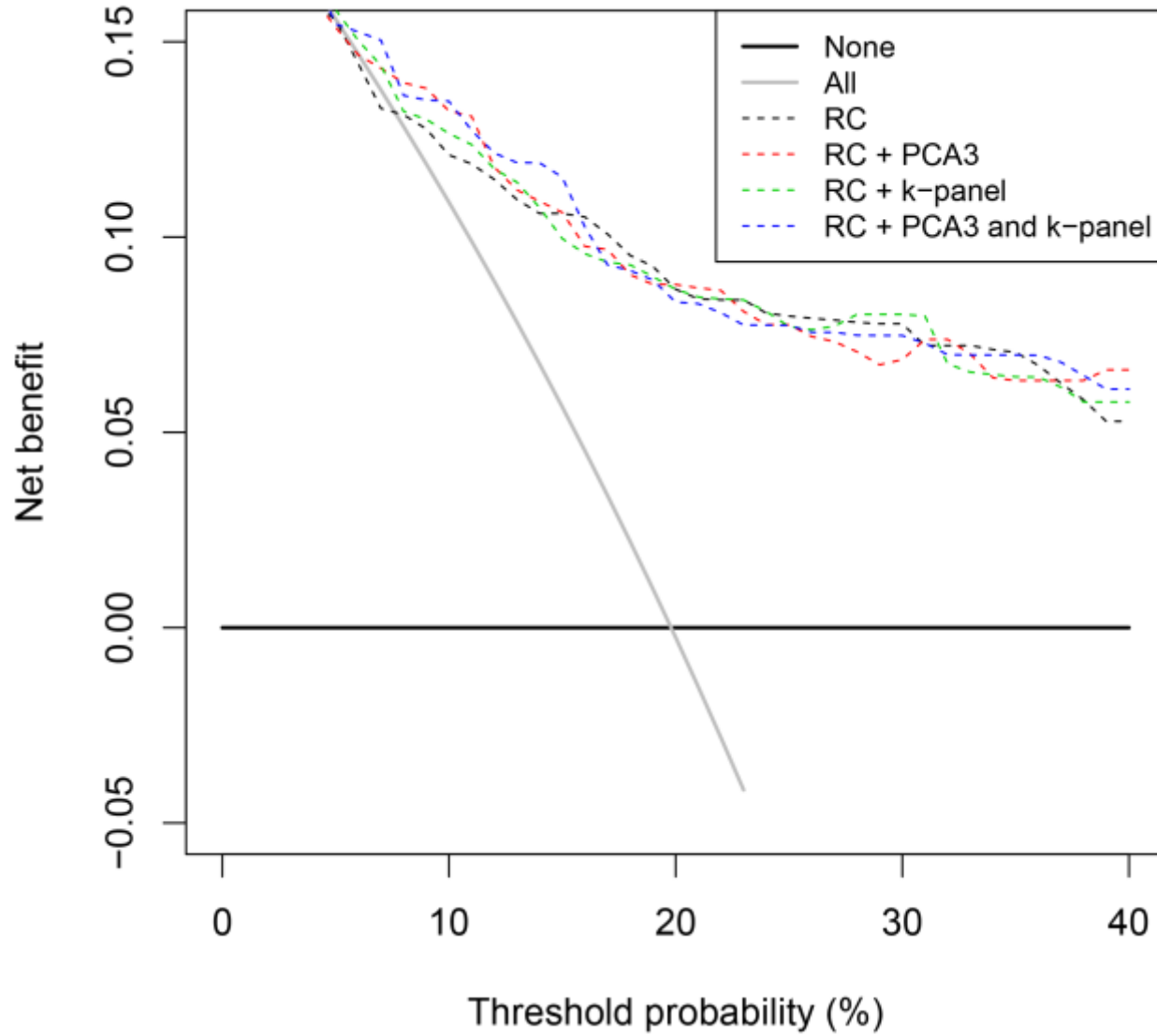
404 ¹ A model including total PSA (ng/ml), DRE (normal/abnormal), assessed DRE volume of the prostate (<30 cc, 30-50 cc, and ≥50 cc)

405 ² A model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), and TRUS assessed prostate volume (ml)

406 ³ Area under the receiver operator curve

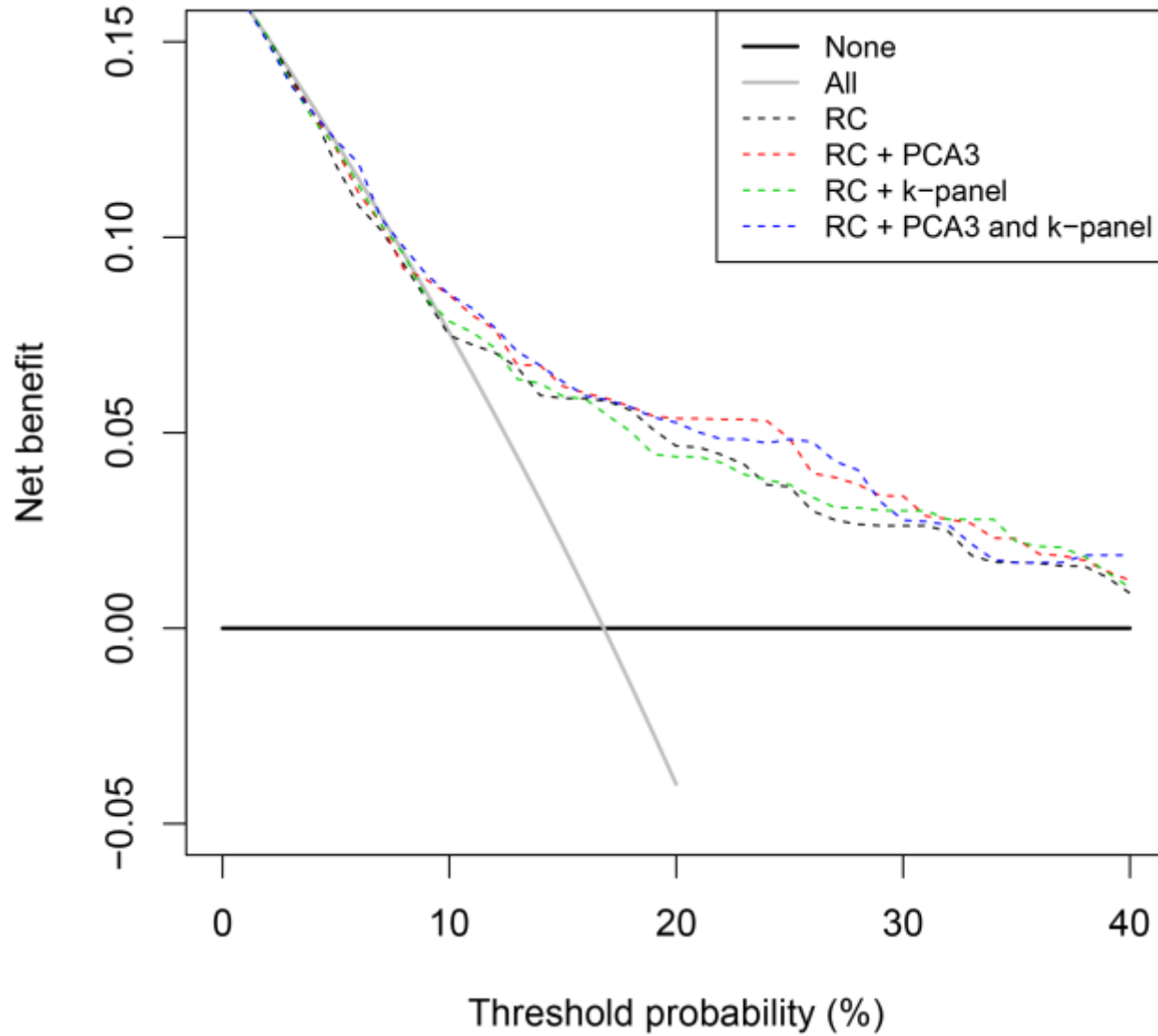
407 ⁴ The reference value for the univariate analysis is total PSA (ng/ml) and DRE (normal/abnormal), for the multivariate analyses it is the original risk calculator

408 Figure 1. Net benefit of prediction models with PCA3 and/or the 4k-panel in the subgroup of men with PSA ≥ 3.0 ng/ml (N=202)



409

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



411

412 APPENDIX

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

414

	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)

415 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)

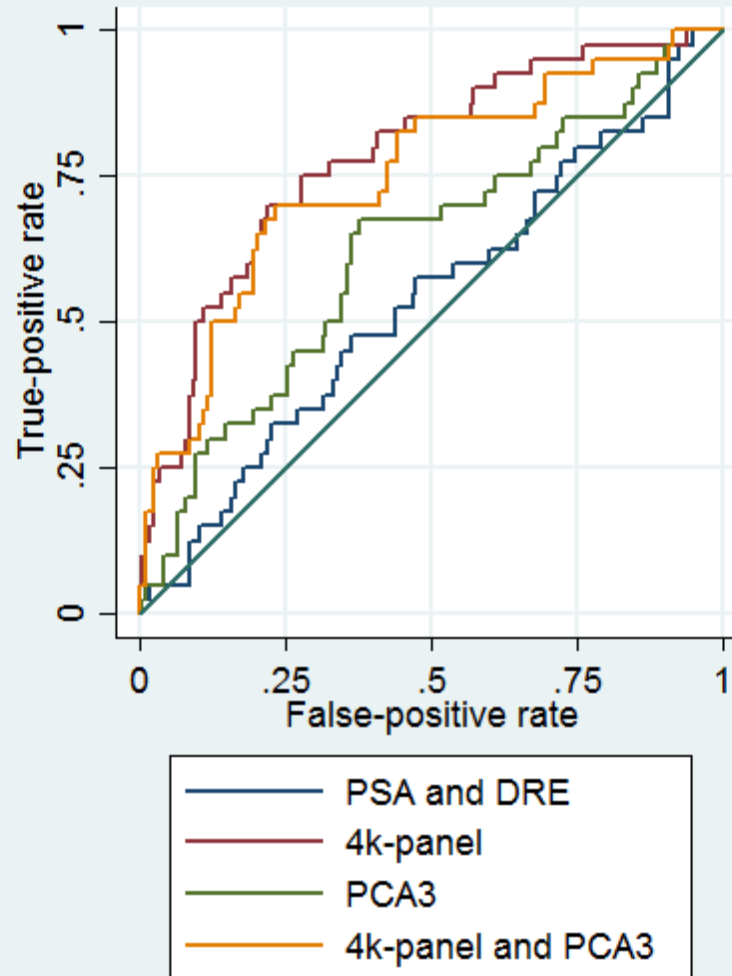
416 ² A model including total PSA (ng/ml), DRE (normal/abnormal), TRUS (normal/abnormal), and TRUS assessed prostate volume (ml)

417 ³ Area under the receiver operator curve

418 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

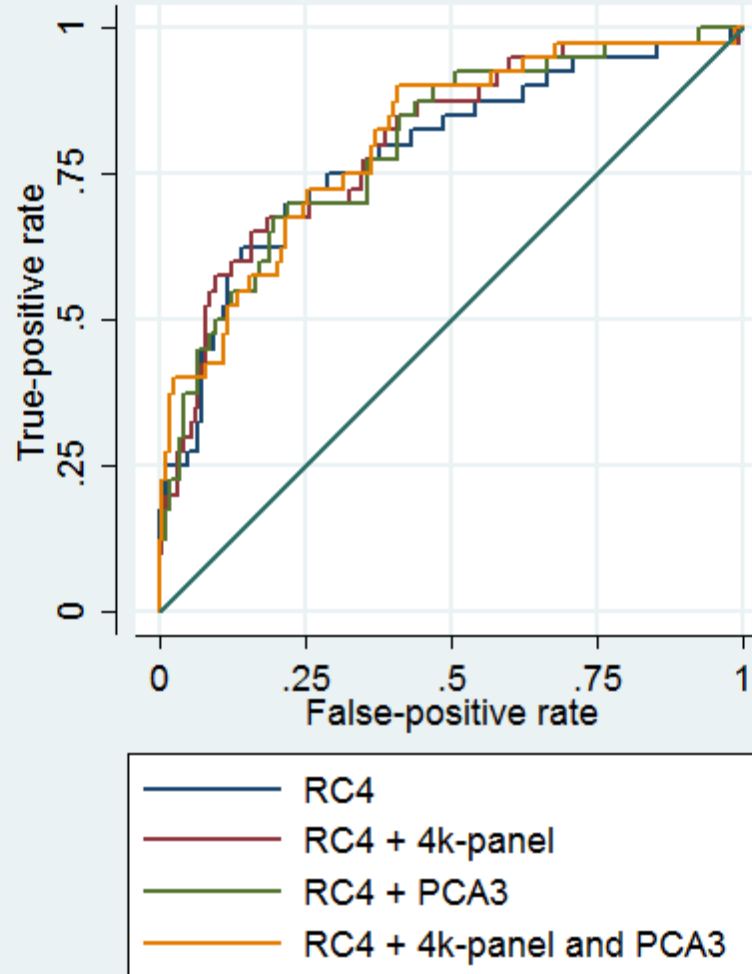
419

420 Figure A1. ROC curves for the subgroup of 202 men rescreened in the ERSPC trial with PSA ≥ 3.0 ng/ml (Table 2).
421 A. Univariate analysis, with PSA (ng/ml) and DRE (normal/abnormal) as a reference



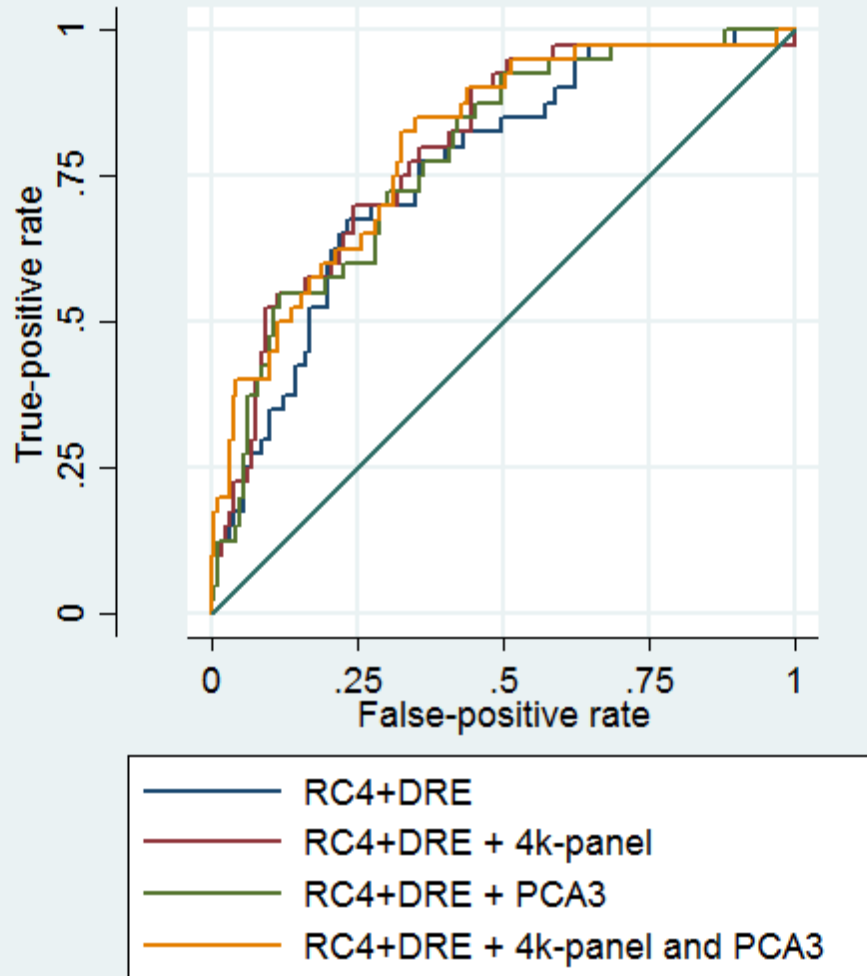
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424 B. Multivariate analysis, with risk calculator 4, a model including total PSA (ng/ml), DRE (normal/abnormal), TRUS
425 (normal/abnormal), and TRUS assessed prostate volume (ml), as a reference



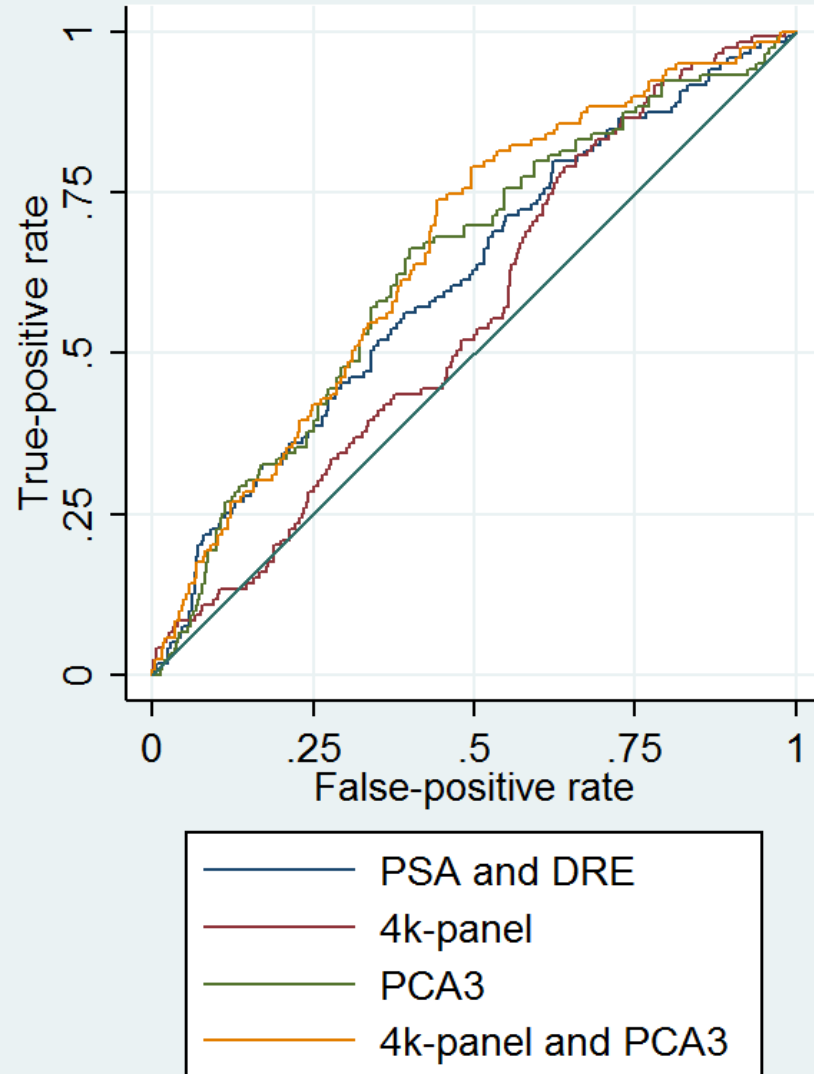
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428 C. Multivariate analysis, with risk calculator 4+DRE, a model including total PSA (ng/ml), DRE (normal/abnormal), assessed DRE
429 volume of the prostate (<30 cc, $30-50$ cc, and ≥ 50 cc), as a reference



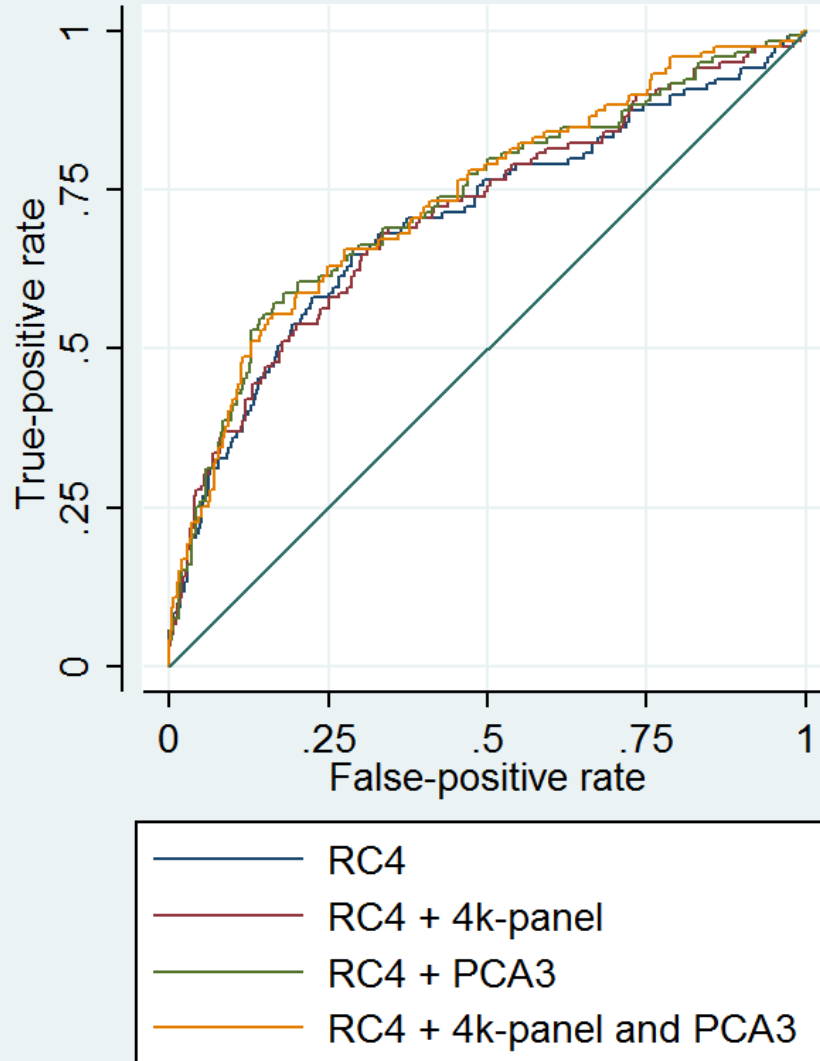
430

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



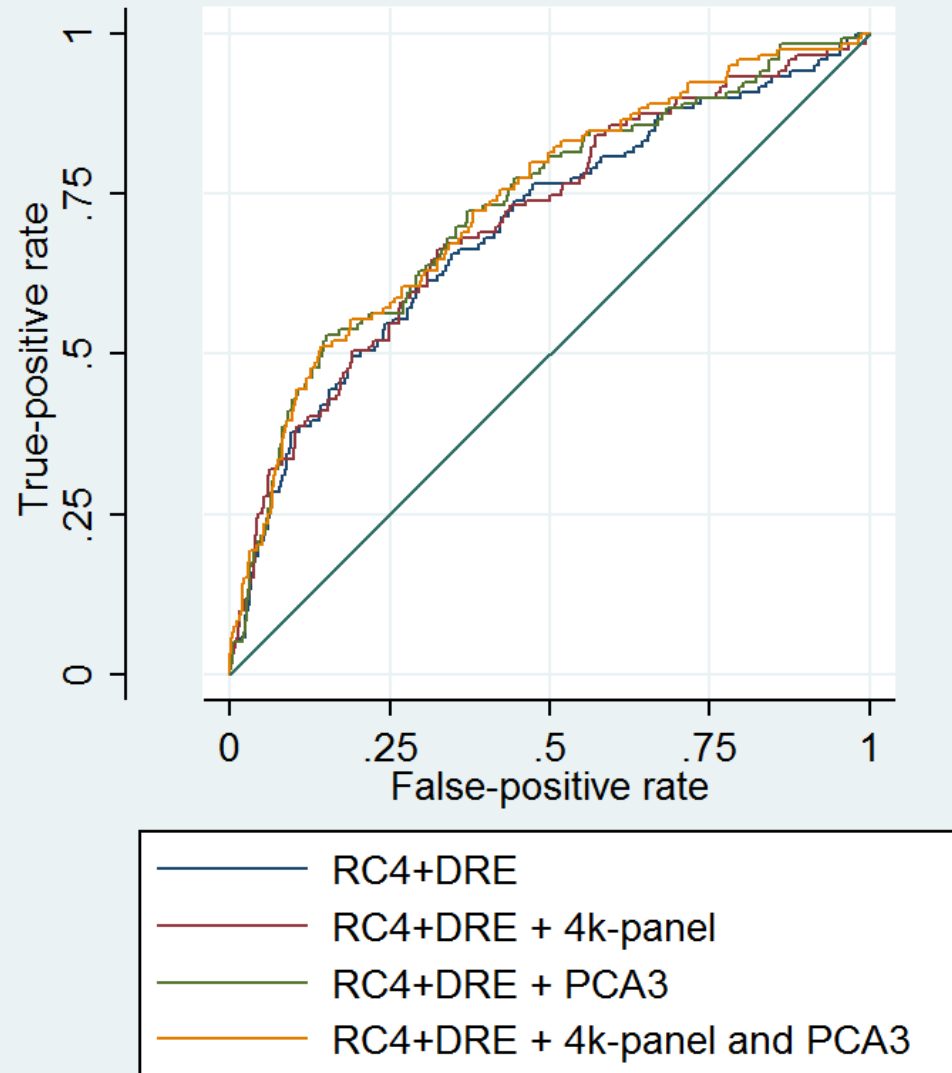
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434 B. Multivariate analysis, with risk calculator 4, a model including total PSA (ng/ml), DRE (normal/abnormal), TRUS
435 (normal/abnormal), and TRUS assessed prostate volume (ml), as a reference



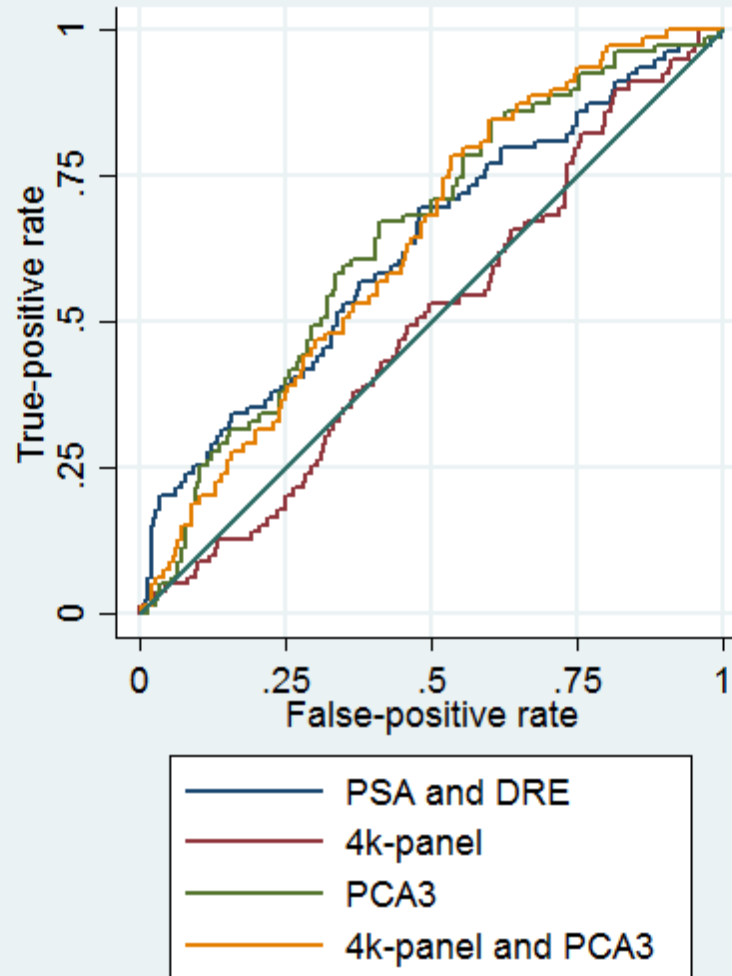
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437 C. Multivariate analysis, with risk calculator 4+DRE, a model including total PSA (ng/ml), DRE (normal/abnormal), assessed DRE
438 volume of the prostate (<30 cc, 30-50 cc, and ≥ 50 cc), as a reference



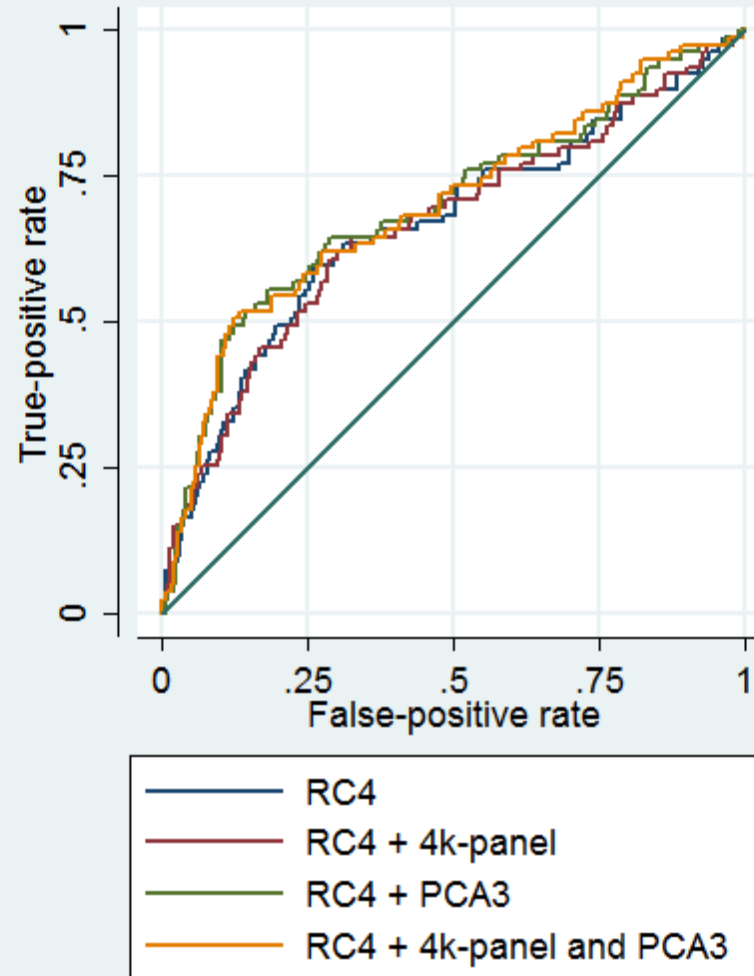
439

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



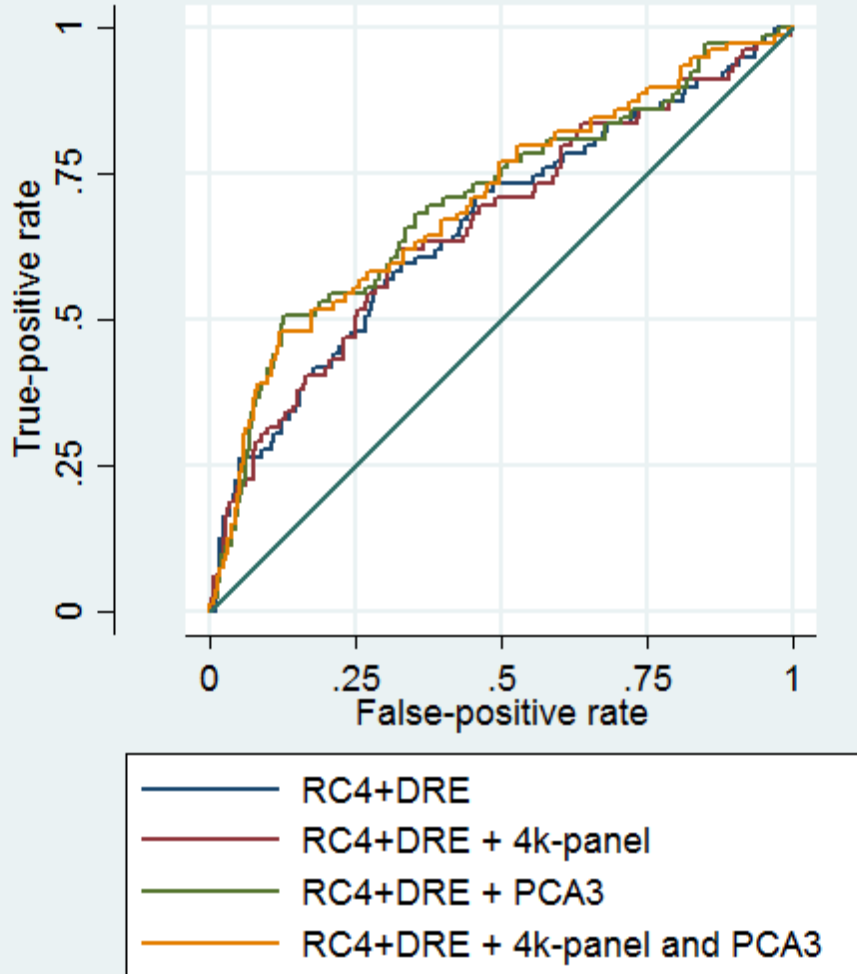
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444 B. Multivariate analysis, with risk calculator 4, a model including total PSA (ng/ml), DRE (normal/abnormal), TRUS
445 (normal/abnormal), and TRUS assessed prostate volume (ml), as a reference



446
447

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



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