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ABSTRACT

Introduction: Risk Calculators (RCs) are easy-to-use tools considering available clinical-variables that could help to select those patients with risk of prostate-cancer (PCa) who should undergo a prostate-biopsy. **Objective:** To perform a comparison for the prediction of significant-PCa (SigPCa) between the European-Randomised Study of Screening for PCa (ERSPC) and the PCa Prevention-Trial (PCPT) RCs in patients with PSA between 3-10ng/ml through an evaluation of the accuracy/variability between two consecutive PSA-values. **Setting:** An observational study in a major university Hospital of the south of Spain. **Methods and participants:** An observational study was performed in patients who underwent a prostate-biopsy. SigPCa probabilities were calculated with the two PSA measures using ERSPC3/4+DRE and PCPTv2+free-PSA RCs. The prediction accuracy of SigPCa was determined by the area under the curve (AUC). Calibration, discrimination, and decision curve analysis were studied. The variability between both RCs-agreement was compared using Cohen's kappa coefficient. **Results:** 510 patients were analysed (87 diagnosed with SigPCa). The median PSA varied from 5.3 to 5ng/ml between both measures. Both RCs overestimated the risk in the case of high-risk probabilities, but ERSPC was better calibrated than PCPT for risks in the clinically useful moderate-risk range. Discrimination ability for SigPCa was similar between models with an AUC=0.73(0.68-0.79) for ERSPC-RC vs. 0.73(0.67-0.79) for PCPT-RC. ERSPC-RC showed less variability than PCPT-RC, with a constant agreement (k=0.7-0.8) for usual range of clinical decision-making. Remarkably, a higher biopsies number would be avoided using the ERSPC-RC, but more SigPCa would be missed. **Conclusions:** Both RCs had similar accuracy for the SigPCa discrimination. However, ERSPC-RC is better calibrated for clinically useful risks and more stable for intra-individual PSA variations.

Strengths and limitations of this study

- This study highlights the need to spread the use of available free tools to improve patient's selection for undergoing prostate biopsy.
- This study is the first to compare two available free risk calculators in patients with a PSA < 10ng/ml analyzing their variability between two consecutive different PSA levels.
- Although the clinical information of this study was extracted from a clinical practice cohort and with information that could be useful for urologists worldwide, this is a retrospective study and the use of TRUS biopsy for PCa diagnosis, even though is the standard in most populations, suffers from random error compared with template biopsy, which could have affected prediction results.

INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed malignancy in males worldwide, and the most frequent in developed countries(1). Its current standard of diagnosis is a prostate biopsy based on PSA levels and digital rectal examination (DRE). However, there are other available and complementary variables that could help to select those patients who should undergo a prostate biopsy (such as age, prostate volume, free PSA, family history, etc.), but these are not always used and/or well-integrated in daily clinical practice(2). In line with this, Risk Calculators (RCs) are easy-to-use tools that can help the clinicians to take advantage of all these available variables (3). The two main RCs are from the European Randomised Study for Screening of Prostate Cancer (ERSPC cohort; ERSPC-RC: http://www.prostatecancer-riskcalculator.com/seven-prostate-cancer-riskcalculators) and from the Prostate Cancer Prevention Trial (PCPT cohort; PCPT-RC: http://deb.uthscsa.edu/URORiskCalc/Pages/calcs.jsp). Both RCs have undergone some modifications, specifically the addition of estimated prostate volume in the ERSPC-RC (4,5). Furthermore, both RCs were originally developed from different patient cohorts and each RC uses different variables.

To date, limited external validations and comparisons have been performed by different groups (6–9). The two most important recent comparisons of the modified RCs were performed by Foley *et al* (6,7) and Poyet *et al* (9). Both found a better discriminatory ability for ERSPC-RC vs PCPTv.2-RC for the diagnoses of significant-PCa (Sig PCa) (AUC around 0.74 vs 0.69, respectively), but they also included patients with a high PSA of up to 50 ng/ml. Despite the possibility of using these RCs in patients with PSA levels up to 50ng/ml, it is clear that the advantages of using both RCs would probably increase in patients with a PSA under 10ng/ml. Furthermore, in the case of the PCPTv.2-RC, the addition of the free PSA value in patients with a PSA under 10ng/ml seems to improve its accuracy (5), and, therefore, given its accessibility, this value should be included in the RC.

The intra-individual and inter-assay variability of PSA is already known (10–12) and, therefore, at least two measures are necessary before a prostate biopsy is indicated. In fact, it has been shown

that approximately 25% of men with initial PSA levels between 4 and 10 ng/mL had normal PSA values upon repeat testing (13). In line with this, despite being primarily based on PSA level, the variability of the two RCs mentioned above has been poorly studied, and might have implications for patient management. Our group has recently evaluated this variability with the ERSPC-RC, which showed stable accuracy over a cohort of patients, but some changes with respect to an individual approach (14). To date, there is no study comparing the accuracy and variability of both RCs, the ERSPC + DRE vs. the PCPTv.2 + free PSA, for the prediction of Sig PCa. Therefore, the aim of this study was to perform a direct comparison between ERSPC + DRE and PCPTv.2 + free PSA RCs in patients with a PSA between 3-10ng/ml, evaluating the accuracy and variability of both methods in the prediction of Sig PCa.

MATERIALS AND METHODS

Study population and design

An observational retrospective study was performed in patients from ONCOVER cohort (1021 biopsies indicated by clinical practise wherein patients donated blood and urine before the biopsy). The study was carried out within the project approved by our Hospital Research Ethics Committee, and informed consent was obtained from all participants. Blood sample was obtained in the morning (between 8:00-10:00 am) after an overnight fasting and then, the prostate biopsy was implemented according to clinical practice. The inclusion criteria for this study were: 1) PSA indication between 3-10 ng/ml; 2) Full clinical and laboratory data to fulfilled ERSPC-RC and PCPT-RC; 3) Age 55-80 years' old; 4) Two consecutives measurements of PSA levels within an interval of 12 weeks. Exclusion criteria included patients with a previously known PCa diagnosis or treatment that could modify PSA levels.

Transrectal prostate biopsy was carried out under local anaesthesia by using a standard peri-prostatic block, a transrectal ultrasound transducer, and an 18G automated needle biopsy instrument. The prostatic volume was measured following the protocol used during transrectal ultrasound (TRUS), and usual recommendations were to take 12 cores in patients undergoing the first biopsy procedure, and a minimum of 16 biopsy cores for those who had a previous biopsy. Biopsy specimens were analysed by expert urologic pathologists according to the International Society of Urological Pathology (ISUP) 2005 modified criteria (15).

Main variables description

Demographics information and medical histories of each patient were obtained. PSA levels were measured twice within a period no longer than 12 weeks, as follows: 1) **PSA 1 and free PSA 1:** for biopsy indication; and, 2) **PSA 2 and free PSA 2:** before undergoing prostate biopsy.

Prostate volume: estimated by TRUS and categorized in three possible values, 25-40-60 ml, as recommended (4) (TRUS volume <30 = 25 mL, 30–50 = 40 mL, and ≥50 = 60 mL).

Significant/ high-grade (HG) prostate cancer (Sig PCa): PCa with a Gleason grade ≥ 7 on biopsy.

ERSPC-RC and PCPT-RC probabilities calculation

ERSPC: The formulas for the ERSPC-RC 3+DRE for patients at initial biopsy and the ERSPC-RC 4+DRE for patients at repeat biopsy were utilized in this study (4). These calculators use PSA, prostate volume, and DRE as variables, with, a negative prostate biopsy in ERSPC 4+ DRE in patients who had a previous biopsy. This provides a probability rating for any PCa or Sig PCa (Gleason ≥ 7).

ERSPC1/Sig PCa (1º Measure): Risk probability calculated by ERSPC-RC3 or 4 (if previous biopsy) ± DRE for any PCa using PSA 1/ Sig PCa – for HG PCa.

ERSPC2/Sig PCa (2º Measure): Risk probability calculated by ERSPC-RC 3 or 4 (if previous biopsy) ± DRE for any PCa using PSA 2/ Sig PCa – for HG PCa.

PCPT: The formulae for the PCPT-RC 2.0 + %free PSA was utilized in this study (5). This calculator use race, age, PSA level, %free PSA level, family history of PCa, DRE and prior prostate biopsy. This gives a probability of negative biopsy, low grade PCa and Sig PCa (gleason ≥ 7).

PCPT1: Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 1 and free PSA 1.

PCPT2: Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 2 and free PSA 2.

The variability of PSA was calculated by the following formula: **|1 Measure – 2 Measure| / 1 Measure**

Statistical analysis

A descriptive study was performed by calculating the median and interquartile ranges (IR) for the quantitative variables, and the absolute frequencies and percentages for the qualitative variables. A Student's T test for paired groups was used to compare the means of the quantitative variables (PSA 1 and 2).

The investigation of the comparative performance in the detection of Sig PCa of both RCs, ERSPC-RC and PCPT-RC, was performed, taking into account these five factors: discrimination capacity, calibration, clinical utility, and consistency against the observed variations in PSA levels for our dataset.

The discrimination ability of the models, i.e., their ability to separate those patients who had Sig PCa from those who do not, was assessed using the area under their Receiver Operator Characteristic (ROC) curve (AUC) (16), as measured in our sample. This is one of the most frequently used measurements of model discrimination, because of its independence of the selection of a specific decision threshold and its robustness against class imbalance. Confidence intervals for these AUCs were computed using bootstrapping. These AUCs were then compared to determine the relative performance of the models using DeLong tests (17). These tests were chosen because of their non-parametric nature, with few assumptions about the data, and their suitability for paired data, as both models were evaluated over the same dataset, properties which make this the most commonly used test to compare AUCs (18). For this comparison, we focused on the first measure of PSA (PSA 1; the value for the indication of the prostate biopsy).

The calibration of the calculators for our cohort was then investigated to determine the agreement between the frequency of the observed outcome (PCa in our case) and the risks predicted by the model. Calibration plots were used for this purpose (19), enabling a visual evaluation of this agreement and the comparison between RCs.

To address the potential clinical utility of the models, we performed decision curve analysis on our data, as proposed by Vickers and Elkin (20). This method has the advantage of not requiring the specification of the relative cost for false-positives and false-negatives, defining a net benefit as a function of the decision threshold at which one would consider obtaining a biopsy.

Finally, the stability of the predictions of both RCs, with regard to the observed intra-patient changes on PSA levels between measurements, was investigated using the Cohen's kappa (k) inter-rater agreement coefficient as a function of the decision threshold. This coefficient was selected due

to its widespread use and robustness against random agreements, and thus, is a better measurement than naïve accuracy.

All the analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill) and R version 3.2.3. A <5% level of significance ($p<0.05$) was used to decide statistically significant differences.

Patient and public involvement

Participants and public were not involved in the development of research questions, study design or recruitment.

RESULTS

Cohort characteristics

In the present study, we analysed 510 patients who met the inclusion criteria previously described. Median age was 65 (60-70) years old, with a family history in 89 patients (17.5%) and a suspicious DRE in 82 patients (16.1%). The median PSA before prostate biopsy indication was 5.3 (4.3-6.9) ng/ml. 176 patients were diagnosed with PCa, 87 of those categorized as Sig PCa. Most patients (n=401; 78.6%) were biopsy-naïve and the median prostate volume was 35 (26-49) cc. Further cohort description according to Sig PCa status is shown in Table 1.

66 patients had a PSA 2 out of the range of 3-10ng/ml due to the variability (50 patients below 3ng/ml and 16 patients above 10ng/ml); thus, in this case, the % free PSA was not calculated, and the risk probability was calculated without the inclusion of this variable. The patients were maintained in the analysis as reflecting the variability of the PSA.

Direct comparison for Sig PCa prediction

Discrimination ability for Sig PCa was no different between the two models [ERSPC1-RC vs. PCPT1-RC: 0.73; 95% CI: (0.68-0.79) vs. 0.73; 95% CI: (0.67-0.79), respectively]. ROC curves are shown in Figure 1. Similarly, no difference was found in the discrimination ability for any PCa. The comparison of the RC for both measures is described in Supplemental Figure 1. Supplemental Table 1 shows multiple comparisons by the DeLong test resulting in no differences between the two RCs for Sig PCa.

The calibration for Sig PCa was more consistent for the ERSPC-RC in the low-risk clinically useful region, compared to that of PCPT-RC (Figure 2). Both models tended to overestimate the risk for a high probability of Sig PCa, and slightly underestimate it for low risk patients, suggesting that the models would benefit from a recalibration for our population, particularly the ERSPC-RC, due to its most apparent parallelism in this region. None of the model predicted very high probabilities for most patients.

Calibration of the different RCs for any PCa showed an overestimation of risk for high-risk probabilities. However, for moderate and low risks, which are the most relevant for clinical practice (since a high risk will lead to a biopsy despite the inaccuracy), ERSPC again showed a more consistent calibration than PCPT. The calibration curves for any PCa are shown in Supplemental Figure 2.

The decision curve analyses revealed that both RCs provided a clinical net benefit in the threshold probability range for Sig PCa (Figure 3). The net benefit was comparable between the two RCs for Sig PCa.

Variability and clinical significance

PSA and free PSA change was significantly different between the two measures, but with low clinical variations [average PSA1 5.69 ng/ml vs. PSA2 5.39 ng/ml ($p < 0.05$) and average free PSA1 16.99% vs. free PSA2 18.03% ($p < 0.05$)]. Median variability of PSA was 14% (6-27%). Taking into account this variability of PSA, ERSPC proved to be more stable than PCPT. The k agreement

between ERSPC1 and ERSPC2 was practically constant, 0.79±0.09 for the usual range of clinical decision (0-0.3). However, PCPT1 and PCPT2 showed wider variations, with a k agreement of approximately 0.55±0.32 in the same range, with a subsequent rapid decrease. The agreement between both models (ERSPC1 vs. PCPT1) proved to be worse for thresholds in this range, peaking 0.47 for a 17% risk, with an average 0.32±0.12 on the interval. The comparison between ERSPC2 and PCPT2 yielded similar results (Figure 4).

Direct comparison of sensibility and specificity of both RCs along the different clinical risk thresholds showed that PCPT-RC has higher sensitivity and lower specificity than ERPSC-RC for a given threshold along the clinically useful region (Figure 5). The balance point is reached at a different risk threshold for each RC. The performances of both RCs at this point are comparable, as shown in Figure 5. Considering the superposition of their respective ROC curves to a good approximation (Figure 1), this means that a transformation of decision thresholds can make both models perform similarly.

DISCUSSION

Currently, considerable research is being carried out to find new diagnostic markers for Sig PCa, in order to reduce the number of biopsies and the over-diagnosis of insignificant PCa (21). These markers are based on body fluids (blood, urine) or image explorations (22,23). Some are recommended by guidelines such as the 4k score test, PCA3, and/or the Prostate Health Index (PHI) in body fluids (only PCA3 and PHI have been approved by the FDA) (24–26), or multiparametric magnetic resonance imaging (mpMRI), with recent evidence of its advantages in biopsy-naïve patients (27). However, costs and availability minimize their implementation worldwide, and, therefore, it is clear that additional and readily available tools, such as RCs, should be implemented in daily clinical practice. The two most used RCs are ERSPC-RC and PCPT-RC, which have been modified and adapted (4,5). Few external validations have been conducted, with varying results (7,9,28). Usually, external validations of RCs show worse performance than the original validations (8), a fact that is corroborated by our study. Therefore, based on all this information, evaluations, validations, and incorporation of RCs are needed (3).

The present study explores and compares, for the first time, both, the PCPT v2 + free PSA and the ERSPC + DRE, not only for accuracy but also for variability and clinical relevance. Our group previously explored the accuracy and variability of the ERSPC + DRE RC (14) but, in this study, we have specifically focused only on patients in the grey zone (PSA 3-10ng/ml) and compared the ERSPC + DRE RC vs. the PCPT v2 + free PSA, an analysis that has not been previously performed. This comparison showed that both RCs had similar accuracy for the discrimination of Sig PCa. However, ERSPC-RC had better calibration and stability for intra-individual PSA variations. Our methodology in calculating the volume is an estimation from the results of the TRUS measure, similar to Poyet *et al*, and following the recommendations of Roobol *et al.* (4). We have focused only on those patients with PSA between 3-10 ng/ml who require additional diagnostic information. The PCPT-RC option with free PSA, which increases the accuracy of discrimination between Sig PCa and no PCa (5), was calculated, as it is an easy-to-use and readily available tool for these patients.

As defined in the methodology, the first measure (ERSPC1 and PCPT1) was the focus of the direct comparison, as this was the used for biopsy indication. The accuracy of both RCs was similar for Sig PCa, showing an accuracy similar to that of Poyet *et al*. (9) for ESRPC and a better accuracy for PCPT1 v2.0 when adding free PSA. As shown in Supplemental Figure 3, the addition of free PSA improved the accuracy of the PCPT-RC [0.65 (0.59-0.71) PCPT1 v.2.0-RC vs. 0.73 (0.67- 0.79) PCPT1 v.2.0 + free PSA –RC; p= 0.02]. Still, these results are far from ideal, and thus, additional data from imaging or fluid markers might be included to improve the accuracy of the RCs. In agreement with the accuracy results, the decision curve analysis was also similar between both RCs. In fact, both RCs showed a net benefit from an early risk threshold, which means that their implementation would improve patient selection.

Studying the variability of the RCs improves our knowledge about their stability, which could be translate into improved decision-making and selection of patients. Our PSA cohort showed a variability that was in the range of those shown in previous literature (11,12,29). Our group and others (14,30) have demonstrated that a higher PSA variability is associated with a reduced risk of Sig PCa in a prostate biopsy, but it does not improve the accuracy of a RC. However, probability stability is important in order to trust RC probabilities at any point. Our study shows good agreement between the two ERSPC + DRE-RC probabilities, with good calibration and stability despite intra-individual PSA variations. PCPTv.2 + free PSA shows worse stability and higher variability, which could be explained simply by the fact that it uses two values (PSA and free PSA) that suffer from this variability (31), while the use of an estimated volume in the ERSPC dilutes the PSA variability. These results should be interpreted with caution, as volume estimation was performed by categorization of TRUS and not by DRE. It is true that this categorization has previously shown good correlation (4). This likely depends on prostate volume (32), as well as low but certain inter-examiner variability (33), which could also increase ERPSC variability in an inter-clinician comparison.

Calibration plots show that both models (PCPT-RC and ERSPC-RC) predict adequately only the actual risk of PCa and Sig PCa for low-risk patients, with a wider useful range in the case of PCa and

a lower range in the case of Sig PCa. For higher risk patients, the calibration curves become irregular. This effect is accentuated for risks close to 1, as both models predict maximum risks of around 0.75 for Sig PCa. Nonetheless, in the usual range for clinical decision (0-0.3), the calibration of both models is acceptable. It was observed that ERSPC-RC outperformed PCPT-RC in two aspects. First, its parallelism was better in the low-risk region, both for PCa and Sig PCa, and showed lower irregularity for higher risks. Conversely, the ERSPC curves were visually more stable with the use of different PSA measurements. This observation was reinforced by the results obtained from the study of Cohen's κ coefficient. ERSPC models obtained from PSA1 and PSA2 for Sig PCa showed a better agreement, which was also more stable through a wider interval of decision thresholds, as compared to the agreement between PCPT models obtained from PSA1 and PSA2. These models showed a smaller and rapidly decreasing agreement as the decision threshold was increased. Moreover, the comparison of coefficients between PCPT1 and PCPT2 and between PCPT2 and ERSPC2 showed that the differences between PCPT1 and PCPT2 were similar to those between ERSPC and PCPT models. Both observations suggest that ERSPC is more insensitive and, therefore, robust to intra-individual variations of PSA than PCPT, while the predictive performance is similar.

Despite the similar decision curve, results from the sensitivity, specificity and ROC curve analysis show that the same risk threshold should not be used for both models. Both RCs are able to have similar performance, and the benefit of using any of them is similar in order to screen patients for a prostate biopsy, if the correct cut-off point is selected.

In clinical practise, the use of these RCs should be the first step in guiding the decision for further management of the patient. Patients with a confirmed, elevated PSA between 3-10ng/ml should be better stratified using other variables within a RC, as men with PSA levels >10ng/mL are likely to proceed to biopsy regardless of other factors. Probably a specific cut-off point in the risk probability should not be used and take advantage of the known probabilities to discuss with the patient the biopsy indication as recommended by the PCPT-RC. In the situation in which the patient is in the low-risk group, according to both RCs (ERSPC and PCPT), the patient could continue with just

follow-up. This fact has also been proposed by Alberts et al. (34) when applying new diagnostic markers, such as mpMRI. Specifically, they showed that following a negative recommendation from the ERSPC-RC would have avoided 62 (51%) of 122 mpMRIs and two (25%) of eight insignificant PCa diagnoses, missing three (10%) of 31 high-grade PCa. As the positive predictive value of these RCs is not as good as their negative predictive value, in case of discordance between both RCs or if there is an indication for a biopsy according to both RCs, other images or fluid biomarkers could increase the accuracy in order to potentially reduce the harm from unnecessary prostate biopsy and over-diagnosis (35). Specifically, Loeb *et al.* (36) has recently demonstrated that the incorporation of PHI into both RCs increases the accuracy of the diagnoses of Sig PCa.

The present study has some limitations. First, despite the prospectively collected information, it is a retrospective study design. Second, prostate volume was an estimation and categorization from a TRUS calculation, and, therefore, it is not the actual approach for which the RC was developed. Third, the PSA values interval was not the same for all patients, which means the results should be interpreted with caution. Four, the use of TRUS biopsy for PCa diagnosis, although is the standard in most populations, suffers from random error compared with template biopsy (37), which could have affected prediction results. However, the clinical information was extracted from a clinical practice cohort and with information that could be useful for urologists worldwide.

Altogether, our results showed that: 1) the use of both RCs (ERSPC and PCPT) could improve the selection of patients who need prostate biopsy, and that both RCs showed similar accuracy for discrimination of Sig PCa; 2) ERSPC-RC had better calibration and stability than PCPT-RC for intra-individual PSA variations; 3) when comparing both RCs sensitivity and specificity, a higher rate of biopsies could be avoided with the ERSPC-RC vs. the PCPT-RC, but with a higher rate of Sig PCa missed. Thus, in those patients with a PSA between 3-10 ng/ml, these tools should be used in order to improve selection and specificity. The RCs specifically should be selected according to the variables available in the clinic. In addition, both RCs could also be used and the decision to undergo a biopsy be shared with the patient.

CONFLICTS OF INTEREST

Nothing to declare

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin [Internet]. 2018/01/04. 2018;68(1):7–30. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29313949
2. Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. Eur Urol [Internet]. 2016/08/25. 2017;71(4):618–29. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27568654
3. Louie KS, Seigneurin A, Cathcart P, Sasieni P. Do prostate cancer risk models improve the predictive accuracy of PSA screening? A meta-analysis. Ann Oncol [Internet]. 2015;26(5):848–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25403590
4. 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 [Internet]. 2012;61(3):577–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22104592
5. Ankerst DP, Hoefler J, Bock S, Goodman PJ, Vickers A, Hernandez J, et al. Prostate Cancer Prevention Trial risk calculator 2.0 for the prediction of low- vs high-grade prostate cancer. Urology [Internet]. 2014;83(6):1362–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24862395
6. Lundon DJ, Kelly BD, Foley R, Loeb S, Fitzpatrick JM, Watson RW, et al. Prostate cancer risk assessment tools in an unscreened population. World J Urol [Internet]. 2015;33(6):827–32. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25091862
7. Foley RW, Maweni RM, Gorman L, Murphy K, Lundon DJ, Durkan G, et al. The ERSPC Risk Calculators Significantly Outperform The PCPT 2.0 In The Prediction Of Prostate Cancer; A Multi-Institutional Study. BJU Int [Internet]. 2016; Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26833820

8. Foley RW, Lundon DJ, Murphy K, Murphy TB, Galvin DJ, Watson RW. Predicting prostate cancer: analysing the clinical efficacy of prostate cancer risk calculators in a referral population. Ir J Med Sci [Internet]. 2015;184(3):701–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25843017

9. Poyet C, Nieboer D, Bhindi B, Kulkarni GS, Wiederkehr C, Wettstein MS, et al. Prostate cancer risk prediction using the novel versions of the European Randomised Study for Screening of Prostate Cancer (ERSPC) and Prostate Cancer Prevention Trial (PCPT) risk calculators: independent validation and comparison in a contemporary Europe. BJU Int [Internet]. 2016;117(3):401–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26332503

10. Murthy V, Rishi A, Gupta S, Kannan S, Mahantshetty U, Tongaonkar H, et al. Clinical impact of prostate specific antigen (PSA) inter-assay variability on management of prostate cancer. Clin Biochem [Internet]. 2015/10/23. 2016;49(1–2):79–84. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26506115

11. Komatsu K, Wehner N, Prestigiacomo AF, Chen Z, Stamey TA. Physiologic (intraindividual) variation of serum prostate-specific antigen in 814 men from a screening population. Urology [Internet]. 1996;47(3):343–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8633399

12. Morote J, Raventós CX, Lorente JA, Enbabo G, López M, de Torres I. Intraindividual variations of total and percent free serum prostatic-specific antigen levels in patients with normal digital rectal examination. Eur Urol [Internet]. 1999;36(2):111–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10420031

13. Lavallée LT, Binette A, Witiuk K, Cnossen S, Mallick R, Fergusson DA, et al. Reducing the Harm of Prostate Cancer Screening: Repeated Prostate-Specific Antigen Testing. Mayo Clin Proc [Internet]. 2015/12/10. 2016;91(1):17–22. Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26688045

14. Gómez-Gómez E, Carrasco-Valiente J, Blanca-Pedregosa A, Barco-Sánchez B, Fernandez-Rueda JL, Molina-Abril H, et al. European Randomized Study of Screening for Prostate Cancer Risk Calculator: External Validation, Variability, and Clinical Significance. Urology. 2017;102.

15. Epstein JI, Allsbrook WC, Amin MB, Egevad LL, Committee IG. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol [Internet]. 2005;29(9):1228–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16096414

16. Fawcett T. An introduction to ROC analysis. Pattern Recognit Lett [Internet]. 2006 Jun 1 [cited 2018 Dec 20];27(8):861–74. Available from: https://www.sciencedirect.com/science/article/pii/S016786550500303X

17. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics [Internet]. 1988 Sep [cited 2018 Dec 20];44(3):837–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3203132

18. Zhou X-H, McClish DK, Obuchowski NA, Electronic Book Collection., Wiley InterScience (Online service). Statistical methods in diagnostic medicine [Internet]. Wiley; 2011 [cited 2018 Dec 20]. 545 p. Available from: https://www.wiley.com/en-us/Statistical+Methods+in+Diagnostic+Medicine%2C+2nd+Edition-p-9780470183144

19. Hendriksen JMT, Geersing GJ, Moons KGM, de Groot JAH. Diagnostic and prognostic prediction models. J Thromb Haemost [Internet]. 2013 Jun [cited 2018 Dec 20];11:129–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23809117

20. Vickers AJ, Elkin EB. Decision Curve Analysis: A Novel Method for Evaluating Prediction Models. Med Decis Mak [Internet]. 2006 Nov 5 [cited 2018 Dec 20];26(6):565–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17099194

21. Loeb S, Bjurlin MA, Nicholson J, Tammela TL, Penson DF, Carter HB, et al. Overdiagnosis and overtreatment of prostate cancer. Eur Urol [Internet]. 2014;65(6):1046–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24439788

22. Loeb S, Lilja H, Vickers A. Beyond prostate-specific antigen: utilizing novel strategies to screen men for prostate cancer. Curr Opin Urol [Internet]. 2016;26(5):459–65. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27262138

23. Johnston E, Pye H, Bonet-Carne E, Panagiotaki E, Patel D, Galazi M, et al. INNOVATE: A prospective cohort study combining serum and urinary biomarkers with novel diffusion-weighted magnetic resonance imaging for the prediction and characterization of prostate cancer. BMC Cancer [Internet]. 2016;16(1):1–11. Available from: http://dx.doi.org/10.1186/s12885-016-2856-2

24. McDonald ML, Parsons JK. 4-Kallikrein Test and Kallikrein Markers in Prostate Cancer Screening. Urol Clin North Am [Internet]. 2016;43(1):39–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26614027

25. Lepor A, Catalona WJ, Loeb S. The Prostate Health Index: Its Utility in Prostate Cancer Detection. Urol Clin North Am [Internet]. 2016;43(1):1–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26614024

26. De Luca S, Passera R, Cappia S, Bollito E, Randone DF, Milillo A, et al. Fluctuation in prostate cancer gene 3 (PCA3) score in men undergoing first or repeat prostate biopsies. BJU Int [Internet]. 2014/04/29. 2014;114(6b):E56-61. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24472071

27. Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. Lancet [Internet]. 2017/01/20. 2017;389(10071):815–22. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28110982

28. Grill S, Fallah M, Leach RJ, Thompson IM, Freedland S, Hemminki K, et al. Incorporation

of detailed family history from the Swedish Family Cancer Database into the PCPT risk calculator. J Urol [Internet]. 2014/09/19. 2015;193(2):460–5. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25242395

29. Sölétormos G, Semjonow A, Sibley PE, Lamerz R, Petersen PH, Albrecht W, et al. Biological variation of total prostate-specific antigen: a survey of published estimates and consequences for clinical practice. Clin Chem [Internet]. 2005;51(8):1342–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15961552

30. Nordström T, Adolfsson J, Grönberg H, Eklund M. Repeat Prostate-Specific Antigen Tests Before Prostate Biopsy Decisions. J Natl Cancer Inst [Internet]. 2016;108(12). Available from: https://www.ncbi.nlm.nih.gov/pubmed/27418620

31. Ankerst DP, Gelfond J, Goros M, Herrera J, Strobl A, Thompson IM, et al. Serial Percent Free Prostate Specific Antigen in Combination with Prostate Specific Antigen for Population Based Early Detection of Prostate Cancer. J Urol [Internet]. 2016/03/12. 2016;196(2):355–60. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26979652

32. Roehrborn CG, Girman CJ, Rhodes T, Hanson KA, Collins GN, Sech SM, et al. Correlation between prostate size estimated by digital rectal examination and measured by transrectal ultrasound. Urology [Internet]. 1997;49(4):548–57. Available from: https://www.ncbi.nlm.nih.gov/pubmed/9111624

33. Roehrborn CG, Sech S, Montoya J, Rhodes T, Girman CJ. Interexaminer reliability and validity of a three-dimensional model to assess prostate volume by digital rectal examination. Urology [Internet]. 2001;57(6):1087–92. Available from: https://www.ncbi.nlm.nih.gov/pubmed/11377314

34. Alberts AR, Schoots IG, Bokhorst LP, van Leenders GJ, Bangma CH, Roobol MJ. Risk-based Patient Selection for Magnetic Resonance Imaging-targeted Prostate Biopsy after Negative Transrectal Ultrasound-guided Random Biopsy Avoids Unnecessary Magnetic Resonance Imaging Scans. Eur Urol [Internet]. 2016;69(6):1129–34. Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26651990

35. Chiu PK, Alberts AR, Venderbos LDF, Bangma CH, Roobol MJ. Additional benefit of using a risk-based selection for prostate biopsy: an analysis of biopsy complications in the Rotterdam section of the European Randomized Study of Screening for Prostate Cancer. BJU Int [Internet]. 2017/06/05. 2017;120(3):394–400. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28498624

36. Loeb S, Shin SS, Broyles DL, Wei JT, Sanda M, Klee G, et al. Prostate Health Index improves multivariable risk prediction of aggressive prostate cancer. BJU Int [Internet]. 2016/11/22. 2017;120(1):61–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27743489

37. Ahmed HU, Hu Y, Carter T, Arumainayagam N, Lecornet E, Freeman A, et al. Characterizing clinically significant prostate cancer using template prostate mapping biopsy. J Urol [Internet]. 2011/06/15. 2011;186(2):458–64. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21679984

Footnotes

- **Contributors** E.G.G, R.M.L, MJ.R.T and J.C.V carried out the conception and design of the study; E.G.G, JJ.S.B, J.C.V, AM.B, J.V.R, I.M.B.A, J.M.L, JM.J.V contributed to the data acquisition; E.G.G, J.C.V, J.L.F.R, RM.L and MJ.R.T carried out the analysis and interpretation of data; E.G.G, J.C.V and RM.L drafted the manuscript; JJ.S.B, JL.F.RM AM.B, J.V.R, J.M.L, JM.J.V, and MJ.R.T carried out a critical revision of the manuscript for important intellectual content; E.G.G, and J.L.F.R performed the statistical analysis; RM.L, MJ.R.T and J.C.V supervised the work.
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- **Competing interests** None declared.
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- **Patient consent for publication** Not required.

Figure legends

Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa.

Figure 2: Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are underestimated from the lower right, where they are overestimated. **A**, Calibration plots for ERSPC1-RC Sig PCa risk estimation. **B**, Calibration plots for PCPT1-RCSig PCa risk estimation.

Figure 3: Results of the decision curve analysis. **A**, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1–RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating all patients (dashed line) and treating none (thin line). **B**, Plot demonstrating net reduction of interventions per 100 patients using the ERSPC-RC (black line) and the PCPT-RC (grey line).

Figure 4: Graphics showing Cohen's k coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. **A**, Agreement between ERSPC1-RC and ERSPC2-RC for Sig PCa. **B**, Agreement between PCPT1-RC and PCPT2-RC for Sig PCa. **C**, Agreement between ERSPC1-RC and PCPT1-RC for Sig PCa.

Figure 5: Graphics showing sensitivities and specificities of both RCs along the clinically useful risk threshold. The ERSPC-RC (black line) and the PCPT-RC (grey line).

Table 1. Clinical and demographic characteristics of the cohort of patients categorized according to cancer status.

Variable	No Sig PCa n=423	Sig PCa n=87	All n=510
Age	64.0 (60.0-69.0)	68.0 (63.0-71.0)	65.0 (60.0-70.0)
Family History	81 (19.1)	8 (9.2)	89 (17.5)
Positive DRE	55 (13.0)	27 (31.0)	82 (16.1)
1 Serum PSA	5.3 (4.3-6.9)	5.8 (4.5-7.2)	5.3 (4.3-6.9)
1 free PSA %	16.2 (12.4-21.4)	12.5 (9-16.6)	15.9 (11.8-20.4)
2 Serum PSA	5.0 (3.7-6.6)	5.4 (4.1-6.7)	5.0 (3.8-6.6)
2 free PSA %	17.9 (13.9-23.4)	12.5 (9.1-16.3)	16.9 (12.8-22.1)
Prostate volume	38.0 (29.0-50.0)	26.0 (20.7-34.0)	35 (26-49)
First Biopsy	322 (76.1)	79 (90.8)	401 (78.6)
PCPT1 Sig PCa	0.08 (0.05-0.13)	0.16 (0.10-0.30)	0.09 (0.06-0.15)
ERSPC1 Sig PCa	0.05 (0.02-0.10)	0.12 (0.05-0.31)	0.05 (0.03-0.12)
PCPT2 Sig PCa	0.07 (0.04-0.11)	0.16 (0.08-0.27)	0.07 (0.05-0.13)
ERSPC2 Sig PCa	0.04 (0.02-0.08)	0.12 (0.05-0.30)	0.05 (0.02-0.11)
PCa	89 (21)	87 (100)	176 (34.5)

PCa= Prostate cancer; **Sig PCa**= significant PCa (Gleason ≥ 7 on biopsy); **No Sig PCa**= No cancer or non-significant PCa; **ERSPC1 / PCPT1 Sig PCa** = Probability of high grade PCa using the first measurement of serum PSA (at the time of biopsy indication by the urologist); **ERSPC2 / PCPT2 Sig PCa** = Probability high grade PCa using the second measurement of serum PSA (just before undergoing prostate biopsy). Median values (interquartile range) are expressed for quantitative variables, and absolute values (percentage) for qualitative variables.

546

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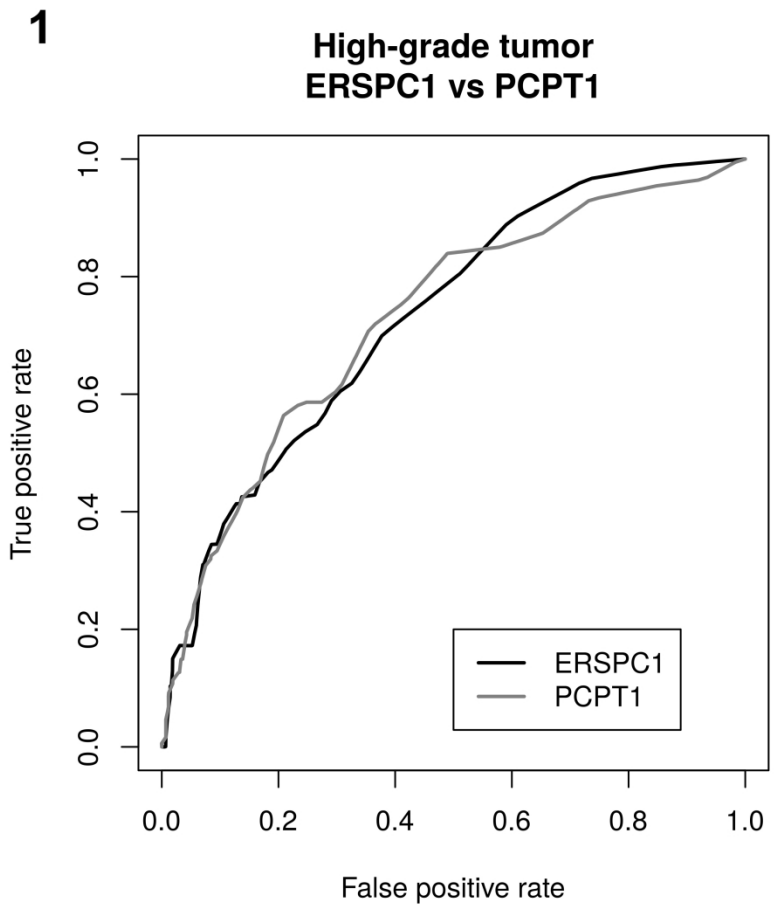
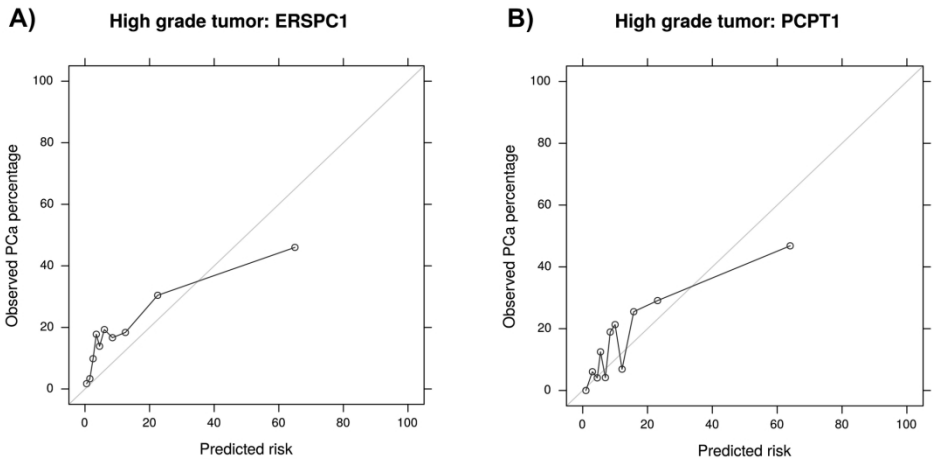


Risk Calculator for Sig PCa	AUC ($CI_{95\%}$)
ERSPC1	0.73 (0.68, 0.79)
PCPT1	0.73 (0.67, 0.79)

Receiver Operating Characteristic curves and Area Under the Curve values for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa.

2



Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are underestimated from the lower right, where they are overestimated. A, Calibration plots for ERSPC1-RC Sig PCa risk estimation. B, Calibration plots for PCPT1-RCSig PCa risk estimation.

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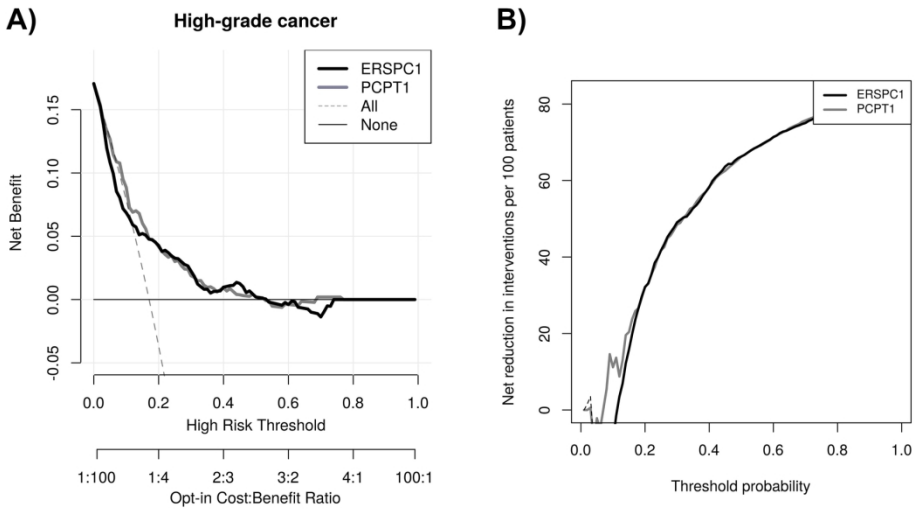

Results of the decision curve analysis. A, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1–RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating all patients (dashed line) and treating none (thin line). B, Plot demonstrating net reduction of interventions per 100 patients using the ERSPC-RC (black line) and the PCPT-RC (grey line).

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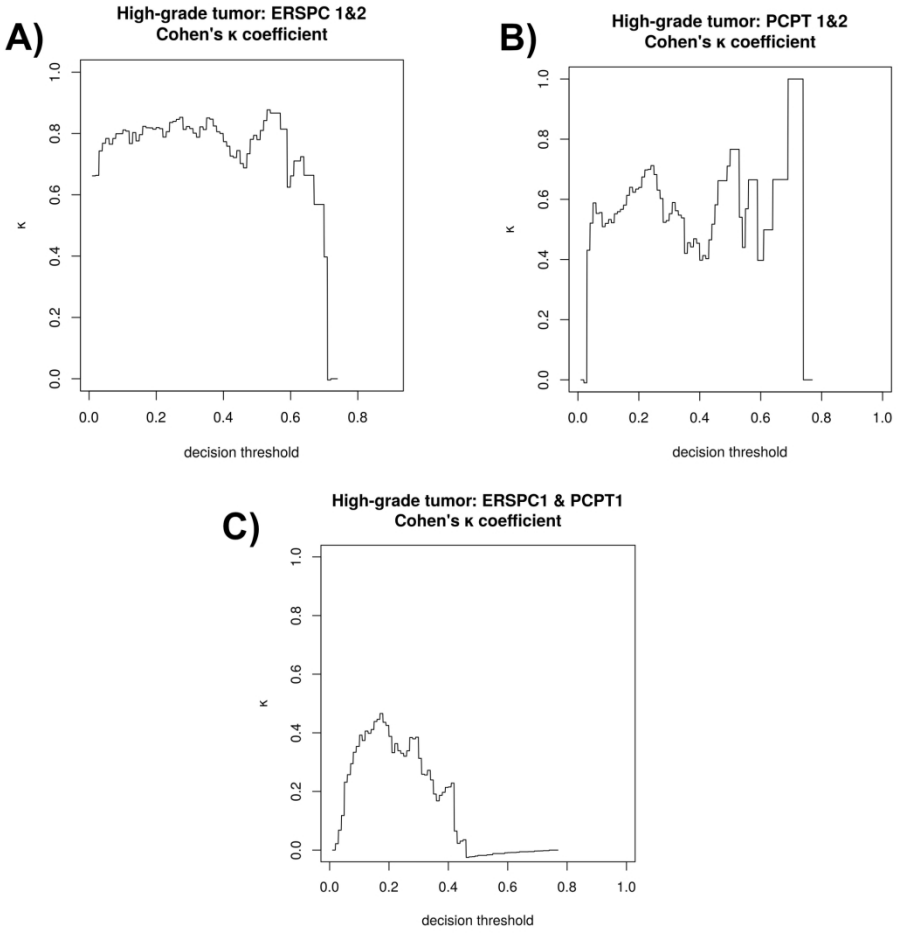
4



Graphics showing Cohen's k coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. A, Agreement between ERSPC1-RC and ERSPC2-RC for Sig PCa. B, Agreement between PCPT1-RC and PCPT2-RC for Sig PCa. C, Agreement between ERSPC1-RC and PCPT1-RC for Sig PCa.

190x190mm (300 x 300 DPI)

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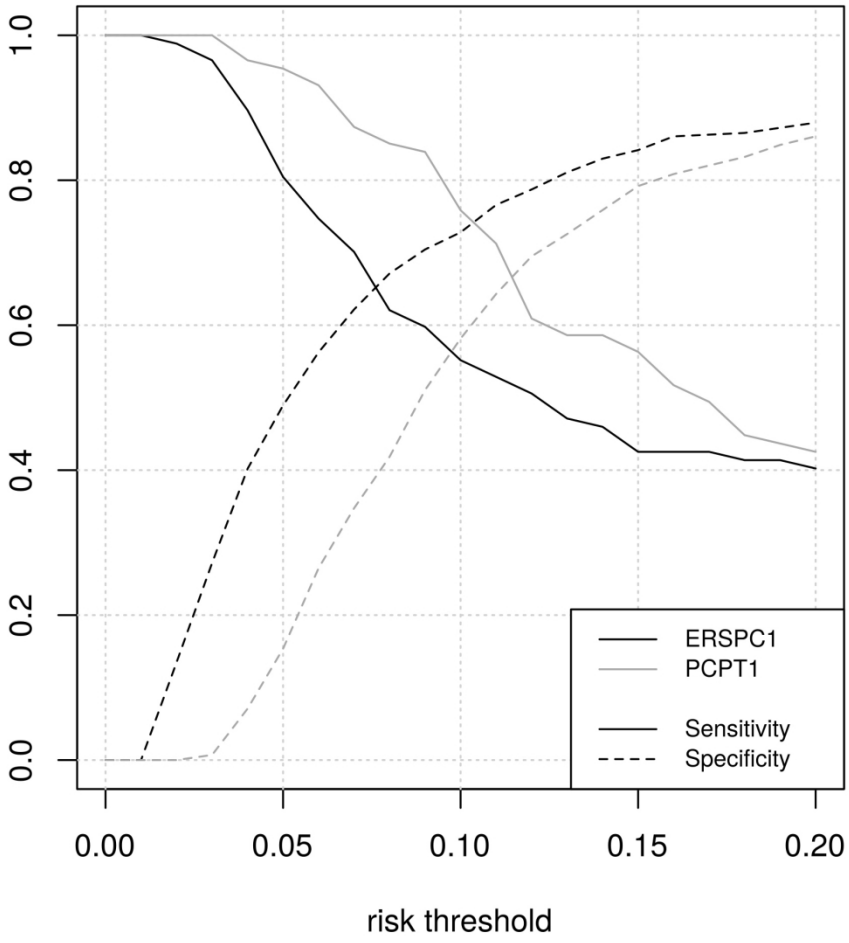
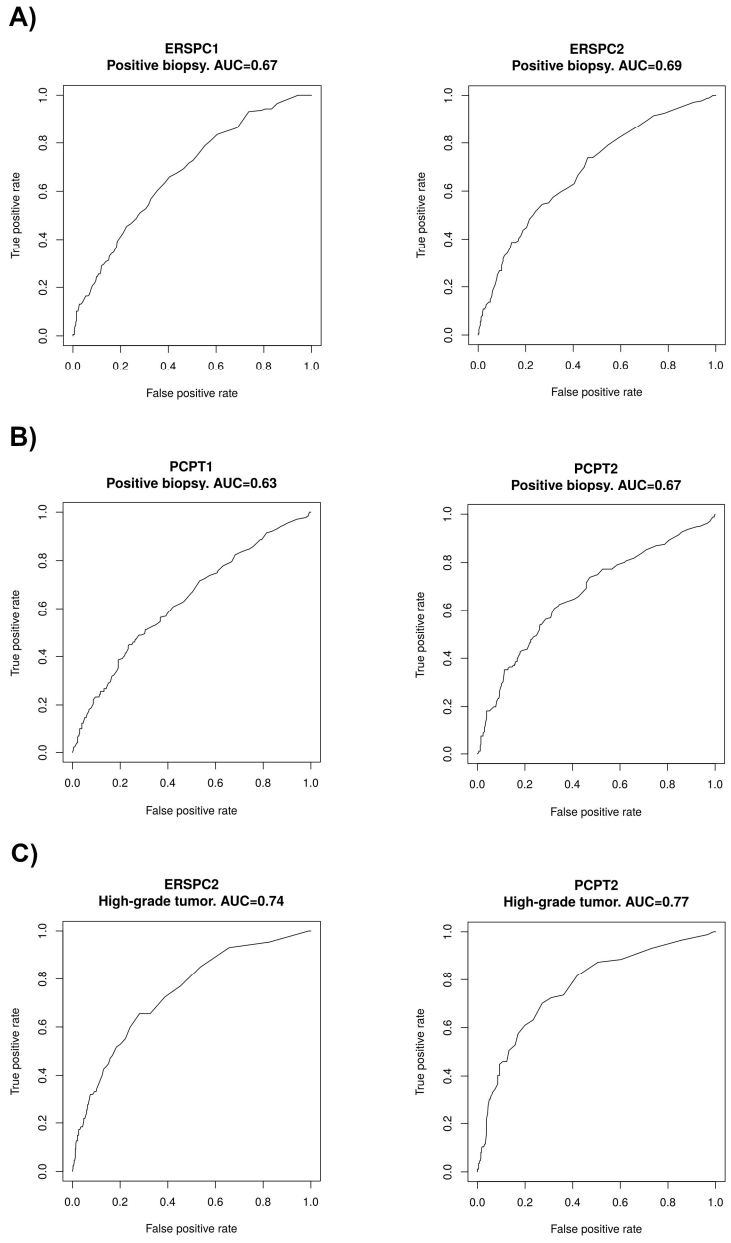


Graphics showing sensitivities and specificities of both RCs along the clinically useful risk threshold. The ERSPC-RC (black line) and the PCPT-RC (grey line).

Supplemental table 1: DeLong p values resulting from the pairwise comparison of the Area under the Receiver Operator Characteristic (ROC) curve (AUC) between Risk Calculators for significant Prostate cancer (Sig PCa) detection.

Sig PCa (p-value)	ERSPC1	ERSPC2	PCPT1	PCPT2
ERSPC1	X	0.51	0.95	0.19
ERSPC2	X	X	0.74	0.25
PCPT1	X	X	X	0.06

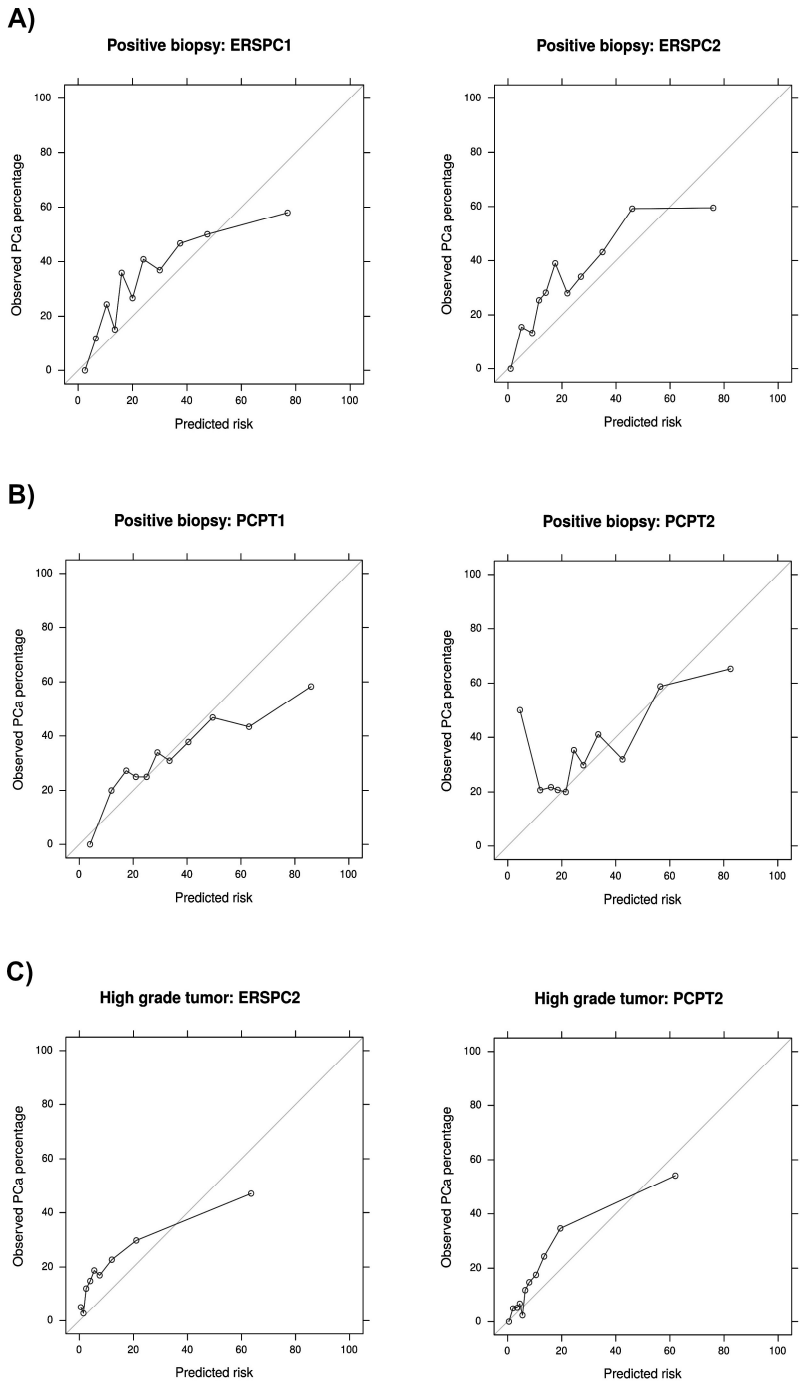
S1



AUC ($CI_{95\%}$)	PB	HG
ERSPC1	0.67 (0.63, 0.72)	0.73 (0.68, 0.79)
ERSPC2	0.69 (0.64, 0.73)	0.74 (0.68, 0.80)
PCPT1	0.63 (0.58, 0.68)	0.73 (0.67, 0.79)
PCPT2	0.67 (0.62, 0.72)	0.77 (0.71, 0.82)

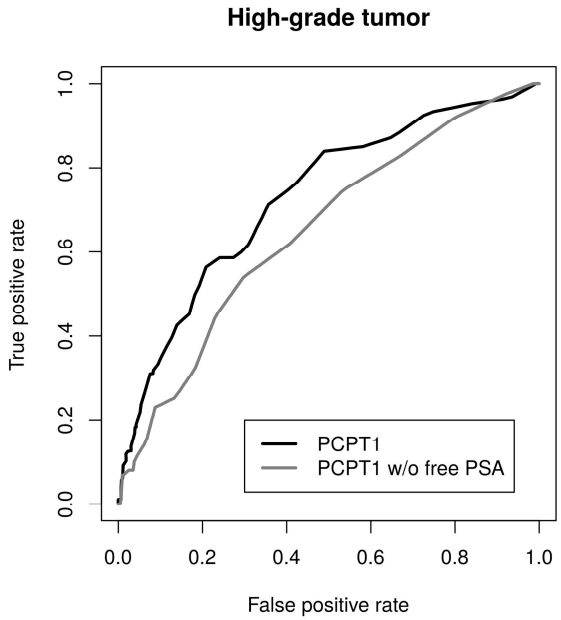
Supplemental Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values: **A**, for the ERSPC1-RC and the ERSPC2-RC for positive biopsy; **B**, for the PCPT1-RC and the PCPT2-RC for positive biopsy; and **C**, for the ERSPC2-RC and the PCPT2-RC for Sig PCa.

S2



Supplemental Figure 2: Calibration plots of the RCs in this cohort, demonstrating the agreement between predicted and observed probabilities: **A**, of a positive biopsy for the ERSPC1-RC and the ERSPC2-RC; **B**, of a positive biopsy for the PCPT1-RC and for the PCPT2-RC; and **C**, of a Sig PCa on the biopsy for the ERSPC2-RC and the PCPT2-RC.

S3



Risk Calculator for Sig PCa	AUC ($CI_{95\%}$)
PCPT1 without free PSA	0.65 (0.59, 0.71)
PCPT1 + free PSA	0.73 (0.67, 0.79)
DeLong p value: 0.02	

Supplemental Figure 3: Receiver Operating Characteristic curves and Area Under the Curve values for the PCPT1-RC without free PSA (black) and for the PCPT1-RC with free PSA (grey) to predict Sig PCa. P-value according to the DeLong test.

BMJ Open

Observational study comparing the accuracy/variability between the ERPSC and the PCPT risk calculators for the prediction of significant prostate cancer in patients with PSA <10ng/ml

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Primary Subject Heading:	Urology
Secondary Subject Heading:	Urology
Keywords:	Significant prostate cancer, risk calculator variability, ERSPC, PCPT

Observational study comparing the accuracy/variability between the ERPSC and the PCPT risk calculators for the prediction of significant prostate cancer in patients with PSA <10ng/ml

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Running title: Risk Calculators for Significant Prostate Cancer

Key Words: Significant prostate cancer; risk calculator variability; ERSPC; PCPT.

Tables and figures: 1 and 5

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ABSTRACT

Introduction: Risk Calculators (RCs) are easy-to-use tools considering available clinical-variables that could help to select those patients with risk of prostate-cancer (PCa) who should undergo a prostate-biopsy. **Objective:** To perform a comparison for the prediction of significant-PCa (SigPCa) between the European-Randomised Study of Screening for PCa (ERSPC) and the PCa Prevention-Trial (PCPT) RCs in patients with PSA between 3-10ng/ml through an evaluation of the accuracy/variability between two consecutive PSA-values. **Setting:** An observational study in a major university Hospital of the south of Spain. **Methods and participants:** An observational study was performed in patients who underwent a prostate-biopsy. SigPCa probabilities were calculated with the two PSA measures using ERSPC3/4+DRE and PCPTv2+free-PSA RCs. The discrimination ability of SigPCa was determined by the area under the curve (AUC). Calibration, discrimination, and decision curve analysis were studied. The variability between both RCs-agreement was compared using Cohen's kappa coefficient. **Results:** 510 patients were analysed (87 diagnosed with SigPCa). The median PSA values were 5.3 and 5ng/ml for PSA1 and PSA2 respectively. Both RCs overestimated the risk in the case of high-risk probabilities, but ERSPC was better calibrated than PCPT for risks in the clinically useful moderate-risk range. Discrimination ability for SigPCa was similar between models with an AUC=0.73(0.68-0.79) for ERSPC-RC vs. 0.73(0.67-0.79) for PCPT-RC. ERSPC-RC showed less variability than PCPT-RC, with a constant agreement (k=0.7-0.8) for usual range of clinical decision-making. Remarkably, a higher biopsies number would be avoided using the ERSPC-RC, but more SigPCa would be missed along all the risk probabilities. **Conclusions:** Both RCs had similar accuracy for the SigPCa discrimination. However, ERSPC-RC has more stable calibration for clinically useful risks and was more stable for intra-individual PSA variations.

Strengths and limitations of this study

- This study highlights the need to spread the use of available free tools which would be useful in patient's selection for undergoing prostate biopsy.
- This study is the first to compare two available free risk calculators in patients with a PSA < 10ng/ml analyzing their variability between two consecutive different PSA levels.
- Although the clinical information of this study was extracted from a clinical practice cohort and with information that could be useful for urologists worldwide, this is a retrospective study and the use of TRUS biopsy for PCa diagnosis, even though is the standard in most populations, suffers from random error compared with template biopsy, which could have affected prediction results.

INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed malignancy in males worldwide, and the most frequent in developed countries(1). Its current standard of diagnosis is a prostate biopsy based on PSA levels and digital rectal examination (DRE). However, there are other available and complementary variables that could help to select those patients who should undergo a prostate biopsy (such as age, prostate volume, free PSA, family history, etc.), but these are not always used and/or well-integrated in daily clinical practice(2). In line with this, Risk Calculators (RCs) are easy-to-use tools that can help the clinicians to take advantage of all these available variables (3). The two main RCs are from the European Randomised Study for Screening of Prostate Cancer (ERSPC cohort; ERSPC-RC: http://www.prostatecancer-riskcalculator.com/seven-prostate-cancer-riskcalculators) and from the Prostate Cancer Prevention Trial (PCPT cohort; PCPT-RC: http://deb.uthscsa.edu/URORiskCalc/Pages/calcs.jsp). Both RCs have undergone some modifications, specifically the addition of estimated prostate volume in the ERSPC-RC (4,5). Furthermore, both RCs were originally developed from different patient cohorts and each RC uses different variables.

To date, limited external validations and comparisons have been performed by different groups (6–9). The two most important recent comparisons of the modified RCs were performed by Foley *et al* (6,7) and Poyet *et al* (9). Both found a better discriminatory ability for ERSPC-RC vs PCPTv.2-RC for the diagnoses of significant-PCa (Sig PCa) (AUC around 0.74 vs 0.69, respectively), but they also included patients with a high PSA of up to 50 ng/ml. Despite the possibility of using these RCs in patients with PSA levels up to 50ng/ml, it is clear that the advantages of using both RCs would probably increase in patients with a PSA under 10ng/ml, where the rate of positive biopsy for PCa clearly decrease, with an important number of unnecessary biopsies. In line with this, EAU guidelines recommends to offer further assessment with other tools to these specific patients with a PSA 2-10 ng/ml(2). Furthermore, in the case of the PCPTv.2-RC, the addition of the free PSA value in patients

with a PSA under 10ng/ml seems to improve its accuracy (5), and, therefore, given its accessibility, this value should be included in the RC.

The intra-individual and inter-assay variability of PSA is already known (10–12) and, therefore, at least two measures are necessary before a prostate biopsy is indicated. In fact, it has been shown that approximately 25% of men with initial PSA levels between 4 and 10 ng/mL had normal PSA values upon repeat testing (13). In line with this, despite being primarily based on PSA level, the variability of the two RCs mentioned above has been poorly studied, and might have implications for patient management. Our group has recently evaluated this variability with the ERSPC-RC, which showed stable accuracy over a cohort of patients, but some changes with respect to an individual approach (14). To date, there is no study comparing the accuracy and variability of both RCs, the ERSPC + DRE vs. the PCPTv.2 + free PSA, for the prediction of Sig PCa. Therefore, the aim of this study was to perform a direct comparison between ERSPC + DRE and PCPTv.2 + free PSA RCs in patients with a PSA between 3-10ng/ml, evaluating the accuracy and variability of both methods in the prediction of Sig PCa.

MATERIALS AND METHODS

Study population and design

An observational retrospective study was performed in patients from ONCOVER cohort (1021 biopsies indicated by clinical practise wherein patients donated blood and urine before the biopsy). The study was carried out within the project approved by our Hospital Research Ethics Committee, and informed consent was obtained from all participants. Blood sample was obtained in the morning (between 8:00-10:00 am) after fasting overnight and then, the prostate biopsy was implemented according to clinical practice. The inclusion criteria for this study were: 1) PSA indication between 3-10 ng/ml; 2) Full clinical and laboratory data to fulfilled ERSPC-RC and PCPT-RC; 3) Age 55-80 years' old; 4) Two consecutives measurements of PSA levels within an interval of 12 weeks. Exclusion criteria included patients with a previously known PCa diagnosis or treatment that could modify PSA levels (Supplemental table 1).

Transrectal prostate biopsy was carried out under local anaesthesia by using a standard peri-prostatic block, a transrectal ultrasound transducer, and an 18G automated needle biopsy instrument. The prostatic volume was measured following the protocol used during transrectal ultrasound (TRUS), and usual recommendations were to take 12 cores in patients undergoing the first biopsy procedure, and a minimum of 16 biopsy cores for those who had a previous biopsy. Biopsy specimens were analysed by expert urologic pathologists according to the International Society of Urological Pathology (ISUP) 2005 modified criteria (15).

Main variables description

Demographics information and medical histories of each patient were obtained. PSA levels were measured twice within a period no longer than 12 weeks, as follows: 1) **PSA 1 and free PSA 1:** for biopsy indication; and, 2) **PSA 2 and free PSA 2:** before undergoing prostate biopsy. Both PSA measures were evaluated in the same laboratory by Chemiluminescent Microparticle Immunoassays

(ng/ml, by a CMIA; Ref. 7k70; Abbott). Median and interquartile range of both measure were 6 (3-8) weeks.

Prostate volume: estimated by TRUS and categorized in three possible values, 25-40-60 ml, as recommended (4) (TRUS volume <30 = 25 mL, 30–50 = 40 mL, and ≥50 = 60 mL).

Significant/ high-grade (HG) prostate cancer (Sig PCa): PCa with a Gleason grade ≥ 7 on biopsy.

ERSPC-RC and PCPT-RC probabilities calculation

ERSPC: The formulas for the ERSPC-RC 3+DRE for patients at initial biopsy and the ERSPC-RC 4+DRE for patients at repeat biopsy were utilized in this study. These calculators use PSA, prostate volume, and DRE as variables, with, a negative prostate biopsy in ERSPC 4+ DRE in patients who had a previous biopsy. This provides a probability rating for any PCa or Sig PCa (Gleason ≥ 7).

ERSPC1/Sig PCa (1º Measure): Risk probability calculated by ERSPC-RC3 or 4 (if previous biopsy) ± DRE for any PCa using PSA 1/ Sig PCa – for HG PCa.

ERSPC2/Sig PCa (2º Measure): Risk probability calculated by ERSPC-RC 3 or 4 (if previous biopsy) ± DRE for any PCa using PSA 2/ Sig PCa – for HG PCa.

PCPT: The formulae for the PCPT-RC 2.0 + %free PSA was utilized in this study. This calculator use race, age, PSA level, %free PSA level, family history of PCa, DRE and prior prostate biopsy. This gives a probability of negative biopsy, low grade PCa and Sig PCa (gleason ≥ 7).

PCPT1: Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 1 and free PSA 1.

PCPT2: Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 2 and free PSA 2.

The variability of PSA was calculated by the following formula: **|Measure 1–Measure2|/Measure 1**

Statistical analysis

A descriptive study was performed by calculating the median and interquartile ranges (IR) for the quantitative variables, and the absolute frequencies and percentages for the qualitative variables. A Student's T test for paired groups was used to compare the means of the quantitative variables (PSA 1 and 2).

The investigation of the comparative performance in the detection of Sig PCa of both RCs, ERSPC-RC and PCPT-RC, was performed, taking into account these four factors: discrimination capacity, calibration, clinical utility, and consistency against the observed variations in PSA levels for our dataset.

The discrimination ability of the models, i.e., their ability to separate those patients who had Sig PCa from those who do not, was assessed using the area under their Receiver Operator Characteristic (ROC) curve (AUC) (16), as measured in our sample. This is one of the most frequently used measurements of model discrimination, because of its independence of the selection of a specific decision threshold and its robustness against class imbalance. Confidence intervals for these AUCs were computed using bootstrapping. These AUCs were then compared to determine the relative performance of the models using DeLong tests (17). These tests were chosen because of their non-parametric nature, with few assumptions about the data, and their suitability for paired data, as both models were evaluated over the same dataset, properties which make this the most commonly used test to compare AUCs (18). For this comparison, we focused on the first measure of PSA (PSA 1; the value for the indication of the prostate biopsy).

The calibration of the calculators for our cohort was then investigated to determine the agreement between the frequency of the observed outcome (PCa in our case) and the risks predicted by the model. Calibration plots were used for this purpose (19), enabling a visual evaluation of this agreement and the comparison between RCs.

To address the potential clinical utility of the models, we performed decision curve analysis on our data, as proposed by Vickers and Elkin (20). This method has the advantage of not requiring the

specification of the relative cost for false-positives and false-negatives, defining a net benefit as a function of the decision threshold at which one would consider obtaining a biopsy.

Finally, the stability of the predictions of both RCs, with regard to the observed intra-patient changes on PSA levels between measurements, was investigated using the Cohen's kappa (k) inter-rater agreement coefficient as a function of the decision threshold. This coefficient was selected due to its widespread use and robustness against random agreements, and thus, is a better measurement than naïve accuracy.

All the analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill) and R version 3.2.3(R Foundation for Statistical Computing, Vienna, Austria: URL https://www.R-project.org/). A <5% level of significance ($p<0.05$) was used to decide statistically significant differences.

Patient and public involvement

Participants and public were not involved in the development of research questions, study design or recruitment.

RESULTS

Cohort characteristics

In the present study, we analysed 510 patients who met the inclusion criteria previously described. Median age was 65 (60-70) years old, with a family history in 89 patients (17.5%) and a suspicious DRE in 82 patients (16.1%). The median PSA before prostate biopsy indication was 5.3 (4.3-6.9) ng/ml. 176 patients were diagnosed with PCa, 87 of those categorized as Sig PCa. Most patients (n=401; 78.6%) were biopsy-naïve and the median prostate volume was 35 (26-49) cc. Further cohort description according to Sig PCa status is shown in Table 1.

66 patients had a PSA2 out of the range of 3-10ng/ml due to the variability (50 patients below 3ng/ml and 16 patients above 10ng/ml); thus, in this case, the % free PSA was not calculated, and the risk probability was calculated without the inclusion of this variable. These patients were maintained in the analysis as reflecting the variability of the PSA, and the application of the models in this real situation, despite it could introduce a bias in terms of calibration and variability.

Direct comparison for Sig PCa prediction

Discrimination ability for Sig PCa was no different between the two models [ERSPC1-RC vs. PCPT1-RC: 0.73; 95% CI: (0.68-0.79) vs. 0.73; 95% CI: (0.67-0.79), respectively]. ROC curves are shown in Figure 1A. Similarly, no difference was found in the discrimination ability for any PCa. The comparison of the RC for both measures is described in Figure 1(B-D) with similar results but a clear tendency of better accuracy for PCPT2-RC vs ERSPC2-RC (p= 0.06). Supplemental Table 2 shows multiple comparisons by the DeLong test resulting in no differences between the two RCs for Sig PCa.

The calibration for Sig PCa was more consistent for the ERSPC-RC in the low-risk clinically useful region, compared to that of PCPT-RC (Figure 2). Both models tended to overestimate the risk for a high probability of Sig PCa, and slightly underestimate it for low risk patients, suggesting that the models would benefit from a recalibration for our population, particularly the ERSPC-RC, due to its most apparent parallelism in this region. None of the model predicted very high probabilities for most patients.

Calibration of the different RCs for any PCa showed an overestimation of risk for high-risk probabilities. However, for moderate and low risks, which are the most relevant for clinical practice (since a high risk will lead to a biopsy despite the inaccuracy), ERSPC visually showed a more consistent and less fluctuating calibration in the useful range than PCPT. The calibration curves for any PCa are shown in Supplemental Figure 1.

The decision curve analyses revealed that both RCs provided a clinical net benefit in the threshold probability range for Sig PCa (Figure 3). The net benefit was comparable between the two RCs for Sig PCa.

As shown in Supplemental Figure 2, the addition of free PSA clearly improved the accuracy of the PCPT-RC [0.65 (0.59-0.71) PCPT1 v.2.0-RC vs. 0.73 (0.67- 0.79) PCPT1 v.2.0 + free PSA –RC; p= 0.02].

Variability and clinical significance

PSA and free PSA change was significantly different between the two measures, but with low clinical variations [average PSA1 5.69 ng/ml vs. PSA2 5.39 ng/ml ($p < 0.05$) and average free PSA1 16.99% vs. free PSA2 18.03% ($p < 0.05$)]. Median variability of PSA was 14% (6-27%). Taking into account this variability of PSA, ERSPC proved to be more stable than PCPT. The k agreement between ERSPC1 and ERSPC2 was practically constant, 0.79±0.09 for the usual range of clinical decision (0-0.3). However, PCPT1 and PCPT2 showed wider variations, with a k agreement of approximately 0.55±0.32 in the same range, with a subsequent rapid decrease. The agreement between both models (ERSPC1 vs. PCPT1) proved to be worse for thresholds in this range, peaking 0.47 for a 17% risk, with an average 0.32±0.12 on the interval. The comparison between ERSPC2 and PCPT2 yielded similar results (Figure 4).

Direct comparison of sensitivity and specificity of both RCs along the different clinical risk thresholds showed that PCPT-RC has higher sensitivity and lower specificity than ERPSC-RC for a given threshold along the clinically useful region (Figure 5). The balance point is reached at a different risk threshold for each RC. The performances of both RCs at this point are comparable, as shown in Figure 5. Considering the superposition of their respective ROC curves to a good approximation (Figure 1), this means that a transformation of decision thresholds can make both models perform similarly.

DISCUSSION

Currently, considerable research is being carried out to find new diagnostic markers for Sig PCa, in order to reduce the number of biopsies and the over-diagnosis of insignificant PCa (21). These markers are based on body fluids (blood, urine) or image explorations (22,23). Some are recommended by guidelines such as the 4k score test, PCA3, and/or the Prostate Health Index (PHI) in body fluids (only PCA3 and PHI have been approved by the FDA) (24–26), or multiparametric magnetic resonance imaging (mpMRI), with recent evidence of its advantages in biopsy-naïve patients (27). However, costs and availability minimize their implementation worldwide, and, therefore, it is clear that additional and readily available tools, such as RCs, should be implemented in daily clinical practice. The two most used RCs are ERSPC-RC and PCPT-RC, which have been modified and adapted (4,5). Few external validations have been conducted, with varying results (7,9,28). Usually, external validations of RCs show worse performance than the original validations (8), a fact that is corroborated by our study. Therefore, based on all this information, evaluations, validations, and incorporation of RCs are needed (3).

The present study explores and compares, for the first time, both, the PCPT v2 + free PSA and the ERSPC + DRE, not only for accuracy but also for variability and clinical relevance. Our group previously explored the accuracy and variability of the ERSPC + DRE RC (14) but, in this study, we have specifically focused only on patients in the grey zone (PSA 3-10ng/ml) and compared the ERSPC + DRE RC vs. the PCPT v2 + free PSA, an analysis that has not been previously performed. This comparison showed that both RCs had similar accuracy for the discrimination of Sig PCa. However, ERSPC-RC had better calibration and stability for intra-individual PSA variations. Our methodology in calculating the volume is an estimation from the results of the TRUS measure, similar to Poyet *et al*, and following the recommendations of Roobol *et al.* (4). We have focused only on those patients with PSA between 3-10 ng/ml who require additional diagnostic information. The PCPT-RC option with free PSA, which increases the accuracy of discrimination between Sig PCa and no PCa (5), was calculated, as it is an easy-to-use and readily available tool for these patients.

As defined in the methodology, the first measure (ERSPC1 and PCPT1) was the focus of the direct comparison, as this was the used for biopsy indication. The accuracy of both RCs was similar for Sig PCa in our study, showing an accuracy similar to other external validations such as Poyet *et al*. (9) and Foley *et al.* (7) for ESRPC (AUC= 0.73 and 0.74, respectively) and a better accuracy for PCPT1 v2.0 when adding free PSA (AUC= 0.70 and 0.69, respectively). Still, these results are far from ideal, and thus, additional data from imaging or fluid markers might be included to improve the accuracy of the RCs. In agreement with the accuracy results, the decision curve analysis was also similar between both RCs. In fact, both RCs showed a net benefit from an early risk threshold, which means that their implementation would be useful in the pathway of patient selection.

Studying the variability of the RCs improves our knowledge about their stability, which could be translate into improved decision-making and selection of patients. Our PSA cohort showed a variability that was in the range of those shown in previous literature (11,12,29). Our group and others (14,30) have demonstrated that a higher PSA variability is associated with a reduced risk of Sig PCa in a prostate biopsy, but it does not improve the accuracy of a RC. However, probability stability is important in order to trust RC probabilities at any point. Our study shows good agreement between the two ERSPC + DRE-RC probabilities, with good calibration and stability despite intra-individual PSA variations. PCPTv.2 + free PSA shows worse stability and higher variability, which could be explained simply by the fact that it uses two values (PSA and free PSA) that suffer from this variability (31), while the use of an estimated volume in the ERSPC dilutes the PSA variability. These results should be interpreted with caution, as volume estimation was performed by categorization of TRUS and not by DRE. It is true that this categorization has previously shown good correlation (4). This likely depends on prostate volume (32), as well as low but certain inter-examiner variability (33), which could also increase ERPSC variability in an inter-clinician comparison.

Calibration plots show that both models (PCPT-RC and ERSPC-RC) predict adequately only the actual risk of PCa and Sig PCa for low-risk patients, with a wider useful range in the case of PCa and a lower range in the case of Sig PCa. For higher risk patients, the calibration curves become irregular.

This effect is accentuated for risks close to 1, as both models predict maximum risks of around 0.75 for Sig PCa. Nonetheless, in the usual range for clinical decision (0-0.3), the calibration is not good, showing visually a more consistent and less fluctuating calibration in the useful range for ERSPC than for PCPT, which could make possible to recalibrate it on our population over that range with better results, but this is left to further research. It was observed that ERSPC-RC outperformed PCPT-RC in two aspects. First, its parallelism was better in the low-risk region, both for PCa and Sig PCa, and showed lower irregularity for higher risks. Conversely, the ERSPC curves were visually more stable with the use of different PSA measurements. This observation was reinforced by the results obtained from the study of Cohen's κ coefficient. ERSPC models obtained from PSA1 and PSA2 for Sig PCa showed a better agreement, which was also more stable through a wider interval of decision thresholds, as compared to the agreement between PCPT models obtained from PSA1 and PSA2. These models showed a smaller and rapidly decreasing agreement as the decision threshold was increased. Moreover, the comparison of coefficients between PCPT1 and PCPT2 and between PCPT2 and ERSPC2 showed that the differences between PCPT1 and PCPT2 were similar to those between ERSPC and PCPT models. Both observations suggest that ERSPC is more insensitive and, therefore, robust to intra-individual variations of PSA than PCPT, while the predictive performance is similar.

Despite the similar decision curve, results from the sensitivity, specificity and ROC curve analysis show that the same risk threshold should not be used for both models. Both RCs are able to have similar performance, and the benefit of using any of them is similar in order to screen patients for a prostate biopsy, if the correct cut-off point is selected. It should be highlighted the importance of having an almost 100% negative predictive value, as the advantage of reducing unnecessary biopsies should not be at the cost of missing or delaying the diagnoses of a Sig PCa.

In clinical practice, the use of these RCs should be the first step in guiding the decision for further management of the patient. Patients with a confirmed, elevated PSA between 3-10ng/ml should be better stratified using other variables within a RC, as men with PSA levels >10ng/mL are likely to proceed to biopsy regardless of other factors. Probably a specific cut-off point in the risk probability

should not be used and take advantage of the known probabilities to discuss with the patient the biopsy indication as recommended by the PCPT-RC. In the situation in which the patient is in the low-risk group, according to both RCs (ERSPC and PCPT), the patient could continue with just follow-up. This fact has also been proposed by Alberts et al. (34) when applying new diagnostic markers, such as mpMRI. Specifically, they showed that following a negative recommendation from the ERSPC-RC would have avoided 62 (51%) of 122 mpMRIs and two (25%) of eight insignificant PCa diagnoses, missing three (10%) of 31 high-grade PCa. As the positive predictive value of these RCs is not as good as their negative predictive value, in case of discordance between both RCs or if there is an indication for a biopsy according to both RCs, other images or fluid biomarkers could increase the accuracy in order to potentially reduce the harm from unnecessary prostate biopsy and over-diagnosis (35). Specifically, Loeb *et al.* (36) has recently demonstrated that the incorporation of PHI into both RCs increases the accuracy of the diagnoses of Sig PCa. Another relevant point should be comment from the tendency of better predictive ability with the second evaluations of PSA, reinforcing the idea of the need of several PSA values to confirm the risk and discarded confounding factors. Furthermore, this analysis could suggest a tend to better discrimination ability of PCPT in the range of lower probabilities (when PSA is low), but further research would be needed to validate this idea. These risk calculators only show a static probability so other longitudinal variables and clinical judgment should be required for their application.

The present study has some limitations. First, despite the prospectively collected information, it is a retrospective study design with a limited number of patients. Second, prostate volume was an estimation and categorization from a TRUS calculation, and, therefore, it is not the actual approach for which the RC was developed. Third, the PSA values interval was not the same for all patients, which means the results should be interpreted with caution. Four, the use of TRUS biopsy for PCa diagnosis, although is the standard in most populations, suffers from random error compared with template biopsy (37), which could have affected prediction results. However, the clinical information

was extracted from a clinical practice cohort and with information that could be useful for urologists worldwide.

Altogether, our results showed that: 1) the use of both RCs (ERSPC and PCPT) could be a useful tool in the selection of patients who need prostate biopsy, and that both RCs showed similar accuracy for discrimination of Sig PCa; 2) ERSPC-RC had more stable calibration and stability than PCPT-RC for intra-individual PSA variations; 3) when comparing both RCs sensitivity and specificity, a higher rate of biopsies could be avoided with the ERSPC-RC vs. the PCPT-RC, but with a higher rate of Sig PCa missed. Thus, in those patients with a PSA between 3-10 ng/ml, these tools should be used in order to improve selection and specificity. The RCs specifically should be selected according to the variables available in the clinic. In addition, both RCs could also be used and the decision to undergo a biopsy be shared with the patient.

CONFLICTS OF INTEREST

Nothing to declare

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin [Internet]. 2018/01/04. 2018;68(1):7–30. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29313949
2. Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. Eur Urol [Internet]. 2016/08/25. 2017;71(4):618–29. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27568654
3. Louie KS, Seigneurin A, Cathcart P, Sasieni P. Do prostate cancer risk models improve the predictive accuracy of PSA screening? A meta-analysis. Ann Oncol [Internet]. 2015;26(5):848–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25403590
4. 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 [Internet]. 2012;61(3):577–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22104592
5. Ankerst DP, Hoefler J, Bock S, Goodman PJ, Vickers A, Hernandez J, et al. Prostate Cancer Prevention Trial risk calculator 2.0 for the prediction of low- vs high-grade prostate cancer. Urology [Internet]. 2014;83(6):1362–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24862395
6. Lundon DJ, Kelly BD, Foley R, Loeb S, Fitzpatrick JM, Watson RW, et al. Prostate cancer risk assessment tools in an unscreened population. World J Urol [Internet]. 2015;33(6):827–32. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25091862
7. Foley RW, Maweni RM, Gorman L, Murphy K, Lundon DJ, Durkan G, et al. The ERSPC Risk Calculators Significantly Outperform The PCPT 2.0 In The Prediction Of Prostate Cancer; A Multi-Institutional Study. BJU Int [Internet]. 2016; Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26833820

8. Foley RW, Lundon DJ, Murphy K, Murphy TB, Galvin DJ, Watson RW. Predicting prostate cancer: analysing the clinical efficacy of prostate cancer risk calculators in a referral population. Ir J Med Sci [Internet]. 2015;184(3):701–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25843017

9. Poyet C, Nieboer D, Bhindi B, Kulkarni GS, Wiederkehr C, Wettstein MS, et al. Prostate cancer risk prediction using the novel versions of the European Randomised Study for Screening of Prostate Cancer (ERSPC) and Prostate Cancer Prevention Trial (PCPT) risk calculators: independent validation and comparison in a contemporary Europe. BJU Int [Internet]. 2016;117(3):401–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26332503

10. Murthy V, Rishi A, Gupta S, Kannan S, Mahantshetty U, Tongaonkar H, et al. Clinical impact of prostate specific antigen (PSA) inter-assay variability on management of prostate cancer. Clin Biochem [Internet]. 2015/10/23. 2016;49(1–2):79–84. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26506115

11. Komatsu K, Wehner N, Prestigiacomo AF, Chen Z, Stamey TA. Physiologic (intraindividual) variation of serum prostate-specific antigen in 814 men from a screening population. Urology [Internet]. 1996;47(3):343–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8633399

12. Morote J, Raventós CX, Lorente JA, Enbabo G, López M, de Torres I. Intraindividual variations of total and percent free serum prostatic-specific antigen levels in patients with normal digital rectal examination. Eur Urol [Internet]. 1999;36(2):111–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10420031

13. Lavallée LT, Binette A, Witiuk K, Cnossen S, Mallick R, Fergusson DA, et al. Reducing the Harm of Prostate Cancer Screening: Repeated Prostate-Specific Antigen Testing. Mayo Clin Proc [Internet]. 2015/12/10. 2016;91(1):17–22. Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26688045

14. Gómez-Gómez E, Carrasco-Valiente J, Blanca-Pedregosa A, Barco-Sánchez B, Fernandez-Rueda JL, Molina-Abril H, et al. European Randomized Study of Screening for Prostate Cancer Risk Calculator: External Validation, Variability, and Clinical Significance. Urology. 2017;102.

15. Epstein JI, Allsbrook WC, Amin MB, Egevad LL, Committee IG. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol [Internet]. 2005;29(9):1228–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16096414

16. Fawcett T. An introduction to ROC analysis. Pattern Recognit Lett [Internet]. 2006 Jun 1 [cited 2018 Dec 20];27(8):861–74. Available from: https://www.sciencedirect.com/science/article/pii/S016786550500303X

17. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics [Internet]. 1988 Sep [cited 2018 Dec 20];44(3):837–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3203132

18. Zhou X-H, McClish DK, Obuchowski NA, Electronic Book Collection., Wiley InterScience (Online service). Statistical methods in diagnostic medicine [Internet]. Wiley; 2011 [cited 2018 Dec 20]. 545 p. Available from: https://www.wiley.com/en-us/Statistical+Methods+in+Diagnostic+Medicine%2C+2nd+Edition-p-9780470183144

19. Hendriksen JMT, Geersing GJ, Moons KGM, de Groot JAH. Diagnostic and prognostic prediction models. J Thromb Haemost [Internet]. 2013 Jun [cited 2018 Dec 20];11:129–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23809117

20. Vickers AJ, Elkin EB. Decision Curve Analysis: A Novel Method for Evaluating Prediction Models. Med Decis Mak [Internet]. 2006 Nov 5 [cited 2018 Dec 20];26(6):565–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17099194

21. Loeb S, Bjurlin MA, Nicholson J, Tammela TL, Penson DF, Carter HB, et al. Overdiagnosis and overtreatment of prostate cancer. Eur Urol [Internet]. 2014;65(6):1046–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24439788

22. Loeb S, Lilja H, Vickers A. Beyond prostate-specific antigen: utilizing novel strategies to screen men for prostate cancer. Curr Opin Urol [Internet]. 2016;26(5):459–65. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27262138

23. Johnston E, Pye H, Bonet-Carne E, Panagiotaki E, Patel D, Galazi M, et al. INNOVATE: A prospective cohort study combining serum and urinary biomarkers with novel diffusion-weighted magnetic resonance imaging for the prediction and characterization of prostate cancer. BMC Cancer [Internet]. 2016;16(1):1–11. Available from: http://dx.doi.org/10.1186/s12885-016-2856-2

24. McDonald ML, Parsons JK. 4-Kallikrein Test and Kallikrein Markers in Prostate Cancer Screening. Urol Clin North Am [Internet]. 2016;43(1):39–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26614027

25. Lepor A, Catalona WJ, Loeb S. The Prostate Health Index: Its Utility in Prostate Cancer Detection. Urol Clin North Am [Internet]. 2016;43(1):1–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26614024

26. De Luca S, Passera R, Cappia S, Bollito E, Randone DF, Milillo A, et al. Fluctuation in prostate cancer gene 3 (PCA3) score in men undergoing first or repeat prostate biopsies. BJU Int [Internet]. 2014/04/29. 2014;114(6b):E56-61. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24472071

27. Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. Lancet [Internet]. 2017/01/20. 2017;389(10071):815–22. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28110982

28. Grill S, Fallah M, Leach RJ, Thompson IM, Freedland S, Hemminki K, et al. Incorporation

of detailed family history from the Swedish Family Cancer Database into the PCPT risk calculator. J Urol [Internet]. 2014/09/19. 2015;193(2):460–5. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25242395

29. Sölétormos G, Semjonow A, Sibley PE, Lamerz R, Petersen PH, Albrecht W, et al. Biological variation of total prostate-specific antigen: a survey of published estimates and consequences for clinical practice. Clin Chem [Internet]. 2005;51(8):1342–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15961552

30. Nordström T, Adolfsson J, Grönberg H, Eklund M. Repeat Prostate-Specific Antigen Tests Before Prostate Biopsy Decisions. J Natl Cancer Inst [Internet]. 2016;108(12). Available from: https://www.ncbi.nlm.nih.gov/pubmed/27418620

31. Ankerst DP, Gelfond J, Goros M, Herrera J, Strobl A, Thompson IM, et al. Serial Percent Free Prostate Specific Antigen in Combination with Prostate Specific Antigen for Population Based Early Detection of Prostate Cancer. J Urol [Internet]. 2016/03/12. 2016;196(2):355–60. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26979652

32. Roehrborn CG, Girman CJ, Rhodes T, Hanson KA, Collins GN, Sech SM, et al. Correlation between prostate size estimated by digital rectal examination and measured by transrectal ultrasound. Urology [Internet]. 1997;49(4):548–57. Available from: https://www.ncbi.nlm.nih.gov/pubmed/9111624

33. Roehrborn CG, Sech S, Montoya J, Rhodes T, Girman CJ. Interexaminer reliability and validity of a three-dimensional model to assess prostate volume by digital rectal examination. Urology [Internet]. 2001;57(6):1087–92. Available from: https://www.ncbi.nlm.nih.gov/pubmed/11377314

34. Alberts AR, Schoots IG, Bokhorst LP, van Leenders GJ, Bangma CH, Roobol MJ. Risk-based Patient Selection for Magnetic Resonance Imaging-targeted Prostate Biopsy after Negative Transrectal Ultrasound-guided Random Biopsy Avoids Unnecessary Magnetic Resonance Imaging Scans. Eur Urol [Internet]. 2016;69(6):1129–34. Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26651990

35. Chiu PK, Alberts AR, Venderbos LDF, Bangma CH, Roobol MJ. Additional benefit of using a risk-based selection for prostate biopsy: an analysis of biopsy complications in the Rotterdam section of the European Randomized Study of Screening for Prostate Cancer. BJU Int [Internet]. 2017/06/05. 2017;120(3):394–400. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28498624

36. Loeb S, Shin SS, Broyles DL, Wei JT, Sanda M, Klee G, et al. Prostate Health Index improves multivariable risk prediction of aggressive prostate cancer. BJU Int [Internet]. 2016/11/22. 2017;120(1):61–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27743489

37. Ahmed HU, Hu Y, Carter T, Arumainayagam N, Lecornet E, Freeman A, et al. Characterizing clinically significant prostate cancer using template prostate mapping biopsy. J Urol [Internet]. 2011/06/15. 2011;186(2):458–64. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21679984

Footnotes

- **Contributors** E.G.G, R.M.L, MJ.R.T and J.C.V carried out the conception and design of the study; E.G.G, JJ.S.B, J.C.V, AM.B, J.V.R, I.M.B.A, J.M.L, JM.J.V contributed to the data acquisition; E.G.G, J.C.V, J.L.F.R, RM.L and MJ.R.T carried out the analysis and interpretation of data; E.G.G, J.C.V and RM.L drafted the manuscript; JJ.S.B, J.L.F.R, AM.B, J.V.R, J.M.L, JM.J.V, and MJ.R.T carried out a critical revision of the manuscript for important intellectual content; E.G.G, and J.L.F.R performed the statistical analysis; RM.L, MJ.R.T and J.C.V supervised the work.
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- **Competing interests** None declared.
- **Ethics approval** This study was performed as part of the ONCOVER project. Ethical approval was obtained by the Reina Sofia Hospital Research Ethics Committee in accordance with the Declaration of Helsinki and informed consent was obtained from all participants for the project.
- **Provenance and peer review** Not commissioned; externally peer reviewed.
- **Data sharing statement** All data is shown within the manuscript
- **Patient consent for publication** Not required.

Figure legends

Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values: **A**, for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa; **B**, for the ERSPC1-RC and the ERSPC2-RC for positive biopsy; **C**, for the PCPT1-RC and the PCPT2-RC for positive biopsy; and **D**, for the ERSPC2-RC and the PCPT2-RC for Sig PCa.

Figure 2: Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are underestimated from the lower right, where they are overestimated. **A**, Calibration plots for ERSPC1-RC Sig PCa risk estimation. **B**, Calibration plots for PCPT1-RCSig PCa risk estimation.

Figure 3: Results of the decision curve analysis. **A**, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1–RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating all patients (dashed line) and treating none (thin line). **B**, Plot demonstrating net reduction of interventions per 100 patients using the ERSPC-RC (black line) and the PCPT-RC (grey line).

Figure 4: Graphics showing Cohen's k coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. **A**, Agreement between ERSPC1-RC and ERSPC2-RC for Sig PCa. **B**, Agreement between PCPT1-RC and PCPT2-RC for Sig PCa. **C**, Agreement between ERSPC1-RC and PCPT1-RC for Sig PCa.

Figure 5: Graphics showing sensitivities and specificities of both RCs along the clinically useful risk threshold. The ERSPC-RC (black line) and the PCPT-RC (grey line).

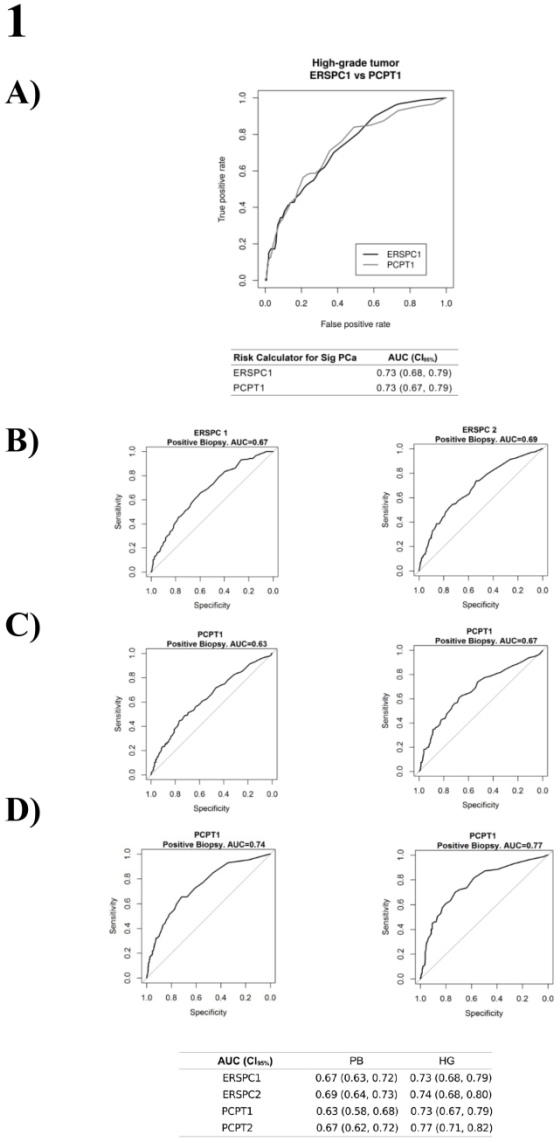
Table 1. Clinical and demographic characteristics of the cohort of patients categorized according to cancer status.

Variable	No Sig PCa n=423	Sig PCa n=87	All n=510
Age	64.0 (60.0-69.0)	68.0 (63.0-71.0)	65.0 (60.0-70.0)
Family History	81 (19.1)	8 (9.2)	89 (17.5)
Positive DRE	55 (13.0)	27 (31.0)	82 (16.1)
1 Serum PSA	5.3 (4.3-6.9)	5.8 (4.5-7.2)	5.3 (4.3-6.9)
1 free PSA %	16.2 (12.4-21.4)	12.5 (9-16.6)	15.9 (11.8-20.4)
2 Serum PSA	5.0 (3.7-6.6)	5.4 (4.1-6.7)	5.0 (3.8-6.6)
2 free PSA %	17.9 (13.9-23.4)	12.5 (9.1-16.3)	16.9 (12.8-22.1)
Prostate volume	38.0 (29.0-50.0)	26.0 (20.7-34.0)	35 (26-49)
First Biopsy	322 (76.1)	79 (90.8)	401 (78.6)
PCPT1 Sig PCa	0.08 (0.05-0.13)	0.16 (0.10-0.30)	0.09 (0.06-0.15)
ERSPC1 Sig PCa	0.05 (0.02-0.10)	0.12 (0.05-0.31)	0.05 (0.03-0.12)
PCPT2 Sig PCa	0.07 (0.04-0.11)	0.16 (0.08-0.27)	0.07 (0.05-0.13)
ERSPC2 Sig PCa	0.04 (0.02-0.08)	0.12 (0.05-0.30)	0.05 (0.02-0.11)
PCa	89 (21)	87 (100)	176 (34.5)

PCa= Prostate cancer; **Sig PCa**= significant PCa (Gleason ≥ 7 on biopsy); **No Sig PCa**= No cancer or non-significant PCa; **ERSPC1 / PCPT1 Sig PCa** = Probability of high grade PCa using the first measurement of serum PSA (at the time of biopsy indication by the urologist); **ERSPC2 / PCPT2 Sig PCa** = Probability high grade PCa using the second measurement of serum PSA (just before undergoing prostate biopsy). Median values (interquartile range) are expressed for quantitative variables, and absolute values (percentage) for qualitative variables.

559

560



Risk Calculator for Sig PCa	AUC ($CI_{95\%}$)
ERSPC1	0.73 (0.68, 0.79)
PCPT1	0.73 (0.67, 0.79)

AUC ($CI_{95\%}$)	PB	HG
ERSPC1	0.67 (0.63, 0.72)	0.73 (0.68, 0.79)
ERSPC2	0.69 (0.64, 0.73)	0.74 (0.68, 0.80)
PCPT1	0.63 (0.58, 0.68)	0.73 (0.67, 0.79)
PCPT2	0.67 (0.62, 0.72)	0.77 (0.71, 0.82)

Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values: A, for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa; B, for the ERSPC1-RC and the ERSPC2-RC for positive biopsy; C, for the PCPT1-RC and the PCPT2-RC for positive biopsy; and D, for the ERSPC2-RC and the PCPT2-RC for Sig PCa.

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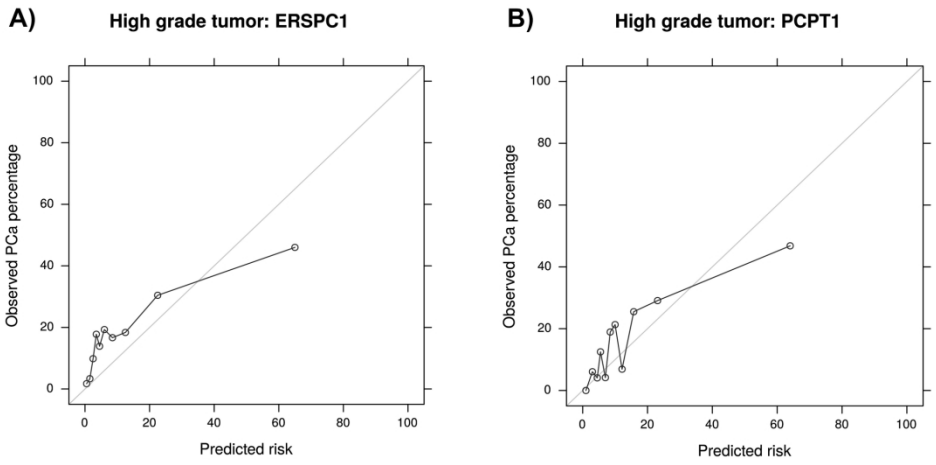

Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are underestimated from the lower right, where they are overestimated. A, Calibration plots for ERSPC1-RC Sig PCa risk estimation. B, Calibration plots for PCPT1-RCSig PCa risk estimation.

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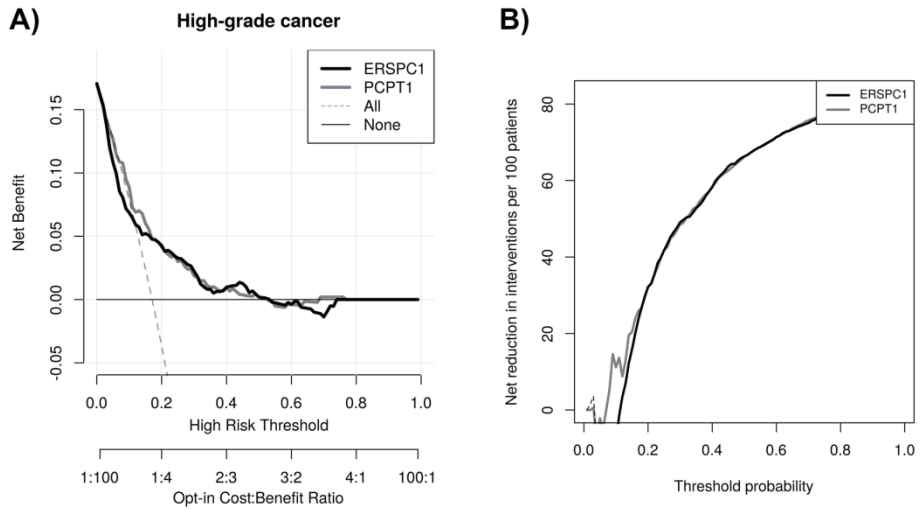

Results of the decision curve analysis. A, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1–RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating all patients (dashed line) and treating none (thin line). B, Plot demonstrating net reduction of interventions per 100 patients using the ERSPC-RC (black line) and the PCPT-RC (grey line).

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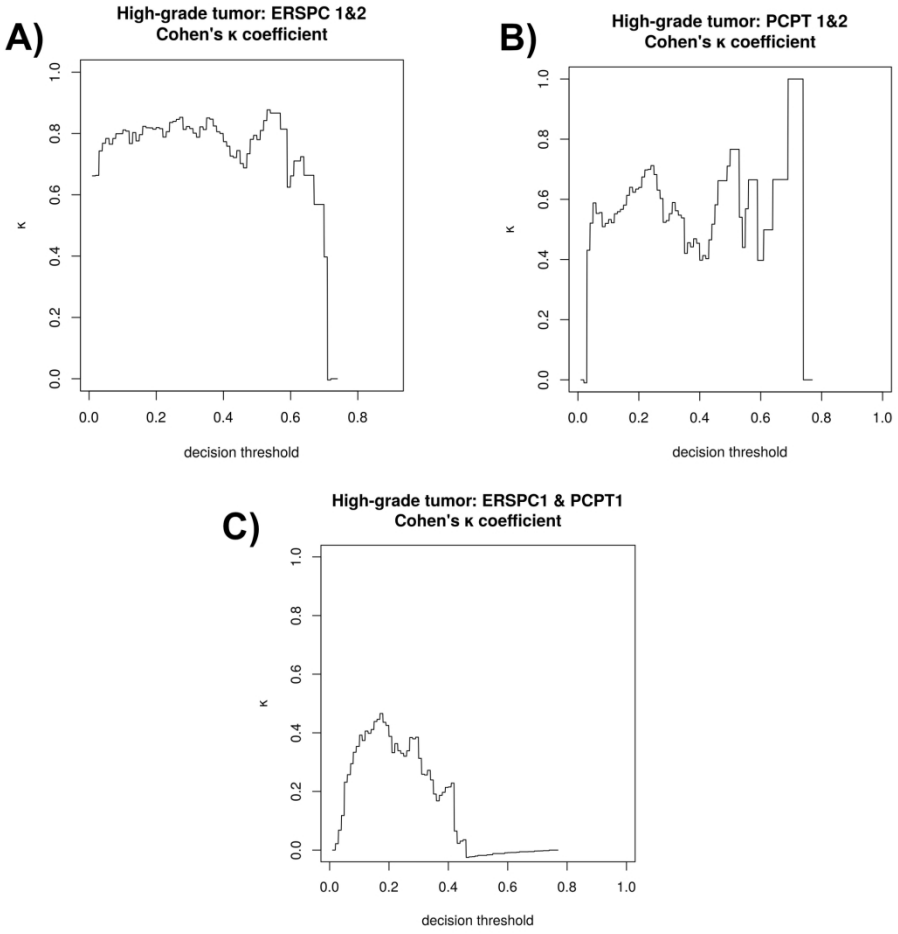

Graphics showing Cohen's k coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. A, Agreement between ERSPC1-RC and ERSPC2-RC for Sig PCa. B, Agreement between PCPT1-RC and PCPT2-RC for Sig PCa. C, Agreement between ERSPC1-RC and PCPT1-RC for Sig PCa.

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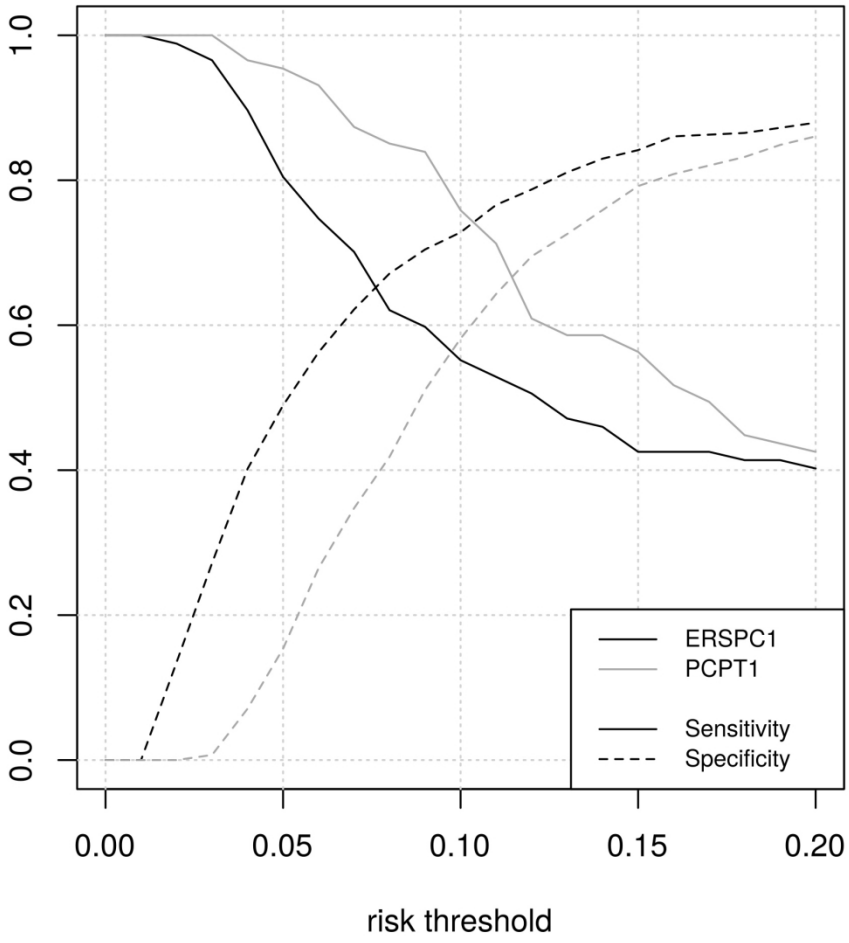


Graphics showing sensitivities and specificities of both RCs along the clinically useful risk threshold. The ERSPC-RC (black line) and the PCPT-RC (grey line).

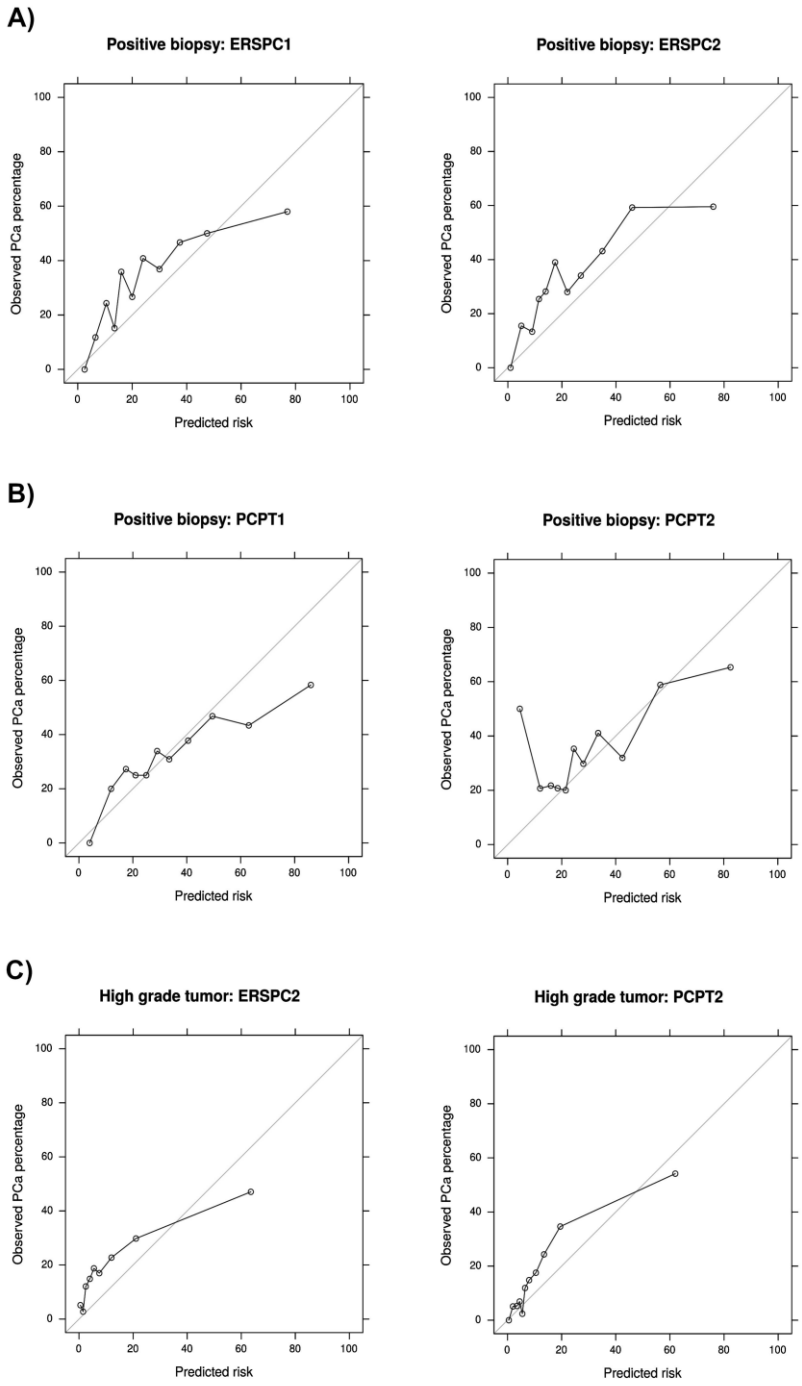
Supplemental Table 1. Patients excluded from the ONCOVER cohort for this study depends on the exclusion criteria.

Exclusion criteria	Number
Under active surveillance	25
2 consecutive PSA levels well recorded or affected	50
Prostate volume not well recorded	177
PSA of biopsy indication out of the range 3-10ng/ml , or Age out of the range 55-80	251
Total exclusions	511

Supplemental table 2: DeLong p values resulting from the pairwise comparison of the Area under the Receiver Operator Characteristic (ROC) curve (AUC) between Risk Calculators for significant Prostate cancer (Sig PCa) detection.

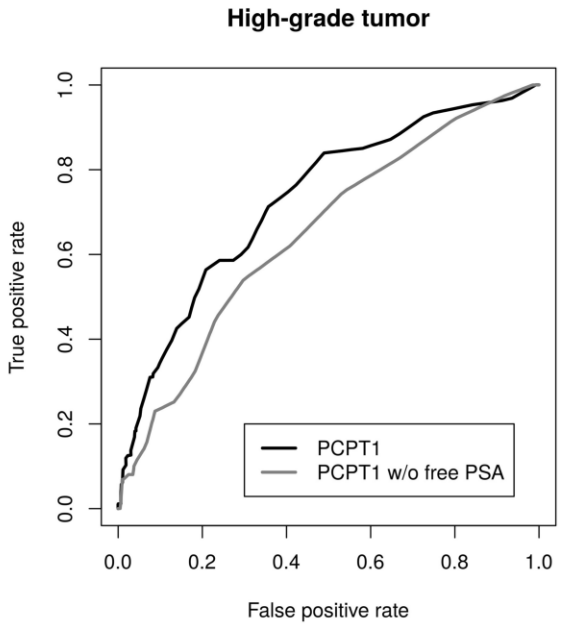
Sig PCa (p-value)	ERSPC1	ERSPC2	PCPT1	PCPT2
ERSPC1	X	0.51	0.95	0.19
ERSPC2	X	X	0.74	0.25
PCPT1	X	X	X	0.06

S1



Supplemental Figure 1: Calibration plots of the RCs in this cohort, demonstrating the agreement between predicted and observed probabilities: **A**, of a positive biopsy for the ERSPC1-RC and the ERSPC2-RC; **B**, of a positive biopsy for the PCPT1-RC and for the PCPT2-RC; and **C**, of a Sig PCa on the biopsy for the ERSPC2-RC and the PCPT2-RC.

S2



Risk Calculator for Sig PCa	AUC ($CI_{95\%}$)
PCPT1 without free PSA	0.65 (0.59, 0.71)
PCPT1 + free PSA	0.73 (0.67, 0.79)
DeLong p value: 0.02	

Supplemental Figure 2: Receiver Operating Characteristic curves and Area Under the Curve values for the PCPT1-RC without free PSA (black) and for the PCPT1-RC with free PSA (grey) to predict Sig PCa. P-value according to the DeLong test.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	*8*
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-10
		(*b*) Describe any methods used to examine subgroups and interactions	8-10
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	8-10
		(*e*) Describe any sensitivity analyses	8-10
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	Supplemental
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	11- Supplemental
Outcome data	15*	Report numbers of outcome events or summary measures	12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supplemental
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Observational study comparing the accuracy/variability between the ERPSC and the PCPT risk calculators for the prediction of significant prostate cancer in patients with PSA <10ng/ml

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Primary Subject Heading:	Urology
Secondary Subject Heading:	Urology
Keywords:	Significant prostate cancer, risk calculator variability, ERSPC, PCPT

Observational study comparing the accuracy/variability between the ERPSC and the PCPT risk calculators for the prediction of significant prostate cancer in patients with PSA <10ng/ml

Authors: Gómez-Gómez E[1,2#], Salamanca-Bustos JJ[2], Carrasco-Valiente J[1,2], Fernández-Rueda J.L[3], Blanca AM[1], Valero-Rosa J[1,2], Bravo-Arrebola I.M[2], Márquez-López J[1,2], Jiménez-Vacas JM[1,4,5], Luque RM[1,4,5#], Requena-Tapia MJ[1,2].

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Running title: Risk Calculators for Significant Prostate Cancer

Key Words: Significant prostate cancer; risk calculator variability; ERSPC; PCPT.

Tables and figures: 1 and 5

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Servicios Sociales e Igualdad, Spain. This work has been awarded by the Real Academia de Medicina de Sevilla 2018. The funding agreement ensured the authors' independence in designing the study, interpreting the data, writing, and publishing the report

ABSTRACT

Introduction: Risk Calculators (RCs) are easy-to-use tools considering available clinical-variables that could help to select those patients with risk of prostate-cancer (PCa) who should undergo a prostate-biopsy. **Objective:** To perform a comparison for the prediction of significant-PCa (SigPCa) between the European-Randomised Study of Screening for PCa (ERSPC) and the PCa Prevention-Trial (PCPT) RCs in patients with PSA between 3-10ng/ml through an evaluation of the accuracy/variability between two consecutive PSA-values. **Setting:** An observational study in a major university Hospital of the south of Spain. **Methods and participants:** An observational study was performed in patients who underwent a prostate-biopsy. SigPCa probabilities were calculated with the two PSA measures using ERSPC3/4+DRE and PCPTv2+free-PSA RCs. The prediction discrimination of SigPCa was determined by the area under the curve (AUC). Calibration, discrimination, and decision curve analysis were studied. The variability between both RCs-agreement was compared using Cohen's kappa coefficient. **Results:** 510 patients were analysed (87 diagnosed with SigPCa). The median PSA value were 5.3 and 5ng/ml for PSA1 and PSA2 respectively. Both RCs overestimated the risk in the case of high-risk probabilities. Discrimination ability for SigPCa was similar between models with an AUC=0.73(0.68-0.79) for ERSPC-RC vs. 0.73(0.67-0.79) for PCPT-RC. ERSPC-RC showed less variability than PCPT-RC, with a constant agreement (k=0.7-0.8) for usual range of clinical decision-making. Remarkably, a higher biopsies number would be avoided using the ERSPC-RC, but more SigPCa would be missed along all the risk probabilities. **Conclusions:** Both RCs had similar accuracy for the SigPCa discrimination. However, ERSPC-RC seems to be more stable for intra-individual PSA variations.

Strengths and limitations of this study

- This study highlights the need to spread the use of available free tools which would be useful in patient's selection for undergoing prostate biopsy.
- This study is the first to compare two available free risk calculators in patients with a PSA < 10ng/ml analyzing their variability between two consecutive different PSA levels.
- Although the clinical information of this study was extracted from a clinical practice cohort and with information that could be useful for urologists worldwide, this is a retrospective study and the use of TRUS biopsy for PCa diagnosis, even though is the standard in most populations, suffers from random error compared with template biopsy, which could have affected prediction results.

INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed malignancy in males worldwide, and the most frequent in developed countries(1). Its current standard of diagnosis is a prostate biopsy based on PSA levels and digital rectal examination (DRE). However, there are other available and complementary variables that could help to select those patients who should undergo a prostate biopsy (such as age, prostate volume, free PSA, family history, etc.), but these are not always used and/or well-integrated in daily clinical practice(2). In line with this, Risk Calculators (RCs) are easy-to-use tools that can help the clinicians to take advantage of all these available variables (3). The two main RCs are from the European Randomised Study for Screening of Prostate Cancer (ERSPC cohort; ERSPC-RC: http://www.prostatecancer-riskcalculator.com/seven-prostate-cancer-riskcalculators) and from the Prostate Cancer Prevention Trial (PCPT cohort; PCPT-RC: http://deb.uthscsa.edu/URORiskCalc/Pages/calcs.jsp). Both RCs have undergone some modifications, specifically the addition of estimated prostate volume in the ERSPC-RC (4,5). Furthermore, both RCs were originally developed from different patient cohorts and each RC uses different variables.

To date, limited external validations and comparisons have been performed by different groups (6–9). The two most important recent comparisons of the modified RCs were performed by Foley *et al* (6,7) and Poyet *et al* (9). Both found a better discriminatory ability for ERSPC-RC vs PCPTv.2-RC for the diagnoses of significant-PCa (Sig PCa) (AUC around 0.74 vs 0.69, respectively), but they also included patients with a high PSA of up to 50 ng/ml. Despite the possibility of using these RCs in patients with PSA levels up to 50ng/ml, it is clear that the advantages of using both RCs would probably increase in patients with a PSA under 10ng/ml, where the rate of positive biopsy for PCa clearly decrease, with an important number of unnecessary biopsies. Furthermore, in the case of the PCPTv.2-RC, the addition of the free PSA value in patients with a PSA under 10ng/ml seems to improve its accuracy (5), and, therefore, given its accessibility, this value should be included in the RC.

The intra-individual and inter-assay variability of PSA is already known (10–12) and, therefore, at least two measures are necessary before a prostate biopsy is indicated. In fact, it has been shown that approximately 25% of men with initial PSA levels between 4 and 10 ng/mL had normal PSA values upon repeat testing (13). In line with this, despite being primarily based on PSA level, the variability of the two RCs mentioned above has been poorly studied, and might have implications for patient management. Our group has recently evaluated this variability with the ERSPC-RC, which showed stable accuracy over a cohort of patients, but some changes with respect to an individual approach (14). To date, there is no study comparing the accuracy and variability of both RCs, the ERSPC + DRE vs. the PCPTv.2 + free PSA, for the prediction of Sig PCa. Therefore, the aim of this study was to perform a direct comparison between ERSPC + DRE and PCPTv.2 + free PSA RCs in patients with a PSA between 3-10ng/ml, evaluating the accuracy and variability of both methods in the prediction of Sig PCa.

MATERIALS AND METHODS

Study population and design

An observational retrospective study was performed in patients from ONCOVER cohort (1021 biopsies indicated by clinical practice wherein patients donated blood and urine before the biopsy). The study was carried out within the project approved by our Hospital Research Ethics Committee, and informed consent was obtained from all participants. Blood sample was obtained in the morning (between 8:00-10:00 am) after fasting overnight and then, the prostate biopsy was implemented according to clinical practice. The inclusion criteria for this study were: 1) PSA indication between 3-10 ng/ml; 2) Full clinical and laboratory data to fulfilled ERSPC-RC and PCPT-RC; 3) Age 55-80 years' old; 4) Two consecutives measurements of PSA levels within an interval of 12 weeks. Exclusion criteria included patients with a previously known PCa diagnosis or treatment that could modify PSA levels (Supplemental table 1).

Transrectal prostate biopsy was carried out under local anaesthesia by using a standard peri-prostatic block, a transrectal ultrasound transducer, and an 18G automated needle biopsy instrument. The prostatic volume was measured following the protocol used during transrectal ultrasound (TRUS), and usual recommendations were to take 12 cores in patients undergoing the first biopsy procedure, and a minimum of 16 biopsy cores for those who had a previous biopsy. Biopsy specimens were analysed by expert urologic pathologists according to the International Society of Urological Pathology (ISUP) 2005 modified criteria (15).

Main variables description

Demographic information and the medical history of each patient was obtained. PSA levels were measured twice within a period no longer than 12 weeks, as follows: 1) **PSA 1 and free PSA 1:** for biopsy indication; and, 2) **PSA 2 and free PSA 2:** before undergoing prostate biopsy. For both PSA measures were evaluated by Chemiluminescent Microparticle Immunoassays (ng/ml, by a CMIA;

Ref. 7k70; Abbott). The median and interquartile range of time between measurements was 6 (3-8) weeks.

Prostate volume: estimated by TRUS and categorized in three possible values, 25-40-60 ml, as recommended (4) (TRUS volume <30 = 25 mL, 30–50 = 40 mL, and ≥50 = 60 mL).

Significant/ high-grade (HG) prostate cancer (Sig PCa): PCa with a Gleason grade ≥ 7 on biopsy.

ERSPC-RC and PCPT-RC probabilities calculation

ERSPC: The formulas for the ERSPC-RC 3+DRE for patients at initial biopsy and the ERSPC-RC 4+DRE for patients at repeat biopsy were utilized in this study. These calculators use PSA, prostate volume, and DRE as variables, with, a negative prostate biopsy in ERSPC 4+ DRE in patients who had a previous biopsy. This provides a probability rating for any PCa or Sig PCa (Gleason ≥ 7).

ERSPC1/Sig PCa (1º Measure): Risk probability calculated by ERSPC-RC3 or 4 (if previous biopsy) ± DRE for any PCa using PSA 1/ Sig PCa – for HG PCa.

ERSPC2/Sig PCa (2º Measure): Risk probability calculated by ERSPC-RC 3 or 4 (if previous biopsy) ± DRE for any PCa using PSA 2/ Sig PCa – for HG PCa.

PCPT: The formulae for the PCPT-RC 2.0 + %free PSA was utilized in this study. This calculator uses race, age, PSA level, %free PSA level, family history of PCa, DRE and prior prostate biopsy. This gives a probability of negative biopsy, low grade PCa and Sig PCa (gleason ≥ 7).

PCPT1: Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 1 and free PSA 1.

PCPT2: Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 2 and free PSA 2.

The variability of PSA was calculated by the following formula: **| Measure 1– Measure2| / Measure 1**

Statistical analysis

A descriptive study was performed by calculating the median and interquartile ranges (IR) for the quantitative variables, and the absolute frequencies and percentages for the qualitative variables. A Student's T test for paired groups was used to compare the means of the quantitative variables (PSA 1 and 2).

The investigation of the comparative performance in the detection of Sig PCa of both RCs, ERSPC-RC and PCPT-RC, was performed, taking into account these four factors: discrimination capacity, calibration, clinical utility, and consistency against the observed variations in PSA levels for our dataset.

The discrimination ability of the models, i.e., their ability to separate those patients who had Sig PCa from those who do not, was assessed using the area under their Receiver Operator Characteristic (ROC) curve (AUC) (16), as measured in our sample. This is one of the most frequently used measurements of model discrimination, because of its independence of the selection of a specific decision threshold and its robustness against class imbalance. Confidence intervals for these AUCs were computed using bootstrapping. These AUCs were then compared to determine the relative performance of the models using DeLong tests (17). These tests were chosen because of their non-parametric nature, with few assumptions about the data, and their suitability for paired data, as both models were evaluated over the same dataset, properties which make this the most commonly used test to compare AUCs (18). For this comparison, we focused on the calculated risk score utilising the first measure of PSA (PSA 1; the value for the indication of the prostate biopsy).

The calibration of the calculators for our cohort was then investigated to determine the agreement between the frequency of the observed outcome (Sig PCa in our case) and the risks predicted by the model. Calibration plots were used for this purpose (19), enabling a visual evaluation of this agreement and the comparison between RCs.

To address the potential clinical utility of the models, we performed decision curve analysis on our data, as proposed by Vickers and Elkin (20). This method has the advantage of not requiring the

specification of the relative cost for false-positives and false-negatives, defining a net benefit as a function of the decision threshold at which one would consider obtaining a biopsy.

Finally, the stability of the predictions of both RCs, with regard to the observed intra-patient changes on PSA levels between measurements, was investigated using the Cohen's kappa (k) inter-rater agreement coefficient as a function of the decision threshold. This coefficient was selected due to its widespread use and robustness against random agreements, and thus, is a better measurement than naïve accuracy.

All the analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill) and R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria: URL https://www.R-project.org/). A <5% level of significance ($p<0.05$) was used to decide statistically significant differences.

Patient and public involvement

Participants and public were not involved in the development of research questions, study design or recruitment.

RESULTS

Cohort characteristics

In the present study, we analysed 510 patients who met the inclusion criteria previously described. Median age was 65 (60-70) years old, with a family history in 89 patients (17.5%) and a suspicious DRE in 82 patients (16.1%). The median PSA before prostate biopsy indication was 5.3 (4.3-6.9) ng/ml. 176 patients were diagnosed with PCa, 87 of those categorized as Sig PCa. Most patients (n=401; 78.6%) were biopsy-naïve and the median prostate volume was 35 (26-49) cc. Further cohort description according to Sig PCa status is shown in Table 1.

66 patients had a PSA 2 out of the range of 3-10ng/ml due to the variability (50 patients below 3ng/ml and 16 patients above 10ng/ml); thus, in this case, the % free PSA was not calculated, and the risk probability was calculated without the inclusion of this variable. The patients were maintained in the analysis as reflecting the variability of the PSA, and the application of the models in this real situations, although acknowledging that it could introduce a bias in terms of calibration and variability.

Direct comparison for Sig PCa prediction

Discrimination ability for Sig PCa was no different between the two models [ERSPC1-RC vs. PCPT1-RC: 0.73; 95% CI: (0.68-0.79) vs. 0.73; 95% CI: (0.67-0.79), respectively]. ROC curves are shown in Figure 1A. Similarly, no difference was found in the discrimination ability for any PCa. The comparison of the RC for both measures is described in Figure 1(B-D) with similar results but a tendency of better accuracy for PCPT2-RC vs ERSPC2-RC (p= 0.25). Supplemental Table 2 shows multiple comparisons by the DeLong test resulting in no differences between the two RCs for Sig PCa.

Both models tended to overestimate the risk for a high probability of Sig PCa, and slightly underestimate it for low risk patients, suggesting that the models would benefit from a recalibration for our population (Figure 2). None of the models predicted very high probabilities for most patients. The calibration curves for any PCa are shown in Supplemental Figure 1.

The decision curve analyses revealed that both RCs provided a clinical net benefit in the threshold probability range for Sig PCa (Figure 3). The net benefit was comparable between the two RCs for Sig PCa.

As shown in Supplemental Figure 2, the addition of free PSA clearly improved the accuracy of the PCPT-RC [0.65 (0.59-0.71) PCPT1 v.2.0-RC vs. 0.73 (0.67- 0.79) PCPT1 v.2.0 + free PSA –RC; p= 0.02].

Variability and clinical significance

PSA and free PSA change was significantly different between the two measures, but with low clinical variations [average PSA1 5.69 ng/ml vs. PSA2 5.39 ng/ml ($p < 0.05$) and average free PSA1 16.99% vs. free PSA2 18.03% ($p < 0.05$)]. Median variability of PSA was 14% (6-27%). Taking into account this variability of PSA, ERSPC proved to be more stable than PCPT. The k agreement between ERSPC1 and ERSPC2 was practically constant, 0.79±0.09 for the usual range of clinical decision (0-0.3). However, PCPT1 and PCPT2 showed wider variations, with a k agreement of approximately 0.55±0.32 in the same range, with a subsequent rapid decrease. The agreement between both models (ERSPC1 vs. PCPT1) proved to be worse for thresholds in this range, peaking 0.47 for a 17% risk, with an average 0.32±0.12 on the interval. The comparison between ERSPC2 and PCPT2 yielded similar results (Figure 4).

Direct comparison of sensitivity and specificity of both RCs along the different clinical risk thresholds showed that PCPT-RC has higher sensitivity and lower specificity than ERPSC-RC for a given threshold along the clinically useful region (Figure 5). The balance point is reached at a different risk threshold for each RC. The performances of both RCs at this point are comparable, as shown in Figure 5. Considering the superposition of their respective ROC curves to a good approximation (Figure 1), this means that a transformation of decision thresholds can make both models perform similarly.

DISCUSSION

Currently, considerable research is being carried out to find new diagnostic markers for Sig PCa, in order to reduce the number of biopsies and the over-diagnosis of insignificant PCa (21). These markers are based on body fluids (blood, urine) or image explorations (22,23). Some are recommended by guidelines such as the 4k score test, PCA3, and/or the Prostate Health Index (PHI) in body fluids (only PCA3 and PHI have been approved by the FDA) (24–26), or multiparametric magnetic resonance imaging (mpMRI), with recent evidence of its advantages in biopsy-naïve patients (27). However, costs and availability minimize their implementation worldwide, and, therefore, it is clear that additional and readily available tools, such as RCs, should be implemented in daily clinical practice. The two most used RCs are ERSPC-RC and PCPT-RC, which have been modified and adapted (4,5). Few external validations have been conducted, with varying results (7,9,28). Usually, external validations of RCs show worse performance than the original validations (8), a fact that is corroborated by our study. Therefore, based on all this information, evaluations, validations, and incorporation of RCs are needed (3).

The present study explores and compares for the first time, both the PCPT v2 + free PSA and the ERSPC + DRE, not only for accuracy but also for variability and clinical relevance. Our group previously explored the accuracy and variability of the ERSPC + DRE RC (14) but, in this study, we have specifically focused only on patients in the grey zone (PSA 3-10ng/ml) and compared the ERSPC + DRE RC vs. the PCPT v2 + free PSA, an analysis that has not been previously performed. This comparison showed that both RCs had similar accuracy for the discrimination of Sig PCa. However, ERSPC-RC had better calibration and stability for intra-individual PSA variations. Our methodology in calculating the volume is an estimation from the results of the TRUS measure, similar to Poyet *et al*, and following the recommendations of Roobol *et al.* (4). We have focused only on those patients with PSA between 3-10 ng/ml who require additional diagnostic information. The PCPT-RC option with free PSA, which increases the accuracy of discrimination between Sig PCa and no PCa (5), was calculated, as it is an easy-to-use and readily available tool for these patients.

As defined in the methodology, the first measure (ERSPC1 and PCPT1) was the focus of the direct comparison, as this was used as the indication for biopsy. The accuracy of both RCs was similar for Sig PCa in our study, showing an accuracy similar to other external validations such as Poyet *et al*. (9) and Foley *et al.* (7) for ESRPC (AUC= 0.73 and 0.74, respectively) and a better accuracy for PCPT1 v2.0 when adding free PSA (AUC= 0.70 and 0.69, respectively). Still, these results are far from ideal, and thus, additional data from imaging or fluid markers might be included to improve the accuracy of the RCs. In agreement with the accuracy results, the decision curve analysis was also similar between both RCs. In fact, both RCs showed a net benefit from an early risk threshold, which means that their implementation would be useful in the pathway of patient selection.

Studying the variability of the RCs improves our knowledge about their stability, which could translate into improved decision-making and selection of patients. Our PSA cohort showed a variability that was in the range of that previously shown in the literature (11,12,29). Our group and others (14,30) have demonstrated that a higher PSA variability is associated with a reduced risk of Sig PCa in a prostate biopsy, but it does not improve the accuracy of a RC. However, probability stability is important in order to trust RC probabilities at any point. Our study shows good agreement between the two ERSPC + DRE-RC probabilities, with good calibration and stability despite intra-individual PSA variations. PCPTv.2 + free PSA shows worse stability and higher variability, which could be explained simply by the fact that it uses two values (PSA and free PSA) that suffer from this variability (31), while the use of an estimated volume in the ERSPC dilutes the PSA variability. These results should be interpreted with caution, as volume estimation was performed by categorization of TRUS and not by DRE. It is true that this categorization has previously shown good correlation (4). This likely depends on prostate volume (32), as well as low but certain inter-examiner variability (33), which could also increase ERPSC variability in an inter-clinician comparison. It should also be taken into account that the clinical translation of this stability is not clear, firstly, because of the limitation of the use of a single estimated prostate volume and because the global accuracy of both RC are not significantly different, and seems to have a tendency to improve in the PCPT2 RC.

Calibration plots show that both models (PCPT-RC and ERSPC-RC) predict adequately only the actual risk of PCa and Sig PCa for low-risk patients, with a wider useful range in the case of PCa and a lower range in the case of Sig PCa. For higher risk patients, the calibration curves become irregular. This effect is accentuated for risks close to 1, as both models predict maximum risks of around 0.75 for Sig PCa. The models would benefit from recalibration for our population in the low-moderate risk region, considering that this is the region of greater interest for the model, as patients with a high predicted risk would probably undergo biopsy anyway. Nonetheless, despite not showing a good calibration in the usual range for clinical decision (0-0.3), visually ERSPC seems to be more consistent with a less fluctuating calibration in this range compared to PCPT, but at this point, this should be confirmed in future studies because no conclusion for direct comparison about calibration could be reached in the present study as quantitative analysis is outside the aim of this research. The comparison of coefficients between PCPT1 and PCPT2 and between PCPT2 and ERSPC2 showed that the differences between PCPT1 and PCPT2 were similar to those between ERSPC and PCPT models. As previously discussed ERSPC seems to be more insensitive and, therefore, robust to intra-individual variations of PSA compared to PCPT, while the predictive performance is similar and the clinical translation not clear yet.

Despite the similar decision curve, results from the sensitivity, specificity and ROC curve analysis show that the same risk threshold should not be used for both models. Both RCs are able to have similar performance, and the benefit of using any of them is similar in order to screen patients for a prostate biopsy, if the correct cut-off point is selected. It should be highlighted the importance of having an almost 100% negative predictive value, as the advantage of reducing unnecessary biopsies should not be at the cost of missing or delaying the diagnoses of a Sig PCa.

In clinical practice, the use of these RCs should be the first step in guiding the decision for further management of the patient. Patients with a confirmed, elevated PSA between 3-10ng/ml should be better stratified using other variables within a RC, as men with PSA levels >10ng/mL are likely to

proceed to biopsy regardless of other factors. Probably a specific cut-off point in the risk probability should not be used and take advantage of the known probabilities to discuss with the patient the biopsy indication as recommended by the PCPT-RC. In the situation in which the patient is in the low-risk group, according to both RCs (ERSPC and PCPT), the patient could continue with just follow-up. This fact has also been proposed by Alberts *et al.* (34) when applying new diagnostic markers, such as mpMRI. Specifically, they showed that following a negative recommendation from the ERSPC-RC would have avoided 62 (51%) of 122 mpMRIs and two (25%) of eight insignificant PCa diagnoses, missing three (10%) of 31 high-grade PCa. As the positive predictive value of these RCs is not as good as their negative predictive value, in case of discordance between both RCs or if there is an indication for a biopsy according to both RCs, other images or fluid biomarkers could increase the accuracy in order to potentially reduce the harm from unnecessary prostate biopsy and over-diagnosis (35). Specifically, Loeb *et al.* (36) has recently demonstrated that the incorporation of PHI into both RCs increases the accuracy of the diagnoses of Sig PCa. Another relevant point should be comment from the tendency of better predictive ability with the second evaluations of PSA, reinforcing the idea of the need of several PSA values to confirm the risk and discarded confounding factors. Furthermore, this analysis could suggest a tend to better discrimination ability of PCPT in the range of lower probabilities (when PSA is low), but further research would be needed to validate this affirmation. These risk calculators only show a static probability so other longitudinal variables and clinical judgment should be required for their application.

The present study has some limitations. First, despite the prospectively collected information, it is a retrospective study design. Second, prostate volume was an estimation and categorization from a TRUS calculation, and, therefore, it is not the actual approach for which the RC was developed. Third, the PSA values interval was not the same for all patients, which means the results should be interpreted with caution. Four, the use of TRUS biopsy for PCa diagnosis, although is the standard in most populations, suffers from random error compared with template biopsy (37), which could have

affected prediction results. However, the clinical information was extracted from a clinical practice cohort and with information that could be useful for urologists worldwide.

Altogether, our results showed that: 1) the use of both RCs (ERSPC and PCPT) could be an useful tool in the selection of patients who need prostate biopsy, and that both RCs showed similar accuracy for discrimination of Sig PCa; 2) ERSPC-RC showed higher stability than PCPT-RC for intra-individual PSA variations; 3) when comparing both RCs sensitivity and specificity, a higher rate of biopsies could be avoided with the ERSPC-RC vs. the PCPT-RC, but with a higher rate of Sig PCa missed. Thus, in those patients with a PSA between 3-10 ng/ml, these tools should be used in order to improve selection and specificity. The RCs specifically should be selected according to the variables available in the clinic. In addition, both RCs could also be used and the decision to undergo a biopsy be shared with the patient.

CONFLICTS OF INTEREST

Nothing to declare

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin [Internet]. 2018/01/04. 2018;68(1):7–30. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29313949
2. Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. Eur Urol [Internet]. 2016/08/25. 2017;71(4):618–29. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27568654
3. Louie KS, Seigneurin A, Cathcart P, Sasieni P. Do prostate cancer risk models improve the predictive accuracy of PSA screening? A meta-analysis. Ann Oncol [Internet]. 2015;26(5):848–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25403590
4. 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 [Internet]. 2012;61(3):577–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22104592
5. Ankerst DP, Hoefler J, Bock S, Goodman PJ, Vickers A, Hernandez J, et al. Prostate Cancer Prevention Trial risk calculator 2.0 for the prediction of low- vs high-grade prostate cancer. Urology [Internet]. 2014;83(6):1362–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24862395
6. Lundon DJ, Kelly BD, Foley R, Loeb S, Fitzpatrick JM, Watson RW, et al. Prostate cancer risk assessment tools in an unscreened population. World J Urol [Internet]. 2015;33(6):827–32. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25091862
7. Foley RW, Maweni RM, Gorman L, Murphy K, Lundon DJ, Durkan G, et al. The ERSPC Risk Calculators Significantly Outperform The PCPT 2.0 In The Prediction Of Prostate Cancer; A Multi-Institutional Study. BJU Int [Internet]. 2016; Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26833820

8. Foley RW, Lundon DJ, Murphy K, Murphy TB, Galvin DJ, Watson RW. Predicting prostate cancer: analysing the clinical efficacy of prostate cancer risk calculators in a referral population. Ir J Med Sci [Internet]. 2015;184(3):701–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25843017

9. Poyet C, Nieboer D, Bhindi B, Kulkarni GS, Wiederkehr C, Wettstein MS, et al. Prostate cancer risk prediction using the novel versions of the European Randomised Study for Screening of Prostate Cancer (ERSPC) and Prostate Cancer Prevention Trial (PCPT) risk calculators: independent validation and comparison in a contemporary Europe. BJU Int [Internet]. 2016;117(3):401–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26332503

10. Murthy V, Rishi A, Gupta S, Kannan S, Mahantshetty U, Tongaonkar H, et al. Clinical impact of prostate specific antigen (PSA) inter-assay variability on management of prostate cancer. Clin Biochem [Internet]. 2015/10/23. 2016;49(1–2):79–84. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26506115

11. Komatsu K, Wehner N, Prestigiacomo AF, Chen Z, Stamey TA. Physiologic (intraindividual) variation of serum prostate-specific antigen in 814 men from a screening population. Urology [Internet]. 1996;47(3):343–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8633399

12. Morote J, Raventós CX, Lorente JA, Enbabo G, López M, de Torres I. Intraindividual variations of total and percent free serum prostatic-specific antigen levels in patients with normal digital rectal examination. Eur Urol [Internet]. 1999;36(2):111–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10420031

13. Lavallée LT, Binette A, Witiuk K, Cnossen S, Mallick R, Fergusson DA, et al. Reducing the Harm of Prostate Cancer Screening: Repeated Prostate-Specific Antigen Testing. Mayo Clin Proc [Internet]. 2015/12/10. 2016;91(1):17–22. Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26688045

14. Gómez-Gómez E, Carrasco-Valiente J, Blanca-Pedregosa A, Barco-Sánchez B, Fernandez-Rueda JL, Molina-Abril H, et al. European Randomized Study of Screening for Prostate Cancer Risk Calculator: External Validation, Variability, and Clinical Significance. Urology. 2017;102.

15. Epstein JI, Allsbrook WC, Amin MB, Egevad LL, Committee IG. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol [Internet]. 2005;29(9):1228–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16096414

16. Fawcett T. An introduction to ROC analysis. Pattern Recognit Lett [Internet]. 2006 Jun 1 [cited 2018 Dec 20];27(8):861–74. Available from: https://www.sciencedirect.com/science/article/pii/S016786550500303X

17. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics [Internet]. 1988 Sep [cited 2018 Dec 20];44(3):837–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3203132

18. Zhou X-H, McClish DK, Obuchowski NA, Electronic Book Collection., Wiley InterScience (Online service). Statistical methods in diagnostic medicine [Internet]. Wiley; 2011 [cited 2018 Dec 20]. 545 p. Available from: https://www.wiley.com/en-us/Statistical+Methods+in+Diagnostic+Medicine%2C+2nd+Edition-p-9780470183144

19. Hendriksen JMT, Geersing GJ, Moons KGM, de Groot JAH. Diagnostic and prognostic prediction models. J Thromb Haemost [Internet]. 2013 Jun [cited 2018 Dec 20];11:129–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23809117

20. Vickers AJ, Elkin EB. Decision Curve Analysis: A Novel Method for Evaluating Prediction Models. Med Decis Mak [Internet]. 2006 Nov 5 [cited 2018 Dec 20];26(6):565–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17099194

21. Loeb S, Bjurlin MA, Nicholson J, Tammela TL, Penson DF, Carter HB, et al. Overdiagnosis and overtreatment of prostate cancer. Eur Urol [Internet]. 2014;65(6):1046–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24439788

22. Loeb S, Lilja H, Vickers A. Beyond prostate-specific antigen: utilizing novel strategies to screen men for prostate cancer. Curr Opin Urol [Internet]. 2016;26(5):459–65. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27262138

23. Johnston E, Pye H, Bonet-Carne E, Panagiotaki E, Patel D, Galazi M, et al. INNOVATE: A prospective cohort study combining serum and urinary biomarkers with novel diffusion-weighted magnetic resonance imaging for the prediction and characterization of prostate cancer. BMC Cancer [Internet]. 2016;16(1):1–11. Available from: http://dx.doi.org/10.1186/s12885-016-2856-2

24. McDonald ML, Parsons JK. 4-Kallikrein Test and Kallikrein Markers in Prostate Cancer Screening. Urol Clin North Am [Internet]. 2016;43(1):39–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26614027

25. Lepor A, Catalona WJ, Loeb S. The Prostate Health Index: Its Utility in Prostate Cancer Detection. Urol Clin North Am [Internet]. 2016;43(1):1–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26614024

26. De Luca S, Passera R, Cappia S, Bollito E, Randone DF, Milillo A, et al. Fluctuation in prostate cancer gene 3 (PCA3) score in men undergoing first or repeat prostate biopsies. BJU Int [Internet]. 2014/04/29. 2014;114(6b):E56-61. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24472071

27. Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. Lancet [Internet]. 2017/01/20. 2017;389(10071):815–22. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28110982

28. Grill S, Fallah M, Leach RJ, Thompson IM, Freedland S, Hemminki K, et al. Incorporation

of detailed family history from the Swedish Family Cancer Database into the PCPT risk calculator. J Urol [Internet]. 2014/09/19. 2015;193(2):460–5. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25242395

29. Sölétormos G, Semjonow A, Sibley PE, Lamerz R, Petersen PH, Albrecht W, et al. Biological variation of total prostate-specific antigen: a survey of published estimates and consequences for clinical practice. Clin Chem [Internet]. 2005;51(8):1342–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15961552

30. Nordström T, Adolfsson J, Grönberg H, Eklund M. Repeat Prostate-Specific Antigen Tests Before Prostate Biopsy Decisions. J Natl Cancer Inst [Internet]. 2016;108(12). Available from: https://www.ncbi.nlm.nih.gov/pubmed/27418620

31. Ankerst DP, Gelfond J, Goros M, Herrera J, Strobl A, Thompson IM, et al. Serial Percent Free Prostate Specific Antigen in Combination with Prostate Specific Antigen for Population Based Early Detection of Prostate Cancer. J Urol [Internet]. 2016/03/12. 2016;196(2):355–60. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26979652

32. Roehrborn CG, Girman CJ, Rhodes T, Hanson KA, Collins GN, Sech SM, et al. Correlation between prostate size estimated by digital rectal examination and measured by transrectal ultrasound. Urology [Internet]. 1997;49(4):548–57. Available from: https://www.ncbi.nlm.nih.gov/pubmed/9111624

33. Roehrborn CG, Sech S, Montoya J, Rhodes T, Girman CJ. Interexaminer reliability and validity of a three-dimensional model to assess prostate volume by digital rectal examination. Urology [Internet]. 2001;57(6):1087–92. Available from: https://www.ncbi.nlm.nih.gov/pubmed/11377314

34. Alberts AR, Schoots IG, Bokhorst LP, van Leenders GJ, Bangma CH, Roobol MJ. Risk-based Patient Selection for Magnetic Resonance Imaging-targeted Prostate Biopsy after Negative Transrectal Ultrasound-guided Random Biopsy Avoids Unnecessary Magnetic Resonance Imaging Scans. Eur Urol [Internet]. 2016;69(6):1129–34. Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26651990

35. Chiu PK, Alberts AR, Venderbos LDF, Bangma CH, Roobol MJ. Additional benefit of using a risk-based selection for prostate biopsy: an analysis of biopsy complications in the Rotterdam section of the European Randomized Study of Screening for Prostate Cancer. BJU Int [Internet]. 2017/06/05. 2017;120(3):394–400. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28498624

36. Loeb S, Shin SS, Broyles DL, Wei JT, Sanda M, Klee G, et al. Prostate Health Index improves multivariable risk prediction of aggressive prostate cancer. BJU Int [Internet]. 2016/11/22. 2017;120(1):61–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27743489

37. Ahmed HU, Hu Y, Carter T, Arumainayagam N, Lecornet E, Freeman A, et al. Characterizing clinically significant prostate cancer using template prostate mapping biopsy. J Urol [Internet]. 2011/06/15. 2011;186(2):458–64. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21679984

Footnotes

- **Contributors** E.G.G, R.M.L, MJ.R.T and J.C.V carried out the conception and design of the study; E.G.G, JJ.S.B, J.C.V, AM.B, J.V.R, I.M.B.A, J.M.L, JM.J.V contributed to the data acquisition; E.G.G, J.C.V, J.L.F.R, RM.L and MJ.R.T carried out the analysis and interpretation of data; E.G.G, J.C.V and RM.L drafted the manuscript; JJ.S.B, J.L.F.R, AM.B, J.V.R, J.M.L, JM.J.V, and MJ.R.T carried out a critical revision of the manuscript for important intellectual content; E.G.G, and J.L.F.R performed the statistical analysis; RM.L, MJ.R.T and J.C.V supervised the work.
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- **Competing interests** None declared.
- **Ethics approval** This study was performed as part of the ONCOVER project. Ethical approval was obtained by the Reina Sofia Hospital Research Ethics Committee in accordance with the Declaration of Helsinki and informed consent was obtained from all participants for the project.
- **Provenance and peer review** Not commissioned; externally peer reviewed.
- **Data sharing statement** All data is shown within the manuscript
- **Patient consent for publication** Not required.

Figure legends

Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values: **A**, for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa; **B**, for the ERSPC1-RC and the ERSPC2-RC for positive biopsy; **C**, for the PCPT1-RC and the PCPT2-RC for positive biopsy; and **D**, for the ERSPC2-RC and the PCPT2-RC for Sig PCa.

Figure 2: Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are underestimated from the lower right, where they are overestimated. **A**, Calibration plots for ERSPC1-RC Sig PCa risk estimation. **B**, Calibration plots for PCPT1-RCSig PCa risk estimation.

Figure 3: Results of the decision curve analysis. **A**, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1–RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating all patients (dashed line) and treating none (thin line). **B**, Plot demonstrating net reduction of interventions per 100 patients using the ERSPC-RC (black line) and the PCPT-RC (grey line).

Figure 4: Graphics showing Cohen's k coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. **A**, Agreement between ERSPC1-RC and ERSPC2-RC for Sig PCa. **B**, Agreement between PCPT1-RC and PCPT2-RC for Sig PCa. **C**, Agreement between ERSPC1-RC and PCPT1-RC for Sig PCa.

Figure 5: Graphics showing sensitivities and specificities of both RCs along the clinically useful risk threshold. The ERSPC-RC (black line) and the PCPT-RC (grey line).

Table 1. Clinical and demographic characteristics of the cohort of patients categorized according to cancer status.

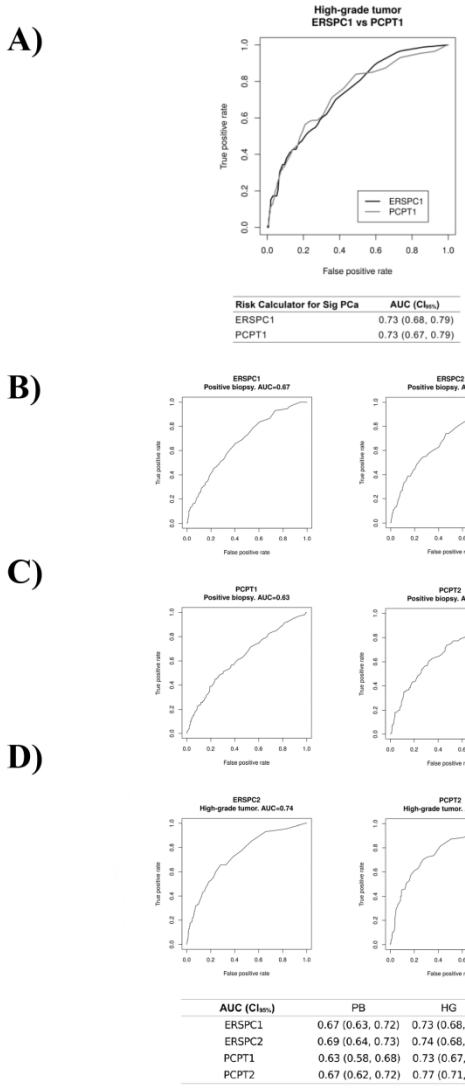
Variable	No Sig PCa n=423	Sig PCa n=87	All n=510
Age	64.0 (60.0-69.0)	68.0 (63.0-71.0)	65.0 (60.0-70.0)
Family History	81 (19.1)	8 (9.2)	89 (17.5)
Positive DRE	55 (13.0)	27 (31.0)	82 (16.1)
1 Serum PSA	5.3 (4.3-6.9)	5.8 (4.5-7.2)	5.3 (4.3-6.9)
1 free PSA %	16.2 (12.4-21.4)	12.5 (9-16.6)	15.9 (11.8-20.4)
2 Serum PSA	5.0 (3.7-6.6)	5.4 (4.1-6.7)	5.0 (3.8-6.6)
2 free PSA %	17.9 (13.9-23.4)	12.5 (9.1-16.3)	16.9 (12.8-22.1)
Prostate volume	38.0 (29.0-50.0)	26.0 (20.7-34.0)	35 (26-49)
First Biopsy	322 (76.1)	79 (90.8)	401 (78.6)
PCPT1 Sig PCa	0.08 (0.05-0.13)	0.16 (0.10-0.30)	0.09 (0.06-0.15)
ERSPC1 Sig PCa	0.05 (0.02-0.10)	0.12 (0.05-0.31)	0.05 (0.03-0.12)
PCPT2 Sig PCa	0.07 (0.04-0.11)	0.16 (0.08-0.27)	0.07 (0.05-0.13)
ERSPC2 Sig PCa	0.04 (0.02-0.08)	0.12 (0.05-0.30)	0.05 (0.02-0.11)
PCa	89 (21)	87 (100)	176 (34.5)

PCa= Prostate cancer; **Sig PCa**= significant PCa (Gleason ≥ 7 on biopsy); **No Sig PCa**= No cancer or non-significant PCa; **ERSPC1 / PCPT1 Sig PCa** = Probability of high grade PCa using the first measurement of serum PSA (at the time of biopsy indication by the urologist); **ERSPC2 / PCPT2 Sig PCa** = Probability high grade PCa using the second measurement of serum PSA (just before undergoing prostate biopsy). Median values (interquartile range) are expressed for quantitative variables, and absolute values (percentage) for qualitative variables.

548

549

1



Risk Calculator for Sig PCa	AUC ($CI_{95\%}$)
ERSPC1	0.73 (0.68, 0.79)
PCPT1	0.73 (0.67, 0.79)

AUC ($CI_{95\%}$)	PB	HG
ERSPC1	0.67 (0.63, 0.72)	0.73 (0.68, 0.79)
ERSPC2	0.69 (0.64, 0.73)	0.74 (0.68, 0.80)
PCPT1	0.63 (0.58, 0.68)	0.73 (0.67, 0.79)
PCPT2	0.67 (0.62, 0.72)	0.77 (0.71, 0.82)

Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values: A, for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa; B, for the ERSPC1-RC and the ERSPC2-RC for positive biopsy; C, for the PCPT1-RC and the PCPT2-RC for positive biopsy; and D, for the ERSPC2-RC and the PCPT2-RC for Sig PCa.

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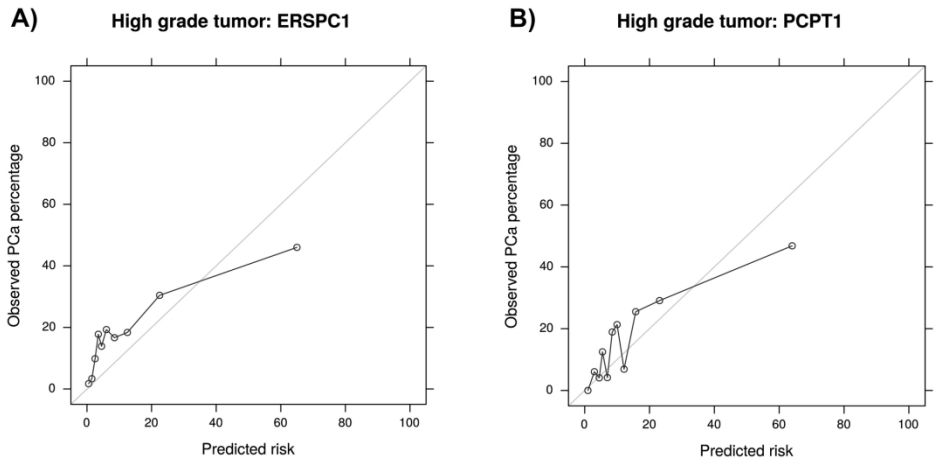

Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are underestimated from the lower right, where they are overestimated. A, Calibration plots for ERSPC1-RC Sig PCa risk estimation. B, Calibration plots for PCPT1-RCSig PCa risk estimation.

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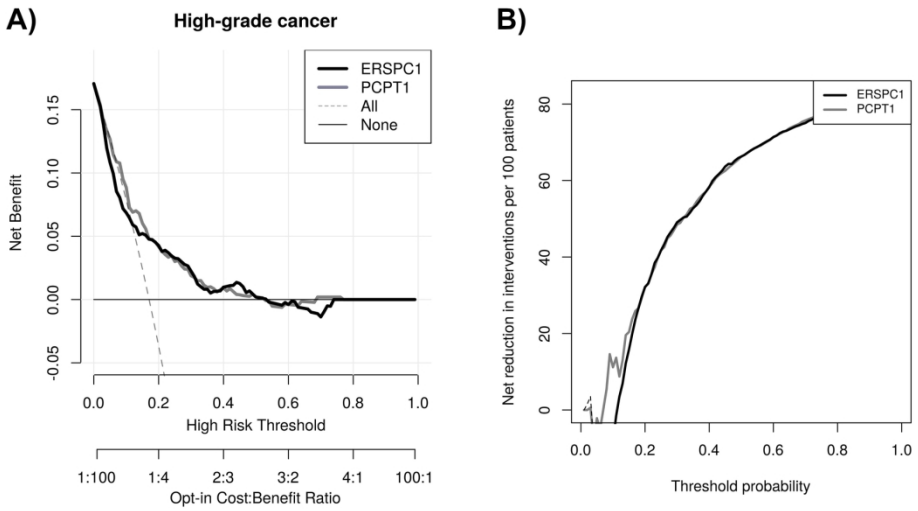

Results of the decision curve analysis. A, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1–RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating all patients (dashed line) and treating none (thin line). B, Plot demonstrating net reduction of interventions per 100 patients using the ERSPC-RC (black line) and the PCPT-RC (grey line).

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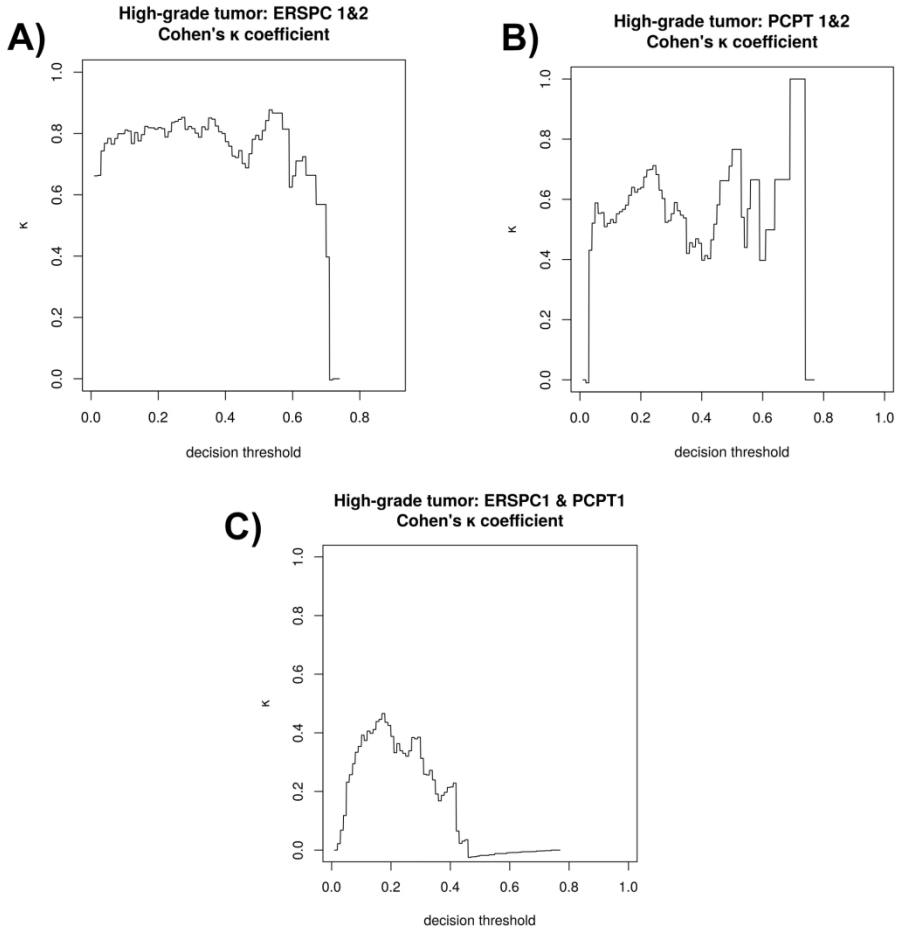
4



Graphics showing Cohen's k coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. A, Agreement between ERSPC1-RC and ERSPC2-RC for Sig PCa. B, Agreement between PCPT1-RC and PCPT2-RC for Sig PCa. C, Agreement between ERSPC1-RC and PCPT1-RC for Sig PCa.

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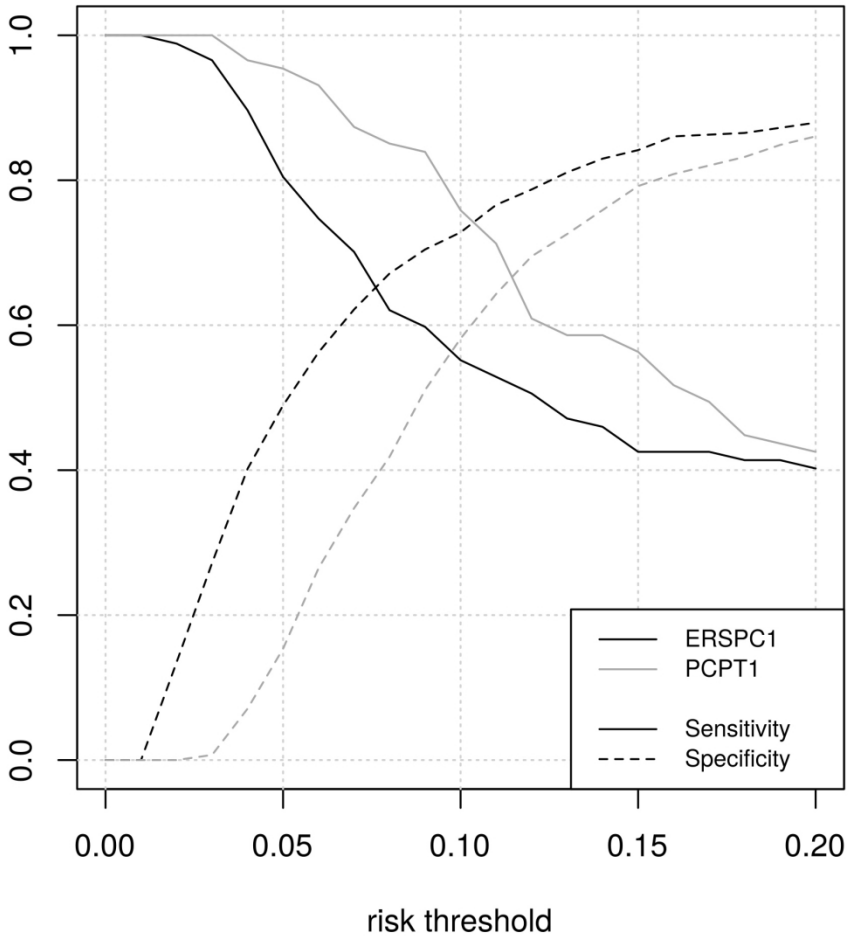


Graphics showing sensitivities and specificities of both RCs along the clinically useful risk threshold. The ERSPC-RC (black line) and the PCPT-RC (grey line).

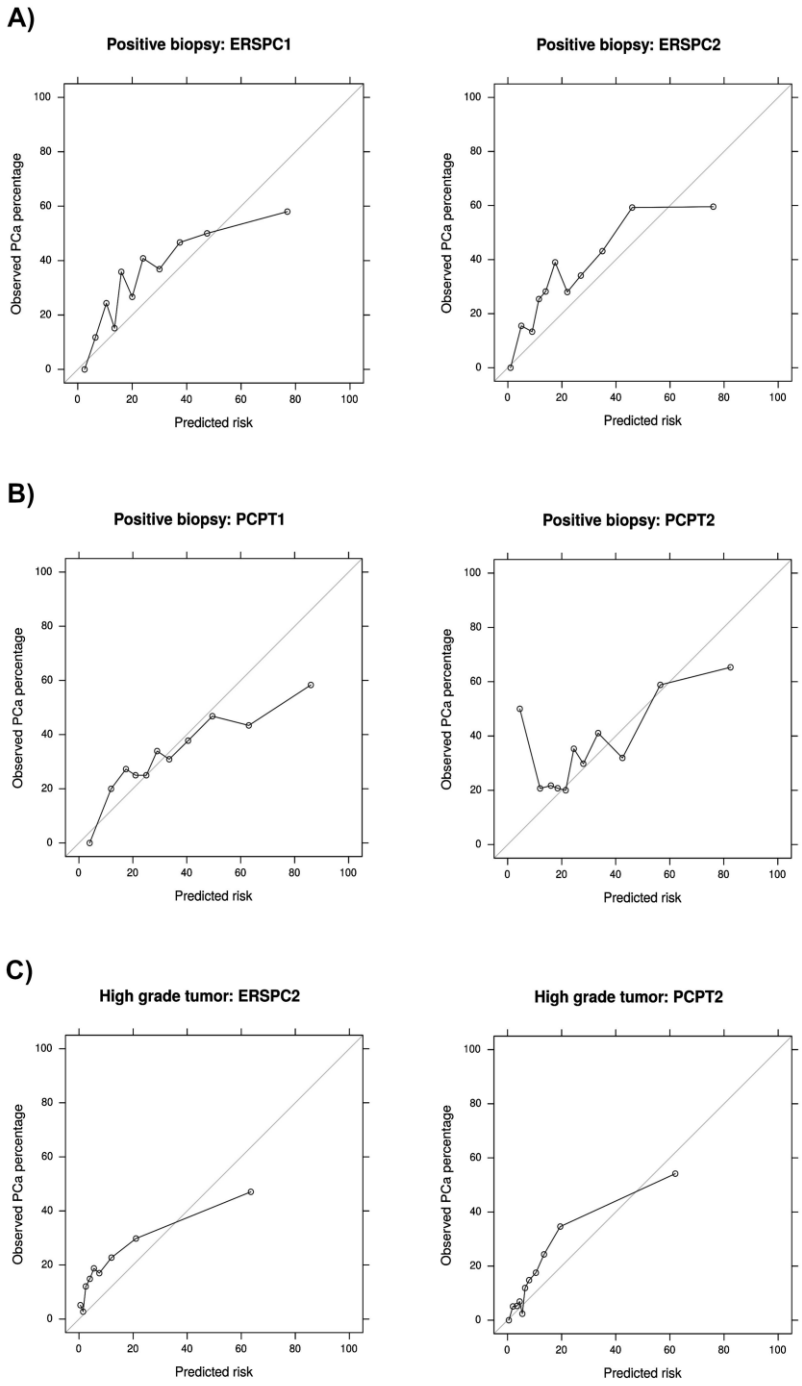
Supplemental Table 1. Patients excluded from the ONCOVER cohort for this study depends on the exclusion criteria.

Exclusion criteria	Number
Under active surveillance	25
2 consecutive PSA levels well recorded or affected	50
Prostate volume not well recorded	177
PSA of biopsy indication out of the range 3-10ng/ml , or Age out of the range 55-80	251
Total exclusions	511

Supplemental table 2: DeLong p values resulting from the pairwise comparison of the Area under the Receiver Operator Characteristic (ROC) curve (AUC) between Risk Calculators for significant Prostate cancer (Sig PCa) detection.

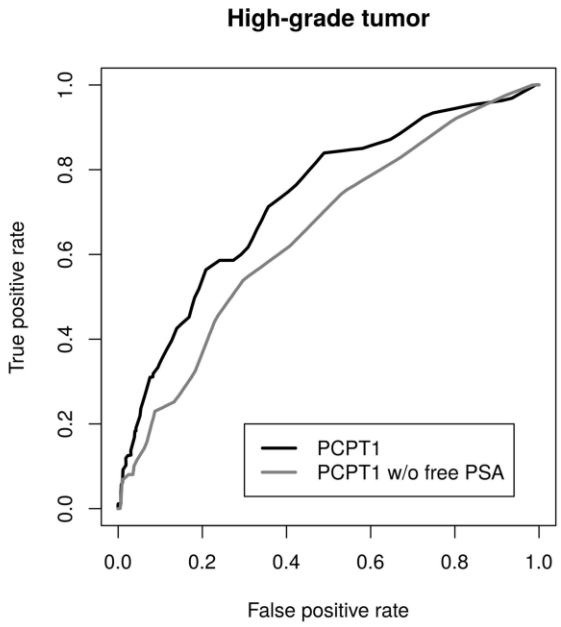
Sig PCa (p-value)	ERSPC1	ERSPC2	PCPT1	PCPT2
ERSPC1	X	0.51	0.95	0.19
ERSPC2	X	X	0.74	0.25
PCPT1	X	X	X	0.06

S1



Supplemental Figure 1: Calibration plots of the RCs in this cohort, demonstrating the agreement between predicted and observed probabilities: **A**, of a positive biopsy for the ERSPC1-RC and the ERSPC2-RC; **B**, of a positive biopsy for the PCPT1-RC and for the PCPT2-RC; and **C**, of a Sig PCa on the biopsy for the ERSPC2-RC and the PCPT2-RC.

S2



Risk Calculator for Sig PCa	AUC ($CI_{95\%}$)
PCPT1 without free PSA	0.65 (0.59, 0.71)
PCPT1 + free PSA	0.73 (0.67, 0.79)
DeLong p value: 0.02	

Supplemental Figure 2: Receiver Operating Characteristic curves and Area Under the Curve values for the PCPT1-RC without free PSA (black) and for the PCPT1-RC with free PSA (grey) to predict Sig PCa. P-value according to the DeLong test.

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	*8*
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-10
		(*b*) Describe any methods used to examine subgroups and interactions	8-10
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	8-10
		(*e*) Describe any sensitivity analyses	8-10
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	Supplemental
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	11- Supplemental
Outcome data	15*	Report numbers of outcome events or summary measures	12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supplemental
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Observational study comparing the accuracy/variability between the ERPSC and the PCPT risk calculators for the prediction of significant prostate cancer in patients with PSA <10ng/ml

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Primary Subject Heading:	Urology
Secondary Subject Heading:	Urology
Keywords:	Significant prostate cancer, risk calculator variability, ERSPC, PCPT

Observational study comparing the accuracy/variability between the ERPSC and the PCPT risk calculators for the prediction of significant prostate cancer in patients with PSA <10ng/ml

Authors: Gómez-Gómez E[1,2#], Salamanca-Bustos JJ[2], Carrasco-Valiente J[1,2], Fernández-Rueda J.L[3], Blanca AM[1], Valero-Rosa J[1,2], Bravo-Arrebola I.M[2], Márquez-López J[1,2], Jiménez-Vacas JM[1,4,5], Luque RM[1,4,5#], Requena-Tapia MJ[1,2].

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Running title: Risk Calculators for Significant Prostate Cancer

Key Words: Significant prostate cancer; risk calculator variability; ERSPC; PCPT.

Tables and figures: 1 and 5

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Servicios Sociales e Igualdad, Spain. This work has been awarded by the Real Academia de Medicina de Sevilla 2018. The funding agreement ensured the authors' independence in designing the study, interpreting the data, writing, and publishing the report.

ABSTRACT

Introduction: Risk Calculators (RCs) are easy-to-use tools considering available clinical variables that could help to select those patients with risk of prostate cancer (PCa) who should undergo a prostate biopsy. **Objective:** To perform a comparison for the prediction of significant-PCa (SigPCa) between the European-Randomised Study of Screening for PCa (ERSPC) and the PCa Prevention-Trial (PCPT) RCs in patients with PSA between 3-10ng/ml through an evaluation of the accuracy/variability between two consecutive PSA-values. **Setting:** An observational study in a major university hospital in the south of Spain. **Methods and participants:** An observational study was performed in patients who underwent a prostate biopsy. SigPCa probabilities were calculated with the two PSA measures using ERSPC3/4+DRE and PCPTv2+free-PSA RCs. The prediction of SigPCa was determined by the area under the curve (AUC). Calibration, discrimination, and decision curve analysis were studied. The variability between both RCs-agreement was compared using Cohen's kappa coefficient. **Results:** 510 patients were analysed (87 diagnosed with SigPCa). The median PSA value were 5.3 and 5ng/ml for PSA1 and PSA2 respectively. Both RCs overestimated the risk in the case of high-risk probabilities. Discriminative ability for SigPCa was similar between models with an AUC=0.73(0.68-0.79) for ERSPC-RC vs. 0.73(0.67-0.79) for PCPT-RC. ERSPC-RC showed less variability than PCPT-RC, with a constant agreement (k=0.7-0.8) for usual range of clinical decision-making. Remarkably, a higher number of biopsies would be avoided using the ERSPC-RC, but more SigPCa would be missed along all the risk probabilities. **Conclusions:** Both RCs performed similar in the prediction of SigPCa. However, ERSPC-RC seems to be more stable for intra-individual PSA variations.

Strengths and limitations of this study

- This study is the first to compare two available free risk calculators in patients with a PSA < 10ng/ml analyzing their variability between two consecutive different PSA levels.
- One of the study limitations is that prostate volume was an estimation and categorization from a TRUS calculation, and, therefore, it is not the actual approach for which the RC was developed.
- Although the clinical information of this study was extracted from a clinical practice cohort and with information that could be useful for urologists worldwide, this is a retrospective study and the use of TRUS biopsy for PCa diagnosis, even though it is the standard in most populations, suffers from random error compared with template biopsy, which could have affected prediction results.

INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed malignancy in males worldwide, and the most frequent in developed countries(1). Its current standard of diagnosis is a prostate biopsy based on PSA levels and digital rectal examination (DRE). However, there are other available and complementary variables that could help to select those patients who should undergo a prostate biopsy (such as age, prostate volume, free PSA, family history, etc.), but these are not always used and/or well-integrated in daily clinical practice(2). In line with this, Risk Calculators (RCs) are easy-to-use tools that can help the clinicians to take advantage of all these available variables (3). The two main RCs are from the European Randomised Study for Screening of Prostate Cancer (ERSPC cohort; ERSPC-RC: http://www.prostatecancer-riskcalculator.com/seven-prostate-cancer-riskcalculators) and from the Prostate Cancer Prevention Trial (PCPT cohort; PCPT-RC: http://deb.uthscsa.edu/URORiskCalc/Pages/calcs.jsp). Both RCs have undergone some modifications, specifically the addition of estimated prostate volume in the ERSPC-RC (4,5). Furthermore, both RCs were originally developed from different patient cohorts and each RC uses different variables.

To date, limited external validations and comparisons have been performed by different groups (6–9). The two most important recent comparisons of the modified RCs were performed by Foley *et al* (6,7) and Poyet *et al* (9). Both found a better discriminative ability for ERSPC-RC vs PCPTv.2-RC for the diagnoses of significant-PCa (Sig PCa) (AUC around 0.74 vs 0.69, respectively), but they also included patients with a high PSA of up to 50 ng/ml. Despite the possibility of using these RCs in patients with PSA levels up to 50ng/ml, it is clear that the advantages of using both RCs would probably increase in patients with a PSA under 10ng/ml, where the rate of positive biopsy for PCa clearly decrease, with an important number of unnecessary biopsies. Furthermore, in the case of the PCPTv.2-RC, the addition of the free PSA value in patients with a PSA under 10ng/ml seems to improve its predictive ability (5), and, therefore, given its accessibility, this value should be included in the RC.

The intra-individual and inter-assay variability of PSA is already known (10–12) and, therefore, at least two measures are necessary before a prostate biopsy is indicated. In fact, it has been shown that approximately 25% of men with initial PSA levels between 4 and 10 ng/mL had normal PSA values upon repeat testing (13). In line with this, despite being primarily based on PSA level, the variability of the two RCs mentioned above has been poorly studied, and might have implications for patient management. Our group has recently evaluated this variability with the ERSPC-RC, which showed stable accuracy over a cohort of patients, but some changes with respect to an individual approach (14). To date, there is no study comparing the accuracy and variability of both RCs, the ERSPC + DRE vs. the PCPTv.2 + free PSA, for the prediction of Sig PCa. Therefore, the aim of this study was to perform a direct comparison between ERSPC + DRE and PCPTv.2 + free PSA RCs in patients with a PSA between 3-10ng/ml, evaluating the accuracy and variability of both methods in the prediction of Sig PCa.

MATERIALS AND METHODS

Study population and design

An observational retrospective study was performed in patients from ONCOVER cohort (1021 biopsies indicated by clinical practice wherein patients donated blood and urine before the biopsy). The study was carried out within the project approved by our Hospital Research Ethics Committee, and informed consent was obtained from all participants. Blood sample was obtained in the morning (between 8:00-10:00 am) after fasting overnight and then, the prostate biopsy was implemented according to clinical practice. The inclusion criteria for this study were: 1) PSA indication between 3-10 ng/ml; 2) Full clinical and laboratory data to fulfill ERSPC-RC and PCPT-RC criteria; 3) Age 55-80 years old; 4) Two consecutives measurements of PSA levels within an interval of 12 weeks. Exclusion criteria included patients with a previously known PCa diagnosis or treatment that could modify PSA levels (Supplemental table 1).

Transrectal prostate biopsy was carried out under local anaesthesia by using a standard peri-prostatic block, a transrectal ultrasound transducer, and an 18G automated needle biopsy instrument. The prostatic volume was measured following the protocol used during transrectal ultrasound (TRUS), and usual recommendations were to take 12 cores in patients undergoing the first biopsy procedure, and a minimum of 16 biopsy cores for those who had a previous biopsy. Biopsy specimens were analysed by expert urologic pathologists according to the International Society of Urological Pathology (ISUP) 2005 modified criteria (15).

Main variables description

Demographic information and the medical history of each patient was obtained. PSA levels were measured twice within a period no longer than 12 weeks, as follows: 1) **PSA 1 and free PSA 1:** for biopsy indication; and, 2) **PSA 2 and free PSA 2:** before undergoing prostate biopsy. For both PSA measures were evaluated by Chemiluminescent Microparticle Immunoassays (ng/ml, by a CMIA;

Ref. 7k70; Abbott). The median and interquartile range of time between measurements was 6 (3-8) weeks.

Prostate volume: estimated by TRUS and categorized in three possible values, 25-40-60 ml, as recommended (4) (TRUS volume <30 = 25 cc, 30–50 = 40 cc, and ≥50 = 60 cc).

Significant/ high-grade (HG) prostate cancer (Sig PCa): PCa with a Gleason grade ≥ 7 on biopsy.

ERSPC-RC and PCPT-RC probabilities calculation

ERSPC: The formulas for the ERSPC-RC 3+DRE for patients at initial biopsy and the ERSPC-RC 4+DRE for patients at repeat biopsy were utilized in this study. These calculators use PSA, prostate volume, and DRE as variables, with, a negative prostate biopsy in ERSPC 4+ DRE in patients who had a previous biopsy. This provides a probability rating for any PCa or Sig PCa (Gleason ≥ 7).

ERSPC1/Sig PCa (1º Measure): Risk probability calculated by ERSPC-RC3 or 4 (if previous biopsy) + DRE; used PSA 1 to calculate the predicted risk for both any PCa and Sig PCa (HG PCa).

ERSPC2/Sig PCa (2º Measure): Risk probability calculated by ERSPC-RC 3 or 4 (if previous biopsy) + DRE; used PSA 2 to calculate the predicted risk for both any PCa and Sig PCa (HG PCa).

PCPT: The formulae for the PCPT-RC 2.0 + %free PSA was utilized in this study. This calculator uses race, age, PSA level, %free PSA level, family history of PCa, DRE and prior prostate biopsy. This gives a probability of negative biopsy, low grade PCa and Sig PCa (Gleason ≥ 7).

PCPT1: Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 1 and free PSA 1.

PCPT2: Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 2 and free PSA 2.

The variability of PSA was calculated by the following formula: **| Measure 1 – Measure2| / Measure 1**

Statistical analysis

A descriptive study was performed by calculating the median and interquartile ranges (IR) for the quantitative variables, and the absolute frequencies and percentages for the qualitative variables. A Student's T test for paired groups was used to compare the means of the quantitative variables (PSA 1 and 2).

The investigation of the comparative performance in the detection of Sig PCa of both RCs, ERSPC-RC and PCPT-RC, was performed, taking into account these four factors: discrimination capacity, calibration, clinical utility, and consistency against the observed variations in PSA levels for our dataset.

The discriminative ability of the models, i.e., their ability to separate those patients who had Sig PCa from those who do not, was assessed using the area under their Receiver Operator Characteristic (ROC) curve (AUC) (16), as measured in our sample. This is one of the most frequently used measurements of model discrimination, because of its independence of the selection of a specific decision threshold and its robustness against class imbalance. Confidence intervals for these AUCs were computed using bootstrapping. These AUCs were then compared to determine the relative performance of the models using DeLong tests (17). These tests were chosen because of their non-parametric nature, with few assumptions about the data, and their suitability for paired data, as both models were evaluated over the same dataset, properties which make this the most commonly used test to compare AUCs (18). For this comparison, we focused on the calculated risk score utilising the first measure of PSA (PSA 1; the value which met the criteria for a prostate biopsy).

The calibration of the calculators for our cohort was then investigated to determine the agreement between the frequency of the observed outcome (Sig PCa in our case) and the risks predicted by the model. Calibration plots were used for this purpose (19), enabling a visual evaluation of this agreement and the comparison between RCs.

To address the potential clinical utility of the models, we performed decision curve analysis on our data, as proposed by Vickers and Elkin (20). This method has the advantage of not requiring the

specification of the relative cost for false-positives and false-negatives, defining a net benefit as a function of the decision threshold at which one would consider obtaining a biopsy.

Finally, the stability of the predictions of both RCs, with regard to the observed intra-patient changes on PSA levels between measurements, was investigated using the Cohen's kappa (k) interrater agreement coefficient as a function of the decision threshold. This coefficient was selected due to its widespread use and robustness against random agreements, and thus, is a better measurement than naïve accuracy.

All the analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill) and R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria: URL https://www.R-project.org/). A <5% level of significance ($p<0.05$) was used to decide statistically significant differences.

Patient and public involvement

Participants and public were not involved in the development of research questions, study design or recruitment.

RESULTS

Cohort characteristics

In the present study, we analysed 510 patients who met the inclusion criteria previously described. Median age was 65 (60-70) years old, with a family history in 89 patients (17.5%) and a suspicious DRE in 82 patients (16.1%). The median PSA before prostate biopsy indication was 5.3 (4.3-6.9) ng/ml. 176 patients were diagnosed with PCa, 87 of those categorized as Sig PCa. Most patients (n=401; 78.6%) were biopsy-naïve and the median prostate volume was 35 (26-49) cc. Further cohort description according to Sig PCa status is shown in Table 1(and in Supplemental data).

66 patients had a PSA 2 out of the range of 3-10ng/ml due to the variability (50 patients below 3ng/ml and 16 patients above 10ng/ml); thus, in this case, the % free PSA was not calculated, and the risk probability was calculated without the inclusion of this variable. The patients were maintained in the analysis as reflecting the variability of the PSA, and the application of the models in this real situation, although acknowledging that it could introduce a bias in terms of calibration and variability.

Direct comparison for Sig PCa prediction

Discrimination ability for Sig PCa was no different between the two models [ERSPC1-RC vs. PCPT1-RC: 0.73; 95% CI: (0.68-0.79) vs. 0.73; 95% CI: (0.67-0.79), respectively]. ROC curves are shown in Figure 1A. Similarly, no difference was found in the discrimination ability for any PCa. The comparison of the RC for both measures is described in Figure 1(B-D) with similar results but a tendency of better accuracy for PCPT2-RC vs ERSPC2-RC (p= 0.25). Supplemental Table 2 shows multiple comparisons by the DeLong test resulting in no differences between the two RCs for Sig PCa.

Both models tended to overestimate the risk for a high probability of Sig PCa, and slightly underestimate it for low risk patients, suggesting that the models would benefit from a recalibration for our population (Figure 2). None of the models predicted very high probabilities for most patients. The calibration curves for any PCa are shown in Supplemental Figure 1.

The decision curve analyses revealed that both RCs provided a clinical net benefit in the threshold probability range for Sig PCa (Figure 3). The net benefit was comparable between the two RCs for Sig PCa.

As shown in Supplemental Figure 2, the addition of free PSA clearly improved the discriminative ability of the PCPT-RC [0.65 (0.59-0.71) PCPT1 v.2.0-RC vs. 0.73 (0.67- 0.79) PCPT1 v.2.0 + free PSA –RC; p= 0.02].

Variability and clinical significance

PSA and free PSA change was significantly different between the two measures, but with low clinical variations [average PSA1 5.69 ng/ml vs. PSA2 5.39 ng/ml ($p < 0.05$) and average free PSA1 16.99% vs. free PSA2 18.03% ($p < 0.05$)]. Median variability of PSA was 14% (6-27%). Taking into account this variability of PSA, ERSPC proved to be more stable than PCPT. The k agreement between ERSPC1 and ERSPC2 was practically constant, 0.79±0.09 for the usual range of clinical decision (0-0.3). However, PCPT1 and PCPT2 showed wider variations, with a k agreement of approximately 0.55±0.32 in the same range, with a subsequent rapid decrease. The agreement between both models (ERSPC1 vs. PCPT1) proved to be worse for thresholds in this range, peaking 0.47 for a 17% risk, with an average 0.32±0.12 on the interval. The comparison between ERSPC2 and PCPT2 yielded similar results (Figure 4).

Direct comparison of sensitivity and specificity of both RCs along the different clinical risk thresholds showed that PCPT-RC has higher sensitivity and lower specificity than ERPSC-RC for a given threshold along the clinically useful region (Figure 5). The balance point is reached at a different risk threshold for each RC. The performances of both RCs at this point are comparable, as shown in Figure 5. Considering the superposition of their respective ROC curves to a good approximation (Figure 1), this means that a transformation of decision thresholds can make both models perform similarly.

DISCUSSION

Currently, considerable research is being carried out to find new diagnostic markers for Sig PCa, in order to reduce the number of biopsies and the over-diagnosis of insignificant PCa (21). These markers are based on body fluids (blood, urine) or image explorations (22,23). Some are recommended by guidelines such as the 4k score test, PCA3, and/or the Prostate Health Index (PHI) in body fluids (only PCA3 and PHI have been approved by the FDA) (24–26), or multiparametric magnetic resonance imaging (mpMRI), with recent evidence of its advantages in biopsy-naïve patients (27). However, costs and availability minimize their implementation worldwide, and, therefore, it is clear that additional and readily available tools, such as RCs, should be implemented in daily clinical practice. The two most used RCs are ERSPC-RC and PCPT-RC, which have been modified and adapted (4,5). Few external validations have been conducted, with varying results (7,9,28). Usually, external validations of RCs show worse performance than the original validations (8), a fact that is corroborated by our study. Therefore, based on all this information, evaluations, validations, and incorporation of RCs are needed (3).

The present study explores and compares for the first time, both the PCPT v2 + free PSA and the ERSPC + DRE, not only for accuracy but also for variability and clinical relevance. Our group previously explored the accuracy and variability of the ERSPC + DRE RC (14) but, in this study, we have specifically focused only on patients in the grey zone (PSA 3-10ng/ml) and compared the ERSPC + DRE RC vs. the PCPT v2 + free PSA, an analysis that has not been previously performed. This comparison showed that both RCs had similar accuracy for the discrimination of Sig PCa. However, ERSPC-RC had better calibration and stability for intra-individual PSA variations. Our methodology in calculating the volume is an estimation from the results of the TRUS measure, similar to Poyet *et al.*, and following the recommendations of Roobol *et al.* (4). We have focused only on those patients with PSA between 3-10 ng/ml who require additional diagnostic information. The PCPT-RC option with free PSA, which increases the accuracy of discrimination between Sig PCa and no PCa (5), was calculated, as it is an easy-to-use and readily available tool for these patients.

As defined in the methodology, the first measure (ERSPC1 and PCPT1) was the focus of the direct comparison, as this was used as the indication for biopsy. The accuracy of both RCs was similar for Sig PCa in our study, showing an accuracy similar to other external validations such as Poyet *et al*. (9) and Foley *et al.* (7) for ESRPC (AUC= 0.73 and 0.74, respectively) and a better accuracy for PCPT1 v2.0 when adding free PSA (AUC= 0.70 and 0.69, respectively). Still, these results are far from ideal, and thus, additional data from imaging or fluid markers might be included to improve the accuracy of the RCs. In agreement with the accuracy results, the decision curve analysis was also similar between both RCs. In fact, both RCs showed a net benefit from an early risk threshold, which means that their implementation would be useful in the pathway of patient selection.

Studying the variability of the RCs improves our knowledge about their stability, which could translate into improved decision-making and selection of patients. Our PSA cohort showed a variability that was in the range of that previously shown in the literature (11,12,29). Our group and others (14,30) have demonstrated that a higher PSA variability is associated with a reduced risk of Sig PCa in a prostate biopsy, but it does not improve the accuracy of a RC. However, probability stability is important in order to trust RC probabilities at any point. Our study shows good agreement between the two ERSPC + DRE-RC probabilities, with good calibration and stability despite intra-individual PSA variations. PCPTv.2 + free PSA shows worse stability and higher variability, which could be explained simply by the fact that it uses two values (PSA and free PSA) that suffer from this variability (31), while the use of an estimated volume in the ERSPC dilutes the PSA variability. These results should be interpreted with caution, as volume estimation was performed by categorization of TRUS and not by DRE. It is true that this categorization has previously shown good correlation (4). This likely depends on prostate volume (32), as well as low but certain inter-examiner variability (33), which could also increase ERPSC variability in an inter-clinician comparison. It should also be taken into account that the clinical translation of this stability is not clear, firstly, because of the limitation of the use of a single estimated prostate volume and because the global accuracy of both RC are not significantly different, and seems to have a tendency to improve in the PCPT2 RC.

Calibration plots show that both models (PCPT-RC and ERSPC-RC) predict adequately only the actual risk of PCa and Sig PCa for low-risk patients, with a wider useful range in the case of PCa and a lower range in the case of Sig PCa. For higher risk patients, the calibration curves become irregular. This effect is accentuated for risks close to 1, as both models predict maximum risks of around 0.75 for Sig PCa. The models would benefit from recalibration for our population in the low-moderate risk region, considering that this is the region of greater interest for the model, as patients with a high predicted risk would probably undergo biopsy anyway. Nonetheless, despite not showing a good calibration in the usual range for clinical decision (0-0.3), visually ERSPC seems to be more consistent with a less fluctuating calibration in this range compared to PCPT, but at this point, this should be confirmed in future studies because no conclusion for direct comparison about calibration could be reached in the present study as quantitative analysis is outside the aim of this research. The comparison of coefficients between PCPT1 and PCPT2 and between PCPT2 and ERSPC2 showed that the differences between PCPT1 and PCPT2 were similar to those between ERSPC and PCPT models. As previously discussed ERSPC seems to be more insensitive and, therefore, robust to intra-individual variations of PSA compared to PCPT, while the predictive performance is similar and the clinical translation not clear yet.

Despite the similar decision curve, results from the sensitivity, specificity and ROC curve analysis show that the same risk threshold should not be used for both models. Both RCs are able to have similar performance, and the benefit of using any of them is similar in order to screen patients for a prostate biopsy, if the correct cut-off point is selected. It should be highlighted the importance of having an almost 100% negative predictive value, as the advantage of reducing unnecessary biopsies should not be at the cost of missing or delaying the diagnoses of a Sig PCa.

In clinical practice, the use of these RCs should be the first step in guiding the decision for further management of the patient. Patients with a confirmed, elevated PSA between 3-10ng/ml should be better stratified using other variables within a RC, as men with PSA levels >10ng/mL are likely to

proceed to biopsy regardless of other factors. Probably a specific cut-off point in the risk probability should not be used and take advantage of the known probabilities to discuss with the patient the biopsy indication as recommended by the PCPT-RC. In the situation in which the patient is in the low-risk group, according to both RCs (ERSPC and PCPT), the patient could continue with just follow-up. This fact has also been proposed by Alberts *et al.* (34) when applying new diagnostic markers, such as mpMRI. Specifically, they showed that following a negative recommendation from the ERSPC-RC would have avoided 62 (51%) of 122 mpMRIs and two (25%) of eight insignificant PCa diagnoses, missing three (10%) of 31 high-grade PCa. As the positive predictive value of these RCs is not as good as their negative predictive value, in case of discordance between both RCs or if there is an indication for a biopsy according to both RCs, other images or fluid biomarkers could increase the accuracy in order to potentially reduce the harm from unnecessary prostate biopsy and over-diagnosis (35). Specifically, Loeb *et al.* (36) has recently demonstrated that the incorporation of PHI into both RCs increases the accuracy of the diagnoses of Sig PCa. Another relevant point should be comment from the tendency of better predictive ability with the second evaluations of PSA, reinforcing the idea of the need of several PSA values to confirm the risk and discarded confounding factors. Furthermore, this analysis could suggest a tend towards better discrimination ability of PCPT in the range of lower probabilities (when PSA is low), but further research would be needed to validate this hypothesis. These risk calculators only show a static probability so other longitudinal variables and clinical judgment should be required for their application.

The present study has some limitations. First, despite the prospectively collected information, it is a retrospective study design. Second, prostate volume was an estimation and categorization from a TRUS calculation, and, therefore, it is not the actual approach for which the RC was developed. Third, the PSA values interval was not the same for all patients, which means the results should be interpreted with caution. Four, the use of TRUS biopsy for PCa diagnosis, although it is the standard in most populations, suffers from random error compared with template biopsy (37), which could

have affected prediction results. However, the clinical information was extracted from a clinical practice cohort and with information that could be useful for urologists worldwide.

Altogether, our results showed that: 1) the use of both RCs (ERSPC and PCPT) could be a useful tool in the selection of patients who need prostate biopsy, and that both RCs performed similar in the prediction of Sig PCa; 2) ERSPC-RC showed higher stability than PCPT-RC for intra-individual PSA variations; 3) when comparing both RCs sensitivity and specificity, a higher rate of biopsies could be avoided with the ERSPC-RC vs. the PCPT-RC, but with a higher rate of Sig PCa missed. Thus, in those patients with a PSA between 3-10 ng/ml, these tools should be used in order to improve selection and specificity. The RCs specifically should be selected according to the variables available in the clinic. In addition, both RCs could also be used and the decision to undergo a biopsy be shared with the patient.

CONFLICTS OF INTEREST

Nothing to declare.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin [Internet]. 2018/01/04. 2018;68(1):7–30. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29313949
2. Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. Eur Urol [Internet]. 2016/08/25. 2017;71(4):618–29. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27568654
3. Louie KS, Seigneurin A, Cathcart P, Sasieni P. Do prostate cancer risk models improve the predictive accuracy of PSA screening? A meta-analysis. Ann Oncol [Internet]. 2015;26(5):848–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25403590
4. 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 [Internet]. 2012;61(3):577–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22104592
5. Ankerst DP, Hoefler J, Bock S, Goodman PJ, Vickers A, Hernandez J, et al. Prostate Cancer Prevention Trial risk calculator 2.0 for the prediction of low- vs high-grade prostate cancer. Urology [Internet]. 2014;83(6):1362–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24862395
6. Lundon DJ, Kelly BD, Foley R, Loeb S, Fitzpatrick JM, Watson RW, et al. Prostate cancer risk assessment tools in an unscreened population. World J Urol [Internet]. 2015;33(6):827–32. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25091862
7. Foley RW, Maweni RM, Gorman L, Murphy K, Lundon DJ, Durkan G, et al. The ERSPC Risk Calculators Significantly Outperform The PCPT 2.0 In The Prediction Of Prostate Cancer; A Multi-Institutional Study. BJU Int [Internet]. 2016; Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26833820

8. Foley RW, Lundon DJ, Murphy K, Murphy TB, Galvin DJ, Watson RW. Predicting prostate cancer: analysing the clinical efficacy of prostate cancer risk calculators in a referral population. Ir J Med Sci [Internet]. 2015;184(3):701–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25843017

9. Poyet C, Nieboer D, Bhindi B, Kulkarni GS, Wiederkehr C, Wettstein MS, et al. Prostate cancer risk prediction using the novel versions of the European Randomised Study for Screening of Prostate Cancer (ERSPC) and Prostate Cancer Prevention Trial (PCPT) risk calculators: independent validation and comparison in a contemporary Europe. BJU Int [Internet]. 2016;117(3):401–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26332503

10. Murthy V, Rishi A, Gupta S, Kannan S, Mahantshetty U, Tongaonkar H, et al. Clinical impact of prostate specific antigen (PSA) inter-assay variability on management of prostate cancer. Clin Biochem [Internet]. 2015/10/23. 2016;49(1–2):79–84. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26506115

11. Komatsu K, Wehner N, Prestigiacomo AF, Chen Z, Stamey TA. Physiologic (intraindividual) variation of serum prostate-specific antigen in 814 men from a screening population. Urology [Internet]. 1996;47(3):343–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8633399

12. Morote J, Raventós CX, Lorente JA, Enbabo G, López M, de Torres I. Intraindividual variations of total and percent free serum prostatic-specific antigen levels in patients with normal digital rectal examination. Eur Urol [Internet]. 1999;36(2):111–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10420031

13. Lavallée LT, Binette A, Witiuk K, Cnossen S, Mallick R, Fergusson DA, et al. Reducing the Harm of Prostate Cancer Screening: Repeated Prostate-Specific Antigen Testing. Mayo Clin Proc [Internet]. 2015/12/10. 2016;91(1):17–22. Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26688045

14. Gómez-Gómez E, Carrasco-Valiente J, Blanca-Pedregosa A, Barco-Sánchez B, Fernandez-Rueda JL, Molina-Abril H, et al. European Randomized Study of Screening for Prostate Cancer Risk Calculator: External Validation, Variability, and Clinical Significance. Urology. 2017;102.

15. Epstein JI, Allsbrook WC, Amin MB, Egevad LL, Committee IG. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol [Internet]. 2005;29(9):1228–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16096414

16. Fawcett T. An introduction to ROC analysis. Pattern Recognit Lett [Internet]. 2006 Jun 1 [cited 2018 Dec 20];27(8):861–74. Available from: https://www.sciencedirect.com/science/article/pii/S016786550500303X

17. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics [Internet]. 1988 Sep [cited 2018 Dec 20];44(3):837–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3203132

18. Zhou X-H, McClish DK, Obuchowski NA, Electronic Book Collection., Wiley InterScience (Online service). Statistical methods in diagnostic medicine [Internet]. Wiley; 2011 [cited 2018 Dec 20]. 545 p. Available from: https://www.wiley.com/en-us/Statistical+Methods+in+Diagnostic+Medicine%2C+2nd+Edition-p-9780470183144

19. Hendriksen JMT, Geersing GJ, Moons KGM, de Groot JAH. Diagnostic and prognostic prediction models. J Thromb Haemost [Internet]. 2013 Jun [cited 2018 Dec 20];11:129–41. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23809117

20. Vickers AJ, Elkin EB. Decision Curve Analysis: A Novel Method for Evaluating Prediction Models. Med Decis Mak [Internet]. 2006 Nov 5 [cited 2018 Dec 20];26(6):565–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17099194

21. Loeb S, Bjurlin MA, Nicholson J, Tammela TL, Penson DF, Carter HB, et al. Overdiagnosis and overtreatment of prostate cancer. Eur Urol [Internet]. 2014;65(6):1046–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24439788

22. Loeb S, Lilja H, Vickers A. Beyond prostate-specific antigen: utilizing novel strategies to screen men for prostate cancer. Curr Opin Urol [Internet]. 2016;26(5):459–65. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27262138

23. Johnston E, Pye H, Bonet-Carne E, Panagiotaki E, Patel D, Galazi M, et al. INNOVATE: A prospective cohort study combining serum and urinary biomarkers with novel diffusion-weighted magnetic resonance imaging for the prediction and characterization of prostate cancer. BMC Cancer [Internet]. 2016;16(1):1–11. Available from: http://dx.doi.org/10.1186/s12885-016-2856-2

24. McDonald ML, Parsons JK. 4-Kallikrein Test and Kallikrein Markers in Prostate Cancer Screening. Urol Clin North Am [Internet]. 2016;43(1):39–46. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26614027

25. Lepor A, Catalona WJ, Loeb S. The Prostate Health Index: Its Utility in Prostate Cancer Detection. Urol Clin North Am [Internet]. 2016;43(1):1–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26614024

26. De Luca S, Passera R, Cappia S, Bollito E, Randone DF, Milillo A, et al. Fluctuation in prostate cancer gene 3 (PCA3) score in men undergoing first or repeat prostate biopsies. BJU Int [Internet]. 2014/04/29. 2014;114(6b):E56-61. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24472071

27. Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. Lancet [Internet]. 2017/01/20. 2017;389(10071):815–22. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28110982

28. Grill S, Fallah M, Leach RJ, Thompson IM, Freedland S, Hemminki K, et al. Incorporation

of detailed family history from the Swedish Family Cancer Database into the PCPT risk calculator. J Urol [Internet]. 2014/09/19. 2015;193(2):460–5. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25242395

29. Sölétormos G, Semjonow A, Sibley PE, Lamerz R, Petersen PH, Albrecht W, et al. Biological variation of total prostate-specific antigen: a survey of published estimates and consequences for clinical practice. Clin Chem [Internet]. 2005;51(8):1342–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15961552

30. Nordström T, Adolfsson J, Grönberg H, Eklund M. Repeat Prostate-Specific Antigen Tests Before Prostate Biopsy Decisions. J Natl Cancer Inst [Internet]. 2016;108(12). Available from: https://www.ncbi.nlm.nih.gov/pubmed/27418620

31. Ankerst DP, Gelfond J, Goros M, Herrera J, Strobl A, Thompson IM, et al. Serial Percent Free Prostate Specific Antigen in Combination with Prostate Specific Antigen for Population Based Early Detection of Prostate Cancer. J Urol [Internet]. 2016/03/12. 2016;196(2):355–60. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26979652

32. Roehrborn CG, Girman CJ, Rhodes T, Hanson KA, Collins GN, Sech SM, et al. Correlation between prostate size estimated by digital rectal examination and measured by transrectal ultrasound. Urology [Internet]. 1997;49(4):548–57. Available from: https://www.ncbi.nlm.nih.gov/pubmed/9111624

33. Roehrborn CG, Sech S, Montoya J, Rhodes T, Girman CJ. Interexaminer reliability and validity of a three-dimensional model to assess prostate volume by digital rectal examination. Urology [Internet]. 2001;57(6):1087–92. Available from: https://www.ncbi.nlm.nih.gov/pubmed/11377314

34. Alberts AR, Schoots IG, Bokhorst LP, van Leenders GJ, Bangma CH, Roobol MJ. Risk-based Patient Selection for Magnetic Resonance Imaging-targeted Prostate Biopsy after Negative Transrectal Ultrasound-guided Random Biopsy Avoids Unnecessary Magnetic Resonance Imaging Scans. Eur Urol [Internet]. 2016;69(6):1129–34. Available from:

https://www.ncbi.nlm.nih.gov/pubmed/26651990

35. Chiu PK, Alberts AR, Venderbos LDF, Bangma CH, Roobol MJ. Additional benefit of using a risk-based selection for prostate biopsy: an analysis of biopsy complications in the Rotterdam section of the European Randomized Study of Screening for Prostate Cancer. BJU Int [Internet]. 2017/06/05. 2017;120(3):394–400. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28498624

36. Loeb S, Shin SS, Broyles DL, Wei JT, Sanda M, Klee G, et al. Prostate Health Index improves multivariable risk prediction of aggressive prostate cancer. BJU Int [Internet]. 2016/11/22. 2017;120(1):61–8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27743489

37. Ahmed HU, Hu Y, Carter T, Arumainayagam N, Lecornet E, Freeman A, et al. Characterizing clinically significant prostate cancer using template prostate mapping biopsy. J Urol [Internet]. 2011/06/15. 2011;186(2):458–64. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21679984

Footnotes

- **Contributors** E.G.G, R.M.L, MJ.R.T and J.C.V carried out the conception and design of the study; E.G.G, JJ.S.B, J.C.V, AM.B, J.V.R, I.M.B.A, J.M.L, JM.J.V contributed to the data acquisition; E.G.G, J.C.V, J.L.F.R, RM.L and MJ.R.T carried out the analysis and interpretation of data; E.G.G, J.C.V and RM.L drafted the manuscript; JJ.S.B, J.L.F.R, AM.B, J.V.R, J.M.L, JM.J.V, and MJ.R.T carried out a critical revision of the manuscript for important intellectual content; E.G.G, and J.L.F.R performed the statistical analysis; RM.L, MJ.R.T and J.C.V supervised the work.
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- **Competing interests** None declared.
- **Ethics approval** This study was performed as part of the ONCOVER project. Ethical approval was obtained by the Reina Sofia Hospital Research Ethics Committee in accordance with the Declaration of Helsinki and informed consent was obtained from all participants for the project.
- **Provenance and peer review** Not commissioned; externally peer reviewed.
- **Data sharing statement** All data is shown within the manuscript
- **Patient consent for publication** Not required.

Figure legends

Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values: **A**, for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa; **B**, for the ERSPC1-RC and the ERSPC2-RC for positive biopsy; **C**, for the PCPT1-RC and the PCPT2-RC for positive biopsy; and **D**, for the ERSPC2-RC and the PCPT2-RC for Sig PCa.

Figure 2: Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are underestimated from the lower right, where they are overestimated. **A**, Calibration plots for ERSPC1-RC Sig PCa risk estimation. **B**, Calibration plots for PCPT1-RC Sig PCa risk estimation.

Figure 3: Results of the decision curve analysis. **A**, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1–RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating all patients (dashed line) and treating none (thin line). **B**, Plot demonstrating net reduction of interventions per 100 patients using the ERSPC-RC (black line) and the PCPT-RC (grey line).

Figure 4: Graphics showing Cohen's k coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. **A**, Agreement between ERSPC1-RC and ERSPC2-RC for Sig PCa. **B**, Agreement between PCPT1-RC and PCPT2-RC for Sig PCa. **C**, Agreement between ERSPC1-RC and PCPT1-RC for Sig PCa.

Figure 5: Graphics showing sensitivities and specificities of both RCs along the clinically useful risk threshold. The ERSPC-RC (black line) and the PCPT-RC (grey line).

Table 1. Clinical and demographic characteristics of the cohort of patients categorized according to cancer status.

Variable	No Sig PCa n=423	Sig PCa n=87	All n=510
Age	64.0 (60.0-69.0)	68.0 (63.0-71.0)	65.0 (60.0-70.0)
Family History	81 (19.1)	8 (9.2)	89 (17.5)
Positive DRE	55 (13.0)	27 (31.0)	82 (16.1)
1 Serum PSA	5.3 (4.3-6.9)	5.8 (4.5-7.2)	5.3 (4.3-6.9)
1 free PSA %	16.2 (12.4-21.4)	12.5 (9-16.6)	15.9 (11.8-20.4)
2 Serum PSA	5.0 (3.7-6.6)	5.4 (4.1-6.7)	5.0 (3.8-6.6)
2 free PSA %	17.9 (13.9-23.4)	12.5 (9.1-16.3)	16.9 (12.8-22.1)
Prostate volume	38.0 (29.0-50.0)	26.0 (20.7-34.0)	35 (26-49)
First Biopsy	322 (76.1)	79 (90.8)	401 (78.6)
PCPT1 Sig PCa	0.08 (0.05-0.13)	0.16 (0.10-0.30)	0.09 (0.06-0.15)
ERSPC1 Sig PCa	0.05 (0.02-0.10)	0.12 (0.05-0.31)	0.05 (0.03-0.12)
PCPT2 Sig PCa	0.07 (0.04-0.11)	0.16 (0.08-0.27)	0.07 (0.05-0.13)
ERSPC2 Sig PCa	0.04 (0.02-0.08)	0.12 (0.05-0.30)	0.05 (0.02-0.11)
PCa	89 (21)	87 (100)	176 (34.5)

PCa= Prostate cancer; **Sig PCa**= significant PCa (Gleason ≥ 7 on biopsy); **No Sig PCa**= No cancer or non-significant PCa; **ERSPC1 / PCPT1 Sig PCa** = Probability of high grade PCa using the first measurement of serum PSA (at the time of biopsy indication by the urologist); **ERSPC2 / PCPT2 Sig PCa** = Probability high grade PCa using the second measurement of serum PSA (just before undergoing prostate biopsy). Median values (interquartile range) are expressed for quantitative variables, and absolute values (percentage) for qualitative variables.

549

550

1

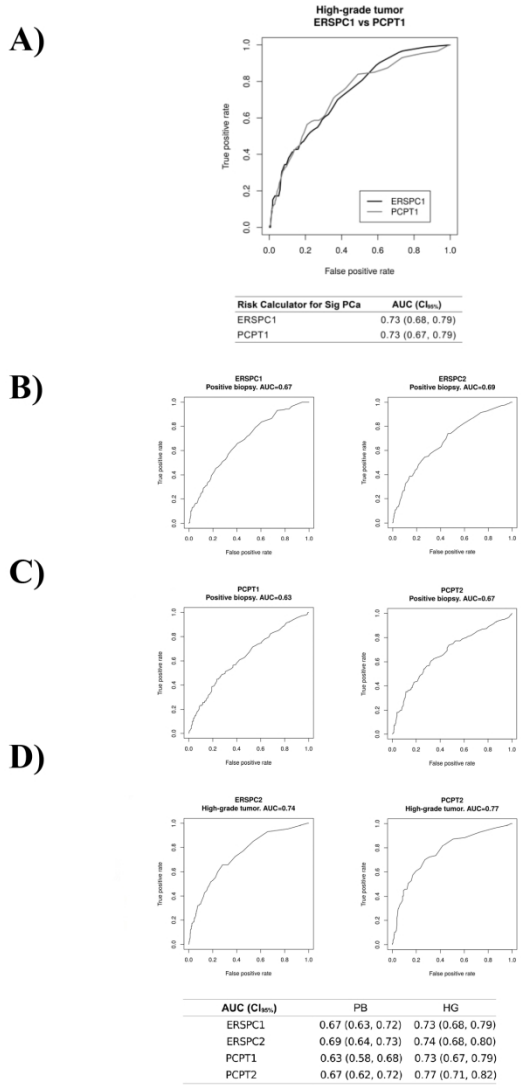

Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values: A, for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa; B, for the ERSPC1-RC and the ERSPC2-RC for positive biopsy; C, for the PCPT1-RC and the PCPT2-RC for positive biopsy; and D, for the ERSPC2-RC and the PCPT2-RC for Sig PCa.

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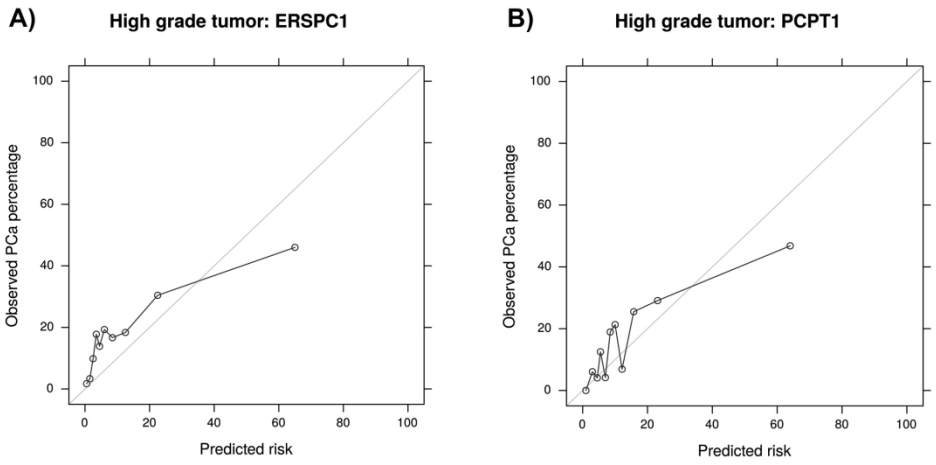

Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are underestimated from the lower right, where they are overestimated. A, Calibration plots for ERSPC1-RC Sig PCa risk estimation. B, Calibration plots for PCPT1-RCSig PCa risk estimation.

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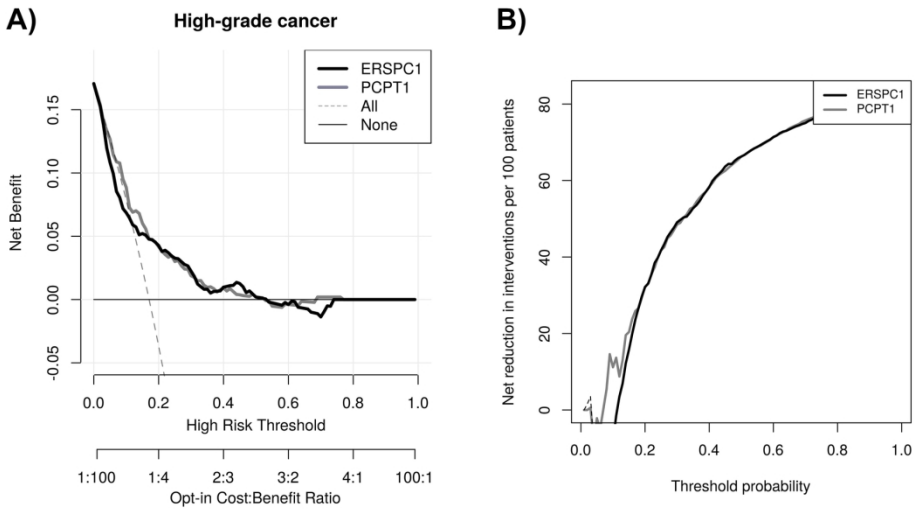

Results of the decision curve analysis. A, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1–RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating all patients (dashed line) and treating none (thin line). B, Plot demonstrating net reduction of interventions per 100 patients using the ERSPC-RC (black line) and the PCPT-RC (grey line).

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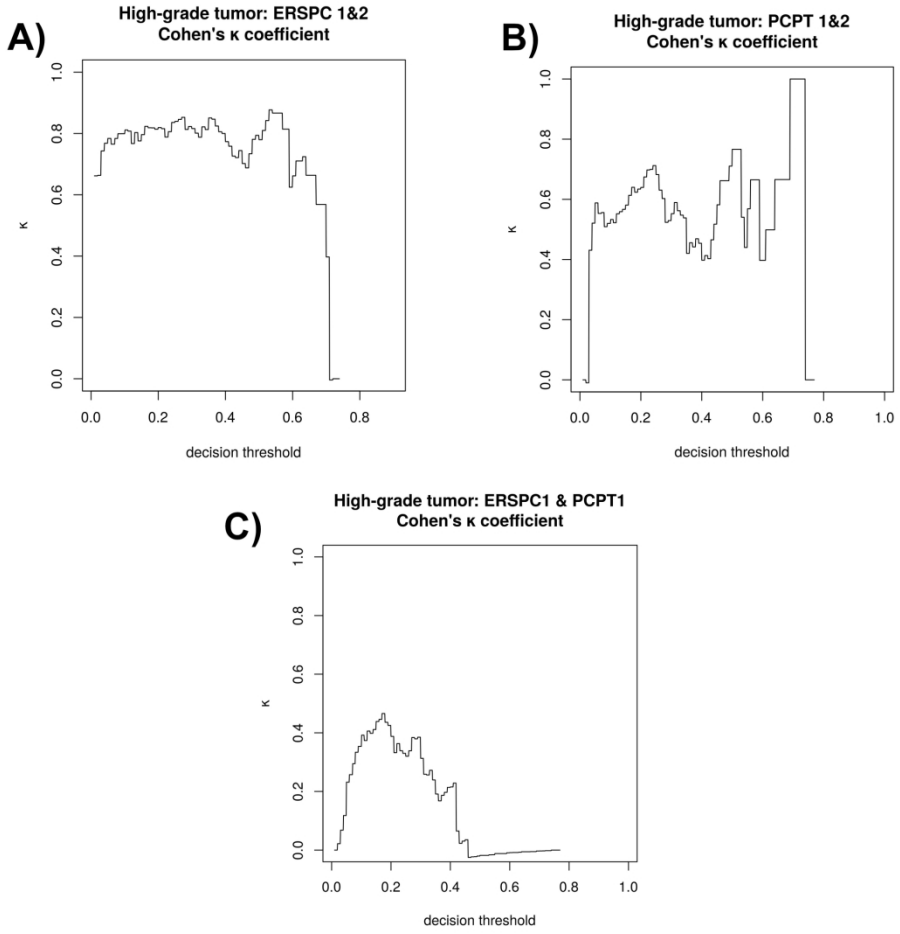
4



Graphics showing Cohen's k coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. A, Agreement between ERSPC1-RC and ERSPC2-RC for Sig PCa. B, Agreement between PCPT1-RC and PCPT2-RC for Sig PCa. C, Agreement between ERSPC1-RC and PCPT1-RC for Sig PCa.

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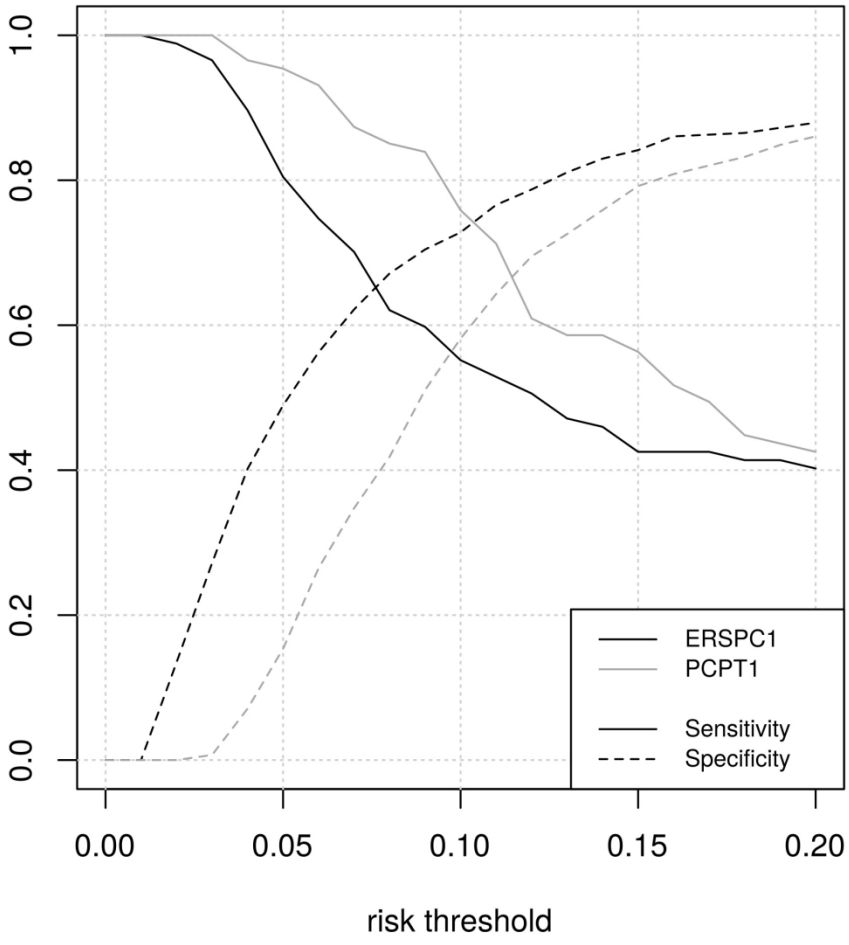


Graphics showing sensitivities and specificities of both RCs along the clinically useful risk threshold. The ERSPC-RC (black line) and the PCPT-RC (grey line).

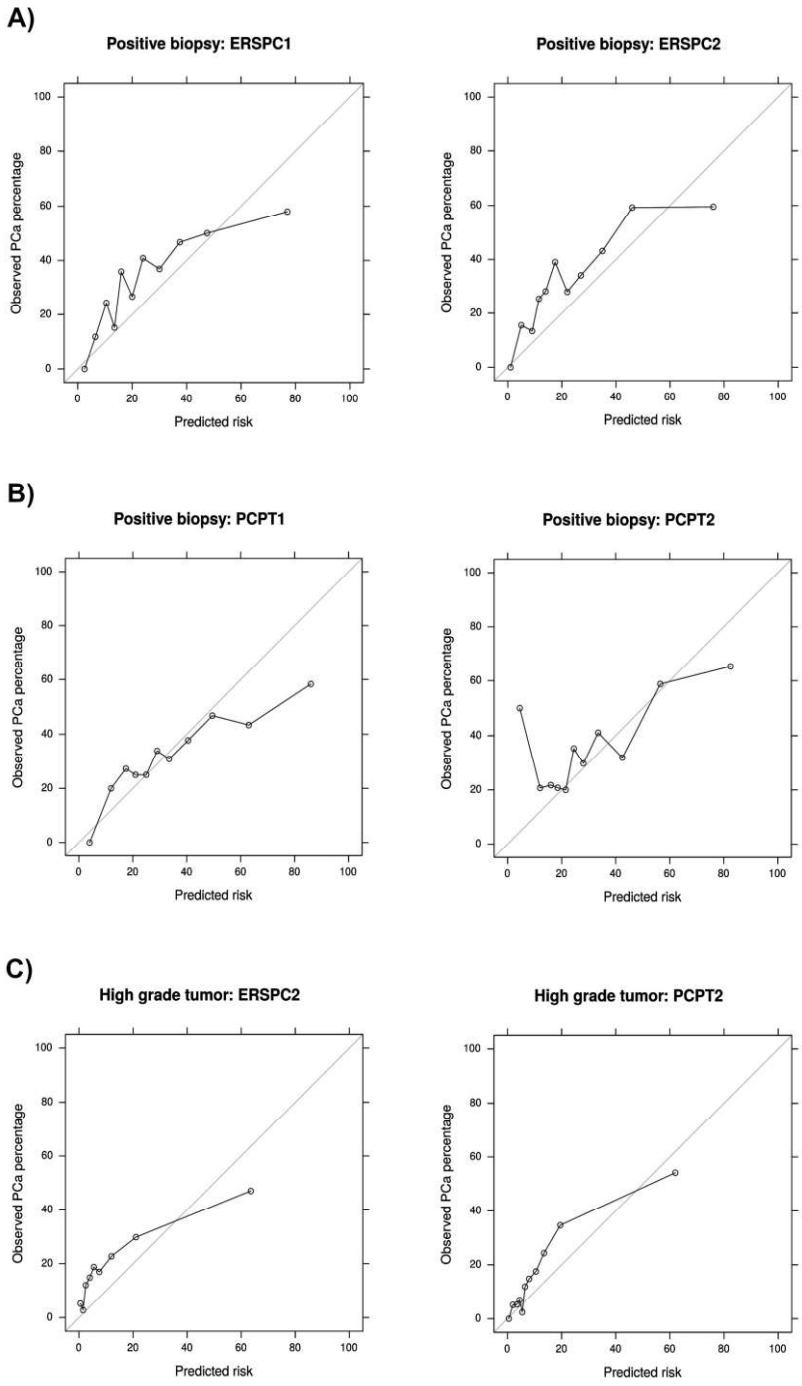
Supplemental Table 1. Patients excluded from the ONCOVER cohort for this study depends on the exclusion criteria.

Exclusion criteria	Number
Under active surveillance	25
2 consecutive PSA levels well recorded or affected	50
Prostate volume not well recorded	177
PSA of biopsy indication out of the range 3-10ng/ml , or Age out of the range 55-80	251
Total exclusions	511

Supplemental table 2: DeLong p values resulting from the pairwise comparison of the Area under the Receiver Operator Characteristic (ROC) curve (AUC) between Risk Calculators for significant Prostate cancer (Sig PCa) detection.

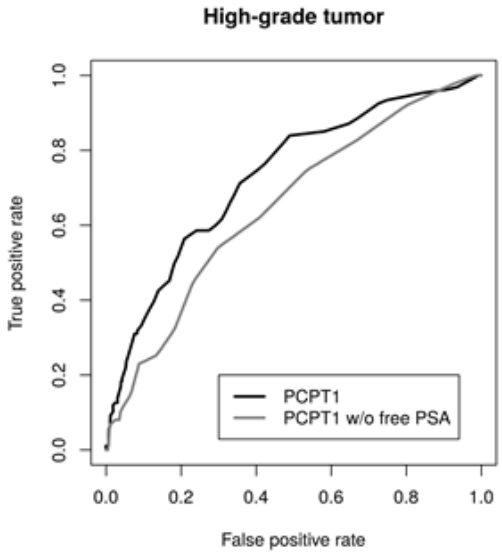
Sig PCa (p-value)	ERSPC1	ERSPC2	PCPT1	PCPT2
ERSPC1	X	0.51	0.95	0.19
ERSPC2	X	X	0.74	0.25
PCPT1	X	X	X	0.06

S1



Supplemental Figure 1: Calibration plots of the RCs in this cohort, demonstrating the agreement between predicted and observed probabilities: **A**, of a positive biopsy for the ERSPC1-RC and the ERSPC2-RC; **B**, of a positive biopsy for the PCPT1-RC and for the PCPT2-RC; and **C**, of a Sig PCa on the biopsy for the ERSPC2-RC and the PCPT2-RC.

S2



Risk Calculator for Sig PCa	AUC ($CI_{95\%}$)
PCPT1 without free PSA	0.65 (0.59, 0.71)
PCPT1 + free PSA	0.73 (0.67, 0.79)
DeLong p value: 0.02	

Supplemental Figure 2: Receiver Operating Characteristic curves and Area Under the Curve values for the PCPT1-RC without free PSA (grey) and for the PCPT1-RC with free PSA (black) to predict Sig PCa. P-value according to the DeLong test.

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N°ID;age;psa1;free.psa1;psa2;free.psa2;prior.biopsy;abnormal.dre;family.history;vol;vol.dre;pb;hg;ers
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80;69;8;35	3;6	65;28	8;TRUE;FA	14;0	02;0	14;0	02;0	9;0
81;59;4	15;11	8;4	8;11	8;FALSE;F.	35;0	1;0	4;0	12;0
82;65;4	78;24	4;4	73;28	3;FALSE;F.	09;0	02;0	09;0	02;0
83;56;3	06;29	7;3	06;32	3;FALSE;T	25;0	12;0	25;0	12;0
84;59;5	57;8	7;5	21;8	8;FALSE;F.	8;0;TRUE;F	45;0	15;0	43;0
85;74;5	45;17;5	42;19	5;FALSE;F.	7;0;TRUE;T	44;0	14;0	44;0	14;0
86;68;5	39;20;5	11;27	7;TRUE;TR	23;0	06;0	23;0	06;0	77;0
87;59;8	28;16	8;10	16;NA;FAL	35;0	11;0	42;0	15;0	75;0
88;66;4	88;17;7	69;21;FALS	09;0	02;0	17;0	04;0	72;0	21;0
89;66;9;9	2;9	4;8	7;FALSE;T	82;0	71;0	83;0	72;0	26;0
90;58;3	32;13	6;1	39;NA;FAL	28;0	07;0	1;0	02;0	67;0
91;63;4	13;10	7;0	68;NA;FAL	3;0;FALSE;	34;0	1;0	04;0;0	46;0
92;70;3	5;11;3	43;11	9;TRUE;FA	22;0	03;0	22;0	03;0	48;0
93;63;7	34;6	5;8	76;7	8;FALSE;T	77;0	63;0	81;0	7;0
94;62;9	6;7	7;7	89;10	3;FALSE;F.	65;0	31;0	58;0	24;0
95;78;5	2;22;5	23;19	6;FALSE;F.	21;0	05;0	21;0	05;0	75;0
96;68;5	31;17	3;5	92;16;FALS	43;0	14;0	47;0	16;0	71;0
97;61;3	22;33	5;3	33;27	9;FALSE;F.	12;0	02;0	12;0	03;0
98;72;4	8;9	2;5	04;7	9;FALSE;F.	4;0	12;0	41;0	13;0
99;77;4	49;18	7;4	1;25	6;FALSE;T	62;0	43;0	58;0	39;0
100;64;7;1	83;13	4;TRUE;FA	24;0	06;0	25;0	06;0	69;0	21;0
101;70;3	07;17	5;2	87;NA;FAL	11;0	02;0	1;0	02;0	72;0
102;75;7	1;19;6	45;24	1;TRUE;FA	08;0	01;0	08;0	01;0	74;0
103;67;9	6;29	4;8	34;30	2;TRUE;FA	08;0	01;0	08;0	01;0
104;57;9	45;22	8;9	75;25	7;TRUE;FA	15;0	03;0	15;0	03;0
105;67;4	28;7;3	78;8	2;FALSE;F.	36;0	1;0	32;0	08;0	14;0
106;55;4	75;27	5;6	4;17	8;FALSE;F.	39;0	12;0	5;0	18;0
107;68;5;1	8;2	1;NA;FALS	2;0	05;0	07;0	01;0	72;0	2;0
108;64;7	2;18	5;9	38;17	5;TRUE;FA	25;0	06;0	26;0	07;0
109;72;8	86;13	1;9	28;13	5;FALSE;T	81;0	7;0	82;0	72;0
110;65;5	8;15	4;5	74;16	3;FALSE;F.	24;0	06;0	24;0	06;0
111;68;6	14;14;5	57;16	5;FALSE;F.	25;0	07;0	23;0	06;0	6;0

112;71;8	7;15;7	04;16	3;TRUE;FA	08;0	01;0	08;0	01;0	7;0
113;59;3	93;19	3;3	98;17	5;FALSE;F	6;1;FALSE	15;0	03;0	15;0
114;72;4	2;6	3;4	82;7	6;FALSE;F	3;0;TRUE;T	35;0	1;0	4;0
115;68;9	9;18	5;7	05;14	8;FALSE;F	3;1;FALSE	41;0	14;0	29;0
116;63;4	5;19	4;1	13;NA;FAL	18;0	04;0	03;0;0	78;0	17;0
117;76;7	6;13	4;5	27;19	9;FALSE;T	34;0	21;0	23;0	12;0
118;59;3	49;20	3;3	46;17;FALS	13;0	03;0	13;0	03;0	81;0
119;67;5	3;33;2	89;NA;FAL	1;0	02;0	04;0	01;0	86;0	1;0
120;64;5	6;50	7;5	55;21	9;TRUE;FA	24;0	05;0	23;0	05;0
121;68;7	55;26	1;6	25;12	08;FALSE;I	16;0	04;0	13;0	03;0
122;63;9	75;10	8;1	19;NA;TRU	26;0	07;0	18;0	01;0	53;0
123;69;6	3;16	1;7	39;18	6;TRUE;TR	3;2;FALSE	14;0	03;0	15;0
124;57;4	5;12	2;4	75;15	1;FALSE;F	37;0	11;0	39;0	12;0
125;74;4	6;19	8;1	02;NA;FAL	18;0	04;0	02;0;0	73;0	19;0
126;56;3	9;18	5;2	67;NA;FAL	33;0	09;0	22;0	05;0	79;0
127;66;5	68;32	8;2	08;NA;FAL	3;0;FALSE	46;0	15;0	16;0	03;0
128;78;8;12	5;7	46;13	5;FALSE;T	9;0;TRUE;T	79;0	67;0	77;0	64;0
129;60;3	6;10	6;3	83;10	7;FALSE;F	3;0	08;0	32;0	08;0
130;58;8	58;13	3;7	47;18;TRU	08;0	01;0	08;0	01;0	71;0
131;58;4	93;9	5;4	6;9	1;FALSE;F	41;0	12;0	38;0	11;0
132;67;3	1;12	9;3	57;13	7;FALSE;F	1;1;FALSE	11;0	02;0	13;0
133;68;7	7;8	5;7	96;10	1;FALSE;F	8;0;TRUE;T	57;0	23;0	58;0
134;59;4	84;21;4	55;25	4;FALSE;F	09;0	02;0	08;0	02;0	77;0
135;60;6	43;11	5;6	91;10	7;FALSE;F	27;0	07;0	29;0	08;0
136;65;9	38;2	3;2	86;NA;FAL	64;0	3;0	23;0	05;0;0	31;0
137;76;6	2;6	8;6	77;6	6;FALSE;F	49;0	17;0	52;0	2;0
138;58;7	34;9	5;7	1;12	5;TRUE;FA	6;0;TRUE;F	25;0	06;0	24;0
139;64;3	57;22	9;3	29;27;FALS	15;0	07;0	13;0	06;0	81;0
140;61;8;12	2;7	68;11	7;FALSE;F	17;0	05;0	16;0	04;0	55;0
141;63;4	39;15;4	33;18	9;FALSE;F	17;0	04;0	17;0	04;0	68;0
142;64;5	1;25;4	17;20	8;FALSE;F	1;0	02;0	07;0	02;0	83;0
143;70;5	6;12	3;1	96;NA;TRU	23;0	06;0	19;0	02;0	56;0
144;58;3	89;20	8;3	21;20	8;FALSE;F	32;0	09;0	27;0	06;0
145;70;7;5	6;2	69;NA;FAL	53;0	36;0	21;0	1;0	04;0	41;0
146;67;4	02;24	3;3	49;23	2;FALSE;F	15;0	03;0	13;0	03;0
147;80;7	4;20	4;11	6;NA;FALS	34;0	2;0	49;0	35;0	68;0
148;74;4	82;17	6;3	62;22	9;TRUE;FA	7;1;FALSE	13;0	01;0	12;0
149;65;5	65;16	9;4	64;18	9;FALSE;F	23;0	06;0	18;0	04;0
150;72;6	6;17;6	28;19	9;FALSE;F	28;0	08;0	26;0	07;0	68;0
151;66;4	2;17	6;4	57;23	4;FALSE;F	16;0	04;0	18;0	04;0
152;55;3	27;11	6;3	22;14	2;FALSE;F	12;0	02;0	12;0	02;0
153;73;7	2;16	9;7	15;13	7;FALSE;F	3;0	09;0	3;0	09;0
154;57;4	3;16	9;4	9;11;FALSE	17;0	04;0	2;0	05;0	76;0
155;59;6	7;5	2;5	35;5	9;FALSE;F	28;0	08;0	22;0	05;0
156;65;7;1	84;12	7;FALSE;F	53;0	21;0	58;0	24;0	44;0	38;0
157;55;3	5;11	8;3	07;9	1;FALSE;F	29;0	07;0	25;0	06;0
158;65;9;6	7;9	35;8	5;FALSE;F	2;0	05;0	21;0	06;0	1;0
159;55;8	9;12;6	88;15	5;TRUE;FA	14;0	03;0	14;0	02;0	6;0
160;61;7	9;13	7;7	19;15	1;FALSE;T	6;1;TRUE;F	57;0	41;0	54;0
161;68;5	4;4	7;2	24;NA;FAL	1;0	02;0	03;0	01;0	02;0
162;59;7	69;14	1;5	7;16	4;FALSE;F	17;0	04;0	11;0	03;0
163;60;4	5;11;4	64;15	9;FALSE;F	37;0	11;0	38;0	11;0	49;0
164;60;5	5;14	7;5	76;15	1;FALSE;T	44;0	27;0	46;0	29;0
165;57;4	7;30;5	34;26	2;FALSE;F	09;0	02;0	1;0	02;0	87;0
166;66;3	4;14	7;3	38;16	5;FALSE;F	12;0	03;0	12;0	03;0
167;70;4;18	6;2	59;NA;FAL	07;0	01;0	04;0	01;0	74;0	2;0

168;73;4	56;21	7;5	29;20;FALS	18;0	04;0	21;0	05;0	72;0
169;64;6	05;11	5;7	85;11	4;FALSE;T	47;0	31;0	57;0	41;0
170;62;4	78;13	3;4	6;15	4;TRUE;FA	13;0	01;0	13;0	01;0
171;75;9	2;9	6;8	04;11	4;FALSE;T	63;0	47;0	58;0	42;0
172;59;6	44;11	8;6	34;14	8;FALSE;T	2;1;TRUE;F	5;0	33;0	49;0
173;70;9	78;4;8	95;4	3;FALSE;F	7;0;TRUE;T	65;0	31;0	62;0	28;0
174;72;3	4;17;3	36;16;FALS	06;0	01;0	05;0	01;0	7;0	22;0
175;70;4	3;24	4;4	78;29	9;FALSE;F	8;1;FALSE	17;0	04;0	19;0
176;68;5;1	3;7	08;16	1;FALSE;T	22;0	12;0	32;0	19;0	69;0
177;55;4	06;28	8;5	76;29	1;FALSE;F	5;0;FALSE	34;0	09;0	46;0
178;58;5	29;20	9;5	82;15	8;FALSE;F	1;0	02;0	12;0	03;0
179;60;5	82;12	7;6	18;15	3;TRUE;FA	13;0	02;0	13;0	02;0
180;62;4	01;13	7;3	92;23	4;FALSE;F	15;0	03;0	15;0	03;0
181;70;6	28;23	2;6	51;19	9;FALSE;T	49;0	32;0	5;0	33;0
182;60;4	7;16	2;5	12;19	1;FALSE;F	19;0	04;0	21;0	05;0
183;66;7	81;18	1;12	69;NA;TRU	08;0	01;0	09;0	02;0	77;0
184;68;3	7;9;2	56;NA;FAL	55;0	35;0	41;0	22;0	28;0	41;0
185;68;3	36;16	9;2	95;NA;FAL	28;0	07;0	24;0	06;0	72;0
186;64;3	58;9	1;3	65;18;FALS	13;0	03;0	14;0	03;0	34;0
187;66;8	6;13	3;9	22;14	2;FALSE;F	36;0	12;0	38;0	13;0
188;56;3	7;5	4;2	08;NA;TRU	22;0	03;0	2;0	02;0	1;0
189;59;5	8;15;6	73;13	2;TRUE;FA	13;0	02;0	14;0	02;0	77;0
190;66;7	83;13	9;8	4;17;TRUE	14;0	02;0	14;0	02;0	69;0
191;56;5	63;10	3;4	32;10	1;FALSE;F	45;0	15;0	36;0	1;0
192;58;3	7;12;2	4;NA;FALS	31;0	08;0	19;0	04;0	58;0	32;0
193;70;5	8;19	7;5	3;22	4;FALSE;F	12;0	03;0	1;0	02;0
194;72;7	4;29;6	99;39;TRU	15;0	03;0	15;0	03;0	86;0	07;0
195;57;7;1	6;8	08;18	3;TRUE;FA	08;0	01;0	08;0	01;0	81;0
196;65;6	36;20	4;6	14;16	2;FALSE;T	49;0	32;0	48;0	31;0
197;62;6	17;20	7;7	18;22	2;TRUE;FA	7;0;FALSE	24;0	05;0	25;0
198;65;3	79;13	1;4	18;13	1;FALSE;F	9;0;TRUE;T	32;0	08;0	35;0
199;57;7	7;10;2	75;NA;FAL	56;0	4;0	22;0	1;0	39;0	38;0
200;70;4	4;15	4;4	02;20	3;FALSE;F	17;0	04;0	15;0	03;0
201;70;5	97;21	8;6	25;24	8;TRUE;FA	08;0	01;0	08;0	01;0
202;59;3	8;9;2	85;NA;FAL	14;0	03;0	1;0	02;0	36;0	47;0
203;62;5	07;11	1;3	51;13	6;FALSE;F	42;0	13;0	29;0	07;0
204;67;5;2	6;3	91;20	4;TRUE;FA	07;0	01;0	07;0	01;0	88;0
205;65;3	22;16	1;3	72;16	1;FALSE;F	27;0	06;0	31;0	08;0
206;70;5	7;21;6	36;20	7;FALSE;F	23;0	06;0	26;0	07;0	77;0
207;59;5	6;14	2;6	95;9	3;FALSE;F	11;0	03;0	15;0	04;0
208;56;5	26;18;4	81;18	5;FALSE;F	43;0	14;0	4;0	12;0	78;0
209;62;3	9;16	2;2	91;NA;FAL	15;0	03;0	1;0	02;0	72;0
210;55;4	59;24	4;5	13;26	1;FALSE;T	37;0	22;0	41;0	25;0
211;61;3	5;15	3;3	61;21;FALS	29;0	15;0	3;0	15;0	69;0
212;61;5;1	8;5	4;12	9;FALSE;F	09;0	02;0	1;0	02;0	64;0
213;65;4	35;14	9;3	5;17	1;FALSE;T	19;0	09;0	14;0	07;0
214;68;4	8;19	9;6	01;18	4;TRUE;FA	07;0	01;0	08;0	01;0
215;74;4	8;12	6;4	61;12	5;FALSE;F	4;0	12;0	38;0	11;0
216;65;7	35;21	7;8	12;20	9;TRUE;FA	14;0	02;0	14;0	02;0
217;66;7	47;14	6;6	64;15	3;FALSE;F	56;0	22;0	51;0	19;0
218;69;8	6;22;10	29;NA;FAL	39;0	25;0	45;0	31;0	77;0	12;0
219;75;7;1	6;5	41;8	3;FALSE;F	53;0	21;0	44;0	14;0	42;0
220;56;6	05;16;6	55;12	8;FALSE;F	2;1;FALSE	25;0	07;0	27;0	08;0
221;73;4	2;33;3	38;42;FALS	18;0	09;0	14;0	06;0	78;0	14;0
222;62;4	6;24	7;3	81;28	3;FALSE;F	5;2;FALSE	08;0	02;0	07;0
223;68;5;1	2;6	23;21	1;FALSE;F	4;2;TRUE;F	09;0	02;0	13;0	03;0

224;68;7	4;10	2;2	89;NA;FAL	3;2;FALSE;	16;0	04;0	04;0	01;0
225;70;4	12;15;3	46;16	07;FALSE;I	2;1;FALSE;	16;0	04;0	13;0	03;0
226;59;8	7;10;13	15;NA;TRU	4;1;FALSE;	14;0	03;0	15;0	04;0	49;0
227;74;6	19;36;2	78;NA;FAL	3;2;TRUE;F	13;0	03;0	04;0	01;0	81;0
228;73;5	3;9	33;5	62;8	8;FALSE;F	6;0;TRUE;T	43;0	14;0	45;0
229;65;9	7;16;5	61;15	5;TRUE;FA	09;0	01;0	08;0	01;0	77;0
230;67;4	3;36	6;7	07;27	8;FALSE;F	08;0	02;0	15;0	04;0
231;57;5	01;24	7;5	63;25	9;FALSE;F	1;0	02;0	11;0	03;0
232;68;4	3;14	4;4	5;11	7;FALSE;F	36;0	1;0	37;0	11;0
233;63;3	21;8	8;1	01;NA;FAL	27;0	06;0	06;0	01;0	28;0
234;56;5	1;11	3;4	88;10	2;FALSE;F	21;0	05;0	2;0	05;0
235;60;5	22;14	7;5	45;13	5;FALSE;F	21;0	05;0	22;0	06;0
236;55;8	12;8	8;4	04;11	8;FALSE;F	59;0	25;0	34;0	09;0
237;63;6	9;11	3;6	64;9	3;FALSE;F	53;0	2;0	51;0	19;0
238;69;6	9;8;6	31;9;FALSI	53;0	2;0	5;0	18;0	18;0	5;0
239;65;9	3;13	1;10	73;NA;TRU	8;1;TRUE;F	14;0	03;0	15;0	03;0
240;55;3	33;9	3;3	02;10	9;FALSE;F	28;0	07;0	25;0	06;0
241;65;6	41;14	6;5	66;16	2;FALSE;F	27;0	07;0	23;0	06;0
242;61;6	7;14;5	13;17	1;FALSE;F	3;1;FALSE;	28;0	08;0	21;0	05;0
243;66;4	73;12;5	54;19	1;FALSE;F	09;0	02;0	11;0	02;0	52;0
244;65;5	2;16	2;4	7;20;FALSI	1;0	02;0	09;0	02;0	7;0
245;67;5	4;17	7;5	12;22	4;FALSE;F	22;0	06;0	21;0	05;0
246;66;5	3;14	6;3	54;18	3;FALSE;F	22;0	05;0	13;0	03;0
247;64;6	1;18	9;4	8;18	1;FALSE;F	25;0	07;0	19;0	05;0
248;72;8	26;20	9;8;21	2;FALSE;F	35;0	11;0	34;0	1;0	77;0
249;74;6	72;20;6	85;20	7;FALSE;T	51;0	34;0	52;0	35;0	71;0
250;73;6	9;6;6	85;15	1;FALSE;F	53;0	2;0	53;0	2;0	05;0
251;79;4	5;29;7	49;27	3;TRUE;TR	22;0	05;0	24;0	08;0	83;0
252;65;8;2	98;22	3;FALSE;F	3;1;TRUE;F	34;0	1;0	29;0	08;0	79;0
253;55;7	1;12	7;3	49;20;FALS	54;0	21;0	29;0	07;0	61;0
254;75;6	7;14	8;6	57;14	3;FALSE;T	74;0	6;0	74;0	59;0
255;65;7	2;9	2;6	17;9	2;TRUE;FA	14;0	02;0	13;0	02;0
256;58;3	94;20	9;3	97;20	4;FALSE;F	33;0	09;0	33;0	09;0
257;65;6	2;20	8;4	6;20	2;FALSE;F	13;0	03;0	08;0	02;0
258;66;3	4;10	7;3	04;10	8;FALSE;T	52;0	32;0	47;0	28;0
259;66;4	48;7	1;3	31;7	8;FALSE;T	61;0	43;0	51;0	31;0
260;60;3	67;12	2;4	48;14	2;FALSE;F	31;0	08;0	37;0	11;0
261;61;4	11;11	5;5	26;12	1;FALSE;F	07;0	02;0	1;0	02;0
262;67;7	6;18	2;6	11;14	4;FALSE;F	32;0	1;0	25;0	07;0
263;62;9	23;11	3;4	67;14	3;FALSE;F	38;0	13;0	19;0	04;0
264;60;7	5;12;2	38;NA;FAL	55;0	39;0	18;0	08;0	45;0	34;0
265;60;8	6;16	7;12	97;NA;TRU	08;0	01;0	09;0	02;0	81;0
266;66;6	81;12;3	42;31	2;FALSE;F	28;0	08;0	13;0	03;0	5;0
267;70;8	9;16	4;5	81;21	3;TRUE;FA	7;2;FALSE;	08;0	01;0	08;0
268;61;6	3;28	7;6	34;36	7;FALSE;F	13;0	03;0	13;0	03;0
269;66;5	22;13;5	17;11	6;FALSE;T	42;0	25;0	42;0	25;0	54;0
270;57;6	7;16;8	03;17	3;FALSE;F	6;1;FALSE;	28;0	08;0	34;0	1;0
271;67;7	6;16;9	99;13	7;TRUE;FA	08;0	01;0	09;0	01;0	71;0
272;56;4	7;16	2;3	48;18	6;TRUE;FA	13;0	01;0	12;0	01;0
273;62;3	95;22	8;3	59;23	3;FALSE;F	1;2;FALSE;	07;0	01;0	06;0
274;62;4	53;14;4	89;17	7;FALSE;F	6;1;FALSE;	18;0	04;0	2;0	05;0
275;62;8	8;8	6;8	57;10	8;TRUE;FA	14;0	03;0	14;0	03;0
276;61;6;2	7;2	31;NA;FAL	25;0	07;0	07;0	01;0	86;0	11;0
277;69;4	6;10;4	67;10	4;FALSE;T	62;0	44;0	63;0	44;0	28;0
278;70;4;3	5;42	2;FALSE;F	07;0	01;0	08;0	02;0	81;0	14;0
279;70;8	3;10	2;7	41;9	4;FALSE;F	35;0	11;0	31;0	09;0

280;75;6	8;11	6;5	44;12	6;FALSE;F	52;0	2;0	44;0	14;0
281;60;6	63;7	3;6	18;7	7;FALSE;F	51;0	19;0	49;0	17;0
282;66;3	6;31;1	82;NA;FAL	3;0	08;0	14;0	03;0	85;0	12;0
283;67;5	2;16	1;4	89;22	6;FALSE;F	42;0	13;0	4;0	12;0
284;60;6	4;8	8;6	52;9	9;FALSE;F	5;0	18;0	51;0	19;0
285;70;3	59;18	6;2	59;NA;FAL	29;0	15;0	2;0	09;0	72;0
286;61;6	3;19	1;6	2;13;FALSE	26;0	07;0	26;0	07;0	78;0
287;67;6	9;10;20	9;NA;TRUE	3;1;FALSE;	14;0	02;0	17;0	06;0	44;0
288;61;5	5;19	1;19	6;NA;FALS	5;0;TRUE;F	44;0	15;0	84;0	6;0
289;55;5	11;15	4;4	4;17	7;TRUE;FA	23;0	04;0	23;0	04;0
290;69;4	8;18;4	76;22;FALS	64;0	45;0	64;0	45;0	7;0	19;0
291;55;3	9;11	2;5	12;12	1;TRUE;FA	12;0	01;0	13;0	02;0
292;64;6;6	6;2	34;NA;TRU	24;0	05;0	2;0	02;0	15;0	48;0
293;66;8	8;10	5;8	64;9	7;FALSE;F	37;0	12;0	36;0	12;0
294;72;3	78;23	3;6	19;36	5;FALSE;F	06;0	01;0	13;0	03;0
295;74;7	5;15	5;6	11;18	3;TRUE;FA	08;0	01;0	08;0	01;0
296;56;3	2;14	6;3	42;13	7;FALSE;F	26;0	06;0	28;0	07;0
297;70;4;47	9;4	14;52	8;FALSE;F	15;0	03;0	16;0	04;0	85;0
298;65;4	88;19	5;4	43;23	9;FALSE;T	1;1;TRUE;F	4;0	23;0	36;0
299;74;7	85;28	3;3	18;28	6;FALSE;F	33;0	1;0	11;0	02;0
300;57;3	3;17	3;4	3;13;FALSE	05;0	01;0	08;0	02;0	77;0
301;61;3	82;19;4	66;16	3;FALSE;F	14;0	03;0	18;0	04;0	78;0
302;60;5	9;12	4;4	47;8	2;FALSE;F	47;0	16;0	37;0	11;0
303;58;4	89;32	9;4	39;25;TRU	13;0	02;0	13;0	01;0	92;0
304;63;8	74;9;9	47;8	1;TRUE;FA	14;0	03;0	15;0	03;0	31;0
305;62;3	66;15;3	32;14	7;FALSE;F	31;0	08;0	28;0	07;0	69;0
306;70;4	2;29;5	21;30	9;FALSE;T	59;0	4;0	67;0	49;0	82;0
307;75;9	54;24	5;8	02;28	6;FALSE;F	21;0	06;0	17;0	05;0
308;69;8	34;19;8	4;16	5;FALSE;F	6;0	26;0	6;0	26;0	75;0
309;56;8	3;5	8;3	54;15	2;FALSE;F	35;0	11;0	13;0	03;0
310;56;5	2;16	2;5	29;17	9;FALSE;F	5;1;FALSE;	21;0	05;0	21;0
311;63;3	9;13;4	25;16	7;FALSE;F	33;0	09;0	35;0	1;0	6;0
312;70;3	82;33;4	12;26	4;TRUE;FA	12;0	01;0	12;0	01;0	86;0
313;56;3	2;5	2;3	72;5	3;FALSE;F	26;0	06;0	31;0	08;0
314;61;7	19;45;4	09;45	2;FALSE;F	15;0	04;0	07;0	01;0	89;0
315;61;4;18	29;18	1;FALSE;F	15;0	03;0	26;0	07;0	71;0	23;0
316;61;5	03;28	1;3	4;25	5;FALSE;F	2;0	05;0	12;0	03;0
317;65;4	51;27;4	1;24	3;FALSE;F	18;0	04;0	16;0	04;0	84;0
318;66;3	45;22	3;3	33;27;FALS	13;0	03;0	12;0	03;0	8;0
319;74;7	9;7	3;3	38;23	3;FALSE;F	58;0	24;0	28;0	07;0
320;71;3	4;17;4	06;21	6;FALSE;F	12;0	03;0	16;0	04;0	7;0
321;69;5	64;18;5	58;14	5;FALSE;T	45;0	28;0	44;0	28;0	7;0
322;55;5;14	5;6	29;9	5;FALSE;F	41;0	13;0	49;0	18;0	64;0
323;57;5	9;26	8;7	04;24	1;FALSE;F	24;0	06;0	29;0	08;0
324;72;4	3;57;4	6;19	3;FALSE;T	35;0	2;0	38;0	22;0	81;0
325;74;7	33;27;3	45;28	9;FALSE;F	16;0	04;0	06;0	01;0	82;0
326;69;4	77;25;4	97;25	7;FALSE;F	39;0	12;0	41;0	13;0	81;0
327;71;9	46;5;2	61;NA;FAL	21;0	06;0	04;0	01;0	02;0	48;0
328;60;3	93;17	3;3	78;14	8;FALSE;F	33;0	09;0	32;0	08;0
329;58;3	48;17	5;4	01;18	7;FALSE;F	06;0	01;0	07;0	01;0
330;66;4;1	94;11	6;FALSE;F	33;0	09;0	33;0	09;0	47;0	38;0
331;63;7;15	8;6	66;16	6;TRUE;FA	4;1;FALSE;	14;0	02;0	14;0	02;0
332;68;4	04;17	8;4	62;17	3;FALSE;F	16;0	03;0	18;0	04;0
333;60;3	8;17	7;2	63;NA;FAL	14;0	03;0	09;0	02;0	76;0
334;61;7	5;19	6;9	55;19	6;TRUE;TR	39;0	19;0	41;0	23;0
335;71;6	6;17	1;1	06;NA;TRU	3;0;TRUE;T	24;0	05;0	18;0	01;0

336;58;7	66;7;8	57;8	5;FALSE;F	57;0	23;0	61;0	27;0	15;0
337;72;4;1	4;4	6;10	6;FALSE;T	57;0	38;0	62;0	44;0	42;0
338;59;3	1;13;4	11;10	9;FALSE;F	26;0	06;0	34;0	09;0	58;0
339;72;5	59;12	5;5	45;12	8;TRUE;FA	13;0	02;0	13;0	02;0
340;72;7	59;12;6	99;21	3;TRUE;FA	08;0	01;0	08;0	01;0	55;0
341;68;3	5;5	9;3	92;6	3;FALSE;F	29;0	07;0	33;0	09;0
342;61;6;1	04;18	5;FALSE;T	3;1;TRUE;T	47;0	3;0	33;0	18;0	76;0
343;64;4	7;22	3;4	97;22	3;FALSE;T	63;0	45;0	65;0	47;0
344;67;4	92;20;5	22;10	3;FALSE;F	4;0	12;0	43;0	14;0	77;0
345;76;6	03;26	7;6	24;26	6;FALSE;F	25;0	07;0	26;0	07;0
346;61;6	3;15;6	26;16	7;TRUE;FA	13;0	02;0	13;0	02;0	71;0
347;60;6	82;15	6;2	49;NA;FAL	75;0	6;0	4;0	22;0	69;0
348;71;6	86;29	6;6	4;31	5;TRUE;FA	14;0	02;0	13;0	02;0
349;59;3	6;16;3	02;17	5;FALSE;F	3;0	08;0	25;0	06;0	73;0
350;76;8	7;42;10	82;NA;FAL	36;0	12;0	44;0	16;0	82;0	1;0
351;63;3	5;11	8;2	53;NA;TRU	22;0	03;0	21;0	02;0	64;0
352;76;4	4;5	8;4	39;5	4;FALSE;T	61;0	42;0	61;0	42;0
353;68;5;1	9;5	19;13	1;FALSE;T	65;0	47;0	66;0	49;0	47;0
354;65;3	3;14	8;3	74;16	3;FALSE;F	12;0	02;0	14;0	03;0
355;70;6	08;17;8	21;21	1;FALSE;F	25;0	07;0	34;0	11;0	69;0
356;62;5	3;26;3	3;24	2;FALSE;F	22;0	05;0	12;0	02;0	85;0
357;78;4	19;19;5	66;17	1;FALSE;T	59;0	4;0	69;0	52;0	68;0
358;69;5	1;13;6	71;9;FALSE	42;0	13;0	52;0	19;0	55;0	31;0
359;61;5	1;24;4	6;27	6;FALSE;F	21;0	05;0	18;0	04;0	84;0
360;67;3	9;18;4	78;17	1;TRUE;FA	12;0	01;0	13;0	01;0	81;0
361;60;8	9;24	8;8	5;27	5;FALSE;F	2;0	05;0	19;0	05;0
362;70;7	7;14	4;9	49;17	3;TRUE;FA	08;0	01;0	08;0	01;0
363;71;3	18;16;3	8;13	6;FALSE;F	11;0	02;0	14;0	03;0	62;0
364;57;5	4;10;4	66;12	6;TRUE;FA	13;0	02;0	13;0	01;0	52;0
365;66;9	39;17	3;6	54;18	3;FALSE;T	9;0;FALSE	83;0	72;0	74;0
366;70;6	9;23;7	04;13	4;TRUE;TR	24;0	07;0	24;0	07;0	79;0
367;57;6	5;20;10	59;NA;TRU	14;0	03;0	16;0	05;0	85;0	09;0
368;63;3	3;22	8;3	6;23	3;FALSE;F	05;0	01;0	06;0	01;0
369;60;4	3;29	4;5	35;28;FALS	08;0	02;0	1;0	02;0	86;0
370;59;3	5;24;3	43;27	4;FALSE;F	06;0	01;0	06;0	01;0	84;0
371;70;5	8;28;5	07;28	9;FALSE;F	5;1;TRUE;T	24;0	06;0	2;0	05;0
372;57;8	88;18;9	06;17	9;FALSE;F	37;0	12;0	38;0	12;0	74;0
373;65;6;8	3;5	91;7	1;TRUE;FA	13;0	02;0	13;0	02;0	3;0
374;68;6	5;24;5	2;28	4;FALSE;F	13;0	03;0	1;0	02;0	82;0
375;67;7;1	13;17	1;TRUE;FA	14;0	02;0	14;0	02;0	69;0	2;0
376;76;4	5;17;4	08;15	4;FALSE;F	18;0	04;0	16;0	04;0	66;0
377;64;6	5;12;7	31;12	8;TRUE;FA	08;0	01;0	08;0	01;0	61;0
378;57;5	5;17;2	25;NA;FAL	22;0	06;0	07;0	01;0	76;0	19;0
379;60;4;2	32;22	2;FALSE;F	6;1;FALSE	15;0	03;0	12;0	03;0	8;0
380;68;5	05;27	3;3	71;21;FALS	41;0	13;0	31;0	08;0	83;0
381;60;5;1	8;4	49;9	5;FALSE;T	65;0	47;0	62;0	43;0	45;0
382;68;5	22;16;4	52;20	7;FALSE;F	21;0	05;0	18;0	04;0	68;0
383;63;6	68;10;5	46;12	6;TRUE;FA	08;0	01;0	08;0	01;0	47;0
384;57;4	17;10;4	42;14	9;FALSE;T	34;0	19;0	36;0	21;0	36;0
385;72;3	4;3	2;3	33;3	6;FALSE;T	52;0	32;0	51;0	31;0;0
386;66;4	43;11;3	1;20	3;FALSE;F	37;0	11;0	26;0	06;0	46;0
387;60;3	82;18;5	07;18	7;FALSE;F	32;0	08;0	42;0	13;0	77;0
388;63;4	3;9;5;9	4;FALSE;F	17;0	04;0	2;0	05;0	32;0	47;0
389;72;5	32;14;5	66;19	2;FALSE;F	22;0	05;0	23;0	06;0	58;0
390;66;6	5;22	7;6	52;21	3;FALSE;F	13;0	03;0	13;0	03;0
391;64;5	4;17	7;5	3;19	6;FALSE;T	68;0	5;0	67;0	5;0

392;57;6	43;8	9;7	06;10;FALS	5;0	18;0	54;0	21;0	27;0
393;70;5	71;11	4;6	61;64	2;FALSE;F.	46;0	15;0	51;0	19;0
394;64;5	4;12;6	35;14	3;FALSE;F.	44;0	14;0	5;0	18;0	52;0
395;58;3	2;14;3	75;14	1;FALSE;F.	26;0	06;0	31;0	08;0	68;0
396;57;3	83;12;3	79;10	8;TRUE;FA	2;0;FALSE;	22;0	03;0	22;0	03;0
397;68;4	1;22	9;3	26;33	7;FALSE;F.	16;0	04;0	12;0	02;0
398;65;6;1	71;16	2;FALSE;F.	1;1;TRUE;T	25;0	07;0	28;0	08;0	45;0
399;56;7	59;13	4;9	05;20	8;TRUE;FA	5;1;TRUE;F	14;0	02;0	14;0
400;61;6	46;13	4;9	98;13	1;TRUE;FA	6;1;TRUE;F	14;0	02;0	15;0
401;57;4	15;6	8;6	31;3;FALSE	6;0;FALSE;	35;0	1;0	5;0	18;0
402;59;7	7;13;7	07;13	8;TRUE;FA	14;0	02;0	14;0	02;0	69;0
403;62;4	8;27;4	82;31	7;FALSE;F.	09;0	02;0	09;0	02;0	85;0
404;56;4	14;23	3;5	76;24	1;FALSE;F.	35;0	1;0	46;0	16;0
405;70;4	13;17;5	05;20	7;TRUE;FA	12;0	01;0	13;0	02;0	73;0
406;57;5	48;13	5;6	07;15	1;TRUE;FA	63;1;FALSE	13;0	02;0	13;0
407;57;5;23	51;NA;FAL	97;2;FALSE	09;0	02;0	04;0	01;0	84;0	13;0
408;75;3	04;33;3	13;34	1;TRUE;TR	69;1;FALSE	21;0	04;0	21;0	04;0
409;65;4	6;30;5	7;26	4;FALSE;F.	08;0	02;0	11;0	03;0	85;0
410;55;7	9;7	9;10	76;NA;TRU	14;0	02;0	15;0	03;0	31;0
411;63;7	8;18	5;6	74;20	4;TRUE;FA	08;0	01;0	08;0	01;0
412;64;6	7;17;6	1;20	3;TRUE;FA	08;0	01;0	08;0	01;0	79;0
413;55;4;13	2;3	95;14	9;FALSE;F.	15;0	03;0	15;0	03;0	59;0
414;58;6	7;11	1;2	24;NA;FAL	28;0	08;0	07;0	01;0	49;0
415;61;4	5;14	6;4	45;13	2;TRUE;FA	13;0	01;0	13;0	01;0
416;70;4	4;18	1;6	4;20	9;FALSE;F.	08;0	02;0	13;0	03;0
417;71;4	76;12	6;5	68;10	5;FALSE;F.	19;0	05;0	23;0	06;0
418;62;4	2;15	2;4	27;17	3;TRUE;FA	12;0	01;0	12;0	01;0
419;70;3	7;26;4	72;26	2;FALSE;F.	14;0	03;0	19;0	04;0	82;0
420;60;6	5;6	8;4	68;9	4;FALSE;F.	51;0	19;0	39;0	12;0
421;59;5	4;14	5;4	13;16	2;FALSE;F.	22;0	06;0	16;0	04;0
422;56;4	08;12	5;3	89;14	1;TRUE;FA	7;1;FALSE;	12;0	01;0	12;0
423;71;4	9;16;4	7;16	3;FALSE;F.	7;1;TRUE;F	2;0	05;0	19;0	04;0
424;78;8	5;12	7;2	46;NA;FAL	6;0	44;0	19;0	09;0	42;0
425;62;4	7;18	2;3	44;24	1;FALSE;F.	19;0	04;0	13;0	03;0
426;69;4	1;23	1;3	35;22	6;FALSE;F.	16;0	04;0	12;0	03;0
427;59;4	5;12	1;5	42;8	6;FALSE;T	24;0;TRUE	62;0	43;0	68;0
428;69;4	11;16;4	98;17	4;FALSE;F.	46;0;TRUE	34;0	09;0	41;0	13;0
429;62;5	1;10;3	8;9	7;FALSE;F.	54;0;FALSE	42;0	13;0	32;0	08;0
430;62;5	8;9;5	92;8	9;FALSE;T	7;0	54;0	71;0	54;0	28;0
431;64;6;32	13;29	3;FALSE;T	27;0	15;0	37;0	23;0	82;0	11;0
432;56;4	6;16;5	72;11	5;FALSE;F.	18;0	04;0	23;0	06;0	68;0
433;63;6;32	6;5	1;28	2;FALSE;F.	12;0	03;0	1;0	02;0	87;0
434;62;7	6;24;4	2;24	2;FALSE;F.	32;0	1;0	16;0	04;0	84;0
435;65;6	37;18	8;9	57;29	9;TRUE;FA	08;0	01;0	08;0	01;0
436;74;8	9;10	9;8	94;9	1;FALSE;T	61;0	46;0	62;0	46;0
437;77;9;25	5;6	19;23	9;FALSE;F.	2;0	05;0	13;0	03;0	8;0
438;56;3	88;25	5;4	85;21	8;FALSE;F.	15;0	03;0	19;0	05;0
439;65;8;1(	96;NA;FAL	34;0	1;0	45;0	16;0	36;0	42;0	22;0
440;70;5	8;15	3;4	99;12	6;FALSE;F.	12;0	03;0	09;0	02;0
441;67;4	06;28	2;1	86;NA;FAL	16;0	04;0	06;0	01;0	8;0
442;70;4	74;16	5;4	49;14;FALS	19;0	05;0	18;0	04;0	68;0
443;70;5	51;17	4;6	11;16	2;FALSE;F.	23;0	06;0	25;0	07;0
444;64;4	77;11	5;1	38;NA;FAL	19;0	05;0	04;0	01;0	5;0
445;77;4	99;10	2;6	12;8	4;FALSE;F.	46;0;TRUE	41;0	13;0	48;0
446;59;7	7;11	7;4	48;15	1;FALSE;F.	64;2;FALSE	17;0	04;0	08;0
447;68;9	12;23	2;8	56;20;TRU	14;0	03;0	14;0	03;0	86;0

448;69;8;7	8;7	36;8	2;FALSE;F	58;0	24;0	55;0	22;0	13;0
449;63;4	36;20	4;2	98;NA;FAL	17;0	04;0	1;0	02;0	75;0
450;62;5	2;12	7;6	31;11	2;TRUE;FA	07;0	01;0	08;0	01;0
451;65;8	37;14;10	21;NA;FAL	35;0	11;0	42;0	15;0	55;0	31;0
452;62;3	5;24	1;3	06;26	4;FALSE;F	13;0	03;0	11;0	02;0
453;70;5	4;30;5	07;28	2;FALSE;F	1;0	02;0	1;0	02;0	84;0
454;59;3	29;16;4	21;15	2;FALSE;F	27;0	07;0	35;0	1;0	74;0
455;64;5	19;16	8;5	78;14	7;FALSE;T	66;0	49;0	7;0	53;0
456;71;9	1;12	9;3	33;20	7;FALSE;F	38;0	13;0	12;0	03;0
457;69;3	89;11;3	92;13	5;FALSE;F	32;0	09;0	33;0	09;0	45;0
458;65;7;1	64;16	6;FALSE;F	15;0	04;0	19;0	05;0	63;0	26;0
459;69;5	81;16	6;4	7;14;FALSE	24;0	06;0	19;0	04;0	69;0
460;71;5	21;29	2;5	41;21	8;FALSE;F	21;0	05;0	22;0	06;0
461;69;4	55;15;4	12;13	5;FALSE;F	18;0	04;0	16;0	04;0	58;0
462;65;4	92;11	9;5	42;12	9;FALSE;F	2;0	05;0	22;0	06;0
463;69;5	3;15;4	15;19;FALS	1;0	02;0	07;0	02;0	64;0	26;0
464;78;6	8;13	2;7	37;13;FALS	75;0	6;0	77;0	63;0	45;0
465;66;3	5;22;3	28;24;FALS	13;0	03;0	12;0	02;0	76;0	19;0
466;62;9	32;18;8	41;19	9;TRUE;FA	08;0	01;0	08;0	01;0	83;0
467;75;5	26;13	6;4	29;21	2;FALSE;F	03;2;FALSE	1;0	02;0	08;0
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469;57;6;1	2;3	49;19	1;FALSE;F	12;0	03;0	06;0	01;0	78;0
470;72;4	8;16	3;4	25;15	5;TRUE;FA	13;0	01;0	12;0	01;0
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472;75;5	15;15	6;6	8;13	9;FALSE;F	21;0	05;0	28;0	08;0
473;64;4	96;24	1;4	97;24	5;FALSE;F	2;0	05;0	2;0	05;0
474;72;6;1	51;NA;FAL	25;0	07;0	08;0	02;0	65;0	23;0	12;0
475;69;8	4;16	7;8	47;21	7;TRUE;FA	5;1;FALSE	14;0	02;0	14;0
476;57;8	07;13	5;7	92;13	7;TRUE;FA	08;0	01;0	08;0	01;0
477;60;3	81;28	7;3	64;30	7;FALSE;F	14;0	03;0	14;0	03;0
478;58;4	55;28	7;4	07;26	2;TRUE;FA	23;0	04;0	22;0	03;0
479;68;7	09;17	7;4	3;23	2;TRUE;FA	14;0	02;0	13;0	01;0
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481;63;7	87;14	3;7	81;15	2;FALSE;F	17;0	04;0	17;0	04;0
482;62;6	9;8	9;6	91;8	9;TRUE;FA	24;0	06;0	24;0	06;0
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491;58;7	9;7	9;8	91;8	8;TRUE;FA	08;0	01;0	08;0	01;0
492;72;4	25;17	1;6	61;15	1;FALSE;F	35;0	1;0	51;0	19;0
493;62;7	06;2	6;7	91;3	6;FALSE;F	54;0	21;0	58;0	24;0;0
494;62;4	59;8	2;5	53;8	3;FALSE;F	38;0	11;0	45;0	15;0
495;64;4	6;19	1;4	15;21	2;FALSE;F	64;1;TRUE	18;0	04;0	16;0
496;56;3	4;12	5;3	34;16	7;FALSE;F	12;0	03;0	12;0	03;0
497;67;3	55;18	8;2	61;NA;FAL	34;1;TRUE	13;0	03;0	09;0	02;0
498;71;4	5;12	6;3	65;9	3;FALSE;F	5;0;TRUE;T	37;0	11;0	3;0
499;64;5	66;33;7	99;32	9;FALSE;F	23;0	06;0	33;0	1;0	87;0
500;66;3	9;17	2;5	08;15	1;FALSE;T	57;0	37;0	66;0	48;0
501;60;4	32;13	4;3	85;14;FALS	35;0	2;0	32;0	17;0	55;0
502;60;6	8;5	2;7	74;4	7;FALSE;F	52;0	2;0	57;0	23;0
503;72;5	07;16	7;4	68;16	2;FALSE;F	2;0	05;0	19;0	04;0

504;56;3	45;10	7;2	78;NA;FAL	29;0	07;0	23;0	05;0	52;0
505;67;6	5;22	5;6	08;24	3;FALSE;F	27;0	07;0	25;0	07;0
506;71;9	4;32	5;10	18;NA;TRU	08;0	01;0	09;0	01;0	9;0
507;79;6	6;10;7	53;9	5;FALSE;T	74;0	59;0	77;0	64;0	25;0
508;55;5	6;15	3;6	15;14	7;FALSE;F	11;0	03;0	12;0	03;0
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510;56;6	7;13;6	25;13	7;FALSE;F	28;0	08;0	26;0	07;0	62;0

pc1;erspc1.hg;erspc2;erspc2.hg;pcptrc.negative.1;pcptrc.low.grade.1;pcptrc.high.grade.1;pcptrc.negativ

47;0	17;0	39;0	45;0	16;0	79;0	17;0	04;0	79;0
33;0	42;0	4;0	28;0	32;0	52;0	21;0	27;0	56;0
52;0	33;0	15;0	58;0	3;0	12;0	82;0	13;0	05;0
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3;0	85;0	14;0	01;0	65;0	25;0	1;0	85;0	14;0
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04;0	17;0	39;0	44;0	42;0	31;0	27;0	7;0	16;0
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82;0	13;0	05;0	85;0	12;0	03;0	74;0	19;0	07;0
83;0	13;0	04;0	84;0	13;0	03;0	76;0	18;0	06;0
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27;0	13;0	75;0	18;0	07;0	75;0	17;0	08;0	77;0
37;0	6;0	24;0	16;0	66;0	21;0	13;0	68;0	19;0
49;0	49;0	8;0	16;0	04;0	71;0	2;0	09;0	8;0
6;0	3;0	1;0	68;0	25;0	07;0	66;0	24;0	1;0
38;0	13;0	72;0	22;0	06;0	77;0	18;0	05;0	77;0
66;0	23;0	11;0	67;0	22;0	11;0	73;0	18;0	09;0
1;0	03;0	86;0	11;0	03;0	78;0	17;0	05;0	77;0
66;0	26;0	08;0	72;0	22;0	06;0	77;0	18;0	05;0
06;0	78;0	17;0	05;0	73;0	19;0	08;0	78;0	17;0

2;0	08;0	69;0	22;0	09;0	65;0	25;0	1;0	63;0
46;0	33;0	21;0	44;0	32;0	24;0	7;0	19;0	11;0
7;0	21;0	09;0	77;0	17;0	06;0	82;0	13;0	05;0
23;0	35;0	42;0	36;0	31;0	33;0	55;0	21;0	24;0
32;0	51;0	31;0	18;0	66;0	22;0	12;0	72;0	18;0
01;0	44;0	55;0	01;0	45;0	54;0	62;0	23;0	15;0
08;0	67;0	24;0	09;0	74;0	19;0	07;0	74;0	19;0
05;0	81;0	14;0	05;0	84;0	12;0	04;0	73;0	2;0
19;0	12;0	65;0	2;0	15;0	7;0	18;0	12;0	65;0
16;0	87;0	1;0	03;0	88;0	09;0	03;0	8;0	16;0
82;0	14;0	04;0	72;0	21;0	07;0	76;0	18;0	06;0
62;0	27;0	11;0	72;0	2;0	08;0	77;0	16;0	07;0
64;0	28;0	08;0	83;0	14;0	03;0	77;0	18;0	05;0
78;0	13;0	09;0	73;0	16;0	11;0	65;0	19;0	16;0
67;0	26;0	07;0	74;0	21;0	05;0	71;0	22;0	07;0
15;0	08;0	63;0	2;0	17;0	7;0	18;0	12;0	63;0
31;0	77;0	16;0	07;0	73;0	17;0	1;0	77;0	16;0
22;0	06;0	77;0	18;0	05;0	76;0	18;0	06;0	77;0
46;0	2;0	75;0	19;0	06;0	77;0	18;0	05;0	77;0
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51;0	39;0	89;0	09;0	02;0	86;0	11;0	03;0	89;0
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19;0	12;0	79;0	13;0	08;0	75;0	15;0	1;0	74;0
45;0	41;0	14;0	46;0	41;0	13;0	76;0	18;0	06;0
1;0	83;0	15;0	02;0	79;0	17;0	04;0	83;0	15;0
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07;0	88;0	05;0	07;0	69;0	14;0	17;0	7;0	14;0
13;0	06;0	81;0	13;0	06;0	76;0	17;0	07;0	75;0
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06;0	86;0	1;0	04;0	87;0	09;0	04;0	8;0	14;0
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84;0	1;0	06;0	86;0	09;0	05;0	76;0	15;0	09;0
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66;0	27;0	07;0	65;0	27;0	08;0	73;0	21;0	06;0
16;0	07;0	77;0	16;0	07;0	69;0	21;0	1;0	68;0
66;0	26;0	08;0	34;0	46;0	2;0	75;0	19;0	06;0
17;0	05;0	79;0	17;0	04;0	77;0	18;0	05;0	78;0
22;0	06;0	8;0	16;0	04;0	77;0	18;0	05;0	8;0
85;0	11;0	04;0	86;0	1;0	04;0	77;0	16;0	07;0
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83;0	12;0	05;0	8;0	13;0	07;0	79;0	14;0	07;0
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86;0	09;0	05;0	85;0	09;0	06;0	76;0	15;0	09;0
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01;0	84;0	13;0	03;0	85;0	12;0	03;0	76;0	18;0
71;0	21;0	08;0	78;0	15;0	07;0	72;0	2;0	08;0

3;0	46;0	24;0	73;0	21;0	06;0	61;0	26;0	13;0
64;0	26;0	1;0	68;0	24;0	08;0	73;0	19;0	08;0
32;0	19;0	73;0	16;0	11;0	78;0	14;0	08;0	73;0
13;0	06;0	7;0	23;0	07;0	6;0	26;0	14;0	7;0
15;0	28;0	45;0	27;0	23;0	47;0	3;0	69;0	21;0
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86;0	11;0	03;0	85;0	1;0	05;0	74;0	19;0	07;0
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41;0	41;0	18;0	27;0	5;0	23;0	65;0	25;0	1;0
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66;0	21;0	13;0	72;0	16;0	12;0	74;0	15;0	11;0
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64;0	25;0	11;0	7;0	22;0	08;0	71;0	2;0	09;0
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77;0	17;0	06;0	75;0	19;0	06;0	72;0	2;0	08;0
15;0	08;0	77;0	15;0	08;0	63;0	22;0	15;0	64;0
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09;0	08;0	82;0	07;0	11;0	71;0	14;0	15;0	63;0
14;0	07;0	77;0	16;0	07;0	62;0	25;0	13;0	64;0
29;0	1;0	82;0	15;0	03;0	75;0	19;0	06;0	81;0
55;0	24;0	21;0	53;0	25;0	22;0	61;0	2;0	19;0
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82;0	15;0	03;0	81;0	15;0	04;0	79;0	17;0	04;0
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87;0	1;0	03;0	89;0	08;0	03;0	73;0	2;0	07;0
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73;0	2;0	07;0	77;0	17;0	06;0	74;0	19;0	07;0
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81;0	14;0	05;0	85;0	11;0	04;0	84;0	12;0	04;0
01;0	78;0	18;0	04;0	79;0	17;0	04;0	72;0	22;0
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03;0	82;0	15;0	03;0	74;0	19;0	07;0	82;0	15;0
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05;0	82;0	13;0	05;0	68;0	23;0	09;0	67;0	24;0
34;0	41;0	25;0	29;0	44;0	27;0	64;0	22;0	14;0

42;0	36;0	22;0	49;0	33;0	18;0	64;0	22;0	14;0
17;0	55;0	28;0	2;0	54;0	26;0	73;0	2;0	07;0
03;0	82;0	15;0	03;0	76;0	18;0	06;0	82;0	15;0
69;0	23;0	08;0	8;0	15;0	05;0	72;0	2;0	08;0
3;0	49;0	21;0	39;0	43;0	18;0	73;0	19;0	08;0
19;0	09;0	76;0	16;0	08;0	73;0	18;0	09;0	76;0
16;0	06;0	59;0	3;0	11;0	73;0	2;0	07;0	73;0
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73;0	21;0	06;0	52;0	28;0	2;0	69;0	23;0	08;0
75;0	19;0	06;0	8;0	15;0	05;0	8;0	15;0	05;0
11;0	77;0	15;0	08;0	7;0	18;0	12;0	7;0	18;0
64;0	26;0	1;0	68;0	24;0	08;0	86;0	11;0	03;0
37;0	86;0	11;0	03;0	79;0	14;0	07;0	86;0	11;0
39;0	39;0	22;0	33;0	43;0	24;0	66;0	22;0	12;0
79;0	16;0	05;0	85;0	1;0	05;0	73;0	19;0	08;0
7;0	17;0	13;0	77;0	14;0	09;0	71;0	16;0	13;0
71;0	23;0	06;0	68;0	26;0	06;0	81;0	16;0	03;0
11;0	04;0	84;0	11;0	05;0	73;0	19;0	08;0	73;0
21;0	75;0	16;0	09;0	81;0	13;0	06;0	72;0	18;0
83;0	11;0	06;0	82;0	14;0	04;0	63;0	22;0	15;0
19;0	04;0	63;0	29;0	08;0	81;0	16;0	03;0	78;0
17;0	05;0	72;0	21;0	07;0	78;0	17;0	05;0	76;0
57;0	32;0	11;0	27;0	51;0	22;0	74;0	19;0	07;0
06;0	02;0	89;0	08;0	03;0	83;0	12;0	05;0	84;0
42;0	27;0	23;0	45;0	32;0	71;0	18;0	11;0	69;0
24;0	07;0	68;0	25;0	07;0	78;0	17;0	05;0	79;0
12;0	06;0	83;0	11;0	06;0	71;0	18;0	11;0	68;0
8;0	11;0	09;0	82;0	11;0	07;0	59;0	23;0	18;0
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05;0	74;0	2;0	06;0	78;0	17;0	05;0	77;0	18;0
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1;0	04;0	84;0	12;0	04;0	76;0	16;0	08;0	76;0
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87;0	1;0	03;0	86;0	11;0	03;0	76;0	18;0	06;0
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5;0	77;0	18;0	05;0	62;0	23;0	15;0	77;0	18;0
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83;0	1;0	07;0	83;0	09;0	08;0	76;0	13;0	11;0
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34;0	08;0	44;0	43;0	13;0	76;0	2;0	04;0	73;0
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14;0	26;0	46;0	28;0	71;0	2;0	09;0	68;0	21;0
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11;0	78;0	14;0	08;0	76;0	15;0	09;0	76;0	15;0
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de.nofreepsa.2;Time between PSA (weeks)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	7
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	*8*
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8-10
		(*b*) Describe any methods used to examine subgroups and interactions	8-10
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	8-10
		(*e*) Describe any sensitivity analyses	8-10
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	11
		(b) Give reasons for non-participation at each stage	11
		(c) Consider use of a flow diagram	Supplemental
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	11- Supplemental
Outcome data	15*	Report numbers of outcome events or summary measures	12
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supplemental
Discussion			
Key results	18	Summarise key results with reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.