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# BMJ Open

## Comparison between the accuracy/variability of ERSPC and the PCPT risk calculators for the prediction of significant prostate cancer in patients with PSA <10ng/ml

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Keywords:	Significant prostate cancer, risk calculator variability, ERSPC, PCPT

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3     **Comparison between the accuracy/variability of ERSPC and the PCPT risk**  
4     **calculators for the prediction of significant prostate cancer in patients with PSA**  
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6     **<10ng/ml**  
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36   **Running title:** Risk Calculators for Significant Prostate Cancer  
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39   **Key Words:** Significant prostate cancer; risk calculator variability; ERSPC; PCPT.  
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For peer review only

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2    1    **ABSTRACT**  
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4    2         **Introduction:** Risk Calculators (RCs) are easy-to-use tools considering available clinical-  
5 variables that could help to select those patients with risk of prostate-cancer (PCa) who should  
6 undergo a prostate-biopsy. **Objective:** To perform a comparison for the prediction of significant-PCa  
7 (SigPCa) between the European-Randomised Study of Screening for PCa (ERSPC) and the PCa  
8 Prevention-Trial (PCPT) RCs in patients with PSA between 3-10ng/ml through an evaluation of the  
9 accuracy/variability between two consecutive PSA-values. **Setting:** An observational study in a  
10 major university Hospital of the south of Spain. **Methods and participants:** An observational study  
11 was performed in patients who underwent a prostate-biopsy. SigPCa probabilities were calculated  
12 with the two PSA measures using ERSPC3/4+DRE and PCPTv2+free-PSA RCs. The prediction  
13 accuracy of SigPCa was determined by the area under the curve (AUC). Calibration, discrimination,  
14 and decision curve analysis were studied. The variability between both RCs-agreement was compared  
15 using Cohen's kappa coefficient. **Results:** 510 patients were analysed (87 diagnosed with SigPCa).  
16 The median PSA varied from 5.3 to 5ng/ml between both measures. Both RCs overestimated the risk  
17 in the case of high-risk probabilities, but ERSPC was better calibrated than PCPT for risks in the  
18 clinically useful moderate-risk range. Discrimination ability for SigPCa was similar between models  
19 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  
20 less variability than PCPT-RC, with a constant agreement ( $k=0.7-0.8$ ) for usual range of clinical  
21 decision-making. Remarkably, a higher biopsies number would be avoided using the ERSPC-RC, but  
22 more SigPCa would be missed. **Conclusions:** Both RCs had similar accuracy for the SigPCa  
23 discrimination. However, ERSPC-RC is better calibrated for clinically useful risks and more stable  
24 for intra-individual PSA variations.

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2 24 **Strengths and limitations of this study**  
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- 4 25 - This study highlights the need to spread the use of available free tools to improve patient's  
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6 26 selection for undergoing prostate biopsy.  
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8 27 - This study is the first to compare two available free risk calculators in patients with a PSA <  
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10 28 10ng/ml analyzing their variability between two consecutive different PSA levels.  
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12 29 - Although the clinical information of this study was extracted from a clinical practice cohort  
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14 30 and with information that could be useful for urologists worldwide, this is a retrospective  
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16 31 study and the use of TRUS biopsy for PCa diagnosis, even though is the standard in most  
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18 32 populations, suffers from random error compared with template biopsy, which could have  
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20 33 affected prediction results.  
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2   35   **INTRODUCTION**  
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5   36   Prostate cancer (PCa) is the second most frequently diagnosed malignancy in males worldwide,  
6   37   and the most frequent in developed countries(1). Its current standard of diagnosis is a prostate biopsy  
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8   38   based on PSA levels and digital rectal examination (DRE). However, there are other available and  
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10   39   complementary variables that could help to select those patients who should undergo a prostate biopsy  
11  
12   40   (such as age, prostate volume, free PSA, family history, etc.), but these are not always used and/or  
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14   41   well-integrated in daily clinical practice(2). In line with this, Risk Calculators (RCs) are easy-to-use  
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16   42   tools that can help the clinicians to take advantage of all these available variables (3). The two main  
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18   43   RCs are from the European Randomised Study for Screening of Prostate Cancer (ERSPC cohort;  
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20   44   ERSPC-RC: <http://www.prostatecancer-riskcalculator.com/seven-prostate-cancer-riskcalculators>)  
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22   45   and from the Prostate Cancer Prevention Trial (PCPT cohort; PCPT-RC:  
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24   46   <http://deb.uthscsa.edu/URORiskCalc/Pages/calcs.jsp>). Both RCs have undergone some  
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26   47   modifications, specifically the addition of estimated prostate volume in the ERSPC-RC (4,5).  
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28   48   Furthermore, both RCs were originally developed from different patient cohorts and each RC uses  
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30   49   different variables.  
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32   50   To date, limited external validations and comparisons have been performed by different groups  
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34   51   (6–9). The two most important recent comparisons of the modified RCs were performed by Foley *et*  
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36   52   *al* (6,7) and Poyet *et al* (9). Both found a better discriminatory ability for ERSPC-RC vs PCPTv.2-  
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38   53   RC for the diagnoses of significant-PCa (Sig PCa) (AUC around 0.74 vs 0.69, respectively), but they  
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40   54   also included patients with a high PSA of up to 50 ng/ml. Despite the possibility of using these RCs  
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42   55   in patients with PSA levels up to 50ng/ml, it is clear that the advantages of using both RCs would  
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44   56   probably increase in patients with a PSA under 10ng/ml. Furthermore, in the case of the PCPTv.2-  
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46   57   RC, the addition of the free PSA value in patients with a PSA under 10ng/ml seems to improve its  
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48   58   accuracy (5), and, therefore, given its accessibility, this value should be included in the RC.  
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51   59   The intra-individual and inter-assay variability of PSA is already known (10–12) and, therefore,  
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53   60   at least two measures are necessary before a prostate biopsy is indicated. In fact, it has been shown  
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2 61 that approximately 25% of men with initial PSA levels between 4 and 10 ng/mL had normal PSA  
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4 62 values upon repeat testing (13). In line with this, despite being primarily based on PSA level, the  
5 variability of the two RCs mentioned above has been poorly studied, and might have implications for  
6 patient management. Our group has recently evaluated this variability with the ERSPC-RC, which  
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8 64 showed stable accuracy over a cohort of patients, but some changes with respect to an individual  
9 approach (14). To date, there is no study comparing the accuracy and variability of both RCs, the  
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11 65 ERSPC + DRE vs. the PCPTv.2 + free PSA, for the prediction of Sig PCa. Therefore, the aim of this  
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13 66 study was to perform a direct comparison between ERSPC + DRE and PCPTv.2 + free PSA RCs in  
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15 68 patients with a PSA between 3-10ng/ml, evaluating the accuracy and variability of both methods in  
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17 69 the prediction of Sig PCa.  
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2 72 **MATERIALS AND METHODS**  
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4 73 **Study population and design**  
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6 74 An observational retrospective study was performed in patients from ONCOVER cohort (1021  
7 biopsies indicated by clinical practise wherein patients donated blood and urine before the biopsy).  
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9 75 The study was carried out within the project approved by our Hospital Research Ethics Committee,  
10 and informed consent was obtained from all participants. Blood sample was obtained in the morning  
11 76 (between 8:00-10:00 am) after an overnight fasting and then, the prostate biopsy was implemented  
12 according to clinical practice. The inclusion criteria for this study were: 1) PSA indication between  
13 77 3-10 ng/ml; 2) Full clinical and laboratory data to fulfilled ERSPC-RC and PCPT-RC; 3) Age 55-80  
14 years' old; 4) Two consecutives measurements of PSA levels within an interval of 12 weeks.  
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16 81 Exclusion criteria included patients with a previously known PCa diagnosis or treatment that could  
17 modify PSA levels.  
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19 82 Transrectal prostate biopsy was carried out under local anaesthesia by using a standard peri-  
20 prostatic block, a transrectal ultrasound transducer, and an 18G automated needle biopsy instrument.  
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22 83 The prostatic volume was measured following the protocol used during transrectal ultrasound (TRUS),  
23 and usual recommendations were to take 12 cores in patients undergoing the first biopsy procedure,  
24 and a minimum of 16 biopsy cores for those who had a previous biopsy. Biopsy specimens were  
25 analysed by expert urologic pathologists according to the International Society of Urological  
26 Pathology (ISUP) 2005 modified criteria (15).  
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50 92 **Main variables description**  
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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  $\geq$ 50 = 60 mL).

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2 98      **Significant/ high-grade (HG) prostate cancer (Sig PCa):** PCa with a Gleason grade  $\geq 7$  on  
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4 99      biopsy.  
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9 101      **ERSPC-RC and PCPT-RC probabilities calculation**

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11 102      **ERSPC:** The formulas for the ERSPC-RC 3+DRE for patients at initial biopsy and the ERSPC-  
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13 103      RC 4+DRE for patients at repeat biopsy were utilized in this study (4). These calculators use PSA,  
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15 104      prostate volume, and DRE as variables, with, a negative prostate biopsy in ERSPC 4+ DRE in patients  
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17 105      who had a previous biopsy. This provides a probability rating for any PCa or Sig PCa (Gleason  $\geq 7$ ).  
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19 106      **ERSPC1/Sig PCa (1° Measure):** Risk probability calculated by ERSPC-RC3 or 4 (if previous  
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21 107      biopsy)  $\pm$  DRE for any PCa using PSA 1/ Sig PCa – for HG PCa.  
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25 108      **ERSPC2/Sig PCa (2° Measure):** Risk probability calculated by ERSPC-RC 3 or 4 (if previous  
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27 109      biopsy)  $\pm$  DRE for any PCa using PSA 2/ Sig PCa – for HG PCa.  
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30 110      **PCPT:** The formulae for the PCPT-RC 2.0 + %free PSA was utilized in this study (5). This  
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32 111      calculator use race, age, PSA level, %free PSA level, family history of PCa, DRE and prior prostate  
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34 112      biopsy. This gives a probability of negative biopsy, low grade PCa and Sig PCa (gleason  $\geq 7$ ).  
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36 113      **PCPT1:** Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 1 and free PSA 1.  
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38 114      **PCPT2:** Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 2 and free PSA 2.  
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41 115      The variability of PSA was calculated by the following formula: **|1 Measure – 2 Measure| / 1**  
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43 116      **Measure**

#### 44 45 117 46 47 48 118      **Statistical analysis**

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50 119      A descriptive study was performed by calculating the median and interquartile ranges (IR) for the  
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52 120      quantitative variables, and the absolute frequencies and percentages for the qualitative variables. A  
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54 121      Student's T test for paired groups was used to compare the means of the quantitative variables (PSA  
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56 122      1 and 2).  
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2 123 The investigation of the comparative performance in the detection of Sig PCa of both RCs,  
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4 124 ERSPC-RC and PCPT-RC, was performed, taking into account these five factors: discrimination  
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6 125 capacity, calibration, clinical utility, and consistency against the observed variations in PSA levels  
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8 126 for our dataset.  
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11 127 The discrimination ability of the models, i.e., their ability to separate those patients who had Sig  
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13 128 PCa from those who do not, was assessed using the area under their Receiver Operator Characteristic  
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15 129 (ROC) curve (AUC) (16), as measured in our sample. This is one of the most frequently used  
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17 130 measurements of model discrimination, because of its independence of the selection of a specific  
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19 131 decision threshold and its robustness against class imbalance. Confidence intervals for these AUCs  
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21 132 were computed using bootstrapping. These AUCs were then compared to determine the relative  
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23 133 performance of the models using DeLong tests (17). These tests were chosen because of their non-  
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25 134 parametric nature, with few assumptions about the data, and their suitability for paired data, as both  
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27 135 models were evaluated over the same dataset, properties which make this the most commonly used  
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29 136 test to compare AUCs (18). For this comparison, we focused on the first measure of PSA (PSA 1; the  
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31 137 value for the indication of the prostate biopsy).  
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36 138 The calibration of the calculators for our cohort was then investigated to determine the agreement  
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38 139 between the frequency of the observed outcome (PCa in our case) and the risks predicted by the model.  
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41 140 Calibration plots were used for this purpose (19), enabling a visual evaluation of this agreement and  
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43 141 the comparison between RCs.  
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46 142 To address the potential clinical utility of the models, we performed decision curve analysis on  
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48 143 our data, as proposed by Vickers and Elkin (20). This method has the advantage of not requiring the  
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50 144 specification of the relative cost for false-positives and false-negatives, defining a net benefit as a  
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52 145 function of the decision threshold at which one would consider obtaining a biopsy.  
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55 146 Finally, the stability of the predictions of both RCs, with regard to the observed intra-patient  
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57 147 changes on PSA levels between measurements, was investigated using the Cohen's kappa ( $\kappa$ ) inter-  
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59 148 rater agreement coefficient as a function of the decision threshold. This coefficient was selected due  
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1  
2 149 to its widespread use and robustness against random agreements, and thus, is a better measurement  
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4 150 than naïve accuracy.  
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7 151 All the analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill) and R version  
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9 152 3.2.3. A <5% level of significance ( $p<0.05$ ) was used to decide statistically significant differences.  
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13 154 **Patient and public involvement**  
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17 155 Participants and public were not involved in the development of research questions, study design  
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19 156 or recruitment.  
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2 159 **RESULTS**  
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4 160 **Cohort characteristics**  
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7 161 In the present study, we analysed 510 patients who met the inclusion criteria previously described.  
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9 162 Median age was 65 (60-70) years old, with a family history in 89 patients (17.5%) and a suspicious  
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11 163 DRE in 82 patients (16.1%). The median PSA before prostate biopsy indication was 5.3 (4.3-6.9)  
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13 164 ng/ml. 176 patients were diagnosed with PCa, 87 of those categorized as Sig PCa. Most patients  
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15 165 (n=401; 78.6%) were biopsy-naïve and the median prostate volume was 35 (26-49) cc. Further cohort  
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17 166 description according to Sig PCa status is shown in Table 1.  
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20 167 66 patients had a PSA 2 out of the range of 3-10ng/ml due to the variability (50 patients below  
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22 168 3ng/ml and 16 patients above 10ng/ml); thus, in this case, the % free PSA was not calculated, and the  
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24 169 risk probability was calculated without the inclusion of this variable. The patients were maintained in  
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26 170 the analysis as reflecting the variability of the PSA.  
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4 173 **Direct comparison for Sig PCa prediction**  
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6 174 Discrimination ability for Sig PCa was no different between the two models [ERSPC1-RC vs.  
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8 175 PCPT1-RC: 0.73; 95% CI: (0.68-0.79) vs. 0.73; 95% CI: (0.67-0.79), respectively]. ROC curves are  
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10 176 shown in Figure 1. Similarly, no difference was found in the discrimination ability for any PCa. The  
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12 177 comparison of the RC for both measures is described in Supplemental Figure 1. Supplemental Table  
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14 178 1 shows multiple comparisons by the DeLong test resulting in no differences between the two RCs  
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16 179 for Sig PCa.  
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20 180 The calibration for Sig PCa was more consistent for the ERSPC-RC in the low-risk clinically  
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22 181 useful region, compared to that of PCPT-RC (Figure 2). Both models tended to overestimate the risk  
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24 182 for a high probability of Sig PCa, and slightly underestimate it for low risk patients, suggesting that  
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26 183 the models would benefit from a recalibration for our population, particularly the ERSPC-RC, due to  
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28 184 its most apparent parallelism in this region. None of the model predicted very high probabilities for  
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30 185 most patients.  
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34 186 Calibration of the different RCs for any PCa showed an overestimation of risk for high-risk  
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36 187 probabilities. However, for moderate and low risks, which are the most relevant for clinical practice  
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38 188 (since a high risk will lead to a biopsy despite the inaccuracy), ERSPC again showed a more consistent  
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40 189 calibration than PCPT. The calibration curves for any PCa are shown in Supplemental Figure 2.  
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43 190 The decision curve analyses revealed that both RCs provided a clinical net benefit in the threshold  
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45 191 probability range for Sig PCa (Figure 3). The net benefit was comparable between the two RCs for  
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47 192 Sig PCa.  
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50 193 **Variability and clinical significance**  
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52 194 PSA and free PSA change was significantly different between the two measures, but with low  
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54 195 clinical variations [average PSA1 5.69 ng/ml vs. PSA2 5.39 ng/ml ( $p < 0.05$ ) and average free PSA1  
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56 196 16.99% vs. free PSA2 18.03% ( $p < 0.05$ )]. Median variability of PSA was 14% (6-27%). Taking into  
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58 197 account this variability of PSA, ERSPC proved to be more stable than PCPT. The k agreement

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2 198 between ERSPC1 and ERSPC2 was practically constant,  $0.79\pm0.09$  for the usual range of clinical  
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4 199 decision (0-0.3). However, PCPT1 and PCPT2 showed wider variations, with a  $k$  agreement of  
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6 200 approximately  $0.55\pm0.32$  in the same range, with a subsequent rapid decrease. The agreement  
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8 201 between both models (ERSPC1 vs. PCPT1) proved to be worse for thresholds in this range, peaking  
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10 202 0.47 for a 17% risk, with an average  $0.32\pm0.12$  on the interval. The comparison between ERSPC2  
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12 203 and PCPT2 yielded similar results (Figure 4).

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16 204 Direct comparison of sensibility and specificity of both RCs along the different clinical risk  
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18 205 thresholds showed that PCPT-RC has higher sensitivity and lower specificity than ERPSC-RC for a  
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20 206 given threshold along the clinically useful region (Figure 5). The balance point is reached at a  
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22 207 different risk threshold for each RC. The performances of both RCs at this point are comparable, as  
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24 208 shown in Figure 5. Considering the superposition of their respective ROC curves to a good  
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26 209 approximation (Figure 1), this means that a transformation of decision thresholds can make both  
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28 209 models perform similarly.

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## 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 3–10 ng/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

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2 264 a lower range in the case of Sig PCa. For higher risk patients, the calibration curves become irregular.  
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4 265 This effect is accentuated for risks close to 1, as both models predict maximum risks of around 0.75  
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6 266 for Sig PCa. Nonetheless, in the usual range for clinical decision (0-0.3), the calibration of both  
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8 267 models is acceptable. It was observed that ERSPC-RC outperformed PCPT-RC in two aspects. First,  
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10 268 its parallelism was better in the low-risk region, both for PCa and Sig PCa, and showed lower  
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12 269 irregularity for higher risks. Conversely, the ERSPC curves were visually more stable with the use of  
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14 270 different PSA measurements. This observation was reinforced by the results obtained from the study  
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16 271 of Cohen's  $\kappa$  coefficient. ERSPC models obtained from PSA1 and PSA2 for Sig PCa showed a better  
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18 272 agreement, which was also more stable through a wider interval of decision thresholds, as compared  
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20 273 to the agreement between PCPT models obtained from PSA1 and PSA2. These models showed a  
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22 274 smaller and rapidly decreasing agreement as the decision threshold was increased. Moreover, the  
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24 275 comparison of coefficients between PCPT1 and PCPT2 and between PCPT2 and ERSPC2 showed  
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26 276 that the differences between PCPT1 and PCPT2 were similar to those between ERSPC and PCPT  
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28 277 models. Both observations suggest that ERSPC is more insensitive and, therefore, robust to intra-  
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30 278 individual variations of PSA than PCPT, while the predictive performance is similar.  
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36 279 Despite the similar decision curve, results from the sensitivity, specificity and ROC curve analysis  
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38 280 show that the same risk threshold should not be used for both models. Both RCs are able to have  
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40 281 similar performance, and the benefit of using any of them is similar in order to screen patients for a  
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42 282 prostate biopsy, if the correct cut-off point is selected.

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44 283 In clinical practise, the use of these RCs should be the first step in guiding the decision for further  
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46 284 management of the patient. Patients with a confirmed, elevated PSA between 3-10ng/ml should be  
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48 285 better stratified using other variables within a RC, as men with PSA levels >10ng/mL are likely to  
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50 286 proceed to biopsy regardless of other factors. Probably a specific cut-off point in the risk probability  
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52 287 should not be used and take advantage of the known probabilities to discuss with the patient the  
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54 288 biopsy indication as recommended by the PCPT-RC. In the situation in which the patient is in the  
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56 289 low-risk group, according to both RCs (ERSPC and PCPT), the patient could continue with just

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2 290 follow-up. This fact has also been proposed by Alberts et al. (34) when applying new diagnostic  
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4 291 markers, such as mpMRI. Specifically, they showed that following a negative recommendation from  
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6 292 the ERSPC-RC would have avoided 62 (51%) of 122 mpMRIs and two (25%) of eight insignificant  
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8 293 PCA diagnoses, missing three (10%) of 31 high-grade PCa. As the positive predictive value of these  
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10 294 RCs is not as good as their negative predictive value, in case of discordance between both RCs or if  
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12 295 there is an indication for a biopsy according to both RCs, other images or fluid biomarkers could  
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14 296 increase the accuracy in order to potentially reduce the harm from unnecessary prostate biopsy and  
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16 297 over-diagnosis (35). Specifically, Loeb *et al.* (36) has recently demonstrated that the incorporation of  
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18 298 PHI into both RCs increases the accuracy of the diagnoses of Sig PCa.  
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23 299 The present study has some limitations. First, despite the prospectively collected information, it  
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25 300 is a retrospective study design. Second, prostate volume was an estimation and categorization from a  
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27 301 TRUS calculation, and, therefore, it is not the actual approach for which the RC was developed. Third,  
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29 302 the PSA values interval was not the same for all patients, which means the results should be  
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31 303 interpreted with caution. Four, the use of TRUS biopsy for PCA diagnosis, although is the standard in  
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33 304 most populations, suffers from random error compared with template biopsy (37), which could have  
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35 305 affected prediction results. However, the clinical information was extracted from a clinical practice  
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37 306 cohort and with information that could be useful for urologists worldwide.  
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41 307 Altogether, our results showed that: 1) the use of both RCs (ERSPC and PCPT) could improve  
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43 308 the selection of patients who need prostate biopsy, and that both RCs showed similar accuracy for  
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45 309 discrimination of Sig PCa; 2) ERSPC-RC had better calibration and stability than PCPT-RC for intra-  
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47 310 individual PSA variations; 3) when comparing both RCs sensitivity and specificity, a higher rate of  
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49 311 biopsies could be avoided with the ERSPC-RC vs. the PCPT-RC, but with a higher rate of Sig PCa  
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51 312 missed. Thus, in those patients with a PSA between 3-10 ng/ml, these tools should be used in order  
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53 313 to improve selection and specificity. The RCs specifically should be selected according to the  
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55 314 variables available in the clinic. In addition, both RCs could also be used and the decision to undergo  
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57 315 a biopsy be shared with the patient.  
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4 317 **CONFLICTS OF INTEREST**  
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4 492 **Footnotes**  
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- 7 494 • **Contributors** E.G.G, R.M.L, MJ.R.T and J.C.V carried out the conception and design of the  
8 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  
9 acquisition; E.G.G, J.C.V, J.L.F.R, RM.L and MJ.R.T carried out the analysis and  
10 interpretation of data; E.G.G, J.C.V and RM.L drafted the manuscript; JJ.S.B, JL.F.R, AM.B,  
11 495 J.V.R, J.M.L, JM.J.V, and MJ.R.T carried out a critical revision of the manuscript for  
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13 496 MJ.R.T and J.C.V supervised the work.

- 14 497  
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18 499 (ERDF/ESF, “Investing in your future”: PI16/00264, CM16/00180), and CIBERobn. CIBER  
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- **Ethics approval** This study was performed as part of the ONCOVER project. Ethical  
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- **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 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).

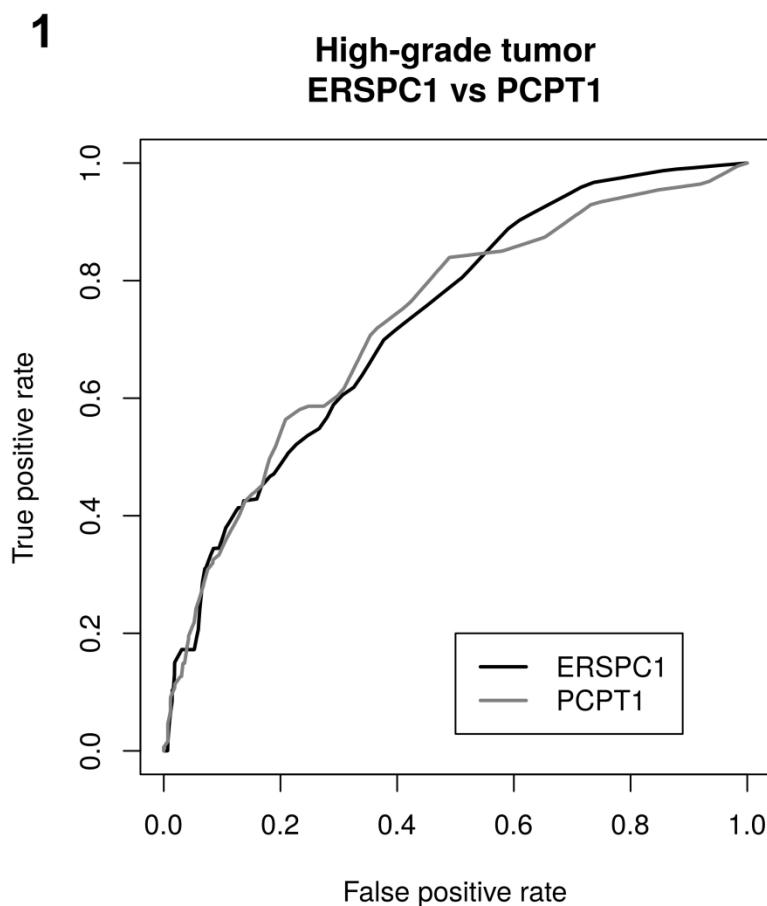
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2 543   **Table 1.** Clinical and demographic characteristics of the cohort of patients categorized according to  
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4 544 cancer status.  
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Variable	No Sig PCa n=423	Sig PCa n=87	All n=510
<b>Age</b>	64.0 (60.0-69.0)	68.0 (63.0-71.0)	65.0 (60.0-70.0)
<b>Family History</b>	81 (19.1)	8 (9.2)	89 (17.5)
<b>Positive DRE</b>	55 (13.0)	27 (31.0)	82 (16.1)
<b>1 Serum PSA</b>	5.3 (4.3-6.9)	5.8 (4.5-7.2)	5.3 (4.3-6.9)
<b>1 free PSA %</b>	16.2 (12.4-21.4)	12.5 (9-16.6)	15.9 (11.8-20.4)
<b>2 Serum PSA</b>	5.0 (3.7-6.6)	5.4 (4.1-6.7)	5.0 (3.8-6.6)
<b>2 free PSA %</b>	17.9 (13.9-23.4)	12.5 (9.1-16.3)	16.9 (12.8-22.1)
<b>Prostate volume</b>	38.0 (29.0-50.0)	26.0 (20.7-34.0)	35 (26-49)
<b>First Biopsy</b>	322 (76.1)	79 (90.8)	401 (78.6)
<b>PCPT1 Sig PCa</b>	0.08 (0.05-0.13)	0.16 (0.10-0.30)	0.09 (0.06-0.15)
<b>ERSPC1 Sig PCa</b>	0.05 (0.02-0.10)	0.12 (0.05-0.31)	0.05 (0.03-0.12)
<b>PCPT2 Sig PCa</b>	0.07 (0.04-0.11)	0.16 (0.08-0.27)	0.07 (0.05-0.13)
<b>ERSPC2 Sig PCa</b>	0.04 (0.02-0.08)	0.12 (0.05-0.30)	0.05 (0.02-0.11)
<b>PCa</b>	89 (21)	87 (100)	176 (34.5)

32  
33 **PCa**= Prostate cancer; **Sig PCa**= significant PCa (Gleason  $\geq$  7 on biopsy); **No Sig PCa**=  
34 No cancer or non-significant PCa; **ERSPC1 / PCPT1 Sig PCa** = Probability of high grade  
35 PCa using the first measurement of serum PSA (at the time of biopsy indication by the  
36 urologist); **ERSPC2 / PCPT2 Sig PCa** = Probability high grade PCa using the second  
37 measurement of serum PSA (just before undergoing prostate biopsy). Median values  
38 (interquartile range) are expressed for quantitative variables, and absolute values  
39 (percentage) for qualitative variables.  
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Risk Calculator for Sig PCa	AUC (CI <sub>95%</sub> )
ERSPC1	0.73 (0.68, 0.79)
PCPT1	0.73 (0.67, 0.79)

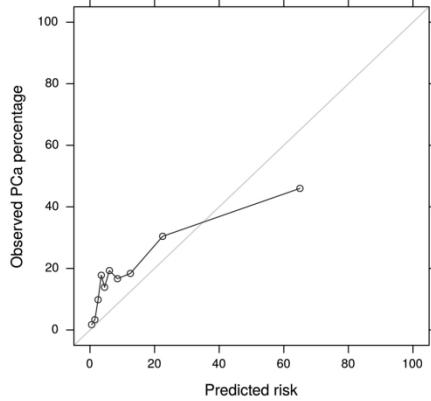
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45           Receiver Operating Characteristic curves and Area Under the Curve values for the ERSPC1 -RC (black) and  
46           PCPT1 -RC (grey) for Sig PCa.  
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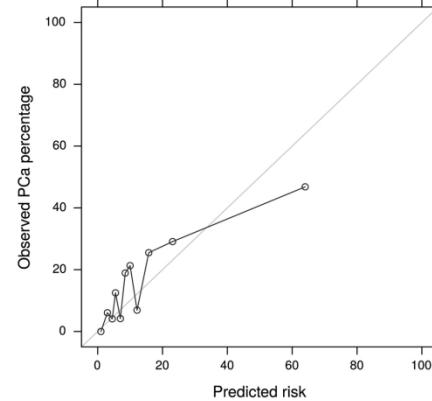
A)

High grade tumor: ERSPC1



B)

High grade tumor: PCPT1

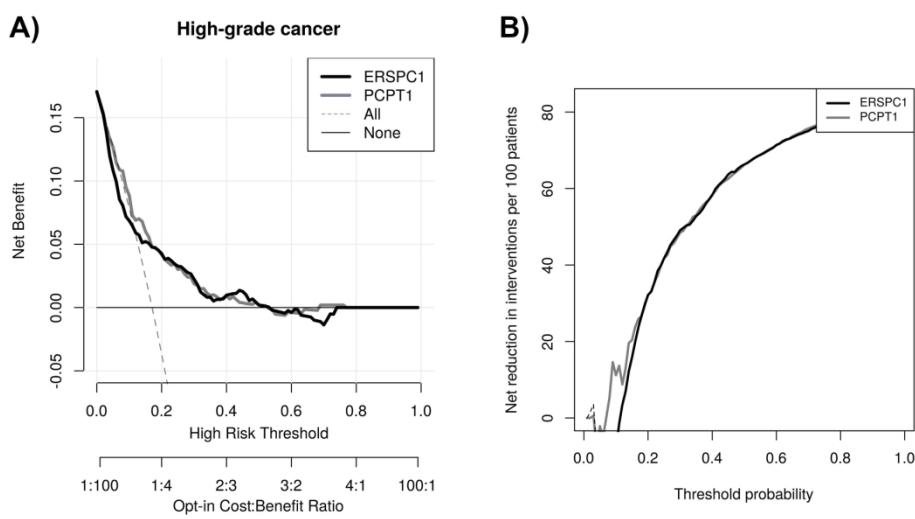


Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are 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.

191x142mm (300 x 300 DPI)

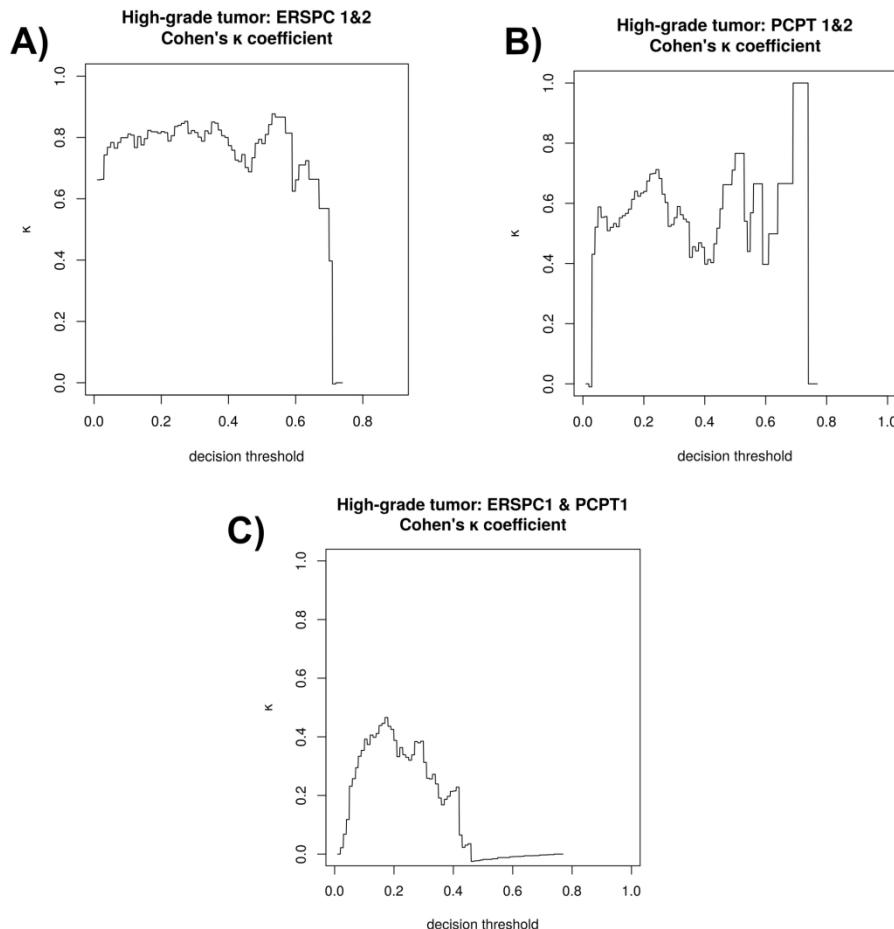
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Results of the decision curve analysis. A, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1-RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating 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).

169x126mm (300 x 300 DPI)

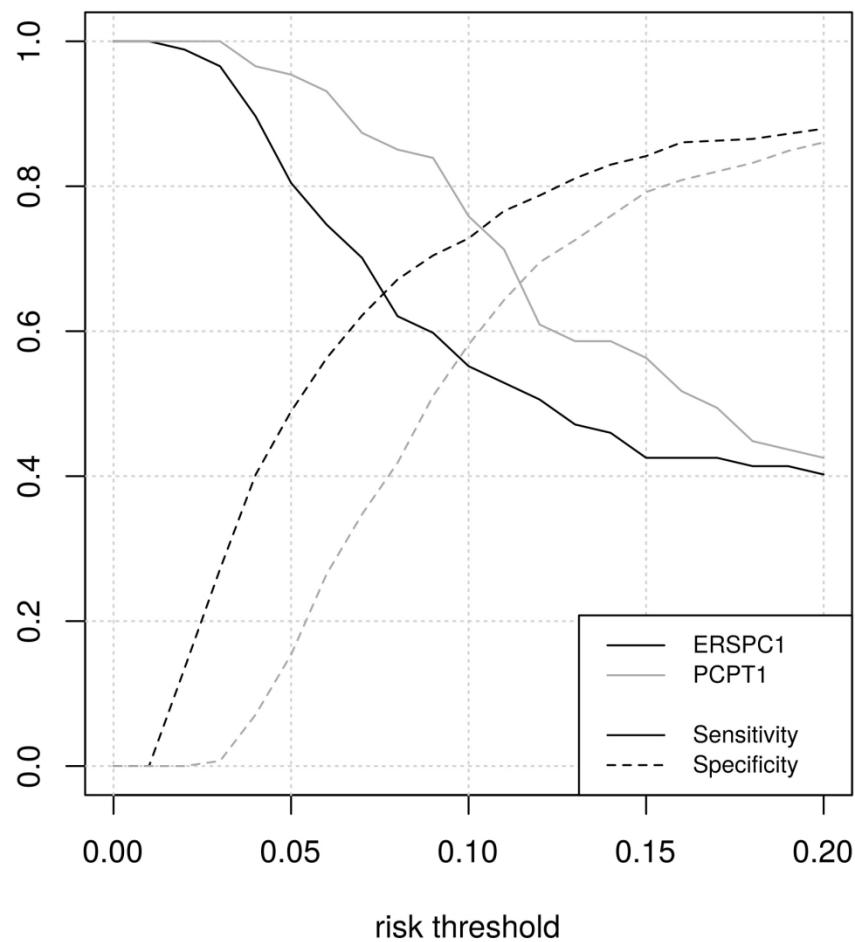
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Graphics showing Cohen's  $\kappa$  coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. A, Agreement between 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)

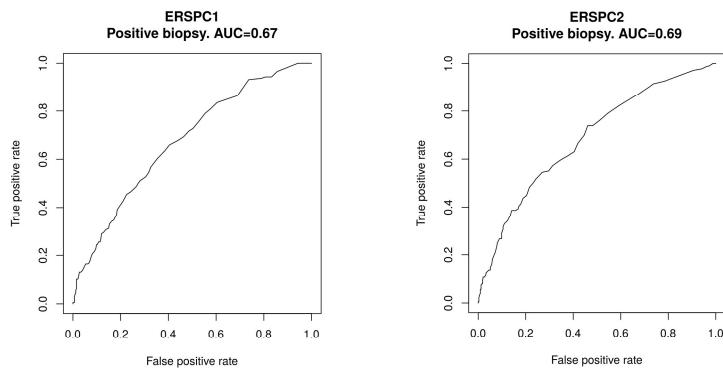
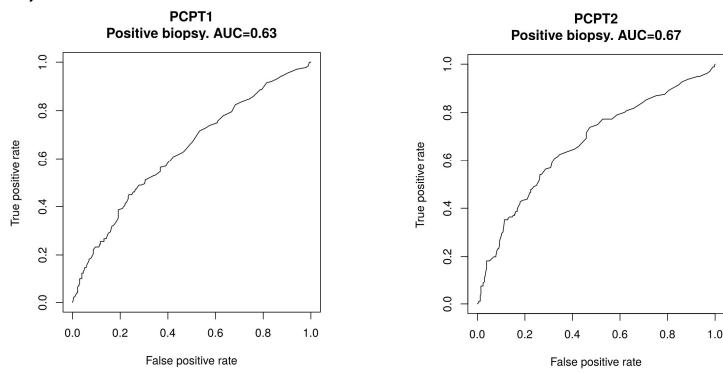
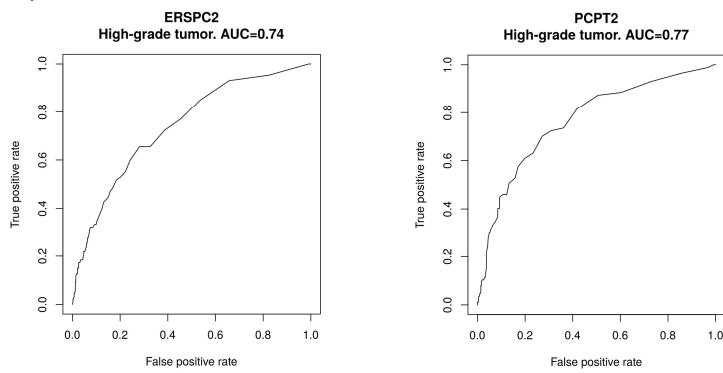
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Graphics showing sensitivities and specificities of both RCs along the clinically useful risk threshold. The ERSPC-RC (black line) and the PCPT-RC (grey line).

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

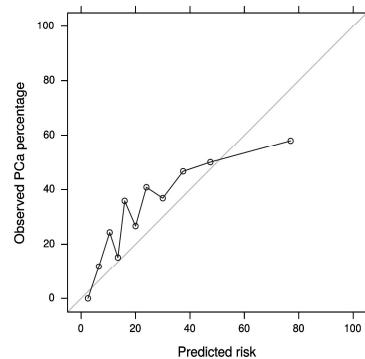
AUC (CI <sub>95%</sub> )	PB	HG
ERSPC1	0.67 (0.63, 0.72)	0.73 (0.68, 0.79)
ERSPC2	0.69 (0.64, 0.73)	0.74 (0.68, 0.80)
PCPT1	0.63 (0.58, 0.68)	0.73 (0.67, 0.79)
PCPT2	0.67 (0.62, 0.72)	0.77 (0.71, 0.82)

**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.

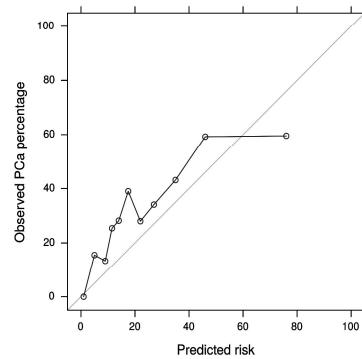
## S2

A)

Positive biopsy: ERSPC1

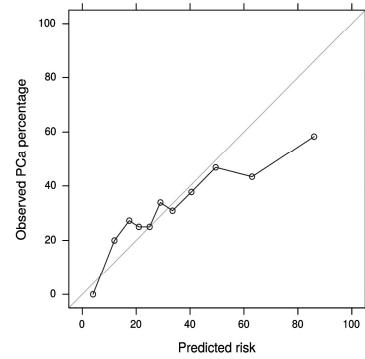


Positive biopsy: ERSPC2

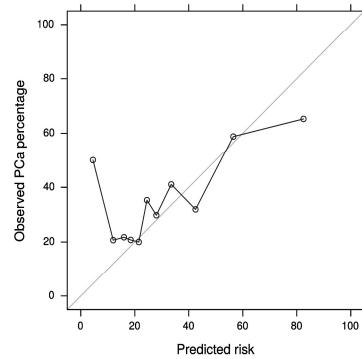


B)

Positive biopsy: PCPT1

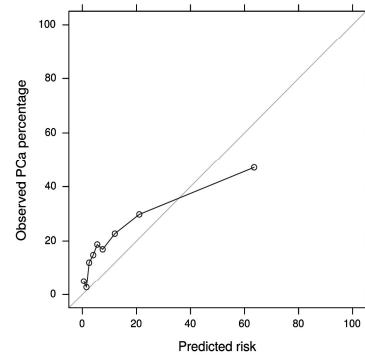


Positive biopsy: PCPT2

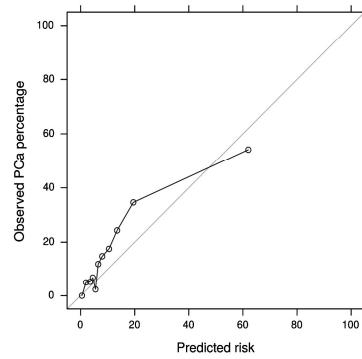


C)

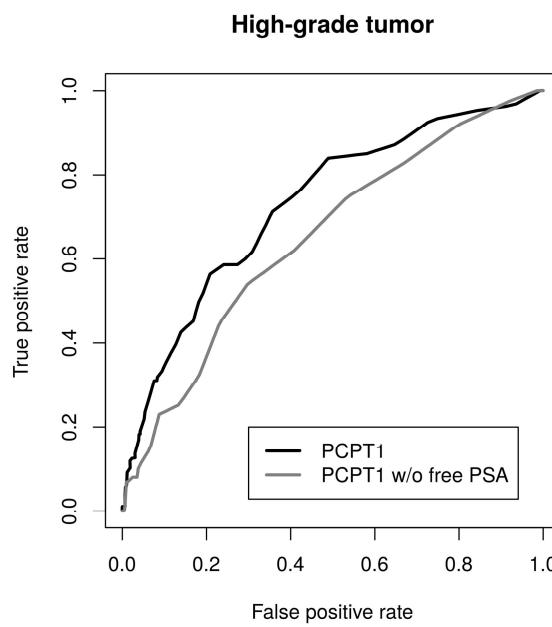
High grade tumor: ERSPC2



High grade tumor: PCPT2



**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.

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Risk Calculator for Sig PCa	AUC (CI <sub>95%</sub> )
PCPT1 without free PSA	0.65 (0.59, 0.71)
PCPT1 + free PSA	0.73 (0.67, 0.79)
DeLong p value: 0.02	

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

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2019-031032.R1
Article Type:	Original research
Date Submitted by the Author:	03-Jul-2019
Complete List of Authors:	Gomez Gomez, Enrique; Hospital Universitario Reina Sofia, Urology; Maimonides Institute for Biomedical Research of Cordoba, Genitourinary diseases Salamanca Bustos, Juan José; Hospital Universitario Reina Sofia, Urology Carrasco Valiente, Julia; Hospital Universitario Reina Sofia, Urology; Maimonides Institute for Biomedical Research of Cordoba, Genitourinary diseases Fernandez Rueda, Jose Luis; Maimonides Institute for Biomedical Research of Cordoba, Innovation and methodology Blanca, Ana; Maimonides Institute for Biomedical Research of Cordoba, Genitourinary diseases Valero Rosa, José; Hospital Universitario Reina Sofia, Urology; Maimonides Institute for Biomedical Research of Cordoba, Genitourinary diseases Bravo Arrebola, Ines; Hospital Universitario Reina Sofia, Urology Marquez López, Javier; Hospital Universitario Reina Sofia, Urology Jimenez Vacas, Juan Manuel; Maimonides Institute for Biomedical Research of Cordoba, Oncobesity and Metabolism Luque, Raul; Maimonides Institute for Biomedical Research of Cordoba, Oncobesity and Metabolism Requena Tapia, Maria José; Clinical Management Unit of Urology at Reina Sofia University Regional Hospital in Cordoba; Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC). Hospital Universitario Reina Sofía. Universidad de Córdoba
<b>Primary Subject Heading</b>:	Urology
Secondary Subject Heading:	Urology
Keywords:	Significant prostate cancer, risk calculator variability, ERSPC, PCPT

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3 **Observational study comparing the accuracy/variability between the ERSPC and**  
4 **the PCPT risk calculators for the prediction of significant prostate cancer in patients**  
5 **with PSA <10ng/ml**  
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10 **Authors:** Gómez-Gómez E<sup>1,2#</sup>, Salamanca-Bustos JJ<sup>2</sup>, Carrasco-Valiente J<sup>1,2</sup>, Fernández-  
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39 **Running title:** Risk Calculators for Significant Prostate Cancer  
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42 **Key Words:** Significant prostate cancer; risk calculator variability; ERSPC; PCPT.  
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45 **Tables and figures:** 1 and 5  
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48 **Acknowledgment**  
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51 (MINECO) and FEDER programme [Projects “Development of methods for early cancer  
52 detection”, (CCB.030PM)], Instituto de Salud Carlos III and co-funded by European  
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3 Servicios Sociales e Igualdad, Spain. This work has been awarded by the Real Academia  
4  
5 de Medicina de Sevilla 2018. The funding agreement ensured the authors' independence  
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7 in designing the study, interpreting the data, writing, and publishing the report  
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## 1    1 ABSTRACT

2    2    **Introduction:** Risk Calculators (RCs) are easy-to-use tools considering available clinical-  
3    3    variables that could help to select those patients with risk of prostate-cancer (PCa) who should  
4    4    undergo a prostate-biopsy. **Objective:** To perform a comparison for the prediction of significant-PCa  
5    5    (SigPCa) between the European-Randomised Study of Screening for PCa (ERSPC) and the PCa  
6    6    Prevention-Trial (PCPT) RCs in patients with PSA between 3-10ng/ml through an evaluation of the  
7    7    accuracy/variability between two consecutive PSA-values. **Setting:** An observational study in a  
8    8    major university Hospital of the south of Spain. **Methods and participants:** An observational study  
9    9    was performed in patients who underwent a prostate-biopsy. SigPCa probabilities were calculated  
10   10    with the two PSA measures using ERSPC3/4+DRE and PCPTv2+free-PSA RCs. The discrimination  
11   11    ability of SigPCa was determined by the area under the curve (AUC). Calibration, discrimination,  
12   12    and decision curve analysis were studied. The variability between both RCs-agreement was compared  
13   13    using Cohen's kappa coefficient. **Results:** 510 patients were analysed (87 diagnosed with SigPCa).  
14   14    The median PSA values were 5.3 and 5ng/ml for PSA1 and PSA2 respectively. Both RCs  
15   15    overestimated the risk in the case of high-risk probabilities, but ERSPC was better calibrated than  
16   16    PCPT for risks in the clinically useful moderate-risk range. Discrimination ability for SigPCa was  
17   17    similar between models with an AUC=0.73(0.68-0.79) for ERSPC-RC vs. 0.73(0.67-0.79) for PCPT-  
18   18    RC. ERSPC-RC showed less variability than PCPT-RC, with a constant agreement ( $k=0.7-0.8$ ) for  
19   19    usual range of clinical decision-making. Remarkably, a higher biopsies number would be avoided  
20   20    using the ERSPC-RC, but more SigPCa would be missed along all the risk probabilities.  
21   21    **Conclusions:** Both RCs had similar accuracy for the SigPCa discrimination. However, ERSPC-RC  
22   22    has more stable calibration for clinically useful risks and was more stable for intra-individual PSA  
23   23    variations.

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2 25 **Strengths and limitations of this study**  
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- 4 26 - This study highlights the need to spread the use of available free tools which would be useful  
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6 27 in patient's selection for undergoing prostate biopsy.  
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8 28 - This study is the first to compare two available free risk calculators in patients with a PSA <  
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10 29 10ng/ml analyzing their variability between two consecutive different PSA levels.  
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12 30 - Although the clinical information of this study was extracted from a clinical practice cohort  
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14 31 and with information that could be useful for urologists worldwide, this is a retrospective  
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16 32 study and the use of TRUS biopsy for PCa diagnosis, even though is the standard in most  
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18 33 populations, suffers from random error compared with template biopsy, which could have  
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20 34 affected prediction results.  
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2   36   **INTRODUCTION**  
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5   37   Prostate cancer (PCa) is the second most frequently diagnosed malignancy in males worldwide,  
6   38   and the most frequent in developed countries(1). Its current standard of diagnosis is a prostate biopsy  
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8   39   based on PSA levels and digital rectal examination (DRE). However, there are other available and  
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10   40   complementary variables that could help to select those patients who should undergo a prostate biopsy  
11   41   (such as age, prostate volume, free PSA, family history, etc.), but these are not always used and/or  
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13   42   well-integrated in daily clinical practice(2). In line with this, Risk Calculators (RCs) are easy-to-use  
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15   43   tools that can help the clinicians to take advantage of all these available variables (3). The two main  
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17   44   RCs are from the European Randomised Study for Screening of Prostate Cancer (ERSPC cohort;  
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19   45   ERSPC-RC: <http://www.prostatecancer-riskcalculator.com/seven-prostate-cancer-riskcalculators>)  
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21   46   and from the Prostate Cancer Prevention Trial (PCPT cohort; PCPT-RC:  
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23   47   <http://deb.uthscsa.edu/URORiskCalc/Pages/calcs.jsp>). Both RCs have undergone some  
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25   48   modifications, specifically the addition of estimated prostate volume in the ERSPC-RC (4,5).  
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27   49   Furthermore, both RCs were originally developed from different patient cohorts and each RC uses  
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29   50   different variables.  
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32   51   To date, limited external validations and comparisons have been performed by different groups  
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34   52   (6–9). The two most important recent comparisons of the modified RCs were performed by Foley *et*  
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36   53   *al* (6,7) and Poyet *et al* (9). Both found a better discriminatory ability for ERSPC-RC vs PCPTv.2-  
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38   54   RC for the diagnoses of significant-PCa (Sig PCa) (AUC around 0.74 vs 0.69, respectively), but they  
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40   55   also included patients with a high PSA of up to 50 ng/ml. Despite the possibility of using these RCs  
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42   56   in patients with PSA levels up to 50ng/ml, it is clear that the advantages of using both RCs would  
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44   57   probably increase in patients with a PSA under 10ng/ml, where the rate of positive biopsy for PCa  
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46   58   clearly decrease, with an important number of unnecessary biopsies. In line with this, EAU guidelines  
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48   59   recommends to offer further assessment with other tools to these specific patients with a PSA 2–10  
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50   60   ng/ml(2). Furthermore, in the case of the PCPTv.2-RC, the addition of the free PSA value in patients  
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2 61 with a PSA under 10ng/ml seems to improve its accuracy (5), and, therefore, given its accessibility,  
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4 62 this value should be included in the RC.  
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6 63 The intra-individual and inter-assay variability of PSA is already known (10–12) and, therefore,  
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8 64 at least two measures are necessary before a prostate biopsy is indicated. In fact, it has been shown  
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10 65 that approximately 25% of men with initial PSA levels between 4 and 10 ng/mL had normal PSA  
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12 66 values upon repeat testing (13). In line with this, despite being primarily based on PSA level, the  
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14 67 variability of the two RCs mentioned above has been poorly studied, and might have implications for  
15  
16 patient management. Our group has recently evaluated this variability with the ERSPC-RC, which  
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18 68 showed stable accuracy over a cohort of patients, but some changes with respect to an individual  
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20 69 approach (14). To date, there is no study comparing the accuracy and variability of both RCs, the  
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22 70 ERSPC + DRE vs. the PCPTv.2 + free PSA, for the prediction of Sig PCa. Therefore, the aim of this  
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24 71 study was to perform a direct comparison between ERSPC + DRE and PCPTv.2 + free PSA RCs in  
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26 72 patients with a PSA between 3-10ng/ml, evaluating the accuracy and variability of both methods in  
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28 73 the prediction of Sig PCa.  
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2 76 **MATERIALS AND METHODS**  
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4 77 **Study population and design**  
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6 78 An observational retrospective study was performed in patients from ONCOVER cohort (1021  
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8 biopsies indicated by clinical practise wherein patients donated blood and urine before the biopsy).  
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10 80 The study was carried out within the project approved by our Hospital Research Ethics Committee,  
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12 and informed consent was obtained from all participants. Blood sample was obtained in the morning  
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14 (between 8:00-10:00 am) after fasting overnight and then, the prostate biopsy was implemented  
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16 according to clinical practice. The inclusion criteria for this study were: 1) PSA indication between  
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18 3-10 ng/ml; 2) Full clinical and laboratory data to fulfilled ERSPC-RC and PCPT-RC; 3) Age 55-80  
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20 years' old; 4) Two consecutives measurements of PSA levels within an interval of 12 weeks.  
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22 Exclusion criteria included patients with a previously known PCa diagnosis or treatment that could  
23  
24 modify PSA levels (Supplemental table 1).  
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29 88 Transrectal prostate biopsy was carried out under local anaesthesia by using a standard peri-  
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31 prostatic block, a transrectal ultrasound transducer, and an 18G automated needle biopsy instrument.  
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33 90 The prostatic volume was measured following the protocol used during transrectal ultrasound (TRUS),  
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35 and usual recommendations were to take 12 cores in patients undergoing the first biopsy procedure,  
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37 and a minimum of 16 biopsy cores for those who had a previous biopsy. Biopsy specimens were  
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39 analysed by expert urologic pathologists according to the International Society of Urological  
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41 Pathology (ISUP) 2005 modified criteria (15).  
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48 96 **Main variables description**  
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50 97 Demographics information and medical histories of each patient were obtained. PSA levels were  
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52 measured twice within a period no longer than 12 weeks, as follows: 1) **PSA 1 and free PSA 1:** for  
53  
54 biopsy indication; and, 2) **PSA 2 and free PSA 2:** before undergoing prostate biopsy. Both PSA  
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56 measures were evaluated in the same laboratory by Chemiluminescent Microparticle Immunoassays  
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2 101 (ng/ml, by a CMIA; Ref. 7k70; Abbott). Median and interquartile range of both measure were 6 (3-  
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4 102 8) weeks.  
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7 103 **Prostate volume:** estimated by TRUS and categorized in three possible values, 25-40-60 ml, as  
8  
9 104 recommended (4) (TRUS volume <30 = 25 mL, 30–50 = 40 mL, and ≥50 = 60 mL).  
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11 105 **Significant/ high-grade (HG) prostate cancer (Sig PCa):** PCa with a Gleason grade ≥ 7 on  
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13 106 biopsy.  
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18 108 **ERSPC-RC and PCPT-RC probabilities calculation**  
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20 109 **ERSPC:** The formulas for the ERSPC-RC 3+DRE for patients at initial biopsy and the ERSPC-  
21  
22 110 RC 4+DRE for patients at repeat biopsy were utilized in this study. These calculators use PSA,  
23  
24 prostate volume, and DRE as variables, with, a negative prostate biopsy in ERSPC 4+ DRE in patients  
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26 111 who had a previous biopsy. This provides a probability rating for any PCa or Sig PCa (Gleason ≥ 7).  
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30 113 **ERSPC1/Sig PCa (1° Measure):** Risk probability calculated by ERSPC-RC3 or 4 (if previous  
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32 114 biopsy) ± DRE for any PCa using PSA 1/ Sig PCa – for HG PCa.  
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34 115 **ERSPC2/Sig PCa (2° Measure):** Risk probability calculated by ERSPC-RC 3 or 4 (if previous  
35  
36 116 biopsy) ± DRE for any PCa using PSA 2/ Sig PCa – for HG PCa.  
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39 117 **PCPT:** The formulae for the PCPT-RC 2.0 + %free PSA was utilized in this study. This calculator  
40  
41 118 use race, age, PSA level, %free PSA level, family history of PCa, DRE and prior prostate biopsy.  
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43 119 This gives a probability of negative biopsy, low grade PCa and Sig PCa (gleason ≥ 7).  
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46 120 **PCPT1:** Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 1 and free PSA 1.  
47

48 121 **PCPT2:** Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 2 and free PSA 2.  
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50 122 The variability of PSA was calculated by the following formula: |Measure 1–  
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52 123 **Measure2/Measure 1**  
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57 125 **Statistical analysis**  
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2 126 A descriptive study was performed by calculating the median and interquartile ranges (IR) for the  
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4 127 quantitative variables, and the absolute frequencies and percentages for the qualitative variables. A  
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6 128 Student's T test for paired groups was used to compare the means of the quantitative variables (PSA  
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8 129 1 and 2).

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11 130 The investigation of the comparative performance in the detection of Sig PCa of both RCs,  
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13 131 ERSPC-RC and PCPT-RC, was performed, taking into account these four factors: discrimination  
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15 132 capacity, calibration, clinical utility, and consistency against the observed variations in PSA levels  
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17 133 for our dataset.

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19 134 The discrimination ability of the models, i.e., their ability to separate those patients who had Sig  
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21 135 PCa from those who do not, was assessed using the area under their Receiver Operator Characteristic  
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23 136 (ROC) curve (AUC) (16), as measured in our sample. This is one of the most frequently used  
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25 137 measurements of model discrimination, because of its independence of the selection of a specific  
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27 138 decision threshold and its robustness against class imbalance. Confidence intervals for these AUCs  
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29 139 were computed using bootstrapping. These AUCs were then compared to determine the relative  
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31 140 performance of the models using DeLong tests (17). These tests were chosen because of their non-  
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33 141 parametric nature, with few assumptions about the data, and their suitability for paired data, as both  
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35 142 models were evaluated over the same dataset, properties which make this the most commonly used  
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37 143 test to compare AUCs (18). For this comparison, we focused on the first measure of PSA (PSA 1; the  
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39 144 value for the indication of the prostate biopsy).

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41 145 The calibration of the calculators for our cohort was then investigated to determine the agreement  
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43 146 between the frequency of the observed outcome (PCa in our case) and the risks predicted by the model.  
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45 147 Calibration plots were used for this purpose (19), enabling a visual evaluation of this agreement and  
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47 148 the comparison between RCs.

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49 149 To address the potential clinical utility of the models, we performed decision curve analysis on  
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51 150 our data, as proposed by Vickers and Elkin (20). This method has the advantage of not requiring the

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2 151 specification of the relative cost for false-positives and false-negatives, defining a net benefit as a  
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4 152 function of the decision threshold at which one would consider obtaining a biopsy.  
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6 153 Finally, the stability of the predictions of both RCs, with regard to the observed intra-patient  
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8 154 changes on PSA levels between measurements, was investigated using the Cohen's kappa (k) inter-  
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10 155 rater agreement coefficient as a function of the decision threshold. This coefficient was selected due  
11  
12 156 to its widespread use and robustness against random agreements, and thus, is a better measurement  
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14 157 than naïve accuracy.  
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16 158 All the analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill) and R  
17  
18 159 version 3.2.3(R Foundation for Statistical Computing, Vienna, Austria: URL [https://www.R-](https://www.R-project.org/)  
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20 160 project.org/). A <5% level of significance ( $p<0.05$ ) was used to decide statistically significant  
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22 161 differences.  
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26 163 **Patient and public involvement**  
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35 164 Participants and public were not involved in the development of research questions, study design  
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37 165 or recruitment.  
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2 168 **RESULTS**  
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4 169 **Cohort characteristics**  
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7 170 In the present study, we analysed 510 patients who met the inclusion criteria previously described.  
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9 171 Median age was 65 (60-70) years old, with a family history in 89 patients (17.5%) and a suspicious  
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11 172 DRE in 82 patients (16.1%). The median PSA before prostate biopsy indication was 5.3 (4.3-6.9)  
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13 173 ng/ml. 176 patients were diagnosed with PCa, 87 of those categorized as Sig PCa. Most patients  
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15 174 (n=401; 78.6%) were biopsy-naïve and the median prostate volume was 35 (26-49) cc. Further cohort  
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17 175 description according to Sig PCa status is shown in Table 1.  
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20 176 66 patients had a PSA2 out of the range of 3-10ng/ml due to the variability (50 patients below  
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22 177 3ng/ml and 16 patients above 10ng/ml); thus, in this case, the % free PSA was not calculated, and the  
23  
24 178 risk probability was calculated without the inclusion of this variable. These patients were maintained  
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26 179 in the analysis as reflecting the variability of the PSA, and the application of the models in this real  
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28 180 situation, despite it could introduce a bias in terms of calibration and variability.  
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4 183 **Direct comparison for Sig PCa prediction**  
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6 184 Discrimination ability for Sig PCa was no different between the two models [ERSPC1-RC vs.  
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8 185 PCPT1-RC: 0.73; 95% CI: (0.68-0.79) vs. 0.73; 95% CI: (0.67-0.79), respectively]. ROC curves are  
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10 186 shown in Figure 1A. Similarly, no difference was found in the discrimination ability for any PCa.  
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12 187 The comparison of the RC for both measures is described in Figure 1(B-D) with similar results but a  
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14 188 clear tendency of better accuracy for PCPT2-RC vs ERSPC2-RC ( $p= 0.06$ ). Supplemental Table 2  
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16 189 shows multiple comparisons by the DeLong test resulting in no differences between the two RCs for  
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18 190 Sig PCa.  
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20 191 The calibration for Sig PCa was more consistent for the ERSPC-RC in the low-risk clinically  
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22 192 useful region, compared to that of PCPT-RC (Figure 2). Both models tended to overestimate the risk  
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24 193 for a high probability of Sig PCa, and slightly underestimate it for low risk patients, suggesting that  
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26 194 the models would benefit from a recalibration for our population, particularly the ERSPC-RC, due to  
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28 195 its most apparent parallelism in this region. None of the model predicted very high probabilities for  
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30 196 most patients.  
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32 197 Calibration of the different RCs for any PCa showed an overestimation of risk for high-risk  
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34 198 probabilities. However, for moderate and low risks, which are the most relevant for clinical practice  
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36 199 (since a high risk will lead to a biopsy despite the inaccuracy), ERSPC visually showed a more  
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38 200 consistent and less fluctuating calibration in the useful range than PCPT. The calibration curves for  
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40 201 any PCa are shown in Supplemental Figure 1.  
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42 202 The decision curve analyses revealed that both RCs provided a clinical net benefit in the threshold  
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44 203 probability range for Sig PCa (Figure 3). The net benefit was comparable between the two RCs for  
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46 204 Sig PCa.  
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48 205 As shown in Supplemental Figure 2, the addition of free PSA clearly improved the accuracy of  
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50 206 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;  
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52 207  $p= 0.02$ ].  
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4 209 **Variability and clinical significance**  
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6 210 PSA and free PSA change was significantly different between the two measures, but with low  
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8 211 clinical variations [average PSA1 5.69 ng/ml vs. PSA2 5.39 ng/ml ( $p < 0.05$ ) and average free PSA1  
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10 212 16.99% vs. free PSA2 18.03% ( $p < 0.05$ )]. Median variability of PSA was 14% (6-27%). Taking into  
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12 213 account this variability of PSA, ERSPC proved to be more stable than PCPT. The  $k$  agreement  
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14 214 between ERSPC1 and ERSPC2 was practically constant,  $0.79 \pm 0.09$  for the usual range of clinical  
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16 215 decision (0-0.3). However, PCPT1 and PCPT2 showed wider variations, with a  $k$  agreement of  
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18 216 approximately  $0.55 \pm 0.32$  in the same range, with a subsequent rapid decrease. The agreement  
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20 217 between both models (ERSPC1 vs. PCPT1) proved to be worse for thresholds in this range, peaking  
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22 218 0.47 for a 17% risk, with an average  $0.32 \pm 0.12$  on the interval. The comparison between ERSPC2  
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24 219 and PCPT2 yielded similar results (Figure 4).  
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27 220 Direct comparison of sensitivity and specificity of both RCs along the different clinical risk  
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29 221 thresholds showed that PCPT-RC has higher sensitivity and lower specificity than ERSPC-RC for a  
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31 222 given threshold along the clinically useful region (Figure 5). The balance point is reached at a  
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33 223 different risk threshold for each RC. The performances of both RCs at this point are comparable, as  
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35 224 shown in Figure 5. Considering the superposition of their respective ROC curves to a good  
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37 225 approximation (Figure 1), this means that a transformation of decision thresholds can make both  
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39 226 models perform similarly.  
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2 228 **DISCUSSION**  
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4 229 Currently, considerable research is being carried out to find new diagnostic markers for Sig PCa,  
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6 230 in order to reduce the number of biopsies and the over-diagnosis of insignificant PCa (21). These  
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8 231 markers are based on body fluids (blood, urine) or image explorations (22,23). Some are  
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10 232 recommended by guidelines such as the 4k score test, PCA3, and/or the Prostate Health Index (PHI)  
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12 233 in body fluids (only PCA3 and PHI have been approved by the FDA) (24–26), or multiparametric  
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14 234 magnetic resonance imaging (mpMRI), with recent evidence of its advantages in biopsy-naïve  
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16 235 patients (27). However, costs and availability minimize their implementation worldwide, and,  
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18 236 therefore, it is clear that additional and readily available tools, such as RCs, should be implemented  
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20 237 in daily clinical practice. The two most used RCs are ERSPC-RC and PCPT-RC, which have been  
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22 238 modified and adapted (4,5). Few external validations have been conducted, with varying results  
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24 239 (7,9,28). Usually, external validations of RCs show worse performance than the original validations  
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26 240 (8), a fact that is corroborated by our study. Therefore, based on all this information, evaluations,  
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28 241 validations, and incorporation of RCs are needed (3).

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30 242 The present study explores and compares, for the first time, both, the PCPT v2 + free PSA and  
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32 243 the ERSPC + DRE, not only for accuracy but also for variability and clinical relevance. Our group  
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34 244 previously explored the accuracy and variability of the ERSPC + DRE RC (14) but, in this study, we  
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36 245 have specifically focused only on patients in the grey zone (PSA 3–10 ng/ml) and compared the  
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38 246 ERSPC + DRE RC vs. the PCPT v2 + free PSA, an analysis that has not been previously performed.  
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40 247 This comparison showed that both RCs had similar accuracy for the discrimination of Sig PCa.  
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42 248 However, ERSPC-RC had better calibration and stability for intra-individual PSA variations. Our  
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44 249 methodology in calculating the volume is an estimation from the results of the TRUS measure, similar  
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46 250 to Poyet *et al*, and following the recommendations of Roobol *et al.* (4). We have focused only on  
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48 251 those patients with PSA between 3–10 ng/ml who require additional diagnostic information. The  
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50 252 PCPT-RC option with free PSA, which increases the accuracy of discrimination between Sig PCa  
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52 253 and no PCa (5), was calculated, as it is an easy-to-use and readily available tool for these patients.

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2 254 As defined in the methodology, the first measure (ERSPC1 and PCPT1) was the focus of the  
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4 255 direct comparison, as this was the used for biopsy indication. The accuracy of both RCs was similar  
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6 256 for Sig PCa in our study, showing an accuracy similar to other external validations such as Poyet *et*  
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8 257 *al.* (9) and Foley *et al.* (7) for ESRPC (AUC= 0.73 and 0.74, respectively) and a better accuracy for  
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10 258 PCPT1 v2.0 when adding free PSA (AUC= 0.70 and 0.69, respectively). Still, these results are far  
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12 259 from ideal, and thus, additional data from imaging or fluid markers might be included to improve the  
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14 260 accuracy of the RCs. In agreement with the accuracy results, the decision curve analysis was also  
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16 261 similar between both RCs. In fact, both RCs showed a net benefit from an early risk threshold, which  
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18 262 means that their implementation would be useful in the pathway of patient selection.  
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23 263 Studying the variability of the RCs improves our knowledge about their stability, which could be  
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25 264 translate into improved decision-making and selection of patients. Our PSA cohort showed a  
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27 265 variability that was in the range of those shown in previous literature (11,12,29). Our group and others  
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29 266 (14,30) have demonstrated that a higher PSA variability is associated with a reduced risk of Sig PCa  
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31 267 in a prostate biopsy, but it does not improve the accuracy of a RC. However, probability stability is  
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33 268 important in order to trust RC probabilities at any point. Our study shows good agreement between  
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35 269 the two ERSPC + DRE-RC probabilities, with good calibration and stability despite intra-individual  
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37 270 PSA variations. PCPTv.2 + free PSA shows worse stability and higher variability, which could be  
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39 271 explained simply by the fact that it uses two values (PSA and free PSA) that suffer from this  
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41 272 variability (31), while the use of an estimated volume in the ERSPC dilutes the PSA variability. These  
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43 273 results should be interpreted with caution, as volume estimation was performed by categorization of  
44  
45 274 TRUS and not by DRE. It is true that this categorization has previously shown good correlation (4).  
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47 275 This likely depends on prostate volume (32), as well as low but certain inter-examiner variability  
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49 276 (33), which could also increase ERPSC variability in an inter-clinician comparison.  
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55 277 Calibration plots show that both models (PCPT-RC and ERSPC-RC) predict adequately only the  
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57 278 actual risk of PCa and Sig PCa for low-risk patients, with a wider useful range in the case of PCa and  
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59 279 a lower range in the case of Sig PCa. For higher risk patients, the calibration curves become irregular.  
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2 280 This effect is accentuated for risks close to 1, as both models predict maximum risks of around 0.75  
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4 281 for Sig PCa. Nonetheless, in the usual range for clinical decision (0-0.3), the calibration is not good,  
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6 282 showing visually a more consistent and less fluctuating calibration in the useful range for ERSPC  
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8 283 than for PCPT, which could make possible to recalibrate it on our population over that range with  
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11 284 better results, but this is left to further research. It was observed that ERSPC-RC outperformed PCPT-  
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13 285 RC in two aspects. First, its parallelism was better in the low-risk region, both for PCa and Sig PCa,  
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15 286 and showed lower irregularity for higher risks. Conversely, the ERSPC curves were visually more  
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18 287 stable with the use of different PSA measurements. This observation was reinforced by the results  
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20 288 obtained from the study of Cohen's  $\kappa$  coefficient. ERSPC models obtained from PSA1 and PSA2 for  
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22 289 Sig PCa showed a better agreement, which was also more stable through a wider interval of decision  
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24 thresholds, as compared to the agreement between PCPT models obtained from PSA1 and PSA2.  
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27 291 These models showed a smaller and rapidly decreasing agreement as the decision threshold was  
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29 increased. Moreover, the comparison of coefficients between PCPT1 and PCPT2 and between PCPT2  
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31 292 and ERSPC2 showed that the differences between PCPT1 and PCPT2 were similar to those between  
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33 293 ERSPC and PCPT models. Both observations suggest that ERSPC is more insensitive and, therefore,  
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36 294 robust to intra-individual variations of PSA than PCPT, while the predictive performance is similar.  
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39 296 Despite the similar decision curve, results from the sensitivity, specificity and ROC curve analysis  
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41 297 show that the same risk threshold should not be used for both models. Both RCs are able to have  
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43 298 similar performance, and the benefit of using any of them is similar in order to screen patients for a  
44  
45 299 prostate biopsy, if the correct cut-off point is selected. It should be highlighted the importance of  
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47 300 having an almost 100% negative predictive value, as the advantage of reducing unnecessary biopsies  
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50 301 should not be at the cost of missing or delaying the diagnoses of a Sig PCa.

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52 302 In clinical practice, the use of these RCs should be the first step in guiding the decision for further  
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54 management of the patient. Patients with a confirmed, elevated PSA between 3-10ng/ml should be  
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57 303 better stratified using other variables within a RC, as men with PSA levels >10ng/mL are likely to  
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59 304 proceed to biopsy regardless of other factors. Probably a specific cut-off point in the risk probability  
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2 306 should not be used and take advantage of the known probabilities to discuss with the patient the  
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4 307 biopsy indication as recommended by the PCPT-RC. In the situation in which the patient is in the  
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6 308 low-risk group, according to both RCs (ERSPC and PCPT), the patient could continue with just  
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8 309 follow-up. This fact has also been proposed by Alberts et al. (34) when applying new diagnostic  
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10 markers, such as mpMRI. Specifically, they showed that following a negative recommendation from  
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12 310 the ERSPC-RC would have avoided 62 (51%) of 122 mpMRIs and two (25%) of eight insignificant  
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14 311 PCA diagnoses, missing three (10%) of 31 high-grade PCa. As the positive predictive value of these  
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16 312 RCs is not as good as their negative predictive value, in case of discordance between both RCs or if  
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18 313 there is an indication for a biopsy according to both RCs, other images or fluid biomarkers could  
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20 314 increase the accuracy in order to potentially reduce the harm from unnecessary prostate biopsy and  
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22 315 over-diagnosis (35). Specifically, Loeb *et al.* (36) has recently demonstrated that the incorporation of  
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24 316 PHI into both RCs increases the accuracy of the diagnoses of Sig PCa. Another relevant point should  
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26 317 be comment from the tendency of better predictive ability with the second evaluations of PSA,  
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28 318 reinforcing the idea of the need of several PSA values to confirm the risk and discarded confounding  
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30 319 factors. Furthermore, this analysis could suggest a tend to better discrimination ability of PCPT in the  
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32 320 range of lower probabilities (when PSA is low), but further research would be needed to validate this  
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34 321 idea. These risk calculators only show a static probability so other longitudinal variables and clinical  
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36 322 judgment should be required for their application.  
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43 324 The present study has some limitations. First, despite the prospectively collected information, it  
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45 325 is a retrospective study design with a limited number of patients. Second, prostate volume was an  
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47 326 estimation and categorization from a TRUS calculation, and, therefore, it is not the actual approach  
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49 327 for which the RC was developed. Third, the PSA values interval was not the same for all patients,  
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51 328 which means the results should be interpreted with caution. Four, the use of TRUS biopsy for PCa  
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53 329 diagnosis, although is the standard in most populations, suffers from random error compared with  
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55 330 template biopsy (37), which could have affected prediction results. However, the clinical information  
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2 331 was extracted from a clinical practice cohort and with information that could be useful for urologists  
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4 332 worldwide.  
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6 333 Altogether, our results showed that: 1) the use of both RCs (ERSPC and PCPT) could be a useful  
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8 334 tool in the selection of patients who need prostate biopsy, and that both RCs showed similar accuracy  
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10 335 for discrimination of Sig PCa; 2) ERSPC-RC had more stable calibration and stability than PCPT-  
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12 336 RC for intra-individual PSA variations; 3) when comparing both RCs sensitivity and specificity, a  
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14 337 higher rate of biopsies could be avoided with the ERSPC-RC vs. the PCPT-RC, but with a higher rate  
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16 338 of Sig PCa missed. Thus, in those patients with a PSA between 3-10 ng/ml, these tools should be used  
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18 339 in order to improve selection and specificity. The RCs specifically should be selected according to  
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20 340 the variables available in the clinic. In addition, both RCs could also be used and the decision to  
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22 341 undergo a biopsy be shared with the patient.  
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## **CONFLICTS OF INTEREST**

28 344 Nothing to declare  
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## 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 Sofía 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).

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**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
<b>Age</b>	64.0 (60.0-69.0)	68.0 (63.0-71.0)	65.0 (60.0-70.0)
<b>Family History</b>	81 (19.1)	8 (9.2)	89 (17.5)
<b>Positive DRE</b>	55 (13.0)	27 (31.0)	82 (16.1)
<b>1 Serum PSA</b>	5.3 (4.3-6.9)	5.8 (4.5-7.2)	5.3 (4.3-6.9)
<b>1 free PSA %</b>	16.2 (12.4-21.4)	12.5 (9-16.6)	15.9 (11.8-20.4)
<b>2 Serum PSA</b>	5.0 (3.7-6.6)	5.4 (4.1-6.7)	5.0 (3.8-6.6)
<b>2 free PSA %</b>	17.9 (13.9-23.4)	12.5 (9.1-16.3)	16.9 (12.8-22.1)
<b>Prostate volume</b>	38.0 (29.0-50.0)	26.0 (20.7-34.0)	35 (26-49)
<b>First Biopsy</b>	322 (76.1)	79 (90.8)	401 (78.6)
<b>PCPT1 Sig PCa</b>	0.08 (0.05-0.13)	0.16 (0.10-0.30)	0.09 (0.06-0.15)
<b>ERSPC1 Sig PCa</b>	0.05 (0.02-0.10)	0.12 (0.05-0.31)	0.05 (0.03-0.12)
<b>PCPT2 Sig PCa</b>	0.07 (0.04-0.11)	0.16 (0.08-0.27)	0.07 (0.05-0.13)
<b>ERSPC2 Sig PCa</b>	0.04 (0.02-0.08)	0.12 (0.05-0.30)	0.05 (0.02-0.11)
<b>PCa</b>	89 (21)	87 (100)	176 (34.5)

PCa= Prostate cancer; Sig PCa= significant PCa (Gleason  $\geq 7$  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.

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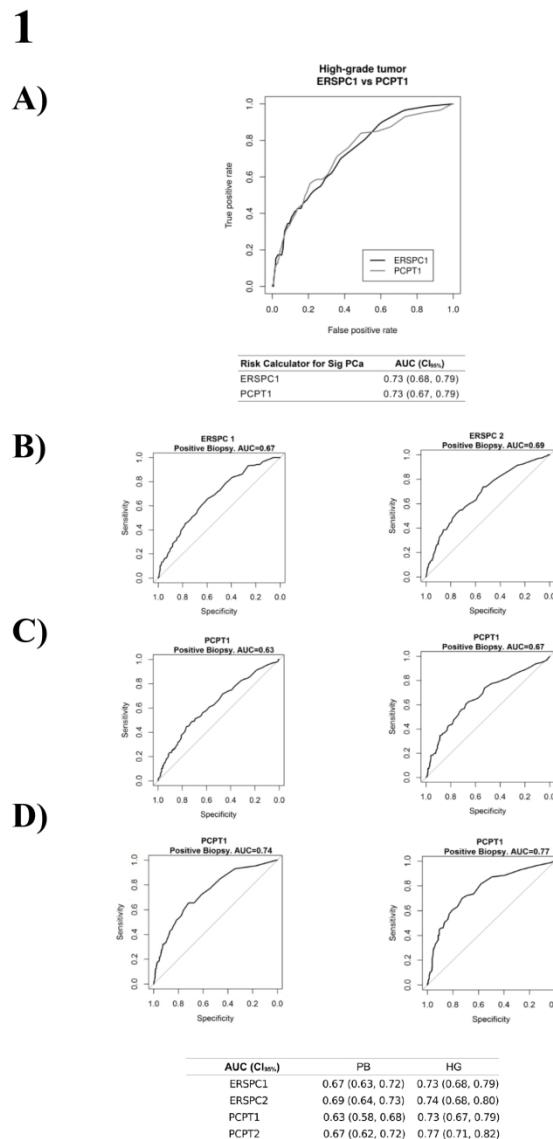


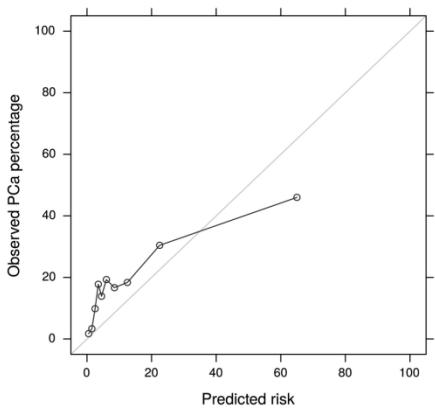
Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values: A, for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa; B, for the ERSPC1-RC and the ERSPC2-RC for positive biopsy; C, for the PCPT1-RC and the PCPT2-RC for positive biopsy; and D, for the ERSPC2-RC and the PCPT2-RC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

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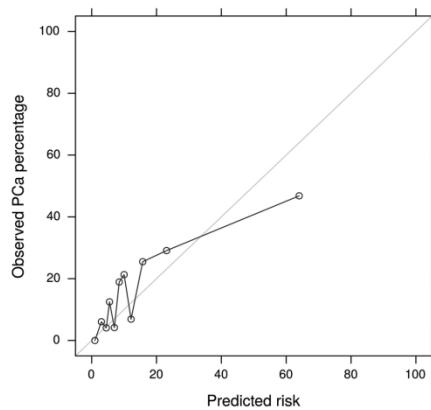
A)

High grade tumor: ERSPC1



B)

High grade tumor: PCPT1

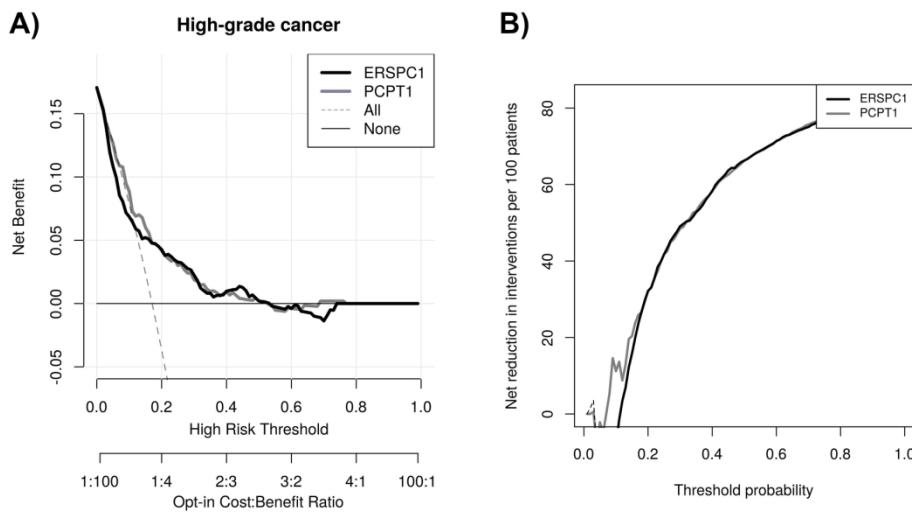


Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are 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.

191x142mm (300 x 300 DPI)

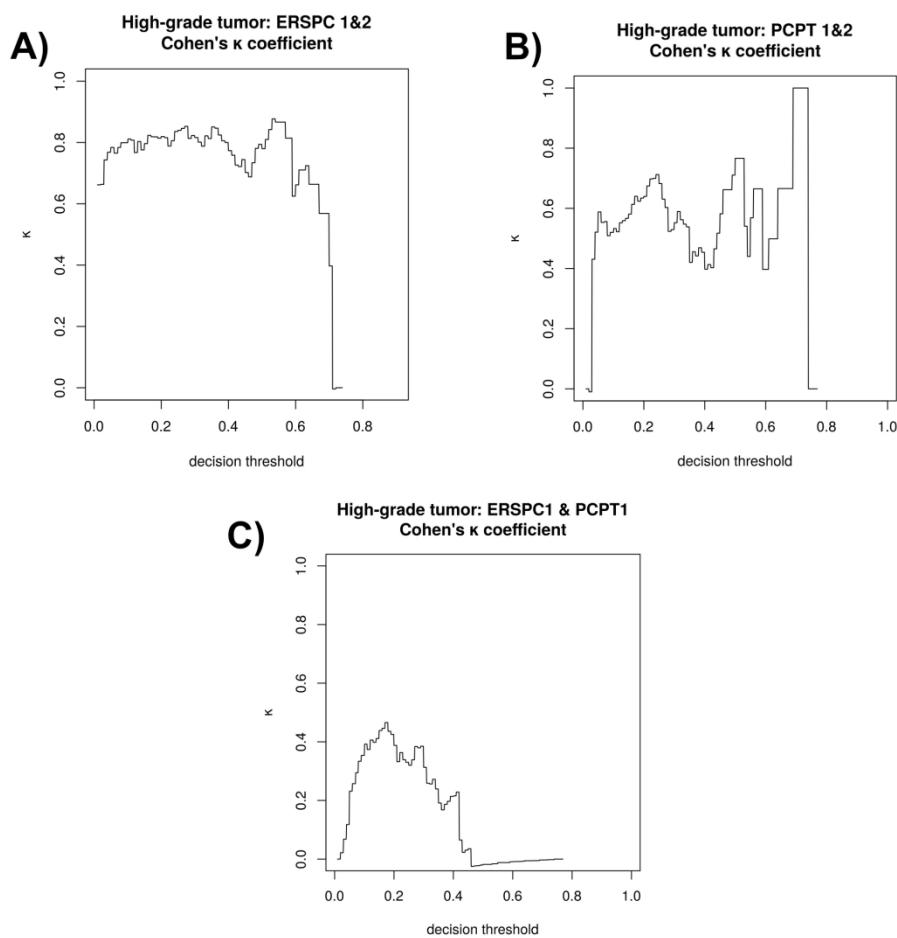
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Results of the decision curve analysis. A, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1-RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating 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).

169x126mm (300 x 300 DPI)

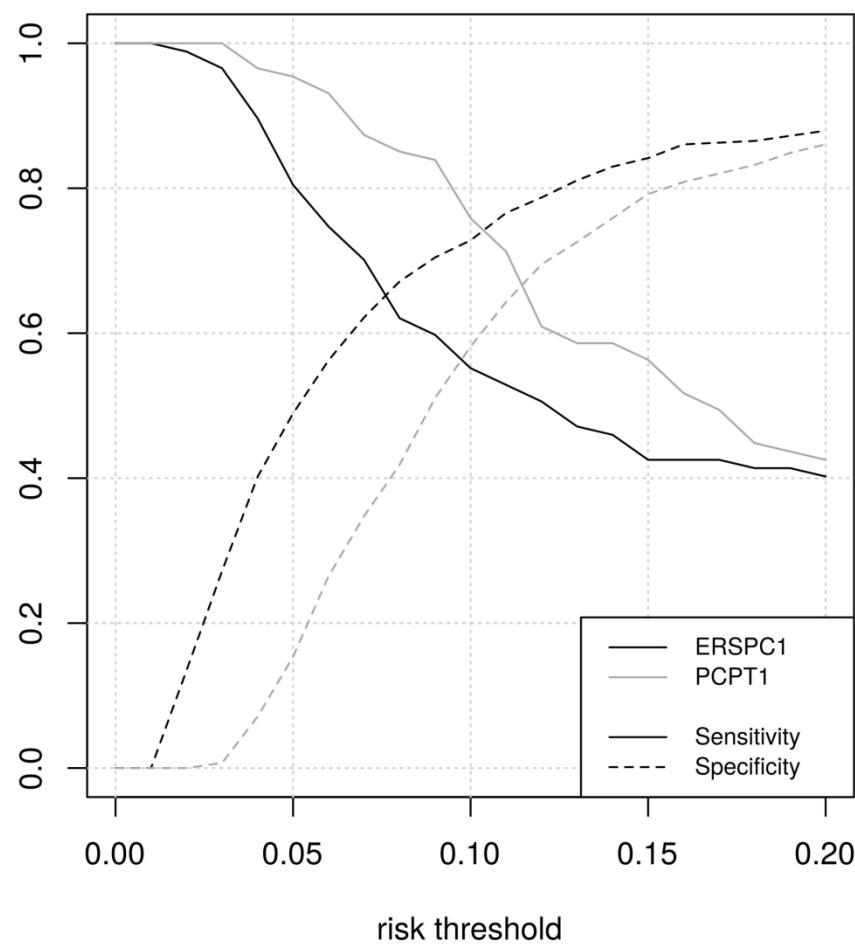
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Graphics showing Cohen's  $\kappa$  coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. A, Agreement between 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).

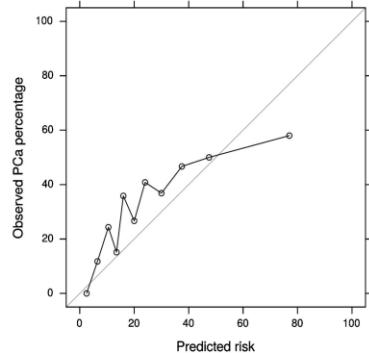
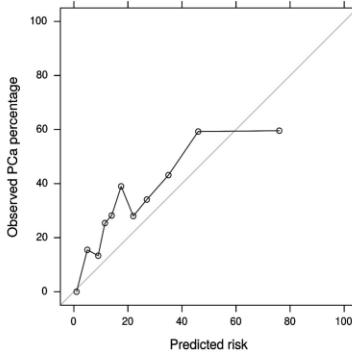
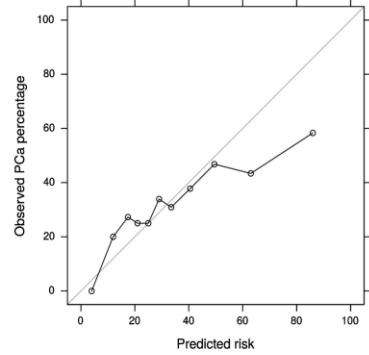
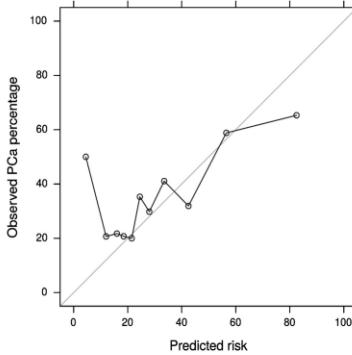
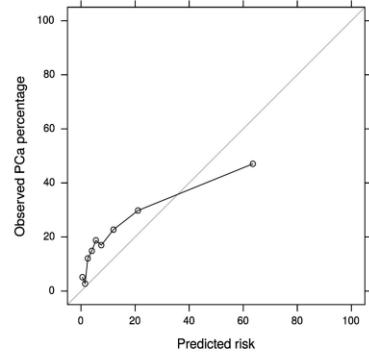
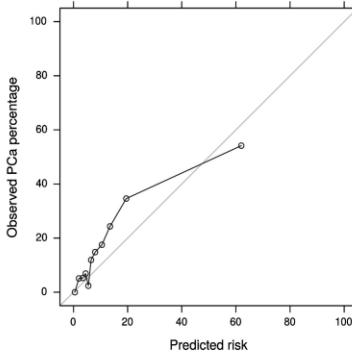
**Supplemental Table 1.** Patients excluded from the ONCOVER cohort for this study

depends on the exclusion criteria.

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

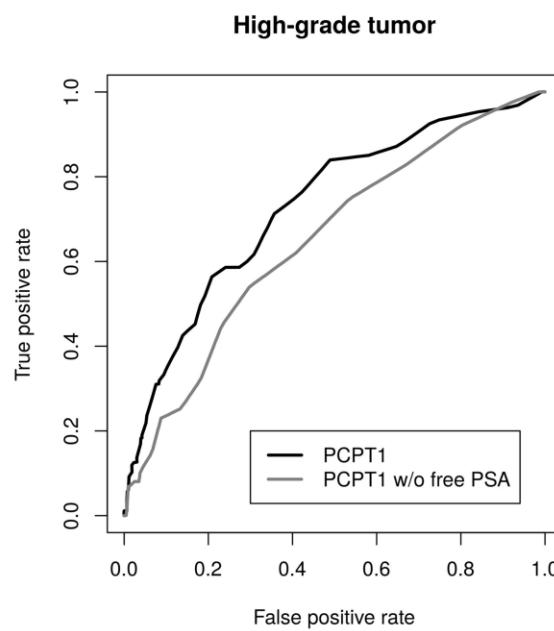
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6 **Supplemental table 2:** DeLong p values resulting from the pairwise comparison of the  
7 Area under the Receiver Operator Characteristic (ROC) curve (AUC) between Risk  
8 Calculators for significant Prostate cancer (Sig PCa) detection.  
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Sig 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****A)****Positive biopsy: ERSPC1****Positive biopsy: ERSPC2****B)****Positive biopsy: PCPT1****Positive biopsy: PCPT2****C)****High grade tumor: ERSPC2****High grade tumor: PCPT2**

**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.

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**S2**



Risk Calculator for Sig PCa	AUC (CI <sub>95%</sub> )
PCPT1 without free PSA	0.65 (0.59, 0.71)
PCPT1 + free PSA	0.73 (0.67, 0.79)

DeLong p value: 0.02

42  
43 **Supplemental Figure 2:** Receiver Operating Characteristic curves and Area Under the Curve  
44 values for the PCPT1-RC without free PSA (black) and for the PCPT1-RC with free PSA (grey) to  
45 predict Sig PCa. P-value according to the DeLong test.

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2                   **STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies***  
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4

Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	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
<b>Introduction</b>			
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
<b>Methods</b>			
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
<b>Results</b>			

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  (b) Give reasons for non-participation at each stage  (c) Consider use of a flow diagram	11  11  Supplemental
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders  (b) Indicate number of participants with missing data for each variable of interest	11  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  (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	12
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supplemental
<b>Discussion</b>			
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
<b>Other information</b>			
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](http://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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Manuscript ID	bmjopen-2019-031032.R2
Article Type:	Original research
Date Submitted by the Author:	28-Aug-2019
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<b>Primary Subject Heading</b>:	Urology
Secondary Subject Heading:	Urology
Keywords:	Significant prostate cancer, risk calculator variability, ERSPC, PCPT

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3 **Observational study comparing the accuracy/variability between the ERSPC and**  
4 **the PCPT risk calculators for the prediction of significant prostate cancer in patients**  
5 **with PSA <10ng/ml**  
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39 **Running title:** Risk Calculators for Significant Prostate Cancer  
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42 **Key Words:** Significant prostate cancer; risk calculator variability; ERSPC; PCPT.  
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44 **Tables and figures:** 1 and 5  
45

46 **Acknowledgment**  
47

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7 in designing the study, interpreting the data, writing, and publishing the report  
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2    1    ABSTRACT  
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4    2    **Introduction:** Risk Calculators (RCs) are easy-to-use tools considering available clinical-  
5 variables that could help to select those patients with risk of prostate-cancer (PCa) who should  
6 undergo a prostate-biopsy. **Objective:** To perform a comparison for the prediction of significant-PCa  
7 (SigPCa) between the European-Randomised Study of Screening for PCa (ERSPC) and the PCa  
8 Prevention-Trial (PCPT) RCs in patients with PSA between 3-10ng/ml through an evaluation of the  
9 accuracy/variability between two consecutive PSA-values. **Setting:** An observational study in a  
10 major university Hospital of the south of Spain. **Methods and participants:** An observational study  
11 was performed in patients who underwent a prostate-biopsy. SigPCa probabilities were calculated  
12 with the two PSA measures using ERSPC3/4+DRE and PCPTv2+free-PSA RCs. The prediction  
13 discrimination of SigPCa was determined by the area under the curve (AUC). Calibration,  
14 discrimination, and decision curve analysis were studied. The variability between both RCs-  
15 agreement was compared using Cohen's kappa coefficient. **Results:** 510 patients were analysed (87  
16 diagnosed with SigPCa). The median PSA value were 5.3 and 5ng/ml for PSA1 and PSA2  
17 respectively. Both RCs overestimated the risk in the case of high-risk probabilities. Discrimination  
18 ability for SigPCa was similar between models with an AUC=0.73(0.68-0.79) for ERSPC-RC vs.  
19 0.73(0.67-0.79) for PCPT-RC. ERSPC-RC showed less variability than PCPT-RC, with a constant  
20 agreement ( $k=0.7-0.8$ ) for usual range of clinical decision-making. Remarkably, a higher biopsies  
21 number would be avoided using the ERSPC-RC, but more SigPCa would be missed along all the risk  
22 probabilities. **Conclusions:** Both RCs had similar accuracy for the SigPCa discrimination. However,  
23 ERSPC-RC seems to be more stable for intra-individual PSA variations.

1  
2 23 **Strengths and limitations of this study**  
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- 4 24 - This study highlights the need to spread the use of available free tools which would be useful  
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6 25 in patient's selection for undergoing prostate biopsy.  
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8 26 - This study is the first to compare two available free risk calculators in patients with a PSA <  
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10 27 10ng/ml analyzing their variability between two consecutive different PSA levels.  
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12 28 - Although the clinical information of this study was extracted from a clinical practice cohort  
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14 29 and with information that could be useful for urologists worldwide, this is a retrospective  
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16 30 study and the use of TRUS biopsy for PCa diagnosis, even though is the standard in most  
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18 31 populations, suffers from random error compared with template biopsy, which could have  
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20 32 affected prediction results.  
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2 34 INTRODUCTION  
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4 35 Prostate cancer (PCa) is the second most frequently diagnosed malignancy in males worldwide,  
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6 36 and the most frequent in developed countries(1). Its current standard of diagnosis is a prostate biopsy  
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8 37 based on PSA levels and digital rectal examination (DRE). However, there are other available and  
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10 38 complementary variables that could help to select those patients who should undergo a prostate biopsy  
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12 39 (such as age, prostate volume, free PSA, family history, etc.), but these are not always used and/or  
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14 40 well-integrated in daily clinical practice(2). In line with this, Risk Calculators (RCs) are easy-to-use  
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16 41 tools that can help the clinicians to take advantage of all these available variables (3). The two main  
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18 42 RCs are from the European Randomised Study for Screening of Prostate Cancer (ERSPC cohort;  
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20 43 ERSPC-RC: <http://www.prostatecancer-riskcalculator.com/seven-prostate-cancer-riskcalculators>)  
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22 44 and from the Prostate Cancer Prevention Trial (PCPT cohort; PCPT-RC:  
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24 45 <http://deb.uthscsa.edu/URORiskCalc/Pages/calcs.jsp>). Both RCs have undergone some  
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26 46 modifications, specifically the addition of estimated prostate volume in the ERSPC-RC (4,5).  
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28 47 Furthermore, both RCs were originally developed from different patient cohorts and each RC uses  
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30 48 different variables.  
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36 49 To date, limited external validations and comparisons have been performed by different groups  
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38 50 (6–9). The two most important recent comparisons of the modified RCs were performed by Foley *et*  
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40 51 *al* (6,7) and Poyet *et al* (9). Both found a better discriminatory ability for ERSPC-RC vs PCPTv.2-  
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42 52 RC for the diagnoses of significant-PCa (Sig PCa) (AUC around 0.74 vs 0.69, respectively), but they  
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44 53 also included patients with a high PSA of up to 50 ng/ml. Despite the possibility of using these RCs  
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46 54 in patients with PSA levels up to 50ng/ml, it is clear that the advantages of using both RCs would  
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48 55 probably increase in patients with a PSA under 10ng/ml, where the rate of positive biopsy for PCa  
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50 56 clearly decrease, with an important number of unnecessary biopsies. Furthermore, in the case of the  
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52 57 PCPTv.2-RC, the addition of the free PSA value in patients with a PSA under 10ng/ml seems to  
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54 58 improve its accuracy (5), and, therefore, given its accessibility, this value should be included in the  
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56 59 RC.  
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1       60       The intra-individual and inter-assay variability of PSA is already known (10–12) and, therefore,  
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3       61       at least two measures are necessary before a prostate biopsy is indicated. In fact, it has been shown  
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5       62       that approximately 25% of men with initial PSA levels between 4 and 10 ng/mL had normal PSA  
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7       63       values upon repeat testing (13). In line with this, despite being primarily based on PSA level, the  
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9       64       variability of the two RCs mentioned above has been poorly studied, and might have implications for  
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11      65       patient management. Our group has recently evaluated this variability with the ERSPC-RC, which  
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13      66       showed stable accuracy over a cohort of patients, but some changes with respect to an individual  
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15      67       approach (14). To date, there is no study comparing the accuracy and variability of both RCs, the  
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17      68       ERSPC + DRE vs. the PCPTv.2 + free PSA, for the prediction of Sig PCa. Therefore, the aim of this  
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19      69       study was to perform a direct comparison between ERSPC + DRE and PCPTv.2 + free PSA RCs in  
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21      70       patients with a PSA between 3-10ng/ml, evaluating the accuracy and variability of both methods in  
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23      71       the prediction of Sig PCa.

1  
2 73 **MATERIALS AND METHODS**  
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4 74 **Study population and design**  
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6 75 An observational retrospective study was performed in patients from ONCOVER cohort (1021  
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8 76 biopsies indicated by clinical practice wherein patients donated blood and urine before the biopsy).  
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10 77 The study was carried out within the project approved by our Hospital Research Ethics Committee,  
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12 78 and informed consent was obtained from all participants. Blood sample was obtained in the morning  
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14 79 (between 8:00-10:00 am) after fasting overnight and then, the prostate biopsy was implemented  
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16 according to clinical practice. The inclusion criteria for this study were: 1) PSA indication between  
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18 80 3-10 ng/ml; 2) Full clinical and laboratory data to fulfilled ERSPC-RC and PCPT-RC; 3) Age 55-80  
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20 81 years' old; 4) Two consecutives measurements of PSA levels within an interval of 12 weeks.  
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22 82 Exclusion criteria included patients with a previously known PCa diagnosis or treatment that could  
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24 83 modify PSA levels (Supplemental table 1).  
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48 93 **Main variables description**  
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50 94 Demographic information and the medical history of each patient was obtained. PSA levels were  
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52 95 measured twice within a period no longer than 12 weeks, as follows: 1) **PSA 1 and free PSA 1:** for  
53  
54 96 biopsy indication; and, 2) **PSA 2 and free PSA 2:** before undergoing prostate biopsy. For both PSA  
55  
56 97 measures were evaluated by Chemiluminescent Microparticle Immunoassays (ng/ml, by a CMIA;  
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1  
2 98 Ref. 7k70; Abbott). The median and interquartile range of time between measurements was 6 (3-8)  
3  
4 99 weeks.  
5

6 100 **Prostate volume:** estimated by TRUS and categorized in three possible values, 25-40-60 ml, as  
7  
8 101 recommended (4) (TRUS volume <30 = 25 mL, 30–50 = 40 mL, and ≥50 = 60 mL).  
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11 102 **Significant/ high-grade (HG) prostate cancer (Sig PCa):** PCa with a Gleason grade ≥ 7 on  
12  
13 103 biopsy.  
14  
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16 104  
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18 105 **ERSPC-RC and PCPT-RC probabilities calculation**  
19

20 106 **ERSPC:** The formulas for the ERSPC-RC 3+DRE for patients at initial biopsy and the ERSPC-  
21  
22 107 RC 4+DRE for patients at repeat biopsy were utilized in this study. These calculators use PSA,  
23  
24 prostate volume, and DRE as variables, with, a negative prostate biopsy in ERSPC 4+ DRE in patients  
25  
26 108 who had a previous biopsy. This provides a probability rating for any PCa or Sig PCa (Gleason ≥ 7).  
27  
28 109

29 110 **ERSPC1/Sig PCa (1° Measure):** Risk probability calculated by ERSPC-RC3 or 4 (if previous  
30  
31 111 biopsy) ± DRE for any PCa using PSA 1/ Sig PCa – for HG PCa.  
32  
33

34 112 **ERSPC2/Sig PCa (2° Measure):** Risk probability calculated by ERSPC-RC 3 or 4 (if previous  
35  
36 113 biopsy) ± DRE for any PCa using PSA 2/ Sig PCa – for HG PCa.  
37  
38

39 114 **PCPT:** The formulae for the PCPT-RC 2.0 + %free PSA was utilized in this study. This calculator  
40  
41 115 uses race, age, PSA level, %free PSA level, family history of PCa, DRE and prior prostate biopsy.  
42  
43 116 This gives a probability of negative biopsy, low grade PCa and Sig PCa (gleason ≥ 7).  
44  
45

46 117 **PCPT1:** Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 1 and free PSA 1.  
47

48 118 **PCPT2:** Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 2 and free PSA 2.  
49

50 119 The variability of PSA was calculated by the following formula: | **Measure 1– Measure2| /**  
51

52 120 **Measure 1**  
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57 122 **Statistical analysis**  
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2 123 A descriptive study was performed by calculating the median and interquartile ranges (IR) for the  
3  
4 124 quantitative variables, and the absolute frequencies and percentages for the qualitative variables. A  
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6 125 Student's T test for paired groups was used to compare the means of the quantitative variables (PSA  
7  
8 126 1 and 2).  
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11 127 The investigation of the comparative performance in the detection of Sig PCa of both RCs,  
12  
13 128 ERSPC-RC and PCPT-RC, was performed, taking into account these four factors: discrimination  
14  
15 129 capacity, calibration, clinical utility, and consistency against the observed variations in PSA levels  
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17 130 for our dataset.  
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20 131 The discrimination ability of the models, i.e., their ability to separate those patients who had Sig  
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22 132 PCa from those who do not, was assessed using the area under their Receiver Operator Characteristic  
23  
24 133 (ROC) curve (AUC) (16), as measured in our sample. This is one of the most frequently used  
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26 134 measurements of model discrimination, because of its independence of the selection of a specific  
27  
28 135 decision threshold and its robustness against class imbalance. Confidence intervals for these AUCs  
29  
30 136 were computed using bootstrapping. These AUCs were then compared to determine the relative  
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32 137 performance of the models using DeLong tests (17). These tests were chosen because of their non-  
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34 138 parametric nature, with few assumptions about the data, and their suitability for paired data, as both  
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36 139 models were evaluated over the same dataset, properties which make this the most commonly used  
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38 140 test to compare AUCs (18). For this comparison, we focused on the calculated risk score utilising the  
39  
40 141 first measure of PSA (PSA 1; the value for the indication of the prostate biopsy).  
41  
42  
43 142 The calibration of the calculators for our cohort was then investigated to determine the agreement  
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45 143 between the frequency of the observed outcome (Sig PCa in our case) and the risks predicted by the  
46  
47 144 model. Calibration plots were used for this purpose (19), enabling a visual evaluation of this  
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49 145 agreement and the comparison between RCs.  
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52 146 To address the potential clinical utility of the models, we performed decision curve analysis on  
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54 147 our data, as proposed by Vickers and Elkin (20). This method has the advantage of not requiring the  
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2 148 specification of the relative cost for false-positives and false-negatives, defining a net benefit as a  
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4 149 function of the decision threshold at which one would consider obtaining a biopsy.  
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6 150 Finally, the stability of the predictions of both RCs, with regard to the observed intra-patient  
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8 changes on PSA levels between measurements, was investigated using the Cohen's kappa (k) inter-  
9 151 rater agreement coefficient as a function of the decision threshold. This coefficient was selected due  
10  
11 152 to its widespread use and robustness against random agreements, and thus, is a better measurement  
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13 153 than naïve accuracy.  
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19 155 All the analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill) and R  
20  
21 156 version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria: URL [https://www.R-](https://www.R-project.org/)  
22  
23 157 project.org/). A <5% level of significance ( $p<0.05$ ) was used to decide statistically significant  
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25 158 differences.  
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32 160 **Patient and public involvement**  
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35 161 Participants and public were not involved in the development of research questions, study design  
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37 162 or recruitment.  
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2 165 **RESULTS**  
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4 166 **Cohort characteristics**  
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6 167 In the present study, we analysed 510 patients who met the inclusion criteria previously described.  
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8 168 Median age was 65 (60-70) years old, with a family history in 89 patients (17.5%) and a suspicious  
9 169 DRE in 82 patients (16.1%). The median PSA before prostate biopsy indication was 5.3 (4.3-6.9)  
10 170 ng/ml. 176 patients were diagnosed with PCa, 87 of those categorized as Sig PCa. Most patients  
11 171 (n=401; 78.6%) were biopsy-naïve and the median prostate volume was 35 (26-49) cc. Further cohort  
12 172 description according to Sig PCa status is shown in Table 1.  
13

14 173 66 patients had a PSA 2 out of the range of 3-10ng/ml due to the variability (50 patients below  
15 174 3ng/ml and 16 patients above 10ng/ml); thus, in this case, the % free PSA was not calculated, and the  
16 175 risk probability was calculated without the inclusion of this variable. The patients were maintained in  
17 176 the analysis as reflecting the variability of the PSA, and the application of the models in this real  
18 177 situations, although acknowledging that it could introduce a bias in terms of calibration and variability.  
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34 179 **Direct comparison for Sig PCa prediction**  
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36 180 Discrimination ability for Sig PCa was no different between the two models [ERSPC1-RC vs.  
37 181 PCPT1-RC: 0.73; 95% CI: (0.68-0.79) vs. 0.73; 95% CI: (0.67-0.79), respectively]. ROC curves are  
38 182 shown in Figure 1A. Similarly, no difference was found in the discrimination ability for any PCa.  
39 183 The comparison of the RC for both measures is described in Figure 1(B-D) with similar results but a  
40 184 tendency of better accuracy for PCPT2-RC vs ERSPC2-RC ( $p=0.25$ ). Supplemental Table 2 shows  
41 185 multiple comparisons by the DeLong test resulting in no differences between the two RCs for Sig  
42 186 PCa.  
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45 187 Both models tended to overestimate the risk for a high probability of Sig PCa, and slightly  
46 188 underestimate it for low risk patients, suggesting that the models would benefit from a recalibration  
47 189 for our population (Figure 2). None of the models predicted very high probabilities for most patients.  
48 190 The calibration curves for any PCa are shown in Supplemental Figure 1.  
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2 191 The decision curve analyses revealed that both RCs provided a clinical net benefit in the threshold  
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4 192 probability range for Sig PCa (Figure 3). The net benefit was comparable between the two RCs for  
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6 193 Sig PCa.  
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9 194 As shown in Supplemental Figure 2, the addition of free PSA clearly improved the accuracy of  
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11 195 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;  
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13 196 p= 0.02].  
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16 197  
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18 198 **Variability and clinical significance**  
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20 199 PSA and free PSA change was significantly different between the two measures, but with low  
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22 200 clinical variations [average PSA1 5.69 ng/ml vs. PSA2 5.39 ng/ml (p < 0.05) and average free PSA1  
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24 201 16.99% vs. free PSA2 18.03% (p < 0.05)]. Median variability of PSA was 14% (6-27%). Taking into  
25  
26 202 account this variability of PSA, ERSPC proved to be more stable than PCPT. The k agreement  
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28 203 between ERSPC1 and ERSPC2 was practically constant, 0.79±0.09 for the usual range of clinical  
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30 204 decision (0-0.3). However, PCPT1 and PCPT2 showed wider variations, with a k agreement of  
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32 205 approximately 0.55±0.32 in the same range, with a subsequent rapid decrease. The agreement  
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34 206 between both models (ERSPC1 vs. PCPT1) proved to be worse for thresholds in this range, peaking  
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36 207 0.47 for a 17% risk, with an average 0.32±0.12 on the interval. The comparison between ERSPC2  
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38 208 and PCPT2 yielded similar results (Figure 4).  
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43 209 Direct comparison of sensitivity and specificity of both RCs along the different clinical risk  
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45 210 thresholds showed that PCPT-RC has higher sensitivity and lower specificity than ERPSC-RC for a  
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47 211 given threshold along the clinically useful region (Figure 5). The balance point is reached at a  
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49 212 different risk threshold for each RC. The performances of both RCs at this point are comparable, as  
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51 213 shown in Figure 5. Considering the superposition of their respective ROC curves to a good  
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53 214 approximation (Figure 1), this means that a transformation of decision thresholds can make both  
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55 215 models perform similarly.  
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**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 3–10 ng/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.

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2 243 As defined in the methodology, the first measure (ERSPC1 and PCPT1) was the focus of the  
3  
4 244 direct comparison, as this was used as the indication for biopsy. The accuracy of both RCs was similar  
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6 245 for Sig PCa in our study, showing an accuracy similar to other external validations such as Poyet *et*  
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8 246 *al.* (9) and Foley *et al.* (7) for ESRPC (AUC= 0.73 and 0.74, respectively) and a better accuracy for  
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10 247 PCPT1 v2.0 when adding free PSA (AUC= 0.70 and 0.69, respectively). Still, these results are far  
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12 248 from ideal, and thus, additional data from imaging or fluid markers might be included to improve the  
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14 249 accuracy of the RCs. In agreement with the accuracy results, the decision curve analysis was also  
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16 250 similar between both RCs. In fact, both RCs showed a net benefit from an early risk threshold, which  
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18 251 means that their implementation would be useful in the pathway of patient selection.  
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23 252 Studying the variability of the RCs improves our knowledge about their stability, which could  
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25 253 translate into improved decision-making and selection of patients. Our PSA cohort showed a  
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27 254 variability that was in the range of that previously shown in the literature (11,12,29). Our group and  
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29 255 others (14,30) have demonstrated that a higher PSA variability is associated with a reduced risk of  
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31 256 Sig PCa in a prostate biopsy, but it does not improve the accuracy of a RC. However, probability  
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33 257 stability is important in order to trust RC probabilities at any point. Our study shows good agreement  
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35 258 between the two ERSPC + DRE-RC probabilities, with good calibration and stability despite intra-  
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37 259 individual PSA variations. PCPTv.2 + free PSA shows worse stability and higher variability, which  
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39 259 could be explained simply by the fact that it uses two values (PSA and free PSA) that suffer from this  
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41 260 variability (31), while the use of an estimated volume in the ERSPC dilutes the PSA variability. These  
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43 261 results should be interpreted with caution, as volume estimation was performed by categorization of  
44  
45 262 TRUS and not by DRE. It is true that this categorization has previously shown good correlation (4).  
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47 263 This likely depends on prostate volume (32), as well as low but certain inter-examiner variability  
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49 264 (33), which could also increase ERPSC variability in an inter-clinician comparison. It should also be  
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51 265 taken into account that the clinical translation of this stability is not clear, firstly, because of the  
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53 266 limitation of the use of a single estimated prostate volume and because the global accuracy of both  
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55 267 RC are not significantly different, and seems to have a tendency to improve in the PCPT2 RC.  
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4 270 Calibration plots show that both models (PCPT-RC and ERSPC-RC) predict adequately only the  
5 actual risk of PCa and Sig PCa for low-risk patients, with a wider useful range in the case of PCa and  
6 a lower range in the case of Sig PCa. For higher risk patients, the calibration curves become irregular.  
7  
8 271 This effect is accentuated for risks close to 1, as both models predict maximum risks of around 0.75  
9 for Sig PCa. The models would benefit from recalibration for our population in the low-moderate risk  
10 region, considering that this is the region of greater interest for the model, as patients with a high  
11 predicted risk would probably undergo biopsy anyway. Nonetheless, despite not showing a good  
12 calibration in the usual range for clinical decision (0-0.3), visually ERSPC seems to be more  
13 consistent with a less fluctuating calibration in this range compared to PCPT, but at this point, this  
14 should be confirmed in future studies because no conclusion for direct comparison about calibration  
15 could be reached in the present study as quantitative analysis is outside the aim of this research. The  
16 comparison of coefficients between PCPT1 and PCPT2 and between PCPT2 and ERSPC2 showed  
17 that the differences between PCPT1 and PCPT2 were similar to those between ERSPC and PCPT  
18 models. As previously discussed ERSPC seems to be more insensitive and, therefore, robust to intra-  
19 individual variations of PSA compared to PCPT, while the predictive performance is similar and the  
20 clinical translation not clear yet.

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22 282 Despite the similar decision curve, results from the sensitivity, specificity and ROC curve analysis  
23 show that the same risk threshold should not be used for both models. Both RCs are able to have  
24 similar performance, and the benefit of using any of them is similar in order to screen patients for a  
25 prostate biopsy, if the correct cut-off point is selected. It should be highlighted the importance of  
26 having an almost 100% negative predictive value, as the advantage of reducing unnecessary biopsies  
27 should not be at the cost of missing or delaying the diagnoses of a Sig PCa.

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29 292 In clinical practice, the use of these RCs should be the first step in guiding the decision for further  
30 management of the patient. Patients with a confirmed, elevated PSA between 3-10ng/ml should be  
31 better stratified using other variables within a RC, as men with PSA levels >10ng/mL are likely to

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2 295 proceed to biopsy regardless of other factors. Probably a specific cut-off point in the risk probability  
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4 296 should not be used and take advantage of the known probabilities to discuss with the patient the  
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6 297 biopsy indication as recommended by the PCPT-RC. In the situation in which the patient is in the  
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8 298 low-risk group, according to both RCs (ERSPC and PCPT), the patient could continue with just  
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10 299 follow-up. This fact has also been proposed by Alberts *et al.* (34) when applying new diagnostic  
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12 300 markers, such as mpMRI. Specifically, they showed that following a negative recommendation from  
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14 301 the ERSPC-RC would have avoided 62 (51%) of 122 mpMRIs and two (25%) of eight insignificant  
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16 302 PCA diagnoses, missing three (10%) of 31 high-grade PCA. As the positive predictive value of these  
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18 303 RCs is not as good as their negative predictive value, in case of discordance between both RCs or if  
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20 304 there is an indication for a biopsy according to both RCs, other images or fluid biomarkers could  
21  
22 305 increase the accuracy in order to potentially reduce the harm from unnecessary prostate biopsy and  
23  
24 306 over-diagnosis (35). Specifically, Loeb *et al.* (36) has recently demonstrated that the incorporation of  
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26 307 PHI into both RCs increases the accuracy of the diagnoses of Sig PCA. Another relevant point should  
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28 308 be comment from the tendency of better predictive ability with the second evaluations of PSA,  
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30 309 reinforcing the idea of the need of several PSA values to confirm the risk and discarded confounding  
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32 310 factors. Furthermore, this analysis could suggest a tend to better discrimination ability of PCPT in the  
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34 311 range of lower probabilities (when PSA is low), but further research would be needed to validate this  
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36 312 affirmation. These risk calculators only show a static probability so other longitudinal variables and  
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38 313 clinical judgment should be required for their application.  
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46 314 The present study has some limitations. First, despite the prospectively collected information, it  
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48 315 is a retrospective study design. Second, prostate volume was an estimation and categorization from a  
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50 316 TRUS calculation, and, therefore, it is not the actual approach for which the RC was developed. Third,  
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52 317 the PSA values interval was not the same for all patients, which means the results should be  
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54 318 interpreted with caution. Four, the use of TRUS biopsy for PCA diagnosis, although is the standard in  
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56 319 most populations, suffers from random error compared with template biopsy (37), which could have  
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2 320 affected prediction results. However, the clinical information was extracted from a clinical practice  
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4 321 cohort and with information that could be useful for urologists worldwide.  
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6 322 Altogether, our results showed that: 1) the use of both RCs (ERSPC and PCPT) could be an useful  
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8 323 tool in the selection of patients who need prostate biopsy, and that both RCs showed similar accuracy  
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10 324 for discrimination of Sig PCa; 2) ERSPC-RC showed higher stability than PCPT-RC for intra-  
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12 325 individual PSA variations; 3) when comparing both RCs sensitivity and specificity, a higher rate of  
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14 326 biopsies could be avoided with the ERSPC-RC vs. the PCPT-RC, but with a higher rate of Sig PCa  
15  
16 327 missed. Thus, in those patients with a PSA between 3-10 ng/ml, these tools should be used in order  
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18 328 to improve selection and specificity. The RCs specifically should be selected according to the  
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20 329 variables available in the clinic. In addition, both RCs could also be used and the decision to undergo  
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22 330 a biopsy be shared with the patient.  
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30 332 **CONFLICTS OF INTEREST**  
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32 333 Nothing to declare  
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4 492 **Footnotes**  
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- 7 494 • **Contributors** E.G.G, R.M.L, MJ.R.T and J.C.V carried out the conception and design of the  
8 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  
9 acquisition; E.G.G, J.C.V, J.L.F.R, RM.L and MJ.R.T carried out the analysis and  
10 interpretation of data; E.G.G, J.C.V and RM.L drafted the manuscript; JJ.S.B, J.L.F.R, AM.B,  
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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 Sofía 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).

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6 545   **Table 1.** Clinical and demographic characteristics of the cohort of patients categorized according to  
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8 cancer status.  
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<b>Variable</b>	<b>No Sig PCa n=423</b>	<b>Sig PCa n=87</b>	<b>All n=510</b>
<b>Age</b>	64.0 (60.0-69.0)	68.0 (63.0-71.0)	65.0 (60.0-70.0)
<b>Family History</b>	81 (19.1)	8 (9.2)	89 (17.5)
<b>Positive DRE</b>	55 (13.0)	27 (31.0)	82 (16.1)
<b>1 Serum PSA</b>	5.3 (4.3-6.9)	5.8 (4.5-7.2)	5.3 (4.3-6.9)
<b>1 free PSA %</b>	16.2 (12.4-21.4)	12.5 (9-16.6)	15.9 (11.8-20.4)
<b>2 Serum PSA</b>	5.0 (3.7-6.6)	5.4 (4.1-6.7)	5.0 (3.8-6.6)
<b>2 free PSA %</b>	17.9 (13.9-23.4)	12.5 (9.1-16.3)	16.9 (12.8-22.1)
<b>Prostate volume</b>	38.0 (29.0-50.0)	26.0 (20.7-34.0)	35 (26-49)
<b>First Biopsy</b>	322 (76.1)	79 (90.8)	401 (78.6)
<b>PCPT1 Sig PCa</b>	0.08 (0.05-0.13)	0.16 (0.10-0.30)	0.09 (0.06-0.15)
<b>ERSPC1 Sig PCa</b>	0.05 (0.02-0.10)	0.12 (0.05-0.31)	0.05 (0.03-0.12)
<b>PCPT2 Sig PCa</b>	0.07 (0.04-0.11)	0.16 (0.08-0.27)	0.07 (0.05-0.13)
<b>ERSPC2 Sig PCa</b>	0.04 (0.02-0.08)	0.12 (0.05-0.30)	0.05 (0.02-0.11)
<b>PCa</b>	89 (21)	87 (100)	176 (34.5)

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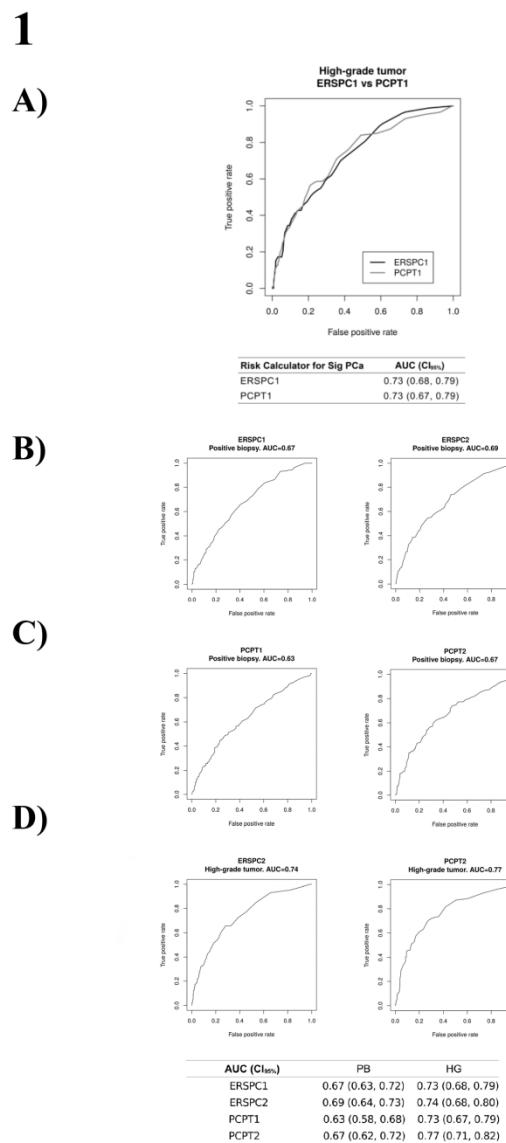


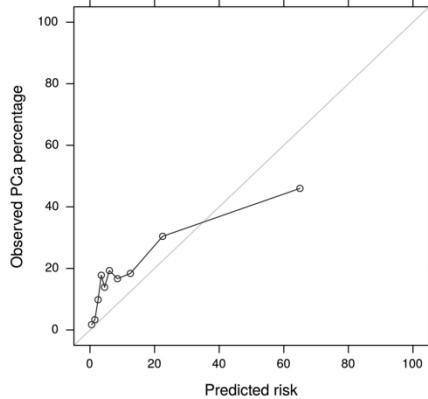
Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values: A, for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa; B, for the ERSPC1-RC and the ERSPC2-RC for positive biopsy; C, for the PCPT1-RC and the PCPT2-RC for positive biopsy; and D, for the ERSPC2-RC and the PCPT2-RC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

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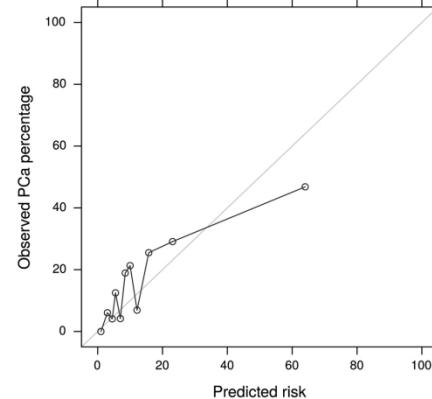
A)

High grade tumor: ERSPC1



B)

High grade tumor: PCPT1

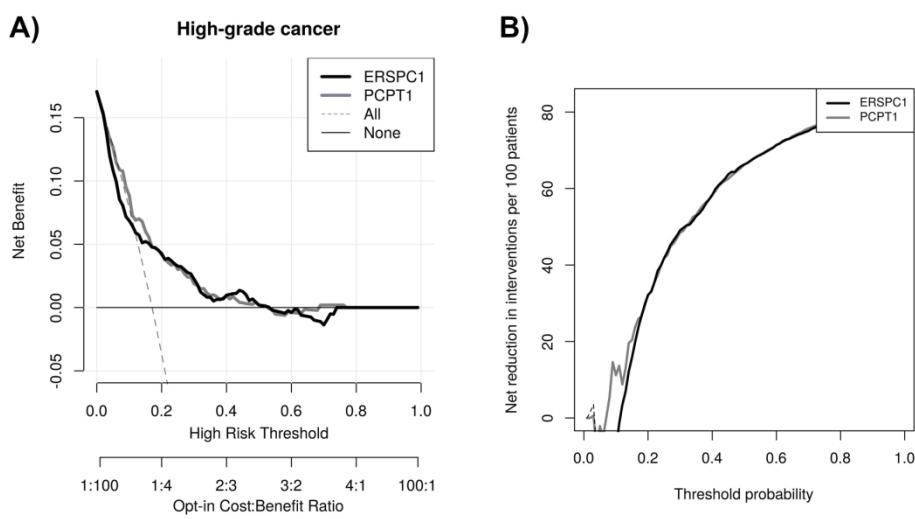


Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are 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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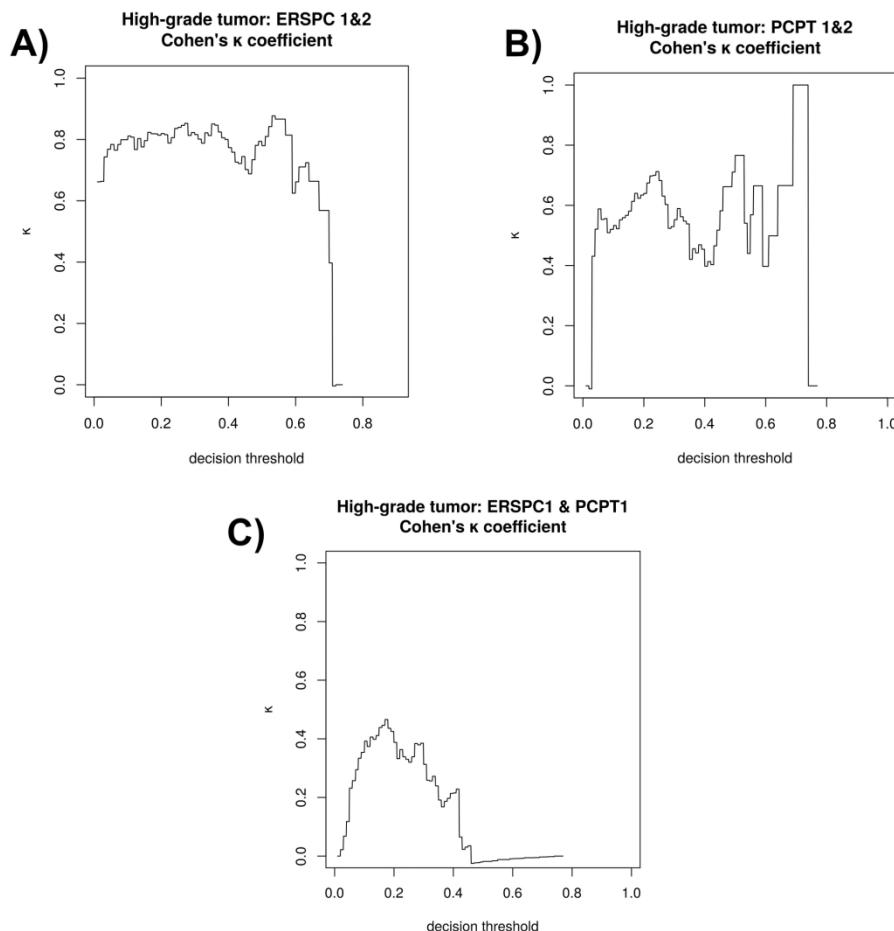
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Results of the decision curve analysis. A, Net benefit for the prediction of Sig PCa on biopsy using the ERSPC1-RC (black line) and the PCPT1-RC (grey line) as a function of the risk threshold, compared to those benefits of the strategies of treating 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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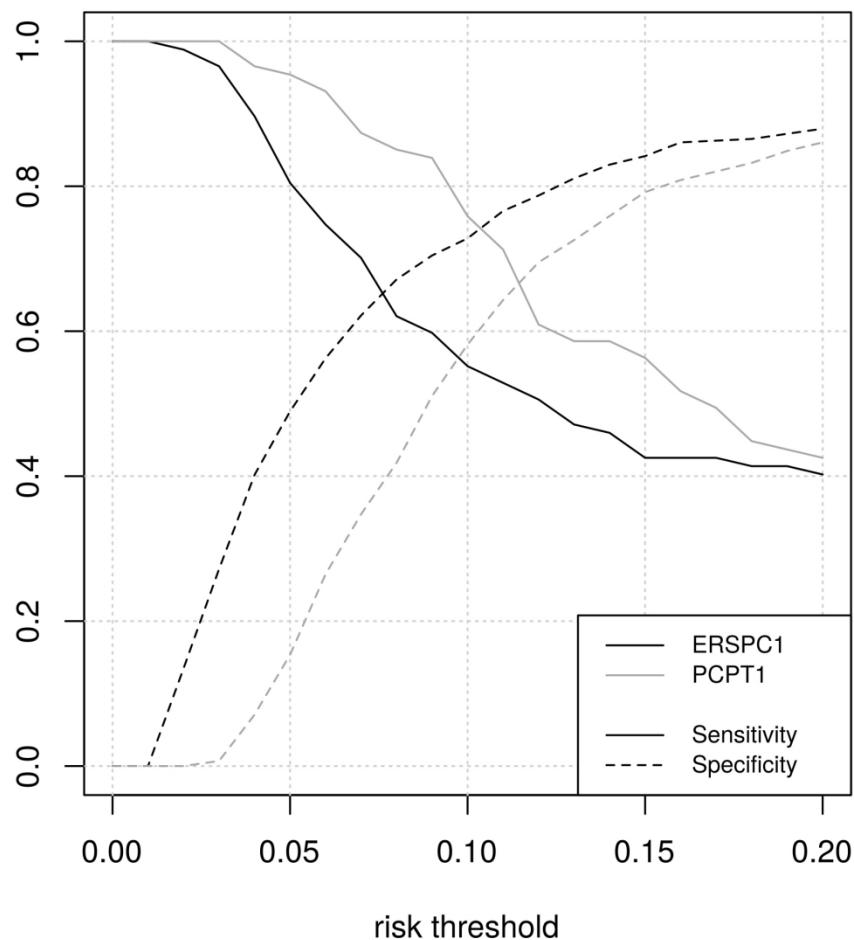
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Graphics showing Cohen's  $\kappa$  coefficient, which evaluated the agreement between RCs, as a function of the decision threshold, with 1 being total agreement and 0 being the worst possible expected agreement between rates. A, Agreement between 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).

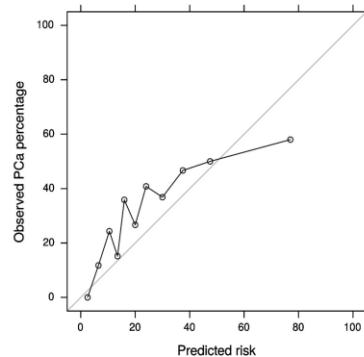
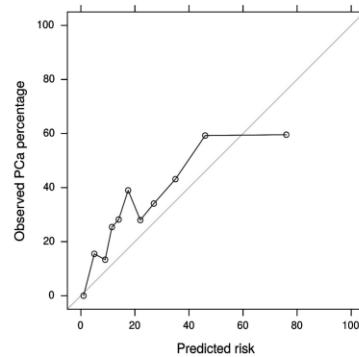
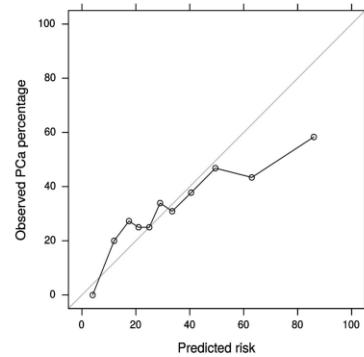
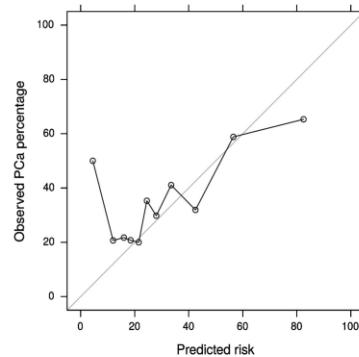
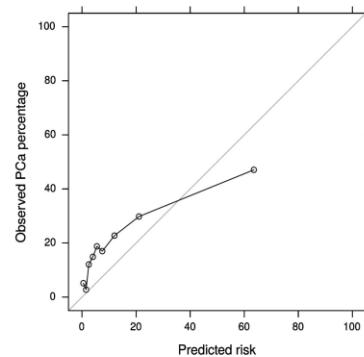
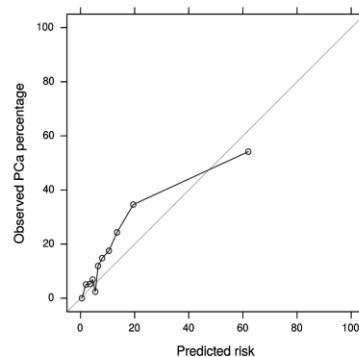
**Supplemental Table 1.** Patients excluded from the ONCOVER cohort for this study

depends on the exclusion criteria.

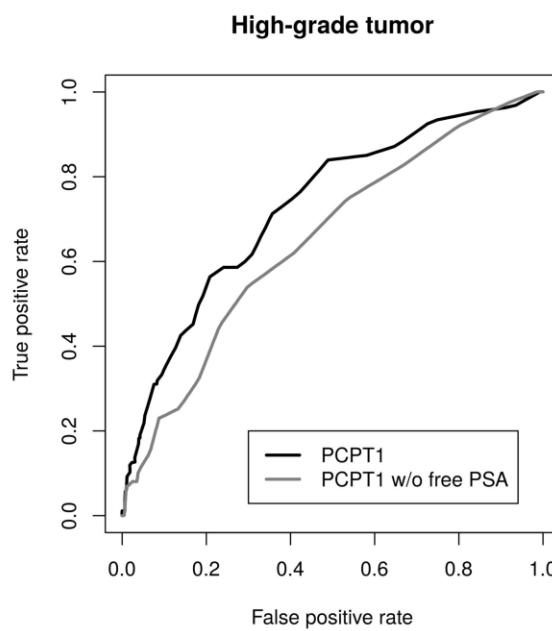
Exclusion criteria	Number
<b>Under active surveillance</b>	25
<b>2 consecutive PSA levels well recorded or affected</b>	50
<b>Prostate volume not well recorded</b>	177
<b>PSA of biopsy indication out of the range 3-10ng/ml , or Age out of the range 55-80</b>	251
<b>Total exclusions</b>	511

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6 **Supplemental table 2:** DeLong p values resulting from the pairwise comparison of the  
7 Area under the Receiver Operator Characteristic (ROC) curve (AUC) between Risk  
8 Calculators for significant Prostate cancer (Sig PCa) detection.  
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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****A)****Positive biopsy: ERSPC1****Positive biopsy: ERSPC2****B)****Positive biopsy: PCPT1****Positive biopsy: PCPT2****C)****High grade tumor: ERSPC2****High grade tumor: PCPT2**

**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.

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Risk Calculator for Sig PCa	AUC (CI <sub>95%</sub> )
PCPT1 without free PSA	0.65 (0.59, 0.71)
PCPT1 + free PSA	0.73 (0.67, 0.79)
DeLong p value: 0.02	

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

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2      **STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies***  
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Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	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
<b>Introduction</b>			
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
<b>Methods</b>			
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
<b>Results</b>			

1	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
2			(b) Give reasons for non-participation at each stage	11
3			(c) Consider use of a flow diagram	Supplemental
4	Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	11
5			(b) Indicate number of participants with missing data for each variable of interest	11- Supplemental
6	Outcome data	15*	Report numbers of outcome events or summary measures	12
7	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
8			(b) Report category boundaries when continuous variables were categorized	
9			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
10	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supplemental
11	<b>Discussion</b>			
12	Key results	18	Summarise key results with reference to study objectives	14
13	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
14	Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16
15	Generalisability	21	Discuss the generalisability (external validity) of the study results	17
16	<b>Other information</b>			
17	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](http://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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<b>Primary Subject Heading</b>:	Urology
Secondary Subject Heading:	Urology
Keywords:	Significant prostate cancer, risk calculator variability, ERSPC, PCPT

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3 **Observational study comparing the accuracy/variability between the ERSPC and**  
4 **the PCPT risk calculators for the prediction of significant prostate cancer in patients**  
5 **with PSA <10ng/ml**  
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42 **Key Words:** Significant prostate cancer; risk calculator variability; ERSPC; PCPT.  
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44 **Tables and figures:** 1 and 5  
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For peer review only

## 1    1 ABSTRACT

2    2    **Introduction:** Risk Calculators (RCs) are easy-to-use tools considering available clinical  
3    3    variables that could help to select those patients with risk of prostate cancer (PCa) who should  
4    4    undergo a prostate biopsy. **Objective:** To perform a comparison for the prediction of significant-PCa  
5    5    (SigPCa) between the European-Randomised Study of Screening for PCa (ERSPC) and the PCa  
6    6    Prevention-Trial (PCPT) RCs in patients with PSA between 3-10ng/ml through an evaluation of the  
7    7    accuracy/variability between two consecutive PSA-values. **Setting:** An observational study in a  
8    8    major university hospital in the south of Spain. **Methods and participants:** An observational study  
9    9    was performed in patients who underwent a prostate biopsy. SigPCa probabilities were calculated  
10   10    with the two PSA measures using ERSPC3/4+DRE and PCPTv2+free-PSA RCs. The prediction of  
11   11    SigPCa was determined by the area under the curve (AUC). Calibration, discrimination, and decision  
12   12    curve analysis were studied. The variability between both RCs-agreement was compared using  
13   13    Cohen's kappa coefficient. **Results:** 510 patients were analysed (87 diagnosed with SigPCa). The  
14   14    median PSA value were 5.3 and 5ng/ml for PSA1 and PSA2 respectively. Both RCs overestimated  
15   15    the risk in the case of high-risk probabilities. Discriminative ability for SigPCa was similar between  
16   16    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  
17   17    showed less variability than PCPT-RC, with a constant agreement ( $k=0.7-0.8$ ) for usual range of  
18   18    clinical decision-making. Remarkably, a higher number of biopsies would be avoided using the  
19   19    ERSPC-RC, but more SigPCa would be missed along all the risk probabilities. **Conclusions:** Both  
20   20    RCs performed similar in the prediction of SigPCa. However, ERSPC-RC seems to be more stable  
21   21    for intra-individual PSA variations.

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2 23 **Strengths and limitations of this study**  
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- 4 24 - This study is the first to compare two available free risk calculators in patients with a PSA <  
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6 25 10ng/ml analyzing their variability between two consecutive different PSA levels.  
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8 26 - One of the study limitations is that prostate volume was an estimation and categorization from  
9 a TRUS calculation, and, therefore, it is not the actual approach for which the RC was  
10 developed.  
11 27  
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13 28  
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15 29 - Although the clinical information of this study was extracted from a clinical practice cohort  
16 and with information that could be useful for urologists worldwide, this is a retrospective  
17 study and the use of TRUS biopsy for PCa diagnosis, even though it is the standard in most  
18 populations, suffers from random error compared with template biopsy, which could have  
19 affected prediction results.  
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2   35   **INTRODUCTION**  
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6   36   Prostate cancer (PCa) is the second most frequently diagnosed malignancy in males worldwide,  
7   37   and the most frequent in developed countries(1). Its current standard of diagnosis is a prostate biopsy  
8   38   based on PSA levels and digital rectal examination (DRE). However, there are other available and  
9  
10   39   complementary variables that could help to select those patients who should undergo a prostate biopsy  
11   40   (such as age, prostate volume, free PSA, family history, etc.), but these are not always used and/or  
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13   41   well-integrated in daily clinical practice(2). In line with this, Risk Calculators (RCs) are easy-to-use  
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15   42   tools that can help the clinicians to take advantage of all these available variables (3). The two main  
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17   43   RCs are from the European Randomised Study for Screening of Prostate Cancer (ERSPC cohort;  
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19   44   ERSPC-RC: <http://www.prostatecancer-riskcalculator.com/seven-prostate-cancer-riskcalculators>)  
20  
21   45   and from the Prostate Cancer Prevention Trial (PCPT cohort; PCPT-RC:  
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23   46   <http://deb.uthscsa.edu/URORiskCalc/Pages/calcs.jsp>). Both RCs have undergone some  
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25   47   modifications, specifically the addition of estimated prostate volume in the ERSPC-RC (4,5).  
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27   48   Furthermore, both RCs were originally developed from different patient cohorts and each RC uses  
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29   49   different variables.  
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32   50   To date, limited external validations and comparisons have been performed by different groups  
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34   51   (6–9). The two most important recent comparisons of the modified RCs were performed by Foley *et*  
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36   52   *al* (6,7) and Poyet *et al* (9). Both found a better discriminative ability for ERSPC-RC vs PCPTv.2-  
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38   53   RC for the diagnoses of significant-PCa (Sig PCa) (AUC around 0.74 vs 0.69, respectively), but they  
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40   54   also included patients with a high PSA of up to 50 ng/ml. Despite the possibility of using these RCs  
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42   55   in patients with PSA levels up to 50ng/ml, it is clear that the advantages of using both RCs would  
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44   56   probably increase in patients with a PSA under 10ng/ml, where the rate of positive biopsy for PCa  
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46   57   clearly decrease, with an important number of unnecessary biopsies. Furthermore, in the case of the  
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48   58   PCPTv.2-RC, the addition of the free PSA value in patients with a PSA under 10ng/ml seems to  
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50   59   improve its predictive ability (5), and, therefore, given its accessibility, this value should be included  
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52   60   in the RC.

1           61       The intra-individual and inter-assay variability of PSA is already known (10–12) and, therefore,  
2           62       at least two measures are necessary before a prostate biopsy is indicated. In fact, it has been shown  
3           63       that approximately 25% of men with initial PSA levels between 4 and 10 ng/mL had normal PSA  
4           64       values upon repeat testing (13). In line with this, despite being primarily based on PSA level, the  
5           65       variability of the two RCs mentioned above has been poorly studied, and might have implications for  
6           66       patient management. Our group has recently evaluated this variability with the ERSPC-RC, which  
7           67       showed stable accuracy over a cohort of patients, but some changes with respect to an individual  
8           68       approach (14). To date, there is no study comparing the accuracy and variability of both RCs, the  
9           69       ERSPC + DRE vs. the PCPTv.2 + free PSA, for the prediction of Sig PCa. Therefore, the aim of this  
10          70       study was to perform a direct comparison between ERSPC + DRE and PCPTv.2 + free PSA RCs in  
11          71       patients with a PSA between 3-10ng/ml, evaluating the accuracy and variability of both methods in  
12          72       the prediction of Sig PCa.

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2 74 **MATERIALS AND METHODS**  
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4 75 **Study population and design**  
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6 76 An observational retrospective study was performed in patients from ONCOVER cohort (1021  
7 biopsies indicated by clinical practice wherein patients donated blood and urine before the biopsy).  
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9 77 The study was carried out within the project approved by our Hospital Research Ethics Committee,  
10 and informed consent was obtained from all participants. Blood sample was obtained in the morning  
11 (between 8:00-10:00 am) after fasting overnight and then, the prostate biopsy was implemented  
12 according to clinical practice. The inclusion criteria for this study were: 1) PSA indication between  
13 3-10 ng/ml; 2) Full clinical and laboratory data to fulfill ERSPC-RC and PCPT-RC criteria; 3) Age  
14 55-80 years old; 4) Two consecutive measurements of PSA levels within an interval of 12 weeks.  
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16 81 Exclusion criteria included patients with a previously known PCa diagnosis or treatment that could  
17 modify PSA levels (Supplemental table 1).  
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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).

48 94 **Main variables description**  
49

50 95 Demographic information and the medical history of each patient was obtained. PSA levels were  
51 measured twice within a period no longer than 12 weeks, as follows: 1) **PSA 1 and free PSA 1:** for  
52 biopsy indication; and, 2) **PSA 2 and free PSA 2:** before undergoing prostate biopsy. For both PSA  
53 measures were evaluated by Chemiluminescent Microparticle Immunoassays (ng/ml, by a CMIA;  
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2 99 Ref. 7k70; Abbott). The median and interquartile range of time between measurements was 6 (3-8)  
3  
4 100 weeks.  
5

6 101 **Prostate volume:** estimated by TRUS and categorized in three possible values, 25-40-60 ml, as  
7  
8 102 recommended (4) (TRUS volume <30 = 25 cc, 30–50 = 40 cc, and ≥50 = 60 cc).  
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11 103 **Significant/ high-grade (HG) prostate cancer (Sig PCa):** PCa with a Gleason grade ≥ 7 on  
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13 104 biopsy.  
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18 106 **ERSPC-RC and PCPT-RC probabilities calculation**  
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20 107 **ERSPC:** The formulas for the ERSPC-RC 3+DRE for patients at initial biopsy and the ERSPC-  
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22 108 RC 4+DRE for patients at repeat biopsy were utilized in this study. These calculators use PSA,  
23  
24 prostate volume, and DRE as variables, with, a negative prostate biopsy in ERSPC 4+ DRE in patients  
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26 109 who had a previous biopsy. This provides a probability rating for any PCa or Sig PCa (Gleason ≥ 7).  
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29 111 **ERSPC1/Sig PCa (1° Measure):** Risk probability calculated by ERSPC-RC3 or 4 (if previous  
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31 112 biopsy) + DRE; used PSA 1 to calculate the predicted risk for both any PCa and Sig PCa (HG PCa).  
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34 113 **ERSPC2/Sig PCa (2° Measure):** Risk probability calculated by ERSPC-RC 3 or 4 (if previous  
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36 114 biopsy) + DRE; used PSA 2 to calculate the predicted risk for both any PCa and Sig PCa (HG PCa).  
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39 115 **PCPT:** The formulae for the PCPT-RC 2.0 + %free PSA was utilized in this study. This calculator  
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41 116 uses race, age, PSA level, %free PSA level, family history of PCa, DRE and prior prostate biopsy.  
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43 117 This gives a probability of negative biopsy, low grade PCa and Sig PCa (Gleason ≥ 7).  
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45 118 **PCPT1:** Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 1 and free PSA 1.  
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48 119 **PCPT2:** Risk probabilities calculated by PCPT 2.0 + %free PSA using PSA 2 and free PSA 2.  
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50 120 The variability of PSA was calculated by the following formula: | **Measure 1 – Measure2| /**  
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52 121 **Measure 1**  
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57 123 **Statistical analysis**  
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2 124 A descriptive study was performed by calculating the median and interquartile ranges (IR) for the  
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4 125 quantitative variables, and the absolute frequencies and percentages for the qualitative variables. A  
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6 126 Student's T test for paired groups was used to compare the means of the quantitative variables (PSA  
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8 127 1 and 2).

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11 128 The investigation of the comparative performance in the detection of Sig PCa of both RCs,  
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13 129 ERSPC-RC and PCPT-RC, was performed, taking into account these four factors: discrimination  
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15 capacity, calibration, clinical utility, and consistency against the observed variations in PSA levels  
16 130 for our dataset.

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20 132 The discriminative ability of the models, i.e., their ability to separate those patients who had Sig  
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22 133 PCa from those who do not, was assessed using the area under their Receiver Operator Characteristic  
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24 (ROC) curve (AUC) (16), as measured in our sample. This is one of the most frequently used  
25 134 measurements of model discrimination, because of its independence of the selection of a specific  
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27 135 decision threshold and its robustness against class imbalance. Confidence intervals for these AUCs  
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29 136 were computed using bootstrapping. These AUCs were then compared to determine the relative  
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31 137 performance of the models using DeLong tests (17). These tests were chosen because of their non-  
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33 138 parametric nature, with few assumptions about the data, and their suitability for paired data, as both  
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35 139 models were evaluated over the same dataset, properties which make this the most commonly used  
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37 140 test to compare AUCs (18). For this comparison, we focused on the calculated risk score utilising the  
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39 141 first measure of PSA (PSA 1; the value which met the criteria for a prostate biopsy).

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43 143 The calibration of the calculators for our cohort was then investigated to determine the agreement  
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45 144 between the frequency of the observed outcome (Sig PCa in our case) and the risks predicted by the  
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47 145 model. Calibration plots were used for this purpose (19), enabling a visual evaluation of this  
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49 146 agreement and the comparison between RCs.

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51 147 To address the potential clinical utility of the models, we performed decision curve analysis on  
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53 148 our data, as proposed by Vickers and Elkin (20). This method has the advantage of not requiring the

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2 149 specification of the relative cost for false-positives and false-negatives, defining a net benefit as a  
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4 150 function of the decision threshold at which one would consider obtaining a biopsy.  
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6 151 Finally, the stability of the predictions of both RCs, with regard to the observed intra-patient  
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8 changes on PSA levels between measurements, was investigated using the Cohen's kappa (k)  
9 152 interrater agreement coefficient as a function of the decision threshold. This coefficient was selected  
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11 153 due to its widespread use and robustness against random agreements, and thus, is a better  
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13 154 measurement than naïve accuracy.  
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16 156 All the analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Ill) and R  
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18 20 157 version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria: URL [https://www.R-](https://www.R-project.org/)  
19  
21 project.org/). A <5% level of significance ( $p<0.05$ ) was used to decide statistically significant  
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23 24 158 differences.  
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32 161 **Patient and public involvement**  
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35 162 Participants and public were not involved in the development of research questions, study design  
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37 163 or recruitment.  
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2 166 **RESULTS**  
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4 167 **Cohort characteristics**  
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6 168 In the present study, we analysed 510 patients who met the inclusion criteria previously described.  
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8 169 Median age was 65 (60-70) years old, with a family history in 89 patients (17.5%) and a suspicious  
9  
10 DRE in 82 patients (16.1%). The median PSA before prostate biopsy indication was 5.3 (4.3-6.9)  
11 170 ng/ml. 176 patients were diagnosed with PCa, 87 of those categorized as Sig PCa. Most patients  
12  
13 171 (n=401; 78.6%) were biopsy-naïve and the median prostate volume was 35 (26-49) cc. Further cohort  
14  
15 172 description according to Sig PCa status is shown in Table 1(and in Supplemental data).  
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18 174 66 patients had a PSA 2 out of the range of 3-10ng/ml due to the variability (50 patients below  
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20 175 3ng/ml and 16 patients above 10ng/ml); thus, in this case, the % free PSA was not calculated, and the  
21  
22 176 risk probability was calculated without the inclusion of this variable. The patients were maintained in  
23  
24 177 the analysis as reflecting the variability of the PSA, and the application of the models in this real  
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26 178 situation, although acknowledging that it could introduce a bias in terms of calibration and variability.  
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30 180 **Direct comparison for Sig PCa prediction**  
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34 180 Discrimination ability for Sig PCa was no different between the two models [ERSPC1-RC vs.  
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36 181 PCPT1-RC: 0.73; 95% CI: (0.68-0.79) vs. 0.73; 95% CI: (0.67-0.79), respectively]. ROC curves are  
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38 182 shown in Figure 1A. Similarly, no difference was found in the discrimination ability for any PCa.  
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40 183 The comparison of the RC for both measures is described in Figure 1(B-D) with similar results but a  
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42 184 tendency of better accuracy for PCPT2-RC vs ERSPC2-RC ( $p= 0.25$ ). Supplemental Table 2 shows  
43  
44 185 multiple comparisons by the DeLong test resulting in no differences between the two RCs for Sig  
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46 186 PCa.  
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49 188 Both models tended to overestimate the risk for a high probability of Sig PCa, and slightly  
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51 189 underestimate it for low risk patients, suggesting that the models would benefit from a recalibration  
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53 189 for our population (Figure 2). None of the models predicted very high probabilities for most patients.  
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55 190 The calibration curves for any PCa are shown in Supplemental Figure 1.  
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2 192 The decision curve analyses revealed that both RCs provided a clinical net benefit in the threshold  
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4 193 probability range for Sig PCa (Figure 3). The net benefit was comparable between the two RCs for  
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6 194 Sig PCa.  
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9 195 As shown in Supplemental Figure 2, the addition of free PSA clearly improved the discriminative  
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11 196 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  
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13 197 PSA –RC; p= 0.02].  
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18 199 **Variability and clinical significance**  
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20 200 PSA and free PSA change was significantly different between the two measures, but with low  
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22 201 clinical variations [average PSA1 5.69 ng/ml vs. PSA2 5.39 ng/ml (p < 0.05) and average free PSA1  
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24 202 16.99% vs. free PSA2 18.03% (p < 0.05)]. Median variability of PSA was 14% (6-27%). Taking into  
25  
26 203 account this variability of PSA, ERSPC proved to be more stable than PCPT. The k agreement  
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28 204 between ERSPC1 and ERSPC2 was practically constant, 0.79±0.09 for the usual range of clinical  
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30 205 decision (0-0.3). However, PCPT1 and PCPT2 showed wider variations, with a k agreement of  
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32 206 approximately 0.55±0.32 in the same range, with a subsequent rapid decrease. The agreement  
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34 207 between both models (ERSPC1 vs. PCPT1) proved to be worse for thresholds in this range, peaking  
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36 208 0.47 for a 17% risk, with an average 0.32±0.12 on the interval. The comparison between ERSPC2  
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38 209 and PCPT2 yielded similar results (Figure 4).  
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43 210 Direct comparison of sensitivity and specificity of both RCs along the different clinical risk  
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45 211 thresholds showed that PCPT-RC has higher sensitivity and lower specificity than ERPSC-RC for a  
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47 212 given threshold along the clinically useful region (Figure 5). The balance point is reached at a  
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49 213 different risk threshold for each RC. The performances of both RCs at this point are comparable, as  
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51 214 shown in Figure 5. Considering the superposition of their respective ROC curves to a good  
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53 215 approximation (Figure 1), this means that a transformation of decision thresholds can make both  
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55 216 models perform similarly.  
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2 218 **DISCUSSION**  
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4 219 Currently, considerable research is being carried out to find new diagnostic markers for Sig PCa,  
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6 220 in order to reduce the number of biopsies and the over-diagnosis of insignificant PCa (21). These  
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8 221 markers are based on body fluids (blood, urine) or image explorations (22,23). Some are  
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10 222 recommended by guidelines such as the 4k score test, PCA3, and/or the Prostate Health Index (PHI)  
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12 223 in body fluids (only PCA3 and PHI have been approved by the FDA) (24–26), or multiparametric  
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14 224 magnetic resonance imaging (mpMRI), with recent evidence of its advantages in biopsy-naïve  
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16 225 patients (27). However, costs and availability minimize their implementation worldwide, and,  
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18 226 therefore, it is clear that additional and readily available tools, such as RCs, should be implemented  
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20 227 in daily clinical practice. The two most used RCs are ERSPC-RC and PCPT-RC, which have been  
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22 228 modified and adapted (4,5). Few external validations have been conducted, with varying results  
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24 229 (7,9,28). Usually, external validations of RCs show worse performance than the original validations  
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26 230 (8), a fact that is corroborated by our study. Therefore, based on all this information, evaluations,  
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28 231 validations, and incorporation of RCs are needed (3).

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34 232 The present study explores and compares for the first time, both the PCPT v2 + free PSA and the  
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36 233 ERSPC + DRE, not only for accuracy but also for variability and clinical relevance. Our group  
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38 234 previously explored the accuracy and variability of the ERSPC + DRE RC (14) but, in this study, we  
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40 235 have specifically focused only on patients in the grey zone (PSA 3–10 ng/ml) and compared the  
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42 236 ERSPC + DRE RC vs. the PCPT v2 + free PSA, an analysis that has not been previously performed.  
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44 237 This comparison showed that both RCs had similar accuracy for the discrimination of Sig PCa.  
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46 238 However, ERSPC-RC had better calibration and stability for intra-individual PSA variations. Our  
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48 239 methodology in calculating the volume is an estimation from the results of the TRUS measure, similar  
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50 239 to Poyet *et al.*, and following the recommendations of Roobol *et al.* (4). We have focused only on  
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52 240 those patients with PSA between 3–10 ng/ml who require additional diagnostic information. The  
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54 241 PCPT-RC option with free PSA, which increases the accuracy of discrimination between Sig PCa  
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56 242 and no PCa (5), was calculated, as it is an easy-to-use and readily available tool for these patients.  
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2 244 As defined in the methodology, the first measure (ERSPC1 and PCPT1) was the focus of the  
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4 245 direct comparison, as this was used as the indication for biopsy. The accuracy of both RCs was similar  
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6 246 for Sig PCa in our study, showing an accuracy similar to other external validations such as Poyet *et*  
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8 247 *al.* (9) and Foley *et al.* (7) for ESRPC (AUC= 0.73 and 0.74, respectively) and a better accuracy for  
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10 248 PCPT1 v2.0 when adding free PSA (AUC= 0.70 and 0.69, respectively). Still, these results are far  
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12 249 from ideal, and thus, additional data from imaging or fluid markers might be included to improve the  
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14 250 accuracy of the RCs. In agreement with the accuracy results, the decision curve analysis was also  
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16 251 similar between both RCs. In fact, both RCs showed a net benefit from an early risk threshold, which  
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18 252 means that their implementation would be useful in the pathway of patient selection.  
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23 253 Studying the variability of the RCs improves our knowledge about their stability, which could  
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25 254 translate into improved decision-making and selection of patients. Our PSA cohort showed a  
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27 255 variability that was in the range of that previously shown in the literature (11,12,29). Our group and  
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29 256 others (14,30) have demonstrated that a higher PSA variability is associated with a reduced risk of  
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31 257 Sig PCa in a prostate biopsy, but it does not improve the accuracy of a RC. However, probability  
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33 258 stability is important in order to trust RC probabilities at any point. Our study shows good agreement  
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35 259 between the two ERSPC + DRE-RC probabilities, with good calibration and stability despite intra-  
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37 260 individual PSA variations. PCPTv.2 + free PSA shows worse stability and higher variability, which  
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39 261 could be explained simply by the fact that it uses two values (PSA and free PSA) that suffer from this  
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41 262 variability (31), while the use of an estimated volume in the ERSPC dilutes the PSA variability. These  
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43 263 results should be interpreted with caution, as volume estimation was performed by categorization of  
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45 264 TRUS and not by DRE. It is true that this categorization has previously shown good correlation (4).  
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47 265 This likely depends on prostate volume (32), as well as low but certain inter-examiner variability  
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49 266 (33), which could also increase ERPSC variability in an inter-clinician comparison. It should also be  
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51 267 taken into account that the clinical translation of this stability is not clear, firstly, because of the  
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53 268 limitation of the use of a single estimated prostate volume and because the global accuracy of both  
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55 269 RC are not significantly different, and seems to have a tendency to improve in the PCPT2 RC.  
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4 271 Calibration plots show that both models (PCPT-RC and ERSPC-RC) predict adequately only the  
5 actual risk of PCa and Sig PCa for low-risk patients, with a wider useful range in the case of PCa and  
6 a lower range in the case of Sig PCa. For higher risk patients, the calibration curves become irregular.  
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8 272 This effect is accentuated for risks close to 1, as both models predict maximum risks of around 0.75  
9 for Sig PCa. The models would benefit from recalibration for our population in the low-moderate risk  
10 region, considering that this is the region of greater interest for the model, as patients with a high  
11 predicted risk would probably undergo biopsy anyway. Nonetheless, despite not showing a good  
12 calibration in the usual range for clinical decision (0-0.3), visually ERSPC seems to be more  
13 consistent with a less fluctuating calibration in this range compared to PCPT, but at this point, this  
14 should be confirmed in future studies because no conclusion for direct comparison about calibration  
15 could be reached in the present study as quantitative analysis is outside the aim of this research. The  
16 comparison of coefficients between PCPT1 and PCPT2 and between PCPT2 and ERSPC2 showed  
17 that the differences between PCPT1 and PCPT2 were similar to those between ERSPC and PCPT  
18 models. As previously discussed ERSPC seems to be more insensitive and, therefore, robust to intra-  
19 individual variations of PSA compared to PCPT, while the predictive performance is similar and the  
20 clinical translation not clear yet.

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22 287 Despite the similar decision curve, results from the sensitivity, specificity and ROC curve analysis  
23 show that the same risk threshold should not be used for both models. Both RCs are able to have  
24 similar performance, and the benefit of using any of them is similar in order to screen patients for a  
25 prostate biopsy, if the correct cut-off point is selected. It should be highlighted the importance of  
26 having an almost 100% negative predictive value, as the advantage of reducing unnecessary biopsies  
27 should not be at the cost of missing or delaying the diagnoses of a Sig PCa.

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29 293 In clinical practice, the use of these RCs should be the first step in guiding the decision for further  
30 management of the patient. Patients with a confirmed, elevated PSA between 3-10ng/ml should be  
31 better stratified using other variables within a RC, as men with PSA levels >10ng/mL are likely to

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2 296 proceed to biopsy regardless of other factors. Probably a specific cut-off point in the risk probability  
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4 297 should not be used and take advantage of the known probabilities to discuss with the patient the  
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6 298 biopsy indication as recommended by the PCPT-RC. In the situation in which the patient is in the  
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8 299 low-risk group, according to both RCs (ERSPC and PCPT), the patient could continue with just  
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11 300 follow-up. This fact has also been proposed by Alberts *et al.* (34) when applying new diagnostic  
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13 301 markers, such as mpMRI. Specifically, they showed that following a negative recommendation from  
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15 302 the ERSPC-RC would have avoided 62 (51%) of 122 mpMRIs and two (25%) of eight insignificant  
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17 303 PCA diagnoses, missing three (10%) of 31 high-grade PCA. As the positive predictive value of these  
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19 304 RCs is not as good as their negative predictive value, in case of discordance between both RCs or if  
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21 305 there is an indication for a biopsy according to both RCs, other images or fluid biomarkers could  
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23 306 increase the accuracy in order to potentially reduce the harm from unnecessary prostate biopsy and  
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25 307 over-diagnosis (35). Specifically, Loeb *et al.* (36) has recently demonstrated that the incorporation of  
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27 308 PHI into both RCs increases the accuracy of the diagnoses of Sig PCA. Another relevant point should  
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29 309 be comment from the tendency of better predictive ability with the second evaluations of PSA,  
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31 310 reinforcing the idea of the need of several PSA values to confirm the risk and discarded confounding  
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33 311 factors. Furthermore, this analysis could suggest a tend towards better discrimination ability of PCPT  
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35 312 in the range of lower probabilities (when PSA is low), but further research would be needed to validate  
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37 313 this hypothesis. These risk calculators only show a static probability so other longitudinal variables  
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39 314 and clinical judgment should be required for their application.  
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46 315 The present study has some limitations. First, despite the prospectively collected information, it  
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48 316 is a retrospective study design. Second, prostate volume was an estimation and categorization from a  
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50 317 TRUS calculation, and, therefore, it is not the actual approach for which the RC was developed. Third,  
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52 318 the PSA values interval was not the same for all patients, which means the results should be  
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54 319 interpreted with caution. Four, the use of TRUS biopsy for PCA diagnosis, although it is the standard  
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56 320 in most populations, suffers from random error compared with template biopsy (37), which could  
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2 321 have affected prediction results. However, the clinical information was extracted from a clinical  
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4 322 practice cohort and with information that could be useful for urologists worldwide.  
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6 323 Altogether, our results showed that: 1) the use of both RCs (ERSPC and PCPT) could be a useful  
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8 324 tool in the selection of patients who need prostate biopsy, and that both RCs performed similar in the  
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10 325 prediction of Sig PCa; 2) ERSPC-RC showed higher stability than PCPT-RC for intra-individual PSA  
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12 326 variations; 3) when comparing both RCs sensitivity and specificity, a higher rate of biopsies could be  
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14 327 avoided with the ERSPC-RC vs. the PCPT-RC, but with a higher rate of Sig PCa missed. Thus, in  
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16 328 those patients with a PSA between 3-10 ng/ml, these tools should be used in order to improve  
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18 329 selection and specificity. The RCs specifically should be selected according to the variables available  
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20 330 in the clinic. In addition, both RCs could also be used and the decision to undergo a biopsy be shared  
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22 331 with the patient.  
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30 333 **CONFLICTS OF INTEREST**  
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32 334 Nothing to declare.  
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**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 Sofía 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).

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**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
<b>Age</b>	64.0 (60.0-69.0)	68.0 (63.0-71.0)	65.0 (60.0-70.0)
<b>Family History</b>	81 (19.1)	8 (9.2)	89 (17.5)
<b>Positive DRE</b>	55 (13.0)	27 (31.0)	82 (16.1)
<b>1 Serum PSA</b>	5.3 (4.3-6.9)	5.8 (4.5-7.2)	5.3 (4.3-6.9)
<b>1 free PSA %</b>	16.2 (12.4-21.4)	12.5 (9-16.6)	15.9 (11.8-20.4)
<b>2 Serum PSA</b>	5.0 (3.7-6.6)	5.4 (4.1-6.7)	5.0 (3.8-6.6)
<b>2 free PSA %</b>	17.9 (13.9-23.4)	12.5 (9.1-16.3)	16.9 (12.8-22.1)
<b>Prostate volume</b>	38.0 (29.0-50.0)	26.0 (20.7-34.0)	35 (26-49)
<b>First Biopsy</b>	322 (76.1)	79 (90.8)	401 (78.6)
<b>PCPT1 Sig PCa</b>	0.08 (0.05-0.13)	0.16 (0.10-0.30)	0.09 (0.06-0.15)
<b>ERSPC1 Sig PCa</b>	0.05 (0.02-0.10)	0.12 (0.05-0.31)	0.05 (0.03-0.12)
<b>PCPT2 Sig PCa</b>	0.07 (0.04-0.11)	0.16 (0.08-0.27)	0.07 (0.05-0.13)
<b>ERSPC2 Sig PCa</b>	0.04 (0.02-0.08)	0.12 (0.05-0.30)	0.05 (0.02-0.11)
<b>PCa</b>	89 (21)	87 (100)	176 (34.5)

PCa= Prostate cancer; Sig PCa= significant PCa (Gleason  $\geq 7$  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.

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For peer review only

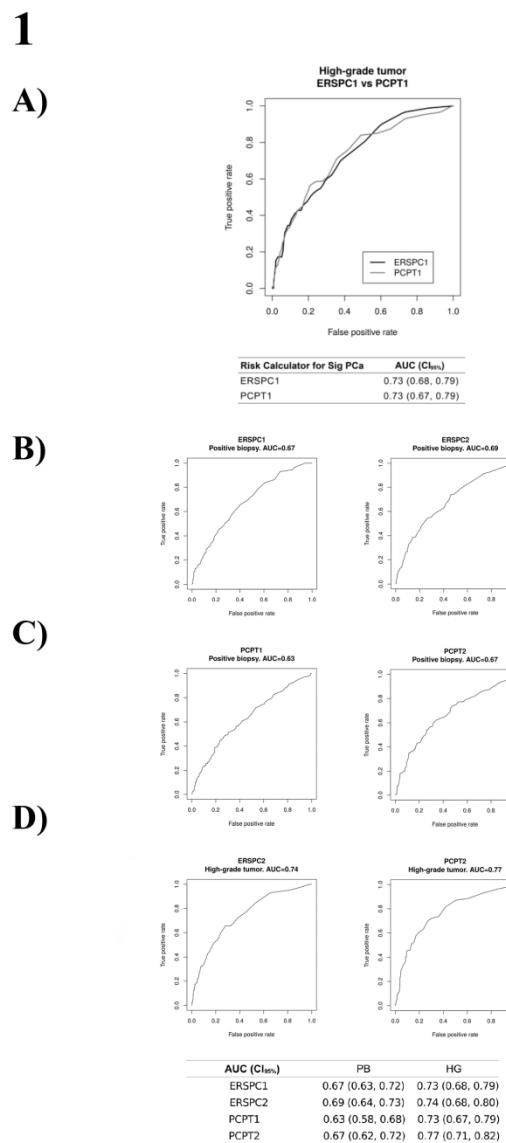


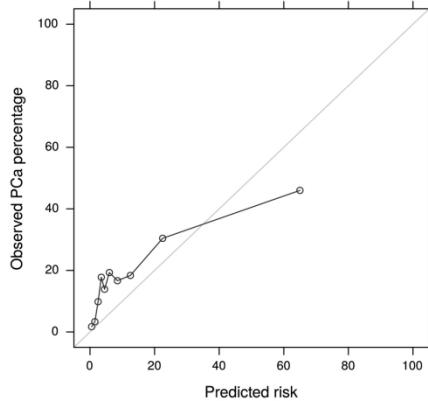
Figure 1: Receiver Operating Characteristic curves and Area Under the Curve values: A, for the ERSPC1 -RC (black) and PCPT1 -RC (grey) for Sig PCa; B, for the ERSPC1-RC and the ERSPC2-RC for positive biopsy; C, for the PCPT1-RC and the PCPT2-RC for positive biopsy; and D, for the ERSPC2-RC and the PCPT2-RC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

145x244mm (300 x 300 DPI)

2

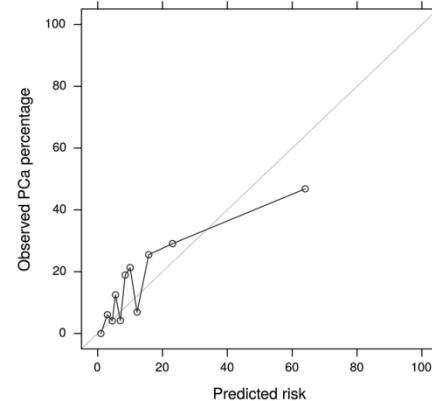
A)

High grade tumor: ERSPC1



B)

High grade tumor: PCPT1

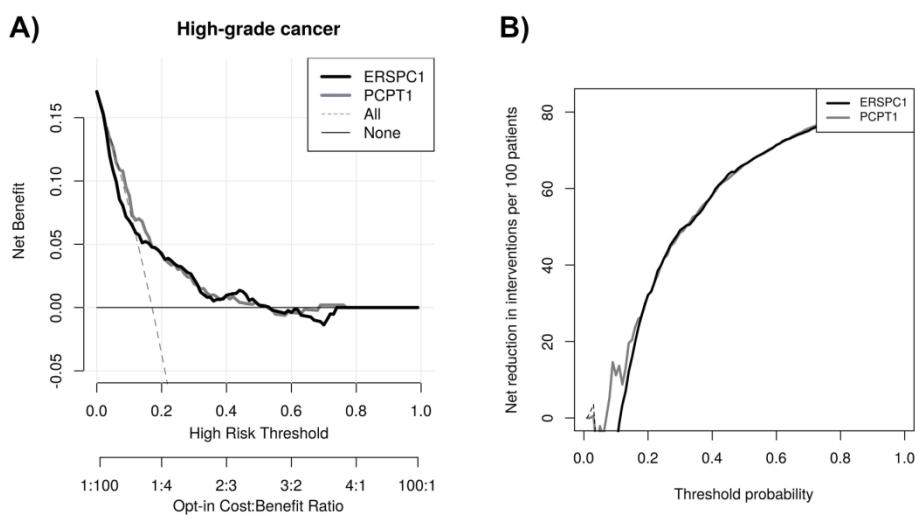


Calibration plots for risk estimation, showing the agreement between predicted risk (horizontal axis) and the actual observed prevalence for people with that risk (vertical axis). The diagonal line shows the ideal behaviour of a perfectly calibrated RC, separating the upper left region where risks are 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.

191x142mm (300 x 300 DPI)

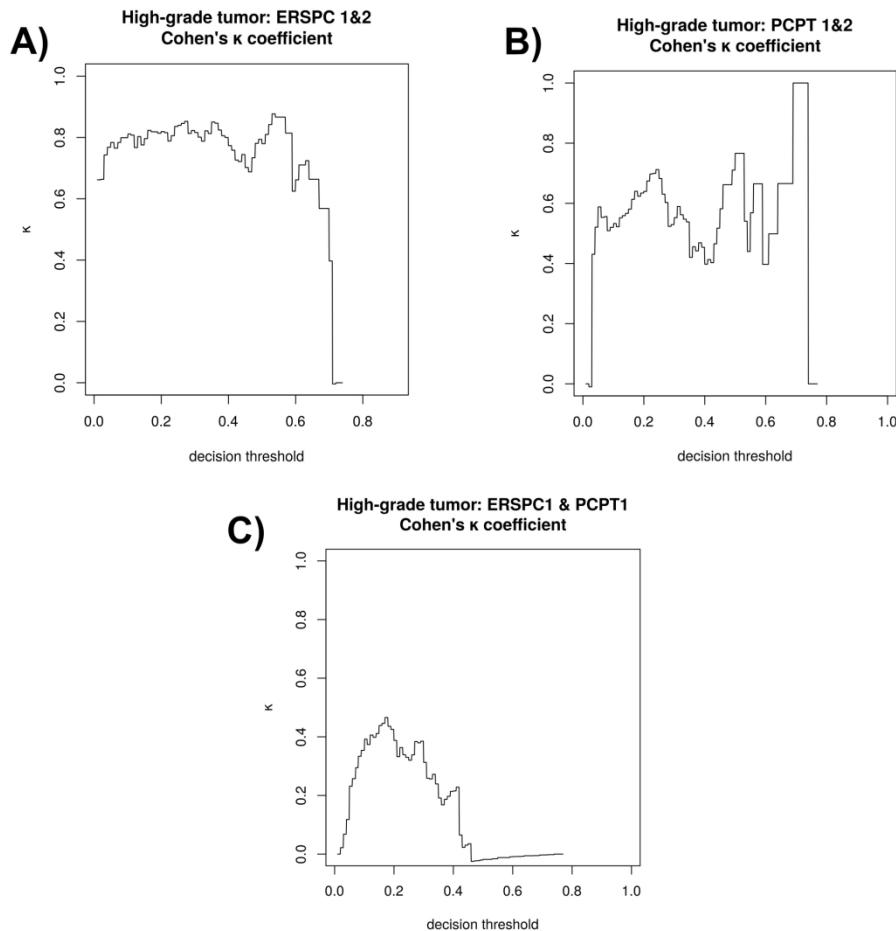
3



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).

169x126mm (300 x 300 DPI)

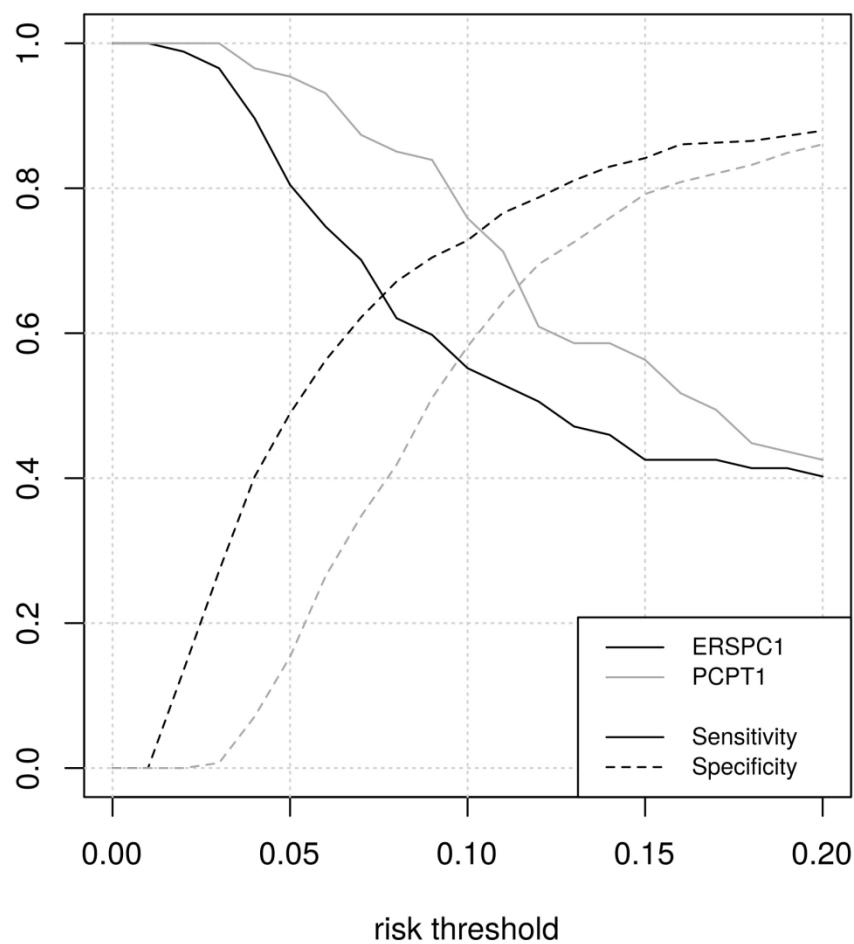
4



Graphics showing Cohen's  $\kappa$  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)

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).

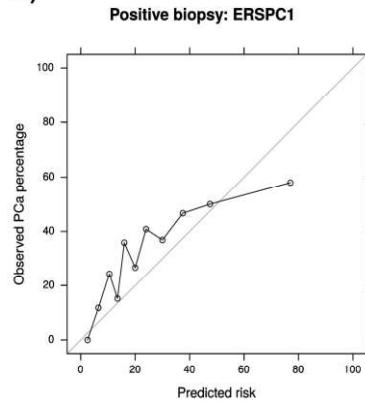
**Supplemental Table 1.** Patients excluded from the ONCOVER cohort for this study

depends on the exclusion criteria.

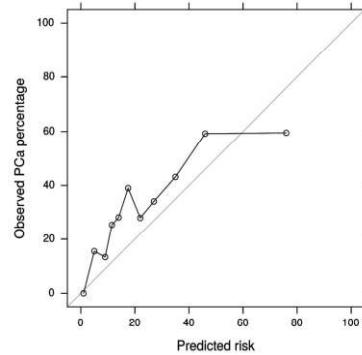
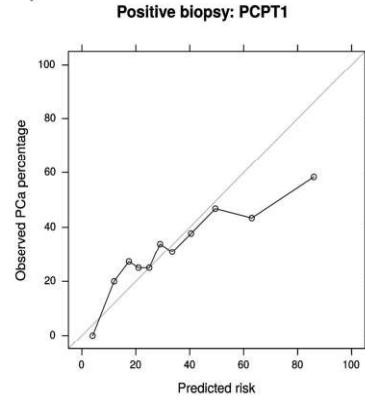
Exclusion criteria	Number
<b>Under active surveillance</b>	25
<b>2 consecutive PSA levels well recorded or affected</b>	50
<b>Prostate volume not well recorded</b>	177
<b>PSA of biopsy indication out of the range 3-10ng/ml , or Age out of the range 55-80</b>	251
<b>Total exclusions</b>	511

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6 **Supplemental table 2:** DeLong p values resulting from the pairwise comparison of the  
7 Area under the Receiver Operator Characteristic (ROC) curve (AUC) between Risk  
8 Calculators for significant Prostate cancer (Sig PCa) detection.  
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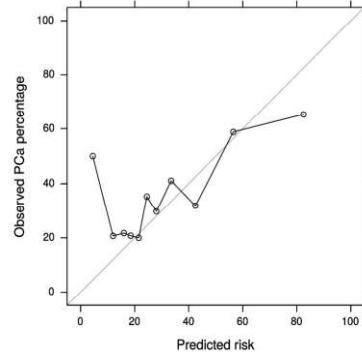
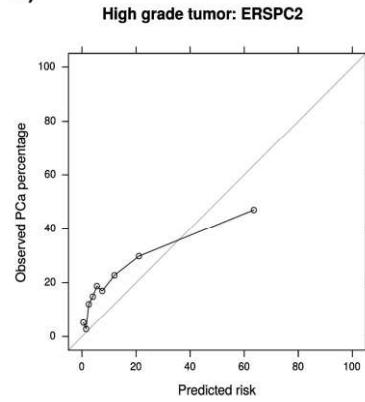
Sig PCa (p-value)	ERSPC1	ERSPC2	PCPT1	PCPT2
ERSPC1	X	0.51	0.95	0.19
ERSPC2	X	X	0.74	0.25
PCPT1	X	X	X	0.06

**S1****A)**

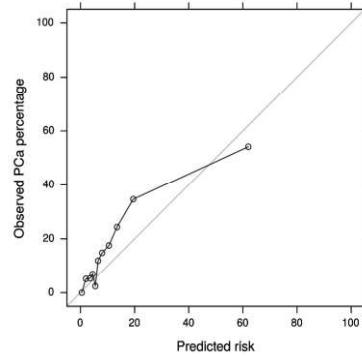
Positive biopsy: ERSPC2

**B)**

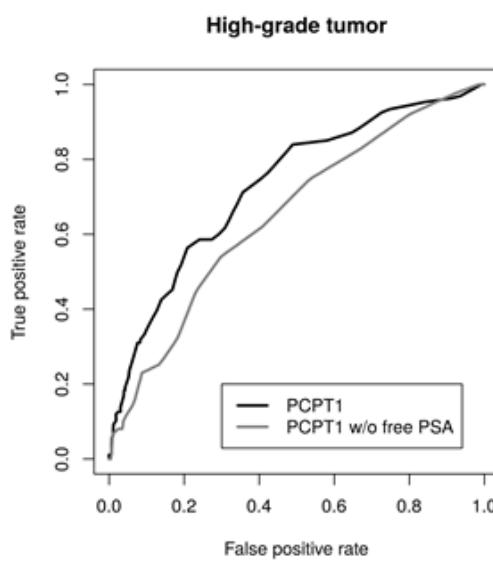
Positive biopsy: PCPT2

**C)**

High grade tumor: PCPT2



**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.

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Risk Calculator for Sig PCa	AUC (CI <sub>95%</sub> )
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.

N♦	ID;age;psa1;free.psa1;psa2;free.psa2;prior.biopsy;abnormal.dre;family.history;vol;vol.dre;pb;hg;ers
1	1;59;3 7;9;3 64;9 3;FALSE;F.31;0 08;0 3;0 08;0 36;0
2	2;77;9 8;10;8 18;12 3;FALSE;T 84;0 74;0 8;0 67;0 25;0
3	3;56;5 9;10;4 76;10 5;TRUE;FA 9;0;FALSE;24;0 05;0 23;0 04;0
4	4;58;3 2;6 5;3 36;8 3;FALSE;T 49;0 3;0 51;0 31;0
5	5;65;6 3;17;4 25;21;FALSE;26;0 07;0 17;0 04;0 72;0 2;0
6	6;73;4 6;8 8;0 45;NA;FALSE;38;0 11;0 02;0;0 2;0 5;0
7	7;62;6;11;4 32;8 1;FALSE;F.48;0 17;0 36;0 1;0 47;0 38;0
8	8;67;3 4;14;3 23;15 1;FALSE;F.28;0 07;0 27;0 06;0 63;0
9	9;67;8 43;14;7 59;31 2;TRUE;FA 25;0 07;0 25;0 06;0 69;0
10	10;59;4 3;14;4 68;17 5;FALSE;F.6;1;FALSE;17;0 04;0 19;0 04;0
11	11;80;3 5;22;3 25;18 1;FALSE;T 53;0 33;0 5;0 3;0 72;0
12	12;61;5 8;27 4;5 32;30 6;FALSE;F.24;0 06;0 22;0 05;0
13	13;63;6 59;16 5;7 56;17 9;TRUE;FA 08;0 01;0 08;0 01;0
14	14;72;3 95;12 9;3 44;17 7;FALSE;T 57;0 37;0 52;0 32;0
15	15;60;6;11 9;6 9;15;FALSE;12;0 03;0 14;0 04;0 54;0 34;0
16	16;67;7 49;14 6;6 81;14 6;TRUE;FA 08;0 01;0 08;0 01;0
17	17;63;5 9;12 6;7 76;11 2;FALSE;F.9;1;FALSE;24;0 06;0 33;0
18	18;69;6 1;41 3;3 57;31 3;FALSE;F.12;0 03;0 06;0 01;0
19	19;70;4 7;13 1;4 77;18 4;FALSE;F.19;0 04;0 19;0 05;0
20	20;72;9 84;20 4;8 15;22 5;TRUE;FA 09;0 01;0 08;0 01;0
21	21;65;6 08;11 8;5 76;11 6;FALSE;F.25;0 07;0 24;0 06;0
22	22;67;5;7;432;9;FALSE;65;0 47;0 6;0 41;0 12;0 45;0 43;0
23	23;80;6 3;7 7;4 69;10 6;TRUE;FA 6;0;TRUE;124;0 05;0 23;0
24	24;64;5 6;23 7;5 45;26 6;TRUE;FA 24;0 05;0 23;0 05;0
25	25;57;9;17 4;3 62;30 1;FALSE;F.5;2;FALSE;2;0 05;0 06;0 01;0
26	26;76;7 54;38 3;7 68;37 6;FALSE;F.2;2;FALSE;16;0 04;0 16;0
27	27;70;4 61;16;3 86;17 3;FALSE;F.3;2;FALSE;08;0 02;0 07;0 01;0
28	28;66;3 7;14 5;3 48;16;TRUE;22;0 03;0 22;0 03;0 68;0
29	29;70;8 6;12 2;6 68;13 7;FALSE;F.61;0 27;0 52;0 19;0
30	30;67;6 57;5 6;8 22;5 7;FALSE;T 5;0 34;0 59;0 43;0
31	31;58;5 3;17 3;4 41;14 9;FALSE;F.43;0 14;0 37;0 11;0
32	32;73;7 8;20 7;11 16;NA;FALSE;35;0 22;0 48;0 34;0 73;0
33	33;64;6 6;16;5 36;17 7;FALSE;F.14;0 03;0 1;0 02;0 7;0
34	34;76;5 43;11 2;5 98;7 3;FALSE;F.44;0 14;0 48;0 17;0
35	35;60;4 29;18 1;4 8;17 2;FALSE;F.08;0 02;0 09;0 02;0
36	36;75;7 16;12 5;8 44;12 5;FALSE;F.54;0 21;0 6;0 26;0
37	37;55;6 56;19 9;6 35;18 8;TRUE;FA 14;0 02;0 13;0 02;0
38	38;61;7;7;649;5 8;FALSE;F.53;0 21;0 51;0 19;0 14;0 55;0
39	39;71;3 5;16 1;3 73;15 8;TRUE;FA 22;0 03;0 22;0 03;0
40	40;66;8 4;14 6;6 58;13 3;TRUE;FA 08;0 01;0 08;0 01;0
41	41;76;7 5;21 4;4 45;33 4;FALSE;F.31;0 09;0 17;0 04;0
42	42;58;4 3;7 7;2 62;NA;FALSE;36;0 1;0 21;0 05;0 23;0
43	43;76;7 8;11 1;6 93;14 4;FALSE;F.33;0 1;0 29;0 08;0
44	44;69;6 67;48 1;4 22;36 4;FALSE;F.14;0 03;0 08;0 02;0
45	45;69;4 18;35;2 7;NA;FALSE;35;0 1;0 22;0 05;0 85;0 11;0
46	46;67;7 27;9 4;6 27;12 1;FALSE;T 77;0 63;0 72;0 57;0
47	47;64;9 18;9 2;9 57;8 9;TRUE;FA 14;0 03;0 15;0 03;0
48	48;65;6 11;23;7 06;32 1;FALSE;F.6;1;FALSE;25;0 07;0 3;0 09;0
49	49;67;4 28;26 4;3 75;24 8;FALSE;F.2;1;FALSE;17;0 04;0 14;0
50	50;69;5 08;14;4 28;18 2;FALSE;T 2;0;FALSE;66;0 48;0 6;0 41;0
51	51;63;7 3;24 7;5 27;30 3;TRUE;FA 08;0 01;0 08;0 01;0
52	52;55;6 75;16 7;6 17;19 1;FALSE;T 31;0 18;0 28;0 16;0
53	53;60;4 09;23 4;4 97;21 1;FALSE;F.07;0 01;0 09;0 02;0
54	54;75;4 5;25;4 89;26 1;FALSE;T 37;0 21;0 4;0 23;0 77;0
55	55;56;5 64;14 7;6 22;11;TRUE;8;1;FALSE;13;0 02;0 13;0 02;0

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3	56;59;4	77;36	8;3	83;26	3;FALSE;F.39;0	12;0	32;0	08;0
4	57;74;5	1;24	8;4	16;31	4;TRUE;FA07;0	01;0	07;0	01;0
5	58;68;4	5;21;5	24;28	8;FALSE;F.08;0	02;0	1;0	02;0	78;0
6	59;70;5	8;11	2;6	19;15	1;FALSE;F.24;0	06;0	26;0	07;0
7	60;63;7	15;14;5	38;28	4;FALSE;F.15;0	04;0	1;0	02;0	63;0
8	61;73;5	9;15;6	64;18	2;FALSE;F.12;0	03;0	14;0	03;0	61;0
9	62;61;6	38;15	8;7	13;29	7;FALSE;F.13;0	03;0	15;0	04;0
10	63;55;3	82;11	5;2	96;NA;FAL14;0	03;0	1;0	02;0	57;0
11	64;58;5	38;6	8;9	63;10	3;TRUE;FA5;0;FALSE;23;0	04;0	26;0	
12	65;63;5	7;19;5	89;14	7;FALSE;F.23;0	06;0	24;0	06;0	77;0
13	66;69;4	35;19	7;4	14;19	3;FALSE;F.17;0	04;0	16;0	04;0
14	67;62;6	98;9	7;3	8;6;FALSE;75;0	61;0	56;0	36;0	33;0
15	68;68;3	3;33	6;5	56;29	6;FALSE;F.05;0	01;0	11;0	02;0
16	69;61;4	51;11	8;4	51;17;FALSE5;1;TRUE;118;0	04;0	18;0	04;0	
17	70;68;3	59;19	7;3	4;22;FALSE06;0	01;0	06;0	01;0	77;0
18	71;71;3	44;16;5	03;15	1;FALSE;F.13;0	03;0	2;0	05;0	68;0
19	72;63;5	09;13	6;5	75;12	6;FALSE;F.21;0	05;0	24;0	06;0
20	73;58;4	27;15	2;3	31;14	1;FALSE;F.17;0	04;0	12;0	03;0
21	74;70;8;12	2;2		78;NA;FAL7;1;FALSE;34;0	1;0	1;0	02;0	49;0
22	75;64;7	65;13;7	38;14	4;TRUE;FA2;2;TRUE;108;0	01;0	08;0	01;0	
23	76;65;4	9;31	1;4	45;24	7;FALSE;F.09;0	02;0	08;0	02;0
24	77;61;6;16	6;6	79;18	2;FALSE;F.25;0	07;0	28;0	08;0	73;0
25	78;61;5;23	1;5	69;35	6;FALSE;F.2;0	05;0	23;0	06;0	83;0
26	79;63;5	08;28	9;4	13;24	2;TRUE;FA13;0	02;0	12;0	01;0
27	80;69;8;35	3;6	65;28	8;TRUE;FA14;0	02;0	14;0	02;0	9;0
28	81;59;4	15;11	8;4	8;11	8;FALSE;F.35;0	1;0	4;0	12;0
29	82;65;4	78;24	4;4	73;28	3;FALSE;F.09;0	02;0	09;0	02;0
30	83;56;3	06;29	7;3	06;32	3;FALSE;T25;0	12;0	25;0	12;0
31	84;59;5	57;8	7;5	21;8	8;FALSE;F.8;0;TRUE;F45;0	15;0	43;0	
32	85;74;5	45;17;5	42;19	5;FALSE;F.7;0;TRUE;144;0	14;0	44;0	14;0	
33	86;68;5	39;20;5	11;27	7;TRUE;TR23;0	06;0	23;0	06;0	77;0
34	87;59;8	28;16	8;10	16;NA;FAL35;0	11;0	42;0	15;0	75;0
35	88;66;4	88;17;7	69;21;FALSE09;0	02;0	17;0	04;0	72;0	21;0
36	89;66;9;9	2;9	4;8	7;FALSE;T82;0	71;0	83;0	72;0	26;0
37	90;58;3	32;13	6;1	39;NA;FAL28;0	07;0	1;0	02;0	67;0
38	91;63;4	13;10	7;0	68;NA;FAL3;0;FALSE;34;0	1;0	04;0;0	46;0	
39	92;70;3	5;11;3	43;11	9;TRUE;FA22;0	03;0	22;0	03;0	48;0
40	93;63;7	34;6	5;8	76;7	8;FALSE;T77;0	63;0	81;0	7;0
41	94;62;9	6;7	7;7	89;10	3;FALSE;F.65;0	31;0	58;0	24;0
42	95;78;5	2;22;5	23;19	6;FALSE;F.21;0	05;0	21;0	05;0	75;0
43	96;68;5	31;17	3;5	92;16;FALSE43;0	14;0	47;0	16;0	71;0
44	97;61;3	22;33	5;3	33;27	9;FALSE;F.12;0	02;0	12;0	03;0
45	98;72;4	8;9	2;5	04;7	9;FALSE;F.4;0	12;0	41;0	13;0
46	99;77;4	49;18	7;4	1;25	6;FALSE;T62;0	43;0	58;0	39;0
47	100;64;7;18	83;13		4;TRUE;FA24;0	06;0	25;0	06;0	69;0
48	101;70;3	07;17	5;2	87;NA;FAL11;0	02;0	1;0	02;0	72;0
49	102;75;7	1;19;6	45;24	1;TRUE;FA08;0	01;0	08;0	01;0	74;0
50	103;67;9	6;29	4;8	34;30	2;TRUE;FA08;0	01;0	08;0	01;0
51	104;57;9	45;22	8;9	75;25	7;TRUE;FA15;0	03;0	15;0	03;0
52	105;67;4	28;7;3	78;8	2;FALSE;F.36;0	1;0	32;0	08;0	14;0
53	106;55;4	75;27	5;6	4;17	8;FALSE;F.39;0	12;0	5;0	18;0
54	107;68;5;178;2		1;NA;FALS2;0	05;0	07;0	01;0	72;0	2;0
55	108;64;7	2;18	5;9	38;17	5;TRUE;FA25;0	06;0	26;0	07;0
56	109;72;8	86;13	1;9	28;13	5;FALSE;T81;0	7;0	82;0	72;0
57	110;65;5	8;15	4;5	74;16	3;FALSE;F.24;0	06;0	24;0	06;0
58	111;68;6	14;14;5	57;16	5;FALSE;F.25;0	07;0	23;0	06;0	6;0

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3	112;71;8	7;15;7	04;16	3;TRUE;FA 08;0	01;0	08;0	01;0	7;0
4	113;59;3	93;19	3;3	98;17	5;FALSE;F.6;1;FALSE;15;0	03;0	15;0	
5	114;72;4	2;6	3;4	82;7	6;FALSE;F.3;0;TRUE;135;0	1;0	4;0	
6	115;68;9	9;18	5;7	05;14	8;FALSE;F.3;1;FALSE;41;0	14;0	29;0	
7	116;63;4	5;19	4;1	13;NA;FAL 18;0	04;0	03;0;0	78;0	17;0
8	117;76;7	6;13	4;5	27;19	9;FALSE;T 34;0	21;0	23;0	12;0
9	118;59;3	49;20	3;3	46;17;FALSE 13;0	03;0	13;0	03;0	81;0
10	119;67;5	3;33;2	89;NA;FAL 1;0	02;0	04;0	01;0	86;0	1;0
11	120;64;5	6;50	7;5	55;21	9;TRUE;FA 24;0	05;0	23;0	05;0
12	121;68;7	55;26	1;6	25;12	08;FALSE;116;0	04;0	13;0	03;0
13	122;63;9	75;10	8;1	19;NA;TRU 26;0	07;0	18;0	01;0	53;0
14	123;69;6	3;16	1;7	39;18	6;TRUE;TF3;2;FALSE;14;0	03;0	15;0	
15	124;57;4	5;12	2;4	75;15	1;FALSE;F.37;0	11;0	39;0	12;0
16	125;74;4	6;19	8;1	02;NA;FAL 18;0	04;0	02;0;0	73;0	19;0
17	126;56;3	9;18	5;2	67;NA;FAL 33;0	09;0	22;0	05;0	79;0
18	127;66;5	68;32	8;2	08;NA;FAL 3;0;FALSE;46;0	15;0	16;0	03;0	
19	128;78;8;125;7	46;13	5;FALSE;T 9;0;TRUE;179;0	67;0	77;0	64;0		
20	129;60;3	6;10	6;3	83;10	7;FALSE;F.3;0	08;0	32;0	08;0
21	130;58;8	58;13	3;7	47;18;TRUI 08;0	01;0	08;0	01;0	71;0
22	131;58;4	93;9	5;4	6;9	1;FALSE;F.41;0	12;0	38;0	11;0
23	132;67;3	1;12	9;3	57;13	7;FALSE;F.1;1;FALSE;11;0	02;0	13;0	
24	133;68;7	7;8	5;7	96;10	1;FALSE;F.8;0;TRUE;157;0	23;0	58;0	
25	134;59;4	84;21;4	55;25	4;FALSE;F.09;0	02;0	08;0	02;0	77;0
26	135;60;6	43;11	5;6	91;10	7;FALSE;F.27;0	07;0	29;0	08;0
27	136;65;9	38;2	3;2	86;NA;FAL 64;0	3;0	23;0	05;0;0	31;0
28	137;76;6	2;6	8;6	77;6	6;FALSE;F.49;0	17;0	52;0	2;0
29	138;58;7	34;9	5;7	1;12	5;TRUE;FA 6;0;TRUE;F 25;0	06;0	24;0	
30	139;64;3	57;22	9;3	29;27;FALSE 15;0	07;0	13;0	06;0	81;0
31	140;61;8;122;7	68;11	7;FALSE;F.17;0	05;0	16;0	04;0	55;0	
32	141;63;4	39;15;4	33;18	9;FALSE;F.17;0	04;0	17;0	04;0	68;0
33	142;64;5	1;25;4	17;20	8;FALSE;F.1;0	02;0	07;0	02;0	83;0
34	143;70;5	6;12	3;1	96;NA;TRU 23;0	06;0	19;0	02;0	56;0
35	144;58;3	89;20	8;3	21;20	8;FALSE;F.32;0	09;0	27;0	06;0
36	145;70;7;5	6;2	69;NA;FAL 53;0	36;0	21;0	1;0	04;0	41;0
37	146;67;4	02;24	3;3	49;23	2;FALSE;F.15;0	03;0	13;0	03;0
38	147;80;7	4;20	4;11	6;NA;FALS 34;0	2;0	49;0	35;0	68;0
39	148;74;4	82;17	6;3	62;22	9;TRUE;FA 7;1;FALSE;13;0	01;0	12;0	
40	149;65;5	65;16	9;4	64;18	9;FALSE;F.23;0	06;0	18;0	04;0
41	150;72;6	6;17;6	28;19	9;FALSE;F.28;0	08;0	26;0	07;0	68;0
42	151;66;4	2;17	6;4	57;23	4;FALSE;F.16;0	04;0	18;0	04;0
43	152;55;3	27;11	6;3	22;14	2;FALSE;F.12;0	02;0	12;0	02;0
44	153;73;7	2;16	9;7	15;13	7;FALSE;F.3;0	09;0	3;0	09;0
45	154;57;4	3;16	9;4	9;11;FALSE 17;0	04;0	2;0	05;0	76;0
46	155;59;6	7;5	2;5	35;5	9;FALSE;F.28;0	08;0	22;0	05;0
47	156;65;7;1	84;12	7;FALSE;F.53;0	21;0	58;0	24;0	44;0	38;0
48	157;55;3	5;11	8;3	07;9	1;FALSE;F.29;0	07;0	25;0	06;0
49	158;65;9;6	7;9	35;8	5;FALSE;F.2;0	05;0	21;0	06;0	1;0
50	159;55;8	9;12;6	88;15	5;TRUE;FA 14;0	03;0	14;0	02;0	6;0
51	160;61;7	9;13	7;7	19;15	1;FALSE;T 6;1;TRUE;F 57;0	41;0	54;0	
52	161;68;5	4;4	7;2	24;NA;FAL 1;0	02;0	03;0	01;0	02;0
53	162;59;7	69;14	1;5	7;16	4;FALSE;F.17;0	04;0	11;0	03;0
54	163;60;4	5;11;4	64;15	9;FALSE;F.37;0	11;0	38;0	11;0	49;0
55	164;60;5	5;14	7;5	76;15	1;FALSE;T 44;0	27;0	46;0	29;0
56	165;57;4	7;30;5	34;26	2;FALSE;F.09;0	02;0	1;0	02;0	87;0
57	166;66;3	4;14	7;3	38;16	5;FALSE;F.12;0	03;0	12;0	03;0
58	167;70;4;186;2	59;NA;FAL 07;0	01;0	04;0	01;0	74;0	2;0	

1								
2								
3	168;73;4	56;21	7;5	29;20;FALSE;18;0	04;0	21;0	05;0	72;0
4	169;64;6	05;11	5;7	85;11	4;FALSE;T 47;0	31;0	57;0	41;0
5	170;62;4	78;13	3;4	6;15	4;TRUE;FA 13;0	01;0	13;0	01;0
6	171;75;9	2;9	6;8	04;11	4;FALSE;T 63;0	47;0	58;0	42;0
7	172;59;6	44;11	8;6	34;14	8;FALSE;T 2;1;TRUE;F 5;0	33;0	49;0	
8	173;70;9	78;4;8	95;4	3;FALSE;F 7;0;TRUE;T 165;0	31;0	62;0	28;0	
9	174;72;3	4;17;3	36;16;FALSE;06;0	01;0	05;0	01;0	7;0	22;0
10	175;70;4	3;24	4;4	78;29	9;FALSE;F 8;1;FALSE;T 17;0	04;0	19;0	
11	176;68;5;173;7	08;16	1;FALSE;T 22;0	12;0	32;0	19;0	69;0	
12	177;55;4	06;28	8;5	76;29	1;FALSE;F 5;0;FALSE;34;0	09;0	46;0	
13	178;58;5	29;20	9;5	82;15	8;FALSE;F 1;0	02;0	12;0	03;0
14	179;60;5	82;12	7;6	18;15	3;TRUE;FA 13;0	02;0	13;0	02;0
15	180;62;4	01;13	7;3	92;23	4;FALSE;F 15;0	03;0	15;0	03;0
16	181;70;6	28;23	2;6	51;19	9;FALSE;T 49;0	32;0	5;0	33;0
17	182;60;4	7;16	2;5	12;19	1;FALSE;F 19;0	04;0	21;0	05;0
18	183;66;7	81;18	1;12	69;NA;TRUE;08;0	01;0	09;0	02;0	77;0
19	184;68;3	7;9;2	56;NA;FALSE;55;0	35;0	41;0	22;0	28;0	41;0
20	185;68;3	36;16	9;2	95;NA;FALSE;28;0	07;0	24;0	06;0	72;0
21	186;64;3	58;9	1;3	65;18;FALSE;13;0	03;0	14;0	03;0	34;0
22	187;66;8	6;13	3;9	22;14	2;FALSE;F 36;0	12;0	38;0	13;0
23	188;56;3	7;5	4;2	08;NA;TRUE;22;0	03;0	2;0	02;0	1;0
24	189;59;5	8;15;6	73;13	2;TRUE;FA 13;0	02;0	14;0	02;0	77;0
25	190;66;7	83;13	9;8	4;17;TRUE;14;0	02;0	14;0	02;0	69;0
26	191;56;5	63;10	3;4	32;10	1;FALSE;F 45;0	15;0	36;0	1;0
27	192;58;3	7;12;2	4;NA;FALSE;31;0	08;0	19;0	04;0	58;0	32;0
28	193;70;5	8;19	7;5	3;22	4;FALSE;F 12;0	03;0	1;0	02;0
29	194;72;7	4;29;6	99;39;TRUE;15;0	03;0	15;0	03;0	86;0	07;0
30	195;57;7;186;8	08;18	3;TRUE;FA 08;0	01;0	08;0	01;0	81;0	
31	196;65;6	36;20	4;6	14;16	2;FALSE;T 49;0	32;0	48;0	31;0
32	197;62;6	17;20	7;7	18;22	2;TRUE;FA 7;0;FALSE;24;0	05;0	25;0	
33	198;65;3	79;13	1;4	18;13	1;FALSE;F 9;0;TRUE;T 132;0	08;0	35;0	
34	199;57;7	7;10;2	75;NA;FALSE;56;0	4;0	22;0	1;0	39;0	38;0
35	200;70;4	4;15	4;4	02;20	3;FALSE;F 17;0	04;0	15;0	03;0
36	201;70;5	97;21	8;6	25;24	8;TRUE;FA 08;0	01;0	08;0	01;0
37	202;59;3	8;9;2	85;NA;FALSE;14;0	03;0	1;0	02;0	36;0	47;0
38	203;62;5	07;11	1;3	51;13	6;FALSE;F 42;0	13;0	29;0	07;0
39	204;67;5;276;3	91;20	4;TRUE;FA 07;0	01;0	07;0	01;0	88;0	
40	205;65;3	22;16	1;3	72;16	1;FALSE;F 27;0	06;0	31;0	08;0
41	206;70;5	7;21;6	36;20	7;FALSE;F 23;0	06;0	26;0	07;0	77;0
42	207;59;5	6;14	2;6	95;9	3;FALSE;F 11;0	03;0	15;0	04;0
43	208;56;5	26;18;4	81;18	5;FALSE;F 43;0	14;0	4;0	12;0	78;0
44	209;62;3	9;16	2;2	91;NA;FALSE;15;0	03;0	1;0	02;0	72;0
45	210;55;4	59;24	4;5	13;26	1;FALSE;T 37;0	22;0	41;0	25;0
46	211;61;3	5;15	3;3	61;21;FALSE;29;0	15;0	3;0	15;0	69;0
47	212;61;5;18;5	4;12	9;FALSE;F 09;0	02;0	1;0	02;0	64;0	
48	213;65;4	35;14	9;3	5;17	1;FALSE;T 19;0	09;0	14;0	07;0
49	214;68;4	8;19	9;6	01;18	4;TRUE;FA 07;0	01;0	08;0	01;0
50	215;74;4	8;12	6;4	61;12	5;FALSE;F 4;0	12;0	38;0	11;0
51	216;65;7	35;21	7;8	12;20	9;TRUE;FA 14;0	02;0	14;0	02;0
52	217;66;7	47;14	6;6	64;15	3;FALSE;F 56;0	22;0	51;0	19;0
53	218;69;8	6;22;10	29;NA;FALSE;39;0	25;0	45;0	31;0	77;0	12;0
54	219;75;7;16;5	41;8	3;FALSE;F 53;0	21;0	44;0	14;0	42;0	
55	220;56;6	05;16;6	55;12	8;FALSE;F 2;1;FALSE;25;0	07;0	27;0	08;0	
56	221;73;4	2;33;3	38;42;FALSE;18;0	09;0	14;0	06;0	78;0	14;0
57	222;62;4	6;24	7;3	81;28	3;FALSE;F 5;2;FALSE;08;0	02;0	07;0	
58	223;68;5;172;6	23;21	1;FALSE;F 4;2;TRUE;F 09;0	02;0	13;0	03;0		

1							
2							
3	224;68;7	4;10	2;2	89;NA;FAL;3;2;FALSE;16;0	04;0	04;0	01;0
4	225;70;4	12;15;3	46;16	07;FALSE;I2;1;FALSE;16;0	04;0	13;0	03;0
5	226;59;8	7;10;13	15;NA;TRU4;1;FALSE;14;0	03;0	15;0	04;0	49;0
6	227;74;6	19;36;2	78;NA;FAL;3;2;TRUE;F13;0	03;0	04;0	01;0	81;0
7	228;73;5	3;9	33;5	62;8	8;FALSE;F6;0;TRUE;143;0	14;0	45;0
8	229;65;9	7;16;5	61;15	5;TRUE;FA09;0	01;0	08;0	01;0
9	230;67;4	3;36	6;7	07;27	8;FALSE;F08;0	02;0	15;0
10	231;57;5	01;24	7;5	63;25	9;FALSE;F1;0	02;0	11;0
11	232;68;4	3;14	4;4	5;11	7;FALSE;F36;0	1;0	37;0
12	233;63;3	21;8	8;1	01;NA;FAL;27;0	06;0	06;0	01;0
13	234;56;5	1;11	3;4	88;10	2;FALSE;F21;0	05;0	2;0
14	235;60;5	22;14	7;5	45;13	5;FALSE;F21;0	05;0	22;0
15	236;55;8	12;8	8;4	04;11	8;FALSE;F59;0	25;0	34;0
16	237;63;6	9;11	3;6	64;9	3;FALSE;F53;0	2;0	51;0
17	238;69;6	9;8;6	31;9;FALSE;53;0	2;0	5;0	18;0	18;0
18	239;65;9	3;13	1;10	73;NA;TRU8;1;TRUE;F14;0	03;0	15;0	03;0
19	240;55;3	33;9	3;3	02;10	9;FALSE;F28;0	07;0	25;0
20	241;65;6	41;14	6;5	66;16	2;FALSE;F27;0	07;0	23;0
21	242;61;6	7;14;5	13;17	1;FALSE;F3;1;FALSE;28;0	08;0	21;0	05;0
22	243;66;4	73;12;5	54;19	1;FALSE;F09;0	02;0	11;0	02;0
23	244;65;5	2;16	2;4	7;20;FALSE;1;0	02;0	09;0	02;0
24	245;67;5	4;17	7;5	12;22	4;FALSE;F22;0	06;0	21;0
25	246;66;5	3;14	6;3	54;18	3;FALSE;F22;0	05;0	13;0
26	247;64;6	1;18	9;4	8;18	1;FALSE;F25;0	07;0	19;0
27	248;72;8	26;20	9;8;21	2;FALSE;F35;0	11;0	34;0	1;0
28	249;74;6	72;20;6	85;20	7;FALSE;T51;0	34;0	52;0	35;0
29	250;73;6	9;6;6	85;15	1;FALSE;F53;0	2;0	53;0	2;0
30	251;79;4	5;29;7	49;27	3;TRUE;TR22;0	05;0	24;0	08;0
31	252;65;8;2;98;22		3;FALSE;F3;1;TRUE;F34;0	1;0	29;0	08;0	79;0
32	253;55;7	1;12	7;3	49;20;FALSE;54;0	21;0	29;0	07;0
33	254;75;6	7;14	8;6	57;14	3;FALSE;T74;0	6;0	74;0
34	255;65;7	2;9	2;6	17;9	2;TRUE;FA14;0	02;0	13;0
35	256;58;3	94;20	9;3	97;20	4;FALSE;F33;0	09;0	33;0
36	257;65;6	2;20	8;4	6;20	2;FALSE;F13;0	03;0	08;0
37	258;66;3	4;10	7;3	04;10	8;FALSE;T52;0	32;0	47;0
38	259;66;4	48;7	1;3	31;7	8;FALSE;T61;0	43;0	51;0
39	260;60;3	67;12	2;4	48;14	2;FALSE;F31;0	08;0	37;0
40	261;61;4	11;11	5;5	26;12	1;FALSE;F07;0	02;0	1;0
41	262;67;7	6;18	2;6	11;14	4;FALSE;F32;0	1;0	25;0
42	263;62;9	23;11	3;4	67;14	3;FALSE;F38;0	13;0	19;0
43	264;60;7	5;12;2	38;NA;FAL;55;0	39;0	18;0	08;0	45;0
44	265;60;8	6;16	7;12	97;NA;TRU08;0	01;0	09;0	02;0
45	266;66;6	81;12;3	42;31	2;FALSE;F28;0	08;0	13;0	03;0
46	267;70;8	9;16	4;5	81;21	3;TRUE;FA7;2;FALSE;08;0	01;0	08;0
47	268;61;6	3;28	7;6	34;36	7;FALSE;F13;0	03;0	13;0
48	269;66;5	22;13;5	17;11	6;FALSE;T42;0	25;0	42;0	25;0
49	270;57;6	7;16;8	03;17	3;FALSE;F6;1;FALSE;28;0	08;0	34;0	1;0
50	271;67;7	6;16;9	99;13	7;TRUE;FA08;0	01;0	09;0	01;0
51	272;56;4	7;16	2;3	48;18	6;TRUE;FA13;0	01;0	12;0
52	273;62;3	95;22	8;3	59;23	3;FALSE;F1;2;FALSE;07;0	01;0	06;0
53	274;62;4	53;14;4	89;17	7;FALSE;F6;1;FALSE;18;0	04;0	2;0	05;0
54	275;62;8	8;8	6;8	57;10	8;TRUE;FA14;0	03;0	14;0
55	276;61;6;2;7;2		31;NA;FAL;25;0	07;0	07;0	01;0	86;0
56	277;69;4	6;10;4	67;10	4;FALSE;T62;0	44;0	63;0	44;0
57	278;70;4;3;5;42		2;FALSE;F07;0	01;0	08;0	02;0	81;0
58	279;70;8	3;10	2;7	41;9	4;FALSE;F35;0	11;0	31;0
59							09;0

1								
2								
3	280;75;6	8;11	6;5	44;12	6;FALSE;F.52;0	2;0	44;0	14;0
4	281;60;6	63;7	3;6	18;7	7;FALSE;F.51;0	19;0	49;0	17;0
5	282;66;3	6;31;1	82;NA;FAL	3;0	08;0	14;0	03;0	85;0
6	283;67;5	2;16	1;4	89;22	6;FALSE;F.42;0	13;0	4;0	12;0
7	284;60;6	4;8	8;6	52;9	9;FALSE;F.5;0	18;0	51;0	19;0
8	285;70;3	59;18	6;2	59;NA;FAL	29;0	15;0	2;0	09;0
9	286;61;6	3;19	1;6	2;13;FALSE	26;0	07;0	26;0	07;0
10	287;67;6	9;10;20	9;NA;TRUE	3;1;FALSE	14;0	02;0	17;0	06;0
11	288;61;5	5;19	1;19	6;NA;FALS	5;0;TRUE	F.44;0	15;0	84;0
12	289;55;5	11;15	4;4	4;17	7;TRUE;FA	23;0	04;0	23;0
13	290;69;4	8;18;4	76;22;FALS	64;0	45;0	64;0	45;0	7;0
14	291;55;3	9;11	2;5	12;12	1;TRUE;FA	12;0	01;0	13;0
15	292;64;6;6	6;2	34;NA;TRU	24;0	05;0	2;0	02;0	15;0
16	293;66;8	8;10	5;8	64;9	7;FALSE;F.37;0	12;0	36;0	12;0
17	294;72;3	78;23	3;6	19;36	5;FALSE;F.06;0	01;0	13;0	03;0
18	295;74;7	5;15	5;6	11;18	3;TRUE;FA	08;0	01;0	08;0
19	296;56;3	2;14	6;3	42;13	7;FALSE;F.26;0	06;0	28;0	07;0
20	297;70;4;479;4		14;52	8;FALSE;F.15;0	03;0	16;0	04;0	85;0
21	298;65;4	88;19	5;4	43;23	9;FALSE;T	1;1;TRUE;F.4;0	23;0	36;0
22	299;74;7	85;28	3;3	18;28	6;FALSE;F.33;0	1;0	11;0	02;0
23	300;57;3	3;17	3;4	3;13;FALSE	05;0	01;0	08;0	02;0
24	301;61;3	82;19;4	66;16	3;FALSE;F.14;0	03;0	18;0	04;0	78;0
25	302;60;5	9;12	4;4	47;8	2;FALSE;F.47;0	16;0	37;0	11;0
26	303;58;4	89;32	9;4	39;25;TRUI	13;0	02;0	13;0	01;0
27	304;63;8	74;9;9	47;8	1;TRUE;FA	14;0	03;0	15;0	03;0
28	305;62;3	66;15;3	32;14	7;FALSE;F.31;0	08;0	28;0	07;0	69;0
29	306;70;4	2;29;5	21;30	9;FALSE;T	59;0	4;0	67;0	49;0
30	307;75;9	54;24	5;8	02;28	6;FALSE;F.21;0	06;0	17;0	05;0
31	308;69;8	34;19;8	4;16	5;FALSE;F.6;0	26;0	6;0	26;0	75;0
32	309;56;8	3;5	8;3	54;15	2;FALSE;F.35;0	11;0	13;0	03;0
33	310;56;5	2;16	2;5	29;17	9;FALSE;F.5;1;FALSE	21;0	05;0	21;0
34	311;63;3	9;13;4	25;16	7;FALSE;F.33;0	09;0	35;0	1;0	6;0
35	312;70;3	82;33;4	12;26	4;TRUE;FA	12;0	01;0	12;0	01;0
36	313;56;3	2;5	2;3	72;5	3;FALSE;F.26;0	06;0	31;0	08;0
37	314;61;7	19;45;4	09;45	2;FALSE;F.15;0	04;0	07;0	01;0	89;0
38	315;61;4;1829;18		1;FALSE;F.15;0	03;0	26;0	07;0	71;0	23;0
39	316;61;5	03;28	1;3	4;25	5;FALSE;F.2;0	05;0	12;0	03;0
40	317;65;4	51;27;4	1;24	3;FALSE;F.18;0	04;0	16;0	04;0	84;0
41	318;66;3	45;22	3;3	33;27;FALSE	13;0	03;0	12;0	03;0
42	319;74;7	9;7	3;3	38;23	3;FALSE;F.58;0	24;0	28;0	07;0
43	320;71;3	4;17;4	06;21	6;FALSE;F.12;0	03;0	16;0	04;0	7;0
44	321;69;5	64;18;5	58;14	5;FALSE;T	45;0	28;0	44;0	28;0
45	322;55;5;145;6		29;9	5;FALSE;F.41;0	13;0	49;0	18;0	64;0
46	323;57;5	9;26	8;7	04;24	1;FALSE;F.24;0	06;0	29;0	08;0
47	324;72;4	3;57;4	6;19	3;FALSE;T	35;0	2;0	38;0	22;0
48	325;74;7	33;27;3	45;28	9;FALSE;F.16;0	04;0	06;0	01;0	82;0
49	326;69;4	77;25;4	97;25	7;FALSE;F.39;0	12;0	41;0	13;0	81;0
50	327;71;9	46;5;2	61;NA;FAL	21;0	06;0	04;0	01;0	02;0
51	328;60;3	93;17	3;3	78;14	8;FALSE;F.33;0	09;0	32;0	08;0
52	329;58;3	48;17	5;4	01;18	7;FALSE;F.06;0	01;0	07;0	01;0
53	330;66;4;194;11		6;FALSE;F.33;0	09;0	33;0	09;0	47;0	38;0
54	331;63;7;158;6		66;16	6;TRUE;FA	4;1;FALSE	14;0	02;0	14;0
55	332;68;4	04;17	8;4	62;17	3;FALSE;F.16;0	03;0	18;0	04;0
56	333;60;3	8;17	7;2	63;NA;FAL	14;0	03;0	09;0	02;0
57	334;61;7	5;19	6;9	55;19	6;TRUE;TF	39;0	19;0	41;0
58	335;71;6	6;17	1;1	06;NA;TRU	3;0;TRUE	124;0	05;0	18;0
59								01;0
60								

1							
2							
3	336;58;7	66;7;8	57;8	5;FALSE;F.57;0	23;0	61;0	27;0
4	337;72;4;1'4;4		6;10	6;FALSE;T 57;0	38;0	62;0	44;0
5	338;59;3	1;13;4	11;10	9;FALSE;F.26;0	06;0	34;0	09;0
6	339;72;5	59;12	5;5	45;12	8;TRUE;FA 13;0	02;0	13;0
7	340;72;7	59;12;6	99;21	3;TRUE;FA 08;0	01;0	08;0	01;0
8	341;68;3	5;5	9;3	92;6	3;FALSE;F.29;0	07;0	33;0
9	342;61;6;1904;18		5;FALSE;T 3;1;TRUE;147;0		3;0	33;0	18;0
10	343;64;4	7;22	3;4	97;22	3;FALSE;T 63;0	45;0	65;0
11	344;67;4	92;20;5	22;10	3;FALSE;F.4;0	12;0	43;0	14;0
12	345;76;6	03;26	7;6	24;26	6;FALSE;F.25;0	07;0	26;0
13	346;61;6	3;15;6	26;16	7;TRUE;FA 13;0	02;0	13;0	02;0
14	347;60;6	82;15	6;2	49;NA;FAL 75;0	6;0	4;0	22;0
15	348;71;6	86;29	6;6	4;31	5;TRUE;FA 14;0	02;0	13;0
16	349;59;3	6;16;3	02;17	5;FALSE;F.3;0	08;0	25;0	06;0
17	350;76;8	7;42;10	82;NA;FAL 36;0	12;0	44;0	16;0	82;0
18	351;63;3	5;11	8;2	53;NA;TRU 22;0	03;0	21;0	02;0
19	352;76;4	4;5	8;4	39;5	4;FALSE;T 61;0	42;0	61;0
20	353;68;5;1'9;5		19;13	1;FALSE;T 65;0	47;0	66;0	49;0
21	354;65;3	3;14	8;3	74;16	3;FALSE;F.12;0	02;0	14;0
22	355;70;6	08;17;8	21;21	1;FALSE;F.25;0	07;0	34;0	11;0
23	356;62;5	3;26;3	3;24	2;FALSE;F.22;0	05;0	12;0	02;0
24	357;78;4	19;19;5	66;17	1;FALSE;T 59;0	4;0	69;0	52;0
25	358;69;5	1;13;6	71;9;FALSE;42;0	13;0	52;0	19;0	55;0
26	359;61;5	1;24;4	6;27	6;FALSE;F.21;0	05;0	18;0	04;0
27	360;67;3	9;18;4	78;17	1;TRUE;FA 12;0	01;0	13;0	01;0
28	361;60;8	9;24	8;8	5;27	5;FALSE;F.2;0	05;0	19;0
29	362;70;7	7;14	4;9	49;17	3;TRUE;FA 08;0	01;0	08;0
30	363;71;3	18;16;3	8;13	6;FALSE;F.11;0	02;0	14;0	03;0
31	364;57;5	4;10;4	66;12	6;TRUE;FA 13;0	02;0	13;0	01;0
32	365;66;9	39;17	3;6	54;18	3;FALSE;T 9;0;FALSE;83;0	72;0	74;0
33	366;70;6	9;23;7	04;13	4;TRUE;TR 24;0	07;0	24;0	07;0
34	367;57;6	5;20;10	59;NA;TRU 14;0	03;0	16;0	05;0	85;0
35	368;63;3	3;22	8;3	6;23	3;FALSE;F.05;0	01;0	06;0
36	369;60;4	3;29	4;5	35;28;FALSE;08;0	02;0	1;0	02;0
37	370;59;3	5;24;3	43;27	4;FALSE;F.06;0	01;0	06;0	01;0
38	371;70;5	8;28;5	07;28	9;FALSE;F.5;1;TRUE;124;0		06;0	2;0
39	372;57;8	88;18;9	06;17	9;FALSE;F.37;0	12;0	38;0	12;0
40	373;65;6;8	3;5	91;7	1;TRUE;FA 13;0	02;0	13;0	02;0
41	374;68;6	5;24;5	2;28	4;FALSE;F.13;0	03;0	1;0	02;0
42	375;67;7;1'13;17		1;TRUE;FA 14;0	02;0	14;0	02;0	69;0
43	376;76;4	5;17;4	08;15	4;FALSE;F.18;0	04;0	16;0	04;0
44	377;64;6	5;12;7	31;12	8;TRUE;FA 08;0	01;0	08;0	01;0
45	378;57;5	5;17;2	25;NA;FAL 22;0	06;0	07;0	01;0	76;0
46	379;60;4;2'32;22		2;FALSE;F.6;1;FALSE;15;0		03;0	12;0	03;0
47	380;68;5	05;27	3;3	71;21;FALSE;41;0	13;0	31;0	08;0
48	381;60;5;1'8;4		49;9	5;FALSE;T 65;0	47;0	62;0	43;0
49	382;68;5	22;16;4	52;20	7;FALSE;F.21;0	05;0	18;0	04;0
50	383;63;6	68;10;5	46;12	6;TRUE;FA 08;0	01;0	08;0	01;0
51	384;57;4	17;10;4	42;14	9;FALSE;T 34;0	19;0	36;0	21;0
52	385;72;3	4;3	2;3	33;3	6;FALSE;T 52;0	32;0	51;0
53	386;66;4	43;11;3	1;20	3;FALSE;F.37;0	11;0	26;0	06;0
54	387;60;3	82;18;5	07;18	7;FALSE;F.32;0	08;0	42;0	13;0
55	388;63;4	3;9;5;9	4;FALSE;F.17;0	04;0	2;0	05;0	32;0
56	389;72;5	32;14;5	66;19	2;FALSE;F.22;0	05;0	23;0	06;0
57	390;66;6	5;22	7;6	52;21	3;FALSE;F.13;0	03;0	13;0
58	391;64;5	4;17	7;5	3;19	6;FALSE;T 68;0	5;0	67;0
59							
60							

1								
2								
3	392;57;6	43;8	9;7	06;10;FALSE;5;0	18;0	54;0	21;0	27;0
4	393;70;5	71;11	4;6	61;64	2;FALSE;F.46;0	15;0	51;0	19;0
5	394;64;5	4;12;6	35;14	3;FALSE;F.44;0	14;0	5;0	18;0	52;0
6	395;58;3	2;14;3	75;14	1;FALSE;F.26;0	06;0	31;0	08;0	68;0
7	396;57;3	83;12;3	79;10	8;TRUE;FA2;0;FALSE;22;0	03;0	22;0	03;0	
8	397;68;4	1;22	9;3	26;33	7;FALSE;F.16;0	04;0	12;0	02;0
9	398;65;6;1	71;16	2;FALSE;F.1;1;TRUE;125;0	07;0	28;0	08;0	45;0	
10	399;56;7	59;13	4;9	05;20	8;TRUE;FA5;1;TRUE;F.14;0	02;0	14;0	
11	400;61;6	46;13	4;9	98;13	1;TRUE;FA6;1;TRUE;F.14;0	02;0	15;0	
12	401;57;4	15;6	8;6	31;3;FALSE;6;0;FALSE;35;0	1;0	5;0	18;0	
13	402;59;7	7;13;7	07;13	8;TRUE;FA14;0	02;0	14;0	02;0	69;0
14	403;62;4	8;27;4	82;31	7;FALSE;F.09;0	02;0	09;0	02;0	85;0
15	404;56;4	14;23	3;5	76;24	1;FALSE;F.35;0	1;0	46;0	16;0
16	405;70;4	13;17;5	05;20	7;TRUE;FA12;0	01;0	13;0	02;0	73;0
17	406;57;5	48;13	5;6	07;15	1;TRUE;FA63;1;FALSE;13;0	02;0	13;0	
18	407;57;5;2	51;NA;FALSE;97;2;FALSE;09;0		02;0	04;0	01;0	84;0	13;0
19	408;75;3	04;33;3	13;34	1;TRUE;TR69;1;FALSE;21;0	04;0	21;0	04;0	
20	409;65;4	6;30;5	7;26	4;FALSE;F.08;0	02;0	11;0	03;0	85;0
21	410;55;7	9;7	9;10	76;NA;TRU14;0	02;0	15;0	03;0	31;0
22	411;63;7	8;18	5;6	74;20	4;TRUE;FA08;0	01;0	08;0	01;0
23	412;64;6	7;17;6	1;20	3;TRUE;FA08;0	01;0	08;0	01;0	79;0
24	413;55;4;12;3	95;14	9;FALSE;F.15;0	03;0	15;0	03;0	59;0	
25	414;58;6	7;11	1;2	24;NA;FALSE;28;0	08;0	07;0	01;0	49;0
26	415;61;4	5;14	6;4	45;13	2;TRUE;FA13;0	01;0	13;0	01;0
27	416;70;4	4;18	1;6	4;20	9;FALSE;F.08;0	02;0	13;0	03;0
28	417;71;4	76;12	6;5	68;10	5;FALSE;F.19;0	05;0	23;0	06;0
29	418;62;4	2;15	2;4	27;17	3;TRUE;FA12;0	01;0	12;0	01;0
30	419;70;3	7;26;4	72;26	2;FALSE;F.14;0	03;0	19;0	04;0	82;0
31	420;60;6	5;6	8;4	68;9	4;FALSE;F.51;0	19;0	39;0	12;0
32	421;59;5	4;14	5;4	13;16	2;FALSE;F.22;0	06;0	16;0	04;0
33	422;56;4	08;12	5;3	89;14	1;TRUE;FA7;1;FALSE;12;0	01;0	12;0	
34	423;71;4	9;16;4	7;16	3;FALSE;F.7;1;TRUE;F.2;0	05;0	19;0	04;0	
35	424;78;8	5;12	7;2	46;NA;FALSE;6;0	44;0	19;0	09;0	42;0
36	425;62;4	7;18	2;3	44;24	1;FALSE;F.19;0	04;0	13;0	03;0
37	426;69;4	1;23	1;3	35;22	6;FALSE;F.16;0	04;0	12;0	03;0
38	427;59;4	5;12	1;5	42;8	6;FALSE;T24;0;TRUE;62;0	43;0	68;0	
39	428;69;4	11;16;4	98;17	4;FALSE;F.46;0;TRUE;34;0	09;0	41;0	13;0	
40	429;62;5	1;10;3	8;9	7;FALSE;F.54;0;FALSE;42;0	13;0	32;0	08;0	
41	430;62;5	8;9;5	92;8	9;FALSE;T7;0	54;0	71;0	54;0	28;0
42	431;64;6;3	13;29	3;FALSE;T27;0	15;0	37;0	23;0	82;0	11;0
43	432;56;4	6;16;5	72;11	5;FALSE;F.18;0	04;0	23;0	06;0	68;0
44	433;63;6;3	6;5	1;28	2;FALSE;F.12;0	03;0	1;0	02;0	87;0
45	434;62;7	6;24;4	2;24	2;FALSE;F.32;0	1;0	16;0	04;0	84;0
46	435;65;6	37;18	8;9	57;29	9;TRUE;FA08;0	01;0	08;0	01;0
47	436;74;8	9;10	9;8	94;9	1;FALSE;T61;0	46;0	62;0	46;0
48	437;77;9;2	5;6	19;23	9;FALSE;F.2;0	05;0	13;0	03;0	8;0
49	438;56;3	88;25	5;4	85;21	8;FALSE;F.15;0	03;0	19;0	05;0
50	439;65;8;1	96;NA;FALSE;34;0		1;0	45;0	16;0	36;0	42;0
51	440;70;5	8;15	3;4	99;12	6;FALSE;F.12;0	03;0	09;0	02;0
52	441;67;4	06;28	2;1	86;NA;FALSE;16;0	04;0	06;0	01;0	8;0
53	442;70;4	74;16	5;4	49;14;FALSE;19;0	05;0	18;0	04;0	68;0
54	443;70;5	51;17	4;6	11;16	2;FALSE;F.23;0	06;0	25;0	07;0
55	444;64;4	77;11	5;1	38;NA;FALSE;19;0	05;0	04;0	01;0	5;0
56	445;77;4	99;10	2;6	12;8	4;FALSE;F.46;0;TRUE;41;0	13;0	48;0	
57	446;59;7	7;11	7;4	48;15	1;FALSE;F.64;2;FALSE;17;0	04;0	08;0	
58	447;68;9	12;23	2;8	56;20;TRUE;14;0	03;0	14;0	03;0	86;0

1							
2							
3	448;69;8;7 8;7	36;8	2;FALSE;F.58;0	24;0	55;0	22;0	13;0
4	449;63;4 36;20	4;2	98;NA;FAL 17;0	04;0	1;0	02;0	75;0
5	450;62;5 2;12	7;6	31;11 2;TRUE;FA 07;0	01;0	08;0	01;0	
6	451;65;8 37;14;10	21;NA;FAL 35;0	11;0 42;0	15;0	55;0	31;0	
7	452;62;3 5;24	1;3	06;26 4;FALSE;F.13;0	03;0	11;0	02;0	
8	453;70;5 4;30;5	07;28	2;FALSE;F.1;0	02;0	1;0	02;0	84;0
9	454;59;3 29;16;4	21;15	2;FALSE;F.27;0	07;0	35;0	1;0	74;0
10	455;64;5 19;16	8;5	78;14 7;FALSE;T 66;0	49;0	7;0	53;0	
11	456;71;9 1;12	9;3	33;20 7;FALSE;F.38;0	13;0	12;0	03;0	
12	457;69;3 89;11;3	92;13	5;FALSE;F.32;0	09;0	33;0	09;0	45;0
13	458;65;7;16 64;16	6;FALSE;F.15;0	04;0 19;0	05;0	63;0	26;0	
14	459;69;5 81;16	6;4	7;14;FALSE;F.24;0	06;0	19;0	04;0	69;0
15	460;71;5 21;29	2;5	41;21 8;FALSE;F.21;0	05;0	22;0	06;0	
16	461;69;4 55;15;4	12;13	5;FALSE;F.18;0	04;0 16;0	04;0	58;0	
17	462;65;4 92;11	9;5	42;12 9;FALSE;F.2;0	05;0	22;0	06;0	
18	463;69;5 3;15;4	15;19;FALSE;1;0	02;0	07;0	02;0	64;0	26;0
19	464;78;6 8;13	2;7	37;13;FALSE;75;0	6;0	77;0	63;0	45;0
20	465;66;3 5;22;3	28;24;FALSE;13;0	03;0 12;0	02;0	76;0	19;0	
21	466;62;9 32;18;8	41;19	9;TRUE;FA 08;0	01;0 08;0	01;0	83;0	
22	467;75;5 26;13	6;4	29;21 2;FALSE;F.03;2;FALSE;1;0	02;0	02;0	08;0	
23	468;56;3 9;11	3;3	54;11 5;FALSE;F.33;0	09;0	29;0	08;0	
24	469;57;6;12;3	49;19	1;FALSE;F.12;0	03;0 06;0	01;0	78;0	
25	470;72;4 8;16	3;4	25;15 5;TRUE;FA 13;0	01;0 12;0	01;0	01;0	
26	471;58;4 59;19	2;4	19;21 9;FALSE;F.18;0	04;0 16;0	04;0	04;0	
27	472;75;5 15;15	6;6	8;13 9;FALSE;F.21;0	05;0 28;0	08;0		
28	473;64;4 96;24	1;4	97;24 5;FALSE;F.2;0	05;0 2;0	05;0		
29	474;72;6;16 51;NA;FAL 25;0	07;0	08;0 02;0	65;0 23;0	12;0		
30	475;69;8 4;16	7;8	47;21 7;TRUE;FA 5;1;FALSE;14;0	02;0	02;0	14;0	
31	476;57;8 07;13	5;7	92;13 7;TRUE;FA 08;0	01;0 08;0	01;0		
32	477;60;3 81;28	7;3	64;30 7;FALSE;F.14;0	03;0 14;0	03;0	03;0	
33	478;58;4 55;28	7;4	07;26 2;TRUE;FA 23;0	04;0 22;0	03;0		
34	479;68;7 09;17	7;4	3;23 2;TRUE;FA 14;0	02;0 13;0	01;0		
35	480;70;8;1'3;5	24;16	4;FALSE;F.34;0	1;0 21;0	05;0	37;0	
36	481;63;7 87;14	3;7	81;15 2;FALSE;F.17;0	04;0 17;0	04;0		
37	482;62;6 9;8	9;6	91;8 9;TRUE;FA 24;0	06;0 24;0	06;0		
38	483;71;4 5;14	1;5	82;19 4;FALSE;F.18;0	04;0 24;0	06;0		
39	484;55;8 48;14	5;7	57;17 8;TRUE;FA 14;0	03;0 14;0	02;0		
40	485;57;7 38;14	2;3	13;16 2;FALSE;F.55;0	22;0 26;0	06;0		
41	486;73;4 8;31;4	9;27	5;FALSE;F.19;0	05;0 2;0	05;0	83;0	
42	487;65;3 75;25	8;4	22;26 7;FALSE;F.06;0	01;0 08;0	02;0		
43	488;57;4;18 11;27;FALSE;07;0	01;0	05;0 01;0	78;0 18;0	04;0		
44	489;59;5 45;19;6	07;19	6;FALSE;F.11;0	02;0 12;0	03;0	79;0	
45	490;73;4 26;15	9;4	66;14 5;FALSE;F.17;0	04;0 18;0	04;0		
46	491;58;7 9;7	9;8	91;8 8;TRUE;FA 08;0	01;0 08;0	01;0		
47	492;72;4 25;17	1;6	61;15 1;FALSE;F.35;0	1;0 51;0	19;0		
48	493;62;7 06;2	6;7	91;3 6;FALSE;F.54;0	21;0 58;0	24;0;0		
49	494;62;4 59;8	2;5	53;8 3;FALSE;F.38;0	11;0 45;0	15;0		
50	495;64;4 6;19	1;4	15;21 2;FALSE;F.64;1;TRUE	18;0 04;0	16;0		
51	496;56;3 4;12	5;3	34;16 7;FALSE;F.12;0	03;0 12;0	03;0		
52	497;67;3 55;18	8;2	61;NA;FAL 34;1;TRUE	13;0 03;0	09;0	02;0	
53	498;71;4 5;12	6;3	65;9 3;FALSE;F.5;0;TRUE	137;0 11;0	3;0		
54	499;64;5 66;33;7	99;32	9;FALSE;F.23;0	06;0 33;0	1;0	87;0	
55	500;66;3 9;17	2;5	08;15 1;FALSE;T 57;0	37;0 66;0	48;0		
56	501;60;4 32;13	4;3	85;14;FALSE;F.35;0	2;0 32;0	17;0	55;0	
57	502;60;6 8;5	2;7	74;4 7;FALSE;F.52;0	2;0 57;0	23;0		
58	503;72;5 07;16	7;4	68;16 2;FALSE;F.2;0	05;0 19;0	04;0		

3	504;56;3	45;10	7;2	78;NA;FAL;29;0	07;0	23;0	05;0	52;0
4	505;67;6	5;22	5;6	08;24	3;FALSE;F;27;0	07;0	25;0	07;0
5	506;71;9	4;32	5;10	18;NA;TRU08;0	01;0	09;0	01;0	9;0
6	507;79;6	6;10;7	53;9	5;FALSE;T;74;0	59;0	77;0	64;0	25;0
7	508;55;5	6;15	3;6	15;14	7;FALSE;F;11;0	03;0	12;0	03;0
8	509;67;3	05;15;3	58;12;FALSE;47;0	28;0	53;0	34;0	66;0	23;0
9	510;56;6	7;13;6	25;13	7;FALSE;F;28;0	08;0	26;0	07;0	62;0

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2								
3	88;0	09;0	03;0	85;0	12;0	03;0	77;0	18;0
4	85;0	1;0	05;0	87;0	08;0	05;0	76;0	15;0
5	16;0	06;0	84;0	12;0	04;0	73;0	19;0	08;0
6	43;0	38;0	19;0	63;0	25;0	12;0	69;0	21;0
7	26;0	11;0	86;0	11;0	03;0	71;0	2;0	09;0
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9	71;0	22;0	07;0	87;0	09;0	04;0	73;0	2;0
10	34;0	09;0	82;0	15;0	03;0	8;0	16;0	04;0
11	07;0	2;0	49;0	31;0	52;0	3;0	18;0	82;0
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13	76;0	18;0	06;0	75;0	19;0	06;0	73;0	19;0
14	39;0	28;0	08;0	47;0	45;0	69;0	19;0	12;0
15	81;0	15;0	04;0	81;0	14;0	05;0	71;0	22;0
16	54;0	34;0	12;0	74;0	2;0	06;0	76;0	18;0
17	18;0	05;0	79;0	16;0	05;0	76;0	18;0	06;0
18	24;0	08;0	63;0	26;0	11;0	75;0	19;0	06;0
19	62;0	28;0	1;0	56;0	32;0	12;0	74;0	19;0
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21	33;0	18;0	77;0	18;0	05;0	65;0	22;0	13;0
22	66;0	22;0	12;0	72;0	18;0	1;0	77;0	15;0
23	86;0	11;0	03;0	83;0	13;0	04;0	74;0	19;0
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27	05;0	05;0	89;0	07;0	04;0	73;0	15;0	12;0
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36	39;0	35;0	22;0	4;0	38;0	63;0	2;0	17;0
37	27;0	06;0	86;0	12;0	02;0	8;0	16;0	04;0
38	39;0	15;0	88;0	11;0	01;0	76;0	18;0	06;0
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42	17;0	08;0	71;0	19;0	1;0	66;0	22;0	12;0
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47	1;0	62;0	24;0	14;0	73;0	18;0	09;0	71;0
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53	87;0	1;0	03;0	78;0	17;0	05;0	78;0	17;0
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56	49;0	26;0	25;0	51;0	25;0	24;0	58;0	2;0
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11	91;0	06;0	03;0	86;0	1;0	04;0	8;0	14;0
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25	18;0	05;0	81;0	15;0	04;0	72;0	22;0	06;0
26	51;0	35;0	14;0	45;0	39;0	16;0	73;0	19;0
27	69;0	79;0	17;0	04;0	66;0	22;0	12;0	79;0
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31	32;0	13;0	51;0	34;0	15;0	7;0	21;0	09;0
32	25;0	07;0	77;0	18;0	05;0	76;0	18;0	06;0
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41	21;0	11;0	75;0	17;0	08;0	66;0	22;0	12;0
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44	67;0	21;0	12;0	55;0	29;0	16;0	65;0	22;0
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47	18;0	55;0	31;0	14;0	7;0	21;0	09;0	68;0
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56	1;0	03;0	86;0	11;0	03;0	78;0	17;0	05;0
57	66;0	26;0	08;0	72;0	22;0	06;0	77;0	18;0
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4	46;0	33;0	21;0	44;0	32;0	24;0	7;0	19;0
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12	16;0	87;0	1;0	03;0	88;0	09;0	03;0	8;0
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14	62;0	27;0	11;0	72;0	2;0	08;0	77;0	16;0
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21	46;0	2;0	75;0	19;0	06;0	77;0	18;0	05;0
22	51;0	33;0	16;0	56;0	29;0	15;0	61;0	26;0
23	51;0	39;0	89;0	09;0	02;0	86;0	11;0	03;0
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37	17;0	81;0	16;0	03;0	79;0	17;0	04;0	81;0
38	48;0	38;0	14;0	64;0	28;0	08;0	75;0	19;0
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40	66;0	27;0	07;0	65;0	27;0	08;0	73;0	21;0
41	16;0	07;0	77;0	16;0	07;0	69;0	21;0	1;0
42	66;0	26;0	08;0	34;0	46;0	2;0	75;0	19;0
43	17;0	05;0	79;0	17;0	04;0	77;0	18;0	05;0
44	22;0	06;0	8;0	16;0	04;0	77;0	18;0	05;0
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46	22;0	09;0	8;0	15;0	05;0	77;0	16;0	07;0
47	27;0	09;0	59;0	3;0	11;0	75;0	19;0	06;0
48	58;0	28;0	14;0	66;0	24;0	1;0	68;0	21;0
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53	11;0	58;0	2;0	22;0	61;0	2;0	19;0	58;0
54	36;0	22;0	19;0	47;0	34;0	64;0	22;0	14;0
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56	08;0	78;0	15;0	07;0	63;0	22;0	15;0	66;0
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60								

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5	32;0	19;0	73;0	16;0	11;0	78;0	14;0	08;0
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7	15;0	28;0	45;0	27;0	23;0	47;0	3;0	69;0
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9	86;0	11;0	03;0	85;0	1;0	05;0	74;0	19;0
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11	57;0	32;0	11;0	43;0	41;0	16;0	68;0	23;0
12	51;0	21;0	83;0	15;0	02;0	74;0	21;0	05;0
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19	36;0	49;0	15;0	49;0	41;0	1;0	77;0	19;0
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22	34;0	14;0	76;0	18;0	06;0	74;0	19;0	07;0
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26	77;0	17;0	06;0	75;0	19;0	06;0	72;0	2;0
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31	14;0	07;0	77;0	16;0	07;0	62;0	25;0	13;0
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36	79;0	15;0	06;0	78;0	17;0	05;0	71;0	2;0
37	43;0	35;0	22;0	45;0	34;0	21;0	75;0	17;0
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41	74;0	18;0	08;0	62;0	26;0	12;0	68;0	21;0
42	48;0	35;0	17;0	65;0	26;0	09;0	68;0	21;0
43	21;0	77;0	18;0	05;0	64;0	23;0	13;0	77;0
44	13;0	06;0	72;0	16;0	12;0	77;0	14;0	09;0
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47	87;0	1;0	03;0	89;0	08;0	03;0	73;0	2;0
48	28;0	18;0	46;0	33;0	21;0	7;0	18;0	12;0
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55	03;0	82;0	15;0	03;0	74;0	19;0	07;0	82;0
56	42;0	3;0	31;0	41;0	28;0	65;0	22;0	13;0
57	05;0	82;0	13;0	05;0	68;0	23;0	09;0	67;0
58	34;0	41;0	25;0	29;0	44;0	27;0	64;0	22;0
59								14;0
60								

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2								
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4	17;0	55;0	28;0	2;0	54;0	26;0	73;0	2;0
5	03;0	82;0	15;0	03;0	76;0	18;0	06;0	82;0
6	69;0	23;0	08;0	8;0	15;0	05;0	72;0	2;0
7	3;0	49;0	21;0	39;0	43;0	18;0	73;0	19;0
8	19;0	09;0	76;0	16;0	08;0	73;0	18;0	09;0
9	16;0	06;0	59;0	3;0	11;0	73;0	2;0	07;0
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11	73;0	21;0	06;0	52;0	28;0	2;0	69;0	23;0
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14	64;0	26;0	1;0	68;0	24;0	08;0	86;0	11;0
15	37;0	86;0	11;0	03;0	79;0	14;0	07;0	86;0
16	39;0	39;0	22;0	33;0	43;0	24;0	66;0	22;0
17	79;0	16;0	05;0	85;0	1;0	05;0	73;0	19;0
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19	71;0	23;0	06;0	68;0	26;0	06;0	81;0	16;0
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21	21;0	75;0	16;0	09;0	81;0	13;0	06;0	72;0
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24	17;0	05;0	72;0	21;0	07;0	78;0	17;0	05;0
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42	11;0	49;0	4;0	78;0	16;0	06;0	63;0	23;0
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36	11;0	03;0	86;0	11;0	03;0	77;0	18;0	05;0
37	13;0	03;0	86;0	12;0	02;0	79;0	17;0	04;0
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39	19;0	07;0	74;0	19;0	07;0	66;0	24;0	1;0
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43	23;0	11;0	62;0	26;0	12;0	69;0	21;0	1;0
44	25;0	14;0	65;0	22;0	13;0	78;0	14;0	08;0
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16	19;0	08;0	79;0	14;0	07;0	76;0	17;0	07;0
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27	67;0	25;0	08;0	72;0	19;0	09;0	67;0	24;0
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41	42;0	3;0	27;0	42;0	31;0	71;0	18;0	11;0
42	07;0	82;0	11;0	07;0	64;0	22;0	14;0	6;0
43	25;0	07;0	47;0	4;0	13;0	73;0	21;0	06;0
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47	33;0	32;0	35;0	2;0	37;0	43;0	57;0	21;0
48	11;0	09;0	77;0	14;0	09;0	58;0	23;0	19;0
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41	12;0	05;0	82;0	13;0	05;0	7;0	21;0	09;0
42	83;0	13;0	04;0	84;0	13;0	03;0	76;0	18;0
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60								

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2									
3	37;0	11;0	82;0	15;0	03;0	81;0	16;0	03;0	82;0
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7	72;0	22;0	06;0	7;0	23;0	07;0	77;0	18;0	05;0
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12									
13									
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22									
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For peer review only

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Section/Topic	Item #	Recommendation	Reported on page #
<b>Title and abstract</b>	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
<b>Introduction</b>			
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
<b>Methods</b>			
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
<b>Results</b>			

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  (b) Give reasons for non-participation at each stage  (c) Consider use of a flow diagram	11  11  Supplemental
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders  (b) Indicate number of participants with missing data for each variable of interest	11  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  (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	12
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supplemental
<b>Discussion</b>			
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
<b>Other information</b>			
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](http://www.strobe-statement.org).