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Accurate diagnosis of prostate cancer by combining Proclarix with magnetic resonance imaging

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Abstract

Objectives: The use of mpMRI has been a significant advance in the diagnosis of clinically significant prostate cancer (ISUP Grade Group ≥2, csPCa) and is recommended in most current guidelines. Proclarix® is a novel CE-marked biomarker test aiding in the identification of csPCa. The aim of the study was the assessment of the clinical performance of Proclarix alone or in combination with mpMRI to predict csPCa.

Patients and Methods: The study included blood samples from 721 men undergoing mpMRI followed by biopsy at University College London (UCL), London, and Vall d'Hebron University Hospital, Barcelona. Samples were tested blindly. The Proclarix-MRI model combining prostate volume, Proclarix and mpMRI results was trained using the UCL cohort (n=159) and validated in the Vall d'Hebron cohort (n=562). Its diagnostic performance was established in correlation to biopsy outcome and compared to available clinical parameters and risk calculators.

Results: Clinical performance of the Proclarix-MRI model in the validation cohort did not significantly differ from the training cohort and resulted in a sensitivity for csPCa of 90%, 90% NPV and 66% PPV. The Proclarix-MRI score's specificity (68%) was significantly (p<0.001) better compared to MRI-ERSPC risk score (51%), Proclarix (27%) or mpMRI (28%) alone. In addition, Proclarix by itself was found to be useful in the MRI PI-RADS 3 subgroup by outperforming PSA density in terms of specificity (25% vs 13%, p=0.004) at 100% sensitivity.

Conclusion: When combined with mpMRI and prostate volume, Proclarix reliably predicted csPCa and ruled out men with no or indolent cancer. A large reduction of two thirds of unneeded biopsies was achieved. Proclarix can further be used with high confidence to reliably detect csPCa in men with an indeterminate PI-RADS 3 mpMRI. Despite these encouraging results, further validation is needed.

Key Words: prostate cancer; biopsy; biomarkers; Proclarix; Proclarix-MRI score; PSA; thrombospondin-1, cathepsin D, diagnosis; mpMRI

INTRODUCTION

The early detection of clinically significant prostate cancer (csPCa), defined here as ISUP Grade Group (GG)≥2, decreases PCa mortality (1). The classic approach for early detection of PCa, based on serum prostate-specific antigen (PSA), digital rectal examination (DRE), and TRUS (Transrectal Ultrasound) systematic-biopsies, has been shown to be sub-optimal due to the high rates of unnecessary biopsies and overdetection of insignificant PCa (iPCa) (2).

The recent improvement of early detection of csPCa has come from multiparametric magnetic resonance imaging (mpMRI) and guided biopsies (3). The PROMIS study clearly demonstrated that mpMRI can reduce unnecessary biopsies and the over-detection of iPCa (4). Besides mpMRI, current guidelines (5) recommend the use of a variety of tools including risk calculators by inputting existing clinical data, e.g MRI-ERSPC risk calculator (including PSA, previous biopsy information, DRE, prostate volume, age, PI-RADS), or novel biomarkers to support a biopsy decision in subjects with serum PSA in the grey zone range (2-10 ng/ml) or equivocal PI-RADS (Prostate Imaging-Report and Data System) (6–8).

There is a clear unmet need for a comprehensive prostate cancer risk assessment to achieve a high specificity and sensitivity for the detection of csPCa to select appropriate men for biopsy alongside a high negative predictive value (NPV) to safely rule-out csPCa in men to give them peace of mind if csPCa risk is low.

In men with negative mpMRI results (PI-RADS 1-2), a low false negative rate is generally observed and less than 15% of csPCa are missed. Using an additional test will likely increase the detection rate of csPCa at the cost of many false positive results. In contrary, mpMRI has a low false positive rate in positive mpMRI (PI-RADS 4-5) and any additional method applied to increase the specificity is likely to increase the rate of missing csPCa.

However, mpMRI is known to have only modest clinical performance when results are indeterminate (PI-RADS 3). Ultimately, this might be the situation either to re-assess the MRI by another physician or to use additional tests such as biomarkers to increase specificity and sensitivity and thus improving decision making.

Proclarix (Proteomedix, Switzerland) is a new blood-based CE-marked test that calculates the csPCa risk score after measuring thrombospondin-1 (THBS1), cathepsin D (CTSD), total PSA (tPSA), free PSA (fPSA) in serum, and combining with age (9). Proclarix was originally developed for use in men with a PSA of 2-10 ng/ml, a prostate volume ≥35 ml and a normal, non-cancer suspicious DRE. The THBS1 and CTSD glycoproteins were identified through a targeted proteomic strategy for biomarker discovery in a PI3K/PTEN mouse model that is involved in the carcinogenesis and progression of PCa (10–12). Steuber *et al.* have recently reported that Proclarix can improve csPCa detection by reducing unnecessary prostate biopsies and seems to have better performance characteristics than other risk calculators (13). The Proclarix-MRI score evaluated here combines the biomarker-based test Proclarix, PI-RADS results from mpMRI together with the patient's prostate volume.

This study was initiated to develop a novel Proclarix-MRI model and evaluate its clinical performance in predicting csPCa in men from two clinical centers. In addition, Proclarix alone was evaluated in the total cohort and with a special focus on resolving indeterminate (PI-RADS 3) mpMRI. The diagnostic performance of Proclarix was evaluated in different clinical settings along the patient journey before and after an mpMRI was performed. Accordingly, comparison of the clinical performance was made against adequate comparators (5). Before mpMRI, Proclarix alone was compared to %fPSA and ERSPC Risk Score (RC). In the setting where an mpMRI was performed but indeterminate, it was compared to PSA density. Finally, the novel Proclarix-MRI model was evaluated in the total cohort and compared to each Proclarix and mpMRI alone as well as to MRI-ERSPC RC.

PATIENTS AND METHODS

Study population

This study is a retrospective two-center study with serum samples and patient data prospectively collected at University College London (UCL) Hospital in the context of the INNOVATE study (14,15) (n=159) and at Vall d'Hebron University Hospital (n=562). Five hemolytic samples (>1.0 g/dL) were excluded from the original Vall d'Hebron cohort (n=567). In the original UCL cohort (n=291) most men with non-suspicious mpMRI results (n=132) did not undergo biopsy and were thus not included in this study. Thus, 159 and 562 patients were selected from the INNNOVATE and Vall d'Hebron cohort.

Both biobanks involved men undergoing mpMRI followed by targeted and systematic biopsies. Serum samples were obtained just prior to prostate biopsy. The mpMRI sequences were interpreted using PI-RADSv.2 by the local experienced radiologists. Histopathologic examination of biopsy specimen was performed according to the established local practice. CsPCa was defined as ISUP GG ≥2 detected on biopsy. A 3T-mpMRI with two to three-core transrectal ultrasound (TRUS) guided-biopsies was carried out in patients with PI-RADSv.2 score of 1 or 2. Patients with PIRADSv.2 of 3 or greater received a 12-core TRUS systematic-biopsy. Biopsies were performed from April 2016 to December of 2019 for the UCL cohort and from January 2018 to March 2020 for the Vall d'Hebron cohort. Demographic and clinical information was collected in an electronic case record form. All men needed to have undergone biopsy to be included in this study. This project was approved by the NRES Committee London-Surrey Borders with REC reference 15/LO/069 for the UCL and by the institutional Ethics Committee PR-AG129/2020 for the Vall d'Hebron cohort.

Testing and risk calculation

Frozen serum was stored locally at -80°C until shipment on dry ice to Proteomedix (Zurich-Schlieren, Switzerland) for analysis. Processing of serum samples, the ELISA kit and calculation of the risk score by laboratory technicians were performed blindly before availability of any clinical information. THBS1 and CTSD were measured using the CE-marked Proclarix kit (Proteomedix, Zurich-Schlieren, Switzerland) as described before (16). Serum tPSA and fPSA were reanalyzed for all samples using the Roche Cobas immunoassay system (Roche Diagnostics, Rotkreuz, Switzerland). Proclarix risk calculation was performed according to the instruction for use. A cut-off of 10% for the Proclarix Risk Score was used as recommended by the manufacturer (17).

The Proclarix-MRI score with a range from 0 to 100% was developed and trained on the UCL cohort of 159 subjects. The model comprises Proclarix RC, PI-RADSv.2 score and prostate volume (ml) as input variables. The cut-off value for the Proclarix-MRI score was set to 17% in order to gain high sensitivity and NPV above 90%, finally resulting in a sensitivity and NPV in the training set of 94% (95%-CI, 89-100%) and 93% (95%-CI, 87-100%), respectively. The risk of csPCa is low in men with a score below the cut-off value of 17% and high with a score at or above the cut-off value.

Statistical analyses

Primary endpoint was the assessment of the clinical performance of Proclarix to diagnose csPCa in the total cohort without taking mpMRI information into account. Its performance was compared to percent free PSA (%fPSA) as well as the ERSPC RC. When specifically investigating patients with PI-RADS 3 mpMRI, results of Proclarix were compared to PSA density using a cut-off of 0.05 ng/ml^2. Furthermore, the reproducibility of the new Proclarix-MRI model was evaluated between the two cohorts and its performance was determined in the combined cohort compared to PI-RADS score, Proclarix, and the MRI-ERSPC RC (8).

Differences in results between sensitivity and specificity of unpaired tests were assessed using Fisher's exact test. For paired test, differences were assessed using the McNemar-test and are presented with the 95%-CI (18). P-values for differences in NPV and positive predictive value (PPV) were determined according to Moskowitz and Pepe (19) and p-values between area under the curve (AUC) of the receiver operating characteristic (ROC) analysis were determined as described in DeLong *et al.* (20), using the algorithm of Sun and Xu (21). Decision curve analysis was conducted as proposed by Vickers *et al.* (22) to assess the clinical usefulness of the different scores by quantifying the net benefits when different thresholds are used. Relevant and acceptable threshold probability was defined as below 20%, ideally 10%, meaning that a urologist would not do more than 10 biopsies to find one high-grade cancer in patients with similar health. Additionally, calibration plot analysis was used for the assessment of prediction of the Proclarix-MRI model, comparing the agreement of observed and predicted outcomes.

Finally, the last endpoint was the assessment of the optimal biopsy strategy regarding the reduction of unnecessary biopsies in the total cohort (n=721). Analyses were performed using R version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study population

Based on mpMRI guided and/or systematic biopsies, csPCa was detected in 302 (42%), iPCa in 92 (13%) and no PCa in 327 (45%) out of 721 men in the total cohort. CsPCa detection rates were similar in the UCL cohort (35%) and in the Vall d'Hebron cohort (41%). The median age, volume and serum PSA values were significantly higher in the Spanish compared to the English cohort (Table 1), while the overall distribution according to the patient disease status was similar in both cohorts (supplementary Fig.1). The percentage of no PCa in men with a negative mpMRI (PI-RADS 1 or 2) was 85% in both cohorts and csPCa was detected only in 3% (1/40) in the UCL and 6% (6 out of 100) in the Vall d'Hebron cohort, respectively. Among men with a positive mpMRI (PI-RADS 3-5) the percentage of men with csPCa was highest among participants with a PI-RADS score of 5 in both cohorts (97%, 36/37 in the UCL and 86%, 91/106 in the Vall d'Hebron cohort), followed by those with a score of 4 (50%, 29/58 and 56%, 105/188) and those with a score of 3 (25%, 6/24 and 17%, 28/168). Conversely, the percentage of men without cancer in those with positive mpMRI was highest among participants with a PI-RADS score of 3, followed by those with a score of 4 and those with a score of 5.

Determining the need for a mpMRI and / or biopsy

The sensitivity of Proclarix for csPCa was 97% [95% CI, 95-99%]. When compared to %fPSA (Table 2) at equal sensitivity (corresponding to a calculated cut-off of 31.5% for %fPSA), Proclarix had a significantly higher specificity (24% [95% CI, 20-28%] vs. 9% [95% CI, 7-12%], p<0.001). The NPV of Proclarix was 91% [95% CI, 86-96%] and the PPV was 48% [95% CI, 44-52%]. Comparing Proclarix to the ERSPC RC (cut-off was set to

obtain a 95% sensitivity to allow a fair comparison), a significantly lower specificity (10% [95% CI, 7-13%], $p<0.001$) was obtained for the ERSPC RC.

Resolving indeterminate mpMRI

Subanalyses of Proclarix for negative (PI-RADS 1+2), indeterminate (PI-RADS 3) and positive mpMRI (PI-RADS 4+5) were performed (Table 3). Proclarix showed consistently a high sensitivity (>96%) at pre-defined cut-off (10%) in all three subpopulations. Due to the very low and high prevalence of csPCa in the negative and in the positive subgroup, respectively, the overall added value of Proclarix was highest in the intermediate mpMRI subgroup. In this challenging subpopulation, at high sensitivities of 100%, Proclarix had significantly higher specificity of 25% [95% CI, 19-32%] compared to PSA density (13%; [95%-CI, 7-18%], $p=0.004$).

Combining Proclarix and MRI

A Proclarix-MRI model combining prostate volume, Proclarix and mpMRI results was trained using the UCL cohort (n=159) and validated in the Vall d'Hebron cohort (n=562). The cut-off was set to 17% to achieve a sensitivity and NPV above 90%. At this threshold, neither sensitivities of 94% (95%-CI, 89-100%) and 90% (95%-CI, 86-94%), nor specificities of 63% (95%-CI, 53-73%) and 68% (95%-CI, 63-73%) in the training and validation cohorts were significantly different. The NPV was high in both cohorts: 93% (95%-CI, 87-100%) and 90% (95%-CI, 87-94%). The AUC of Proclarix-MRI in the validation cohort was 0.93 (95%-CI, 0.89-0.97) and 0.88 (95%-CI, 0.85-0.90) and thus slightly lower ($p=0.049$) in the training cohort. All four undetected csPCa cases in the training cohort were GG2, while in the validation cohort fifteen GG2, four GG3, four GG4 and one GG5 cases were misdiagnosed (supplementary Table 1). Additionally, the calibration plot of the Proclarix-MRI model in the validation cohort (Fig. 2A) shows a very accurate prediction of the model. The calibration

slope is always within the 95%CI of the Proclarix-MRI calibration slope throughout the whole range of predicted values.

Proclarix-MRI was compared to Proclarix, mpMRI and MRI-ERSPC RC (supplementary Table 1 a). ROCs of the different markers are presented in Fig. 1. In the validation cohort, the AUC of Proclarix-MRI (AUC of 0.88) was significantly higher compared to the one of mpMRI (AUC of 0.83, p=0.003), Proclarix (AUC of 0.75, p<0.001) and MRI-ERSPC RC (AUC of 0.85, p=0.037). This observation translated also into the decision curve analysis (Fig. 2B), where the Proclarix-MRI provides a slight increased net benefit for threshold probabilities of >10% compared to the other tests.

In a next step, the performance of the different methods was compared using pre-defined cut-offs. The sensitivity of Proclarix-MRI (90%, 95%-CI 86-94%) was lower than the one of Proclarix and mpMRI (p=0.002) but similar to the one of MRI-ERSPC RC (p=0.134). The specificity of Proclarix-MRI was 68% (95%-CI, 63-73) and significantly higher (p<0.001) than Proclarix, 27% (95%-CI, 22-31), mpMRI 28% (95%-CI,23-33) and MRI-ERSPC 51% (95%-CI, 46-57%). The total of net-interventions avoided for Proclarix-MRI was 249, 97 for Proclarix, 100 for mpMRI, and 188 for MRI-ERSPC RC.

Biopsy strategy

Fig. 3 illustrates different biopsy strategies in this two-cohort study. If no test is used to stratify for biopsy, all 721 men underwent biopsy, independent of significant (n=302), insignificant (n=92) or no cancer (n=327). Assuming a biopsy strategy where Proclarix is used to rule out biopsy without csPCa, this would result in 15% (109) fewer biopsies while still detecting 97% (n=292) of csPCa cases. Specifically, 16% of biopsies with iPCa and 26% of negative biopsies could be avoided. Using mpMRI to stratify patients for biopsy would identify 98% (n=295) of csPCa cases and reduce overall biopsies by 19% (n=140). Fifteen

percent of men with iPCa and 36% of men without cancer could forego a biopsy using this strategy. Using MRI-ERSPC RC would have resulted in identifying 93% (n=282) of csPCa cases, reduce iPCa detection by 45% (n=41), half of the negative biopsies (55%) and overall biopsies by 33% (n=241). When using the Proclarix-MRI score to decide who needs a biopsy, 90% (n=274) of men with csPCa would be correctly identified. The over-detection of iPCa would be reduced by half (54%) and more than two third (70%) of negative biopsies could be saved. Overall, a strategy using the Proclarix-MRI score would have reduced the number of biopsies by 43% (n=308).

DISCUSSION

This clinical study was performed to evaluate the clinical performance of Proclarix alone or in combination with mpMRI in predicting csPCa and thus to rule out men with a low probability of life-threatening disease. Proclarix was originally developed in a combined cohort from Martini-Klinik Hamburg, Germany and Medical University Innsbruck, Austria (9). This study also included samples from two clinical sites, UCL and Vall d'Hebron. Even though age, tPSA and prostate volume are significantly different among these cohorts, no difference in PSA density and Proclarix risk score was observed illustrating the high reproducibility of these parameters in different cohorts.

One of the most relevant improvements in the early detection of csPCa was the implementation of mpMRI and guided biopsies (3). Today, mpMRI is included in most national guidelines and used widely. One limitation of further acceptance of mpMRI might be the inter-observer variability, especially in non-specialized centers.

It was demonstrated in this study that Proclarix could more effectively resolve indeterminate mpMRI than PSA density. When compared to PSA density, Proclarix could save twice as much of the unneeded biopsies by missing no csPCa. Thus, in this difficult to diagnose patient population, where the decision to biopsy is difficult, Proclarix could be a safe option to help physician and patient in the decision making.

Furthermore, we showed that the efficacy of using mpMRI can be improved through the integration with clinical information (prostate volume) and blood-based biomarkers (Proclarix). Since the latter two are purely quantitative measurements and accurate enough to provide reproducible results, this new strategy has the potential to further improve the reproducibility of imaging-based diagnostics. The novel model developed generates a risk score by integrating the values of Proclarix, mpMRI and prostate volume, for the detection of csPCa. Results showed in the calibration plot that this Proclarix-MRI model overall

accurately predicts csPCa. Additionally, its high sensitivity can reliably rule out patients with no or insignificant cancer, also indicated by its high NPV. The Proclarix-MRI score was compared to Proclarix, mpMRI as well as MRI-ERSPC RC and was superior compared to both in determining who can forego a biopsy. These findings could also be shown in the DCA, where Proclarix-MRI showed a higher net benefit for threshold probabilities of >10% compared to the other tests.

Other biomarker tests have recently been combined with mpMRI. The PHI test that measures [-2] proPSA in addition to tPSA and fPSA showed that adding PHI to mpMRI improved overall and significant cancer prediction compared to mpMRI and PSA alone. Only 1/21 (4.8%) csPCa was missed and 31/73 (42%) men potentially spared a re-biopsy (23). SelectMDx, a urine-based test combining HOXC6 and DLX1 expression levels with known risk factors such as age, PSA, PSA density, family history of PCa and DRE information, was evaluated together with mpMRI (24). However, mpMRI had the highest net benefit in that study. Grönberg H *et al.* reported that using a combination of the Stockholm3 blood test and mpMRI reduced overall biopsy procedures by 38% (25). The recently updated MRI-ERSPC RC for previously biopsied men used here as a comparator was previously shown to avoid one-third of biopsies following MRI (8). These results are similar to the ones in this study, where MRI-ERSPC RC achieved a 33% reduction of the biopsies.

Here we present a biopsy strategy where all men would perform the Proclarix-MRI model before undergoing biopsy. By integrating imaging, biomarker and clinical data, the best clinical performance was achieved. Today, these data are easy to obtain, and the method can be readily locally implemented.

A limitation of the present study is that although the serum samples were collected prospectively in the biobanks, the samples were measured retrospectively. The only criteria

was that all men needed to have undergone biopsy, which resulted in the exclusion of 132 samples from the INNOVATE cohort.

Results indicate that the proposed Proclarix-MRI model performs well even in a cohort where biopsies can already be spared based on established clinical practice. No central reading for pathology, ultrasound, and mpMRI was organized and might introduce small inter-site variability. CsPCa definition in prostate biopsies may overestimate the true rate of csPCa allowed in radical prostatectomy specimens.

In conclusion, with its high sensitivity and NPV value, as well as its superiority to ERPSC and %fPSA, the use of Proclarix as a rule out test was confirmed in this study. In indeterminate mpMRI with PI-RADS 3, Proclarix outperformed PSA density as well as MRI-ERSPC RC by ruling out one out four unneeded biopsy and missing no csPCa. The integration of Proclarix, mpMRI and prostate volume into a dedicated biopsy decision algorithm, named the Proclarix-MRI score, significantly outperformed Proclarix and mpMRI alone. While additional prospective validation is needed to support our findings, the diagnostic strategy relying on the Proclarix-MRI score would lower the overall biopsy rate by 40%. The over-detection of men with iPCa would be cut in half and two out of three negative biopsies overall were saved. Overall, the results provide strong support for the use of the Proclarix score in routine PCa diagnostic practice to improve the biopsy decision.

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Table 1: Population characteristics.

	Total	Vall d'Hebron	UCL	p-value
Patients, *n*	721	562	159	*NA*
Age, years	68 (62-73)	69 (63-74)	65 (59-70)	<0.001
tPSA, ng/ml	6.6 (4.7-10.4)	7.0 (4.9-11.2)	5.7 (4.2-7.9)	0.001
Proclarix, (score)	29.0 (16.5-49.3)	28.6 (15.4-50.0)	30.6 (20.2-46.7)	0.621
Volume, (ml)	52 (38-73)	55 (40-76)	44 (32-63)	<0.001
PSA density	0.129 (0.081-0.208)	0.131 (0.080-0.207)	0.121 (0.082-0.208)	0.476
No PCa, n (%)	327 (45.4)	260 (46.3)	67 (42.1)	0.356
GG <2, n (%)	92 (12.8)	72 (12.8)	20 (12.6)	0.938
GG ≥2, n (%)	302 (41.9)	230 (40.9)	72 (45.3)	0.325
PI-RADS=1-2, n (%)	140 (19.4)	100 (17.8)	40 (25.2)	0.038
PI-RADS=3, n (%)	192 (26.6)	168 (29.9)	24 (15.1)	<0.001
PI-RADS=4, n (%)	246 (34.1)	188 (33.5)	58 (36.5)	0.477
PI-RADS=5, n (%)	143 (19.8)	106 (18.9)	37 (23.3)	0.218

Median (25%, 75%); tPSA = total prostate specific antigen; PCa = prostate cancer; GG = grade group

Table 2: Performance characteristics of Proclarix in the combined UCL and Vall d'Hebron cohort, compared to %fPSA and the ERSPC RC.

	Proclarix	**%fPSA**	*p-value*[1)]	**ERSPC RC**	*p-value*[2)]
Cut-off	*10%*	*31.5%*		*2%*	
AUC	0.74 (0.71-0.78)	0.69 (0.65-0.73)	0.003	0.70 (0.66-0.74)	0.038
Sensitivity, %	97 (95-99)	97 (95-99)	1.000	95 (92-97)	0.127
Specificity, %	24 (20-28)	9 (7-12)	<0.001	10 (7-13)	<0.001
NPV, %	91 (85-96)	80 (68-91)	0.055	73 (62-85)	0.005
PPV, %	48 (44-52)	43 (40-47)	<0.001	43 (39-47)	<0.001
Missed csPCa					
GG2	6	3		11	
GG3	1	4		4	
GG4	2	1		0	
GG5	1	2		0	

Value (95% confidence interval); NPV = negative predictive value; PPV = positive predictive value; mpMRI = multi parametric magnetic resonance imaging, GG = grade group

[1)] Comparison Proclarix-MRI score and Proclarix

[2)] Comparison Proclarix-MRI score and ERSPC risk calculator

[3)] Comparison Proclarix-MRI score and mpMRI

[4)] PI-RADS score 1-2 negative, 3-5 positive

Table 3: Performance of Proclarix (cut-off=10%) per PI-RADS segment in the combined cohort (n=721)

mpMRI *PI-RADS*	**Negative (n=140)** *1-2*	**Indeterminate (n=192)** *3*	**Positive (n=389)** *4-5*
AUC	0.45 (0.26-0.64)	0.72 (0.64-0.79)	0.74 (0.69-0.79)
Sensitivity, %	100 (100-100)	100 (100-100)	96 (94-98)
Specificity, %	26 (18-33)	25 (19-32)	20 (13-26)
NPV, %	100 (100-100)	100 (100-100)	71 (56-86)
PPV, %	7 (2-11)	22 (16-29)	71 (66-76)
Missed csPCa, n			
GG2, n	0	0	6
GG3, n	0	0	1
GG4, n	0	0	2
GG5, n	0	0	1

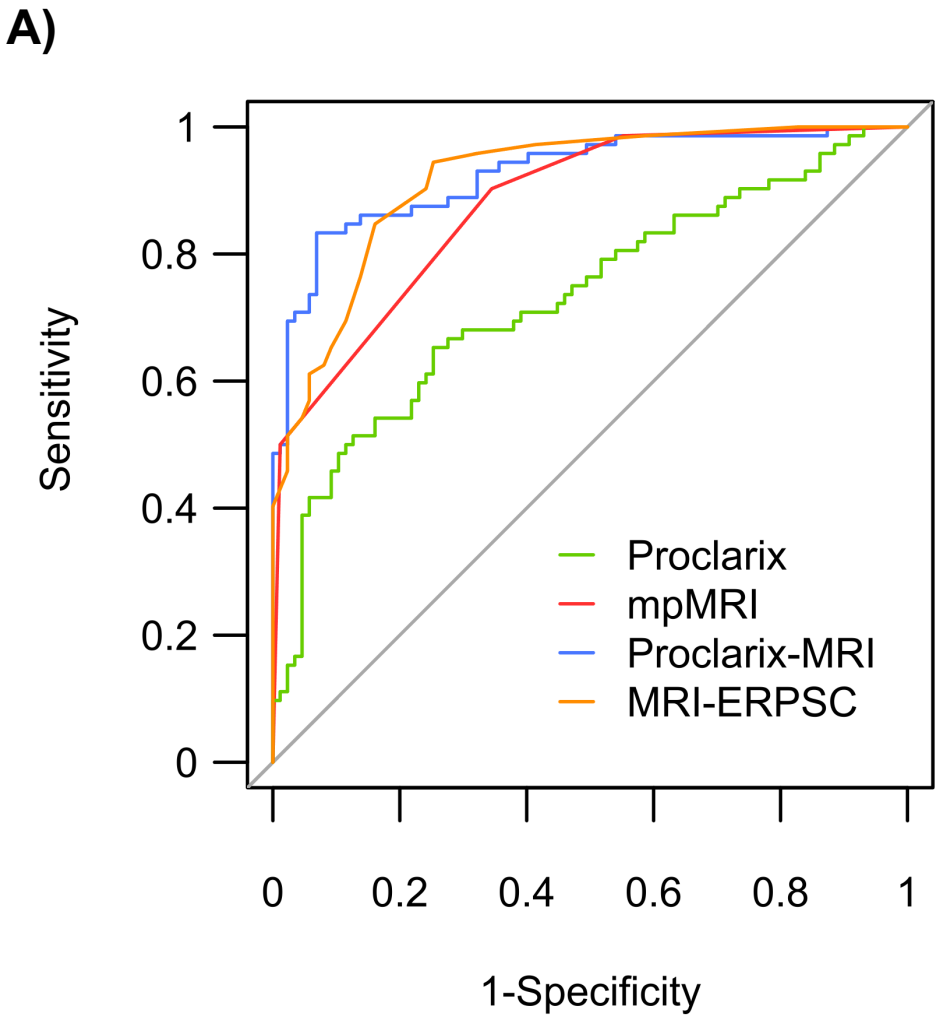
Value (95% confidence interval); NPV = negative predictive value; PPV = positive predictive value; mpMRI = multi parametric magnetic resonance imaging, GG = grade group

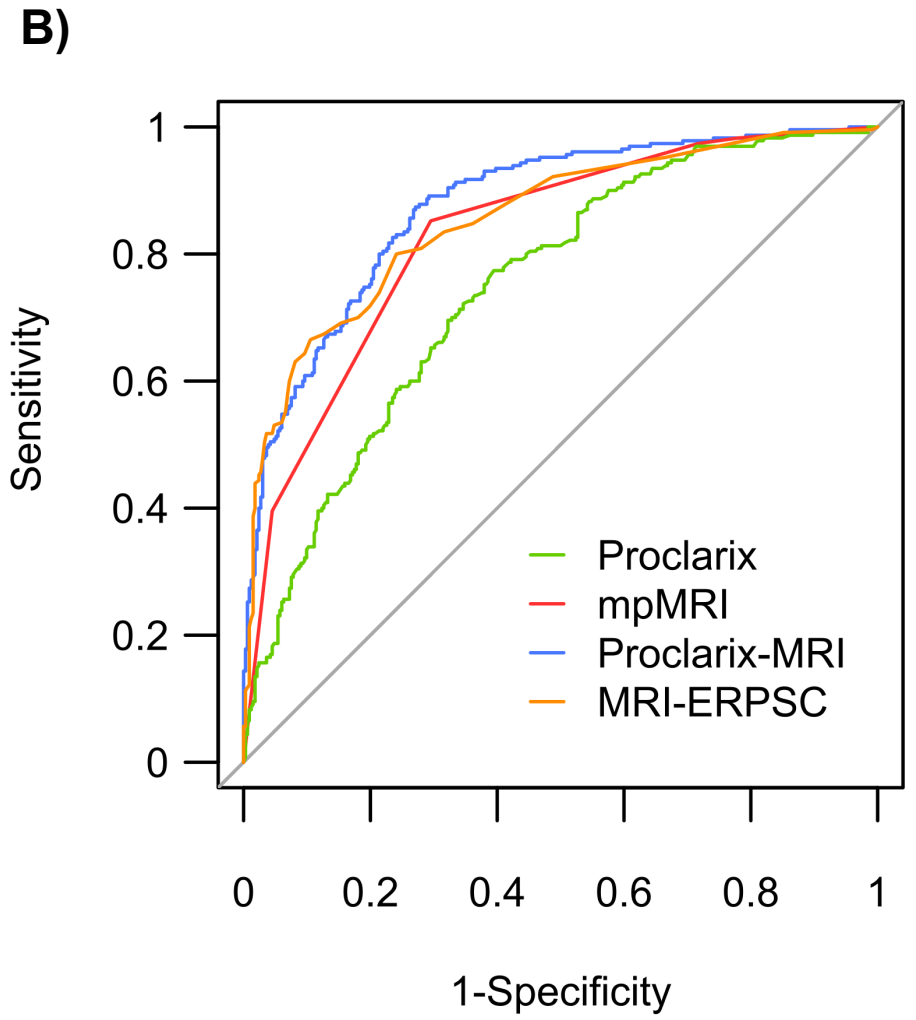
Figure 1: ROC analysis in the A) training UCL (n=159) and B) validation Vall d'Hebron (n=562) cohorts for Proclarix (green line), mpMRI (red line), the Proclarix-MRI score (blue line), and MRI-ERSPC risk calculator (orange line).

Figure 2: A) Calibration plot of the Proclarix-MRI model in the Vall d'Hebron validation cohort (n=562). The dotted line indicates the perfect correspondence between observed and predicted risk. The grey shaded area is the 95%CI of the black line showing the calibration of the Proclarix-MRI model. At the bottom of the graph, histogram shows the frequency distribution of the Proclarix-MRI model. B) DCA in the validation Vall d'Hebron validation cohort (n=562) for Proclarix (green line), mpMRI (red line), the Proclarix-MRI score (blue line), and MRI-ERSPC risk calculator (orange line).

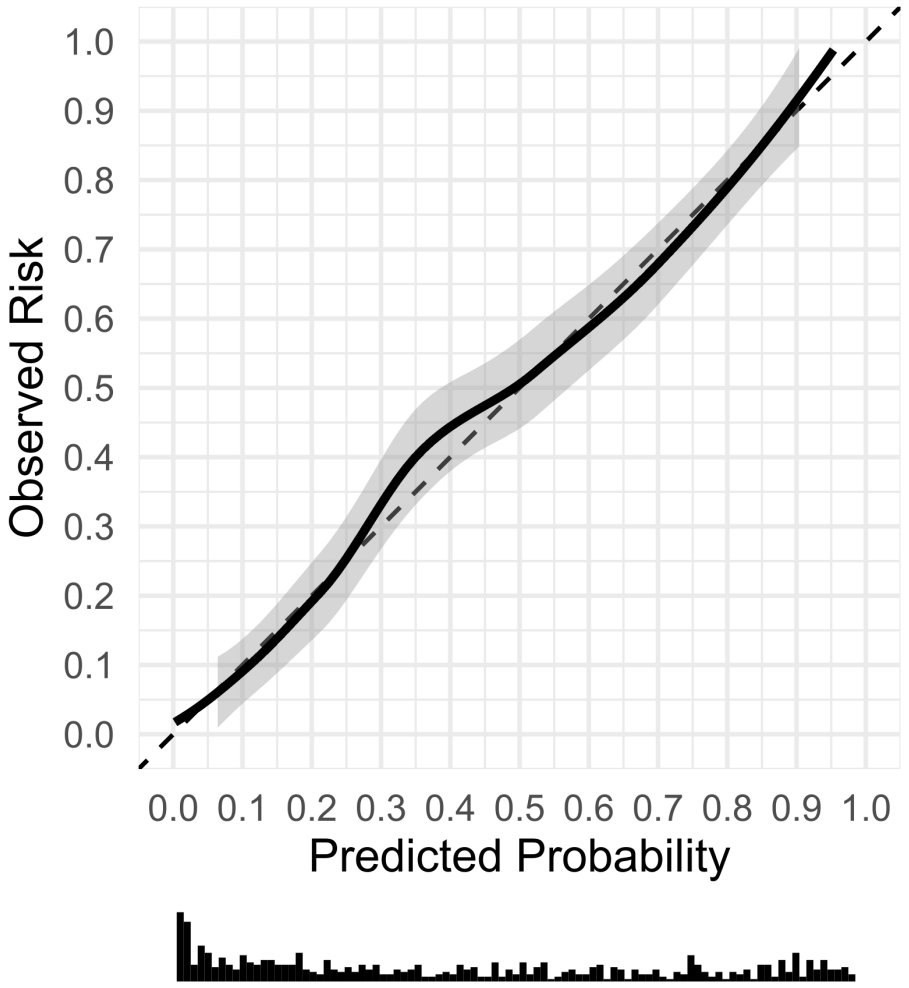
Figure 3: Different strategies to stratify men for biopsy are shown using no test, Proclarix, mpMRI, MRI-ERSPC risk calculator or the Proclarix-MRI score resulting in different numbers of biopsies detecting csPCa (blue), iPCa (light blue), no cancer (grey) as well as avoiding biopsies (green).

Supplementary Figure 1: Patients according to disease status in the **A)** UCL (n=159) and **B)** Vall d'Hebron (n=562) cohorts. Percentages of men with clinically significant (csPCa), insignificant iPCa, or no cancer, identified according to PI-RADS v2 scores, are shown.





A)



B)