

Prospective validation of a urine-based proteomics test for predicting clinically significant prostate cancer in biopsy-naive patients

Enrique Gómez Gómez

`Enrique.gomez.gomez.sspa@juntadeandalucia.es`

Reina Sofía University Hospital, Maimonides Institute of Biomedical Research of Cordoba (IMIBIC),
University of Cordoba (UCO) <https://orcid.org/0000-0002-8753-3306>

Ana Morillo

Kirubel Nigusu Jobre

Department of Biomarker Research, Mosaiques diagnostics GmbH <https://orcid.org/0009-0001-5548-049X>

Guillermo Lendinez

Ana Blanca

Maimonides Institute of Biomedical Research of Cordoba (IMIBIC)

Daniel Lopez Ruiz

Jose Parada

Isabel Heidegger

Medical University Innsbruck <https://orcid.org/0000-0003-1517-5746>

Zoran Culig

Medical University of Innsbruck <https://orcid.org/0000-0002-5001-6153>

Antonio Lopez-Beltran

S. Orsola-Malpighi Hospital <https://orcid.org/0000-0003-3161-8164>

Julia Carrasco-Valiente

Harald Mischak

Mosaiques Diagnostics

Rafael Antonio Medina-López

Virgen del Rocio University Hospital (HVR), Department of Urology; Institute of Biomedicine of Seville (IBIS) <https://orcid.org/0000-0002-4514-4329>

Juan Campos Hernandez

Maria Frantzi

Department of Biomarker Research, Mosaiques diagnostics GmbH

Keywords: Biomarker, mpMRI, Proteomics, Prostate cancer, Urine

Posted Date: March 8th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8933198/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: Yes there is potential conflict of interest.

Abstract

Background and objective: Prostate cancer (PCa) remains a major health burden, while existing diagnostic approaches are limited by overdiagnosis that leads to unnecessary biopsies, and by underdetection of clinically significant disease (csPCa). This study aims to prospectively validate a urine-based 19-biomarker model (19-BM) using capillary electrophoresis–mass spectrometry (CE-MS) for predicting csPCa in patients at risk.

Methods: 161 biopsy-naïve patients at risk for csPCa underwent pre-biopsy CE-MS analysis in urine, multiparametric magnetic resonance imaging (mpMRI), and biopsy at two major hospitals. The 19-BM performance was assessed in comparison with Prostate-Specific Antigen (PSA), European Randomized Study of Screening for Prostate Cancer (ERSPC), and mpMRI. Performance metrics included Area Under the ROC Curve (AUC), sensitivity, specificity, negative, and positive predictive values. 100 patients receiving radical prostatectomy were evaluated to investigate the correlation of the 19-BM scores with the prostatectomy pathology.

Key findings and limitations: The 19-BM scores achieved an AUC of 0.79, outperforming PSA (AUC 0.54, $p < 0.0001$), ERSPC (AUC 0.63, $p = 0.0108$), PSAD (AUC 0.61, $p = 0.0021$) and mpMRI (AUC 0.65, $p = 0.0069$). In patients with low Prostate Imaging Reporting and Data System scores (PI-RADS), 19-BM showed a sensitivity of 75% and specificity 90%. Prospective validation of a previous nomogram combining 19-BM with mpMRI yielded an AUC of 0.82 (with specificity of 91%, sensitivity 62%).

Conclusions: After prospective validation, the 19-BM demonstrates robust performance in predicting csPCa. The urine-based 19BM can serve as a valuable complement to current diagnostic methods, potentially reducing the need for unnecessary invasive procedures, also when combined with mpMRI.

Advancing practice

What does this study add?

This study prospectively validates a urine-based biomarker model (19-BM) as a robust tool for predicting clinically significant prostate cancer (csPCa) in prostate cancer diagnostic pathway. The 19-BM outperforms clinically applicable diagnostic approaches, demonstrating strong discriminative ability. When combined with mpMRI, the 19-BM further improves diagnostic accuracy, supporting its role as a non-invasive tool that enables patient stratification to assist clinical decision-making in patients at risk for csPCa.

Patient summary

In this paper, we investigated whether a urine test could enhance the diagnostic pathway for detection of csPCa, and potentially reduce unnecessary biopsies.

1. Introduction

Prostate cancer (PCa) is a major global health concern¹. It is the second most frequently diagnosed cancer among men worldwide, with approximately 1.4 million new cases and 375,000 deaths recorded in 2020². For localized PCa, the prognosis is generally favorable; therefore, early and accurate detection is critical¹. Till recently, diagnostic pathways started with serum prostate-specific antigen (PSA) testing and digital rectal examination (DRE)^{3,4}. However, the limited specificity of PSA results in a high number of unnecessary biopsies⁵ and contributes to the overdiagnosis and overtreatment of clinically insignificant prostate cancer (insPCa)³. Additionally, DRE alone demonstrates low sensitivity and specificity for excluding PCa⁶. To improve diagnostic accuracy, multiparametric magnetic resonance imaging (mpMRI) has increasingly been incorporated into patients' risk stratification and biopsy guidance^{4,7,8}. Despite this, mpMRI faces limitations, including technical quality⁹, inter-reader variability¹⁰ and a modest positive predictive value (PPV) of approximately 35% for csPCa. Consequently, suspicious mpMRI findings—classified according to the Prostate Imaging Reporting and Data System (PI-RADS) often require additional targeted biopsies for confirmation¹¹. Emerging urinary biomarkers are being investigated to improve early detection, refine risk stratification, and guide biopsy decisions, aiming to reduce unnecessary invasive procedures¹². CE-MS-based proteomics has proven to be a powerful platform for urinary biomarker discovery, due to its non-invasive nature, proteome stability, and high-resolution analytical performance¹³. The 19-biomarker model (19-BM), initially developed using this approach, demonstrated high accuracy in distinguishing csPCa from insPCa¹³. Subsequent validation studies have reported high sensitivity and specificity, outperforming PSA and the European Randomized Study of Screening for Prostate Cancer (ERSPC-3/4) risk calculator^{3,13}. This present study aims to prospectively validate the urine-based 19-BM, employing CE-MS, for the prediction of csPCa in biopsy-naïve patients within the current diagnostic pathway.

2. Patients and Methods

2.1 Study design

This study followed the guidelines for reporting tumor marker prognostic studies (REMARK)¹⁴ and for biomarker identification and reporting in clinical proteomics¹⁵. An overview of the study is provided in **Figure 1. Prospective validation**: The validation study examined patients suspected of having csPCa who underwent mpMRI and Trans Rectal Ultrasound (TRUS)-guided prostate biopsy at two major Hospitals, between January 2022 and March 2025 (**Figure 1A**). The research protocol was approved by the local Hospital Research Ethics Committee (n° 340, ref 5454), and all participants provided informed consent. A sample size of 170 patients was estimated to provide 80% power at a two-sided significance level of 0.05, assuming a loss to follow-up of <5%, resulting in an expected analyzable cohort of 161 patients. This calculation takes into account that the distribution and percentage of csPCa according to the

International Society of Urological Pathology (ISUP) criteria ≥ 2 will be at least 30% and that approximately 40-50% of patients are expected to present as low risk in the marker. The patient population included individuals referred for prostate biopsy based on clinical factors, with an ERSPC 3/4+DRE of ≥ 4 for csPCa and PSA < 20ng/ml. All patients underwent mpMRI, and those with PIRADS ≤ 2 were only selected if PSAD $\geq 0.15\text{ng/cc}^2$. Urine samples were prospectively collected before biopsy and subsequent CE-MS analysis was performed. Participants without suspicious mpMRI findings underwent systematic 12-core TRUS biopsy, and those with suspicious lesions on mpMRI received combined targeted +/- systematic biopsies. An expert urologic pathologist evaluated the biopsy specimens using the 2019 International Society of Urological Pathology's modified criteria¹⁶. csPCa was defined as an ISUP ≥ 2 . Patient characteristics are summarized in **Table 1**, including summary statistics per clinical centre (**Supplementary Table S1**). ***Evaluation of prostatectomy cohort:*** Patients receiving radical prostatectomy (RP; **Figure 1B**) as part of the FIS trial¹⁷ funded by Science and Innovation Ministry (ISC III, FEDER) were considered to assess the correlation between 19-BM scores with the pathology at RP. Ethical approval was obtained from the Reina Sofia Hospital Research Ethics Committee (approval number PI22/01769), and informed consent was obtained from all participants. A summary of the patient variables is provided in **Supplementary Table S2**.

2.2 Prostate mpMRI Scan

Prostate mpMRIs were conducted using 3T systems (MagnetomVida, Siemens AG, Erlangen, Germany) with the 18-channel and a 3T MRI system (Ingenia, Philips, Eindhoven, Netherlands) 32-channel phased-array body coils. The imaging protocol included T1-weighted, T2-weighted in three orthogonal planes, diffusion-weighted for Virgen del Rocio Hospital, and dynamic contrast-enhanced techniques, which was performed only at Reina Sofia Hospital. Lesions were identified and localized based on the prostate sectors as recommended by the PIRADS version 2.1 by JP and DLR with more than 10 years of experience in genitourinary mpMRI. Furthermore, a final assessment category was assigned for each patient. The images were analyzed using the Picture Archiving and Communication System (PACS) (Vue PACS, Philips), Syngo.via, MRI prostate software and Siemens systems. Grouping of the patients based on PIRADS score is provided in **Supplementary Table S3**.

2.3 Urine Collection, Storage, and Proteomics Analysis

Urine samples were collected before biopsy, adhering to established protocols without preceding any prostate massage or DRE¹³. Voided urine specimens were obtained using sterile containers and promptly stored at -20°C until processing. Urine fractionation and peptide extraction were performed following well-established standard operating protocols (SOP)¹³ also described in detail in **Supplementary Text**. CE-MS analysis and data processing followed the previously developed SOPs¹⁸ and ISO13485 standards, generating quality-controlled urinary datasets^{19,20}. In brief, mass over charge signals were converted into a single mass, with each peptide accompanied by molecular mass,

migration time and normalized signal intensity²¹. Normalization was based on 29 internal calibrants (**Supplementary Text**)²².

2.4 Model Scoring and Statistical Analysis

Descriptive statistics (proportions, means, standard deviations, medians, and interquartile ranges) were calculated using RStudio (v2024.12.1+563) and are summarized in **Table 1**. Biomarker scores were generated using SVM-based MosaCluster software (v1.7.0) based on the 19BM model¹³ that integrates 19 peptide markers (listed in **Supplementary Table S4**). Diagnostic performance was assessed by sensitivity, specificity, ROC curves with confidence intervals, and AUC, using a validated cutoff of -0.07 ¹³. Comparisons of AUCs were performed using the DeLong test, and classification scores across PI-RADS and ISUP grade groups were compared using the Kruskal–Wallis test in R studio (Version 2024.12.1+563). Diagnostic nomograms incorporating the 19-BM biomarker, ERSPC scores, and clinical variables and/or mpMRI findings were developed using multiple linear regression. Clinical utility was evaluated by decision curve analysis²³ to estimate net benefit across biopsy decision thresholds. The performance of PSA density (PSAD) and the urinary 19-BM score for prostate cancer detection was further evaluated by clinical significance. PSAD was calculated as serum PSA divided by prostate volume (ng/mL/cc), and 19-BM scores were analyzed as continuous variables. Test performance across quartile-based cutoffs was assessed by relative positive fractions in three diagnostic groups (ISUP 1, biopsy-selected patients, and ISUP ≥ 2), normalized to the lowest cutoff. Test value distributions were visualized using kernel density estimates, with clinically relevant cutoffs indicated.

3. Results

3.1 Cohort characterization

Patients with csPCa had higher PSA density ($p=0.017$) and ERSPC risk scores ($p=0.004$, **Table 1**) compared with those with insPCa. DRE findings differed significantly between groups ($p<0.001$), with a higher proportion of pathological DREs in csPCa. PI-RADS scores were also significantly higher in the significant PCa group ($p=0.006$). No significant differences were observed for age, PSA level, prostate volume, family history, biopsy type, lesion location, or prior biopsy status (all $p>0.05$; **Table 1**).

3.2 Prospective validation of 19-BM

The 19-BM scores significantly distinguished patients with csPCa (ISUP ≥ 2 ; $p<0.001$; **Figure 2A**). A statistically significant difference in 19-BM scores was also shown across patients with different ISUP grades ($p<0.001$; Kruskal-Wallis test), suggesting that 19BM scores are associated with increasing tumor grade (**Figure 2B**). Urinary 19-BM, PSA, ERSPC, and mpMRI were comparatively analyzed for their effectiveness in identifying csPCa. Urinary 19-BM exhibited the highest discriminative ability with an AUC of 0.79 (95% CI:0.72–0.86; **Figure 2C**) with a sensitivity of 94% and a specificity of 52% significantly outperforming PSA [AUC 0.54 (0.45-0.63; 95% CI) $p<0.0001$], ERSPC [AUC 0.63 (0.55-0.72; 95% CI)

p=0.0108], PSAD [AUC 0.61 (0.53-0.69; 95% CI)p=0.0021] and mpMRI [AUC 0.65 (0.57-0.72; 95% CI) p=0.0069](**Figure 2C**). Sub-analysis in patients with low PIRADS scores (n=19), demonstrated a specificity of 90%, sensitivity of 75%, NPV of 93% and PPV of 67%, indicating notable performance within the low PIRADS subgroup.

3.2 Validation of nomogram with mpMRI

The diagnostic precision of the 19-BM, along with mpMRI, in identifying csPCa was validated with an estimated AUC of 0.82 (p = 0.19, 95% CI: 0.76 – 0.89), indicating a strong discrimination of csPCa from insPCa. The combined nomogram exhibited a specificity of 90% and a sensitivity of 64%, and NPV of 91% (82.3-96.8; 95% CI) and PPV of 62% (50.8-71.6; 95% CI), respectively. This nomogram has superior diagnostic ability but not significantly better than 19-BM alone (AUC difference=0.03; p=0.19, 95% CI: -0.02–0.08). The diagnostic performance of the nomogram was compared with another nomogram combining ERSPC scores with mpMRI using ROC curve analysis (**shown in Figure 3A**). The AUC values for the nomograms were: 19-BM and mpMRI (0.82) and ERSPC and mpMRI (0.70). These findings reveal that the 19-BM and mpMRI Nomogram is significantly better than the ERSPC and mpMRI nomogram (AUC difference =0.13; p=0.003, 0.04–0.21; 95% CI). The decision curve analysis (**Figure 3B**) indicated that the 19-BM model consistently provided a greater net benefit compared to the other available variables by their own.

3.4 19-BM reduces the over-detection of insignificant PCa in the current diagnostic pathway

To investigate added value over the state of the art for guiding the diagnostic pathway, we have compared the levels of relative positive fractions of insPCa and csPCa, as described in the methods, between the PSAD, 19-BM, and the nomogram reported herein within the current diagnostic path, defined by a PSA level of >3ng/ml (**Figure 4A-B-C-D**). Across increasing cutoff values, both PSAD, the urinary 19-BM score, and the nomogram, without and with mpMRI, demonstrated declining relative positive fractions in all diagnostic groups. However, the urinary 19BM score showed better separation between insignificant and csPCa than PSAD. For PSAD (**Figure 4A**), the relative positive fractions for ISUP 1 and ISUP ≥2 cancers declined in a largely parallel manner, indicating limited discrimination and a higher risk of over-detection of insignificant PCa. In contrast, the 19-BM scores exhibited a bigger decline in positivity among ISUP 1 cancers, while maintaining higher positivity for ISUP ≥2 cancers across clinically relevant cutoffs (**Figure 4B**). At thresholds where detection of csPCa remained substantial, the proportion of positive tests among insignificant cancers was markedly lower for the 19BM score compared with PSAD density, indicating that the urinary 19BM score is associated with reduced over-detection of insignificant prostate cancer while preserving detection of clinically significant disease. Improved proportions for insPCa correctly classified as negative based on the nomogram were demonstrated both when including and also without mpMRI (**Figure 4C-D**).

3.5 Prediction of ISUP at RP

As the correlation between ISUP grade in biopsy and radical prostatectomy (RP) specimen is not always perfect, the 19-BM scores were further correlated with the pathology after radical prostatectomy. 100 patients receiving RP (as summarized in **Figure 1B**) were considered¹⁷. The analysis revealed a discrimination between csPCa and insPCa (**Supplementary Figure SF1**) and, also, a clear positive correlation with an increased ISUP and adverse pathology in RP specimens (defined as pT3 and/or $\geq$ ISUP 3) (**Supplementary Figure SF2**; $p < 0.01$).

Discussion

This prospective multicenter validation study confirms the diagnostic potential of the urine-based (19-BM as a non-invasive tool for predicting csPCa in biopsy-naïve patients, including those with low PIRADS scores. Our findings demonstrate that 19-BM achieved superior diagnostic accuracy compared with current diagnostic pathways, introducing further options in this process. Importantly, the integration of 19-BM with mpMRI further enhanced predictive performance, supporting its potential role as a complementary test in the diagnostic pathway. This is of added value with the focus on introducing structure early diagnoses, and trying to further improve the detection of csPCa while reducing unnecessary biopsies and overdiagnoses^{24,25}. The 19-BM demonstrated an AUC of 0.79, with a sensitivity of 91% and a specificity of 64%, outperforming PSA (AUC 0.46), ERSPC (AUC 0.64), and mpMRI (AUC 0.65). These findings are consistent with previous validation studies showing that urinary proteomic signatures can accurately differentiate between clinically significant and indolent prostate cancer. The robust discrimination observed across independent cohorts reinforces the reproducibility and reliability of the 19-BM model, particularly given the use of standardized CE-MS workflows under ISO13485-certified conditions. This standardization minimizes analytical variability and supports the model's potential for clinical translation.

The combination of 19-BM with mpMRI in a diagnostic nomogram yielded an improved AUC of 0.82, yet not significantly better than the 19-BM alone. Both net benefit analysis and distribution of positive fractions for ins and csPCa suggests that incorporating 19-BM into existing diagnostic algorithms could reduce unnecessary biopsies while maintaining a high detection rate for csPCa. The superiority of the nomogram including the 19-BM with mpMRI compared with the nomogram including ERSPC and mpMRI model (AUC 0.70) underscores the added value of proteomic information over conventional clinical parameters and other urinary biomarkers tested in similarly designed cohorts²⁶. A particularly noteworthy finding is the performance of 19-BM in patients with low PIRADS scores (≤ 3), where the AUC reached 0.73 with 75% sensitivity and 90% specificity. Detecting significant disease in this subgroup remains challenging, as mpMRI alone exhibits limited sensitivity for low-grade lesions. The ability of 19-BM to identify a subset of patients with csPCa despite non-suspicious mpMRI findings suggests that urinary proteomics may capture tumor-related molecular alterations not visible via imaging^{27,28}. This highlights the potential of 19-BM as a rule-in or triage test in cases where imaging is inconclusive or negative but clinical suspicion persists. Our findings align with recent literature supporting the integration of urinary biomarkers such as SelectMDx, PCA3, and ExoDx into clinical workflows, though

19-BM appears to offer a broader proteomic signature with superior discriminatory power²⁹. An additional advantage is the non-invasive nature of the approach considering that urine sampling does not require any prior prostate massage or digital rectal examination. Moreover, unlike gene-expression-based assays, the proteomic approach directly reflects disease-related physiological processes, offering stability and reproducibility advantages in clinical practice^{30,31}.

Nevertheless, several limitations should be acknowledged. Although the study employed a prospective design and included independent validation across two major centers, the sample size—particularly within the low PI-RADS subgroup—was relatively small, which may have limited statistical power. In addition, heterogeneity between the two hospitals was observed, as reflected by differences in the detection rate of clinically significant prostate cancer (csPCa) (63.7% in one center versus 30% in the other). However, this variability may reflect the pragmatic study design and the consistent performance of the biomarker across settings. Finally, future studies should focus on cost-effectiveness analyses and integration into risk stratification algorithms.

In summary, this study provides strong prospective evidence supporting the clinical validity of the urine-based 19-BM proteomic test for detecting clinically significant prostate cancer in current diagnostic pathways. The 19-BM outperformed PSA, ERSPC, and mpMRI alone and, when combined with mpMRI, achieved the highest overall accuracy and clinical net benefit. The model's strong performance in biopsy-naïve patients with low PIRADS scores further underscores its potential as a complementary non-invasive tool to optimize patient selection for biopsy, reduce overtreatment, and improve prostate cancer diagnostic precision.

Conclusion

The urine-based 19-BM demonstrates robust performance in predicting clinically significant prostate cancer. This proteomics test, when combined with magnetic resonance imaging, can serve as a valuable complement to current diagnostic methods, potentially reducing the need for unnecessary invasive procedures.

Declarations

Funding/ Support

This study received partial funding from PI22/01769 (FIS [Ministry of Science and Innovation, ISC III, FEDER]), from ProSTRAT-AI (01DS23014; Federal Ministry of Education and Research/BMBF/EUREKA Network), and also from PROMOTE (101169245: HORIZON – MSCA-2023-DN-01-01). Dr. Kirubel Nigusu Jobre obtained funding through the European Union's Horizon 2020 Research and Innovation Programme with the Marie Skłodowska-Curie Grant Agreement No. 101169245. The writers state that the sponsor did not take part in the research or influenced its results.

Conflict of interest statement

Prof. Harald Mischak has ownership stakes in Mosaiques Diagnostics GmbH. Dr. Maria Frantzi and Dr. Kirubel Nigusu Jobre are employed by Mosaiques Diagnostics. The other authors have no conflict of interest to declare.

Ethics statement

The research protocol for this study was evaluated and sanctioned by the “Reina Sofia Hospital Research Ethics Committee,” with Approval No. [(nº 340, ref 5454), PI22/01769]. Informed consent in written form was acquired from all participants.

Authors contributions

Kirubel Nigusu Jobre, Ana C. Morillo, Maria Frantzi, and Enrique Gómez Gómez made significant contributions to the conceptualization and design of the manuscript. The data analysis and interpretation were conducted by Kirubel Nigusu Jobre, Ana C. Morillo, Maria Frantzi, and Enrique Gómez Gómez. Contributions to the data acquisition were provided by Guillermo Lendinez, Ana Blanca, Daniel Lopez Ruiz, Jose Parada, Julia Carrasco-Valiente, and Antonio Lopez Beltran. Statistical analysis was carried out by Maria Frantzi and Kirubel Nigusu Jobre. The initial draft of the manuscript was created with the input of Kirubel Nigusu Jobre, Ana C. Morillo, Maria Frantzi, and Enrique Gómez Gómez. All authors engaged in the critical revision and correction of the manuscript concerning scientific and factual accuracy. The supervision of the study was conducted by Enrique Gómez Gómez, Rafael A. Medina, Pablo Campos Hernandez, Isabel Heidegger, Zoran Culig, and Harald Mischak. Every author reviewed and approved the final manuscript version. Each author contributed equally to the manuscript and consented to its final version.

References

1. Cornford P, Van Den Bergh RCN, Briers E, et al. EAU-EANM-ESTRO-ESUR-ISUP-SIOG Guidelines on Prostate Cancer—2024 Update. Part I: Screening, Diagnosis, and Local Treatment with Curative Intent. *Eur Urol*. 2024;86(2):148-163. doi:10.1016/j.eururo.2024.03.027
2. Culp MB, Soerjomataram I, Efsthathiou JA, Bray F, Jemal A. Recent Global Patterns in Prostate Cancer Incidence and Mortality Rates. *Eur Urol*. 2020;77(1):38-52. doi:10.1016/j.eururo.2019.08.005
3. Frantzi M, Heidegger I, Roesch MC, et al. Validation of diagnostic nomograms based on CE-MS urinary biomarkers to detect clinically significant prostate cancer. *World J Urol*. 2022;40(9):2195-2203. doi:10.1007/s00345-022-04077-1
4. Israël B, Hannink G, Barentsz JO, Van Der Leest MMG. Implications of the European Association of Urology Recommended Risk Assessment Algorithm for Early Prostate Cancer Detection. *Eur Urol Open Sci*. 2022;43:1-4. doi:10.1016/j.euros.2022.06.006
5. Fenton JJ, Weyrich MS, Durbin S, Liu Y, Bang H, Melnikow J. Prostate-Specific Antigen-Based Screening for Prostate Cancer: Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA*. 2018;319(18):1914-1931. doi:10.1001/jama.2018.3712

6. Naji L, Randhawa H, Sohani Z, et al. Digital Rectal Examination for Prostate Cancer Screening in Primary Care: A Systematic Review and Meta-Analysis. *Ann Fam Med*. 2018;16(2):149-154. doi:10.1370/afm.2205
7. Turkbey B, Rosenkrantz AB, Haider MA, et al. Prostate Imaging Reporting and Data System Version 2.1: 2019 Update of Prostate Imaging Reporting and Data System Version 2. *Eur Urol*. 2019;76(3):340-351. doi:10.1016/j.eururo.2019.02.033
8. Drost FJH, Osses DF, Nieboer D, et al. Prostate MRI, with or without MRI-targeted biopsy, and systematic biopsy for detecting prostate cancer. Cochrane Urology Group, ed. *Cochrane Database Syst Rev*. 2019;2019(4). doi:10.1002/14651858.CD012663.pub2
9. Giganti F, Ng A, Asif A, et al. Global Variation in Magnetic Resonance Imaging Quality of the Prostate. *Radiology*. 2023;309(1):e231130. doi:10.1148/radiol.231130
10. Sonn GA, Fan RE, Ghanouni P, et al. Prostate Magnetic Resonance Imaging Interpretation Varies Substantially Across Radiologists. *Eur Urol Focus*. 2019;5(4):592-599. doi:10.1016/j.euf.2017.11.010
11. Westphalen AC, McCulloch CE, Anaokar JM, et al. Variability of the Positive Predictive Value of PI-RADS for Prostate MRI across 26 Centers: Experience of the Society of Abdominal Radiology Prostate Cancer Disease-focused Panel. *Radiology*. 2020;296(1):76-84. doi:10.1148/radiol.2020190646
12. Farha MW, Salami SS. Biomarkers for prostate cancer detection and risk stratification. *Ther Adv Urol*. 2022;14:17562872221103988. doi:10.1177/17562872221103988
13. Frantzi M, Gomez Gomez E, Blanca Pedregosa A, et al. CE-MS-based urinary biomarkers to distinguish non-significant from significant prostate cancer. *Br J Cancer*. 2019;120(12):1120-1128. doi:10.1038/s41416-019-0472-z
14. Hayes DF, Sauerbrei W, McShane LM. REMARK guidelines for tumour biomarker study reporting: a remarkable history. *Br J Cancer*. 2023;128(3):443-445. doi:10.1038/s41416-022-02046-4
15. Mischak H, Allmaier G, Apweiler R, et al. Recommendations for biomarker identification and qualification in clinical proteomics. *Sci Transl Med*. 2010;2(46):46ps42. doi:10.1126/scitranslmed.3001249
16. van Leenders GJLH, van der Kwast TH, Grignon DJ, et al. The 2019 International Society of Urological Pathology (ISUP) Consensus Conference on Grading of Prostatic Carcinoma. *Am J Surg Pathol*. 2020;44(8):e87-e99. doi:10.1097/PAS.0000000000001497
17. Frantzi M, Morillo AC, Lendinez G, et al. Validation of a Urine-Based Proteomics Test to Predict Clinically Significant Prostate Cancer: Complementing mpMRI Pathway. *Pathobiol J Immunopathol Mol Cell Biol*. Published online November 11, 2024:1-10. doi:10.1159/000542465
18. Mischak H, Vlahou A, Ioannidis JPA. Technical aspects and inter-laboratory variability in native peptide profiling: the CE-MS experience. *Clin Biochem*. 2013;46(6):432-443. doi:10.1016/j.clinbiochem.2012.09.025

19. Latosinska A, Siwy J, Mischak H, Frantzi M. Peptidomics and proteomics based on CE-MS as a robust tool in clinical application: The past, the present, and the future. *Electrophoresis*. 2019;40(18-19):2294-2308. doi:10.1002/elps.201900091
20. Mavrogeorgis E, Mischak H, Latosinska A, Siwy J, Jankowski V, Jankowski J. Reproducibility Evaluation of Urinary Peptide Detection Using CE-MS. *Mol Basel Switz*. 2021;26(23):7260. doi:10.3390/molecules26237260
21. Frantzi M, Metzger J, Banks RE, et al. Discovery and validation of urinary biomarkers for detection of renal cell carcinoma. *J Proteomics*. 2014;98:44-58. doi:10.1016/j.jprot.2013.12.010
22. Siwy J, Mullen W, Golovko I, Franke J, Zürgbig P. Human urinary peptide database for multiple disease biomarker discovery. *Proteomics Clin Appl*. 2011;5(5-6):367-374. doi:10.1002/prca.201000155
23. Vickers AJ, Holland F. Decision curve analysis to evaluate the clinical benefit of prediction models. *Spine J Off J North Am Spine Soc*. 2021;21(10):1643-1648. doi:10.1016/j.spinee.2021.02.024
24. Arvendell M, Phillips L, Delilovic S, et al. Men's Attitudes Towards Participation in Organised Prostate Cancer Testing: An Abductive Thematic Analysis. *Eur Urol Open Sci*. 2025;71:156-164. doi:10.1016/j.euros.2024.12.007
25. Roobol MJ, De Vos II, Månsson M, et al. European Study of Prostate Cancer Screening – 23-Year Follow-up. *N Engl J Med*. 2025;393(17):1669-1680. doi:10.1056/NEJMoa2503223
26. Lendínez-Cano G, Ojeda-Claro AV, Gómez-Gómez E, et al. Prospective study of diagnostic accuracy in the detection of high-grade prostate cancer in biopsy-naïve patients with clinical suspicion of prostate cancer who underwent the Select MDx test. *The Prostate*. 2021;81(12):857-865. doi:10.1002/pros.24182
27. Oderda M, Marquis A, Bertero L, et al. Histopathologic Features and Transcriptomic Signatures Do Not Solve the Issue of Magnetic Resonance Imaging-Invisible Prostate Cancers: A Matched-Pair Analysis. *The Prostate*. 2025;85(4):374-384. doi:10.1002/pros.24838
28. Parry MA, Srivastava S, Ali A, et al. Genomic Evaluation of Multiparametric Magnetic Resonance Imaging-visible and -nonvisible Lesions in Clinically Localised Prostate Cancer. *Eur Urol Oncol*. 2019;2(1):1-11. doi:10.1016/j.euo.2018.08.005
29. Groen L, Schalken J. Liquid Biopsy for Prostate and Bladder Cancer: Progress and Pitfalls. *Eur Urol Focus*. 2022;8(4):904-906. doi:10.1016/j.euf.2022.08.013
30. Heidegger I, Frantzi M, Salcher S, et al. Prediction of Clinically Significant Prostate Cancer by a Specific Collagen-related Transcriptome, Proteome, and Urinome Signature. *Eur Urol Oncol*. 2025;8(3):652-662. doi:10.1016/j.euo.2024.05.014
31. Frantzi M, Tymoszek P, Salcher S, et al. Prediction of Prostate Cancer Biochemical Recurrence After Radical Prostatectomy by Collagen Models Using Multiomic Profiles. *Eur Urol Oncol*. Published online April 30, 2025:S2588-9311(25)00094-X. doi:10.1016/j.euo.2025.03.016

Tables

Table 1 is available in the Supplementary Files section.

Figures

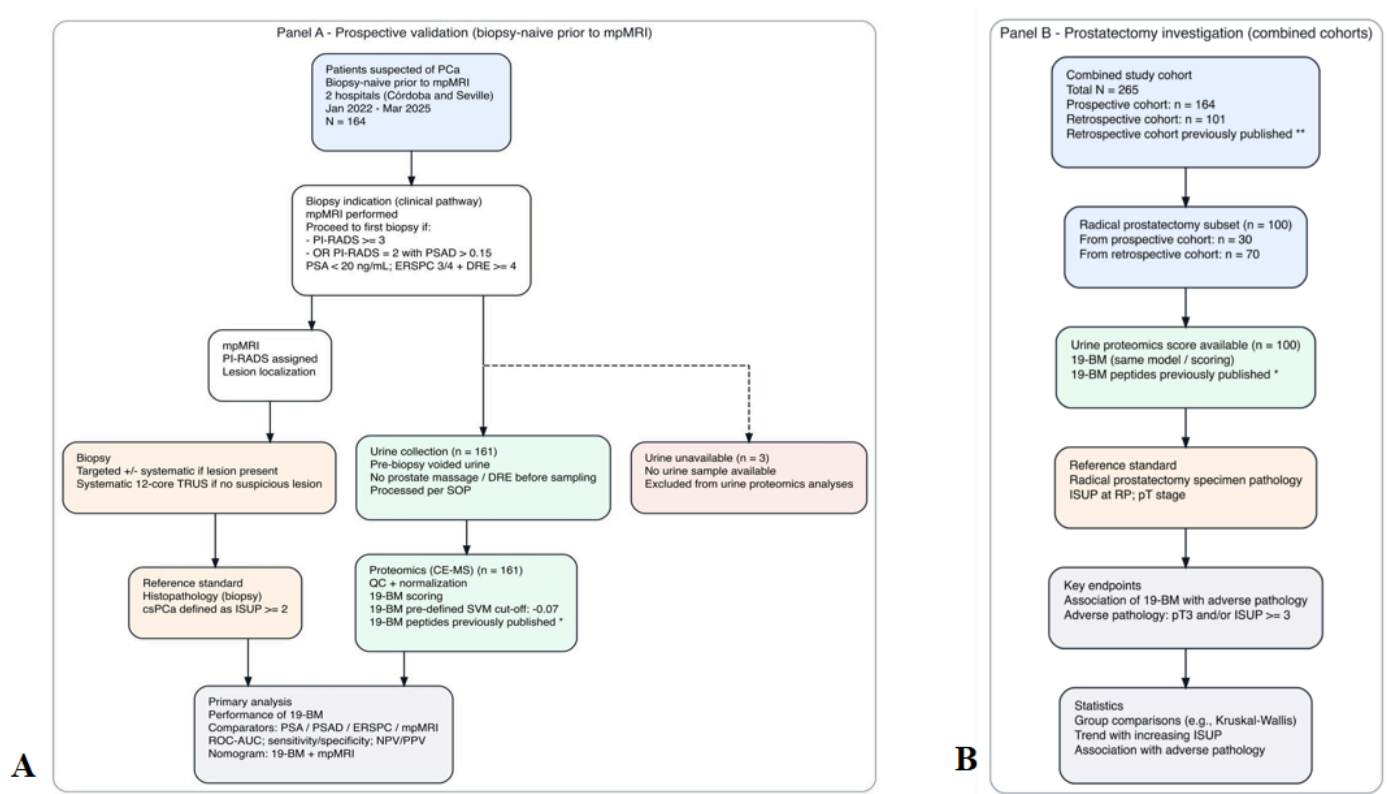


Figure 1

Study overview including A) biopsy-naïve patients at risk for clinically significant PCa (csPCa) and B) patients receiving radical prostatectomy (RP). *Abbreviations: mpMRI= multiparametric Magnetic Resonance Imaging; PCa= Prostate cancer; PI-RADS= Prostate Imaging–Reporting and Data System; PSAD= Prostate-Specific Antigen Density; PSA= Prostate-Specific Antigen; ERSPC= European Randomized Study of Screening for Prostate Cancer; DRE= Digital Rectal Examination; TRUS= Transrectal Ultrasound; SOP= Standard Operating Procedure; CsPCa= Clinically Significant Prostate Cancer; CE-MS= Capillary Electrophoresis–Mass Spectrometry; QC= Quality Control; SVM= Support Vector Machine; ROC-AUC= Area Under the Receiver Operating Characteristic Curve; NPV= Negative Predictive Value; PPV= Positive Predictive Value; ISUP= International Society of Urological Pathology; RP= Radical Prostatectomy; pT stage= pathological Tumor Stage.*

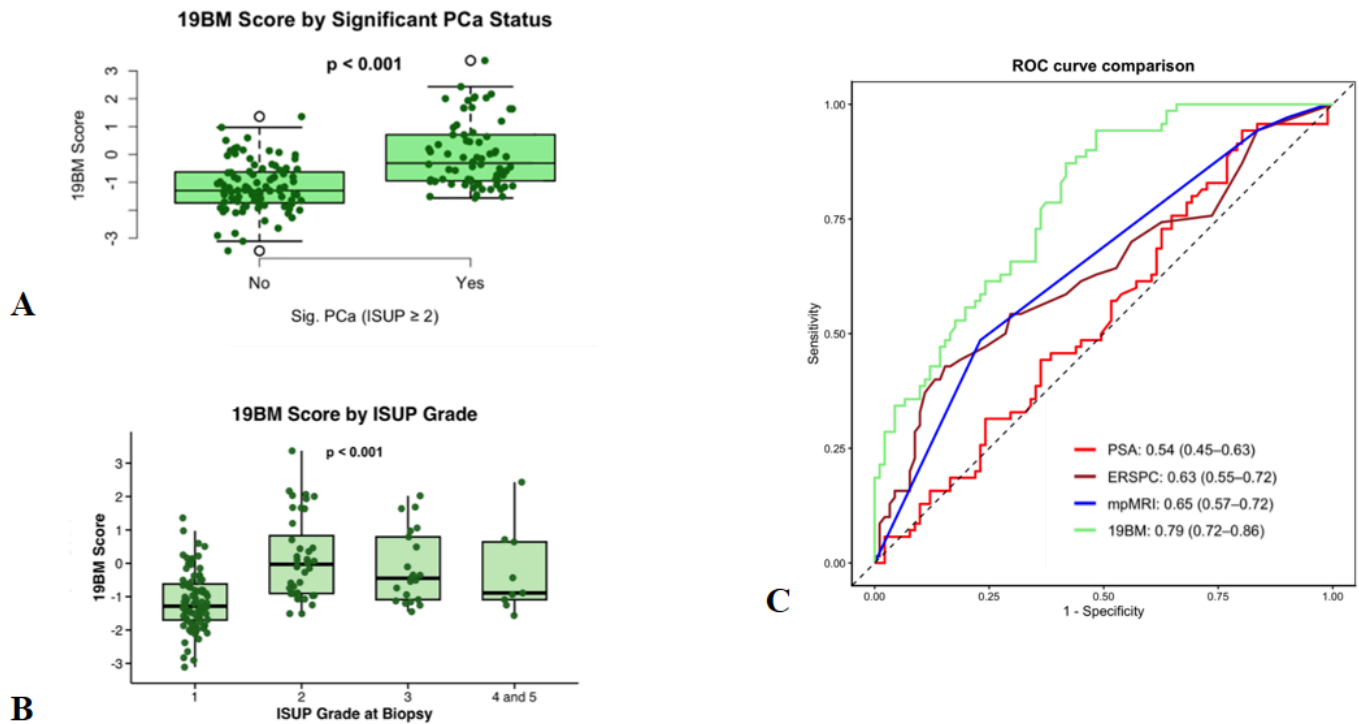


Figure 2

A) Comparison of 19-BM Scores according to cancer significance (csPCa, ISUP ≥ 2) using Kruskal-Wallis test, $p < 0.001$. **B)** Panel B illustrates the distribution of 19-BM scores among ISUP grades 1 to 4. The box plot for each grade shows the median, the interquartile range, and individual data points. **C)** ROC curve comparison of 19-BM, PSA, mpMRI and ERSPC in the evaluation of diagnostic performance.

Abbreviations: PCa= Prostate cancer; ISUP= International Society of Urological Pathology; ROC= Receiver Operating Characteristic; PSA= Prostate-Specific Antigen; ERSPC= European Randomized Study of Screening for Prostate Cancer; mpMRI= multiparametric Magnetic Resonance Imaging.

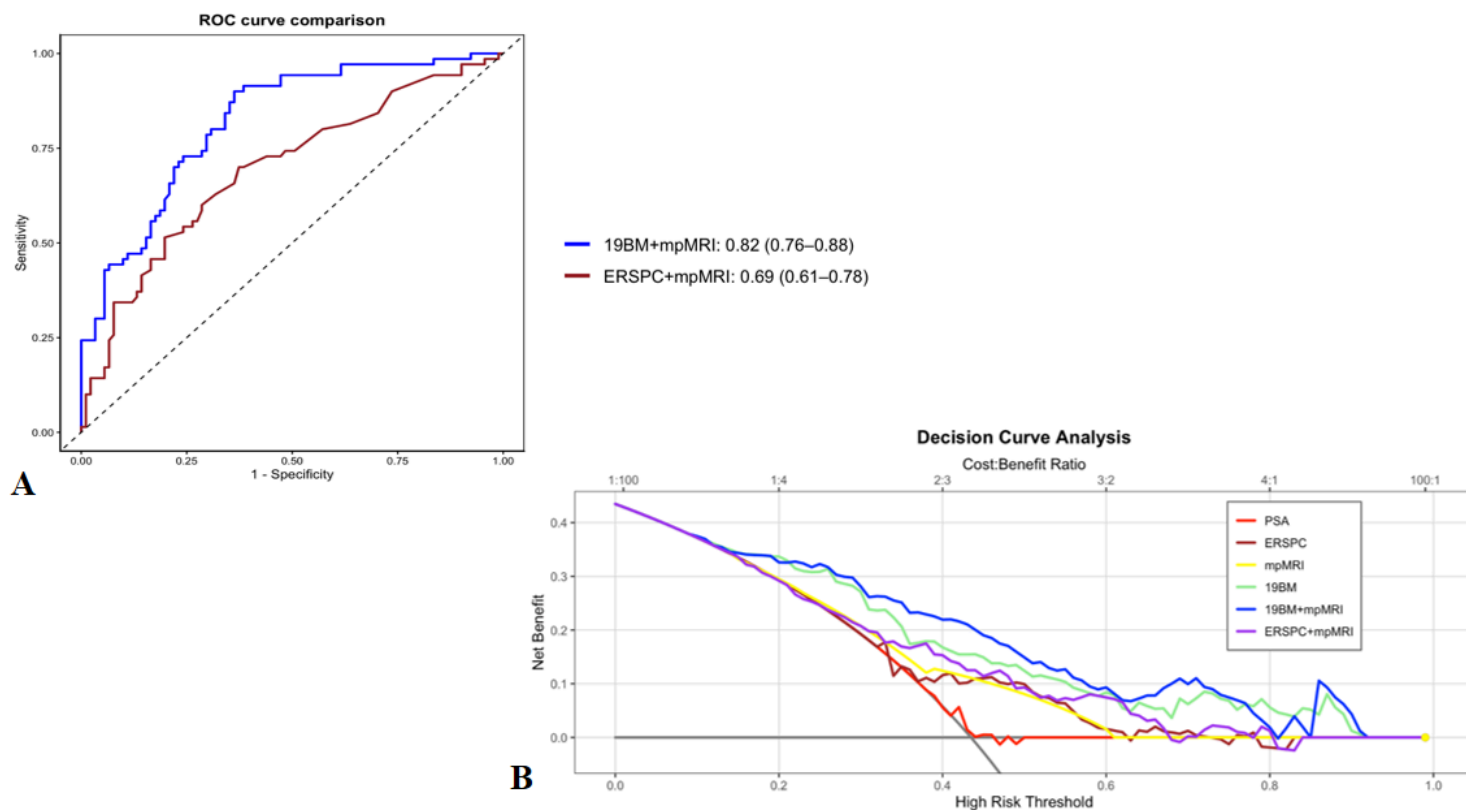


Figure 3

A) ROC curves comparing the diagnostic performance of 19-BM and mpMRI nomogram with ERSPC and mpMRI nomogram. **B)** Analysis of decision curves comparing the net advantages of various diagnostic approaches for prostate cancer at different high-risk thresholds. Abbreviations: ROC= Receiver Operating Characteristic; mpMRI= multiparametric Magnetic Resonance Imaging; ERSPC= European Randomized Study of Screening for Prostate Cancer; PSA= Prostate-Specific Antigen.

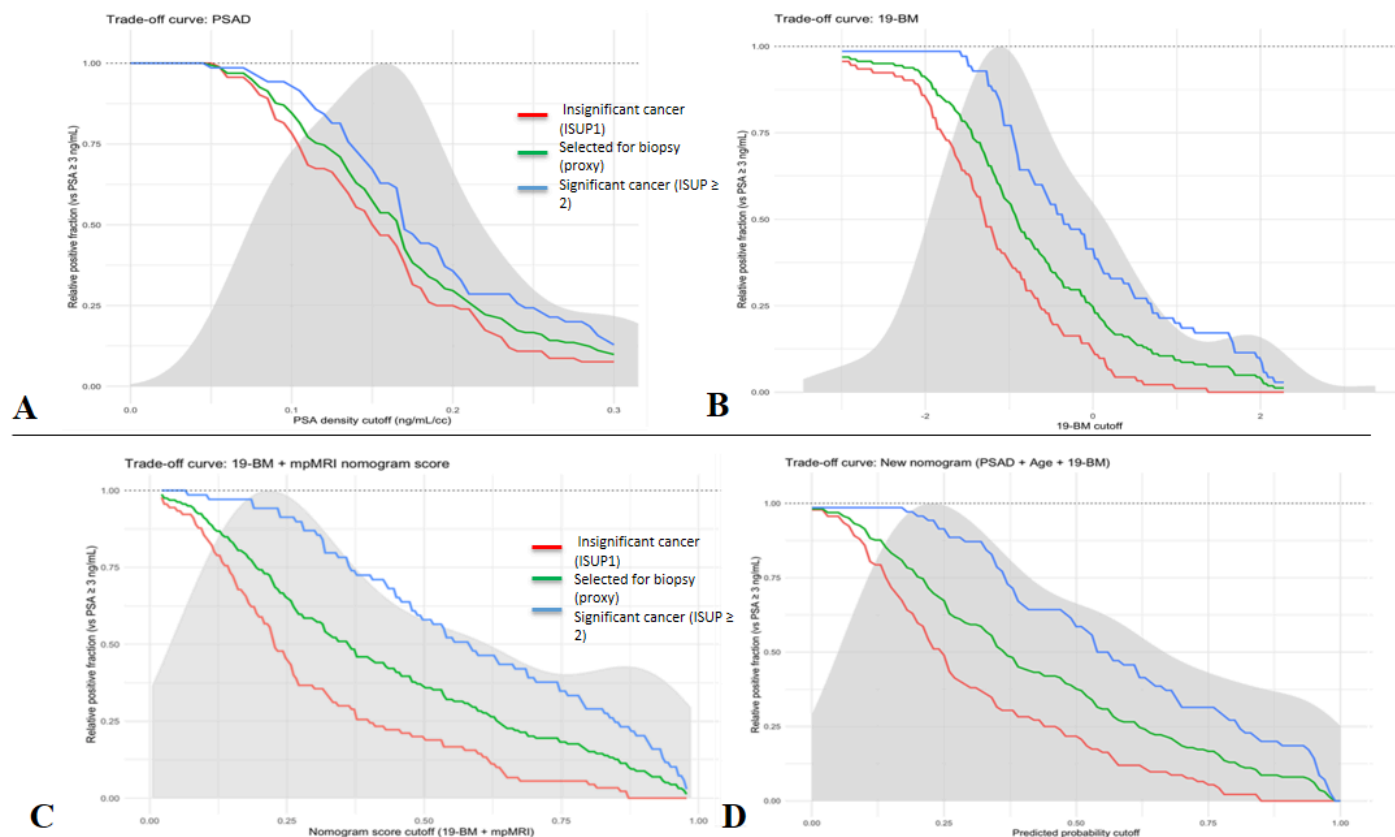


Figure 4

Performance of PSAD and 19-BM score for PCa risk stratification. **A)** Relative positive fraction (sensitivity) for detecting PCa across increasing PSAD cutoffs (ng/ml/cc). Curves are shown for insignificant cancer (ISUP grade group 1; red), patients selected for biopsy (proxy; green), and csPCa (ISUP ≥ 2 ; blue). The grey shaded area represents the distribution of PSAD in the cohort. Vertical dashed lines indicate pre-specified or clinically relevant PSAD. **B)** Relative positive fraction across increasing 19-BM. Curves represent the same outcome groups as in panel **A**. The grey shaded area shows the distribution of urinary biomarker scores in the cohort, and vertical dashed lines denote selected decision thresholds. **C)** Relative positive fraction across increasing monogram scores with and **D)** without mpMRI. *Abbreviations: PSAD= Prostate-Specific Antigen Density; ISUP= International Society of Urological Pathology; mpMRI= multiparametric Magnetic Resonance Imaging.*

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Figsup1.png](#)
- [MorilloJobreetal.SupplementaryText.docx](#)
- [MorilloJobreetal.SupplementaryTables.docx](#)
- [Figsup2.png](#)

- [MorilloJobreetal.Table1.docx](#)