Experimental Study of Proclarix as a Tumor Marker for the Early Detection of Clinically Significant Prostate Cancer

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Doctoral Thesis

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"Research is to see what everybody else has seen, and to think what nobody else has thought."

Albert Szent-Györgyi

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LIST OF ABBREVIATIONS

4Kscore: 4-kallikrein panel

5-ARIs: 5-alpha reductase inhibitors

AUC: area under the curve

BCR: biochemical recurrence

CsPCa: clinically significant prostate cancer

CT: computed tomography

CTSD: cathepsin D

DRE: digital rectal examination

EAU: European Association of Urology

ERSPC: European Randomized Study of Screening for Prostate Cancer

fPSA: percentage of free prostate-specific antigen

GG: grade group

GS: Gleason score

IPCa: insignificant prostate cancer

ISUP: International Society of Urological Pathology

MpMRI: multiparametric magnetic resonance imaging

mRNA: messenger RNA

NPV: negative predictive value

PCa: Prostate Cancer

Prostate Cancer Antigen 3: PCA3

PHI: Prostate Health Index

PI-RADS: Prostate Imaging-Reporting Data System

PPV: positive predictive value

PSA: Prostate-specific antigen

PSAD: Prostate-specific antigen density

PV: prostate volume

THBS1: Thrombospondin-1

TNM: Tumor-Node-Metastasis

TRUS: Transrectal ultrasound

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SUMMARY

1.1 INTRODUCTION

Early detection of clinically significant prostate cancer (csPCa) decreases the specific mortality of the disease. The initial suspicion of PCa is based on an elevation of serum prostate-specific antigen (PSA) and/or an abnormal digital rectal examination (DRE), and requires further confirmation with prostate biopsy. Nonetheless, this current diagnostic approach often leads to a high rate of unnecessary prostate biopsies and an overdetection of insignificant PCa. Proclarix is a recently introduced blood-based marker that provides a risk score for the detection of csPCa (ranging from 0 to 100%) based on the serum determination of Thrombospondin-1, Cathepsin D, total prostate-specific antigen (PSA) and free PSA, in addition to age. This new test has been developed and validated to differentiate men without PCa or insignificant PCa from those with csPCa with serum PSA between 2 and 10ng/mL, prostate volume ≥ 35mL and normal DRE. We aimed to assess the clinical value of incorporating Proclarix into the diagnostic pathway along with multiparametric magnetic resonance imaging (mpMRI) and determine its potential for improving the selection of appropriate candidates for prostate biopsies. Finally, it is also needed to assess the relationship between Proclarix and the aggressiveness of PCa.

1.2. HYPOTHESIS

Proclarix is a new tumor marker developed with the aim of enhancing the early detection of clinically significant prostate cancer. It reduces the demand of

multiparametric magnetic resonance imaging in men with suspicion of PCa and improve the selection of candidates for prostate biopsy. It is also correlated with PCa aggressiveness.

1.3. OBJECTIVES

To contrast the hypotheses proposed in the previous section, we defined the following main objective:

- To investigate the clinical utility and effectiveness of Proclarix in men with suspected csPCa before and after mpMRI.

Moreover, we also proposed the following secondary objectives:

1. To analyze through a systematic review of the literature, how Proclarix was discovered, its characteristics, and the clinical development until the beginning of this project.
2. To evaluate the role of Proclarix in reducing the number of unnecessary prostate biopsies in men with suspected PCa.
3. To compare the performance of Proclarix with PSA density and the Rotterdam-MRI risk-calculator in identifying suitable candidates for prostate biopsy.
4. To analyze the relationship between Proclarix and PCa aggressiveness.

1.4. MATERIALS AND METHODS

The current doctoral is being presented as a compendium of seven publications.

The first publication consisted on a systematic review of the literature and aimed to analyze the current clinical utility of Proclarix for the diagnosis of csPCa. A bibliographic search in PubMed, Cochrane and Trip databased was carried out by two independent reviewers. The Medical Subject Heading (MeSH) terms 'prostate', 'Thrombospondin-1', 'Cathepsin-D' and 'Proclarix' were used. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and the Population, Intervention, Comparison and Outcomes (PICO) selection criteria were followed. The Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool was used to analyze the quality of the included studies. Finally, four articles were included to analyzed the clinical usefulness of Proclarix.

Publications from two to seven analyzed a cohort of 751 men with suspected PCa from two European centers. A prospectively constructed database and a collection of frozen serum samples were used. In case of Vall d´Hebron, 606 men were recruited (https://biobancos. Isciii.es/; Reference collection: 0003439). Database and frozen serums of 159 participants in the INNOVATE study were provided by the University College of London (UCL). The inclusion criteria were men with serum PSA levels ≥ 3ng/mL and/or abnormal DRE, who were scheduled for a 3-tesla mpMRI prior to prostate biopsy. Biopsies were performed from 15 January 2018 to 20 March 2020 in Vall d'Hebron University Hospital and from April 2016 to December 2019 in UCL. Men with PCa on active surveillance and those with symptomatic benign prostatic hyperplasia treated with 5- α-reductase inhibitors were excluded. 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.

The aggressiveness of PCa was assessed from four available surrogate endpoints: the grade group (GG) in prostate biopsies, the clinical stage, the risk of biochemical of localized PCa and the type of pathology in surgical specimen. The GG was defined by the International Society of Urological Pathology categories. The Tumor-Node-Metastasis (TNM) system was used for clinical staging (cT based on DRE, whereas cN and cM were established with computed tomography and 99-technetium bone scintigraphy). The risk of biochemical recurrence of localized PCa after primary treatment was defined by combining PSA, GG, and cT, following D'Amico risk group classification criteria. Finally, an unfavorable pathology in a surgical specimen was defined as GG > 2 or pT ≥ 3.

Our study was conducted in line with Good Clinical Practice guidelines and the ethical principles laid down in the latest version of the Declaration of Helsinki (2013). Before inclusion, all participants signed a written informed consent about the collection and storage of material and personal data in accordance with national bylaws.

1.5. RESULTS

The systematic review of the literature reported that initial studies have shown potential benefit of Proclarix in patients with specific characteristics (PSA levels between 2 and 10ng/mL, normal DRE and prostate volume ≥ 35mL).

The second publication demonstrated that Proclarix can be used be used in all men with suspected PCa, regardless of their serum PSA levels or prostate volume. The area under the curve was similar in patients with serum PSA between 2 and 10ng/mL, normal DRE and prostate volume ≥ 35mL, and those without these specific characteristics (0.701 (95% CI 0.637 – 0.765) and 0.754 (95% CI 0.701 – 0.807), p = 0.038.

By incorporating Proclarix into a diagnostic algorithm along with serum PSA and DRE, using a 10% threshold, a decrease of 25.4% in requests of mpMRI was observed. Additionally, unnecessary prostate biopsies were reduced by 17.7% with a 2.6% of csPCa misdiagnosis.

The third publication reported that Proclarix outperformed the Rotterdam-MRI-risk-calculator and PSA density in the detection of csPCa in men with PI-RADS ≤ 3 lesions on mpMRI. Nonetheless, no tool guaranteed 100% detection of csPCa in PI-RADS 4 and 5 lesions. These findings were consistent with the results reported in the fourth and fifth publications in which Proclarix successfully reduced 21.3% of unnecessary prostate biopsies in men with PI-RADS 3 lesions without missing any cases of csPCa. In contrast, PSA density avoided 26.3% but misdiagnosed 16% of csPCa, and the MRI-Rotterdam-risk-calculator reduced the number of unnecessary prostate biopsies by 7.1% while missing 4% of csPCa. Thus, Proclarix demonstrated a better performance than PSA density and the Rotterdam-MRI-risk-calculator in the selection of candidates for prostate biopsy, especially in men with PI-RADS 3 lesions.

The sixth publication analyzed the combination of Proclarix, mpMRI and prostate volume, resulting in a reduction of two-thirds of unnecessary prostate biopsies. The Proclarix-MRI model exhibited a sensitivity of 90% for detecting csPCa with a negative predictive value of 90% and a positive predictive value of 66%. Notably, the Proclarix-MRI model demonstrated a significantly higher specificity (68%, $p < 0.001$) compared to the MRI-Rotterdam risk-calculator (51%), Proclarix (27%) or mpMRI (28%) alone.

The last publication reported a correlation between Proclarix and the four surrogates of aggressiveness of PCa (GG, the clinical stage, the risk of biochemical recurrence and the pathology in the surgical specimen).

1.6. CONCLUSIONS

Proclarix is a recently approved CE-test that provides the risk of csPCa and can be used in all men with suspected PCa without restrictions on serum PSA or prostate volume. Moreover, Proclarix was able to reduce the number or prostate biopsies in patients with suspected PCa before and after mpMRI. Thus, combining Proclarix with mpMRI has been shown to increase the efficacies of both mpMRI and Proclarix alone. In men with negative mpMRI (PI-RADS < 3), Proclarix demonstrated a 100% sensitivity in the detection of csPCa and was able to avoid one third of prostate biopsies. On the other hand, patients with a positive mpMRI, Proclarix demonstrated the ability to reduce unnecessary prostate biopsies by 43%. In the most challenging scenario of lesions PI-RADS 3, Proclarix was able to reduce 21.3% of unnecessary prostate biopsies without missing any csPCa. Compared to other prediction tools such as PSA density and the Rotterdam-MRI-risk-calculator, Proclarix was found to be more effective in the detection of csPCa. Lastly, Proclarix was correlated with PCa aggressiveness.

RESUMEN

1.1 INTRODUCCIÓN

La detección temprana del cáncer de próstata (CaP) clínicamente significativo disminuye la mortalidad específica de la enfermedad. La sospecha inicial del CaP se basa en un aumento del antígeno prostático específico (*prostate-specific antigen,* PSA) y/o un tacto rectal anormal, y requiere de confirmación adicional con una biopsia prostática. Sin embargo, este enfoque diagnóstico actual a menudo conduce a una alta tasa de biopsias de próstata innecesarias y a una sobre detección de CaP insignificante. Proclarix es un marcador que proporciona el riesgo de detección del CaPcs (del 0 al 100%) basado en la determinación sérica de Trombospondina-1, Catepsina D, PSA total y PSA libre, además de la edad. Este nuevo test se ha desarrollado y validado para diferenciar varones sin CaP o con CaP insignificante de aquellos con CaPcs que tengan unos valores de PSA sérico entre 2 y 10ng/mL, un volumen prostático ≥ 35mL y un tacto rectal normal. Nuestro objetivo fue evaluar el valor clínico de incorporar Proclarix en el algoritmo diagnóstico del CaP junto con la resonancia magnética multiparamétrica (RMNmp) y determinar su potencial de cara a mejorar la selección de candidatos apropiados para la realización de biopsias prostáticas. Por último, también quisimos evaluar la relación entre Proclarix y la agresividad del CaP.

1.2. HIPÓTESIS

Proclarix es un nuevo marcador desarrollado con el objetivo de mejorar la detección precoz del cáncer de próstata clínicamente significativo. Reduce la necesidad

de realizar resonancias magnéticas multiparamétricas en varones con sospecha de CaP y mejora la selección de candidatos para la realización de biopsias prostáticas. También está correlacionado con la agresividad del cáncer de próstata.

1.3. OBJETIVOS

Para contrarastar las hipótesis propuestas en la sección anterior, definismo el siguiente objetivo principal:

- Investigar la utilidad clínica y efectividad de Proclarix en varones con sospecha de cáncer de próstata clínicamente significativo antes y después de la RMNmp.

Además, también propusimos los siguientes objectivos secundarios:

1. Analizar mediante una revisión sistemática de la literatura cómo se descubrió Proclarix, sus características y su desarrollo clínico hasta el inicio de este proyecto.
2. Evaluar el papel de Proclarix en la reducción del número de biopsias de próstata innecesarias en varones con sospecha de cáncer de próstata.
3. Comparar el rendimiento de Proclarix con la densidad de PSA y la calculadora de riesgo de Rotterdam-RMNmp para identificar aquellos candidatos adecuados para la realización de biopsias de próstata.
4. Analizar la relación entre Proclarix y la agresividad del cáncer de próstata

1.4. MATERIAL Y MÉTODOS

La presente tesis doctoral se presenta como un compendio de siete publicaciones.

La primera publicación consistió en una revisión sistemática de la literatura con el objetivo de analizar la utilidad clínica actual de Proclarix para el diagnóstico del CaP clínicamente significativo. Se llevó a cabo una búsqueda bibliográfica en las bases de datos de PubMed, Cochrane y Trip por dos revisores independientes, utilizado los siguientes términos MeSH (*Medical Subject Heading*): "próstata", "Trombospondina-1", "Catepsina D" y "Proclarix". Se siguieron las pautas PRISMA (*Preferred Reporting Items for Systematic Reviews and Meta-Analyses*) y los criterios de selección PICO (*Population, Intervention, Comparison and Outcomes*). La herramienta QUADAS-2 (Quality Assessment of Diagnotic Acurracy Studies 2) fue utilizada para analizar la calidad de los estudios incluidos. Finalmente, cuatro artículos cumplieron los criterios de selección y fueron incluidos en la revisión sistemática.

Las publicaciones de la dos a la siete analizaron una cohorte de 751 varones con sospecha de CaP en dos centros europeos. Se utilizó una base de datos creada prospectivamente y una colección de muestras de suero congeladas. En el Hospital Universitario Vall d'Hebron, se reclutaron 606 varones (https://biobancos. Isciii.es/; Referencia de la colección: 0003439). La base de datos y los sueros congelados de 159 participantes del estudio INNOVATE, fueron proporcionados por la University College of London (UCL). Los criterios de inclusión fueron varones con niveles de PSA sérico ≥ 3ng/mL y/o Un tacto rectal anormal, programados para una RMNmp previa a la biopsia prostática. Las biopsias se realizaron desde el 15 de enero de 2018 hasta el 20 de marzo de 2020 en el Hospital Universitario Vall d'Hebron y desde abril de 2016 hasta diciembre

de 2019 en UCL. Se excluyeron aquellos varones bajo vigilancia activa y aquellos con hiperplasia prostática benigna sintomática tratada con inhibidores de la 5-α-reductasa. En la cohorte original de UCL (n = 291), la mayoría de los hombres con resultados de mpMRI no sospechosos (n = 132), no se sometieron a biopsia y, por lo tanto, no se incluyeron en este estudio.

La agresividad del CaP se evaluó a partir de cuatro subrogados: el grupo de grado (GG) de la biopsia prostática, el estadio clínico, el riesgo de recurrencia bioquímica del CaP localizado y el tipo de patología de la pieza quirúrgica. El GG fue definido por las categorías de la sociedad internacional de patología urológica (*International Society of Urological Pathology*, ISUP). Se utilizó el sistema tumor, ganglio linfático, metástasis (*Tumor-Node-Metastasis*) para la clasificación clínica (cT basado en el tacto rectal, mientras que cN y cM se establecieron con la tomografía computarizada y la gammagrafía ósea con tecnecio-99). El riesgo de recidiva bioquímica del CaP localizado después de tratamiento primario, se determinó combinando el PSA, el GG y el cT, según los criterios D'Amico. Finalmente, una patología desfavorable de la pieza quirúrgica fue definida como un GG > 2 o un pT ≥ 3.

Nuestro estudio se llevó a cabo de acuerdo con las pautas de buenas prácticas clínicas y los principios éticos establecidos en la última versión de la Declaración de Helsinki (2013). Antes de la inclusión, todos los participantes firmaron un consentimiento informado por escrito sobre la recolección y almacenamiento de material y datos personales de acuerdo con las leyes nacionales.

1.5. RESULTADOS

La revisión sistemática de la literatura reportó que los estudios iniciales demostraron un beneficio inicial de Proclarix en aquellos pacientes con características específicas (valores de PSA entre 2 y 10ng/mL, un tacto rectal normal y un volumen prostático ≥ 35mL).

La segunda publicación demostró que Proclarix puede ser utilizado en todos los varones con sospecha de CaP, independientemente de los niveles de PSA sérico y del volumen prostático. El área bajo la curva fue similar en pacientes con niveles de PSA sérico entre 2 y 10ng/mL, un tacto rectal normal y un volumen prostático ≥ 35mL, y aquellos sin estas características específicas (0.701 (IC 95% 0.637 – 0.765) y 0.754 (IC 95% 0.701 – 0.807), p = 0.038. Al incorporar Proclarix en un algoritmo diagnóstico junto con PSA sérico y el tacto rectal, utilizando el umbral del 10%, se observó una disminución del 25.4% en las solicitudes de RMNmp. Además, las biopsias de próstata innecesarias se redujeron en un 17.7% con un 2.6% de diagnósticos erróneos de CaP clínicamente significativo.

En la tercera publicación se evidenció que Proclarix superó a la calculadora de riesgo de Rotterdam-RMN y a la densidad de PSA en la detección del CaP clínicamente significativo en varones con lesiones PI-RADS ≤ 3 en la RMNmp. Sin embargo, ninguna herramienta garantizó una detección del 100% de CaP clínicamente significativo en lesiones PI-RADS 4 y 5. Estos hallazgos fueron consistentes con los resultados informados en la cuarta y quinta publicaciones, en las cuales Proclarix redujo con éxito un 21.3% de biopsias prostáticas innecesarias en varones con lesiones PI-RADS 3 sin dejar de diagnosticar ningún caso de CaP clínicamente significativo. Por otro lado, la

densidad de PSA evitó un 26.3% pero diagnosticó erróneamente un 16% de CaP clínicamente significativo, y la calculadora de riesgo de Rotterdam-RMN redujo el número de biopsias de próstata innecesarias en un 7.1% dejando de diagnosticar un 4% de CaP clínicamente significativo. Por lo tanto, Proclarix demostró un mejor rendimiento que la densidad de PSA y la calculadora de riesgo Rotterdam-RMN en la selección de candidatos para las biopsias prostáticas, especialmente en aquellos varones con lesiones PI-RADS 3.

La sexta publicación analizó la combinación de Proclarix, RMNmp y el volumen prostático, objetivando una reducción de dos tercios de las biopsias de próstata innecesarias. El modelo Proclarix-RMN exhibió una sensibilidad del 90% para detector el CaP clínicamente significativo con un valor predictivo negativo del 90% y un valor predictivo positivo del 66%. Notablemente, el modelo Proclarix-RMN demostró una especificidad significativamente mayor (68%, p <0.001) en comparación con la calculadora de riesgo de Rotterdam-RMN (51%), Proclarix (27%) o RMNmp (28%) por sí solo.

La última publicación informó sobre una correlación entre Proclarix y los cuatro subrogados de la agresividad del CaP (GG, el estadio clínico, el riesgo de recurrencia bioquímica y la patología de la pieza quirúrgica).

1.6. CONCLUSIONES

Proclarix es un test recientemente aprobado por la *Conformité Européenne* (CE) que proporciona el riesgo de CaP clínicamente significativo y puede ser utilizada en todos los varones con sospecha de CaP sin restricciones en el PSA sérico o el volumen

prostático. Además, Proclarix logró reducir el número de biopsias de próstata en pacientes con sospecha de CaP antes y después de la RMNmp. Por lo tanto, la combinación de Proclarix con la mpMRI ha demostrado aumentar la eficacia tanto de la RMNmp como de Proclarix. En varones con RMNmp negativas (PI-RADS < 3), Proclarix demostró una sensibilidad del 100% en la detección del CaP clínicamente significativo y pudo evitar un tercio de las biopsias de próstata. Por otro lado, en pacientes con RMNmp positivas, Proclarix disminuyó un 43% el número de biopsias de próstata innecesarias. En pacientes con lesiones PI-RADS 3, Proclarix pudo reducir un 21.3% de biopsias de próstata innecesarias sin dejar de detectar el CaP clínicamente significativo. Comparado con las otras herramientas de predicción como la densidad de PSA y la calculadora de riesgo de Rotterdam-MRI, Proclarix fue más efectivo en la detección del CaP clínicamente significativo. Por último, Proclarix se correlacionó con la agresividad del CaP.

1. INTRODUCTION

1.1. PROSTATE CANCER

1.1.1. Epidemiology of Prostate Cancer

Prostate cancer (PCa) is the second most frequently occurring cancer in men and the fourth most common cancer overall [1]. In 2020 it was estimated that 1.4 million patients were diagnosed with PCa and was the cause of 359,000 deaths worldwide [2]. A significant variability in the incidence of the disease has been observed in different geographic areas of the world. This variability is probably influenced by the prostatic-specific antigen (PSA) testing rate and by recommendations from international organizations. Specifically, the incidence rate is higher in Australia, North America and Western Europe, while in Asia and North Africa it is lower [3]. Nevertheless, there is a relatively small variation in PCa mortality around the world, with higher rates observed in populations of African descent such as the Caribbean, sub-Saharan Africa and Polynesia [4]. The standardized incidence and mortality rates of PCa by country in 2020 are shown in Figures 1 and 2, respectively [5].

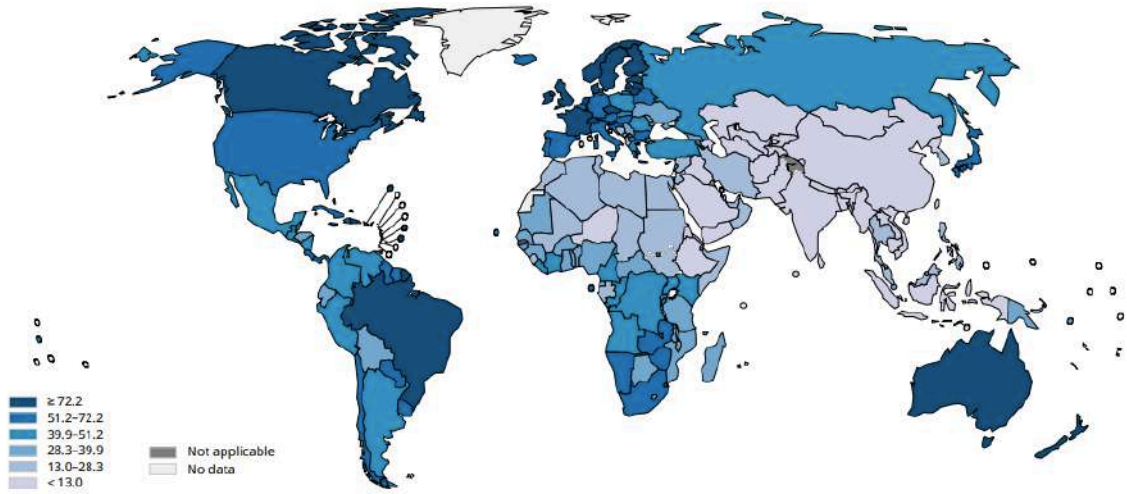

Figure 1. Incidence rates of prostate cancer standardized per age per country [5]

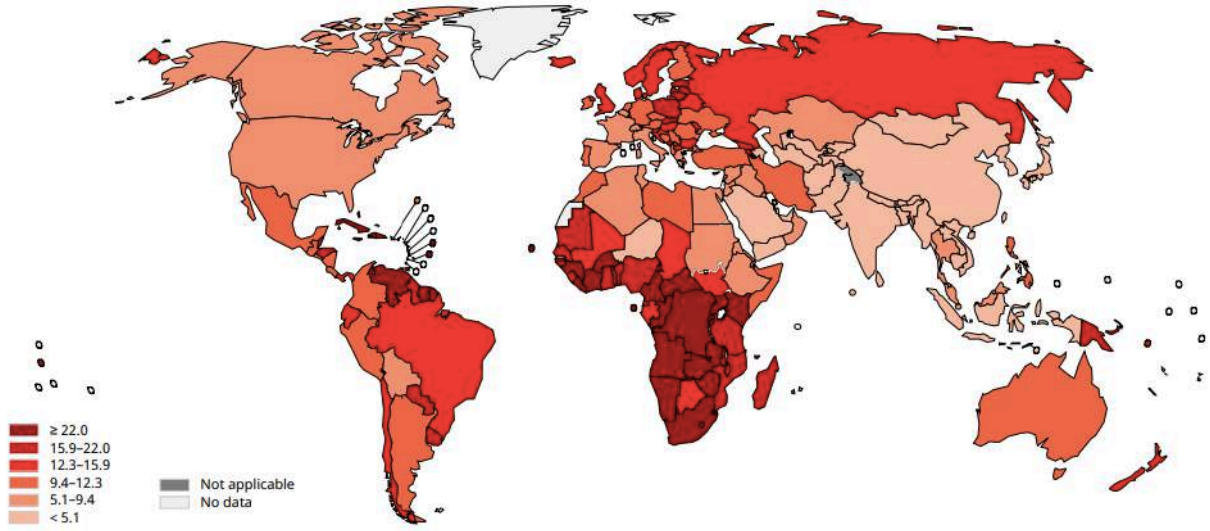

Figure 2. Mortality rates of prostate cancer standardized per age per country [5]

1.1.2. Etiology of Prostate Cancer

Multiple risk factors have been identified for PCa such as age, ethnic background, family history and diet [6]. Numerous genomic studies have been conducted and found hundreds of loci associated with more aggressive tumors. The most frequently identified genes were: BRCA2 (4.5%), CHEK2 (2.2%), ATM (1.8%), and BRCA1 (1.1%) [7]. On the other hand, it was also reported that men with mutations in the BRCA1 and BRCA2 genes were more susceptible to developing tumors with more aggressive pathology (International Society of Urological Pathology (ISUP) grade group $\geq$ 4), at more advanced stages (T3/T4), with lymph node involvement and metastasis at the time of diagnosis [8].

Despite the extensive analysis of multiple exogenous factors that may increase the risk of developing PCa, there are currently no dietary or pharmacological measures that have been shown to effectively prevent the onset of the disease. Studies have shown that individual components of metabolic syndrome such as hypertension or higher waist

circumference, are associated with an increased risk of PCa [9]. Moreover, it has been reported that an androgen deprivation therapy may promote the development of metabolic syndrome in patients with PCa. Thus, it is recommended to counsel patients on the prevention, early detection and treatment of specific metabolic alterations [10]

Multiple studies have examined the association between 5-alpha-reductase inhibitors (5-ARIs) and PCa. Some studies have suggested that 5-ARIs may decrease the risk of developing the disease, while others have reported an increased risk of high-grade PCa in men taking these medications [11–14]. Nevertheless, the exact relationship between 5-ARIs and PCa is still not fully understood, and more research is needed to determine long-term effects of these drugs on prostate health. As with any medications, it is crucial to discuss thoroughly the potential risks and benefits of 5-ARIs prior to initiating treatment. On the other hand, hypogonadal men who receive testosterone supplements did not exhibit an increased risk of PCa [15]. Conversely, in 2018 it was also reported that men with very low concentrations of free testosterone (in the lowest 10%) had a decreased risk of developing the disease [16].

1.1.3. Prostate Cancer diagnosis

The majority of cases of PCa are asymptomatic and not clinically apparent at the time of diagnosis. Therefore, an early detection of the disease is crucial as it reduces its progression and mortality rates [17]. The suspicion of PCa is usually stablished through an elevation of serum PSA levels and/or an abnormal digital rectal examination (DRE). However, definitive diagnosis of the neoplasm requires demonstration through a prostate biopsy [18].

The digital rectal examination is probably the most classic exploration for the detection of PCa. In approximately 18% of cases, PCa is diagnosed by a suspected DRE alone, regardless of PSA levels [19]. Nonetheless, its sensitivity and specificity are low, making its use in population screening debatable, since it can lead to unnecessary prostate biopsies which can result in an overdetection and overtreatment of PCa.

This classic diagnostic pathway has a low specificity which has led to an excessive number of unnecessary biopsies and an overdetection of insignificant PCa (iPCa). However, the introduction of multiparametric magnetic resonance imaging (mpMRI) has emerged as a promising imaging tool for the detection of clinically significant prostate cancer (csPCa) and can aid in guiding prostate biopsies. This imaging technique could have the potential to reduce diagnostic errors and improve the accuracy of csPCa detection, ultimately leading to better patient outcomes. MpMRI consists of both anatomic (T1-weighted and T2-weighted MRI) and functional sequences, including diffusion-weighted imaging and dynamic contrast-enhanced MRI. These sequences enable the evaluation of both structural and functional characteristics of prostate tissue, providing a more comprehensive assessment of potential cancerous lesions [20, 21].

The Prostate Imaging-Reporting Data System (PI-RADS) is an essential tool used to establish and standardize images from mpMRI and interpretation of PCa diagnosis [22]. It was first introduced in 2012 and updated in 2019 to increase the accuracy between mpMRI and pathologic results [22, 23]. The updated version aimed to simplify and standardize the terminology used in radiology reports, making it easier to stratify patients based on their level of suspicion and facilitate the use of mpMRI for targeted prostate biopsy [23]. It assigns a score from 1 to 5 to each lesion detected in the image, with higher scores indicating a greater likelihood of csPCa, as shown in Table 1.

Table 1. PI-RADS v2 Assessment Categories [23]

PI-RADS	**Assessment category**
PI-RADS 1	Very low (clinically significant cancer is highly unlikely to be present)
PI-RADS 2	Low (clinically significant cancer is unlikely to be present)
PI-RADS 3	Intermediate (the presence of clinically significant cancer is equivocal)
PI-RADS 4	High (clinically significant cancer is likely to be present)
PI-RADS 5	Very high (clinically significant cancer is highly likely to be present)

PI-RADS = Prostate Imaging-Reporting Data System

Numerous studies have been conducted in order to establish the role of mpMRI in the diagnostic algorithm of PCa. In 2017, the PROMIS trial was published, which reported that biopsies guided by mpMRI had a 93% sensitivity (CI 95%: 0.88-0.96) in comparison to only 48% (CI 95%: 0.42-0.55) in patients who underwent through systematic biopsies exclusively. Furthermore, the use of mpMRI had the potential to reduce the number of primary biopsies by up to 27% and diagnose approximately 18% more csPCa [24]. More recently, a randomized clinical trial was conducted on a cohort of over 12.000 men with PSA levels greater than 3ng/mL. Patients were randomized to undergo either systematic guided biopsies (10-12 cores) or mpMRI prior to targeted and systematic biopsies. The study reported that the percentage of men undergoing prostate biopsies was twice as high in the systematic biopsy group compared to the mpMRI group. Moreover, the detection of iPCa was 12% in patients who did not undergo mpMRI compared to 4% in those who received targeted biopsies [25].

It has been shown that each increase in the PI-RADS score is usually associated with higher ISUP grade group tumors in the prostate biopsies and more advanced tumors in surgical specimens [26]. Additionally, it has been demonstrated that the aggressiveness of PCa increases with higher PI-RADS categories [27]. This information

can aid in the decision-making process of whether to perform prostate biopsies based on PI-RADS categories. Thus, the incorporation of mpMRI in the diagnostic pathway of PCa has resulted in a decrease of men undergoing prostate biopsies and a reduction in the number of diagnosed iPCa, while simultaneously increasing the detection of csPCa [25, 28].

Nomograms are graphical tools that use statistical models to predict the probability or risk of a specific outcome. They have become an important tool in the diagnosis of PCa since they provide an individualized risk estimation based on different variables. The European Randomized Study of Screening for Prostate Cancer (ERSPC) study which began in the early 1990s and involved over 182.000 European men, has played a significant role in developing several algorithms for estimating the risk of PCa. Nonetheless, the most scientifically sound and extensively validated is considered the Rotterdam Prostate Cancer Risk calculator, based on the ERSPC Rotterdam data [29]. This predictive model, recommended by international guidelines, uses several patient characteristics to assess the probability of detecting any PCa or csPCa defined as those with an ISUP grade group (GG) two or higher on prostate biopsy [18]. This tool is designed to provide personalized risk assessment based on multiple parameters such as age, serum PSA levels, DRE, prostate volume (PV) and previous biopsy results. Moreover, with its rise in popularity, mpMRI has been incorporated into the latest ERSPC risk calculator [30, 31]. This predictive model has been externally validated through several studies, which have yielded mixed results, demonstrating adequate discrimination for csPCa but varying depending on the cohort analyzed [32–34]. The Rotterdam PCa risk calculator serves as a valuable tool for aiding clinicians and patients in making informed decisions regarding PCa screening and diagnosis. Additionally, it offers user-friendly web

and smartphone applications, facilitating its implementation in clinical practice [35] as in the case of recent ERPSC MRI risk calculator. However, it is important to note that none of these predictive models has been evaluated by PI-RADS category [15].

Screening of PCa remains a highly controversial topic [36]. A Cochrane review of randomized PCa screening trials with PCa mortality as endpoint was published in 2013 [37] and updated in 2019 [38, 39]. The review found that the screening was associated with an increased diagnosis of PCa and a detection of more localized disease and less advanced PCa. Moreover, no survival benefit was observed as a result of screening. However, the ERPSC MRI reported a significant reduction in PCa mortality through screening when using PSA level cut-off of 3 - 4ng/mL. Current European guidelines recommend that early PSA testing should be based on the individual risk of men [40]. Accordingly, population screening should be performed in:

- Men aged 50 years or older
- Men aged 45 years or older with family history of PCa
- Men of African descent aged 45 years or older
- Men carrying BRCA2 mutations aged 40 years or older

Nonetheless, it is important to discuss with patients the potential disadvantages of increased incidence and risk of over-treatment as well as the benefits associated with decreased disease-specific mortality.

1.1.4. Classification and staging of prostate cancer

A tumor classification system aims to categorize individuals with comparable clinical outcomes. This enables discussion of prognosis with patients, facilitates the design clinical trials that involve relatively homogeneous groups and assists in the development of treatment recommendations for these specific populations. On the other hand, staging is performed in order to determine the extent of disease spread. Information from staging is essential because it influences treatment decisions and affects prognosis.

In prostate cancer, staging is based on the Tumor-Node-Metastasis (TNM) system which was first implemented in 1978 [41]. The TNM staging is a schematic representation of anatomic tumor extent and pathological tumor grade is reflected of intrinsic features of tumor aggressiveness. The classification of PCa according to the TNM system is displayed in Table 2.

Table 2 Clinical TNM classification of PCa (Classification of Malignant Tumors, 8th Edition [41])

T - Primary** Tumor **(stage based on digital rectal examination [DRE] only)
TX Primary tumor cannot be assessed
T0 No evidence of primary tumor
T1 Clinically inapparent tumor that is **not palpable**
T1a Tumor incidental histological finding in 5% or less of tissue resected
T1b Tumor incidental histological finding in more than 5% of tissue resected
T1c Tumor identified by needle biopsy
T2 Tumor that is palpable and confined within the prostate
T2a Tumor involves one half of one lobe or less
T2b Tumor involves more than half of one lobe, but not both lobes
T2c Tumor involves both lobes
T3 Tumor extends **palpably** through the prostatic capsule
T3a Extracapsular extension (unilateral or bilateral)
T3b Tumor invades seminal vesicle(s)
T4 Tumor is fixed or invades adjacent structures other than seminal vesicles: external sphincter, rectum, elevator muscles, and/or pelvic wall
N - Regional (pelvic) Lymph Nodes[1]
NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastasis
N1 Regional lymph node metastasis
M - Distant Metastasis[2]
M0 No distant metastasis
M1 Distant metastasis
M1a Non-regional lymph node(s)
M1b Bone(s)
M1c Other site(s)

1 Metastasis no larger than 0.2 cm can be designated pNmi.

2 When more than one site of metastasis is present, the most advanced category is used. (p)M1c is the most advanced category

In addition to clinical staging, other prognostic parameters, such as serum PSA levels or the pathologic differentiation grade, are also considered in the prognosis of the disease. One of the first models that included these parameters was described by D'Amico in 1998, combining PSA levels, clinical information on tumor extent and

pathology, in order to predict the risk of biochemical recurrence of localized and locally-advanced PCa [42]. This model has become a widely accepted tool for prognostic evaluation in clinical practice and it is included in the current European Association of Urology (EAU) guidelines. Notably, the cT-stage is based on findings obtained through DRE, rather than imaging studies. As such, if a DRE does not reveal any palpable abnormalities, the tumor should be categorized as cT1c. Conversely, if a lesion is palpable in both lobes of the prostate, it should be considered as cT2c, regardless of the results of mpMRI. In addition, cN and cM stages are established using computed tomography (CT) and 99-tecnhetium bone scintigraphy, recommended in patients with intermediate or high-risk PCa (Gleason score > 2 or PSA > 10ng/mL). The D'Amico risk group classification for biochemical recurrence of localized and locally-advanced PCa have been adapted by the EAU PCa guidelines is outlined in Table 3.

Table 3. EAU risk groups for biochemical recurrence of localized and locally-advanced PCa

	Low-risk	**Intermediate-risk**	**High-risk**	
PSA level	PSA <10ng/mL	PSA 10-20ng/mL	PSA>20	Any PSA
Pathology	And GS <7 (ISUP 1) and cT1-2a*	Or GS 7 (ISUP 2/3) or cT2b*	Or GS >7 (ISUP 4/5) or cT2c*	Any GS (any ISUP) cT3-4 or cN+**
	Localized			**Locally advanced**

GS = Gleason score; ISUP = International Society for Urological Pathology; PSA = prostate-specific antigen

**Based on digital rectal examination*

***Based on CT/bone scan*

1.1.5. Aggressiveness of prostate cancer

A tumor aggressiveness refers to the potential of the cancer to grow and spread rapidly. Determining the aggressiveness of PCa is crucial in order to guide treatment decisions and predicting outcomes for patients. Nonetheless, accurately determining the aggressiveness of a tumor, requires a post-treatment analysis which may not be

feasible in all cases. Thus, in some studies, surrogate endpoints have been used to estimate the aggressiveness of the disease. While these markers may provide an indication of tumor aggressiveness, they are not always definitive and may not accurately reflect the true nature of the tumor [18].

The Gleason score (GS) is a grading system, developed in 1974 by Donald Gleason, to evaluate the aggressiveness of the tumor based on the architecture pattern of the prostate gland [43]. It is based on the appearance of the cancer cells under a microscope and assigns a grade to the two most predominant patterns found in a prostate biopsy core. The grades range from 1 to 5, being 1 the least aggressive and 5 the most aggressive. It is recommended to include the higher grade, even if it is less than 5% of the biopsy material [18]. The sum of the two grades determines the overall GS, which ranges from 2 to 10. A low GS (2 - 6) indicates a less aggressive cancer, while a high GS (8 - 10) determines a more aggressive tumor.

In 2014, the International Society for Urological Pathology approved a new grading system based on GS that limits the pathologic classification to five categories [44]. This change was made in order to align the grading of PCa with other carcinoma classifications. Moreover, punctuations lower than six are no longer used, allowing to reduce overtreatment of insignificant PCa, as they are not considered cancerous. Finally, it differentiates between GS 3 + 4 and 4 + 3 which determines whether or not the cancer is clinically significant. The ISUP grade system is represented in Table 4.

Table 4. International Society of Urological Pathology 2014 grade system

Gleason score	ISUP grade
2-6	1
7 (3+4)	2
7 (4+3)	3
8 (4+4 or 3+5 or 5+3)	4
9-10 (4+5 or 5+4 or 5+5)	5

ISUP = International Society for Urological Pathology

The ISUP grading system is commonly employed as a surrogate endpoint of PCa aggressiveness due to its correlation with disease progression independent of the treatment administered. By using the ISUP system, clinicians can more accurately assess the aggressiveness of PCa and determine the appropriate course of treatment.

Histopathological examination of radical prostatectomy (RP) specimens is an essential component for the diagnostic and prognostic evaluation of PCa. It provides valuable information about the stage, histopathological type, grade and surgical margins of the tumor which are essential for clinical decision-making. Grading conventional prostatic adenocarcinoma using Gleason system is considered the most important prognostic factor for clinical behavior and treatment response [44]. The ISUP grade group in RP specimen is typically determined in a similar way as in biopsies, with the exception that minor high-grade components (< 5%) are excluded from the ISUP grade group. For instance, in a carcinoma that is almost entirely composed of ISUP 3, the presence of a minor (< 5%) ISUP 4 or 5 component is not included in the ISUP score, but its presence is noted in the report [45]. In cases of multifocality, the ISUP grade group of the index lesion is given, which refers to the tumor with the highest grade, stage or volume. Overall, the histopathological examination of the gland is essential in providing

clinicians with relevant information on the prognostic characteristics of PCa, which is critical in making informed decisions about the appropriate course of the treatment.

Numerous studies have evaluated the concordance between preoperative biopsy and postprostatectomy specimen GS, reporting a correlation ranging between 30% and 60%. Upgrading of the GS represents a substantial risk of delaying appropriate treatment in patients with PCa, particularly in the active surveillance group, and has been described in over 50% of cases in some studies [46, 47]. Therefore, it is crucial to consider the pathology of the surgical specimen when determining the aggressiveness of the disease, as it can be a determining factor in several patients. It has been shown that men with a Gleason score of 3 + 4 (ISUP 2) in the surgical specimen exhibit a favorable prognosis compared to patients with a Gleason score of 4 + 3, which behave similarly to tumors with Gleason score of 4 + 4 (ISUP 4) [48]. In addition, Gandaglia et al. reported that patients with a Gleason score of 4 + 3 in the RP specimen had lower biochemical recurrence-free rates after surgery compared to those with a score of 3 + 4 [49]. Thus, the pathology obtained in the RP can help determine the aggressiveness of the tumor depending if the patient has a favorable or unfavorable specimen. An ISUP 2 or lower and a pT of less than 3 is considered favorable, while an ISUP greater than 2 or a pT of 3 or higher is considered unfavorable.

1.2. TUMOR MARKERS FOR THE EARLY DETECTION OF CLINICALLY SIGNIFICANT PROSTATE CANCER

The gold standard for the diagnosis of PCa is a transrectal ultrasound guided needle biopsy of the prostate. The decision to perform a diagnostic biopsy is based on a combination of factors, including an elevated serum PSA level and/or an abnormal DRE. Recently, screening for PCa using serum PSA has come under considerable criticism due to the risk of overdiagnosis and overtreatment of iPCa [50, 51]. To address this issue, mpMRI has been validated as a screening tool to reduce unnecessary prostate biopsies and improve diagnostic accuracy of csPCa [24]. Nonetheless, there are still lots of unneeded biopsies being performed, this is why identifying a biomarker to detect csPCa is important. In this direction, several adjunctive biomarkers have been developed to predict the risk of csPCa and prevent unnecessary prostate biopsies [52]. This could also avoid the risk of harm, such as anxiety, bleeding, infection, requiring hospitalization, and the psychological implications of being diagnosed with iPCa cancer.

Liquid-based biomarkers, such as those acquired from blood or urine, are well placed to act as PCa-specific biomarkers. The identification of biomarkers in liquid biopsies has significant advantages over tissue-based techniques as they can be obtained easily in a less invasive manner. Liquid biopsies can also be routinely taken pre-, post- or on-treatment, meaning continual patient monitoring can be achieved, while tissue biopsies give only a limited snapshot of the disease. Tumor heterogeneity is a significant problem for tissue-based biopsy tests, as results can only be determined from the area that the tissue samples are acquired from [53, 54]. Liquid biopsies, in comparison, have the potential to give a comprehensive view of both primary and metastatic cancers.

The ideal biomarker for the diagnosis of csPCa should have high sensitivity and specificity, be reproducible, and have quantifiable measures that are easy to use. It should also be cost-effective, provide clear results for clinicians and be easily applied to different racial groups [52]. Unfortunately, there are few comparative trials among these biomarkers, leaving clinicians uncertain about which one provides the most useful information.

1.2.1. Prostate-specific antigen and derivates

The prostate-specific antigen is a glycoprotein that belongs to the kallikrein-like serine protease glycoprotein encoded by the prostate-specific gene kallikrein 3 [55]. PSA is mainly secreted by prostatic epithelial cells, and in healthy individuals, the levels of this glycoprotein in blood samples are typically low. The primary physiological function of PSA is to liquefy semen through the process of proteolysis [55].

The discovery of purified human PSA was reported in 1979 [56] and subsequently found in the sera of patients with advanced PCa in 1980 [57]. The serum PSA test was initially approved by the Food and Drug Administration (FDA) to monitor patients with PCa in 1981 [58]. However, when introduced into clinical practice in 1987, it was not designed as a screening marker to detect PCa, but rather to monitor the progression of PCa and the response to therapy. It was not until 1991 that it was approved by FDA as a screening tool for the detection of PCa [59].

Elevated levels of serum PSA are commonly observed in PCa, but are also present in some benign conditions such as benign prostatic hyperplasia or prostatitis [60]. While there is no recognized defined cut-off for diagnosis of PCa, many clinicians consider PSA

levels ≤ 4.0ng/mL as normal, within higher levels indicating a need for further investigation [61]. Overdiagnosis in relation to PSA screening programs, has been reported to range from 20 to 66% [62, 63]. The observed increase in the number of patients receiving treatment while simultaneously reducing the number of PCa cases diagnosed with later-stage disease, led to concerns about overtreatment [64]. Besides the cost implications, overtreatment of PCa can have significant impacts effects on the mental and physical health of patients. As previously noted, diagnostic procedures such as prostatic biopsy carry inherent risks of complications. Additionally, surgical or radiotherapeutic interventions may result in severe side effects affecting up to half of the patients, including urinary incontinence, sexual dysfunction and diminished colonic and renal function [65].

PSA levels can also serve as a prognostic tool for newly diagnosed PCa patients. Generally, higher PSA values are associated with poorer outcomes [66, 67], thus men with PSA levels above 20ng/mL at diagnosis, have been shown to have a significant decrease in 5-year survival rates. Elevated serum PSA concentrations may suggest the presence of more aggressive or occult metastatic disease, thereby indicating the potential benefit of more intensive treatments for these patients [67]. However, while there is a correlation between serum PSA levels at diagnosis and patient outcome, PSA alone has limited prognostic accuracy. Therefore, to enhance prognostic accuracy within the clinic, tumor histological and clinical factors should be evaluated in conjunction with PSA when predicting outcome [68].

In the early nineties, to improve the accuracy of PSA, Benson et al. introduced the PSA density (PSAD), which considers the concentration of PSA over the prostate volume. The primary purpose was to differentiate between benign prostatic hyperplasia

and small-volume organ-confined PCa [69]. PSAD is calculated dividing serum PSA level by prostate volume, determined using either transrectal ultrasound or mpMRI. Several studies have indicated that PSAD can have the potential to identify men with csPCa, thereby influencing biopsy decisions [70, 71]. As serum PSA increase, PSAD becomes a more effective marker for predicting csPCa [72]. Furthermore, it has also been shown that PSAD may be useful in predicting the presence of adverse pathology and determining the aggressiveness of PCa in patients undergoing radical prostatectomy [73, 74]. These results suggest that PSAD may play a role in risk stratification, particularly in deciding which patients are suitable for active surveillance [75, 76]. Overall, PSAD represents a simple, inexpensive tool that could serve to avoid unnecessary prostate biopsies and identify patients requiring further diagnostic investigation.

PSA can exist in multiple forms in the bloodstream, including free PSA and complexed PSA. Free PSA is not bound to any carrier proteins, while complexed PSA is bound to protease inhibitors [77]. Measuring the levels of these molecular forms can provide valuable information in addition to the total PSA [78, 79]. Free PSA (fPSA) levels are generally expressed as a percentage of total PSA, known as percent fPSA. In general, men with PCa have lower percent fPSA levels compared to men without PCa [68]. This can help differentiate between PCa and benign prostatic hyperplasia [78]. Unfortunately, there are limitations to the assessment of percent fPSA as a diagnostic tool, since this free form is less stable than complexed PSA in the blood, it requires prompt sample processing after collection [80]. Additionally, invasive procedures such as DRE and biopsy procedures lead to a temporary rise in the levels of free PSA in the blood [81]. High prostate volumes can also lead to decreased percent fPSA values, which

means that percent fPSA is considered reliable only in patients with prostate volume less than 40mL [82].

Studies have suggested that the percent fPSA measurement can be particularly useful for men with PSA levels between 4 and 10ng/mL, with a diagnostic sensitivity ranging from 75% to 95% and a specificity from 32% to 93% [83]. Nonetheless, a meta-analysis conducted to assess the accuracy of percent fPSA for the diagnosis of PCa in men with PSA levels from 4 to 10ng/mL concluded that the biomarker had low sensitivity and specificity and needed to be combined with additional diagnostic methods [83]. However, Oto et al., recently analyzed the combination of percent fPSA with total PSA and age in a predictive model and concluded that it increased the diagnostic potential of total PSA [84]. To address the challenges associated with percent fPSA, several studies have investigated the use of molecular forms of PSA in diagnostic assays, including intact PSA and [2]proPSA [85]. The Prostate Health Index (PHI) assay, the 4-kallikrein panel (4Kscore) and the Stockholm-3 (STHLM3) model are different tests that incorporate various molecular forms of PSA.

1.2.2. Prostate Health Index

The Prostate Health Index (PHI) is a mathematical formula derived from the relative concentrations of three different PSA forms: total PSA, free PSA and [-2]proPSA. This blood test determines the risk of PCa in men with serum PSA between 2 and 10ng/mL and a non-suspicious DRE. It has been shown to outperform the total PSA and the percent fPSA for the prediction of prostate biopsy outcome. Thus, being able to reduce unnecessary biopsies and improve the accuracy of PCa detection [86–89].

Multiple studies have demonstrated the correlation between PHI and GS in biopsy naïve patients, obtaining higher PHI values in men with an increased probability of a GS $\geq$ 7 in the biopsy [90–92]. Moreover, when compared to mpMRI, Ferro et al., demonstrated that PHI significantly outperformed PI-RADS score to predict positive biopsy results with a comparable performance in the identification of csPCa [93]. On the other hand, a recently published study suggests that the combination of mpMRI and PHI can improve the estimation of the risk category of PCa at the initial diagnosis by using an artificial neural networking analysis. This personalized approach to treatment could potentially lead to better outcomes for patients with PCa [94]. The PHI score has been shown to have a significant impact on patient management in the clinical setting. Specifically, low PHI levels lead to the deferral of biopsies, whereas an elevated score suggests an intermediate or high-probability of PCa and indicates the necessity of a prostate biopsy [95].

From a health-economic perspective, recent studies have demonstrated the cost-effectiveness of incorporating PHI in the decision-making process regarding whether a prostatic biopsy is required [96, 97]. In addition to reducing the number of unnecessary biopsies, the PHI test may also be useful in predicting biochemical recurrence (BCR) following radical prostatectomy [98, 99]. Various studies have demonstrated that higher preoperative PHI scores are associated with an increased risk of BCR after surgery [89, 100]. Furthermore, PHI has been analyzed as an independent predictor of BCR demonstrating a greater accuracy than current predictors such as serum PSA, clinical and pathological stage, and the grade group [99]. This could be useful in order to determine which patients could benefit from adjuvant therapy and follow-up schedule after radical prostatectomy [89]. Moreover, Roobol et al. compared two risk calculators

(the European Randomized Study of Screening of Prostate Cancer and the Risk Calculator 4) incorporating PHI [101]. They concluded that both models exhibited similar performance, but the inclusion of PHI in the risk calculators resulted in a greater reduction in the number of unnecessary biopsies when compared to a strategy based on serum PSA [101]. However, there are some challenges associated with the use of this test in the clinic. Similar to fPSA, molecular instability has also been observed in several studies involving [-2]proPSA. For proper measurement of this molecule, blood samples should be centrifuged within three hours of blood draw. Serum may be stored at room temperature or refrigerated for a maximum of 48 hours. Nonetheless, if the serum is to be stored for an extended period of time, it should be frozen to prevent any potential degradation [102].

1.2.3. Four kallikrein test

The four kallikrein (4K) score is a diagnostic test that combines four different kallikrein proteins (total PSA, free PSA, intact PSA and human kallikrein 2) along with patient age and DRE, to provide a probability, ranging from 0 to 100%, for detecting csPCa [103]. Its primary aim is to reduce disease overdetection by assisting clinicians in deciding which patients require a prostate biopsy. Its use is currently recommended in men undergoing either an initial or a repeat biopsy. The 4Kscore has been correlated with the GS obtained in the biopsy, with higher values observed in patients with csPCa compared to those with iPCa [104, 105]. In a large prospective multi-institutional trial, the 4K score was found to distinguish patients with GS of 7 or higher from those with a score less than 7. By using a cut-off value of 6%, it was reported that 30% of biopsies

could be avoided, while delaying a diagnosis of high-grade PCa in only 1.3% of patients [106]. Further studies have demonstrated the potential of 4K score to predict the presence of csPCa [106–110]. Additionally, in a cohort of 925 men with a previous negative biopsy, the 4K score showed a significantly higher predictive accuracy for PCa detection than PSA, with an area under the curve (AUC) of 0.68 compared to 0.58 [111].

In a cohort of 266 biopsy-naïve men with clinical suspicion of PCa, the combination of 4K score with mpMRI was evaluated, revealing a significant reduction in the number of unnecessary biopsies [112]. The study showed that men with PI-RADS 1 and 2 lesions, the highest negative predictive value (NPV) was observed for those with low or intermediate 4K score risk. The optimal biopsy strategy, consisted on an initial 4K score test, followed by mpMRI if the 4Kscore was $\geq$ 7.5% and a subsequent biopsy if the mpMRI was positive (PI-RADS 3 to 5) or the 4K score was $\geq$ 18%. This approach could potentially avoid 34% of biopsies and lead to missing 2.7% of csPCa [112].

Numerous studies have demonstrated that incorporating the 4K score into the decision-making process, can have a significant impact on both clinicians and patients by reducing the number of biopsies performed, while also increasing the likelihood of identifying aggressive PCa [113]. The 4K score has also been shown to significantly decrease costs and improve the quality of patient care [114, 115]. Additionally, in a retrospective case-control study with a 15-year follow-up, 4K score was found to significantly improve the prediction of metastasis in patients aged 50 to 70 years, compared to serum PSA alone [116]. Nevertheless, in a post-operative setting, this blood-test was reported to not be useful for counselling men after radical prostatectomy. Multiples studies stated that 4Kscore did not improve the value of post-

surgery risk models and could not be used in the prediction of biochemical recurrence [117].

1.2.4. SelectMDx

The SelectMDx assay is a urine-based test that aims to provide the probability of detecting PCa after a biopsy, in addition to the likelihood of low-grade versus high-grade disease. This test measures the levels of messenger RNA (mRNA) of two genes: HOXC6 and DLX1, which are known to be overexpressed in aggressive PCa [118, 119]. The mRNA values are quantified following a post-prostate massage urine specimen and normalized to KLK3 mRNA, the gene that encodes PSA. The mRNA values of HOXC6 and DLX1 are then combined into a single RNA value, which is then used in addition to known clinical risk factors such as age, PSAD and DRE, to determine the percent likelihood of detecting a PCa with a GG of 2 or higher on initial prostate biopsy.

Two independent clinical trials have demonstrated that the mRNA levels of HOXC6 and DLX1 can be used to achieve a sensitivity and a negative predictive value (NPV) of 90% or higher [118, 120]. Van Neste et al. conducted a study to evaluate the performance of SelectMDx by analyzing a cohort of over 900 men scheduled for either initial or repeat prostate biopsy. The result of the study revealed an AUC of 0.76, along with a sensitivity and a NPV of 91% and 94%, respectively [118]. The authors of the study suggested that the implementation of this test could potentially result in a reduction of up to 42% in the total number of biopsies performed while also decreasing the number of unnecessary biopsies performed by 53% [118]. The combination of clinical risk factors with mRNA values resulted in an increase in the AUC to 0.9. These results were further

validated by Haese et al. during their evaluation of 916 men undergoing initial prostate biopsy. They reported that SelectMDx had an AUC of 0.85 with 93% sensitivity, 47% specificity and 95% NPV [120]. Moreover, when the analysis was limited to patients with serum PSA levels lower than 10ng/mL, an AUC of 0.82 was obtained, along with 89% sensitivity, 53% specificity and 95% NPV [120]. The outcomes of the aforementioned studies provide strong evidence of the high sensitivity and NPV of the test in detecting csPCa prior to initial biopsy. In addition, SelectMDx has also been shown to outperform other tests such as PHI in screening for the presence of high-grade PCa before biopsy [121].

It has been shown that this test can potentially aid in the disease risk stratification of patients with mpMRI. In a study involving over 300 men, SelectMDx was more sensitive but less specific than mpMRI in detecting both PCa and csPCa at diagnostic biopsy [122]. In a prospective study conducted on prostate-biopsy-naïve men in the Netherlands with serum PSA levels ≥ 3, it was found that 31% of patients were diagnosed with high-grade PCa. While using SelectMDx alone missed 10% of high-grade PCa, mpMRI missed 13%. Furthermore, a study from 2022 indicated that SelectMDx exhibited comparable diagnostic accuracy to PSAD in detecting csPCa and enabled the avoidance of the same number of prostate biopsies in patients with PI-RADS 3 lesions [123]. In addition, SelectMDx was shown to outperform other biomarkers such as PCA3 and PSA in detecting PI-RADS 4 and 5 lesions on mpMRI [124].

1.2.5. Prostate Cancer Antigen 3

Prostate Cancer Antigen 3 (PCA3) is a prostate specific noncoding mRNA that has been found to be overexpressed in more than 90% of all prostate tumors compared to benign prostate tissue [125–127]. Several studies have reported the use of quantifying PCA3 RNA in post-prostate massage urine [127, 128]. The Progensa PCA3 assay was approved by the FDA in 2012 as a diagnostic test for use in men aged 50 or older with elevated serum PSA, and a previous negative prostate biopsy result [129]. The sensitivity and specificity of the assay, depend on the cut-off score used, which remains a topic of debate. A PCA3 score below 25 has been link to a decreased probability of PCa upon subsequent repeat biopsy. Nonetheless, a PCA3 score of 35 is associated with a sensitivity ranging between 58% and 85%, with a specificity of 58% to 76% [130, 131]. Multiple studies have demonstrated that PCA3 provides an acceptable diagnostic accuracy and can aid in decision-making regarding whether or not to perform an initial biopsy, thus reducing the number of unnecessary biopsies [132]. The incorporation of PCA3 scores into individual risk estimation models, which include clinical factors, age and patient race, has been shown to improve the stratification of PCa [133]. Wei et al. found that PCA3 measurement can reduce the under-detection of high-grade disease in initial prostatic biopsies, while also minimizing the over-detection of low-grade PCa in repeat biopsies [133]. Other studies have also demonstrated that PCA3 can complement serum PSA and other clinical information to provide a more accurate prediction of repeat biopsy outcome [134, 135]. Additionally, Deras et al. were able to demonstrate that PCA3 is an independent marker that is not influenced by prostate volume, serum PSA levels or the number of prior biopsies [136].

As with the previously discussed biomarkers, studies have investigated the combination of PCA3 score with mpMRI to improve the accuracy of PCa diagnosis. In patients with a suspicious lesion identified by mpMRI, the PCA3 score was found to be higher compared to those without suspicious lesions. These results suggest that the PCA3 test could be useful in identifying patients who should undergo mpMRI [137]. Additionally, combining PCA3 with mpMRI has been shown to enhance the predictive accuracy of mpMRI [138, 139]. Ongoing research is developing new methods for detecting PCA3, with the aim of making the assay accessible as a point-of-care test in developing countries [140–143].

Multiple studies have suggested that PCA3 has potential to aid in decision-making between active surveillance and radical treatment options. It has been proposed that a PCA3 threshold score of 20 could be used to identify men with iPCa who would be eligible for active surveillance, while a threshold score of 50 could identify men at higher risk of having csPCa who may benefit from radical therapy [144]. However, the correlation between PCA3 and PCa aggressiveness is under debate, with some studies exhibiting a relationship between PCA3 levels and Gleason score [145–149], whilst others found no association [150, 151]. Nonetheless, comparative analyses have indicated that PHI outperformed PCA3 as a diagnostic test, since it exhibited an increased accuracy in predicting PCa in both initial and repeat biopsies [152], and demonstrated a better performance in detecting aggressive disease [153]. While it is unlikely that PCA3 will replace serum PSA as the frontline biomarker for the detection of PCa, the measurement of both PCA3 and serum PSA may enhance the specificity of csPCa diagnosis.

1.2.6. TMPRSS2-ERG

The translocation that fusions the androgen-regulated transmembrane protease serine 2 (TMPRSS2) and erythroblastosis virus E26 oncogene homolog (ERG) known as TMPRSS2-ERG, was identified by Tomlins et al. [154]. This chromosomal rearrangement could influence PCa prognosis through an androgen regulation mechanism and has been evaluated as a urine biomarker following prostatic massage. The TMPRSS2-ERG fusion gene has been correlated with the Gleason score [155]. MyProstateScore test, previously named as the Michigan Prostate Score, was developed combining serum PSA with the mRNA expression of two urinary markers: Prostate Cancer Antigen 3 and TMPRSS2-ERG fusion gene. It ranges from 0 to 100 and reflects the probability of finding any PCa or high-grade PCa upon biopsy. Studies have shown that both PSA and TMPRSS2-ERG are independent predictors of the Gleason score obtained in the biopsy and the clinical tumor stage, whereas PCA3 did not correlate with biopsy results nor the clinical stage [130, 156]. Furthermore, Ferro et al. concluded that PCA3 improved the accuracy of predicting PCa in men with a serum PSA ranging from 2 to 10 ng/mL at initial biopsy. This test outperformed percent fPSA in these patients [157]. In a validation study involving 1.525 men, MyProstateScore was able to avoid unnecessary biopsy in 33% of patients while missing 3% of GG $\geq$ 2 PCa [158]. Moreover, in a recent study based on 540 men, Tosoian et al. reported that MyProstateScore was significantly higher in patients with GG ≥ 2 PCa than those with negative or GG1 biopsy in the overall population and when stratified by PI-RADS score [159]. Nonetheless, in the PI-RADS 3 population, MyProstateScore showed the best clinical performance for predicting GG ≥ 2 PCa with an AUC of 0.73 compared to 0.55 for serum PSA and 0.62 for PSA density. In

addition, it has been observed that when using a cut-off of ≤ 25, nearly 39% of men with PI-RADS 3 lesions could potentially avoid unnecessary biopsies, while missing 6% of patients with GG ≥ 2 PCa.

1.2.7. ExoDx

The ExoDx prostate Intelliscore is a non-DRE urine exosome-based assay that measures PCA3 and TMPRSS-ERG exosomal RNA levels along with a control gene: SPEDF. In addition, the test integrates clinical variables such as serum PSA, race, age and family history of PCa, in order to accurately predict the likelihood of detecting a GG ≥ 2 PCa on biopsy. Currently, this assay is indicated in men over 50 years of age with serum PSA levels between 2 and 10ng/mL who are scheduled for an initial prostate biopsy due to an abnormal DRE or elevated serum PSA [160]. The initial study demonstrated a NPV of 97.5% for the detection of high-risk PCa, with an AUC of 0.803 [161]. In a multicenter study, the ExoDx assay combined with clinical variables, was found to be significantly superior in predicting the presence of a GG ≥ 2 PCa and negative biopsy results than either ExoDx assay or the clinical variables alone. The study used a predefined cut-off point of 15.6 and reported a NPV of 91%, a sensitivity of 92% and an 8% rate of missing a GG ≥ 2 PCa [160].

1.2.8. Stockholm-3 model

The Stockholm-3 model (STHLM3) is a risk-based model developed to improve the early detection of csPCa as an alternative to serum PSA [162]. It combines five

plasma protein markers (total PSA, free PSA, human kallikrein 2, macrophage inhibitory cytokine-1 and microseminoprotein-β), over 101 genetic markers (single nucleotide polymorphisms) and clinical data such as age, previous biopsy results, family history and the use of 5-ARI, to determine the risk of having a PCa GS ≥ 7 [162]. In a large population cohort in Sweden including men aged 50 to 69 years, the STHLM3 was shown to reduce the number of biopsies by one third while maintaining the same sensitivity to detect csPCa compared to serum PSA results [162]. This new test was also analyzed in combination with mpMRI and was found to perform as well as serum PSA in detecting csPCa, while also reducing the number of mpMRI by 36% and biopsy procedures by 8% [163]. These studies suggest that STHLM3 can improve the PCa diagnostic process compared to PSA, by reducing the number of false-positive and low-grade PCa detected on biopsy. Nevertheless, it should be noted that STHLM3 showed improved effectiveness but at additional costs compared to serum PSA [164].

1.2.9. Other biomarkers

Several tissue-based biomarkers have been developed to aid in the decision-making and determine the need for active treatment or active surveillance. Oncotype DX genomic prostate score utilizes a real-time polymerase chain reaction to measure the expression of 12 genes involved in different neoplastic pathways, compared to 5 reference genes [165]. The resulting score provides information on the risk of PCa death, metastasis within ten years and the likelihood of Gleason score upgrading after radical prostatectomy [165–167]. On the other hand, Prolaris evaluates the tumor aggressiveness using tissue from either prostate biopsies or radical prostatectomy

specimens. It measures the expression of 31 genes known to be upregulated in aggressive PCa, as well as 15 reference genes, and generates a cell-cycle progression score [168]. The Prolaris score has been found to be associated with the risk of biochemical recurrence, metastatic disease and death from PCa [169, 170]. Finally, Decipher is another tissue-based assay used for risk stratification after surgery. It was developed based on prostate tissue from 545 men who underwent radical prostatectomy, of which, 213, developed metastatic disease. Decipher measures the expression of 22 genes that were overexpressed in patients who developed metastatic disease compared to controls [171]. The primary use of this marker is to assess the risk of disease progression in patients with high-risk features identified on radical prostatectomy specimens. Thus, being able to stratify patients with adverse pathology, guiding the decision between adjuvant radiation therapy and active surveillance [172, 173].

1.2.10. Proclarix

Proclarix (Proteomedix, Schlieren, Switzerland) is a recently introduced blood-test based on the combination of serum trombospondin-1 (THBS1), cathepsin D (CTSD), total PSA, and percent free-PSA in addition to age, providing a risk score of csPCa [174–176]. THBS1 and CTSD were initially identified using a discovery mass spectrometry-based proteomics approach [177] and were subsequently observed in a PTEN knockout mouse model silencing the PI3K/PTEN cancer pathway that is involved in the carcinogenesis and progression of PCa [178] and in serum of men with and without PCa [179]. Clinical testing of individual immunoassays for the quantification of several

glycoproteins was performed, and THBS1 and CTSD were ultimately selected because their measurement improved the accuracy of the percentage of fPSA in distinguishing men with and without csPCa [180]. This novel diagnostic test has been developed and validated in men with serum PSA levels between 2 and 10ng/L, prostate volume ≥ 35cc and normal DRE, to distinguish men without PCa or iPCa from those with csPCa. The recommended threshold for Proclarix is 10%, with a 90% sensitivity to detect csPCa [175, 176, 181].

2. RATIONALE OF THE STUDY

Prostate cancer is the second most-frequently diagnosed neoplasm among men, accounting for approximately 15% of all cancers reported worldwide [182]. Suspicion of PCa continues to be based on serum determination of PSA and DRE. Nonetheless, definitive diagnosis of the neoplasm requires its demonstration by a prostate biopsy. Unfortunately, this diagnostic approach has a low specificity, resulting in an excessive number of unnecessary biopsies and an overdetection of iPCa [183]. Many tools have been employed in order to enhance the specificity of serum PSA, such as PSA density [70], age-related ranges of PSA [184], percentage of free PSA [185], and more recently, through some novel blood or urine markers. However, mpMRI has change the paradigm of early detection of PCa, establishing the risk of csPCa through the PI-RADS categories [186]. In addition, this imaging tool allows MRI-targeted biopsies of suspicious areas which increase the sensitivity of systematic biopsies for csPCa. The NPV of mpMRI (PI-RADS <3) reaches up to 90% (80-95%) [23, 187]. Prostate biopsies are recommended in men with PI–RADS > 3 when the risk of csPCa is below 20%, and those with PI–RADS 4 and 5 in whom csPCa is detected around 50% and 80%, respectively [186, 188]. In these challenging scenarios where the rate of csPCa detection is low, parameters such as PSAD, modern markers or predictive models may be helpful [189, 190]. Nonetheless, the use of mpMRI is hampered by the cost and access to imaging in many hospitals. Therefore, an appropriate selection of candidates for mpMRI and after mpMRI specially in men with PI-RADS 3 lesions derivate biopsies may contribute to this strategy [191, 192].

Proclarix is a newly introduced test that has been developed to detect csPCa in men with serum PSA levels between 2 and 10ng/mL, a PV ≥ 35mL and a normal DRE, with a recommended threshold of 10% [175, 176, 181]. Since this new marker has been

recently introduced with a lack of clinical development according to the currently recommended approach of csPCa early detection, based on pre-biopsy mpMRI and avoiding prostate biopsies when PI-RADS < 3 lesions [188], it is justified a project aiming to know its potential clinical usefulness in this new scenario of csPCa early detection. It is necessary to determinate how a new marker can decrease the mpMRI demand and improve the selection of candidates for prostate biopsy. Finally, it is also needed to assess the relationship between Proclarix and the aggressiveness of PCa.

3. HYPOTHESIS

Proclarix is a new tumor marker that has been developed with the aim of enhancing the early detection of clinically significant prostate cancer (csPCa). It is expected that this marker will lower the demand of multiparametric magnetic resonance imaging (mpMRI) in men with suspicion of PCa and improve the selection of candidates for prostate biopsy based on the current recommended PCa diagnostic approach, as well as being correlated with PCa aggressiveness.

4. OBJECTIVES

Objectives

To contrast the hypotheses proposed in the previous section, we defined the following main objective:

- To investigate the clinical utility and effectiveness of Proclarix in men with suspected csPCa before and after mpMRI.

Moreover, we also proposed the following secondary objectives:

1. To analyze through a systematic review of the literature, how Proclarix was discovered, its characteristics, and the clinical development until the beginning of this project.

2. To evaluate the role of Proclarix in reducing the number of unnecessary prostate biopsies in men with suspected PCa.

3. To compare the performance of Proclarix with PSA density and the Rotterdam-MRI risk-calculator in identifying suitable candidates for prostate biopsy.

4. To analyze the relationship between Proclarix and PCa aggressiveness.

5. COMPENDIUM OF PUBLICATIONS

The current doctoral thesis is being presented as a compendium of publications.

5.1. PUBLICATION 1

Title: Proclarix, A New Biomarker for the Diagnosis of Clinically Significant Prostate Cancer: A Systematic Review

Authors: Míriam Campistol; Juan Morote; Lucas Regis; Ana Celma; Jacques Planas; Enrique Trilla

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5.2. PUBLICATION 2

Title: The Efficacy of Proclarix to Select Appropriate Candidates for Magnetic Resonance Imaging and Derived Prostate Biopsies in Men with Suspected Prostate Cancer

Authors: Juan Morote; Míriam Campistol; Anna Celma; Lucas Regis; Inés de Torres; María E. Semidey; Sarai Roche; Richard Mast; Anna Santamaría; Jacques Planas; Enrique Trilla

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The Efficacy of Proclarix to Select Appropriate Candidates for Magnetic Resonance Imaging and Derived Prostate Biopsies in Men with Suspected Prostate Cancer

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Purpose: To analyze how Proclarix is valuable to appropriately select candidates for multiparametric magnetic resonance imaging (mpMRI) and derived biopsies, among men with suspected prostate cancer (PCa). Proclarix is a new marker computing the clinically significant PCa (csPCa) risk, based on serum thosmbospondin-1, cathepsin D, prostate-specific antigen (PSA) and percent free PSA, in addition to age, that has been developed in men with serum PSA 2 to 10 ng/mL, prostate volume ≥35 mL, and normal digital rectal examination (DRE).

Materials and Methods: Proclarix score (0%–100%) is analyzed in a prospective frozen serum collection of 517 correlative men scheduled for guided and/or systematic biopsies after mpMRI. Outcome variables were csPCa detection (grade group ≥2), insignificant PCa (iPCa) overdetection and avoided mpMRIs.

Results: The area under the curve of Proclarix was 0.701 (95% CI 0.637–0.765) among 281 men with serum PSA 2 to 10 ng/mL, prostate volume ≥35 mL, and -normal DRE, and 0.754 (95% CI 0.701–0.807) in the others, p=0.038. Net benefit of Proclarix existed in all men. After selecting 10% threshold, Proclarix was integrated in an algorithm which also used the serum PSA level and DRE. A reduction of 25.4% of mpMRIs request was observed and 17.7% of prostate biopsies. Overdetection of iPCa was reduced in 18.2% and 2.6% of csPCa were misdiagnosed.

Conclusions: Proclarix is valuable in all men with suspected PCa. An algorithm integrating Proclarix score, serum PSA, and DRE can avoid mpMRI requests, unnecessary prostate biopsies and iPCa overdetection, with minimal loss of csPCa detection.

Keywords: Clinically significant; Diagnosis; Multiparametric magnetic resonance imaging; Proclarix; Prostate cancer

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INTRODUCTION

Early detection of clinically significant prostate cancer (csPCa) can decrease the specific mortality of PCa [1]. PCa is suspected through the elevation of serum prostate-specific antigen (PSA) and/or abnormal digital rectal examination (DRE), and classically systematic biopsies have confirmed the diagnosis [2]. This approach has been criticised for the high rate of unnecessary biopsies and the overdetection of insignificant PCa (iPCa) [3]. Many tools have been used to increase the specificity of PCa suspicion; however, the true improvement of early detection of csPCa has come from multiparametric magnetic resonance imaging (mpMRI) and guided biopsies [2]. mpMRI can achieve a negative predictive value of up to 90%, while guided biopsies increase the sensitivity for csPCa, especially when they are associated with systematic biopsies [4]. However, this approach is hampered by the cost and access to imaging in many sites. Therefore, an appropriate selection of candidates for mpMRI and derived biopsies may contribute to this strategy [5,6], by using appropriate biomarkers and risk calculators possible tools [7].

Proclarix (Proteomedix, Schlieren, Switzerland) is a new blood-based CE-marker test based on the combination of serum thrombospondin-1 (THBS1), cathepsin D (CTSD), total PSA, and percent free-PSA in addition to age, providing a risk score of csPCa [8-10]. THBS1 and CTSD were identified from a mass spectrometry-based proteomics discovery approach [11] in a PTEN knockout mouse model of the PI3K/PTEN cancer pathway, which is involved in the carcinogenesis and progression of PCa [12,13]. Both glycoproteins, determined through specific immunoassays, improve the accuracy of percent-free PSA and age to distinguish men with csPCa [14]. Proclarix was developed in men with serum PSA between 2 and 10 ng/mL, prostate volume ≥35 mL, and normal DRE, and 10% threshold has been recommended due to its 90% sensitivity for csPCa [10]. The current challenge is how to make the best use of Proclarix in the current setting of csPCa diagnosis.

Our primary endpoint is to analyze the role of Proclarix to select suitable candidates for mpMRI and derived prostate biopsies among men with suspected PCa. Secondary endpoints are (1) to analyse associations of Proclarix with clinicopathological features of men with suspected PCa, (2) to know if Proclarix is valuable in men with suspected PCa and serum PSA outside the 2 to 10 ng/mL range, or prostate volume <35 mL, or abnormal DRE, and (3) to design an algorithm with Proclarix and clinical data to appropriately select candidates for mpMRI and derived prostate biopsies.

MATERIALS AND METHODS

1. Design, setting, and participants

A retrospective analysis was carried out in a prospective database and frozen serum collection of 567 men with suspected PCa, 433 (76.4%) biopsy naïve, scheduled for prostate biopsy after mpMRI [2], in Vall d'Hebron University Hospital, from 11 January 2018 to 12 March 2020. Blood samples were obtained immediately before prostate biopsy, and serum was stored at -80°C (Collection 0003439; https://biobancos.isciii.es). Men with PCa on active surveillance and those with symptomatic benign prostatic hyperplasia on 5-[Symbol - a]-reductase inhibitors were previously excluded. The clinicopathological characteristics of this cohort study are summarized in Supplement Table 1.

2. Intervention

THBS-1, CTSD, total PSA, and free PSA were determined with specific immunoassays at Proteomedix (Zurich-Schlieren, Switzerland). Then, THBS-1 and CTD levels, percent free PSA, and age were computed in an algorithm that reported a score ranging from 0% to 100%.

3. MpMRI technique and evaluation

Magnetic resonance was acquired on a 3-T scanner, using a surface phased-array coil (Magneton Trio; Siemens Corp., Erlanger, Germany). The acquisition protocol included T2-weighted imaging, diffusion-weighted imaging and dynamic contrast-enhanced imaging, according to the European Society of Urogenital Radiology guidelines [15]. Two expert radiologists analysed images and reported them according to Prostate Imaging-Reporting and Data System (PI-RADS) v.2.0 [16].

4. Prostate biopsy procedure and pathologic analysis

Guided biopsies obtained 2 to 3 cores from each PI-RADS v.2.0 ≥3 lesion through the TRUS-MRI cognitive-fusion technique [17]. A 12-core systematic biopsy was also performed in all men. All biopsies were performed through transrectal approach by one experienced urol-

Table 1. Behaviour of Proclarix, and univariate analysis regarding to clinical characteristics of the study population and its pathologic features

Characteristic	Men	Proclarix (%)	p-value
Biopsy result	567 (100.0)		
Benign	271 (47.8)	21.3 (9.5–34.6)	-
Prostate cancer	296 (52.2)	39.6 (24.9–65.7)	<0.001[a]
Grade group in biopsy	296 (100.0)		
1	66 (22.3)	26.6 (14.6–40.6)	0.026[a]
2	87 (29.4)	39.1 (23.2–56.0)	<0.001
3	61 (20.6)	38.4 (25.5–53.6)	0.861
4	51 (17.2)	53.6 (31.2–51.0)	0.018
5	31 (10.5)	74.5 (46.3–98.0)	0.047
Clinical stage (TNM)	296 (100.0)		
Localized (cT1-2 N0 M0)	263 (88.9)	37.3 (22.6–57.1)	<0.001[a]
Locally advanced (cT3-4 N0 M0)	22 (7.4)	60.1 (36.1–94.9)	<0.001
Disseminated (cT1-4 N0-1 M0-1)	11 (3.7)	97.4 (51.6–100)	<0.001
Localized prostate cancer recurrence risk	263 (100.0)		
Low	56 (21.3)	24.8 (14–4–37.7)	0.198[a]
Intermediate	136 (51.7)	34.1 (23.6–53.4)	<0.001
High	71 (27.0)	57.3 (31.4–80.9)	<0.001
Type of prostate cancer	296 (100.0)		
Insignificant	66 (22.2)	26.5 (14.6–40.6)	0.024[a]
Clinically significant	230 (77.8)	45.8 (28.3–70.5)	<0.001
Type of pathology	80 (100.0)		
Favorable	8 (10.0)	14.9 (6.1–38.7)	0.258[a]
Unfavorable	72 (90.0)	30.3 (20.0–47.2)	0.048

Values presented as number (%) or median (interquartile range).
[a]p-value referred to benign biopsy result.

ogist (A.C.) using a BK Focus 400 ultrasound scanner (BK Medical Inc., Herlev, Denmark). Biopsy samples were sent separately to the pathology department, where two expert pathologists analysed them (M.E.S. and I.T.). The International Society of Urological Pathology (ISUP) grade groups (GG) were used for grading tumours [18]. csPCa was defined when GG ≥2 [19]. In men subjected to radical prostatectomy, favorable pathology was defined when GG <2 and pT <3, and unfavorable pathology was defined when GG ≥2 or pT ≥3.

5. Endpoint variables

csPCa detection, iPCa overdetection, avoided mpMRIs, avoided prostate biopsies, misdiagnosis of csPCa.

6. Populations included in the study

The development population was defined as those men who had the same characteristic as those included in the development of Proclarix, PSA 2 to 10 ng/mL, and prostate volume ≥35 mL, and normal DRE (Subset 1). An additional population was men who did not meet any of these conditions (Subset 2).

7. Statistical analysis

Quantitative variables were expressed as medians and interquartile ranges. Qualitative variables were expressed as rates. Comparisons between quantitative variables were performed with the Mann–Whitney U-test and the Kruskal–Wallis test. Qualitative variables were compared with the chi-square test and the Fisher correction if necessary. Receiver operating characteristic (ROC) curves were constructed and areas under the curve (AUC) were evaluated and compared with the DeLong test. Binary logistic regression analysis was performed to assess predictors of csPCa and generate predictive models. Decision curve analyses (DCAs) were generated to assess net benefits between predictors. Significant differences were assessed when the p-value was less than 5%. SPSS v.25 (IBM Corp., Armonk, NY, USA) and R programming language v.3.3.1 (The R Statistical Foundation, Vienna, Austria) were used.

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Table 2. Analysis of predictors for clinically significant prostate cancer detection in the entire study population

Predictor	Univariate analysis		Multivariate analysis	
	Odds ratio (95% confidence interval)	p-value	Odds ratio (95% confidence interval)	p-value
Age (ref. previous year)	1.079 (1.052–1.106)	<0.001	1.040 (0.879–1.069)	0.186
Prostate cancer family history (ref. no)	1.344 (0.708–2.550)	0.366	1.534 (0.794–2.963)	0.203
Type of biopsy (ref. initial)	0.785 (0.502–1.225)	0.286	0.757 (0.477–1.201)	0.237
Digital rectal examination (ref. normal)	2.607 (1.290–4.682)	<0.001	1.980 (0.785–3.717)	0.094
Prostate-specific antigen (ref. previous ng/mL)	1.028 (1.013–1.053)	<0.001	1.001 (0.991–1.011)	0.858
Proclarix (ref. previous percent value)	1.034 (1.023–1.044)	<0.001	1.042 (1.028–1.057)	<0.001

8. Ethics statement

Our study was conducted in line with Good Clinical Practice guidelines and the ethical principles laid down in the latest version of the Declaration of Helsinki (2013). Before inclusion, all participants signed a written informed consent about the collection and storage of material and personal data in accordance with national bylaws. All anamnestic, clinical and laboratory data containing sensitive information about patients were de-identified in order to ensure analysis of anonymous data only. The present study protocol was reviewed and approved by Vall d'Hebron Ethics Commitee (Reg. No. PR-AG129/2020).

RESULTS

1. Behaviour of Proclarix regarding the clinicopathological characteristics of men with suspected PCa

The associations between Proclarix and clinical and pathological features of the study cohort are summarized in Table 1. The median score of Proclarix in men with a benign result of prostate biopsy was 21.3%, which is significantly lower than the median of 39.6% observed in men with PCa, p<0.001. The median score of Proclarix in men with GG1 PCa was 26.6%, which is significantly higher than that observed in men with benign prostate biopsy, p=0.026. Therefore, the Proclarix score increased with GG, p<0.05, except between GG2 and GG3 tumours, p=0.861. The median Proclarix score in clinically localized PCa was 37.3%, 60.1% in locally advanced PCa, and 97.4% in metastatic PCa, p<0.001. The median Proclarix score in low-risk, clinically localized PCa was 24.8%, which is similar to the median in men without PCa, p=0.198. The median Proclarix score increased to 34.1% in intermediate-risk PCa and 57.3% in high-risk PCa, p<0.001. The median Proclarix score was 45.8% in men with csPCa and 26.5% in iPCa, p<0.001. Among 80 men subjected to radical prostatectomy, the median Proclarix score was 14.9% when the pathology was favorable, being it similar to that observed in men with benign tissue at prostate biopsy, and the median Proclarix score was 30.3% when the pathology was unfavorable, p=0.048.

2. Analysis of Proclarix as a predictor of csPCa in men with suspected PCa before mpMRI

Univariate analysis including age, PCa family history, type of biopsy (initial *versus* repeat), DRE, serum PSA, and Proclarix as potential predictors of csPCa, showed age, DRE, serum PSA, and Proclarix were significantly associated with csPCa, Table 2. Thereafter, a logistic regression analysis showed that the quantitative Proclarix score was the only independent predictor of csPCa, odds ratio (OR) 1.042 (95% confidence interval [CI] 1.028–1.057), p<0.001, Table 2. The ROC curve of the Proclarix score presented in Fig. 1A, had an AUC of 0.767 (95% CI 0.730–0.805). DCA showing the net benefit of Proclarix is presented in Fig. 1B.

In our entire study cohort, the recommended 10% threshold of Proclarix presented a sensitivity for csPCa of 97.4%, specificity of 26.7%, a negative predictive value of 93.8%, and a positive predictive value of 47.6%. In summary, 16.9% of mpMRI requests and derived prostate biopsies will be avoided, as will 2.6% of csPCa misdiagnosis, Table 3. The characteristics of six men, identified with false-negative Proclarix results, are summarized in Supplement Table 2: two men had GG 2, one had GG 3, two men had GG4, and one had GG5.

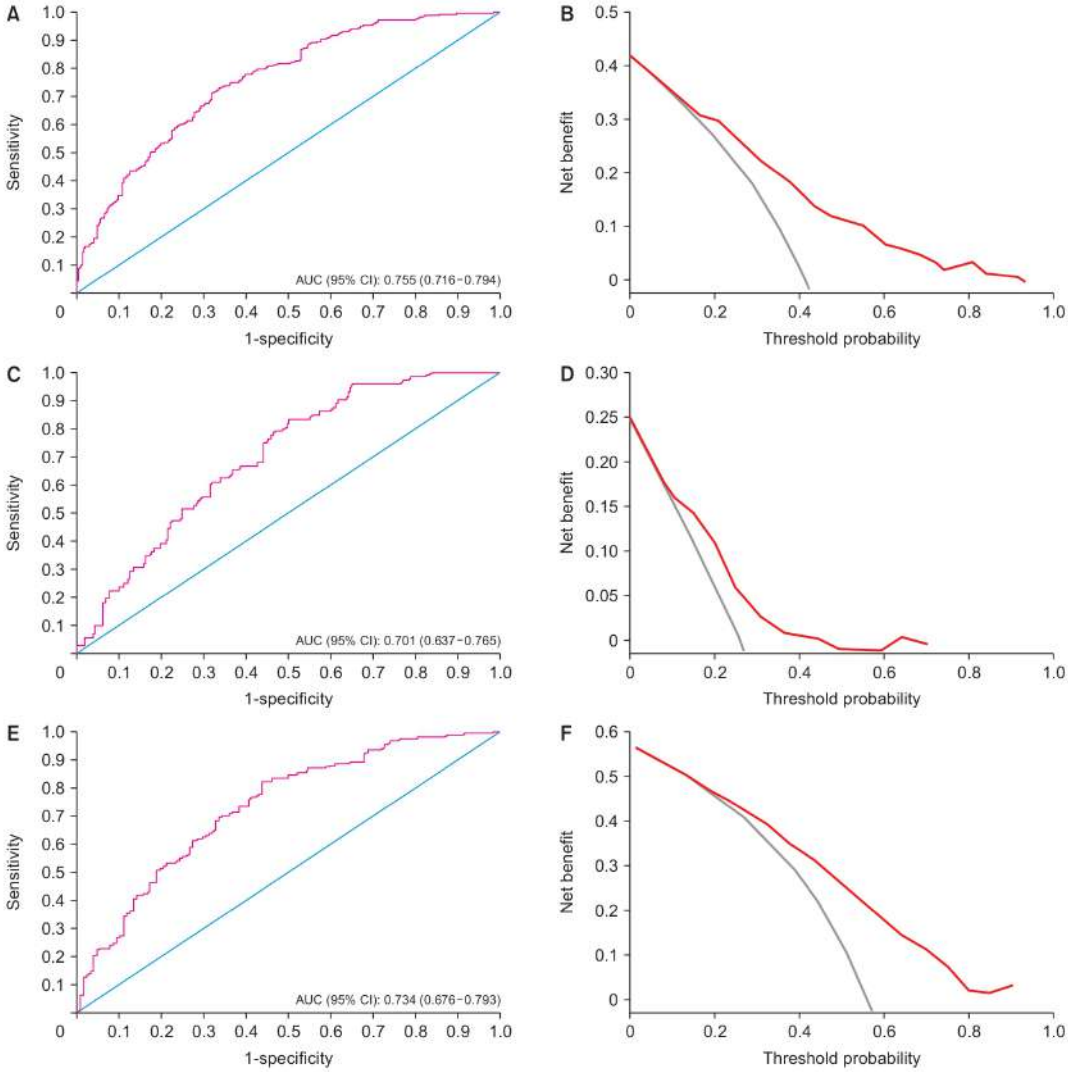

Fig. 1. Receiver operating characteristic curves of Proclarix score for clinically significant prostate cancer, and decision curve analysis showing its net benefit in front of biopsying all men with suspected prostate cancer in the overall population (A, B), in men with serum prostate-specific antigen 2 to 10 ng/mL, prostate volume ≥35 mL, and normal digital rectal examination (Subset 1) (C, D), and men with serum prostate-specific antigen out of the interval 2 to 10 ng/mL, or prostate volume <35 mL, or abnormal digital rectal examination (Subset 2) (E, F). AUC: areas under the curve.

3. Behaviour of Proclarix as a predictor of csPCa in men with suspected PCa regarding the level of serum PSA, prostate volume, and DRE

Among our correlative series of 567 men with suspected PCa, 281 (49.6%) had serum PSA between 2 and 10 ng/mL, prostate volume ≥35 mL, and normal DRE (Subset 1), while 286 (50.4%) has serum PSA outside the 2 to 10 ng/mL range, or prostate volume <35 mL, or abnormal DRE (Subset 2). The characteristics of both subsets are presented in Supplement Table 3. We highlight that the median Proclarix score was 21.0% in Subset 1 and 41.0% in Subset 2, p<0.001, and the rate of csPCa was 25.6% in Subset 1 and 55.2% in Subset 2, p<0.001.

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Table 3. Parameters of efficacy of Proclarix score (threshold 10%) to detect clinically significant prostate cancer in overall study population

Parameter	Value
Sensitivity	224/230 (97.4)
Specificity	90/337 (26.7)
Negative predictive value	90/96 (93.8)
Positive predictive value	224/471 (47.6)
Accuracy	314/567 (55.4)
Avoided magnetic resonance imaging	96/567 (16.9)
Undetected clinically significant prostate cancer	6/230 (2.6)
Odds ratio (95% confidence interval)	13.603 (5.838–31.698)
p-value	<0.001

Values are presented as number (%).

Logistic regression analysis for csPCa was performed in both subsets of men and is shown in Table 4. We highlight that Proclarix score was the only significant and independent predictor of csPCa, OR 1.037 (95% CI 1.018–1.056), p<0.001, in Subset 1, and OR 1.057 (95% CI 1.022–1.083), p<0.001, in Subset 2. The ROC analyses of the Proclarix score in the men of Subset 1 is presented in Fig. 1C, with AUC=0.701 (95% CI 0.637–0.765). DCA showing the net benefit of Proclarix is presented in Fig. 1D. Among men of Subset 2, the ROC analyses of Proclarix score is presented in Fig. 1E, with AUC=0.754 (95% CI 0.701–0.807; p=0.038). DCA showing the net benefit of Proclarix is presented in Fig. 1F (p=0.038).

The parameters of the efficacy of Proclarix using the threshold of 10% in both subsets of men are summa-

Table 4. Logistic regression analysis of candidate predictors for clinically significant prostate cancer detection regarding the characteristic of men with suspected prostate cancer

Predictor	Subset 1		Subset 2	
	Odds ratio (95% confidecne interval)	p-value	Odds ratio (95% confidence interval)	p-value
Age (ref. previous year)	1.005 (0.960–1.051)	0.845	1.032 (0.945–1.102)	0.189
Prostate cancer family history (ref. no)	1.397 (0.541–3.602)	0.490	1.707 (0.654–4.455)	0.274
Type of biopsy (ref. initial)	1.225 (0.619–2.422)	0.560	0.510 (0.270–0.963)	0.138
Digital rectal examination (ref. normal)	-		1.637 (0.980–3.610)	0.089
Prostate-specific antigen (ref. previous ng/mL)	0.957 (0.817–1.120)	0.581	1.001 (0.990–1.011)	0.912
Proclarix (ref. previous percent)	1.037 (1.018–1.056)	<0.001	1.057 (1.022–1.083)	<0.001

-: not available.
Subset 1 (men with serum prostate-specific antigen 2 to 10 ng/mL, and prostate volume ≥35 mL, and normal digital rectal examination), and Subset 2 (men who do not meet any of the previous characteristics).

Table 5. Parameters of efficacy for Proclarix, using a threshold of 10%, regarding the characteristics of men

Parameter	Subset 1	Subset 2
Sensitivity	69/72 (95.8)	155/158 (98.1)
Specificity	68/209 (32.5)	22/128 (17.2)
Negative predictive value	68/71 (95.8)	22/25 (88.0)
Positive predictive value	69/219 (31.5)	155/261 (59.4)
Correct classification	137/281 (48.8)	177/286 (61.9)
Avoided magnetic resonance imaging	71/281 (25.3)	25/286 (8.7)
Undetected clinically significant prostate cancer	3/72 (4.2)	3/158 (1.9)
Odds ratio (95% confidence interval)	11.092 (3.369–36.519)	10.720 (3.130–36.735)
p-value	<0.001	<0.001
Prostate cancer detection	117/281 (41.6)	179/286 (62.6)
Clinically significant prostate cancer detection	72/281 (25.6)	158/286 (55.2)
Insignificant prostate cancer detection	45/281 (16.0)	21/286 (7.3)

Values are presented as number (%).
Subset 1 (men with serum prostate-specific antigen 2 to 10 ng/mL, and prostate volume ≥35 mL, and normal digital rectal examination), and Subset 2 (men who do not meet any of the previous characteristics).

rized in Table 5. We highlight that Proclarix presented a sensitivity of 95.8% in Subset 1 and 98.1% in Subset 2, and specificities of 32.5% and 17.2%, respectively. From the clinical point of view, Proclarix will avoid 25.3% of mpMRI and derived prostate biopsies in Subset 1 with 4.2% misdiagnosis of csPCa, while in Subset 2, these rates were 8.7% and 1.9%, respectively.

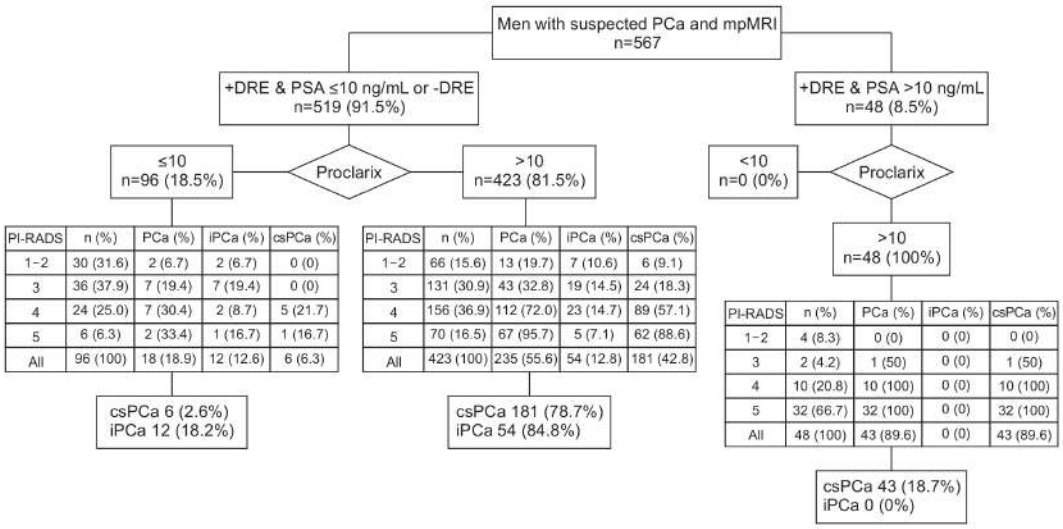
4. Design of an algorithm integrating the characteristics of serum PSA, DRE, and Proclarix to select appropriate candidates for mpMRI and derived prostate biopsies

We detected 48 men (8.5%) with +DRE and PSA >10 ng/mL in whom mpMRI and guided biopsies do not increase the efficacy of systematic biopsies [20]. Proclarix was >10% in all these men and csPCa was detected in 43 (89.6%); Fig. 2. We also confirmed that guided biopsies did not increase the rate of csPCa detection. Both systematic and guided biopsies detected 8 men with GG=2, 10 with GG=3, 11 with GG=4, and 14 with GG=5. Therefore, we propose that men with +DRE and PSA >10 ng/mL will be directly schedule for systematic biopsies (Fig. 3). Among the remaining 519 men who had normal DRE, or abnormal DRE with serum PSA ≤10 ng/mL, Proclarix was ≤10% in 96 (18.5%). iPCa was detected in 12 (18.2% of all iPCa) and csPCa was detected in 6 (2.6% of all csPCa). Proclarix was >10% in 423 men (81.5%). iPCa was detected in 54 (84.8% of all iPCa), and csPCa was detected in 181 (78.7% of all csPCa) (Fig. 2). We propose avoiding mpMRI and derived prostate biopsies among men with Proclarix <10% and performing guided and/or systematic prostate biopsies in those with Proclarix >10% (Fig. 3). This algorithm will avoid 25.4% of mpMRIs, 17.5% of prostate biopsies, and 18.2% of iPCa overdiagnosis, with 2.6% misdiagnoses of csPCa (Fig. 3).

Among 423 men with PI-RADS ≥3 in whom guided and systematic biopsies were performed, csPCa was detected in 181 (42.8%). In 121 men, both biopsies identified csPCa (66.9%), only the guided biopsies identified csPCa in 31 (17.1%), and only the systematic biopsies identified csPCa in 28 (15.5%), p=0.458.

DISCUSSION

The new marker Proclarix has been associated with PCa grading, but used to compare men without PCa or GG 1 with those with GG 2 or 3 and those with GG 4 or 5 [10,21]. The present study confirms that Proclarix score is associated with the GG, but it cannot



PI-RADS	n (%)	PCa (%)	iPCa (%)	csPCa (%)
1–2	30 (31.6)	2 (6.7)	2 (6.7)	0 (0)
3	36 (37.9)	7 (19.4)	7 (19.4)	0 (0)
4	24 (25.0)	7 (30.4)	2 (8.7)	5 (21.7)
5	6 (6.3)	2 (33.4)	1 (16.7)	1 (16.7)
All	96 (100)	18 (18.9)	12 (12.6)	6 (6.3)

PI-RADS	n (%)	PCa (%)	iPCa (%)	csPCa (%)
1–2	66 (15.6)	13 (19.7)	7 (10.6)	6 (9.1)
3	131 (30.9)	43 (32.8)	19 (14.5)	24 (18.3)
4	156 (36.9)	112 (72.0)	23 (14.7)	89 (57.1)
5	70 (16.5)	67 (95.7)	5 (7.1)	62 (88.6)
All	423 (100)	235 (55.6)	54 (12.8)	181 (42.8)

PI-RADS	n (%)	PCa (%)	iPCa (%)	csPCa (%)
1–2	4 (8.3)	0 (0)	0 (0)	0 (0)
3	2 (4.2)	1 (50)	0 (0)	1 (50)
4	10 (20.8)	10 (100)	0 (0)	10 (100)
5	32 (66.7)	32 (100)	0 (0)	32 (100)
All	48 (100)	43 (89.6)	0 (0)	43 (89.6)

Fig. 2. Multiparametric magnetic resonance imaging reports (PI-RADSv.2) and prostate biopsy results regarding the proposed algorithm in men with suspected PCa, based on serum PSA >3.0 ng/mL and/or abnormal DRE, in whom mpMRI and guided and/or systematic biopsies were performed. PCa: prostate cancer, mpMRI: multiparametric magnetic resonance imaging, DRE: digital rectal examination, PSA: prostate-specific antigen, csPCa: clinically significant PCa, iPCa: insignificant PCa.

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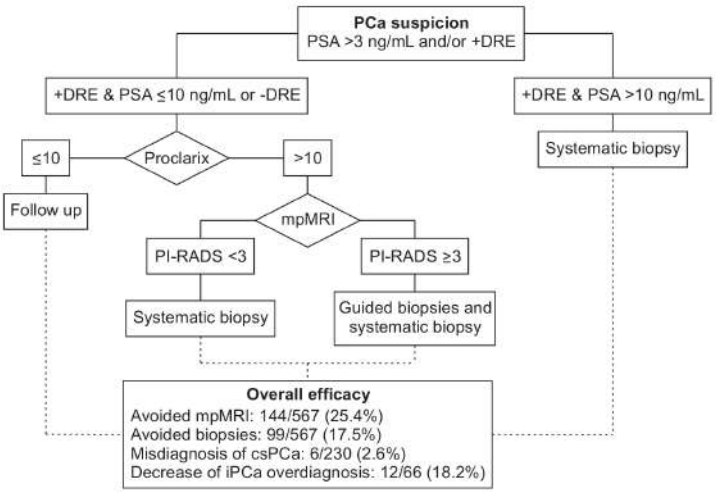

Fig. 3. Overall efficacy of a proposed algorithm, which uses Proclarix evaluation, after PCa suspicion, in men with abnormal DRE and serum PSA ≤10 ng/mL, and those with normal DRE. Men with abnormal DRE and serum PSA >10 ng/mL are directly scheduled to systematic biopsy without previous mpMRI. PCa: prostate cancer, PSA: prostate-specific antigen, DRE: digital rectal examination, mpMRI: multiparametric magnetic resonance imaging, csPCa: clinically significant PCa, iPCa: insignificant PCa.

distinguish between GG 2 and GG 3. We also report that Proclarix is associated with the clinical stage of PCa and the risk of recurrence of treated localized PCa. Nowadays, only the recently published PROPOSe study has analyzed the relationship between Proclarix score and the results of mpMRI. The authors analyze the biopsy results in 121 men with positive mpMRI, suggesting that Proclarix represents a valuable rule-out test in the diagnostic algorithm for PCa, alone or in combination with mpMRI [21].

Proclarix has been used in men with PSA between 2 and 10 ng/mL, prostate volume <35 mL, or abnormal DRE [10-14,21]. We have tested Proclarix in men outside of these characteristics, representing half of our correlative case mix of men with suspected PCa. This is especially relevant with the prostate volume, which is currently not known before mpMRI because transrectal ultrasonography is not usually performed for this purpose [22]. Both populations are different in terms of csPCa incidence, which was 25.3% and 55.5%, respectively. The sensitivity of Proclarix was very high in both subsets of men, 95.8% in men with PSA between 2 and 10 ng/mL, prostate volume <35 mL, or abnormal DRE and 98.1% in the others. However, the specificities were 32.5% and 17.2% respectively. To summarize, Proclarix showed net benefit in both subsets of men with suspected PCa; however, Proclarix was able to reduce 25.3% of mpMRI and derived prostate biopsies in men with serum PSA 2 to 10 ng/mL, and prostate volume ≥35 mL, and normal DRE, while it reduced 8.7% of mpMRI request in those men who did not meet any of these characteristics. The misdiagnosis rate of csPCa was 4.2% and 1.9%, respectively.

We intended to analyze how Proclarix can be used to select appropriate candidates for mpMRI. Because there is evidence that men with abnormal DRE and serum PSA >10 ng/mL do not benefit from mpMRI and guided biopsies [20,23], we propose that these men be scheduled directly for systematic prostate biopsy. The rate of csPCa in these men, who represents around 10% of all men with suspected PCa, was 89.6%; that is, 18.7% of all detected csPCa. Then, we propose that Proclarix will be evaluated in men with normal DRE, and those with abnormal DRE and serum PSA ≤10 ng/mL. Among these men, around 20% had a Proclarix of ≤10%, which was our target for avoiding mpMRI and derived prostate biopsies. Here, the misdiagnosis of csPCa represented 2.6% of all csPCa detected, and the overdiagnosis of iPCa was 18.2% of all iPCa detected. Finally, all men with Proclarix >10% will be scheduled for guided and/or systematic prostate biopsy. This overall approach will reduce the request for mpMRIs by 25.4%, the number of prostate biopsies by 17.5%, the overdetection of iPCa by 18.2%, and the misdiagnosis of csPCa will be 2.6%.

The comparison between Proclarix and other markers is difficult [24-28]. SelectMDx seems more sensitive than mpMRI but less specific [24]. In a cohort of 599 biopsy naïve men scheduled to guided and/or systematic biopsies, SelectMDx will avoid 38% of prostate biopsies

with 10% of csPCa misdiagnosis [25]. 4K test has been shared with mpMRI and clinical variables in a predictive nomogram [26]. In a study of 266 biopsy-naïve men in whom 74 csPCa were detected (27.8%), 4K <7.5 will avoid 32 (12%) mpMRI and 1 csPCa between 74 (1.4%) csPCa will be misdiagnosed [27]. Prostate Health Index has been analyzed only in biopsied men with PI-RADS ≥3 [28]. Comparisons between markers can be only effective in head-to-head studies. We believe that the major strength of Proclarix when used to select appropriate candidates for mpMRI and derived prostate biopsies, is its high sensitivity for csPCa. Nevertheless, the final benefit of any strategy for csPCa detection should be analyzed in terms of health benefit, through appropriate studies of cost-effectiveness analyzing the quality-adjusted life years and healthcare cost [29].

Limitations of our study are its retrospective design and the definition of csPCa used in prostate biopsies which may not represent the true pathology. External and multicenter validation of these results is necessary.

CONCLUSIONS

Proclarix is associated with the clinical stage and grading of PCa, the risk of biochemical recurrence of treated localized PCa, and the type of pathology from surgical specimens. Proclarix is valuable for csPCa detection in all men with suspected PCa, independently of their PSA level, prostate volume, or DRE. Proclarix can be integrated into an algorithm to select appropriate candidates for mpMRI and derived prostate biopsies.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: JM, MC. Data curation: AC, LR, SR, LdT, MS,. Formal analysis: JM. Methodology: JM, AS, JP. Software: Filemaker14, SPSS 25, Bookends13.2.3. Supervision: JM, ET. Writing – original draft: JM. Writing – review & editing: JM, ET.

Supplementary Materials

Supplementary materials can be found *via* https://doi.org/10.5534/wjmh.210117.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Hugosson J, Roobol MJ, Månsson M, Tammela TLJ, Zappa M, Nelen V, et al.; ERSPC investigators. A 16-yr follow-up of the European randomized study of screening for prostate cancer. Eur Urol 2019;76:43-51.
2. Mottet N, van den Bergh RCN, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer-2020 update. Part 1: screening, diagnosis, and local treatment with curative intent. Eur Urol 2021;79:243-62.
3. US Preventive Services Task Force, Grossman DC, Curry SJ, Owens DK, Bibbins-Domingo K, Caughey AB, et al. Screening for prostate cancer: US Preventive Services Task Force recommendation statement. JAMA 2018;319:1901-13.
4. Sathianathen NJ, Omer A, Harriss E, Davies L, Kasivisvanathan V, Punwani S, et al. Negative predictive value of multiparametric magnetic resonance imaging in the detection of clinically significant prostate cancer in the prostate imaging reporting and data system era: a systematic review and meta-analysis. Eur Urol 2020;78:402-14.
5. Vickers AJ, Russo G, Lilja H, Evans C, Schalken JA, Klein E, et al. How should molecular markers and magnetic resonance imaging be used in the early detection of prostate cancer? Eur Urol Oncol 2021. doi: 10.1016/j.euo.2021.01.010 [Epub].
6. Drost FH, Osses D, Nieboer D, Bangma CH, Steyerberg EW, Roobol MJ, et al. Prostate magnetic resonance imaging, with or without magnetic resonance imaging-targeted biopsy, and systematic biopsy for detecting prostate cancer: a Cochrane systematic review and meta-analysis. Eur Urol 2020;77:78-94.
7. Osses DF, Roobol MJ, Schoots IG. Prediction medicine: biomarkers, risk calculators and magnetic resonance imaging as risk stratification tools in prostate cancer diagnosis. Int J Mol Sci 2019;20:1637.

5.3. PUBLICATION 3

Title: Comparison of Proclarix, PSA Density and MRI-ERSPC Risk Calculator to Select Patients for Prostate Biopsy after mpMRI

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Comparison of Proclarix, PSA Density and MRI-ERSPC Risk Calculator to Select Patients for Prostate Biopsy after mpMRI

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Simple Summary: The selection of proper candidates for prostate biopsy after magnetic resonance imaging (MRI) has usually been studied in the overall population with suspected prostate cancer (PCa). However, the performance of these tools can change regarding the Prostate Imaging-Reporting and Data System (PI-RADS) categories. We compared three different tools: PSA density, MRI-ERSPC risk calculator and Proclarix in 567 men with suspected PCa (PSA > 3 ng/mL and/or abnormal rectal examination) in one academic institution. All patients underwent multiple transrectal ultrasound guided biopsies after a multiparametric MRI was performed. We concluded that in the overall population, MRI-ERSPC RC outperformed PSA density and Proclarix, whereas in patients with lesions PI-RADS < 3 Proclarix was better than the other tools. However, no tool guaranteed 100% detection of clinically significant PCa in PI-RADS 4 and 5.

Abstract: Tools to properly select candidates for prostate biopsy after magnetic resonance imaging (MRI) have usually been analyzed in overall populations with suspected prostate cancer (PCa). However, the performance of these tools can change regarding the Prostate Imaging-Reporting and Data System (PI-RADS) categories due to the different incidence of clinically significant PCa (csPCa). The objective of the study was to analyze PSA density (PSAD), MRI-ERSPC risk calculator (RC), and Proclarix to properly select candidates for prostate biopsy regarding PI-RADS categories. We performed a head-to-head analysis of 567 men with suspected PCa, PSA > 3 ng/mL and/or abnormal rectal examination, in whom two to four core transrectal ultrasound (TRUS) guided biopsies to PI-RADS $\geq$ three lesions and/or 12-core TRUS systematic biopsies were performed after 3-tesla mpMRI between January 2018 and March 2020 in one academic institution. The overall detection of csPCa was 40.9% (6% in PI-RADS < 3, 14.8% in PI-RADS 3, 55.3% in PI-RADS 4, and 88.9% in PI-RADS 5). MRI-ERSPC model exhibited a net benefit over PSAD and Proclarix in the overall population. Proclarix outperformed PSAD and MRI-ERSPC RC in PI-RADS $\leq$ 3. PSAD outperformed MRI-ESRPC RC and Proclarix in PI-RADS > 3, although none of them exhibited 100% sensitivity for csPCa in this setting. Therefore, tools to properly select candidates for prostate biopsy after MRI must be analyzed regarding the PI-RADS categories. While MRI-ERSPC RC outperformed PSAD and Proclarix in the overall population, Proclarix outperformed in PI-RADS $\leq$ 3, and no tool guaranteed 100% detection of csPCa in PI-RADS 4 and 5.

Keywords: clinically significant prostate cancer; PSA density; Proclarix; MRI-ERSPC; magnetic resonance imaging

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1. Introduction

Early detection of clinically significant prostate cancer (csPCa) decreases the specific mortality of PCa [1]. The classic diagnostic approach to PCa, based on systematic biopsies after elevated serum prostate-specific antigen (PSA) and/or abnormal digital rectal examination (DRE) [2], has been disapproved due to high rates of unnecessary biopsies and an over detection of insignificant PCa (iPCa) [3]. Recent improvements in early detection of csPCa come from multiparametric magnetic resonance imaging (mpMRI) and guided biopsies [2].

The efficacy of this new diagnostic strategy for csPCa can still be improved by a proper selection of candidates for prostate biopsy, particularly in uncertain cases [3]. The current negative predictive value (NPV) of mpMRI, when the Prostate Imaging-Reporting and Data System (PI-RADS) category is below 3, reaches 95% [4,5], and that is why most clinicians recommend avoiding biopsies in these cases. Conversely, men with PI-RADS categories greater than 3 have a likelihood of csPCa ranging from 55% to 95% [6], and almost all clinicians recommend scheduling prostate biopsies in these circumstances. The probability of csPCa in PI-RADS category 3 is not higher than 20%, making this an uncertain case [7,8].

Among the proposed tools for improving the proper selection of candidates for prostate biopsy after mpMRI, PSA density (PSAD) has recently emerged, as MRI provides the most accurate measurement of prostate volume without additional cost [9]. PSAD has been analyzed according to PI-RADS categories and different thresholds have been proposed depending on the results [10]. Currently, there is no ideal marker to use after mpMRI [11]; the new marker Proclarix might be an adequate candidate due to its high sensitivity for csPCa but has not yet been analyzed by PI-RADS category [12,13]. This test has recently been introduced, providing a risk score of csPCa from 1 to 100% with a cut-off at 10%, obtaining a high sensitivity and a high negative predictive value (90 and 95% respectively) [14]. It is based on the serum determination of Thrombospondin-1, Cathepsin D, PSA and % fPSA, together with age. In addition, predictive models are attractive tools when they incorporate easily assessed clinical variables, when they are externally validated, and when web or smartphone applications (apps) are provided for their easy use in clinical practice, as in the case of the recent MRI-ERSPC risk calculator (RC). However, none of these has been evaluated by the PI-RADS category [15].

Since the incidence of csPCa increases by PI-RADS category, we hypothesized that changes in the predictive value of tools to improve the proper selection of candidates for prostate biopsy will be expected [10]. Therefore, we primarily seek to change the evaluation paradigm of these tools after verifying our hypothesis. We analyzed the usefulness of PSAD, the MRI-ERSPC RC, and the new marker Proclarix in a population of men with suspected PCa, as well as evaluating them by PI-RADS category.

2. Materials and Methods

2.1. Design, Setting and Participants

This is a prospective comparative study between PSAD, MRI-ERSPC RC and Proclarix in 567 consecutive men with suspected PCa due to PSA levels > 3 ng/mL and/or abnormal DRE scheduled for a 3-tesla mpMRI prior to biopsy from 15 January 2018 to 20 March 2020 in one academic institution. Twelve core transrectal ultrasound (TRUS) systematic-biopsies were performed in all participants, and two to four core TRUS cognitive fusion biopsies were taken in those patients with suspicious lesions (PI-RADSv.2 $\geq$ 3). Men with PCa on active surveillance and those with symptomatic benign prostatic hyperplasia treated with 5-α-reductase inhibitors were excluded. This project was approved by the Institutional Ethics Committee (PR-AG129/2020), and informed consent was obtained from study participants.

2.2. Intervention

Proclarix was assessed from serum samples obtained just before prostate biopsies were performed and stored at -80 °C (Collection 0003439; https://biobancos.isciii.es (accessed on 13 December 2021)). Thrombospondin 1 (THBS-1), Cathepsin D (CTD), total PSA, and

free PSA were determined at Proteomedix (Zurich-Schlieren, Switzerland). THBS-1 and CTD levels were measured with specific immunoassays described previously [16]. Total PSA and free PSA were analyzed for all samples with the Roche Cobas immunoassay system (Roche Diagnostics, Rotkreuz, Switzerland), and age was calculated using an algorithm that reported a score ranging from 0% to 100% [14,17]. PSAD (ng/mL/cc) was estimated from the PSA level determined in the Proclarix assessment and the prostate volume reported in the pre-biopsy mpMRI. The MRI-ERSPC likelihood of high-grade PCa (Gleason $\geq 3 + 4$) was estimated for every man through the SWOP web application (Prostate Cancer Research Foundation, Reeuwijk) at www.prostatecancer-riskcalculator.com (accessed on 21 January 2022) [18]. The MRI-ERSP RC includes serum PSA (0.5 to 50 ng/mL), repeat biopsy (yes/no), DRE (normal/abnormal), prostate volume (10–110 cc), age (50–75 years), and PI-RADSv.1 [15]. For these calculations, the MRI-based prostate volume was introduced as well as the PI-RADSv.2 categories [19]. When the observed values were not within the accepted range, the closest minimum or maximum accepted value was entered.

2.3. Endpoint Measurements

The CsPCa detection rate and avoidable prostate biopsies were the primary endpoint measurements. CsPCa was confirmed when the ISUP (International Society of Uropathology) grade group was ≥2 [20,21].

2.4. Statistical Analyses

The association between quantitative variables was assessed with the Mann–Whitney U test and the Kruskal–Wallis test. The associations between qualitative variables were analyzed with a Chi-square test. The odds ratios (OR) and 95% confidence interval (95% CI) were also estimated. Receiver operating characteristic (ROC) curves were constructed and areas under the curve (AUC) were estimated and compared with the DeLong test [22,23]. A decision curve analysis (DCA) was performed to study the net benefits [24] and clinical utility curves (CUC) were generated to assess the difference between missed csPCa and avoided biopsies across the continuous likelihood of csPCa [25]. The performance of predictors with the selected thresholds were analyzed based on sensitivity, specificity, positive and negative predictive values (PPV and NPV), accuracy, rates of avoided biopsies, and rate of missed csPCa. A p-value of less than 5% was considered significant. SPSS v.25 (IBM Corp., Armonk, NY, USA) and R programming language v.3.3.1 (The R Statistical Foundation, Vienna, Austria) were used.

3. Results

3.1. Characteristics of the Study Population and Distribution of Overall PCa, csPCa, and iPCa by PI-RADS Category

The characteristics of the study population are summarized in Table 1. We highlight a median age of 69 years and a PSA of 7.0 ng/mL. In addition, 19.2% of patients had an abnormal DRE, 23.5% were repeated biopsies, and 8.6% had a family history of PCa. The distribution by PI-RADS categories was 17.6% with PI-RADS < 3, 29.8% with PI-RADS 3, 33.5% with PI-RADS 4, and 19% with PI-RADS 5. The overall rate of detected PCa was 52.6%, 40.9% of csPCa, and 11.7% of iPCa. CsPCa was detected in 6% of men with PI-RADS < 3, 14.8% in PI-RADS 3, 55.3% in PI-RADS 4, and 88.9% in PI-RADS 5, $p < 0.001$.

Table 1. Characteristics of the study cohort.

Characteristic	Measurement
Number of cases	567
Median age, years (IQR)	69 (63–74)
Median total PSA, ng/mL (IQR)	7.0 (4.9–11.2)
Abnormal DRE, *n* (%)	109 (19.2)
Median free PSA, ng/mL (IQR)	1.1 (0.7–1.7)
Median prostate volume, mL (IQR)	55 (40–76)
Median percent free PSA, % (IQR)	15.1 (10.7–20.6)
Median PSA density, ng/mL/cc (IQR)	0.13 (0.09–0.21)
Repeat biopsy, *n* (%)	133 (23.5)
Family history of PCa, *n* (%)	48 (8.6%)
PI-RADS, *n* (%)	
1–2	100 (17.6)
3	169 (29.8)
4	190 (33.5)
5	108 (19.0)
Overall PCa detection, *n* (%)	298 (52.6)
csPCa detection, *n* (%)	232 (40.9)
iPCa detection, *n* (%)	66 (11.7)

IQR = Interquartile range; PCa = Prostate Cancer; csPCa = clinically significant PCa; iPCa = insignificant PCa.

3.2. Overall Efficacy, Net Benefit, and Clinical Utility of mpMRI, PSAD, MRI-ERSPC RC, and Proclarix, and Overall Performances after the Selection of Appropriate Thresholds

ROC curves analyzing the efficacy of mpMRI, PSAD MRI-ERSPC RC, and Proclarix for the detection of in the overall population study are presented in Figure 1a. MRI-ERSPC RC showed an AUC of 0.856 (95% CI: 0.824–0.888); mpMRI, 0.831 (95% CI: 0.705–0.786); Proclarix, 0.745 (95% CI: 0.705–0.786); and PSAD, 0.740 (95% CI: 0.698–0.782), with $p = 0.038$. DCAs showed the highest net benefit for mpMRI at threshold probabilities between 0.1 and 0.45, while MRI-ERSPC RC when the threshold probability was higher, Figure 1b. CUCs showed the largest area between csPCa missed and avoided biopsy rates at all threshold probabilities, as shown in Figure 1c.

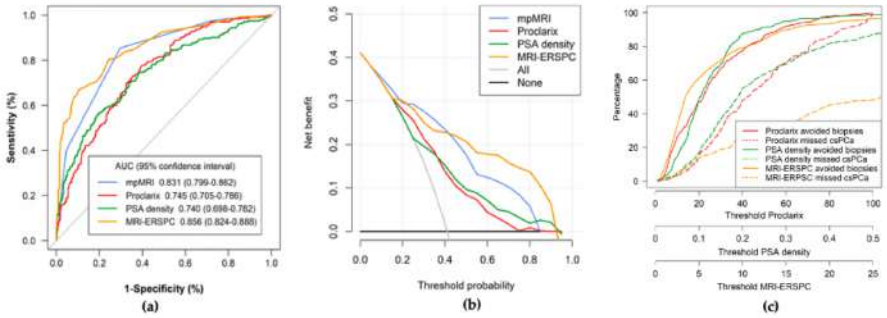

Figure 1. Efficacy (**a**), net benefit (**b**) and clinical utility (**c**) of Proclarix, PSAD and MRI-ERSPC model for csPCa detection in the overall population.

Based on the highest possible sensitivity for csPCa, the selected threshold for mpMRI was PI-RADS 2, with 10% for Proclarix, 0.07 ng/mL/cc for PSAD, and 3% for MRI-ERSPC RC. The performances of these tools based on the selected thresholds are summarized in Table 2. We note that mpMRI exhibited a sensitivity of 97.4%, avoiding 17.6% of prostate biopsies. These parameters were 97.4% and 16.8%, respectively, for Proclarix, 90.1% and 21.0% for PSAD, and 94.4% and 20.6% for MRI-ERSPC RC. The NPVs were 94%, 93.7%,

80.7%, and 88.9%, respectively. The Grade Group (GG) of missed csPCa for each tool are also summarized in Table 2.

Table 2. Overall performance of mpMRI, Proclarix, PSAD, and MRI-ERSPC model for csPCa detection.

Parameter	mpMRI	Proclarix	PSAD	MRI-ERSPC
Cut-off	1–2 PI-RADS	10%	0.07 ng/mL/cc	3%
Sensitivity (%)	226/232 (97.4)	226/232 (97.4)	209/232 (90.1)	219/232 (94.4)
Specificity (%)	94/335 (28.1)	89/335 (26.6)	96/335 (28.7)	104/335 (31.0)
Negative predictive value (%)	94/100 (94.0)	89/95 (93.7)	96/119 (80.7)	109/117 (88.9)
Positive predictive value (%)	226/467 (48.4)	226/472 (47.9)	209/448 (46.7)	219/450 (48.7)
Accuracy (%)	320/567 (56.4)	315/567 (55.6)	305/567 (53.8)	323/567 (57.3)
Avoidable biopsies	100/567 (17.6)	95/567 (16.8)	119/567 (21.0)	117/567 (20.6)
Misdiagnosis of csPCa (%)	6/232 (2.6)	6/232 (2.6)	23/232 (9.9)	13/232 (5.6)
GG2	4	3	10	8
GG3	1	2	6	1
GG4	1	1	4	2
GG5	0	0	3	0

mpMRI = multiparametric magnetic resonance imaging; PSAD = prostate-specific antigen density; csPCa = clinically significant prostate cancer; PI-RADS = prostate imaging-report and data system; GG = grade group.

3.3. Efficacy, Net Benefit, Clinical Utility, and Performance of PSAD, MRI-ERSPC RC, and Proclarix by PI-RADS Category

We will now analyze the behavior of MRI-ERSPC RC, PSAD, and Proclarix for the detection of by PI-RADS category using the previously selected thresholds. ROC curves and the AUCs for every tool are presented in Figure 2. We note different morphologies of these curves and AUCs by PI-RADS categories and in those observed in the overall population. The AUCs of MRI-ERSPC RC in men with PI-RADS < 3 was 0.516 (95% CI: 0.338–0.693), Figure 2a; 0.657 (95% CI: 0.547–0.766) in men with PI-RADS 3, Figure 2b; 0.676 (95% CI: 0.601–0.752) in men with PI-RADS 4, Figure 2c; and 0.765 (95% CI: 0.605–0.926) in men with PI-RADS 5, Figure 2d, with $p = 0.031$. We found that the largest AUC in men with PI-RADS $\leq$ 3 was for Proclarix, at 0.610 (95% CI: 0.416–0.803) in men with PI-RADS < 3, Figure 2a and 0.703 (95% CI: 0.620–0.786) in those with PI-RADS 3, Figure 2b, with $p = 0.039$. In contrast, PSAD exhibited the highest AUC in men with PI-RADS >3, at 0.704 (95% CI: 0.631–0.777) in men with PI-RADS 4, Figure 2c and 0.826 (95% CI: 0.706–0.945) in those with PI-RADS 5, Figure 2d, with $p = 0.028$. DCAs by PI-RADS category showed a net benefit of Proclarix over PSAD and MIR-ERSPC RC in men with PI-RADS $\leq$ 3, especially at low threshold probabilities of csPCa, while neither tool exhibited a clear net benefit in men with PI-RADS 4 and 5. The CUCs by PI-RADS category are shown in Figure 3a–d. We noted that the area between the rates of avoided biopsies and missed csPCa was greater for Proclarix in men with PI-RADS $\leq$ 3 and for PSAD in men with PI-RADS > 3.

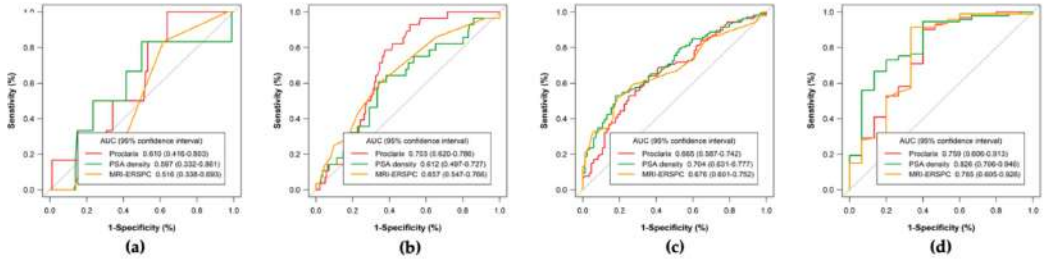

Figure 2. Efficacy of Proclarix, PSAD, and MRI-ERSPC model for csPCa detection regarding PI-RADS categories. PI-RADS < 3 (**a**), PI-RADS 3 (**b**), PI-RADS 4 (**c**), and PI-RADS 5 (**d**).

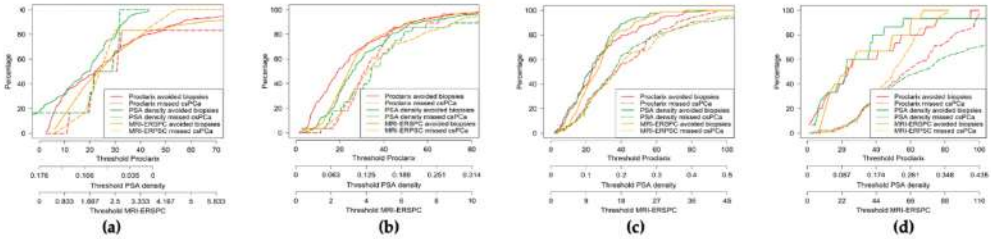

Figure 3. Clinical utility of Proclarix, PSAD, and MRI-ERSPC model for csPCa detection regarding PI-RADS categories. PI-RADS < 3 (**a**), PI-RADS 3 (**b**), PI-RADS 4 (**c**), and PI-RADS 5 (**d**).

Proclarix, PSAD, and MRI-ERSPC RC using the selected thresholds with the highest sensitivity for csPCa by PI-RADS category are summarized in Table 3. We found that Proclarix was able to detect 100% of csPCa in men with negative mpMRI and men with PI-RADS 3, avoiding 30% and 21.3% of prostate biopsies, respectively. Proclarix was also able to reduce 12.1% of prostate biopsies in men with PI-RADS 4 but misdiagnosed 4.8% of csPCa; these rates were 5.6% and 1%, respectively, in men with PI-RADS 5. PSAD was able to avoid between 29% and 9.3% of prostate biopsies by PI-RADS categories but missed between 50% and 4.2% of csPCa, respectively. MRI-ERSPC RC was able to avoid between 63% and 0% of prostate biopsies by PI-RADS categories but missed between 83.3% and 0% of csPCa, respectively. The GG distribution of misdiagnosed csPCa by PI-RADS category for each tool is also shown in Table 3.

Table 3. Characteristics of Proclarix, PSAD and MRI-ERSPC regarding PI-RADS category.

PI-RADS	Sensitivity	Specificity	NPV	PPV	Accuracy	Avoidable Biopsies	Misdiagnosis of csPCa	GG2	GG3	GG4	GG5
Proclarix (cut-off 10%)											
1–2	6/6(100)	30/94 (31.9)	30/30 (100)	6/70 (8.6)	36/100 (36)	30/100 (30)	0/6 (0)	0	0	0	0
3	25/25 (100)	36/144 (25.0)	36/36 (100)	25/133 (19.8)	61/169 (36.1)	36/169 (21.3)	0/25 (0)	0	0	0	0
4	100/105 (95.2)	18/85 (21.2)	18/23 (78.3)	100/167 (59.9)	118/190 (62.1)	23/190 (12.1)	5/105 (4.8)	2	1	1	1
5	95/96 (99.0)	5/12 (41.7)	5/6 (83.3)	95/102 (93.1)	100/108 (92.6)	6/108 (5.6)	1/96 (1.0)	1	0	0	0
PSAD (cut-off 0.07 ng/mL/cc)											
1–2	3/6 (50)	26/94 (27.7)	26/29 (89.7)	3/71 (4.2)	29/100 (29.0)	29/100 (29.0)	3/6 (50.0)	1	1	1	0
3	21/25 (84.0)	41/144 (28.5)	41/45 (91.1)	21/124 (16.0)	62/169 (36.7)	45/169 (26.2)	4/25 (16.0)	4	0	0	0
4	93/105 (88.6)	13/85 (27.1)	23/35 (65.7)	93/155 (60.0)	116/190 (61.1)	35/190 (18.4)	12/105 (11.4)	4	3	3	2
5	92/96 (95.8)	6/12 (50.0)	6/10 (60.0)	92/98 (93.9)	98/108 (90.7)	10/108 (9.3)	4/96 (4.2)	0	2	1	1
MRI-ERSPC model (cut-off 3%)											
1–2	1/6 (16.7)	58/94 (61.7)	58/63 (92.1)	1/37 (2.7)	59/100 (59)	63/100 (63)	5/6 (83.3)	3	1	1	0
3	21/25 (84)	46 (31.9)	46/50 (92)	21/119 (17.6)	67/169 (39.6)	50/169 (29.6)	4/25 (16)	4	0	0	0
4	103/105 (98.1)	2/86 (2.3)	2/4 (50)	103/186 (15.3)	104/190 (54.8)	4/190 (2.1)	2/105 (1.9)	1	1	0	2
5	96/99 (100)	NA	NA	96/108 (88.9)	96/108 (87.9)	0/108 (0)	0/96 (0)	0	0	0	0

PI-RADS = prostate imaging-report and data system; NPV = negative predictive value; PPV = positive predictive value; csPCa = clinically significant prostate cancer; GG = grade group.

4. Discussion

This is the first head-to-head study between PSAD, the externally validated MRI-ERSPC predictive model, and the new marker Proclarix for the proper selection of candidates for prostate biopsy after mpMRI. Morote et al. [26] analyzed the behavior of Proclarix in the same series of patients but in the subgroup of men with PI-RADS 3 category. The re-

sults obtained demonstrated that Proclarix outperformed PSAD in the detection of csPCa in this specific scenario (PI-RADS 3 category), considered the most uncertain of PI-RADS. This present work incorporated an evaluation of Proclarix in all PI-RADS categories compared to PSAD and the predictive model. New and clinically relevant information is provided from the analysis of these tools by PI-RADS category, in addition to the study carried out in the overall population of men with suspected PCa, which has been the most frequent method to report their performances. Clinicians need to know when, where, and how they should use these tools to avoid unnecessary prostate biopsies in exchange for acceptable rates of failure to detect csPCa. The relevant questions that clinicians ask are: (1) How many biopsies are we willing to perform to improve the current negative predictive value of mpMRI?; (2) What is the csPCa loss rate that we are willing to accept by PI-RADS category?; and (3) At what cost? For these purposes, we demonstrate that analysis by PI-RADS category is required.

When the entire population of men with suspected PCa was analyzed, the MRI-ERSPC model was the most efficient tool for csPCa detection. In addition, the MRI-ERSPC model exhibited a net benefit over PSAD and Proclarix and outperformed both according to the differential area between rates of avoided biopsies and missed csPCa shown in the CUCs. However, the performance of these tools changed when analyzed by the PI-RADS category. The new marker Proclarix, which is very sensitive for csPCa [12,13], was the most efficient and clinically useful tool in men with PI-RADS ≤ 3. Proclarix increased the negative predictive value of mpMRI from 94% to 100%, while a prostate biopsy was required in 70% of men with negative mpMRI [4,5]. In contrast, PSAD recommended prostate biopsy in 69% of men with negative mpMRI, leaving 50% of csPCa undetected [10,26]. Finally, MRI-ERSPC RC recommended biopsy in 37% of men with negative mpMRI but missed 83.3% of csPCa. In men with the challenging PI-RADS category 3, Proclarix was also the most efficient tool, and exhibited 100% sensitivity for csPCa while avoiding 21.3% of prostate biopsies. PSAD would avoid 26.2% of prostate biopsies but would miss 16% of csPCa. MRI-ERSPC RC would avoid 29.6% of prostate biopsies but would also miss 16% of csPCa. In men with PI-RADS 4, PSAD was the most efficient tool, avoiding 18.4% of prostate biopsies but missing 11.4% of csPCa. Proclarix would avoid 12.1% of prostate biopsies but would miss 4.8% of csPCa. MRI-ERSPC RC would avoid 9.3% of prostate biopsies and would miss 4.2% of csPCa. Finally, in men with PI-RADS 5, in whom 88.9% of csPCa was detected, PSAD was also the most efficient tool, avoiding 9.3% of prostate biopsies while missing 4.2% of csPCa. Proclarix would avoid 5.6% of prostate biopsies and would miss 1% of csPCa. MRI-ERSPC RC would not avoid any prostate biopsies. The PI-RADS > 3 are categories with high and very high-risk of csPCa in addition to an increased aggressiveness [27–29]. Therefore, clinicians are unwilling to miss any csPCa to avoid some prostate biopsies; therefore, only tools that guarantee 100% sensitivity for csPCa are acceptable in this category.

This study has some limitations. Although 567 men with suspected PCa was a sizeable cohort and there was an accurate representation of the incidence of the PI-RADS category, the low cases of csPCa in men with negative mpMRI and PI-RADS category 3 is a limitation. MRI-ERSP RC was designed to use PI-RADS v.1 in men up to 75 years old with serum PSA up to 20 ng/mL and prostate volume up to 110 ccs; however, we used PI-RADS v.2 and did not limit age or prostate volumes. Additionally, since it is a prospective study in a single center, the risk of bias could be higher. An external and multicenter analysis should be performed. Finally, although the used definition of csPCa in prostate biopsies is widespread, it does not represent the true pathology observed in surgical specimens.

5. Conclusions

This study suggests a change in the paradigm of evaluating tools for the proper selection of candidates for prostate biopsy after mpMRI. Evaluations in the entire population of men with suspected PCa are insufficient. We suggest that evaluations of these tools regarding PI-RADS categories are needed to provide clinicians with sufficient and useful

information to meet their expectations for the early detection of csPCa. MRI-ERSPC RC, was the most effective tool for the adequate selection of candidates for prostate biopsy when the entire population was analyzed. However, Proclarix was the most useful in men with PI-RADS $\leq$ 3. None of the tools exhibited the 100% sensitivity desired for csPCa in high and very high-risk PI-RADS categories. Taking into consideration the results of this study, Proclarix seems to be a relevant tool. It is especially useful in those men with PI-RADS $\leq$ 3 lesions in the mpMRI to decide whether to biopsy the patient.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Hugosson, J.; Roobol, M.J.; Månsson, M.; Tammela, T.L.J.; Zappa, M.; Nelen, V.; Kwiatkowski, M.; Lujan, M.; Carlsson, S.V.; Talala, K.M.; et al. A 16-Yr Follow-up of the European Randomized Study of Screening for Prostate Cancer. *Eur. Urol.* **2019**, *76*, 43–51. [CrossRef] [PubMed]
2. Mottet, N.; van den Bergh, R.C.N.; Briers, E.; van den Broeck, T.; Cumberbatch, M.G.; de Santis, M.; Fanti, S.; Fossati, N.; Gandaglia, G.; Gillessen, S.; et al. EAU-EANM-ESTRO-ESUR-SIOG Guidelines on Prostate Cancer-2020 Update. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. *Eur. Urol.* **2021**, *79*, 243–262. [CrossRef]
3. Grossman, D.C.; Curry, S.J.; Owens, D.K.; Bibbins-Domingo, K.; Caughey, A.B.; Davidson, K.W.; Doubeni, C.A.; Ebell, M.; Epling, J.W.; Kemper, A.R.; et al. Screening for Prostate Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA* **2018**, *319*, 1901–1913. [CrossRef] [PubMed]
4. Sathianathen, N.J.; Omer, A.; Harriss, E.; Davies, L.; Kasivisvanathan, V.; Punwani, S.; Moore, C.M.; Kastner, C.; Barrett, T.; van den Bergh, R.C.; et al. Negative Predictive Value of Multiparametric Magnetic Resonance Imaging in the Detection of Clinically Significant Prostate Cancer in the Prostate Imaging Reporting and Data System Era: A Systematic Review and Meta-Analysis. *Eur. Urol.* **2020**, *78*, 402–414. [CrossRef] [PubMed]
5. Moldovan, P.C.; van den Broeck, T.; Sylvester, R.; Marconi, L.; Bellmunt, J.; van den Bergh, R.C.N.; Bolla, M.; Briers, E.; Cumberbatch, M.G.; Fossati, N.; et al. What Is the Negative Predictive Value of Multiparametric Magnetic Resonance Imaging in Excluding Prostate Cancer at Biopsy? A Systematic Review and Meta-Analysis from the European Association of Urology Prostate Cancer Guidelines Panel. *Eur. Urol.* **2017**, *72*, 250–266. [CrossRef] [PubMed]
6. Mazzone, E.; Stabile, A.; Pellegrino, F.; Basile, G.; Cignoli, D.; Cirulli, G.O.; Sorce, G.; Barletta, F.; Scuderi, S.; Bravi, C.A.; et al. Positive Predictive Value of Prostate Imaging Reporting and Data System Version 2 for the Detection of Clinically Significant Prostate Cancer: A Systematic Review and Meta-Analysis. *Eur Urol. Oncol.* **2021**, *4*, 697–713. [CrossRef]
7. Schoots, I.G. MRI in Early Prostate Cancer Detection: How to Manage Indeterminate or Equivocal PI-RADS 3 Lesions? *Transl Androl. Urol.* **2018**, *7*, 70–82. [CrossRef] [PubMed]
8. Maggi, M.; Panebianco, V.; Mosca, A.; Salciccia, S.; Gentilucci, A.; di Pierro, G.; Busetto, G.M.; Barchetti, G.; Campa, R.; Sperduti, I.; et al. Prostate Imaging Reporting and Data System 3 Category Cases at Multiparametric Magnetic Resonance for Prostate Cancer: A Systematic Review and Meta-Analysis. *Eur. Urol. Focus* **2020**, *6*, 463–478. [CrossRef]
9. Dianat, S.S.; Rancier Ruiz, R.M.; Bonekamp, D.; Carter, H.B.; Macura, K.J. Prostate Volumetric Assessment by Magnetic Resonance Imaging and Transrectal Ultrasound: Impact of Variation in Calculated Prostate-Specific Antigen Density on Patient Eligibility for Active Surveillance Program. *J. Comput. Assist. Tomogr.* **2013**, *37*, 589–595. [CrossRef]
10. Morote, J.; Celma, A.; Diaz, F.; Regis, L.; Roche, S.; Mast, R.; Semidey, M.E.; de Torres, I.M.; Planas, J.; Trilla, E. Prostatic-Specific Antigen Density Behavior According to Multiparametric Magnetic Resonance Imaging Result. *Urol. Oncol.* **2020**, *38*, 410–417. [CrossRef]
11. Osses, D.F.; Roobol, M.J.; Schoots, I.G. Prediction Medicine: Biomarkers, Risk Calculators and Magnetic Resonance Imaging as Risk Stratification Tools in Prostate Cancer Diagnosis. *Int. J. Mol. Sci.* **2019**, *20*, 1637. [CrossRef]

12. Steuber, T.; Tennstedt, P.; Macagno, A.; Athanasiou, A.; Wittig, A.; Huber, R.; Golding, B.; Schiess, R.; Gillessen, S. Thrombospondin 1 and Cathepsin D Improve Prostate Cancer Diagnosis by Avoiding Potentially Unnecessary Prostate Biopsies. *BJU Int.* **2019**, *123*, 826–833. [CrossRef]
13. Steuber, T.; Heidegger, I.; Kafka, M.; Roeder, M.A.; Chun, F.; Preisser, F.; Palisaar, R.-J.; Hanske, J.; Budaeus, L.; Schiess, R.; et al. PROPOSe: A Real-Life Prospective Study of Proclarix, a Novel Blood-Based Test to Support Challenging Biopsy Decision-Making in Prostate Cancer. *Eur. Urol. Oncol.* **2021**. [CrossRef]
14. Klocker, H.; Golding, B.; Weber, S.; Steiner, E.; Tennstedt, P.; Keller, T.; Schiess, R.; Gillessen, S.; Horninger, W.; Steuber, T. Development and Validation of a Novel Multivariate Risk Score to Guide Biopsy Decision for the Diagnosis of Clinically Significant Prostate Cancer. *BJUI Compass* **2020**, *1*, 15–20. [CrossRef]
15. Alberts, A.R.; Roobol, M.J.; Verbeek, J.F.M.; Schoots, I.G.; Chiu, P.K.; Osses, D.F.; Tijsterman, J.D.; Beerlage, H.P.; Mannaerts, C.K.; Schimmöller, L.; et al. Prediction of High-Grade Prostate Cancer Following Multiparametric Magnetic Resonance Imaging: Improving the Rotterdam European Randomized Study of Screening for Prostate Cancer Risk Calculators. *Eur. Urol.* **2019**, *75*, 310–318. [CrossRef]
16. Endt, K.; Goepfert, J.; Omlin, A.; Athanasiou, A.; Tennstedt, P.; Guenther, A.; Rainisio, M.; Engeler, D.S.; Steuber, T.; Gillessen, S.; et al. Development and Clinical Testing of Individual Immunoassays for the Quantification of Serum Glycoproteins to Diagnose Prostate Cancer. *PLoS ONE* **2017**, *12*, e0181557. [CrossRef]
17. Macagno, A.; Athanasiou, A.; Wittig, A.; Huber, R.; Weber, S.; Keller, T.; Rhiel, M.; Golding, B.; Schiess, R. Analytical Performance of Thrombospondin-1 and Cathepsin D Immunoassays Part of a Novel CE-IVD Marked Test as an Aid in the Diagnosis of Prostate Cancer. *PLoS ONE* **2020**, *15*, e0233442. [CrossRef]
18. Roobol, M.J.; Steyerberg, E.W.; Kranse, R.; Wolters, T.; van den Bergh, R.C.N.; Bangma, C.H.; Schröder, F.H. A Risk-Based Strategy Improves Prostate-Specific Antigen-Driven Detection of Prostate Cancer. *Eur. Urol.* **2010**, *57*, 79–85. [CrossRef]
19. Weinreb, J.C.; Barentsz, J.O.; Choyke, P.L.; Cornud, F.; Haider, M.A.; Macura, K.J.; Margolis, D.; Schnall, M.D.; Shtern, F.; Tempany, C.M.; et al. PI-RADS Prostate Imaging—Reporting and Data System: 2015, Version 2. *Eur. Urol.* **2016**, *69*, 16–40. [CrossRef]
20. Epstein, J.I.; Egevad, L.; Amin, M.B.; Delahunt, B.; Srigley, J.R.; Humphrey, P.A. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. *Am. J. Surg. Pathol.* **2016**, *40*, 244–252. [CrossRef]
21. Epstein, J.I.; Zelefsky, M.J.; Sjoberg, D.D.; Nelson, J.B.; Egevad, L.; Magi-Galluzzi, C.; Vickers, A.J.; Parwani, A.V.; Reuter, V.E.; Fine, S.W.; et al. A Contemporary Prostate Cancer Grading System: A Validated Alternative to the Gleason Score. *Eur. Urol.* **2016**, *69*, 428–435. [CrossRef]
22. Creelman, C.D.; Wayne, D. ROC Curves for Discrimination of Linear Extent. *J. Exp. Psychol.* **1968**, *77*, 514–516. [CrossRef] [PubMed]
23. DeLong, E.R.; DeLong, D.M.; Clarke-Pearson, D.L. Comparing the Areas under Two or More Correlated Receiver Operating Characteristic Curves: A Nonparametric Approach. *Biometrics* **1988**, *44*, 837–845. [CrossRef]
24. Vickers, A.J.; Elkin, E.B. Decision Curve Analysis: A Novel Method for Evaluating Prediction Models. *Med. Decis. Mak.* **2006**, *26*, 565–574. [CrossRef]
25. Borque-Fernando, Á.; Esteban-Escaño, L.M.; Rubio-Briones, J.; Lou-Mercadé, A.C.; García-Ruiz, R.; Tejero-Sánchez, A.; Muñoz-Rivero, M.V.; Cabañuz-Plo, T.; Alfaro-Torres, J.; Marquina-Ibáñez, I.M.; et al. A Preliminary Study of the Ability of the 4Kscore Test, the Prostate Cancer Prevention Trial-Risk Calculator and the European Research Screening Prostate-Risk Calculator for Predicting High-Grade Prostate Cancer. *Actas Urológicas Españolas* **2016**, *40*, 155–163. [CrossRef]
26. Morote, J.; Campistol, M.; Triquell, M.; Celma, A.; Regis, L.; de Torres, I.; Semidey, M.E.; Mast, R.; Santamaria, A.; Planas, J.; et al. Improving the Early Detection of Clinically Significant Prostate Cancer in Men in the Challenging Prostate Imaging-Reporting and Data System 3 Category. *Eur. Urol. Open Sci.* **2022**, *37*, 38–44. [CrossRef]
27. Boesen, L.; Nørgaard, N.; Løgager, V.; Balslev, I.; Bisbjerg, R.; Thestrup, K.C.; Jakobsen, H.; Thomsen, H.S. Prebiopsy Biparametric Magnetic Resonance Imaging Combined with Prostate-Specific Antigen Density in Detecting and Ruling out Gleason 7–10 Prostate Cancer in Biopsy-Naïve Men. *Eur. Urol. Oncol.* **2019**, *2*, 311–319. [CrossRef]
28. Abreu-Gomez, J.; Wu, M.; McInnes, M.D.F.; Thornhill, R.E.; Flood, T.A.; Schieda, N. Shape Analysis of Peripheral Zone Observations on Prostate DWI: Correlation to Histopathology Outcomes after Radical Prostatectomy. *Am. J. Roentgenol.* **2020**, *214*, 1239–1247. [CrossRef]
29. Boschheidgen, M.; Schimmöller, L.; Arsov, C.; Ziayee, F.; Morawitz, J.; Valentin, B.; Radke, K.L.; Giessing, M.; Esposito, I.; Albers, P.; et al. MRI Grading for the Prediction of Prostate Cancer Aggressiveness. *Eur. Radiol.* **2021**, *32*, 2351–2359. [CrossRef]

5.4. PUBLICATION 4

Title: Who with suspected prostate cancer can benefit from Proclarix after multiparametric magnetic resonance imaging?

Authors: Juan Morote; Míriam Campistol; Lucas Regis; Anna Celma; Inés de Torres; Maria E. Semidey; Sarai Roche; Richard Mast; Anna Santamaria; Jacques Planas; Enrique Trilla

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Who with suspected prostate cancer can benefit from Proclarix after multiparametric magnetic resonance imaging?

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Abstract

Proclarix is a new blood-based test to assess the likelihood of clinically significant prostate cancer (csPCa) defined as >2 grade group. In this study, we analyzed whether Proclarix and PSA density (PSAD) could improve the selection of candidates for prostate biopsy after multiparametric magnetic resonance imaging (mpMRI). Proclarix and PSAD were assessed in 567 consecutive men with suspected PCa in whom pre-biopsy 3 Tesla mpMRI, scoring with Prostate Imaging-Report and Data System (PI-RADS) v.2, and guided and/or systematic biopsies were performed. Proclarix and PSAD thresholds having csPCa sensitivity over 90% were found at 10% and 0.07 ng/(mL*cm^3), respectively. Among 100 men with negative mpMRI (PI-RADS <3), csPCa was detected in 6 cases, which would have been undetected if systematic biopsies were avoided. However, Proclarix suggested performing a biopsy on 70% of men with negative mpMRI. In contrast, PSAD only detected 50% of csPCa and required 71% of prostate biopsies. In 169 men with PI-RADS 3, Proclarix avoided 21.3% of prostate biopsies and detected all 25 cases of csPCa, while PSAD avoided 26.3% of biopsies, but missed 16% of csPCa. In 190 men with PI-RADS 4 and 108 with PI-RADS 5, Proclarix avoided 12.1% and 5.6% of prostate biopsies, but missed 4.8% and 1% of csPCa, respectively. PSAD avoided 18.4% and 9.3% of biopsies, but missed 11.4% and 4.2% csPCa, respectively. We conclude that Proclarix outperformed PSAD in the selection of candidates for prostate biopsy, especially in men with PI-RADS ≤3.

Keywords

Clinically significant prostate cancer, magnetic resonance imaging, proclarix, thrombospondin-1, cathepsin D, prostate-specific antigen density

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Introduction

Early detection of clinically significant prostate cancer (csPCa) decreases the specific mortality of PCa.[1] Currently, suspicion of PCa is established from a persistent elevation of serum prostate-specific antigen (PSA) and/or abnormal digital rectal examination (DRE) and then followed up with systematic prostate biopsies;[2] however, this approach has been disapproved due to the high rates of unnecessary biopsies and the overdetection of

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insignificant PCa (iPCa).[3] Multiparametric magnetic resonance imaging (mpMRI) and guided biopsies have enabled recent improvements in the early detection of csPCa.[2] Nevertheless, the efficacy of this new strategy could be further improved with a more accurate selection of candidates for prostate biopsy, especially when low or moderate likelihood of csPCa is suggested by mpMRI.[2] A Prostate Imaging-Reporting and Data System (PI-RADS) score of <3 indicates a negative mpMRI, and the current negative predictive value of mpMRI is 80%–95%.[4] Additionally, PI-RADS category 3 suggests a moderate risk of csPCa that does not exceed 20%.[5] In these challenging scenarios where the rate of csPCa detection is low, PSA density (PSAD), modern markers, or predictive models can be helpful.[6,7]

Proclarix is a blood-based marker test that was recently introduced.[8] Proclarix provides a multivariate risk score for csPCa to guide biopsy decision making. This risk score is based on the combination of age and serum measurements of thrombospondin-1 (THBS1), cathepsin D (CTSD), total PSA (tPSA), and free PSA (fPSA).[9,10] THBS1 and CTSD were initially identified using a discovery mass spectrometry–based proteomics approach[11] and were subsequently observed in a PTEN knockout mouse model silencing the PI3 K/PTEN cancer pathway that is involved in the carcinogenesis and progression of PCa[12] and in human serum of men with and without PCa.[13] Clinical testing of individual immunoassays for the quantification of several glycoproteins was performed, and THBS1 and CTSD were ultimately selected because their measurement improved the accuracy of the percentage of fPSA in distinguishing men with and without csPCa.[14] This novel diagnostic test has been developed and validated to distinguish men without PCa or iPCa from those with csPCa among men with serum PSA between 2 and 10 ng/mL, prostate volume ≥35 cc, and normal DRE, with a recommended threshold of 10%.[9,10,15] Moreover, PSAD, which is a classic tool to improve the specificity of PSA, has been reinforced because MRI provides the most accurate measurement of prostate volume without additional cost.[16]

Because the performance of Proclarix according to the PI-RADS category has not yet been studied, our objective is to compare the performance of Proclarix and PSAD in the selection of candidates for prostate biopsy after mpMRI.

Materials and methods

Design, setting, participants, and intervention

This was a prospective head-to-head evaluation of Proclarix in a frozen serum collection (https://biobancos.isciii.es/; Reference collection: 0003439) and PSAD in 567 consecutive men with PSA >3 ng/mL and/or abnormal DRE in whom pre-biopsy 3-Tesla mpMRI was performed (Magnetom Trio, Siemens Corp., Germany). From January 2018 to March 2020 at a single academic institution, men with tumors with a score of ≥3 on PI-RADS v.2 received 2- or 3-core transrectal ultrasound (TRUS)-guided cognitive fusion biopsies to all detected lesions plus a 12-core TRUS systematic biopsy, while those with a PI-RADS v.2 score of <3 received only a 12-core TRUS systematic biopsy (BK Focus 400 ultrasound scanner, BK Medical Inc., Denmark). Blood samples were obtained immediately prior to prostate biopsy, and serum was stored at −80°C. This project was approved by the institutional ethics committee (PRAG129/2020) and informed consent was signed by all participants.

Laboratory method for Proclarix evaluation and prostate-specific antigen density assessment

THBS1 and CTSD were measured using the Proclarix kit (Proteomedix, Zürich-Schlieren, Switzerland) as previously described.[8] Serum tPSA and fPSA were re-analyzed for all samples using the Roche Cobas immunoassay system (Roche Diagnostics, Rotkreuz, Switzerland). All measurements were performed in the Proteomedix laboratory in Zürich-Schlieren, Switzerland, with Proteomedix bearing the costs for measurements and reagents. Serum THBS1, CTD, tPSA, percent fPSA, and age were entered into an algorithm that reported a score from 0% to 100%.[10]

PSAD was estimated from the MRI-derived prostate volume and the tPSA measured in Proclarix evaluation.

Endpoint measurements and definition of clinically significant prostate cancer

The endpoint measurements were csPCa detection rates, rates of avoided prostate biopsies, and rates of overdetection of iPCa. Tumors with an International Society of Uro-Pathology grade group of ≥2 were defined as csPCa.[17]

Statistical analysis

Comparisons were performed with the Mann–Whitney U test for quantitative variables and with the Chi square and Kruskal–Wallis tests for qualitative variables. Receiver operating characteristic (ROC) curves and areas under the curve (AUCs) were used to analyze efficacies, and the DeLong test for their comparisons. Decision curve analysis (DCA) was used to evaluate net benefits. SPSS v.25 (IBM Corp., Armonk, NY, USA) and R programming language v.3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) were used.

Results

PCa was detected in 298 men (52.6%), of whom 232 (40.9% of all participants) were diagnosed with csPCa and 66 (11.6%) had iPCa. The characteristics of the entire study cohort and a comparison of these characteristics in men without PCa or with iPCa and those with csPCa are presented in Table 1. We note that men with csPCa had significantly higher age, serum PSA, and PSAD; a lower percentage of fPSA; and higher rates of abnormal DRE, PCa family history, and positive mpMRI (PI-RADS ≥3). The median Proclarix score was 20.9% in men without PCa or with iPCa and 43.5% in those with csPCa ($P<0.001$). ROC curves of mpMRI, Proclarix, and PSAD for csPCa detection in the overall study cohort are presented in Figure 1(a). The AUC of Proclarix in the overall study cohort was slightly higher than that of PSAD (0.745 vs. 0.740; $P=0.465$). The morphologies of ROC curves suggest that Proclarix was more specific than PSAD at high sensitivities. This was more pronounced in men with PI-RADS scores of 1–3, for whom Proclarix outperformed mpMRI and PSAD (Figure 1(b) to (d)). A 10% risk score of Proclarix and 0.07 ng/(mL*cm^3) of PSAD were selected as thresholds with sensitivities for csPCa over 90%, and they were used across PI-RADS categories.

Table 2 shows the performance of Proclarix and PSAD both in all men and according to the PI-RADS categories. We note that Proclarix was able to detect all the 6% of csPCa detected in the systematic biopsies performed in the 100 men with negative mpMRI, although prostate biopsy was required in 70% of them. In contrast, PSAD detected 50% of csPCa and required 71% of systematic biopsies. In the subset of 169 men with a PI-RADS of 3, Proclarix avoided 21.3% of biopsies and detected all 25 cases of csPCa, while PSAD avoided 26.2% of biopsies, but missed 16.0% of csPCa. In men with PI-RADS of 4 and 5, Proclarix avoided 12.1% and 5.6% of biopsies, but misdiagnosed 4.8% and 1.0% of csPCa, respectively. PSAD avoided 18.4% and 9.3% of biopsies and misdiagnosed 11.4% and 4.2% of csPCa, respectively. The net benefit of Proclarix and PSAD on the biopsy of all men is presented in DCAs of Figure 2(a) and according to the PI-RADS categories in Figure 2(b) to (e).

Discussion

The present study confirms that Proclarix is a very sensitive marker of csPCa (grade group of ≥2).[8–10] Proclarix outperformed PSAD and improved the negative predictive value of mpMRI from 94% to 100%.[4,6] In men with tumors in the equivocal PI-RADS category 3, in whom at least 70% of prostate biopsies are not required,[5,7] Proclarix was able to avoid more than 20% of prostate biopsies without missing csPCa. PSAD was able to avoid 26% of prostate biopsies, but it missed 16% of csPCa. To miss csPCa detection in men with tumors in PI-RADS categories >3 is dangerous due to the higher aggressiveness of the tumors detected compared to those in lower PI-RADS categories. This is why clinicians usually refuse to avoid prostate biopsies in this setting.[5,7] Thus, only tools with 100% sensitivity for csPCa in these settings should be offered to

Table 1. Characteristics of the study cohort and comparison between the characteristics of men without PCa or iPCa and that of those with csPCa (>2 grade group).

Characteristic	All men	Without PCa or iPCa	With csPCa	*P* value
Number of cases	567	335	232	—
Median age, years (IQR)	69 (63–74)	67 (61–72)	72 (67–76)	0.001
Median total PSA, ng/mL (IQR)	7.0 (4.9–11.2)	6.1 (4.5–9.8)	8.0 (5.9–14.2)	0.001
Abnormal DRE, n (%)	109 (19.2)	30 (9.0)	79 (34.1)	0.001
Median free PSA, ng/mL (IQR)	1.1 (0.7–1.7)	1.1 (0.7–1.7)	1.1 (0.7–1.8)	0.832
Median prostate volume, mL (IQR)	55 (40–76)	63 (45–85)	48 (35–63)	0.001
Median percent free PSA, % (IQR)	15.1 (10.7–20.6)	17.2 (12.4–23.4)	12.1 (8.8–17.3)	0.001
Median PSA density, ng/(mL*cm^3) (IQR)	0.13 (0.09–0.21)	0.10 (0.07–0.16)	0.19 (0.12–0.34)	0.001
Repeat biopsy, n (%)	133 (23.5)	88 (26.3)	45 (19.4)	0.035
Family history of PCa, n (%)	48 (8.6%)	24 (7.9)	25 (10.8)	0.089
Proclarix, % (IQR)	28.7 (15.5–50.0)	20.9 (10.1–34.7)	43.5 (26.6–67.2)	0.001
PI-RADS, n (%)				
1–2	100 (17.6)	94 (28.1)	6 (2.6)	0.001
3	169 (29.8)	144 (43.0)	25 (14.8)	
4	190 (33.5)	85 (25.4)	105 (45.3)	
5	108 (19.0)	12 (3.5)	96 (41.4)	
Overall PCa detection, n (%)	298 (52.6)			
csPCa detection, n (%)	232 (40.9)			
iPCa detection, n (%)	66 (11.6)			

iPCa: insignificant PCa; IQR: interquartile range; PCa: prostate cancer; PI-RADS: Prostate Imaging-Report and Data System.

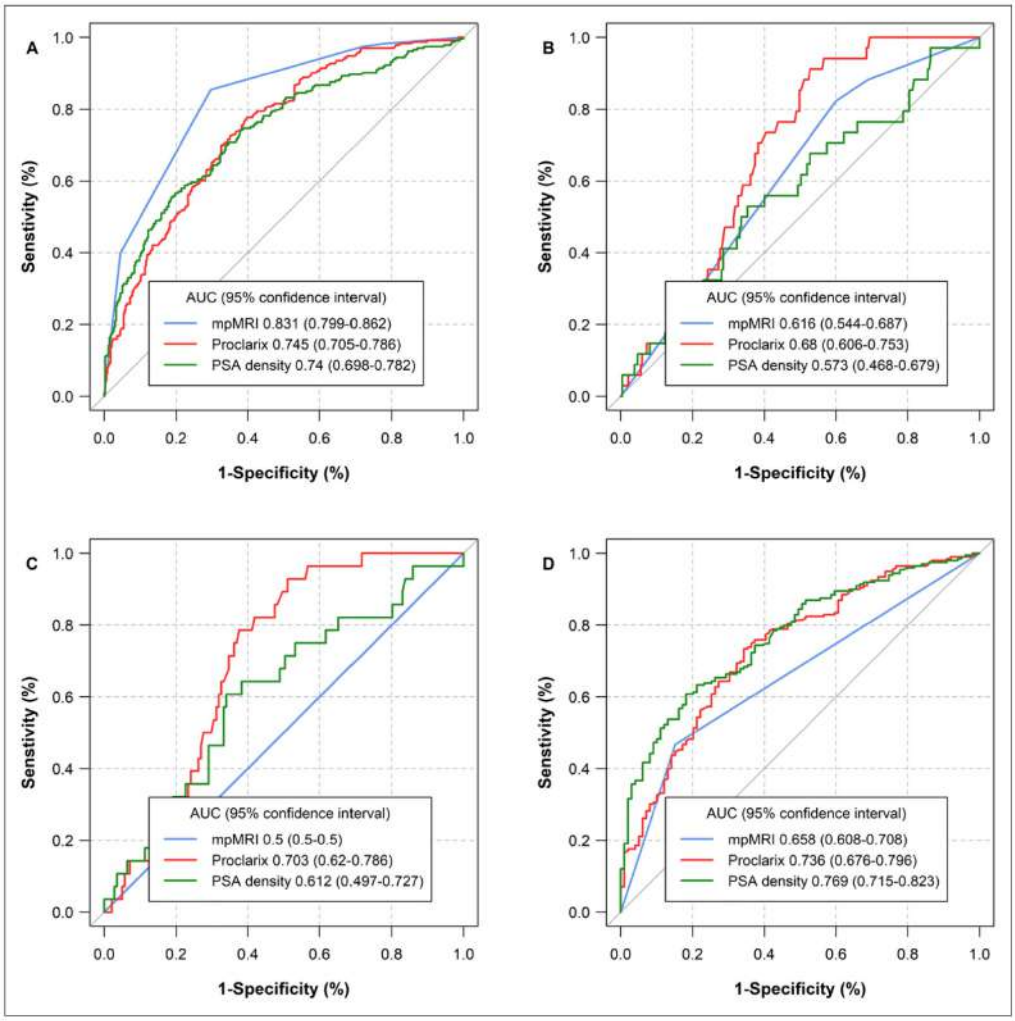

Figure 1. Efficacy of proclarix, PSAD and mpMRI for the detection of csPCa (≥ grade group) in the entire study cohort (a), in men with negative mpMRI (b), PI-RADS 3 (c), and PI-RADS >3 (d).
csPCa: clinically significant prostate cancer; mpMRI: multiparametric magnetic resonance imaging; PI-RADS: Prostate Imaging-Report and Data System; PSAD: Proclarix and PSA density.

clinicians. Therefore, we believe that neither Proclarix nor PSAD should be recommended in men with PI-RADS scores above 3.

PROPOSe was the first study analyzing Proclarix in men with pre-biopsy mpMRI. The authors reported results from 108 men with positive mpMRI in whom guided and systematic biopsies were performed.[14] After fixing a sensitivity for ≥2 grade group PCa at 97%, the specificity of Proclarix was 26% and that of PSAD was 8%, and their negative predictive values were 96% and 88%, respectively. Unfortunately, men with negative mpMRI were not biopsied and the analysis according to the PI-RADS categories was not performed. The authors concluded that Proclarix outperformed PSAD. We believe that additional analyses regarding the PI-RADS categories are important because the overall results do not represent the specific efficacies in every PI-RADS category. Moreover, the

Table 2. Performance of proclarix at 10% threshold and PSAD at 0.07 ng/(mL*cm^2) threshold for csPCa (≥2 grade group) detection in all population study and according to the PI-RADS categories.

	Proclarix (threshold 10%)					PSA density (threshold 0.07 ng/(mL*cm3))				
Parameter	All men (n = 567)	PI-RADS 1–2 (n = 100)	PI-RADS 3 (n = 169)	PI-RADS 4 (n = 190)	PI-RADS 5 (n = 108)	All men (n = 567)	PI-RADS 1–2 (n = 100)	PI-RADS 3 (n = 169)	PI-RADS 4 (n = 190)	PI-RADS 5 (n = 108)
Sensitivity	97.4 (226/232)	100 (6/6)	100 (25/25)	95.2 (100/105)	99.0 (95/96)	90.1 (209/232)	50 (3/6)	84.0 (21/25)	88.6 (93/105)	95.8 (92/96)
Specificity	26.6 (89/335)	31.9 (30/94)	25.0 (36/144)	21.2 (18/85)	41.7 (5/12)	28.7 (96/335)	27.7 (26/94)	28.5 (41/144)	27.1 (13/85)	50.0 (6/12)
Negative PV	93.7 (89/95)	100 (30/30)	100 (36/36)	78.3 (18/23)	83.3 (5/6)	80.7 (96/119)	89.7 (26/29)	91.1 (41/45)	65.7 (23/35)	60.0 (6/10)
Positive PV	47.9 (226/472)	8.6 (6/70)	19.8 (25/133)	59.91 (00/167)	93.1 (95/102)	46.7 (209/448)	4.2 (3/71)	16.0 (21/124)	60.0 (93/155)	93.9 (92/98)
Accuracy	55.6 (315/567)	36 (36/100)	36.1 (61/169)	62.1 (118/190)	92.6 (100/108)	53.8 (305/567)	29.0 (29/100)	36.7 (62/169)	61.1 (116/190)	90.7 (98/108)
Avoided biopsies	16.8 (95/567)	30 (30/100)	21.3 (36/169)	12.1 (23/190)	5.6 (6/108)	21.0 (119/567)	29.0 (29/100)	26.2 (45/169)	18.4 (35/190)	9.3 (10/108)
Missed csPCa	2.6 (6/232)	0 (0/6)	0 (0/25)	4.8 (5/105)	1.0 (1/96)	9.9 (23/232)	50.0 (3/6)	16.0 (4/25)	11.4 (12/105)	4.2 (4/96)

csPCa: clinically significant prostate cancer; PI-RADS: Prostate Imaging-Report and Data System; PV: predictive value.

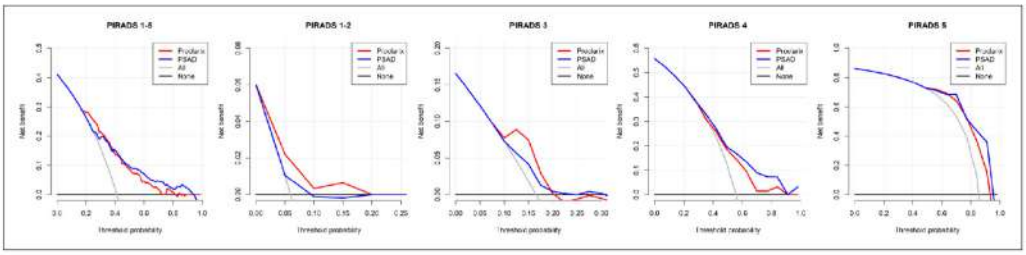

Figure 2. Net benefit of proclarix and PSAD instead of biopsy all men in the entire study cohort (PI-RADS 1–5), and according to the PI-RADS categories.
PI-RADS: Prostate Imaging-Report and Data System; PSAD: Proclarix and PSA density.

case-mix of PI-RADS in every series can change depending on the characteristics of the analyzed population and the interpretation of mpMRI.[7]

Comparisons between Proclarix and other markers are needed.[18–20] In our opinion, a major strength of Proclarix is its high sensitivity for tumors with a grade group of ≥2, which guarantees the detection of most of these tumors. Nevertheless, a cost-benefit analysis must be performed to determine the final benefit of markers as complementary tools of mpMRI.[20]

The limitations of our study include its partially retrospective design and the lack of external validation. Prospective and multicenter studies mimicking real clinical practice are needed, especially studies comparing the existing markers. However, a common limitation of these studies is measuring the rate of csPCa in prostate biopsies, which does not represent the true pathology observed in the whole prostate gland. A strength of our study was to perform systematic biopsies in men with negative mpMRI (PI-RADS <3) which allowed the possibility to know that Proclarix increased the negative predictive value of mpMRI from 94% to 100%.

Finally, we note that Proclarix can improve the selection of candidates for prostate biopsy after mpMRI, especially in men with low or moderate risk of csPCa defined by a PI-RADS score ≤3.

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Declaration of conflicting interests

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References

1. Hugosson J, Roobol MJ, Månsson M, et al. A 16-yr follow-up of the European randomized study of screening for prostate cancer. *Eur Urol* 2019; 76: 43–51.
2. Mottet N, van den Bergh RCN, Briers E, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer-2020 update. Part 1: screening, diagnosis, and local treatment with curative intent. *Eur Urol* 2021; 79: 243–262.
3. Grossman DC, Curry SJ, Owens DK, et al. Screening for prostate cancer: US preventive services task force recommendation statement. *JAMA* 2018; 319: 1901–1913.
4. Sathianathen NJ, Omer A, Harriss E, et al. Negative predictive value of multiparametric magnetic resonance imaging in the detection of clinically significant prostate cancer in the prostate imaging reporting and data system era: a systematic review and meta-analysis. *Eur Urol* 2020; 78: 402–414.
5. Morote J, Celma A, Roche S, et al. Who benefits from multiparametric magnetic resonance imaging after suspicion of prostate cancer. *Eur Urol Oncol* 2019; 2: 664–669.
6. Morote J, Celma A, Diaz F, et al. Prostatic-specific antigen density behavior according to multiparametric magnetic resonance imaging result. *Urol Oncol* 2020; 38: 410–417.
7. Osses DF, Roobol MJ and Schoots IG. Prediction medicine: biomarkers, risk calculators and magnetic resonance imaging as risk stratification tools in prostate cancer diagnosis. *Int J Mol Sci* 2019; 20: E1637.
8. Macagno A, Athanasiou A, Wittig A, et al. Analytical performance of thrombospondin-1 and cathepsin D immunoassays part of a novel E-IVD marked test as an aid in the diagnosis of prostate cancer. *PLoS One* 2020; 15: e0233442.
9. Steuber T, Tennstedt P, Macagno A, et al. Thrombospondin 1 and cathepsin D improve prostate cancer diagnosis by avoiding potentially unnecessary prostate biopsies. *BJU Int* 2019; 123: 826–833.
10. Klocker H, Golding B, Weber S, et al. Development and validation of a novel multivariate risk score to guide biopsy decision for the diagnosis of clinically significant prostate cancer. *BJUI Compass* 2020; 1: 15–20.
11. Schiess R, Wollscheid B and Aebersold R. Targeted proteomic strategy for clinical biomarker discovery. *Mol Oncol* 2009; 3: 33–44.
12. Pourmand G, Ziaee AA, Abedi AR, et al. Role of PTEN gene in progression of prostate cancer. *Urol J* 2007; 4: 95–100.
13. Cima I, Schiess R, Wild P, et al. Cancer genetics-guided discovery of serum biomarker signatures for diagnosis and prognosis of prostate cancer. *Proc Natl Acad Sci* 2011; 108: 3342–3347.
14. Endt K, Goepfert J, Omlin A, et al. Development and clinical testing of individual immunoassays for the quantification of serum glycoproteins to diagnose prostate cancer. *PLoS One* 2017; 12: e0181557.
15. Steuber T, Heidegger I, Kafka M, et al. PROPOSe: a real-life prospective study of proclarix, a novel blood-based test to support challenging biopsy decision-making in prostate cancer. *Eur Urol Oncol* 2021; 10.1016/j.euo.2020.12.003. 10.
16. Dianat SS, Rancier-Ruiz RM, Bonekamp D, et al. Prostate volumetric assessment by magnetic resonance imaging and transrectal ultrasound: impact of variation in calculated prostate-specific antigen density on patient eligibility for active surveillance program. *J Comput Assist Tomogr* 2013; 37: 589–595.
17. Epstein JI. A new contemporary prostate cancer grading system. *Ann Pathol* 2015; 35: 474–476.
18. Wagaskar VG, Sobotka S, Ratnani P, et al. A 4 K score/MRI-based nomogram for predicting prostate cancer, clinically significant prostate cancer, and unfavorable prostate cancer. *Cancer Rep* 2021; 4: e1357.
19. Fan YH, Pan PH, Cheng WM, et al. The prostate health Index aids multi-parametric MRI in diagnosing significant prostate cancer. *Sci Rep* 2021; 11: 1286.
20. Govers TM, Hessels D, Vlaeminck-Guillem V, et al. Cost-effectiveness of SelectMDx for prostate cancer in four European countries: a comparative modeling study. *Prostate Cancer Prostatic Dis* 2019; 22: 101–119.

5.5. PUBLICATION 5

Title: Improving the Early Detection of Clinically Significant Prostate Cancer in Men in the Challenging Prostate Imaging-Reporting and Data System 3 Category

Authors: Juan Morote; Míriam Campistol; Marina Triquell; Anna Celma; Lucas Regis; Inés de Torres; Maria E. Semidey; Richard Mast; Anna Santamaria; Jacques Planas; Enrique Trilla

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Prostatic Disease

Improving the Early Detection of Clinically Significant Prostate Cancer in Men in the Challenging Prostate Imaging-Reporting and Data System 3 Category

Juan Morote[a,b,c,*], *Miriam Campistol*[b], *Marina Triquell*[b], *Anna Celma*[a,b], *Lucas Regis*[a,b], *Inés de Torres*[b,d,e], *Maria E. Semidey*[b,c,d], *Richard Mast*[c], *Anna Santamaria*[b], *Jacques Planas*[a,b], *Enrique Trilla*[a,e]

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Abstract

Background: Prostate Imaging-Reporting and Data System (PI-RADS) category 3 is a challenging scenario for detection of clinically significant prostate cancer (csPCa) and some tools can improve the selection of appropriate candidates for prostate biopsy.
Objective: To assess the performance of the European Randomized Study of Screening for Prostate Cancer (ERSPC) magnetic resonance imaging (MRI) model, the new Proclarix test, and prostate-specific antigen density (PSAD) in selecting candidates for prostate biopsy among men in the PI-RADS 3 category.
Design, setting, and participants: We conducted a head-to-head prospective analysis of 567 men suspected of having PCa for whom guided and systematic biopsies were scheduled between January 2018 and March 2020 in a single academic institution. A PI-RADS v.2 category 3 lesion was identified in 169 men (29.8%).
Outcome measurement and statistical analysis: csPCa, insignificant PCa (iPCa), and unnecessary biopsy rates were analysed. csPCa was defined as grade group $\geq$2. Receiver operating characteristic (ROC) curves, decision curve analysis curves, and clinical utility curves were plotted.
Results and limitations: PCa was detected in 53/169 men (31.4%) with a PI-RADS 3 lesion, identified as csPCa in 25 (14.8%) and iPCa in 28 (16.6%). The area under the ROC curve for csPCa detection was 0.703 (95% confidence interval [CI] 0.621–0.768) for Proclarix, 0.657 (95% CI 0.547–0.766) for the ERSPC MRI model, and 0.612 (95% CI 0.497–0.727) for PSAD (p = 0.027). The threshold with the highest sensitivity was 10% for Proclarix, 1.5% for the ERSPC MRI model, and 0.07 ng/ml/cm^3 for PSAD, which yielded sensitivity of 100%, 91%, and 84%, respectively. Some 21.3%, 26.2%, and 7.1% of biopsies would be avoided with Proclarix, PSAD, and the ERSPC MRI

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model, respectively. Proclarix showed a net benefit over PSAD and the ERSPC MRI model. Both Proclarix and PSAD reduced iPCa overdetection from 16.6% to 11.3%, while the ERSPC MRI model reduced iPCa overdetection to 15.4%.

Conclusions: Proclarix was more accurate in selecting appropriate candidates for prostate biopsy among men in the PI-RADS 3 category when compared to PSAD and the ERSPC MRI model. Proclarix detected 100% of csPCa cases and would reduce prostate biopsies by 21.3% and iPCa overdetection by 5.3%.

Patient summary: We compared three methods and found that the Proclarix test can optimise the detection of clinically significant prostate cancer in men with a score of 3 on the Prostate Imaging-Reporting and Data System for magnetic resonance imaging scans.

1. Introduction

Early detection of clinically significant prostate cancer (csPCa) decreases PCa-specific mortality [1]. Currently, suspicion of PCa is still based on detection of elevated serum levels of prostate-specific antigen (PSA) and/or an abnormal digital rectal examination (DRE) [2]. Suspected PCa has typically been confirmed via systematic biopsies of the prostate, but this approach results in a high rate of unnecessary biopsies and overdetection of insignificant PCa (iPCa) [3]. True improvement in the early detection of csPCa has come from multiparametric magnetic resonance imaging (mpMRI) and guided biopsies [2]. At present, the negative predictive value of mpMRI can reach 95%, so prostate biopsies can usually be avoided in men with a Prostate Imaging-Reporting and Data System (PI-RADS) score <3 [4,5]. By contrast, most clinicians recommend prostate biopsy in men with PI-RADS >3 because the likelihood of PCa ranges from 62% to 92% [6]. PI-RADS category 3 is the most challenging scenario: 60–85% of prostate biopsies are unnecessary and up to 60% of PCa cases detected are insignificant [7,8]. Therefore, tools such as PSA density (PSAD), modern markers, and predictive models are recommended for appropriate selection of candidates for prostate biopsy [2].

Proclarix is a new blood-based test that estimates the likelihood of csPCa by computing the risk according to measurement results for thrombospondin-1 (THBS1), cathepsin D (CTSD), total PSA, and percentage free PSA in serum, as well as patient age [9]. Recent studies have suggested that Proclarix can improve csPCa detection by reducing unnecessary biopsies in men with or without mpMRI [10]. However, data on the behaviour of Proclarix by PI-RADS category are lacking. Meanwhile, PSAD has become relevant as prostate volume can be accurately measured on MRI [11–13]. The externally validated European Randomized Study of Screening for Prostate Cancer (ERSPC) predictive model has recently incorporated the PI-RADS version 1 score and age in the "3+DRE" and "4+DRE" risk calculators [14]. However, no specific analyses of its behaviour regarding PI-RADS categories have been carried out.

In this study we compare the behaviour of PSAD, Proclarix, and the ERSPC MRI predictive model according to PI-RADS categories. Our main objective was to analyse the usefulness of these three tools for appropriate selection of candidates for prostate biopsy in the challenging setting of PI-RADS category 3.

2. Patients and methods

2.1. Design, setting, and participants

This was a prospective head-to-head study in which the likelihood of csPCa was assessed using the Proclarix test, PSAD, and the ERSPC MRI model. A cohort of 567 men with a suspicion of PCa because of PSA >3 ng/ml and/or abnormal DRE underwent prebiopsy 3-T mpMRI and had guided and systematic prostate biopsies scheduled between January 12, 2018 and March 15, 2020 at a single academic institution. A PI-RADS v.2 category 3 lesion was identified in 169 men (29.8%). Men with PCa on active surveillance and those with symptomatic benign prostatic hyperplasia treated with 5α-reductase inhibitors were previously excluded. Two- to three-core transrectal ultrasound-guided biopsies of suspected lesions and 12-core systematic biopsies were performed in all men using the transrectal approach. The study was approved by our institutional ethics committee (PR-AG129/2020).

2.2. Testing

Blood was obtained immediately before prostate biopsy and frozen serum was stored locally at −80 °C for 63–811 d (C. 0003439; https://biobancs.isiii.es) and then shipped on dry ice to Proteomedix (Zurich-Schlieren, Switzerland). Processing of serum samples, the ELISA kit, and calculation of the risk score by laboratory technicians were performed blind before any clinical information was available. THBS1 and CTSD were measured using a Proclarix kit (Proteomedix, Zurich-Schlieren, Switzerland) according to the kit instructions [15]. Serum total PSA and free PSA were reanalysed for all samples using a Roche Cobas immunoassay system (Roche Diagnostics, Rotkreuz, Switzerland). Proclarix risk calculation was performed according to the instructions and the results ranged from 0% to 100%. PSAD was calculated from the prostate volume measured on MRI and the total PSA value from the Proclarix test. The ERSPC MRI risk of high-grade PCa was calculated for every man using the Prostate Cancer Research Foundation (Reeuwijk, The Netherlands) web application at www.prostatecancer-riskcalculator.com. The ERSPC MRI risk calculator includes PSA (0.5–50 ng/ml), repeat biopsy (yes/no), DRE (normal/abnormal), prostate volume (10–110 cm^3, which can now be obtained from MRI), age (50–75 years), and PI-RADS version 1 score [14]. We introduced the MRI-based prostate volume and the PI-

RADS version 2 categories. When the observed values were not within the acceptable range, we entered the minimum or maximum acceptable value, whichever was closer to the observed value.

2.3. Outcome measurements

The main outcome measured was the rate of csPCa detection. csPCa was defined as International Society of Urological Pathology grade group ≥2 [16]. The rate of prostate biopsies avoided and the rate of iPCa overdetection were secondary outcome measurements.

2.4. Statistical analysis

Quantitative variables are presented as the median and interquartile range (IQR). Qualitative variables are presented as the frequency and proportion. Associations between variables were analysed using the Mann-Whitney *U* test and the Kruskal-Wallis test. Associations between variables were also analysed with the χ^2 test. The odds ratio and 95% confidence interval (CI) were estimated. Receiver operating characteristic (ROC) curves were constructed and areas under the ROC curve (AUCs) were estimated and compared with the DeLong test. The PSAD, Proclarix, and ERSPC MRI model thresholds were selected to analyse the optimal sensitivity for csPCa. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), accuracy, and rates of biopsies avoided, overdetection of iPCa, and misdiagnosis of csPCa were estimated. Decision curve analysis (DCA) was carried out to assess the net benefits. Clinical utility curves (CUCs) were used to check the correlation of rates of csPCa misdiagnosis and biopsies avoided regarding the thresholds on a continuous basis. A p value of <5% was considered significant. SPSS version 25 (IBM Corp., Armonk, NY, USA) and R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analyses.

3. Results

3.1. Efficacy of Proclarix, PSAD, and the ERSPC MRI predictive model for csPCa detection across the PI-RADS categories

The distribution of PCa, csPCa, and iPCa among the 567 participants by PI-RADS category is presented in Table 1. csPCa was detected in 6% of men with PI-RADS <3, 14.8% of men with PI-RADS 3, 54.7% of men with PI-RADS 4, and 88% of men with PI-RADS 5 findings ($p < 0.001$). Fig. 1 shows the efficacy of Proclarix, PSAD, and the ERSPC MRI predictive model for the overall population and by PI-RADS category.

3.2. Characteristics of the cohort of men with PI-RADS 3 findings

Men with a PI-RADS 3 lesion represented 29.8% of all men with suspected PCa for whom prebiopsy mpMRI data were available (Table 1). The median age for this group was 66 yr and the median serum PSA was 6.0 ng/ml (Table 2). The rate of abnormal DRE was 7.1%, the rate of PCa family history was 6.5%, and the rate of men with prior negative biopsy results was 28.4%. Prostate biopsy showed benign tissue in 116 men (68.6%) and PCa in 53 men (31.4%), of whom 25 (47.2%) had csPCa and 28 (52.8%) had iPCa. The csPCa and iPCa detection rates were 14.8% and 16.6%, respectively ($p = 0.573$). csPCa was detected in both guided and systematic biopsies in 16 men (64%), and exclusively in five systematic biopsies (20%) and four guided biopsies (16%; $p = 0.236$).

3.3. Efficacy, net benefit, and clinical utility of Proclarix, PSAD and MRI-ERSPC predictive model for csPCa detection in PI-RADS 3

ROC curves for csPCa detection according to Proclarix, PSAD, and the ERSPC MRI model are presented in Fig. 2A. The AUC was 0.703 (95% CI 0.621–0.768) for Proclarix, 0.612 (95% CI 0.497–0.727) for PSAD, and 0. 657 (95% CI 0.547–0.766) for the ERSPC MRI model ($p = 0.027$). DCA showed a net benefit for Proclarix versus PSAD and the ERSPC MRI model at low thresholds within 0.09% versus 0.17% and 0.2%, respectively (Fig. 2B). CUCs showing the rates of csPCa missed and biopsies avoided in relation to the thresholds for the three tools are presented in Fig. 2C. Analysis of Proclarix scores, PSAD values, and ERSPC MRI likelihood values showed that the thresholds with the highest sensitivity for csPCa were 10%, 0.07 ng/ml/cm^3, and 1.5%, which yielded sensitivity of 100% (25/25), 84% (21/25), and 96% (24/25), respectively. The corresponding specificity was 5% (36/144) for Proclarix, 28.5% (41/144) for PSAD, and 7.6% (11/144) for the ERSPC MRI model. The NPV and PPV were 100% (36/36) and 19.8% (25/133) for Proclarix, 91.1% (41/45) and 16% (21/124) for PSAD, and 91.7% (11/12) and 15.3% (24/157) for the ERSPC MRI model, respectively. The diagnostic accuracy was 36.1% (61/169) with Proclarix, 36.7% (62/169) with PSAD, and 20.7% (35/169) with the ERSPC MRI model. In terms of clinical efficacy, Proclarix would avoid 21.3% (36/169) of prostate biopsies and reduce overdetection of iPCa from 16.6% to 11.2% (19/169) without misdiagnosing csPCa. PSAD would avoid 26.2% (45/169) of prostate biopsies, reduce overdetection of iPCa from 16.6% to 11.2% (19/169), but misdiagnose 16% (four out of 25) of csPCa cases. The ERSPC MRI predictive model would avoid only 7.1% (12/169) of prostate biopsies, reduce overdetection of iPCa from 16.6% to 15.3% (26/169), and misdiagnose 4% (two out of 25) of csPCa cases, as shown in Table 3. The performance of Proclarix, PSAD, and the ERSPC MRI model according to biopsy status (biopsy-naïve vs repeat biopsy)

Table 1 – Distribution of men with suspected PCa by PI-RADS category and the corresponding rates of PCa, csPCa, and iPCa

PI-RADS category	Men, *n* (%)	PCa, *n* (%)	csPCa, *n* (%)	iPCa, *n* (%)
1	77 (13.6)	10 (13.0)	4 (5.2)	6 (7.8)
2	23 (4.1)	5 (21.7)	2 (8.7)	3 (13.0)
3	169 (29.8)	53 (31.4)	25 (14.8)	28 (16.6)
4	190 (33.5)	129 (67.9)	94 (54.7)	25 (13.2)
5	108 (19.0)	99 (91.7)	95 (88.0)	4 (3.7)
All	567 (100)	296 (52.2)	230 (40.6)	66 (11.6)

PI-RADS = Prostate Imaging-Reporting and Data System; PCa = prostate cancer; csPCa = clinically significant PCa; iPCa = insignificant PCa.

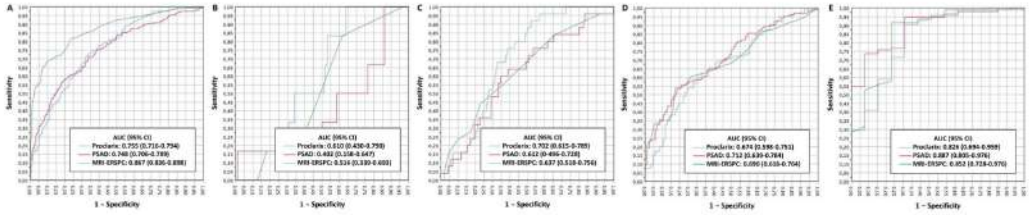

Fig. 1 – Efficacy of Proclarix, PSAD, and the ERSPC MRI predictive model for detection of clinically significant prostate cancer in the overall population and by PI-RADS category. Receiver operating characteristic curves and area under the curve (AUC) for (A) the overall population, (B) men with PI-RADS <3, (C) men with PI-RADS 3, (D) men with PI-RADS 4, and (E) men with PI-RADS 5 findings. PSAD = prostate-specific antigen density; ERSPC = European Randomized Study of Screening for Prostate Cancer; MRI = magnetic resonance imaging; PI-RADS = Prostate Imaging-Reporting and Data System; CI = confidence interval.

Table 2 – Characteristics of men in PI-RADS category 3

Characteristic	Result
Number of cases	169
Median age, yr (IQR)	66 (60–72)
Median total PSA, ng/ml (IQR)	6.0 (3.6–10.2)
Abnormal digital rectal examination, *n* (%)	12 (7.1)
Median free PSA, ng/ml (IQR)	1.1 (0.7–1.6)
Median prostate volume, ml (IQR)	66 (45–85)
Median percentage free PSA, % (IQR)	16.4 (11.5–20.7)
Median PSA density, ng/ml/cm^3 (IQR)	0.11 (0.07–0.16)
Repeat biopsy, *n* (%)	48 (28.4)
Family history of PCa, *n* (%)	11 (6.5)
Overall PCa detection, *n* (%)	53 (31.4)
csPCa detection, *n* (%)	25 (14.8)
iPCa detection, *n* (%)	28 (16.6)

PI-RADS = Prostate Imaging-Reporting and Data System; IQR = interquartile range; PSA = prostate-specific antigen; PCa = prostate cancer; csPCa = clinically significant PCa; iPCa = insignificant PCa.

is presented in Table 4. Proclarix outperformed PSAD and the ERSPC MRI model, detecting all csPCa cases in both subsets and avoiding 24.8% of prostate biopsies in the biopsy-naïve group and 12.5% in the repeat biopsy group.

4. Discussion

The efficacy of diagnostic tools changes in relation to the incidence of the disease in question. This is why we observed differences in utility for the three tools analysed across the PI-RADS categories. We noted a progressive increase in the csPCa detection rate across the PI-RADS categories, as well as differences in efficacy. Currently, most clinicians accept that prostate biopsies can be avoided for men with negative mpMRI because of its high NPV [4.5]. In the present study, the NPV of 94% observed represents an overall csPCa misdiagnosis rate of 2.6% and a 17.6% reduction in prostate biopsies, so it is acceptable to avoid prostate biopsies in these men. By contrast, many clinicians would not accept a test that does not guarantee 100% sensitivity for csPCa in men with PI-RADS >3 lesions. Thus, it makes sense to focus our attention on men with the recognised challenging PI-RADS 3 category [7,8].

The incidence of PI-RADS 3 findings in our series of patients with suspected PCa was 29.8%, which is within the range of 14–46% reported in the literature. This incidence mainly depends on the proportions of biopsy-naïve men and men with a prior negative biopsy, which is approximately 30% in mixed samples [7]. The incidence of csPCa detected among the 169 men with PI-RADS 3 findings (14.8%) is also within the wide range (5–30%) reported in the literature [8]. We observed a net benefit of Proclarix over PSAD and the ERSPC MRI predictive model for determining the likelihood of csPCa, especially at low thresholds, for which high sensitivity is observed. This finding is consis-

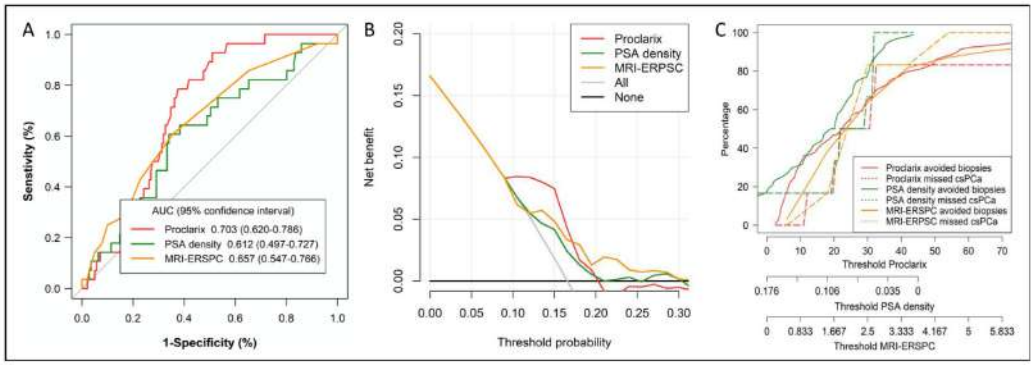

Fig. 2 – Analysis of the efficacy, net benefit, and clinical utility of Proclarix, PSA density, and the ERSPC MRI predictive model for detection of clinically significant prostate cancer in men with a PI-RADS 3 lesion. (A) Receiver operating characteristic curves and area under the (AUC), (B) decision curve analysis, and (C) clinical utility curves. PSA = prostate-specific antigen; ERSPC = European Randomized Study of Screening for Prostate Cancer; MRI = magnetic resonance imaging; PI-RADS = Prostate Imaging-Reporting and Data System.

Table 3 – Performance of Proclarix, PSAD, and the ERSPC MRI predictive model for csPCa detection in men in the PI-RADS 3 category using the most sensitive thresholds

Parameter	csPCs detection, *n*/*N* (%)		
	Proclarix (cutoff = 10%)	PSAD (cutoff = 0.07 ng/ml/cm^3)	ERSPC MRI RC (cutoff = 1.5%)
Sensitivity	25/25 (100)	21/25 (84.0)	24/25 (96.0)
Specificity	36/144 (25.0)	41/144 (28.5)	11/144 (7.6)
Negative predictive value	36/36 (100)	41/45 (91.1)	11/12 (91.7)
Positive predictive value	25/133 (19.8)	21/124 (16.0)	24/157 (15.3)
Accuracy	61/169 (36.1)	62/169 (36.7)	35/169 (20.7)
Prostate biopsies avoided	36/169 (21.3)	45/169 (26.2)	12/169 (7.1)
Decrease in iPCa 9/169 (5.3)	2/169 (1.2)	overdetection	9/169 (5.3)
Misdiagnosis of csPCa	0/25 (0)	4/25 (16.0)	1/25 (4.0)

PI-RADS = Prostate Imaging-Reporting and Data System; PCa = prostate cancer; iPCa = insignificant PCa; csPCa = clinically significant PCa; PSAD =prostate-specific antigen density; ERSPC = European Randomized Study of Screening for Prostate Cancer; MRI = magnetic resonance imaging; RC = risk calculator.

tent with the ROC curves, according to which Proclarix outperformed PSAD and the ERSPC MRI predictive model, showing 100% sensitivity, although its AUC of 0.703 seems suboptimal. This is positive for clinicians, given that Proclarix can detect all csPCa cases while avoiding 21.3% of all prostate biopsies (24.8% in biopsy-naïve men and 12.5% in those scheduled for repeat biopsy). Although PSAD seems slightly more specific than Proclarix, avoiding 26.3% of biopsies, it misdiagnoses 16% of csPCa cases, which is hardly acceptable. This PSAD performance is similar to previous findings [11–13]. The accuracy of the ERSPC MRI predictive model was notably lower compared to Proclarix and PSAD, except at high thresholds, at which the sensitivity is very low. Finally, the reduction in overdetection of iPCa was 5.3% for Proclarix and PSAD (decrease from 16.6% to 11.2%) and 1.2% for the ERSPC MRI model.

Modern markers have been analysed in the context of the current pathway for csPCa diagnosis and are intended to avoid mpMRI scans and subsequent prostate biopsies or to select appropriate candidates for prostate biopsy after mpMRI [17]. Some of these markers are combined with clinical independent predictors in predictive models [15,18]. The Prostate Health Index (PHI) and PCA3 [19], PHI [20,21], 4K [22–25], and the Stockholm 3 test [26] have been analysed, although their specific behaviours regarding PI-RADS categories has never been reported as a main research objective. Data for PHI and SelectMDx could be extracted from analyses of overall series published in the literature [18,27,28] and one specific series of men with PI-RADS 3 findings [29]. Supplementary Table 1 summarises the clinical utility of PHI [27] and SelectMDx [18,28,29] for csPCa detection in men with suspected PCa and a PI-RADS 3 lesion in comparison to Proclarix in the present study. Fan et al. [27] analysed the PHI performance for a group of 56 men and observed a rate of avoidable biopsies of 67.9% while misdiagnosing one out of 16 men (6.3%) men with detected csPCa using a cutoff point of 50. Maggi et al. [18] reported that SelectMDx with a threshold of 13% misdiagnosed 12/14 men (85.7%) diagnosed with csPCa among a sample of 54, avoiding 33.3% of prostate biopsies. Hendriks et al. [28] found that SelectMDx with a threshold of 13% misdiagnosed 7/9 men (77.8%) diagnosed with csPCa among a sample of 38 men, avoiding 40.2% of prostate biopsies. We recently observed that SelectMDx with a threshold of 13% misdiagnosed four out of six men (66.7%) with csPCa in a sample of 62 men, avoiding 40.6% of prostate biopsies [29]. The present study shows that Proclarix is very sensitive for csPCa, making it reliable enough to reassure clinicians. PHI seems more sensitive than SelectMDx but less sensitive than Proclarix. Multicentre validation studies should be performed to confirm the effectiveness of any marker and cost-benefit studies regarding the quality-adjusted life years gained are desirable [30].

The present study was carried out on the largest published sample of men with suspected PCa and PI-RADS 3 findings; however, the sample size may still be a limitation because of the low incidence of csPCa. Unfortunately, we found no way to estimate the appropriate cohort size to

Table 4 – Performance of Proclarix, PSAD, and the ERSPC MRI model for csPCa detection using the most sensitive threshold in biopsy-naïve men and men undergoing repeat biopsy

Parameter	csPCs detection, *n*/*N* (%)					
	Proclarix (cutoff = 10%)		PSAD (cutoff = 0.07 ng/ml/cm^3)		ERSPC MRI RC (cutoff = 1.5%)	
	Initial Bx	Repeat Bx	Initial Bx	Repeat Bx	Initial Bx	Repeat Bx
Sensitivity	18/18 (100)	7/7 (100)	15/18 (83.3)	6/7 (85.7	17/18 (94.4)	7/7 (100)
Specificity	30/103 (29.1)	6/41 (14.6)	33/103(32.0)	8/41 (19.5)	6/103 (5.8)	5/41 (12.2)
Negative predictive value	30/30 (100)	6/6 (100	33/36 (91.7)	8/9 (88.9)	6/7 (85.7)	5/5 (100)
Positive predictive value	18/91 (19.8)	7/42 (16.7)	15/85 (17.6)	6/39 (15.4)	17/114 (14.9)	7/43 (16.3)
Accuracy	48/121 (39.7)	13/48 (27.1)	48/121(39.7)	14/48 (29.2	23/121 (19.0)	13/48 (27.1)
Prostate biopsies avoided	30/121 (24.8)	6/48 (12.5)	36/121 (29.8)	9/48 (18.8)	7/121 (5.8)	5/48 (10.4
Decrease in iPCa overdetection	7/121 (5.8)	2/48 (4.2)	7/121 (5.8)	2/48 (4.2)	1/121 (0.8)	1/48 (2.1)
Misdiagnosis of csPCa	0/18 (0)	0/7 (0)	3/18 (16.7)	1/7 (14.3)	1/8 (5.6)	0/7 (0)

PSAD = prostate-specific antigen density; PCa = prostate cancer; csPCa = clinically significant PCa; iPCa = insignificant PCa; Bx = biopsy; ESPRC = European Randomized Study of Screening for Prostate Cancer; MRI = magnetic resonance imaging; RC = risk calculator.

assess the efficacy of certain tools owing to the lack of previous data. Although all studies use the same definition of csPCa, we understand that the true incidence of csPCa observed in surgical specimens may be overestimated by prostate biopsies. In the era of MRI and guided biopsies it seems important to analyse the efficacy of any tool for improving the detection of csPCa regarding PI-RADS categories. Results for the overall efficacy, net benefits, and clinical utility may result in confusion for clinicians. The overall analyses are important, but they do not guarantee the same effectiveness across the PI-RADS categories [12,13,28].

5. Conclusions

The efficacy of tools for the appropriate selection of candidates for prostate biopsy varies regarding PI-RADS categories. Proclarix performed better than PSAD and the ERSPC MRI predictive model in the challenging scenario of PI-RADS category 3. Proclarix was able to reach 100% detection of csPCa, avoiding almost a quarter of unnecessary prostate biopsies and reducing iPCa overdetection from 16.6% to 11.2%.

Author contributions: Juan Morote had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Morote.
Acquisition of data: Celma, Regis, Planas, Semidey, de Torres, Mast.
Analysis and interpretation of data: Morote, Campistol, Triquell.
Drafting of the manuscript: Morote, Campistol, Triquell.
Critical revision of the manuscript for important intellectual content: Trilla, Santamaria.*Statistical analysis*: Morote.*Obtaining funding*: Morote.
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Supervision: Morote, Trilla.
Other: None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euros.2021.12.009.

References

[1] Hugosson J, Roobol MJ, Månsson M, et al. A 16-yr follow-up of the European Randomized Study of Screening for Prostate Cancer. Eur Urol 2019;76:43–51.
[2] Mottet N, van den Bergh RCN, Briers E, et al. EAU-EANM-ESTRO-ESUR-SIOG guidelines on prostate cancer—2020 update. Part 1: screening, diagnosis, and local treatment with curative intent. Eur Urol 2021;79:243–62.
[3] US Preventive Services Task Force, Grossman DC, Curry SJ, et al. Screening for prostate cancer: US Preventive Services Task Force recommendation statement. JAMA 2018;319:1901–13.
[4] Moldovan PC, Van den Broeck T, Sylvester R, et al. What is the negative predictive value of multiparametric magnetic resonance imaging in excluding prostate cancer at biopsy? A systematic review and meta-analysis from the European Association of Urology Prostate Cancer Guidelines Panel. Eur Urol 2017;71:618–29.
[5] Sathianathen NJ, Omer A, Harriss E, et al. Negative predictive value of multiparametric magnetic resonance imaging in the detection of clinically significant prostate cancer in the Prostate Imaging Reporting and Data System era: a systematic review and meta-analysis. Eur Urol 2020;78:402–14.
[6] Mazzone E, Stabile A, Pellegrino F, et al. Positive predictive value of Prostate Imaging Reporting and Data System version 2 for the detection of clinically significant prostate cancer: a systematic review and meta-analysis. Eur Urol Oncol. In press. http://doi.org/10.1016/j.eururo.2021.07.027.
[7] Schoots IG. MRI in early prostate cancer detection: how to manage indeterminate or equivocal PI-RADS 3 lesions. Transl Androl Urol 2018;7:70–82.
[8] Görtz M, Radtke JP, Hatiboglu G, et al. The value of prostate-specific antigen density for Prostate Imaging-Reporting and Data System 3 lesions on multiparametric magnetic resonance imaging: a strategy to avoid unnecessary prostate biopsies. Eur Urol Focus 2021;7:325–31.
[9] Klocker H, Golding B, Weber S, et al. Development and validation of a novel multivariate risk score to guide biopsy decision for the diagnosis of clinically significant prostate cancer. BJUI Compass 2020;1:15–20.
[10] Steuber T, Heidegger I, Kafka M, et al. PROPOSe: a real-life prospective study of Proclarix, a novel blood-based test to support challenging biopsy decision-making in prostate cancer. Eur Urol Oncol. In press. http://doi.org/10.1016/j.euo.2020.12.003.
[11] Boesen L, Nørgaard N, Løgager V, et al. Prebiopsy biparametric magnetic resonance imaging combined with prostate-specific antigen density in detecting and ruling out Gleason 7–10 prostate cancer in biopsy-naïve men. Eur Urol Oncol 2019;2:311–9.
[12] Morote J, Celma A, Planas J. Re: Lars Boesen, Nis Nørgaard, Vibeke Løgager, et al. Prebiopsy biparametric magnetic resonance imaging combined with prostate-specific antigen density in detecting and ruling out Gleason 7–10 prostate cancer in biopsy-naïve men. Eur Urol Oncol 2019;2:311–9.
[13] Morote J, Celma A, Díaz F, et al. Prostatic-specific antigen density behavior according to multiparametric magnetic resonance imaging result. Urol Oncol 2020;38:410–7.
[14] Alberts AR, Roobol MJ, Verbeek JFM, et al. Prediction of high-grade prostate cancer following multiparametric magnetic resonance imaging: improving the Rotterdam European Randomized Study of Screening for Prostate Cancer risk calculators. Eur Urol 2019;75:310–8.
[15] Macagno A, Athanasiou A, Wittig A, et al. Analytical performance of thrombospondin-1 and cathepsin D immunoassays part of a novel CE-IVD marked test as an aid in the diagnosis of prostate cancer. PLoS One 2020;15:e0233442.
[16] Epstein JI, Egevad L, Amin MB, et al. The 2014 International Society of Urological Pathology (ISUP) consensus conference on Gleason grading of prostatic carcinoma: definition of grading patterns and proposal for a new grading system. Am J Surg Pathol 2016;40:244–52.
[17] Vickers AJ, Russo G, Lilja H, et al. How should molecular markers and magnetic resonance imaging be used in the early detection of prostate cancer. Eur Urol Oncol. In press. http://doi.org/10.1016/j.euo.2021.01.010.
[18] Fan YH, Pan PH, Cheng WM, et al. The Prostate Health Index aids multi-parametric MRI in diagnosing significant prostate cancer. Sci Rep 2021;11:1286.

[19] Porpiglia F, Russo F, Manfredi M, et al. The roles of multiparametric MRI, PCA3, and PHI: which is the best predictor of prostate cancer after a negative biopsy? Results of a prospective study. J Urol 2014;192:60–6.
[20] Roobol MJ, Vedder MM, Nieboer D, et al. Comparison of two prostate cancer risk calculators that include the Prostate Health Index. Eur Urol Focus 2015;1:185–90.
[21] Gnanapragasam VJ, Burling K, George A, et al. The Prostate Health Index adds predictive value to multi-parametric MRI in detecting significant prostate cancers in a repeat biopsy population. Sci Rep 2016;6:35364.
[22] Punnen S, Nahar B, Soodana-Prakash N, et al. Optimizing patient's selection for prostate biopsy: a single institution experience with multi-parametric MRI and the 4Kscore test for the detection of aggressive prostate cancer. PLoS One 2018;13:e0201384.
[23] Falagario UG, Martini A, Wajswol E, et al. Avoiding unnecessary magnetic resonance imaging (MRI) and biopsies: negative and positive predictive value of MRI according to prostate-specific antigen density, 4Kscore and risk calculators. Eur Urol Oncol 2020;3:700–4.
[24] Marzouk K, Ehdaie B, Vertosick E, Zappala S, Vickers A. Developing an effective strategy to improve the detection of significant prostate cancer by combining the 4Kscore and multiparametric MRI. Urol Oncol 2019;37:672–7.
[25] Verbeek JFM, Bangma CH, Kweldam CF, et al. Reducing unnecessary biopsies while detecting clinically significant prostate cancer including cribriform growth with the ERSPC Rotterdam risk calculator and 4Kscore. Urol Oncol 2019;37:138–44.
[26] Grönberg H, Eklund M, Picker W, et al. Prostate cancer diagnostics using a combination of the Stockholm3 blood test and multiparametric magnetic resonance imaging. Eur Urol 2018;74:722–8.
[27] Maggi M, Del Giudice F, Falagario UG, et al. SelectMDx and multiparametric magnetic resonance imaging of the prostate for men undergoing primary prostate biopsy: a prospective assessment in a multi-institutional study. Cancers 2021;13:2047.
[28] Hendriks RJ, van der Leest MMG, Israël B, et al. Clinical use of the SelectMDx urinary-biomarker test with or without mpMRI in prostate cancer diagnosis: a prospective, multicenter study in biopsy-naïve men. Prostate Cancer Prostat Dis 2021;24:1110–9.
[29] Morote J, Díaz F, Celma A, et al. Behaviour of SelectMDx and prostate-specific antigen density in the challenging scenario of Prostate Imaging-Reporting and Data System category 3 lesions. Eur Urol. In press. https://doi.org/10.1016/j.eururo.2021.09.019.
[30] Govers TM, Hessels D, Vlaeminck-Guillem V, et al. Cost-effectiveness of SelectMDx for prostate cancer in four European countries: a comparative modeling study. Prostate Cancer Prostat Dis 2018;22:101–9.

5.6. PUBILCATION 6

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

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5.7. PUBLICATION 7

Title: Relationship between Proclarix and the Aggressiveness of Prostate Cancer

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6. OVERALL SUMMARY OF THE RESULTS

Proclarix was initially designed for men with PSA levels between 2 and 10ng/mL, normal digital rectal examination and prostate volume ≥ 35mL for the detection of csPCa. However, subsequent testing of Proclarix in men who did not meet these specific characteristics showed similar sensitivities and a net benefit in both subset of men. Thus, demonstrating that Proclarix is valuable tool for all men with suspected PCa regardless of their PSA level, prostate volume, or DRE.

When compared with other predictive tools, the ERPSC MRI predictive model exhibited a net benefit over PSA density and Proclarix in the overall population. However, when analyzed by PI-RADS categories, Proclarix outperformed both ERPSC MRI and PSA density in patients with lesions PI-RADS 3 or lower in the selection of candidates for prostate biopsy. Nonetheless, PSA density outperformed MRI ERSPC and Proclarix in PI-RADS > 3 lesions.

When analyzing the specific subgroup of patients with lesions PI-RADS 3, Proclarix showed a net benefit over PSA density and the ERSPC MRI model. The percentage of prostate biopsies avoided with Proclarix, PSA density and the ERSPC MRI model would be 21.3%, 26.2% and 7.1%, respectively. Proclarix avoid 21.3% of prostate biopsies and reduce overdetection of insignificant PCa from 16.6% to 11.2% without misdiagnosing csPCa. PSA density would avoid 26.2% of prostate biopsies, reduce overdetection of insignificant PCa from 16.6% to 11.2%, but misdiagnose 16% of csPCa. Finally, the ERSPC MRI predictive model would avoid only 7.1% of prostate biopsies, reduce the overdetection of insignificant PCa from 16.6% to 15.3%, and misdiagnose 4% of csPCa cases. Thus, Proclarix outperformed PSA density and the ERSPC MRI predictive model in those patients with lesions PI-RADS 3 by significantly reducing the number of unnecessary biopsies while maintaining accurate detection of csPCa.

Proclarix was correlated with the four surrogates of aggressive analyzed. On the first hand, Proclarix score was significantly higher in patients with csPCa (median 60.1%) compared to those with insignificant PCa (median 37.3%) and those without PCa (median 20.7%). On the other hand, Proclarix showed a significant increase with higher GG. Patients with GG 1 tumors had the lowest median Proclarix score (29.4%), while those with GG 5 exhibited the highest Proclarix score (62.8%). Moreover, Proclarix levels were higher in patients with locally advanced PCa (60.1%) and metastatic PCa (97.4%), compared to those with localized PCa (37.3%). Men with higher risk of biochemical recurrence after primary treatment obtained higher Proclarix score (58.7%) than those with intermediate-risk (35%) and those with low-risk (24.9%). Finally, Proclarix score was significantly higher in patients with unfavorable pathology in surgical specimens (35.7%) compared to those with favorable pathology (23.7%). Thus, Proclarix showed potential as a useful tool for predicting the aggressiveness of PCa and could complement mpMRI findings in assessing the significance of tumors and guiding treatment decisions.

7. OVERALL SUMMARY OF THE DISCUSSION

7.1. GENERAL DISCUSSION

The definition of csPCa is a constantly evolving process that has undergone several modifications throughout the years. In 1994, Epstein et al. introduced the first criteria to define csPCa and developed a predictive model to identify patients who would not require definitive therapy based on prostatectomy specimen [194]. According to their study, csPCa was determined based the following criteria: a tumor volume greater than 0.2cm^3, a Gleason grade higher than 7 and the presence of extracapsular extension. On the contrary, they considered a clinically insignificant PCa when the clinical stage was a T1c, the tumor was confined to the prostate with a volume less than 0.2 cm^3, there was no Gleason pattern 4 or 5, and no involvement of seminal vesicles or lymph nodes. In 2011, Ahmed et al. proposed two definitions for csPCa based on three biopsy variables [195]. The first parameter was the total cancer core length with values of ≥ 10 mm and ≥ 6 mm depending on individual preferences, comorbidity, age, and life expectancy. The second parameter was two lesion volume thresholds measured using the maximum cancer core length of ≥ 6mm and ≥ 4mm in one core. Finally, they combined dominant and non-dominant Gleason score 4 using the csPCa definition. Thus, Ahmed et al. concluded that analyzing the prostate biopsy sample could be useful in increasing the proportion of men who choose, or are advised, to undergo active surveillance while also ensuring that those who require therapy do undergo it. In 2016, Epstein et al., introduced a new grading system for PCa that aimed to improve the traditional Gleason score. This new system featured a simplified grading scale ranging from 1 to 5, where grade 1 represented the lowest grade and grade 5 indicated the highest grade. The intention behind this system was to reduce overtreatment of iPCa [196]. Recent

literature has established the definition of csPCa using the ISUP grade group, considering a csPCa when classified as ISUP GG 2 or higher[196, 197]. Thus, in our study, all published articles used this definition, considering a csPCa when the ISUP GG score was 2 or higher. Finally, in their review, Alchin et al. described that the Gleason score upgrade after radical prostatectomy can vary from 29.6% to 45.6%, depending on the study. This finding can be predictive of subsequent biochemical recurrence and oncological failure [198].

Early detection of csPCa can lead to a decrease in the mortality rate associated with the disease [17]. The initial suspicion of PCa is based on an elevation of serum PSA and/or an abnormal DRE, and requires further confirmation with prostate biopsy [40]. Nonetheless, this current diagnostic approach often leads to a high rate of unnecessary prostate biopsies and an overdetection of iPCa [65]. Different tests have been developed to predict the presence of csPCa and to help clinical decision making on who to biopsy and who to re-biopsy after an initially negative biopsy result.

Proclarix is a recently introduced CE-marked test that provides the risk score for csPCa (ranging from 0% to 100%) based on serum levels of Thrombospondin-1, Cathepsin D, PSA, and percentage of free PSA in addition to age [175, 181]. To date, three studies have evaluated the effectiveness of Proclarix in detecting csPCa [175, 176, 181], all of which included patients with specific characteristics such as serum PSA levels between 2 and 10ng/mL, normal DRE and a prostate volume higher than 35cc. Using a cut-off value of 10% Proclarix demonstrated a sensitivity of 90% for detecting csPCa while its specificity ranged from 22% to 43%, with a NPV of 95% [175, 176, 181].

Our study was the first to evaluate the performance of Proclarix in men who did not fit the specific characteristics used in prior investigations (i.e., serum PSA levels <

2ng/mL or > 10ng/mL, abnormal DRE and prostate volume lower than 35mL) [199]. Despite differences in csPCa incidence between the two populations (25.3% and 55.5%, respectively), Proclarix exhibited a sensitivity in both subsets of men, reaching 95.8% in men with the specific characteristics and 98.1% in the others, with specificities of 32.5% and 17.2%, respectively. Proclarix demonstrated a net benefit in both subsets of men with suspected PCa, reducing the need for mpMRI and derived prostate biopsies by 25.3% in men with serum PSA levels between 2 - 10 ng/mL, prostate volume over 35cc, and normal DRE, and by 8.7% in men who did not meet any of these criteria. The misdiagnosis rate of csPCa was 4.2% and 1.9%, respectively. Thus, our study demonstrated that Proclarix had a high sensitivity for detecting csPCa in men with suspected PCa, regardless of their serum PSA level, prostate volume or DRE [200].

In recent years, the use of mpMRI has been introduced in the diagnostic algorithm of csPCa in order to improve its accuracy and to guide prostate biopsies, and it is now recommended in most current guidelines [112, 201]. The NPV of mpMRI when PI-RADS is below 3, reaches 91% [188, 202], this is why most clinicians recommend avoiding biopsies in these cases. Conversely, when PI-RADS categories are greater than 3, the likelihood of csPCa is high, ranging from 55% to 95% [186], and performing prostate biopsies is usually recommended in these circumstances. However, the selection of candidates for prostate biopsy, especially those with low or moderate likelihood of csPCa suggested by mpMRI, remains challenging [201], particularly in lesions PI-RADS 3 where 60 - 85% of prostate biopsies are unnecessary and up to 60% of PCa detected are insignificant [203, 204].

Modern markers have been analyzed in the context of the current pathway for csPCa diagnosis and are intended to avoid mpMRI and subsequent prostate biopsies or

to select appropriate candidates for prostate biopsy after mpMRI [205]. Some of these markers are combined with clinical independent predictors in predictive models [174, 206]. The Prostate Health Index (PHI) and PCA3 [207], PHI [101, 208], 4K [107, 108, 112, 209], and the Stockholm 3 test [70] have been analyze, although their specific behaviors regarding PI-RADS categories has never been reported as a main research objective. The comparison between Proclarix and other markers is difficult [112, 122, 124, 210] and can only be effective in head-to-head studies.

When analyzing Proclarix performance in the detection of csPCa, only Steuber et al. included the use of mpMRI for fusion biopsy in 121 patients of their cohort [181]. The authors concluded that the performance of Proclarix improved when used in conjunction with mpMRI in the decision to biopsy the patient. Other markers have been also analyzed in combination with mpMRI. On the first hand, the PHI test provides a risk assessment for PCa in men with similar characteristics to the initial validation cohort of Proclarix (serum PSA between 2 and 10ng/mL and a nonsuspicious DRE). Studies have demonstrated that combining PHI with mpMRI improves the prediction of overall and csPCa prediction, compared to mpMRI and serum PSA alone [93]. Moreover, PHI outperformed the total PSA and the percent of free PSA for the prediction of prostate biopsy outcome; thus, being able to reduce the number of unnecessary biopsies and improving the accuracy of csPCa detection [86–89]. Nonetheless, Steuber et al. concluded that PSA density performed better than PHI in detecting csPCa and was able to spare more prostate biopsies [181]. Similarly, SelectMDx, a urine-based marker, has been shown to effectively differentiate high-grade PCa from insignificant disease [118, 120]. When used in conjunction with mpMRI it also exhibited a higher sensitivity but with a lower specificity [211], and was able to avoid 38% of unnecessary prostate

biopsies while misdiagnosing 10% of csPCa [124]. The four-kallikrein (4K) score was developed to identify csPCa in patients with suspicious DRE and elevated serum PSA. It has been integrated with mpMRI and clinical variables to create a predictive nomogram [26]. In a study of 266 biopsy-naïve men who underwent mpMRI, using the 4K score would avoid 12% of unnecessary biopsies while misdiagnosing 1.4% of csPCa [210].

There are limited head-to-head studies comparing different biomarkers. In a recently published study, the effectiveness of Proclarix and PHI in the detection of csPCa was investigated. They reported that both biomarkers accurately detect the presence of csPCa, with Proclarix exhibiting higher specificity and positive predictive value compared to PHI, while maintaining similar sensitivities. Additionally, when Proclarix and PHI were combined, a synergistic effect was observed, leading to an improvement in the diagnostic performance of the individual tests alone with the highest clinical benefit [212].

Unfortunately, there is lack of studies examining the role of these novel biomarkers according to PI-RADS categories. Nonetheless, we performed a head-to-head study comparing Proclarix with PSA density, and the externally validated ERSPC MRI [212–214]. When the entire population of men with suspected PCa was analyzed, the ERSPC MRI model was the most efficient tool for the detection of csPCa, outperforming both Proclarix and PSA density. However, the performance of these tools varied when analyzed by PI-RADS category. In men with lesions PI-RADS ≤ 3 Proclarix was found to be the most efficient and clinically useful tool, increasing the NPV of mpMRI from 94% to 100% and avoiding unnecessary prostate biopsies in 70% of men with negative mpMRI [188, 202]. In contrast, PSA density recommended prostate biopsy in 69% of men with negative mpMRI, leaving 50% of csPCa undetected [189, 213].

Finally, ERPSC MRI risk-calculator recommended biopsy in 37% of men with negative mpMRI but missed 83.3% of csPCa. In patients with challenging PI-RADS 3 lesions, Proclarix was again found to be the most efficient tool, exhibiting 100% sensitivity for detecting csPCa while avoiding 21.3% of prostate biopsies. In comparison, PSA density would avoid 26.2% of prostate biopsies but would miss 16% of csPCa while ERPSC MRI would avoid 29.6% of prostate biopsies but also miss 16% of csPCa. In men with PI-RADS 4 lesions, PSA density was the most efficient tool, avoiding 18.4% of prostate biopsies but missing 11.4% of csPCa. Proclarix would avoid 12.1% of prostate biopsies but would miss 4.8% of csPCa while ERPSC MRI risk-calculator would avoid 9.3% of prostate biopsies and would miss 4.2% of csPCa. In men with PI-RADS 5 lesions, in where 88.9% of csPCa was detected, PSA density was again the most efficient tool, avoiding 9.3% of prostate biopsies while missing 4.2% of csPCa. Proclarix would avoid 5.6% of prostate biopsies and miss 1% of csPCa while ERPSC MRI risk-calculator would not avoid any prostate biopsies. It is worth noting that PI-RADS categories greater than 3 are associated with high and very high-risk of csPCa, along with increase aggressiveness of the disease [214, 215]. Therefore, tools that ensure 100% sensitivity for the detection of csPCa are preferred in this category, even if it means performing some unnecessary prostate biopsies.

On the other hand, a PI-RADS 3 lesion described in the mpMRI suggests a moderate risk of csPCa that does not exceed 20% [216]. It is a very challenging scenario since the rate of csPCa detection is low. Thus, PSA density, predictive models and several markers have been analyzed in order to increase its specificity and avoid unnecessary prostate biopsies [189, 217]. We carried out the largest published sample of men with suspected PCa and PI-RADS 3 findings on mpMRI in order to compare the behavior of

PSA density, Proclarix and ERPSC MRI predictive model. The incidence of csPCa detected among 169 men with PI-RADS 3 findings was 14.3%, which is within the range reported in the literature (5 - 30%) [204]. We observed a net benefit of Proclarix over PSA density and the ERPSC MRI predictive model for determining the likelihood of csPCa [213, 218]. Proclarix demonstrated a 100% sensitivity with an AUC of 0.703, meaning that Proclarix was able to detect all csPCa while avoiding 21.3% of prostate biopsies (24.8% in biopsy-naïve men and 12.5% in those scheduled for repeat biopsy). In contrast, while PSA density showed slightly higher specificity than Proclarix, avoiding 26.3% of biopsies, it misdiagnosed 16% of csPCa, which is deemed unacceptable. This PSA density performance is similar to previous findings [189, 214]. The accuracy of the ERPSC MRI predictive model was notably lower compared to Proclarix and PSA density, except at high thresholds, where the sensitivity was very low. Additionally, Proclarix and PSA density were found to decrease the overdetection of iPCa by 5.3%, whereas the ERPSC MRI predictive model only showed a reduction of 1.2%. Overall, these findings suggest that Proclarix may be the most effective tool for detecting csPCa in patients with PI-RADS 3 lesions on mpMRI. While PSAD showed comparable performance, it had a higher rate of misdiagnosis, and the ERPSC MRI predictive model was less accurate. Very few biomarkers have been analyzed by PI-RADS categories. A recent analysis conducted by Tosoian et al., evaluated the effectiveness of MyProstateScore testing in men with PI-RADS 3 lesions identified on mpMRI. The study demonstrated that MyProstateScore exhibited superior performance compared to PSA density in the detection of csPCa in this specific subgroup of patients [159]. Thus, Proclarix and MyProstateScore may be valuable tools to improve the specificity of biopsies and reduce unnecessary procedures in patients with PI-RADS 3 findings on mpMRI.

Integrating clinical information such as prostate volume and biomarkers could potentially improve the efficacy of mpMRI. 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. Steuber et al. evaluated this strategy in the PROPOSe study and found that the Proclarix-MRI model accurately discriminated among patients with indeterminate mpMRI categories, allowing one-third to safely avoid biopsies without missing csPCa [181]. Our study yielded similar results, showing that combining prostate volume and Proclarix score with mpMRI, improved the efficacy of csPCa detection, the NPV and specificity further increased 97% and 33% respectively. 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 overdetection of men with iPCa would be cut in half and two out of three negative biopsies overall would be saved. Proclarix-MRI showed a higher net benefit for threshold probabilities of > 10% compared to the other tests, significantly outperforming PSA density, ERPSC MRI predictive model, Proclarix and mpMRI alone.

We intended to analyze how Proclarix could be used to select appropriate candidates for mpMRI in the diagnostic evaluation for PCa. Based on existing evidence suggesting limited benefit from mpMRI in men with abnormal DRE and serum PSA >10ng/mL [216, 219], we proposed that this specific subgroup of patients should be directly scheduled for systematic prostate biopsy. Among these men, who represent approximately 10% of all patients with suspected PCa, the rate of csPCa was found to be 89.6% accounting for 18.7% of all csPCa detected. Additionally, we suggest that Proclarix

should be evaluated in men with normal DRE findings, as well as those with abnormal DRE and serum PSA levels ≤ 10ng/mL. Among these individuals, approximately 20% had a Proclarix score ≤ 10%, which served as our threshold for avoiding mpMRI and derived prostate biopsies. In this subset of men, the misdiagnosis rate of csPCa was found to be 2.6% of all csPCa cases detected, while the overdiagnosis of iPCa was 18.2% of all iPCa detected. Finally, all men with a Proclarix score > 10% should be scheduled for guided and/or systematic prostate biopsies. Implementing this proposed diagnostic algorithm using Proclarix, would result in a reduction in mpMRI requests by 25.4%, a decrease in the number of prostate biopsies by 17.5%, a reduction in the rate of overdiagnosis of iPCa by 18.2%, and a misdiagnosis of csPCa of 2.6%. These findings highlight the potential clinical benefits of incorporating Proclarix into the diagnostic pathway of csPCa [199]. The proposed diagnostic algorithm for patients with suspicion of PCa using Proclarix is shown in Figure 2 of the second publication [199].

Previous studies have explored the association between Proclarix and PCa grading, but have typically only compared men without PCa or those with GG 1 to those with GG 2 or 3 and those with GG 4 or 5 [176, 181]. Our study confirms that Proclarix score is indeed associated with GG, but we were not able to distinguish between GG 2 and GG 3. Additionally, our study also found an association between Proclarix and the clinical stage of PCa as well as the risk of recurrence of treated localized PCa [199]. The correlation between the GG and other biomarkers has been also analyzed. Multiples studies have demonstrated a correlation between PHI and the GG in biopsy-naïve patients, obtaining higher PHI values in men with an increased probability of a Gleason score ≥ 7 in the biopsy [90–92, 220]. On the hand, the 4K has been extensively studied using two surrogate endpoints of aggressiveness: the grade group and the pathology

observed in surgical specimens. In relation to the GG obtained from biopsy samples, the 4K score obtained higher values in those patients with csPCa, compared to those with iPCa [104, 105]. Multiple studies have confirmed the accuracy of 4K score in detecting high-grade PCa (Gleason score ≥ 7) and its potential to reduce the number of unnecessary biopsies [104, 105, 221]. Nevertheless, 4K score was analyzed in a postoperative setting and was reported to not be useful for counseling men after radical prostatectomy. Several studies have stated that the incorporation of 4K score did not improve the value of post-surgery risk models and could not be used in the prediction of biochemical recurrence [117].

In our opinion, a major strength of Proclarix is its high sensitivity for csPCa, which guarantees the detection of most of these tumors and is effective in order to select appropriate candidates for mpMRI and derived prostate biopsies. Furthermore, we acknowledge the need for additional head-to-head studies comparing Proclarix with other biomarkers in order to gain a better understating of their relative effectiveness and utility in the detection of csPCa. Nevertheless, it is crucial to conduct a comprehensive cost-benefit analysis to determine the ultimate advantage of using these markers as complementary tools of mpMRI. Additionally, the final benefit of any strategy for csPCa detection should be analyzed in terms of health benefit, through appropriate studies on cost-effectiveness analyzing the quality-adjusted life years and healthcare cost.

7.2. LIMITATIONS AND STRENGHTS

Our study has certain limitations that should be acknowledged. Firstly, the serum samples were collected prospectively in the biobanks, but the measurements of the samples were performed retrospectively. This may introduce biases and limitations in data collection and analysis. Additionally, the only criterion for inclusion was the requirement for men to have undergone a prostate biopsy, resulting in the exclusion of 132 samples from the INNOVATE cohort. Despite this limitation, the results indicate that the proposed Proclarix-MRI model performs well even in a cohort where biopsies could potentially be avoided based on established clinical practice. There was no central reading conducted for pathology, ultrasound, and mpMRI, which may introduce a small inter-site variability in the results.

Additionally, the lack of external validation represents another limitation, as it reduces the generalizability of our findings. Prospective and multicenter studies mimicking real clinical practice are warranted to validate and further explore the utility of Proclarix. Furthermore, conducting comparative studies among existing markers would provide valuable insights into their relative effectiveness.

On the other hand, it is important to note, that a common limitation of the study is the assessment of csPCa in prostate biopsies. This approach may not fully represent the true pathology observed in the entire prostate gland. Although the definition of csPCa remains consistent across all studies, the true incidence of csPCa observed in surgical specimens may be overestimated when relying solely on prostate biopsies. In the era of MRI and guided biopsies it is crucial to assess the efficacy of any diagnostic tool in improving csPCa detection regarding PI-RADS categories. While overall analyses are important, they may not guarantee the same level of effectiveness across different

PI-RADS categories. This can lead to confusion among clinicians regarding the net benefits and clinical utility of the diagnostic tool.

Another significant limitation of the study was the small number of patients who underwent radical prostatectomy. This limited sample size may have affected the generalizability and statistical power of the study's findings. Therefore, further investigations with larger sample size are warranted to enhance the assessment of Proclarix performance with the pathological analysis the surgical specimen. In conclusion, although our study has certain limitations in terms of its design and lacks of external validation, it provides valuable insights into the potential benefits of Proclarix.

8. CONCLUSIONS

1. Proclarix, a predictive model for csPCa, has been developed from a discovery model based on mass spectrometry proteomic approach, which identified THBS1 and CTSD as tumor markers of csPCa in a PTEN knockout mouse model silencing PI3K/PTEN. Proclarix uses serum quantification of THBS1 and CTSD, along with PSA, percent free PSA, and age of men with suspected PCa with serum PSA levels between 2 and 10ng/mL and a prostate volume of 35mL or higher. Proclarix is reported as a percent probability ranging from 0 to 100%, with a recommended threshold of 10% set by the manufacturer.

2. Proclarix can be used be used in all men with suspected PCa, without limitations on serum PSA levels or prostate volume, in order to reduce the number of mpMRI and prostate biopsies.

3. When used in combination with mpMRI, Proclarix contributes to a significant reduction in unnecessary prostate biopsies, especially in men with negative mpMRI results, where it demonstrates a high sensitivity in detecting csPCa while avoiding a considerable portion of biopsies. Additionally, Proclarix aids in reducing unnecessary prostate biopsies in men with positive mpMRI findings while potentially missing 2.6% of csPCa.

4. Lastly, Proclarix is also a marker of PCa aggressiveness since it relates with the four surrogates of aggressiveness: the ISUP grade group, the clinical stage, the risk of localized PCa and the pathology after radical prostatectomy.

9. FUTURE LINES

Future studies should aim to adopt prospective and multicenter designs involving larger and more diverse patients to confirm and expand upon current findings. Despite notable progress in the development of diagnostic markers for PCa, there is a lack of comprehensive data from clinical trials with head-to-head comparison of these assays regarding their ability to predict csPCa in initial or subsequent prostate biopsies. Several promising molecular biomarkers have been used for the detection of csPCa prior to prostate biopsy, but no definitive conclusions have been drawn regarding the superiority of any specific biomarker. In order to avoid the risks associated with overdiagnosis and overtreatment of PCa, as well as the consequences of unnecessary prostate biopsies, it is necessary to conduct further cross-validation studies and head-to-head comparisons of potential biomarkers in well-designed prospective clinical trials. In light of these considerations, continued research efforts are essential to validate the potential benefits of Proclarix and refine its clinical utility. By employing rigorous methodologies and expanding the scope of investigation, we can further elucidate the role of Proclarix and contribute to more informed decision-making in the management of prostate cancer.

10.BIBLIOGRAPHICAL REFERENCES

[1] Culp MBB, Soerjomataram I, Efstathiou JA, et al. Recent Global Patterns in Prostate Cancer Incidence and Mortality Rates. *Eur Urol* 2020; 77: 38–52.

[2] About | JCO Global Oncology, https://ascopubs.org/go/about/about-go?cmpid=js_jgo_jgo_awareness_psrh_googleadwords_oncphy_int-cancer-research_intl_031023____aware_text_&gclid=CjwKCAjw_YShBhAiEiwAMomsEMOvYDoJI7dlkgzg32rWqrPA73lM2H8VekUkdJYBWGVFkqBtjpPiQRoC6csQAvD_BwE (accessed 27 March 2023).

[3] Fleshner K, Carlsson S V, Roobol MJ. The effect of the USPSTF PSA screening recommendation on prostate cancer incidence patterns in the USA. *Nature Publishing Group*. Epub ahead of print 2016.

[4] Haas GP, Delongchamps N, Brawley OW, et al. The Worldwide Epidemiology of Prostate Cancer: Perspectives from Autopsy Studies. *Can J Urol* 2008; 15: 3866.

[5] Prostate Source: Globocan 2020 Number of new cases in 2020, both sexes, all ages, https://gco.iarc.fr/today (2020, accessed 30 March 2023).

[6] Hemminki K. Familial risk and familial survival in prostate cancer. *World J Urol* 2012; 30: 143–148.

[7] Giri VN, Hegarty SE, Hyatt C, et al. Germline genetic testing for inherited prostate cancer in practice: Implications for genetic testing, precision therapy, and cascade testing. *Prostate* 2019; 79: 333–339.

[8] Castro E, Goh C, Olmos D, et al. Germline BRCA Mutations Are Associated With Higher Risk of Nodal Involvement, Distant Metastasis, and Poor Survival Outcomes in Prostate Cancer. *J Clin Oncol* 2013; 31: 1748–1757.

[9] Zhang JQ, Geng H, Ma M, et al. Metabolic Syndrome Components are Associated with Increased Prostate Cancer Risk. *Med Sci Monit* 2015; 21: 2387–2396.

[10] Morote J, Gómez-Caamaño A, Alvarez-Ossorio JL, et al. The metabolic syndrome and its components in patients with prostate cancer on androgen deprivation therapy. *J Urol* 2015; 193: 1963–1969.

[11] Knijnik PG, Brum PW, Cachoeira ET, et al. The impact of 5-alpha-reductase inhibitors on mortality in a prostate cancer chemoprevention setting: a meta-analysis. *World J Urol* 2021; 39: 365–376.

[12] Hu et al. Association between 5-ARI and PCa incidence and mortality. DOI: 10.21037/tau-20-843.

[13] Thompson IM, Goodman PJ, Tangen CM, et al. The influence of finasteride on the development of prostate cancer. *N Engl J Med* 2003; 349: 215–224.

[14] Andriole GL, Bostwick DG, Brawley OW, et al. Effect of Dutasteride on the Risk of Prostate Cancer. *New England Journal of Medicine* 2010; 362: 1192–1202.

[15] Haider A, Zitzmann M, Doros G, et al. Incidence of prostate cancer in hypogonadal men receiving testosterone therapy: observations from 5-year median followup of 3 registries. *J Urol* 2015; 193: 80–86.

[16] Watts EL, Appleby PN, Perez-Cornago A, et al. Low Free Testosterone and Prostate Cancer Risk: A Collaborative Analysis of 20 Prospective Studies. *Eur Urol* 2018; 74: 585–594.

[17] Hugosson J, Roobol MJ, Månsson M, et al. A 16-yr Follow-up of the European Randomized study of Screening for Prostate Cancer. *Eur Urol* 2019; 76: 43–51.

[18] Cornford P, van den Bergh RCN, Briers E, et al. EAU-EANM-ESTRO-ESUR-SIOG Guidelines on Prostate Cancer. Part II-2020 Update: Treatment of Relapsing and Metastatic Prostate Cancer. *Eur Urol* 2021; 79: 263–282.

[19] Carvalhal GF, Smith DS, Mager DE, et al. Digital rectal examination for detecting prostate cancer at prostate specific antigen levels of 4 ng./ml. or less. *Journal of Urology* 1999; 161: 835–839.

[20] Turkbey B, Brown AM, Sankineni S, et al. Multiparametric Prostate Magnetic Resonance Imaging in the Evaluation of Prostate Cancer. *CA Cancer J Clin* 2016; 66: 326.

[21] Turkbey B, Albert PS, Kurdziel K, et al. Imaging localized prostate cancer: current approaches and new developments. *AJR Am J Roentgenol* 2009; 192: 1471–1480.

[22] Barentsz JO, Richenberg J, Clements R, et al. ESUR prostate MR guidelines 2012. Epub ahead of print 2012. DOI: 10.1007/s00330-011-2377-y.

[23] Weinreb JC, Barentsz JO, Choyke PL, et al. PI-RADS Prostate Imaging - Reporting and Data System: 2015, Version 2. *Eur Urol* 2016; 69: 16–40.

[24] Ahmed HU, El-Shater Bosaily A, Brown LC, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. *Lancet* 2017; 389: 815–822.

[25] Eklund M, Jäderling F, Discacciati A, et al. MRI-Targeted or Standard Biopsy in Prostate Cancer Screening. *N Engl J Med* 2021; 385: 908–920.

[26] Mazzone E, Gandaglia G, Ploussard G, et al. Risk Stratification of Patients Candidate to Radical Prostatectomy Based on Clinical and Multiparametric Magnetic Resonance Imaging Parameters: Development and External Validation of Novel Risk Groups. *Eur Urol* 2022; 81: 193–203.

[27] Morote J, Borque-Fernando A, Triquell M, et al. Multiparametric Magnetic Resonance Imaging Grades the Aggressiveness of Prostate Cancer. *Cancers (Basel)* 1828; 2022: 1828.

[28] Eldred-Evans D, Burak P, Connor MJ, et al. Population-Based Prostate Cancer Screening With Magnetic Resonance Imaging or Ultrasonography: The IP1-PROSTAGRAM Study. *JAMA Oncol* 2021; 7: 395–402.

[29] Roobol MJ, Schröder FH, Hugosson J, et al. Importance of prostate volume in the European Randomised Study of Screening for Prostate Cancer (ERSPC) risk calculators: results from the prostate biopsy collaborative group. *World J Urol* 2012; 30: 149–155.

[30] Roobol MJ, Steyerberg EW, Kranse R, et al. A Risk-Based Strategy Improves Prostate-Specific Antigen–Driven Detection of Prostate Cancer. *Eur Urol* 2010; 57: 79–85.

[31] Alberts AR, Roobol MJ, Verbeek JFM, et al. Prediction of High-grade Prostate Cancer Following Multiparametric Magnetic Resonance Imaging: Improving the Rotterdam European Randomized Study of Screening for Prostate Cancer Risk Calculators. *Eur Urol* 2019; 75: 310–318.

[32] Gayet M, Mannaerts CK, Nieboer D, et al. Prediction of Prostate Cancer: External Validation of the ERSPC Risk Calculator in a Contemporary Dutch Clinical Cohort. *Eur Urol Focus* 2018; 4: 228–234.

[33] Poyet C, Nieboer D, Bhindi B, et al. Prostate cancer risk prediction using the novel versions of the European Randomised Study for Screening of Prostate Cancer (ERSPC) and Prostate Cancer Prevention Trial (PCPT) risk calculators: independent

validation and comparison in a contemporary European cohort. *BJU Int* 2016; 117: 401–408.

[34] De Nunzio C, Lombardo R, Tema G, et al. External validation of Chun, PCPT, ERSPC, Kawakami, and Karakiewicz nomograms in the prediction of prostate cancer: A single center cohort-study. *Urologic Oncology: Seminars and Original Investigations* 2018; 36: 364.e1-364.e7.

[35] Prostate Cancer Research Foundation R. MRI-ERSPC Risk Calculator. SWOP web application .

[36] Etzioni R, Gulati R, Cooperberg M, et al. Limitations of basing screening policies on screening trials: The US Preventive Services Task Force and Prostate Cancer Screening. *Med Care* 2013; 51: 295–300.

[37] Cochrane Library Cochrane Database of Systematic Reviews Screening for prostate cancer (Review). Epub ahead of print 2013. DOI: 10.1002/14651858.CD004720.pub3.

[38] Ilic D, Djulbegovic M, Jung JH, et al. Prostate cancer screening with prostate-specific antigen (PSA) test: a systematic review and meta-analysis. *BMJ*; 362. Epub ahead of print 5 September 2018. DOI: 10.1136/BMJ.K3519.

[39] Hayes JH, Barry MJ. Screening for Prostate Cancer With the Prostate-Specific Antigen Test: A Review of Current Evidence. *JAMA* 2014; 311: 1143–1149.

[40] Mottet N, Bellmunt J, Bolla M, et al. EAU-ESTRO-SIOG Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. *Eur Urol* 2017; 71: 618–629.

[41] Brierley J.D., Gospodarowicz M.K., Wittekind C. TNM Classification of Malignant Tumours, 8 th edition due December 2016. *Union for International Cancer Control* 2017; 1–272.

[42] D'Amico A V., Whittington R, Bruce Malkowicz S, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA* 1998; 280: 969–974.

[43] Gleason DF, Mellinger GT, Ardving LJ. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. *J Urol* 1974; 111: 58–64.

[44] Epstein JI, Egevad L, Amin MB, et al. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. *Am J Surg Pathol* 2016; 40: 244–252.

[45] 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: E87–E99.

[46] D'ELIA C, CERRUTO MA, ClOFFI A, et al. Upgrading and upstaging in prostate cancer: From prostate biopsy to radical prostatectomy. *Mol Clin Oncol* 2014; 2: 1145–1149.

[47] Epstein JI, Feng Z, Trock BJ, et al. Upgrading and downgrading of prostate cancer from biopsy to radical prostatectomy: incidence and predictive factors using the modified Gleason grading system and factoring in tertiary grades. *Eur Urol* 2012; 61: 1019–1024.

[48] Pierorazio PM, Walsh PC, Partin AW, et al. Prognostic Gleason grade grouping: data based on the modified Gleason scoring system. *BJU Int* 2013; 111: 753–760.

[49] Gandaglia G, Ploussard G, Isbarn H, et al. What is the optimal definition of misclassification in patients with very low-risk prostate cancer eligible for active surveillance? Results from a multi-institutional series. *Urol Oncol* 2015; 33: 164.e1-164.e9.

[50] Andriole GL, David Crawford E, Grubb RL, et al. Mortality Results from a Randomized Prostate-Cancer Screening Trial A bs tr ac t. *N Engl J Med* 2009; 18: 1310–1319.

[51] Negoita S, Feuer EJ, Mariotto A, et al. Annual Report to the Nation on the Status of Cancer, part II: Recent changes in prostate cancer trends and disease characteristics. *Cancer* 2018; 124: 2801–2814.

[52] Kohaar I, Petrovics G, Srivastava S. A Rich Array of Prostate Cancer Molecular Biomarkers: Opportunities and Challenges. *Int J Mol Sci*; 20. Epub ahead of print 2 April 2019. DOI: 10.3390/IJMS20081813.

[53] Wei L, Wang J, Lampert E, et al. Intratumoral and Intertumoral Genomic Heterogeneity of Multifocal Localized Prostate Cancer Impacts Molecular Classifications and Genomic Prognosticators. *Eur Urol* 2017; 71: 183–192.

[54] Salami SS, Hovelson DH, Kaplan JB, et al. Transcriptomic heterogeneity in multifocal prostate cancer. *JCI Insight*; 3. Epub ahead of print 2 November 2018. DOI: 10.1172/JCI.INSIGHT.123468.

[55] Balk SP, Ko YJ, Bubley GJ. Biology of prostate-specific antigen. *J Clin Oncol* 2003; 21: 383–391.

[56] Wang MC, Valenzuela LA, Murphy GP, et al. Purification of a Human Prostate Specific Antigen. *Journal of Urology* 2017; 197: S148–S152.

[57] Papsidero L, Wang M, Valenzuela L, et al. A prostate antigen in sera of prostatic cancer patients. *Cancer Res*.

[58] Kuriyama M, Wang MC, Papsidero LD, et al. Quantitation of Prostate-specific Antigen in Serum by a Sensitive Enzyme Immunoassay1, http://aacrjournals.org/cancerres/article-pdf/40/12/4658/2407069/cr0400124658.pdf (accessed 16 April 2023).

[59] Catalona WJ, Smith DS, Ratliff TL, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. *N Engl J Med* 1991; 324: 1156–1161.

[60] Nadler RB, Humphrey PA, Smith DS, et al. Effect of inflammation and benign prostatic hyperplasia on elevated serum prostate specific antigen levels. *J Urol* 1995; 154: 407–413.

[61] Parkes C, Wald NJ, Murphy P, et al. Prospective observational study to assess value of prostate specific antigen as screening test for prostate cancer. *BMJ* 1995; 311: 1340.

[62] Fenton JJ, Weyrich MS, Durbin S, et al. Prostate-Specific Antigen-Based Screening for Prostate Cancer: Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA* 2018; 319: 1914–1931.

[63] Draisma G, Etzioni R, Tsodikov A, et al. Lead time and overdiagnosis in prostate-specific antigen screening: importance of methods and context. *J Natl Cancer Inst* 2009; 101: 374–383.

[64] Klotz L. Prostate cancer overdiagnosis and overtreatment. *Curr Opin Endocrinol Diabetes Obes* 2013; 20: 204–209.

[65] Grossman DC, Curry SJ, Owens DK, et al. Screening for Prostate Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA* 2018; 319: 1901–1913.

[66] Gasinska A, Jaszczynski J, Rychlik U, et al. Prognostic Significance of Serum PSA Level and Telomerase, VEGF and GLUT-1 Protein Expression for the Biochemical Recurrence in Prostate Cancer Patients after Radical Prostatectomy. *Pathology Oncology Research* 2020; 26: 1049.

[67] Martinez MAV, Correa E, Jeurkar C, et al. The prognostic significance of PSA as an indicator of age standardized relative survival: An analysis of the SEER database 2004-2014. *https://doi.org/101200/JCO20183615_suppl.e18768* 2018; 36: e18768–e18768.

[68] Duffy MJ. Biomarkers for prostate cancer: prostate-specific antigen and beyond. *Clin Chem Lab Med* 2020; 58: 326–339.

[69] Benson MC, Ihn Seong Whang, Pantuck A, et al. Prostate specific antigen density: a means of distinguishing benign prostatic hypertrophy and prostate cancer. *J Urol* 1992; 147: 815–816.

[70] Nordström T, Akre O, Aly M, et al. Prostate-specific antigen (PSA) density in the diagnostic algorithm of prostate cancer. *Prostate Cancer Prostatic Dis* 2018; 21: 57–63.

[71] Aminsharifi A, Howard L, Wu Y, et al. Prostate Specific Antigen Density as a Predictor of Clinically Significant Prostate Cancer When the Prostate Specific

Antigen is in the Diagnostic Gray Zone: Defining the Optimum Cutoff Point Stratified by Race and Body Mass Index. *J Urol* 2018; 200: 758–766.

[72] Jue JS, Barboza MP, Prakash NS, et al. Re-examining Prostate-specific Antigen (PSA) Density: Defining the Optimal PSA Range and Patients for Using PSA Density to Predict Prostate Cancer Using Extended Template Biopsy. *Urology* 2017; 105: 123–128.

[73] Sfoungaristos S, Perimenis P. PSA density is superior than PSA and Gleason score for adverse pathologic features prediction in patients with clinically localized prostate cancer. *Can Urol Assoc J* 2012; 6: 46–50.

[74] Jin BS, Kang SH, Kim DY, et al. Pathological upgrading in prostate cancer patients eligible for active surveillance: Does prostate-specific antigen density matter? *Korean J Urol* 2015; 56: 624–629.

[75] Ha YS, Yu J, Salmasi AH, et al. Prostate-specific antigen density toward a better cutoff to identify better candidates for active surveillance. *Urology* 2014; 84: 365–372.

[76] Washington SL, Baskin AS, Ameli N, et al. MRI-Based Prostate-Specific Antigen Density Predicts Gleason Score Upgrade in an Active Surveillance Cohort. *AJR Am J Roentgenol* 2020; 214: 574–578.

[77] Shariat SF, Canto EI, Kattan MW, et al. Beyond Prostate-Specific Antigen: New Serologic Biomarkers for Improved Diagnosis and Management of Prostate Cancer. *Rev Urol* 2004; 6: 58.

[78] Catalona WJ, Partin AW, Slawin KM, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign

prostatic disease: A prospective multicenter clinical trial. *J Am Med Assoc* 1998; 279: 1542–1547.

[79] Partin AW, Brawer MK, Bartsch G, et al. Complexed prostate specific antigen improves specificity for prostate cancer detection: results of a prospective multicenter clinical trial. *J Urol* 2003; 170: 1787–1791.

[80] Piironen T, Pettersson K, Suonpää M, et al. In vitro stability of free prostate-specific antigen (PSA) and prostate-specific antigen (PSA) complexed to alpha 1-antichymotrypsin in blood samples. *Urology* 1996; 48: 81–87.

[81] Ornstein DK, Rao GS, Smith DS, et al. Effect of Digital Rectal Examination and Needle Biopsy on Serum Total and Percentage of Free Prostate Specific Antigen Levels. *J Urol* 1997; 157: 195–198.

[82] The Influence of Prostate Volume on the Ratio of Free to Total Prostate Specific Antigen in Serum of Patients with Prostate Carcinoma and Benign Prostate Hyperplasia. DOI: 10.1002/(SICI)1097-0142(19970101)79:1.

[83] Huang Y, Li ZZ, Huang YL, et al. Value of free/total prostate-specific antigen (f/t PSA) ratios for prostate cancer detection in patients with total serum prostate-specific antigen between 4 and 10 ng/mL: A meta-analysis. *Medicine*; 97. Epub ahead of print 1 March 2018. DOI: 10.1097/MD.0000000000010249.

[84] Oto J, Fernández-Pardo Á, Royo M, et al. A predictive model for prostate cancer incorporating PSA molecular forms and age. *Sci Rep*; 10. Epub ahead of print 1 December 2020. DOI: 10.1038/S41598-020-58836-4.

[85] Ayyildiz SN, Ayyildiz A. PSA, PSA derivatives, proPSA and prostate health index in the diagnosis of prostate cancer. *Turk J Urol* 2014; 40: 82.

[86] Loeb S, Sanda MG, Broyles DL, et al. The Prostate Health Index Selectively Identifies Clinically Significant Prostate Cancer HHS Public Access. *J Urol* 2015; 193: 1163–1169.

[87] Catalona WJ, Partin AW, Sanda MG, et al. A Multi-Center Study of [−2]Pro-Prostate-Specific Antigen (PSA) in Combination with PSA and Free PSA for Prostate Cancer Detection in the 2.0 to 10.0 ng/mL PSA Range. *J Urol* 2011; 185: 1650.

[88] Bruzzese D, Mazzarella C, Ferro M, et al. Prostate health index vs percent free prostate-specific antigen for prostate cancer detection in men with 'gray' prostate-specific antigen levels at first biopsy: systematic review and meta-analysis. *Transl Res* 2014; 164: 444–451.

[89] Ferro M, De Cobelli O, Lucarelli G, et al. Beyond PSA: The role of prostate health index (phi). *International Journal of Molecular Sciences*; 21. Epub ahead of print 2 February 2020. DOI: 10.3390/ijms21041184.

[90] Fossati N, Buffi NM, Haese A, et al. Preoperative prostate-specific antigen isoform p2PSA and its derivatives, %p2PSA and prostate health index, predict pathologic outcomes in patients undergoing radical prostatectomy for prostate cancer: Results from a multicentric european prospective study. *Eur Urol* 2015; 68: 132–138.

[91] Wang W, Wang M, Wang L, et al. Diagnostic ability of %p2PSA and prostate health index for aggressive prostate cancer: a meta-analysis. DOI: 10.1038/srep05012.

[92] Dolejsova O, Kucera R, Fuchsova R, et al. The ability of prostate health index (Phi) to predict gleason score in patients with prostate cancer and discriminate patients between gleason score 6 and gleason score higher than 6—a study on

320 patients after radical prostatectomy. *Technol Cancer Res Treat*; 17. Epub ahead of print 2018. DOI: 10.1177/1533033818787377.

[93] Ferro M, Crocetto F, Bruzzese D, et al. Prostate Health Index and Multiparametric MRI: Partners in Crime Fighting Overdiagnosis and Overtreatment in Prostate Cancer. *Cancers 2021, Vol 13, Page 4723* 2021; 13: 4723.

[94] Gentile F, La Civita E, Della Ventura B, et al. A Combinatorial Neural Network Analysis Reveals a Synergistic Behaviour of Multiparametric Magnetic Resonance and Prostate Health Index in the Identification of Clinically Significant Prostate Cancer. *Clin Genitourin Cancer* 2022; 20: e406–e410.

[95] White J, Shenoy BV, Tutrone RF, et al. Clinical utility of the Prostate Health Index (phi) for biopsy decision management in a large group urology practice setting. *Prostate Cancer Prostatic Dis* 2018; 21: 78–84.

[96] Huang D, Yang X, Wu Y, et al. Cost-Effectiveness Analysis of Prostate Health Index in Decision Making for Initial Prostate Biopsy. *Front Oncol*; 10. Epub ahead of print 24 November 2020. DOI: 10.3389/FONC.2020.565382.

[97] Teoh JYC, Leung CH, Wang MH, et al. The cost-effectiveness of prostate health index for prostate cancer detection in Chinese men. *Prostate Cancer Prostatic Dis* 2020; 23: 615–621.

[98] Maxeiner A, Kilic E, Matalon J, et al. The prostate health index PHI predicts oncological outcome and biochemical recurrence after radical prostatectomy - analysis in 437 patients. *Oncotarget* 2017; 8: 79279–79288.

[99] Lughezzani G, Lazzeri M, Buffi NM, et al. Preoperative prostate health index is an independent predictor of early biochemical recurrence after radical

prostatectomy: Results from a prospective single-center study. *Urologic Oncology: Seminars and Original Investigations* 2015; 33: 337.e7-337.e14.

[100] Lepor A, Catalona WJ, Loeb S. The Prostate Health Index: Its Utility In Prostate Cancer Detection. DOI: 10.1016/j.ucl.2015.08.001.

[101] Roobol MJ, Vedder MM, Nieboer D, et al. Comparison of Two Prostate Cancer Risk Calculators that Include the Prostate Health Index. *Eur Urol Focus* 2015; 1: 185–190.

[102] Semjonow A, Köpke T, Eltze E, et al. Pre-analytical in-vitro stability of [-2]proPSA in blood and serum. *Clin Biochem* 2010; 43: 926–928.

[103] Vickers AJ, Cronin AM, Aus G, et al. A panel of kallikrein markers can reduce unnecessary biopsy for prostate cancer: data from the European Randomized Study of Prostate Cancer Screening in Göteborg, Sweden. *BMC Med*; 6. Epub ahead of print 8 July 2008. DOI: 10.1186/1741-7015-6-19.

[104] Parekh DJ, Punnen S, Sjoberg DD, et al. A Multi-institutional Prospective Trial in the USA Confirms that the 4Kscore Accurately Identifies Men with High-grade Prostate Cancer. *Eur Urol* 2015; 68: 464–470.

[105] Bryant RJ, Sjoberg DD, Vickers AJ, et al. Predicting High-Grade Cancer at Ten-Core Prostate Biopsy Using Four Kallikrein Markers Measured in Blood in the ProtecT Study. *JNCI J Natl Cancer Inst* 2015; 107: 95.

[106] Nordström T, Vickers A, Assel M, et al. Comparison between the four-kallikrein panel and Prostate Health Index (PHI) for predicting prostate cancer. DOI: 10.1016/j.eururo.2014.08.010.

[107] Verbeek JFM, Bangma CH, Kweldam CF, et al. Reducing unnecessary biopsies while detecting clinically significant prostate cancer including cribriform growth

with the ERSPC Rotterdam risk calculator and 4Kscore. *Urologic Oncology: Seminars and Original Investigations* 2019; 37: 138–144.

[108] Marzouk K, Ehdaie B, Vertosick E, et al. Developing an effective strategy to improve the detection of significant prostate cancer by combining the 4Kscore and multiparametric MRI. *Urol Oncol* 2019; 37: 672.

[109] Punnen S, Nahar B, Prakash NS, et al. The 4Kscore Predicts the Grade and Stage of Prostate Cancer in the Radical Prostatectomy Specimen: Results from a Multi-institutional Prospective Trial. *Eur Urol Focus* 2017; 3: 94–99.

[110] Zappala SM, Dong Y, Linder V, et al. The 4Kscore blood test accurately identifies men with aggressive prostate cancer prior to prostate biopsy with or without DRE information. *Int J Clin Pract*; 71. Epub ahead of print 1 June 2017. DOI: 10.1111/IJCP.12943.

[111] Gupta A, Roobol MJ, Savage CJ, et al. A four-kallikrein panel for the prediction of repeat prostate biopsy: data from the European Randomized Study of Prostate Cancer screening in Rotterdam, Netherlands. *Br J Cancer* 2010; 103: 708–714.

[112] Falagario UG, Martini A, Wajswol E, et al. Avoiding Unnecessary Magnetic Resonance Imaging (MRI) and Biopsies: Negative and Positive Predictive Value of MRI According to Prostate-specific Antigen Density, 4Kscore and Risk Calculators. *Eur Urol Oncol* 2020; 3: 700–704.

[113] Konety B, Zappala SM, Parekh DJ, et al. The 4Kscore® Test Reduces Prostate Biopsy Rates in Community and Academic Urology Practices. *Rev Urol* 2015; 17: 231.

[114] Voigt JD, Dong Y, Linder V, et al. Use of the 4Kscore test to predict the risk of aggressive prostate cancer prior to prostate biopsy: Overall cost savings and improved quality of care to the us healthcare system. *Rev Urol* 2017; 19: 1–10.

[115] Voigt JD, Zappala SM, Vaughan ED, et al. The Kallikrein Panel for prostate cancer screening: its economic impact. *Prostate* 2014; 74: 250–259.

[116] Stattin P, Vickers AJ, Sjoberg DD, et al. Improving the Specificity of Screening for Lethal Prostate Cancer Using Prostate-specific Antigen and a Panel of Kallikrein Markers: A Nested Case-Control Study HHS Public Access. *Eur Urol* 2015; 68: 207–213.

[117] Haese A, Tin AL, Carlsson S V., et al. A pre-specified model based on four kallikrein markers in blood improves predictions of adverse pathology and biochemical recurrence after radical prostatectomy. *Br J Cancer* 2020; 123: 604–609.

[118] Van Neste L, Hendriks RJ, Dijkstra S, et al. Detection of High-grade Prostate Cancer Using a Urinary Molecular Biomarker-Based Risk Score. *Eur Urol* 2016; 70: 740–748.

[119] Leyten GHJM, Hessels D, Smit FP, et al. Identification of a Candidate Gene Panel for the Early Diagnosis of Prostate Cancer. *Clin Cancer Res* 2015; 21: 3061–3070.

[120] Haese A, Trooskens G, Steyaert S, et al. Multicenter Optimization and Validation of a 2-Gene mRNA Urine Test for Detection of Clinically Significant Prostate Cancer before Initial Prostate Biopsy. *J Urol* 2019; 202: 256–262.

[121] Hoyer G, Crawford ED, Arangua P, et al. SelectMDx versus Prostate Health Index in the identification of high-grade prostate cancer. *https://doi.org/101200/JCO2019377_suppl30* 2019; 37: 30–30.

[122] Maggi M, Giudice F Del, Falagario UG, et al. SelectMDx and Multiparametric Magnetic Resonance Imaging of the Prostate for Men Undergoing Primary Prostate Biopsy: A Prospective Assessment in a Multi-Institutional Study. *Cancers (Basel)* 2021; 13: 2047.

[123] Morote J, Díaz F, Celma A, et al. Behavior of SelectMDx and Prostate-specific Antigen Density in the Challenging Scenario of Prostate Imaging-Reporting and Data System Category 3 Lesions. *Eur Urol* 2022; 81: 124–125.

[124] Hendriks RJ, van der Leest MMG, Israël B, et al. Clinical use of the SelectMDx urinary-biomarker test with or without mpMRI in prostate cancer diagnosis: a prospective, multicenter study in biopsy-naïve men. *Prostate Cancer Prostatic Dis* 2021; 24: 1110–1119.

[125] Bussemakers MJG, Van Bokhoven A, Verhaegh GW, et al. DD3: a new prostate-specific gene, highly overexpressed in prostate cancer. *Cancer Res* 1999; 59: 5975–5979.

[126] de Kok JB, Verhaegh GW, Roelofs RW, et al. *DD3 PCA3 , a Very Sensitive and Specific Marker to Detect Prostate Tumors*. 2002.

[127] Hessels D, Klein Gunnewiek JMT, Van Oort I, et al. DD3PCA3-based molecular urine analysis for the diagnosis of prostate cancer. *Eur Urol* 2003; 44: 8–16.

[128] Nakanishi H, Groskopf J, Fritsche HA, et al. PCA3 molecular urine assay correlates with prostate cancer tumor volume: implication in selecting candidates for active surveillance. *J Urol* 2008; 179: 1804–1810.

[129] Groskopf J, Aubin SMJ, Deras IL, et al. APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. *Clin Chem* 2006; 52: 1089–1095.

[130] Leyten GHJM, Hessels D, Jannink SA, et al. Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. *Eur Urol* 2014; 65: 534–542.

[131] Marks LS, Fradet Y, Lim Deras I, et al. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology* 2007; 69: 532–535.

[132] Muñoz Rodríguez SV, García-Perdomo HA. Diagnostic accuracy of prostate cancer antigen 3 (PCA3) prior to first prostate biopsy: A systematic review and meta-analysis. *Can Urol Assoc J*; 14. Epub ahead of print 2020. DOI: 10.5489/CUAJ.6008.

[133] Wei JT, Feng Z, Partin AW, et al. Can urinary PCA3 supplement PSA in the early detection of prostate cancer? *J Clin Oncol* 2014; 32: 4066–4072.

[134] Pepe P, Aragona F. Materials and Methods PCA3 Score vs PSA Free/Total Accuracy in Prostate Cancer Diagnosis at Repeat Saturation Biopsy.

[135] Auprich M, Augustin H, Budäus L, et al. A comparative performance analysis of total prostate-specific antigen, percentage free prostate-specific antigen, prostate-specific antigen velocity and urinary prostate cancer gene 3 in the first, second and third repeat prostate biopsy. *BJU Int* 2012; 109: 1627–1635.

[136] Deras IL, Aubin SMJ, Blase A, et al. PCA3: a molecular urine assay for predicting prostate biopsy outcome. *J Urol* 2008; 179: 1587–1592.

[137] Leyten GHJM, Wierenga EA, Michiel Sedelaar, et al. Value of PCA3 to predict biopsy outcome and its potential role in selecting patients for multiparametric MRI. *Int J Mol Sci* 2013; 14: 11347–11355.

[138] De Luca S, Passera R, Cattaneo G, et al. High prostate cancer gene 3 (PCA3) scores are associated with elevated Prostate Imaging Reporting and Data System (PI-RADS) grade and biopsy Gleason score, at magnetic resonance

imaging/ultrasonography fusion software-based targeted prostate biopsy after a previous negative standard biopsy. *BJU Int* 2016; 118: 723–730.

[139] Kaufmann S, Bedke J, Gatidis S, et al. Prostate cancer gene 3 (PCA3) is of additional predictive value in patients with PI-RADS grade III (intermediate) lesions in the MR-guided re-biopsy setting for prostate cancer. *World J Urol* 2016; 34: 509–515.

[140] Htoo KPP, Yamkamon V, Yainoy S, et al. Colorimetric detection of PCA3 in urine for prostate cancer diagnosis using thiol-labeled PCR primer and unmodified gold nanoparticles. *Clin Chim Acta* 2019; 488: 40–49.

[141] Fu X, Wen J, Li J, et al. Highly sensitive detection of prostate cancer specific PCA3 mimic DNA using SERS-based competitive lateral flow assay. *Nanoscale* 2019; 11: 15530–15536.

[142] Soares JC, Soares AC, Rodrigues VC, et al. Detection of the Prostate Cancer Biomarker PCA3 with Electrochemical and Impedance-Based Biosensors. *ACS Appl Mater Interfaces* 2019; 11: 46645–46650.

[143] Yamkamon V, Phyu Pyar Htoo K, Yainoy S, et al. Urinary PCA3 detection in prostate cancer by magnetic nanoparticles coupled with colorimetric enzyme-linked oligonucleotide assay. *EXCLI J* 2020; 19: 501–513.

[144] Van Poppel H, Haese A, Graefen M, et al. The relationship between Prostate CAncer gene 3 (PCA3) and prostate cancer significance. *BJU Int* 2012; 109: 360–366.

[145] Haese A, de la Taille A, van Poppel H, et al. Clinical Utility of the PCA3 Urine Assay in European Men Scheduled for Repeat Biopsy. *Eur Urol* 2008; 54: 1081–1088.

[146] Chunhua L, Zhao H, Zhao H, et al. Clinical Significance of Peripheral Blood PCA3 Gene Expression in Early Diagnosis of Prostate Cancer. *Transl Oncol* 2018; 11: 628–632.

[147] Tosoian JJ, Patel HD, Mamawala M, et al. Longitudinal assessment of urinary PCA3 for predicting prostate cancer grade reclassification in favorable-risk men during active surveillance. *Prostate Cancer Prostatic Dis* 2017; 20: 339–342.

[148] Merola R, Tomao L, Antenucci A, et al. PCA3 in prostate cancer and tumor aggressiveness detection on 407 high-risk patients: A National Cancer Institute experience. *Journal of Experimental and Clinical Cancer Research* 2015; 34: 1–6.

[149] Chevli KK, Duff M, Walter P, et al. Urinary PCA3 as a predictor of prostate cancer in a cohort of 3,073 men undergoing initial prostate biopsy. *J Urol* 2014; 191: 1743–1748.

[150] Foj L, Milà M, Mengual L, et al. Real-time PCR PCA3 assay is a useful test measured in urine to improve prostate cancer detection. *Clin Chim Acta* 2014; 435: 53–58.

[151] Hessels D, Van Gils MPMQ, Van Hooij O, et al. Predictive value of PCA3 in urinary sediments in determining clinico-pathological characteristics of prostate cancer. *Prostate* 2010; 70: 10–16.

[152] Scattoni V, Lazzeri M, Lughezzani G, et al. Head-to-Head Comparison of Prostate Health Index and Urinary PCA3 for Predicting Cancer at Initial or Repeat Biopsy. *J Urol* 2013; 190: 496–501.

[153] Cantiello F, Russo GI, Ferro M, et al. Prognostic accuracy of Prostate Health Index and urinary Prostate Cancer Antigen 3 in predicting pathologic features after radical prostatectomy. *Urol Oncol* 2015; 33: 163.e15-163.e23.

[154] Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science (1979)* 2005; 310: 644–648.

[155] Fine SW, Gopalan A, Leversha MA, et al. TMPRSS2-ERG gene fusion is associated with low Gleason scores and not with high grade morphologic features. *Mod Pathol* 2010; 23: 1325–1333.

[156] Tomlins SA, Day JR, Lonigro RJ, et al. Urine TMPRSS2:ERG Plus PCA3 for Individualized Prostate Cancer Risk Assessment HHS Public Access. *Eur Urol* 2016; 70: 45–53.

[157] Ferro M, Bruzzese D, Perdonà S, et al. Prostate Health Index (Phi) and Prostate Cancer Antigen 3 (PCA3) Significantly Improve Prostate Cancer Detection at Initial Biopsy in a Total PSA Range of 2-10 ng/ml. *PLoS One* 2013; 8: 67687.

[158] Tosoian JJ, Trock BJ, Morgan TM, et al. Use of the MyProstateScore Test to Rule Out Clinically Significant Cancer: Validation of a Straightforward Clinical Testing Approach. *J Urol* 2021; 205: 732–739.

[159] Tosoian JJ, Singhal U, Davenport MS, et al. Urinary MyProstateScore (MPS) to Rule out Clinically-Significant Cancer in Men with Equivocal (PI-RADS 3) Multiparametric MRI: Addressing an Unmet Clinical Need. *Urology* 2022; 164: 184–190.

[160] McKiernan J, Noerholm M, Tadigotla V, et al. A urine-based Exosomal gene expression test stratifies risk of high-grade prostate Cancer in men with prior negative prostate biopsy undergoing repeat biopsy. *BMC Urol*; 20. Epub ahead of print 1 September 2020. DOI: 10.1186/S12894-020-00712-4.

[161] Donovan MJ, Noerholm M, Bentink S, et al. A molecular signature of PCA3 and ERG exosomal RNA from non-DRE urine is predictive of initial prostate biopsy result. *Prostate Cancer Prostatic Dis* 2015; 18: 370–375.

[162] Grönberg H, Adolfsson J, Aly M, et al. Prostate cancer screening in men aged 50-69 years (STHLM3): a prospective population-based diagnostic study. *Lancet Oncol* 2015; 16: 1667–1676.

[163] Nordström T, Discacciati A, Bergman M, et al. Prostate cancer screening using a combination of risk-prediction, MRI, and targeted prostate biopsies (STHLM3-MRI): a prospective, population-based, randomised, open-label, non-inferiority trial. *Lancet Oncol* 2021; 22: 1240–1249.

[164] Risør BW, Tayyari Dehbarez N, Fredsøe J, et al. Cost-Effectiveness Analysis of Stockholm 3 Testing Compared to PSA as the Primary Blood Test in the Prostate Cancer Diagnostic Pathway: A Decision Tree Approach. *Appl Health Econ Health Policy* 2022; 20: 867–880.

[165] Knezevic D, Goddard AD, Natraj N, et al. Analytical validation of the Oncotype DX prostate cancer assay - a clinical RT-PCR assay optimized for prostate needle biopsies. *BMC Genomics*; 14. Epub ahead of print 8 October 2013. DOI: 10.1186/1471-2164-14-690.

[166] Cullen J, Rosner IL, Brand TC, et al. A Biopsy-based 17-gene Genomic Prostate Score Predicts Recurrence After Radical Prostatectomy and Adverse Surgical Pathology in a Racially Diverse Population of Men with Clinically Low- and Intermediate-risk Prostate Cancer. *Eur Urol* 2015; 68: 123–131.

[167] Albala D, Kemeter MJ, Febbo PG, et al. Health Economic Impact and Prospective Clinical Utility of Oncotype DX® Genomic Prostate Score. *Rev Urol* 2016; 18: 123.

[168] Warf B. Analytical Validation of a Cell Cycle Progression Signature Used as a Prognostic Marker in Prostate Cancer. *J Mol Biomark Diagn* 2015; 6: 239.

[169] Cuzick J, Swanson GP, Fisher G, et al. Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study. *Lancet Oncol* 2011; 12: 245–55.

[170] Freedland SJ, Gerber L, Reid J, et al. Prognostic utility of cell cycle progression score in men with prostate cancer after primary external beam radiation therapy. *Int J Radiat Oncol Biol Phys* 2013; 86: 848–853.

[171] Erho N, Crisan A, Vergara IA, et al. Discovery and validation of a prostate cancer genomic classifier that predicts early metastasis following radical prostatectomy. *PLoS One*; 8. Epub ahead of print 24 June 2013. DOI: 10.1371/JOURNAL.PONE.0066855.

[172] Klein EA, Haddad Z, Yousefi K, et al. Decipher Genomic Classifier Measured on Prostate Biopsy Predicts Metastasis Risk. *Urology* 2016; 90: 148–152.

[173] Nguyen PL, Shin H, Yousefi K, et al. Impact of a Genomic Classifier of Metastatic Risk on Postprostatectomy Treatment Recommendations by Radiation Oncologists and Urologists. *Urology* 2015; 86: 35–40.

[174] Macagno A, Athanasiou A, Wittig A, et al. Analytical performance of thrombospondin-1 and cathepsin D immunoassays part of a novel CE-IVD marked test as an aid in the diagnosis of prostate cancer. *PLoS One* 2020; 15: e0233442.

[175] Steuber T, Tennstedt P, Macagno A, et al. Thrombospondin 1 and cathepsin D improve prostate cancer diagnosis by avoiding potentially unnecessary prostate biopsies. *BJU Int* 2019; 123: 826–833.

[176] Klocker H, Golding B, Weber S, et al. Development and validation of a novel multivariate risk score to guide biopsy decision for the diagnosis of clinically significant prostate cancer. *BJUI Compass* 2020; 1: 15–20.

[177] Schiess R, Wollscheid B, Aebersold R. Targeted proteomic strategy for clinical biomarker discovery. *Mol Oncol* 2009; 3: 33–44.

[178] Pourmand G, Ziaee A-A, Reza Abedi A, et al. Role of PTEN Gene in Progression of Prostate Cancer. *Urological Oncology 95 Urology Journal* 2007; 4: 95–100.

[179] Cima I, Schiess R, Wild P, et al. Cancer genetics-guided discovery of serum biomarker signatures for diagnosis and prognosis of prostate cancer. *Proc Natl Acad Sci U S A* 2011; 108: 3342–3347.

[180] Endt K, Goepfert J, Omlin A, et al. Development and clinical testing of individual immunoassays for the quantification of serum glycoproteins to diagnose prostate cancer. *PLoS One* 2017; 12: e0181557.

[181] Steuber T, Heidegger I, Kafka M, et al. PROPOSe: A Real-life Prospective Study of Proclarix, a Novel Blood-based Test to Support Challenging Biopsy Decision-making in Prostate Cancer. *Eur Urol Oncol* 2022; 5: 321–327.

[182] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; 68: 394–424.

[183] Hoffman RM. Screening for Prostate Cancer. *New England Journal of Medicine* 2011; 365: 2013–2019.

[184] Loeb S, Roehl KA, Antenor JA V., et al. Baseline prostate-specific antigen compared with median prostate-specific antigen for age group as predictor of

prostate cancer risk in men younger than 60 years old. *Urology* 2006; 67: 316–320.

[185] Catalona WJ, Partin AW, Slawin KM, et al. Use of the percentage of free prostate-specific antigen to enhance differentiation of prostate cancer from benign prostatic disease: A prospective multicenter clinical trial. *J Am Med Assoc* 1998; 279: 1542–1547.

[186] Mazzone E, Stabile A, Pellegrino F, et al. Positive Predictive Value of Prostate Imaging Reporting and Data System Version 2 for the Detection of Clinically Significant Prostate Cancer: A Systematic Review and Meta-analysis. *Eur Urol Oncol* 2021; 4: 697–713.

[187] Polanec SH, Bickel H, Wengert GJ, et al. Can the addition of clinical information improve the accuracy of PI-RADS version 2 for the diagnosis of clinically significant prostate cancer in positive MRI? *Clin Radiol* 2020; 75: 157.e1-157.e7.

[188] Sathianathen NJ, Omer A, Harriss E, et al. Negative Predictive Value of Multiparametric Magnetic Resonance Imaging in the Detection of Clinically Significant Prostate Cancer in the Prostate Imaging Reporting and Data System Era: A Systematic Review and Meta-analysis. *Eur Urol* 2020; 78: 402–414.

[189] Morote J, Celma A, Diaz F, et al. Prostatic-specific antigen density behavior according to multiparametric magnetic resonance imaging result. *Urol Oncol* 2020; 38: 410–417.

[190] Osses DF, Roobol MJ, Schoots IG. Prediction Medicine: Biomarkers, Risk Calculators and Magnetic Resonance Imaging as Risk Stratification Tools in Prostate Cancer Diagnosis. *Int J Mol Sci*; 20. Epub ahead of print 1 April 2019. DOI: 10.3390/IJMS20071637.

[191] Osses DF, Roobol MJ, Schoots IG. Molecular Sciences Prediction Medicine: Biomarkers, Risk Calculators and Magnetic Resonance Imaging as Risk Stratification Tools in Prostate Cancer Diagnosis. DOI: 10.3390/ijms20071637.

[192] Schoots IG. MRI in early prostate cancer detection: how to manage indeterminate or equivocal PI-RADS 3 lesions? *Transl Androl Urol* 2018; 7: 70–82.

[193] Lu Z, Kim W, Wilbur WJ. Evaluation of query expansion using MeSH in PubMed. *Inf Retr Boston* 2009; 12: 69–80.

[194] Epstein JI, Walsh PC, Carmichael M, et al. Pathologic and Clinical Findings to Predict Tumor Extent of Nonpalpable (Stage T1 c) Prostate Cancer. *JAMA* 1994; 271: 368–374.

[195] Ahmed HU, Hu Y, Carter T, et al. Characterizing clinically significant prostate cancer using template prostate mapping biopsy. *Journal of Urology* 2011; 186: 458–464.

[196] Epstein JI, Zelefsky MJ, Sjoberg DD, et al. A Contemporary Prostate Cancer Grading System: A Validated Alternative to the Gleason Score. *Eur Urol* 2016; 69: 428.

[197] Rouvière O, Puech P, Renard-Penna R, et al. Use of prostate systematic and targeted biopsy on the basis of multiparametric MRI in biopsy-naive patients (MRI-FIRST): a prospective, multicentre, paired diagnostic study. *Lancet Oncol* 2019; 20: 100–109.

[198] Alchin DR, Murphy D, Lawrentschuk N. Risk factors for Gleason Score upgrading following radical prostatectomy. *Minerva Urologica e Nefrologica* 2017; 69: 459–465.

[199] Morote J, Campistol M, Celma A, et al. The Efficacy of Proclarix to Select Appropriate Candidates for Magnetic Resonance Imaging and Derived Prostate Biopsies in Men with Suspected Prostate Cancer. Epub ahead of print 2022. DOI: 10.5534/wjmh.210117.

[200] Morote J, Campistol M, Celma A, et al. The Efficacy of Proclarix to Select Appropriate Candidates for Magnetic Resonance Imaging and Derived Prostate Biopsies in Men with Suspected Prostate Cancer. *World J Mens Health*; 40. Epub ahead of print 27 December 2021. DOI: 10.5534/WJMH.210117.

[201] Mottet N, van den Bergh RCN, Briers E, et al. EAU-EANM-ESTRO-ESUR-SIOG Guidelines on Prostate Cancer—2020 Update. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. *Eur Urol* 2021; 79: 243–262.

[202] Moldovan PC, Van den Broeck T, Sylvester R, et al. What Is the Negative Predictive Value of Multiparametric Magnetic Resonance Imaging in Excluding Prostate Cancer at Biopsy? A Systematic Review and Meta-analysis from the European Association of Urology Prostate Cancer Guidelines Panel. *Eur Urol* 2017; 72: 250–266.

[203] Schoots IG. MRI in early prostate cancer detection: how to manage indeterminate or equivocal PI-RADS 3 lesions? *Transl Androl Urol* 2018; 7: 70.

[204] Görtz M, Radtke JP, Hatiboglu G, et al. The Value of Prostate-specific Antigen Density for Prostate Imaging-Reporting and Data System 3 Lesions on Multiparametric Magnetic Resonance Imaging: A Strategy to Avoid Unnecessary Prostate Biopsies. *Eur Urol Focus* 2021; 7: 325–331.

[205] Vickers AJ, Russo G, Lilja H, et al. How Should Molecular Markers and Magnetic Resonance Imaging Be Used in the Early Detection of Prostate Cancer? *Eur Urol Oncol* 2022; 5: 135–137.

[206] Fan YH, Pan PH, Cheng WM, et al. The Prostate Health Index aids multi-parametric MRI in diagnosing significant prostate cancer. *Sci Rep*; 11. Epub ahead of print 1 December 2021. DOI: 10.1038/S41598-020-78428-6.

[207] Porpiglia F, Russo F, Manfredi M, et al. The roles of multiparametric magnetic resonance imaging, PCA3 and prostate health index-which is the best predictor of prostate cancer after a negative biopsy? *J Urol* 2014; 192: 60–66.

[208] Gnanapragasam VJ, Burling K, George A, et al. The Prostate Health Index adds predictive value to multi-parametric MRI in detecting significant prostate cancers in a repeat biopsy population. *Sci Rep*; 6. Epub ahead of print 17 October 2016. DOI: 10.1038/SREP35364.

[209] Punnen S, Nahar B, Soodana-Prakash N, et al. Optimizing patient's selection for prostate biopsy: A single institution experience with multi-parametric MRI and the 4Kscore test for the detection of aggressive prostate cancer. *PLoS One* 2018; 13: e0201384.

[210] Wagaskar VG, Sobotka S, Ratnani P, et al. A 4K score/MRI-based nomogram for predicting prostate cancer, clinically significant prostate cancer, and unfavorable prostate cancer. *Cancer Rep (Hoboken)*; 4. Epub ahead of print 1 August 2021. DOI: 10.1002/CNR2.1357.

[211] Maggi M, Giudice F Del, Falagario UG, et al. SelectMDx and Multiparametric Magnetic Resonance Imaging of the Prostate for Men Undergoing Primary

Prostate Biopsy: A Prospective Assessment in a Multi-Institutional Study. *Cancers (Basel)* 2021; 13: 2047.

[212] Terracciano D, La Civita E, Athanasiou A, et al. New strategy for the identification of prostate cancer: The combination of Proclarix and the prostate health index. *Prostate* 2022; 82: 1469–1476.

[213] Morote J, Campistol M, Triquell M, et al. Improving the Early Detection of Clinically Significant Prostate Cancer in Men in the Challenging Prostate Imaging-Reporting and Data System 3 Category. *Eur Urol Open Sci* 2022; 37: 38–44.

[214] Boesen L, Nørgaard N, Løgager V, et al. Prebiopsy Biparametric Magnetic Resonance Imaging Combined with Prostate-specific Antigen Density in Detecting and Ruling out Gleason 7–10 Prostate Cancer in Biopsy-naïve Men. *Eur Urol Oncol*; 2. Epub ahead of print 2019. DOI: 10.1016/j.euo.2018.09.001.

[215] Boschheidgen M, Schimmöller L, Arsov C, et al. MRI grading for the prediction of prostate cancer aggressiveness. *Eur Radiol*. Epub ahead of print 2021. DOI: 10.1007/s00330-021-08332-8.

[216] Morote J, Celma A, Roche S, et al. Who Benefits from Multiparametric Magnetic Resonance Imaging After Suspicion of Prostate Cancer? *Eur Urol Oncol* 2019; 2: 664–669.

[217] Osses DF, Roobol MJ, Schoots IG. Prediction Medicine: Biomarkers, Risk Calculators and Magnetic Resonance Imaging as Risk Stratification Tools in Prostate Cancer Diagnosis. *Int J Mol Sci*; 20. Epub ahead of print 1 April 2019. DOI: 10.3390/IJMS20071637.

[218] Morote J, Pye H, Campistol M, et al. Accurate diagnosis of prostate cancer by combining Proclarix with magnetic resonance imaging. *BJU Int*. Epub ahead of print 21 March 2023. DOI: 10.1111/BJU.15998.

[219] Morote J, Celma A, Planas J. Are Multiparametric Magnetic Resonance Imaging and Guided Biopsies Needed in Men with Normal Digital Rectal Examination and Prostatic-specific Antigen >20 ng/ml? *Eur Urol Oncol* 2021; 4: 334–335.

[220] Lazzeri M, Haese A, De La Taille A, et al. Serum isoform [-2]proPSA derivatives significantly improve prediction of prostate cancer at initial biopsy in a total PSA range of 2-10 ng/ml: A multicentric european study. *Eur Urol* 2013; 63: 986–994.

[221] Carlsson S, Maschino A, Schröder F, et al. Predictive value of four kallikrein markers for pathologically insignificant compared with aggressive prostate cancer in radical prostatectomy specimens: results from the European Randomized Study of Screening for Prostate Cancer section Rotterdam. *Eur Urol* 2013; 64: 693–699.

11. APPENDICES

11.1. APPENDIX 1: CEIC APPROVAL

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INFORME DEL COMITÉ ÉTICO DE INVESTIGACIÓN CLÍNICA Y COMISIÓN DE PROYECTOS DE INVESTIGACIÓN DEL HOSPITAL UNIVERSITARI VALL D'HEBRON

Doña Mireia Navarro Sebastián, Secretaria del Comité Ético de Investigación Clínica del Hospital Universitari Vall d'Hebron,

CERTIFICA

Que el Comité Ético de Investigación Clínica del Hospital Universitario Vall d'Hebron, en el cual la Comisión de proyectos de investigación está integrada, se reunió en sesión ordinaria nº 232 el pasado 29/05/2015 y evaluó el proyecto de investigación PR(AG)96/2015, con fecha 01/03/2015, titulado "*An integrated approach to solve clinical needs in aggressive prostate cancer: identification of markers for the early detection, predictors of resistance and targets for the recurrent disease.Un enfoque integrado para resolver las necesidades clínicas del cáncer de próstata agresivo: identificación de marcadores para la detección precoz, predictores de resistencia y dianas para la enfermedad recurrente.*" que tiene como investigador principal al Dr. Joan Morote Robles del Servicio de Urología de nuestro Centro.

El resultado de la evaluación fue el siguiente:

DICTAMEN FAVORABLE

El Comité tanto en su composición como en los PNT cumple con las normas de BPC (CPMP/ICH/135/95) y con el Real Decreto 223/2004, y su composición actual es la siguiente:

Presidenta: Gallego Melcón, Soledad. Médico
Vicepresidente: Segarra Sarries, Joan. Abogado

Secretaria: Navarro Sebastián, Mireia. Química
Vocales: Armadans Gil, Lluís. Médico
Azpiroz Vidaur, Fernando. Médico
Corona Pérez-Cardona, Pablo. Médico
Cucurull Folguera, Esther. Médico Farmacóloga
Latorre Arteche, Francisco. Médico
De Torres Ramírez, Inés M. Médico
Fernández Liz, Eladio. Farmacéutico de Atención Primaria
Ferreira González, Ignacio. Médico
Fuentelsaz Gallego, Carmen. Diplomada Enfermería
Fuentes Camps, Inmaculada. Médico Farmacóloga
Guardia Massó, Jaume. Médico
Hortal Ibarra, Juan Carlos. Profesor de Universidad de Derecho
Montoro Ronsano, J. Bruno. Farmacéutico Hospital
Rodríguez Gallego, Alexis. Médico Farmacólogo
Sánchez Raya, Judith. Médico
Solé Orsola, Marta. Diplomada Enfermería
Suñé Martín, Pilar. Farmacéutica Hospital
Vargas Blasco, Víctor, Médico
Vilca Yengle, Luz Mª. Médico

En dicha reunión del Comité Ético de Investigación Clínica se cumplió el quórum preceptivo legalmente.

En el caso de que se evalúe algún proyecto del que un miembro sea investigador/colaborador, éste se ausentará de la reunión durante la discusión del proyecto.

Lo que firmo en Barcelona a 29 de mayo de 2015

MIREIA NAVARRO SEBASTIAN

Firmado digitalmente por MIREIA NAVARRO SEBASTIAN
Nombre de reconocimiento (DN): c=ES, ou=Vegeu https://www.catcert.cat/verCIC (c)03, ou=Serveis Públics de Certificació CPISA-2, sn=NAVARRO SEBASTIAN, givenName=MIREIA, serialNumber=38121226Z, cn=MIREIA NAVARRO SEBASTIAN
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Sra. Mireia Navarro

Secretaria CEIC

11.2. APPENDIX 2: MATERIAL TRANSFER AGREEMENT

MATERIAL TRANSFER AGREEMENT

This Material Transfer Agreement (hereinafter, the "**Agreement**"), effective as of February 15, 2020 (hereinafter referred to as the "**Effective Date**"), is made by and between:

FUNDACIÓ HOSPITAL UNIVERSITARI VALL D'HEBRON - INSTITUT DE RECERCA (hereinafter referred to as "**VHIR**"), a non-for-profit Spanish foundation with tax identification number G-60594009, based in Barcelona, Spain, Passeig Vall d'Hebron, 119-129, Mediterranea Building, 2nd floor, represented by Dr. Joan X. Comella Carnicé, acting in his capacity as Director;

HOSPITAL UNIVERSITARI VALL D'HEBRON (hereinafter referred to as "**HUVH**"), with address in Passeig Vall d'Hebron 119-129, Barcelona (08035) represented by Dr. Albert Salazar I Soler, acting as HUVH Manager

VHIR and HUVH are collectively referred to hereinafter as "**Provider**".

and

ProteoMediX AG (herein after referred to as "**COMPANY**" or "**RECIPIENT**"), a corporation with tax number CHE-115.542.635 and, with a corporate address of Wagistrasse 23, 8952 Schlieren, Switzerland and represented by Dr. Ralph Schiess acting in his capacity as CSO.

VHIR, HUVH and COMPANY are collectively referred to hereinafter as the "**Parties**" and individually as a "**Party**".

RECITALS

I. **WHEREAS**, VHIR's overall purpose is to support, promote, and foster research, scientific and technological knowledge, teaching, and training within the context of the Hospital Universitari Vall d'Hebron and its areas of influence, and the Universitat Autònoma de Barcelona.

II. **WHEREAS** VHIR, through Prof. Juan Morote, investigator of VHIR's Unit of Urology, has title to certain materials including the know-how and all proprietary information in relation thereto, which are described in **Appendix 1** of the present document (hereinafter referred to as the "**Material**").

III. **WHEREAS** Recipient wishes to obtain from Provider, and Provider wishes to provide the Material for the sole purpose of carrying out the project described in **Appendix 2** (hereinafter referred to as, the "**Project**").

NOW THEREFORE, in consideration of the mutual promises, covenants and conditions set forth herein, the Parties agree as follows:

1. PURPOSE OF THE AGREEMENT

1.1 The purpose of this Agreement is to establish the terms and conditions under which Provider shall provide the Material to the Recipient for its use in connection with the Project.

2. USE OF THE MATERIAL

2.1 Recipient represents and warrants that it shall use the Material for the purpose of carrying out the Project and agrees and understands that it shall not be entitled to carry out any other analysis, extraction of samples or replica of the Material without the prior written consent of the Provider. Moreover, Recipient undertakes not to use the Material in human subjects, in clinical trials or for diagnostic purposes involving human subjects without the prior written consent of the Provider, and always under the appropriate governmental authorisations, when applicable.

2.2 Recipient agrees and undertakes to use the Material in compliance with all applicable laws and regulations, including but not limited to Public Health Service and National Institutes of Health regulations and guidelines relating to research involving the use of animals.

2.3 Material will not be distributed or released by COMPANY to any person other than co-workers working at COMPANY or one of COMPANY'S contractors (for the measurement of total and free PSA) ANALYTICA Medizinische Laboratorien AG or Labormedizinisches Zentrum Dr Risch, both accredited diagnostic laboratories, provided that such laboratory is under contractual obligations at least as protective to VHIR's and HUVH's rights and the use of the Material as provided herein. Otherwise, Material shall be stored and used only at the premises of the COMPANY, who undertakes not to change the location of Material or the custody of the same without the Provider's prior consent in writing.

2.4 Recipient also undertakes to limit access to the Material only to those of its employees, or agents, taking part in the Project who require access for development of their responsibilities thereunder. In this regard, Recipient ensures that all of its employees and agents having access to the Material shall comply with the provisions of this Agreement, having agreed to be bound by the terms of this Agreement or by entering into an agreement of similar scope and obligations.

2.5 In any case, the results of the research carried out by the Recipient in execution of the Project, including all creations, discoveries, know-how, information, and/or inventions

relating to the Material obtained, directly or indirectly, by Recipient as a consequence of the use of the Material, including any Industrial and/or Intellectual Property Rights (hereinafter, the "**Results**"), shall be deemed confidential and shall be subject to clause 6 below.

3. DELIVERY OF THE MATERIAL AND TRANSFER OF RISK

3.1 Upon execution of this Agreement, Provider agrees to deliver the Material to the Recipient in the term, formal conditions and with the information and/or documentation indicated in Appendix 1.

3.2 Once delivery of the Material has been made, any risk related to the Material shall be transferred to Recipient, who shall be responsible for its use, storage and, given the case, disposal, always subject to the applicable laws and regulations.

4. COMPENSATION

4.1. Recipient agrees to duly compensate the Provider for the costs associated with archiving and processing of samples in an amount of 70 euros/2 cc of serum per included men, that will be paid at the signature of this Agreement.

4.2. Recipient agrees to manage the shipping and assume every cost associated with the transfer of the Material.

5. WARRANTY AND LIABILITY

5.1. The Material provided under this Agreement is understood to be experimental in nature. Provider makes no representations and extends no warranties of any kind, express or implied, including but not limited to warranties of merchantability, fitness for a particular purpose, non-infringement of any patent, copyright, trademark or any other proprietary rights of a third party, or lack of health and safety risks in the use of the Materials or the Results.

5.2. Provider shall not be liable for any damages arising from the use, handling, storage or disposal of the Material or its Results by Recipient.

5.3. Recipient shall indemnify and hold Provider harmless for any loss, claim, damage or liability, of whatever kind or nature in connection with this Agreement, or which may arise from, the use, handling, storage or disposal of the Material by Recipient.

6. CONFIDENTIALITY

6.1 Subject to the other provisions of this Agreement, both Parties agree to treat (i) any and all information which is disclosed between the Parties orally, electronically, visually, or in a document or other tangible form and which is identified as confidential or which may reasonably be inferred to be confidential; and (ii) test results, error data, feedback, or other reports, in connection with the Material and the Results (hereinafter **"Confidential Information"**), with all cautions reasonably necessary and practicable to prevent its disclosure to persons other than those of their employees, agents or contractors who need to have access to the Confidential Information for the purposes of, or as envisaged by this Agreement.

6.2 Each Party warrants that all such employees shall be obliged to maintain the confidentiality of the Confidential Information and to use it only in accordance with the provisions of this Agreement, and each Party shall use all reasonable endeavours to avoid and act against non-compliance by its employees.

6.3 The confidentiality obligations hereinabove mentioned shall not apply to:

a) Information which the receiving Party can establish by written record was in its possession before the date hereof and not obtained, directly or indirectly, from the other Party.

b) Information which is or becomes in the future public knowledge through no fault or omission of either Party, its employees or its directors.

c) Information lawfully obtained by either Party after the date hereof from a third party with the right to disclose it.

6.4 The confidentiality obligations contained in this Agreement shall not prevent either Party from disclosing Confidential Information as a result of an administrative or judicial order to regulatory authorities. However, the Party so required shall notify the other Party as soon as practicable and, in addition, shall make its best efforts to procure confidential treatment by the requesting authority.

6.5 The obligations assumed under this clause shall remain in full force and effect even after termination of this Agreement, as long as the information remains secret and confidential.

7. INDUSTRIAL AND/OR INTELLECTUAL PROPERTY RIGHTS

7.1. Recipient acknowledges and agrees that the Material is the proprietary information of VHIR, that the property of VHIR's title to it shall remain fully vested with VHIR and nothing in this Agreement shall be construed as an assignment or transmission of any industrial and/or intellectual property rights worldwide, including without limitation

any and all patents, applications for patents, utility patents, design patents, copyrights, trademarks, trade secrets, moral rights, database rights, topography rights, character rights, rights of publication, trade dress, and any other worldwide intangible or tangible right related to Material belonging to VHIR which are not granted herein (hereinafter, "**Intellectual and/or Industrial Property Rights**"). The transfer of the Material shall not be construed to grant an option or license to the Recipient under any patent, trade secret or other rights now or hereinafter held by VHIR, other than the non-exclusive, non-transferable, revocable right to use the Material for the purposes indicated herein.

7.2. VHIR acknowledges that the diagnostic test Proclarix to be used in the Project represents a significant investment on the part of Recipient and is considered proprietary to Recipient.

7.3. VHIR shall be the only party to have the right to file patent applications on inventions related to Trial results and own such rights, while Proteomedix shall be the only party to have the right to file patent applications on inventions related to Proclarix test results and shall own such rights.

7.4 The Parties acknowledge that publication of the Results of any data derived from the use of the Material is the principal objective of their collaboration. Both Parties will use their reasonable efforts to encourage and assist in prompt publication of the results arising from the use of the Material.

8. VALIDITY AND TERM

8.1. This Agreement shall become effective on the date hereof and shall be valid for an initial term of one year, unless either Party provides 30 days prior written notice of its intention not to renew this Agreement.

Notwithstanding the foregoing, either Party may unilaterally terminate this Agreement or any renewal of it prior to its expiry by giving a 30 days prior written notice thereof to the other Party.

8.2. Any breach of this Agreement by any of the Parties may be remedied within 30 days of receiving written notice thereof. Any uncured breaches or breaches which, by nature, are not capable of being remedied shall entitle the other Party to claim proper fulfilment or alternatively, to terminate the Agreement, and in any case, to be indemnified for damages resulting from the aforementioned breach.

8.3. Upon termination or expiration of the Agreement, howsoever caused, Recipient will automatically discontinue its use of the Material and will, unless otherwise agreed, return or destroy any remaining Material.

8.4. Upon termination of the Agreement, any right of the Parties derived of the Intellectual and/or Industrial Rights relating to the Results, as well as clauses 5, 7, 8 and 9, shall survive termination.

9. PERSONAL DATA PROTECTION

For the development of the object of this Agreement, no provision is made for the processing of personal data of one of the Parties on behalf of the other.

Notwithstanding the foregoing, the Parties undertake to comply with current legislation on the Protection of Personal Data. Therefore, in accordance with the provisions of Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 and the legislation in force on the matter, the Parties agree that if, for the successful conclusion of this Agreement, one of them should process personal data on behalf of the other, they will grant, as Controller and Processor respectively, a contract of data processor that will be annexed to the present, with the content and requirements demandable by the regulations in force.

It shall be the duty of the Controller to inform the Data Processor when he is required to process data on his own behalf, and to submit him to a processing order contract, prior to the Data Processor beginning to process data on behalf of the Data Processor, and under no circumstances may such obligations fall on, delegate, transfer, impute or pass on to the Data Processor. Likewise, and beforehand, the Controller must inform and request the express consent of the interested parties whose data will be processed by the Controller.

10. MISCELLANEOUS

Any formal notice required or permitted by this Agreement must be delivered in writing and sent by certified mail, return receipt requested, addressed to the other Party at the address shown at the beginning of this Agreement or at such other address for which such Party gives notice hereunder.

Should any part, article, paragraph, sentence or clause of this Agreement be deemed vague, invalid or inapplicable, such part shall be eliminated and the rest of the Agreement shall remain valid and in force.

This Agreement may not be changed or modified in any way, orally or otherwise, unless such amendment is made in writing and signed by both Parties.

This Agreement may not be assigned by either of the Parties in any case without the express prior written consent of the other Party.

Should either of the Parties fail to execute any of the provisions of this Agreement, such failure shall not be deemed to constitute a waiver of such provisions nor of any other provision set forth herein nor a waiver of that Party's right to execute such provisions thereafter.

Neither Party shall be liable for its inability to perform any of the obligations under this Agreement provided that the cause of such inability is due to causes beyond its reasonable control such as, but not restricted to, fire, floods, strikes, or other industrial disturbances, restrictions, the unavailability of fuel or power supply, accidents, war (declared or undeclared), an embargo, isolation, mutiny, insurrection or a change of government.

11. GOVERNING LAW AND JURISDICTION

11.1. This Agreement shall be governed by and construed under the laws of Spain.

11.2. With express waiver to any other jurisdiction that may correspond to the Parties, any dispute or controversy in relation to, in connection with or resulting from this Agreement shall be exclusively resolved by the courts of the city of Barcelona.

IN WITNESS WHEREOF, the Parties have caused this **MATERIAL TRANSFER AGREEMENT** to be executed by their duly authorised representatives as of the Effective Date.

VHIR
Director
Dr. Joan X. Comella Carnicé

HUVH
General Manager
Dr. Albert Salazar i Soler

ProteoMediX AG
CSO
Dr. Ralph Schiess

ProteoMediX AG
COO/CFO
Christian Brühlmann

Acknowledge and approved:

JUAN MOROTE ROBLES - DNI 37724480H

Firmado digitalmente por JUAN MOROTE ROBLES - DNI 37724480H
Fecha: 2020.02.20 12:18:46 +01'00'

Dr. Morote Robles
Principal Investigator **VHIR**

APPENDIX 1: MATERIALS OBJECT OF THE PRESENT MATERIAL TRANSFER AGREEMENT

421 pcs. 2 ml frozen Human Serum Samples according to inclusion- /exclusion criteria specified in project.

APPENDIX 2: PROJECT

Clinical Research Committee – Study Proposal

Title: **Role of Prostarix with significant prostate cancer in the multiparametric magnetic resonance imaging era**

Country: **Spain**

Principal Investigator: **Juan Morote Robles – Head of Urology and Kidney Transplant Department – Vall d'Hebron Hospital Campus – Full professor of Urology – Universitat Autònoma de Barcelona**

Key information	Description
Rationale	Multiparametric magnetic resonance imaging of the prostate (mpMRI), in men with suspected prostate cancer (PCa) based on elevated prostate-specific antigen (PSA) and/or suspicious digital rectal examination (DRE), avoids unnecessary biopsies when negative (**PIRADSv2 <3**) and detect suspicious lesions (in which prostate biopsies (PB) can be targeted. However, the negative predictive value (NPV) of mpMRI ranges from 80% in initial PB (PBI), and 80% in repetitive PB (RPB) (1). Per clinical practice protocol in Vall d'Hebron Hospital, all men with positive mpMRI (**PIRADSv2 >=3**) undergo targeted PB (TPB) consisted of obtaining two cores from each suspicious lesion up to a maximum of 3 lesions and systematic (1) core transrectal ultrasound guided biopsy (SPB). However, in men with **PIRADSv2** <3 it has been estimated that only about **20% of biopsied males have SPCa** (confidential data pending of publication Vall d'Hebron. PPV= 0.7%, NPV=11%). Therefore, it is a scenario in which specificity must be improved. Here the PIRAD cut-off of 3 (2) doesn't achieve a substantial improvement, since it's essential not to lose more than 1-2% of SPCa. In men with **PIRADSv2** <3 it has been estimated that the **overall detection of SPCa, after a repeated biopsy if the initial one was negative, is 78% in PIRADSv2 = 4 and above 94% in PIRADSv2** =5 (confidential data pending publication Vall d'Hebron). In 5%, the detection of SPCa is greater than 63% in PIRADSv2 =4 and 85% in PIRADSv2 =5. In 50%, the probability of detecting a SPCa is 41%.If a n PIRADSv2 =4 and 59.3% when it is PIRADSv2 =5. Based on these estimates, the Vall d'Hebron hospital indicates performing **RPB** after 3 months in all male with PIRADSv2=3 and negative PB. Prostarix, a test combining biomarkers TMEM1, CTSD (3), KFS4, SOPS4 and age in a dedicated software algorithm is an aid to the discrimination of men in whom a biopsy should be performed to identify high grade PCa. Since recently the Prostarix has demonstrated an improvement in the identification of subjects deserving a biopsy for the diagnosis of high grade PCa compared to SOPS4 (4). In this context, it is hypothesized that Prostarix could be combined with mpMRI, as a model which can detect SPCa and avoid a significant percentage of unnecessary PB in patients with clinical suspicion of PCa.
Main Objective	