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# New strategy for the identification of prostate cancer: The combination of Proclarix and the prostate health index

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## Abstract

**Objectives:** Prostate health index (PHI) and, more recently, Proclarix have been proposed as serum biomarkers for prostate cancer (PCa). In this study, we aimed to evaluate Proclarix and PHI for predicting clinically significant prostate cancer (csPCa).

**Patients and Methods:** Proclarix and PHI were measured using samples of 344 men from two different centers. All patients underwent prostate biopsy, and among those, 188 men with PCa on biopsy had an additional radical prostatectomy (RP). All men had a prostate-specific antigen (PSA) between 2 and 10 ng/ml. Evaluation of area under the curve (AUC) and performance at predefined cut-offs of Proclarix and PHI risk scores as well as the linear combination thereof was performed to predict csPCa. PSA density was used as an independent comparator.

**Results:** The cohort median age and PSA were 65 (interquartile range [IQR]: 60–71) and 5.6 (IQR: 4.3–7.2) ng/ml, respectively. CsPCa was diagnosed in 161 (47%) men based on the RP specimen. ROC analysis showed that Proclarix and PHI accurately predicted csPCa with no significant difference (AUC of 0.79 and 0.76, $p = 0.378$) but significantly better when compared to PSA density (AUC of 0.66, $p < 0.001$). When using specific cut-offs, Proclarix (cut-off 10) revealed higher specificity and positive predictive value than PHI (cut-off 27) at similar sensitivities. The combination of Proclarix and PHI provided a significant increase in the AUC ($p \leq 0.007$) compared to the individual tests alone and the highest clinical benefit was achieved.

**Conclusion:** Results of this study show that both Proclarix and PHI accurately detect the presence of csPCa. The model combining Proclarix and PHI revealed the synergistic effect and improved the diagnostic performance of the individual tests.

KEYWORDS

biomarkers; cathepsin D, CTSD; PHI; Proclarix; prostate cancer; thrombospondin 1, THBS1

  

## 1 | INTRODUCTION

Early detection of prostate cancer (PCa) is widely performed using screening of total prostate-specific antigen (PSA) and percentage of its free fraction (%fPSA). However, this setting is associated with a significant number of men undergoing unnecessary biopsy of the prostate and increased detection of benign prostatic hyperplasia (BPH) as well as indolent cancer with a grade group (GG) according to ISUP equal to one. This limited diagnostic accuracy has been widely described,[1] and new biomarkers and tools have been proposed for better discrimination of detection of clinically significant PCa (csPCa, defined as GG ≥ 2), versus those with indolent tumors.[2]

However, only a few blood-based in vitro diagnostic certified (IVD) tests are available, and thus widely implemented in a routine diagnostic-laboratories set-up, in contrast to laboratory-developed tests, which are designed and manufactured to be used only within a single laboratory. Currently, one of the most used alternatives to PSA is the prostate health index (PHI; Beckman Coulter). PHI is comprised of the measurement of PSA, %fPSA, and [−2]proPSA, which are combined into a score correlating with the probability of detecting PCa. Its clinical evidence has been shown in multiple studies.[3,4] PHI has been approved by the FDA and is CE-marked. More recently, a newly blood-based IVD CE-marked test, Proclarix (Proteomedix Switzerland), became available in Europe. Proclarix combines thrombospondin-1 (THBS1), cathepsin D (CTSD), total PSA (tPSA), free PSA (fPSA), and patient age to compute a risk score.[5,6] Its clinical performance to increase prediction of csPCa compared to %fPSA has been recently demonstrated and published in a prospective study.[5]

Our hypothesis was that the combination of the kallikrein markers contained in PHI and the cancer-related markers of Proclarix discovered using a PTEN knock-out mouse model could further improve the diagnostic performance of the individual marker combinations. Thus, in this study, we aim to evaluate the performance and the potential synergistic effect of Proclarix and PHI in a combined retrospective serum sample cohort ($n$ = 344) collected from men before undergoing a biopsy of the prostate at two clinical centers. The results are also compared to PSA density (level of serum PSA divided by the prostate volume).

Biopsy-specimen-based grading is often hampered by limited accuracy due to sampling error resulting in frequent up- and downgrading. To improve the clinical endpoint, only samples from men that had a prostatectomy after a positive biopsy were included in this study.

## 2 | PATIENTS AND METHODS

### 2.1 | Study design

The primary endpoint of the study was the evaluation of the clinical performance of Proclarix and PHI for predicting csPCa, defined as Grade Group (GG), according to ISUP greater than or equal to two. Specifically, the potential synergistical value for predicting csPCa by combining Proclarix and PHI was evaluated using a model containing only both scores as input parameters.

### 2.2 | Study population

A total of 344 serum samples were obtained from two centers: 159 from the Department of Translational Medical Sciences, University Federico II, Naples, Italy, and 185 from the Martini-Klinik, University Hospital Hamburg-Eppendorf, Hamburg, Germany. All samples were collected consecutively from May 2020 to July 2021 (Naples) and from 2013 to 2016 (Hamburg) before undergoing a prostate biopsy. While only transrectal ultrasound (TRUS)-biopsy was performed in Hamburg, men in Naples underwent mpMRI-guided biopsy. Additionally, 97 Naples patients and 91 Hamburg patients with diagnosed PCa at biopsy underwent radical prostatectomy (RP). Serum samples were obtained just before prostate biopsy.

Histopathological examination of biopsy specimens was performed at each study site by the local pathologist. GG grading according to ISUP was determined using biopsy samples (biopsy GG) as well as—if applicable—biopsy adjudicated with RP findings (pathological GG). CsPCa was defined as GG ≥ 2.[6] Only subjects with elevated total PSA values between 2 and 10 ng/ml were included and patients with missing required clinical data or receiving drugs (i.e., 5-alpha reductase inhibitors) were excluded from the study. Prostate volume was calculated by TRUS. The use of material from each biobank was approved by the local ethics committees and all patients had given a general written informed consent for the storage and future studies of their samples.

### 2.3 | Determination of Proclarix, PHI, and PSA density

Serum aliquots were stored at −80°C until they were processed. Measurement of the Proclarix was performed blinded using the CTSD and THBS1 ELISA from the CE-marked Proclarix kit (Proteomedix) as described before.[7] CTSD and THBS1 ELISA for the Hamburg samples were conducted in Proteomedix laboratories facilities (Zurich-Schlieren, Switzerland) and for the Naples samples at the University Federico II (Naples, Italy). Total PSA and free PSA values were also determined blinded on a Cobas system (Roche) for Hamburg samples, and on a Access2 Immunoassay System analyzer (Beckman-Coulter) calibrated against the WHO standard for the Naples samples. In both cases, the Proclarix score was determined according to the instruction for use using the online risk-score calculator (www.proclarix.com/riskcalculator). PHI measurements were conducted at University Federico II (Naples, Italy) for all samples, according to the manufacturer's instructions for use. PSA density was calculated by dividing the level of serum total PSA by the prostate volume determined by TRUS.

### 2.4 | Proclarix and PHI combination model

To assess a possible synergy between Proclarix and PHI for the detection of csPCa, a dedicated linear model was built. The model returns a score with a range from 0 to 100, which correlates to the probability of detecting csPCa.

### 2.5 | Statistical analyses

Patients were divided into two groups: one control group comprising patients with a negative biopsy and non-csPCa (GG1) and the csPCa group including only patients diagnosed with cancer defined as GG ≥ 2.

Analysis of Proclarix was performed using a cut-off of 10[5,8] as recommended by the manufacturers. A threshold of 27 for the detection of csPCa versus non-csPCa or negative biopsy (control group) was applied for PHI as described previously.[9] Sensitivities and specificities were compared using the McNemar test,[10] while *p* values for NPV and PPV were calculated according to Moskowitz and Pepe.[11] Predicted discrimination was visualized using receiver operating characteristics (ROC) curves. When comparing the area under the curve (AUC) of the ROC, analysis was determined as described in DeLong et al.,[12] using the algorithm of Sun and Xu.[13] Decision curve analysis was conducted as proposed by Vickers and Elkinet[14] to assess the clinical usefulness of the different scores by quantifying the net benefits when a different threshold is used. Finally, $p < 0.05$ were considered significant and all analyses were performed using R version 4.0.2 (R Foundation for Statistical Computing).

## 3 | RESULTS

### 3.1 | Study population

Patient characteristics are summarized in Table 1. The proportion of the subjects diagnosed with csPCa was higher when using the pathological GG than when using the biopsy GG (47% vs. 34%, respectively). Regardless of whether pathological or biopsy GG was applied, the patient population with csPCa was significantly different ($p < 0.001$) from the control population with regard to age, prostate volume, PSA density, Proclarix, and PHI scores, while, as expected, total PSA did not differ between groups ($p = 0.201$).

### 3.2 | Evaluation of Proclarix and PHI score

When comparing ROC using biopsy GG, the AUC of Proclarix (0.76, 95%CI: 0.71–0.81) was nearly identical to PHI (0.75, 95%CI: 0.70–0.81) ($p = 0.843$). When using pathological GG, the AUC of Proclarix (0.79, 95%CI: 0.74–0.84) was slightly higher but still not significantly different ($p = 0.378$) than the AUC of PHI (0.76, 95% CI: 0.71–0.81) as shown in Figure 1. The AUC of PSA density was significantly lower when compared to Proclarix both at biopsy (0.66, 95%CI: 0.60–0.72, $p = 0.007$) and at RP (0.66, 95%CI: 0.60–0.72, $p < 0.001$). The evaluation of the clinical performance of Proclarix, PHI, and PSA density is summarized in Supporting Information: Table 1.

When using a cut-off of 10 for Proclarix and 27 for PHI, both scores showed a comparable high sensitivity >95% ($p \geq 0.157$). Proclarix missed six patients diagnosed with csPCa when using biopsy GG and seven when using pathological GG, most of them

**TABLE 1** Population characteristics of the cohort

| Variable | Total | Biopsy grade group | | | Pathological grade group | | |
|---|---|---|---|---|---|---|---|
| | | Benign or insignificantPCa | Clinically significantPCa | *p* value[a] | Benign or insignificantPCa | Clinically significantPCa | *p* value[a] |
| Patients, *n* | 344 | 231 | 113 | NA | 183 | 161 | NA |
| Age, years | 65 (60–71) | 64 (58–69) | 68 (63–72) | <0.001 | 64 (58–69) | 67 (62–72) | <0.001 |
| tPSA, ng/ml | 5.6 (4.3–7.2) | 5.5 (4.2–7.1) | 5.9 (4.6–7.5) | 0.222 | 5.4 (4.1–7.1) | 5.7 (4.6–7.5) | 0.201 |
| Volume, ml | 50.5 (39.0–68.0) | 55.0 (42.0–70.0) | 42.8 (35.0–56.0) | <0.001 | 55.0 (42.0–73.0) | 45.0 (35.2–60.0) | <0.001 |
| PSA density | 0.11 (0.08–0.15) | 0.10 (0.07–0.13) | 0.12 (0.08–0.18) | <0.001 | 0.09 (0.08–0.13) | 0.12 (0.09–0.17 | <0.001 |
| Proclarix score | 21.4 (11.4–35.8) | 16.4 (8.4–26.3) | 34.2 (22.5–47.6) | <0.001 | 14.3 (6.9–24.0) | 32.4 (19.5–45.7) | <0.001 |
| PHI score | 46.2 (35.0–61.6) | 41.4 (31.9–53.6) | 60.5 (45.2–75.7) | <0.001 | 38.2 (30.8–50.4) | 56.9 (44.2–74.1) | <0.001 |

*Note*: Median (25%, 75%).

Abbreviations: PCa, prostate cancer; RP, radical prostatectomy; tPSA, total prostate-specific antigen.

[a]Comparison between "No or insignificant PCa" and "Clinically significant PCa" population.

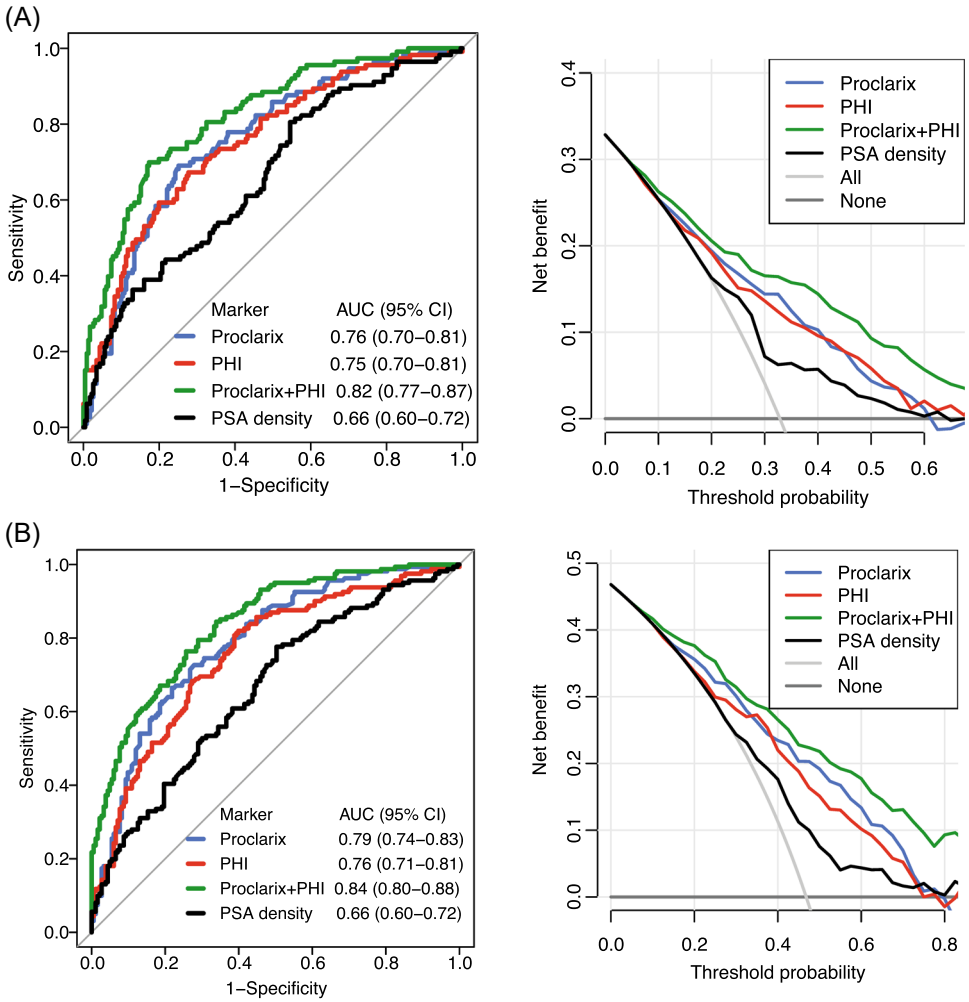


**FIGURE 1** Receiver operating characteristic analysis (left) and decision curve analysis (right) according to the (A) biopsy grade group or (B) pathological grade group are shown for Proclarix (blue), PHI (red), the combination of Proclarix and PHI (green), and PSA density (black). For the decision curve analysis, as a comparison, the light gray line represents the strategy of performing a biopsy in all men, and the dark gray horizontal line represents the strategy of no men undergoing biopsy. PHI, prostate health index; PSA, prostate-specific antigen. [Color figure can be viewed at wileyonlinelibrary.com]

being GG2 ($n = 4$ and $n = 7$ respectively) and none having GG5. On the other hand, PHI missed only two and four patients respectively, with one of them having in both cases GG5. The specificity of Proclarix (29%, 95%CI 23%–34% and 36%, 95%CI: 29%–42% for biopsy and pathological GG respectively) was significantly ($p < 0.001$) higher than PHI (12%, 95%CI: 8%–16%, and 14%, 95%CI: 9%–19% respectively).

The clinical benefit of both scores was further assessed by clinical utility curves (CUCs, Figure 2). CUCs illustrate the relation between the rate of avoided biopsies and the corresponding proportion of missed csPCa. Using the recommended cut-off for Proclarix (i.e., 10) translates into a rather high clinical benefit (roughly one-third avoided biopsies) with less than 10% missed csPCa. It is thus an ideal cut-off. Using a cut-off of 27, PHI leads to a low clinical benefit (less than 10% avoided biopsies) but a high sensitivity for csPCa.

### 3.3 | Combining Proclarix and PHI

Two linear models using Proclarix and PHI were created based on the whole cohort ($n = 344$), either using the biopsy or the pathological GG as a reference. The AUC of the combined models was 0.82 (95%CI: 0.77–0.87) at the biopsy endpoint and 0.84 (95%CI: 0.80–0.88) when correlated to RP outcome. The odds ratio (OR) and AUC of the uni- and multivariate analyses are summarized in Table 2A,B. Both Proclarix and PHI had a significant contribution to the combined model ($p < 0.001$). When looking at the different AUC, the combined model significantly improved the diagnostic accuracy compared to Proclarix ($p \leq 0.007$) and to PHI ($p \leq 0.002$) alone. This observation translated also in the DCA, where the combination of markers provided an increased net benefit for threshold probabilities of >10% (Figure 1) compared to the markers alone. When using an appropriate cut-off value, the models could potentially avoid more than 50% of

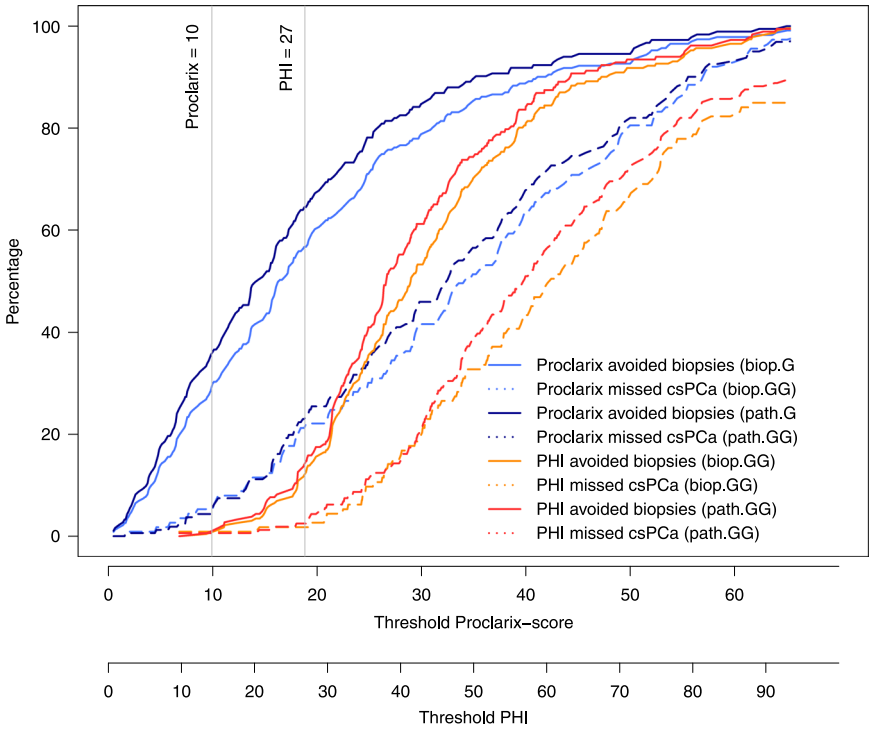


**FIGURE 2** Clinical utility curve analyses are shown for Proclarix (blue lines) and PHI (red/orange lines). The figure shows the relation between the rates of avoided biopsies (continuous lines) and the corresponding risk of missed csPCa (dotted lines), depending on the cut-off used for decision-making. Performance at fixed cut-offs for the Proclarix (threshold = 10) and PHI (threshold = 27) are visualized with the gray lines. csPCa, clinically significant prostate cancer. [Color figure can be viewed at wileyonlinelibrary.com]

**TABLE 2** Comparison of AUC and odds ratios for Proclarix, PHI, and Proclarix + PHI according to the **(A)** biopsy grade group and **(B)** pathological grade group

| Predictors for csPCa | Score increase | OR (95% CI) | *p* value | AUC (95% CI) | *p* value[a] |
|---|---|---|---|---|---|
| (A) | | | | | |
| Univariate | | | | | |
| Proclarix | 1 | 1.06 (1.04–1.07) | <0.001[b] | 0.76 (0.71–0.81) | 0.002 |
| PHI | 1 | 1.04 (1.03–1.06) | <0.001[b] | 0.75 (0.70–0.81) | 0.001 |
| Multivariate | | | | | |
| Proclarix + PHI | 1 | 1.05 (1.04–1.06) | <0.001[b] | 0.82 (0.78–0.87) | Ref. |
| Proclarix | 1 | 1.05 (1.04–1.07) | <0.001[c] | NA | NA |
| PHI | 1 | 1.04 (1.03–1.05) | <0.001[c] | NA | NA |
| (B) | | | | | |
| Univariate | | | | | |
| Proclarix | 1 | 1.07 (1.05–1.09) | <0.001[b] | 0.79 (0.74–0.84) | 0.007 |
| PHI | 1 | 1.05 (1.03–1.06) | <0.001[b] | 0.76 (0.71–0.81) | <0.001 |
| Multivariate | | | | | |
| Proclarix + PHI | 1 | 1.07 (1.05–1.08) | <0.001[b] | 0.84 (0.80–0.88) | Ref. |
| Proclarix | 1 | 1.07 (1.05–1.09) | <0.001[c] | NA | NA |
| PHI | 1 | 1.05 (1.03–1.06) | <0.001[c] | NA | NA |

[a]Comparison to Proclarix + PHI using the DeLong method.

[b]Univariate logistic regression.

[c]Multivariate logistic regression.

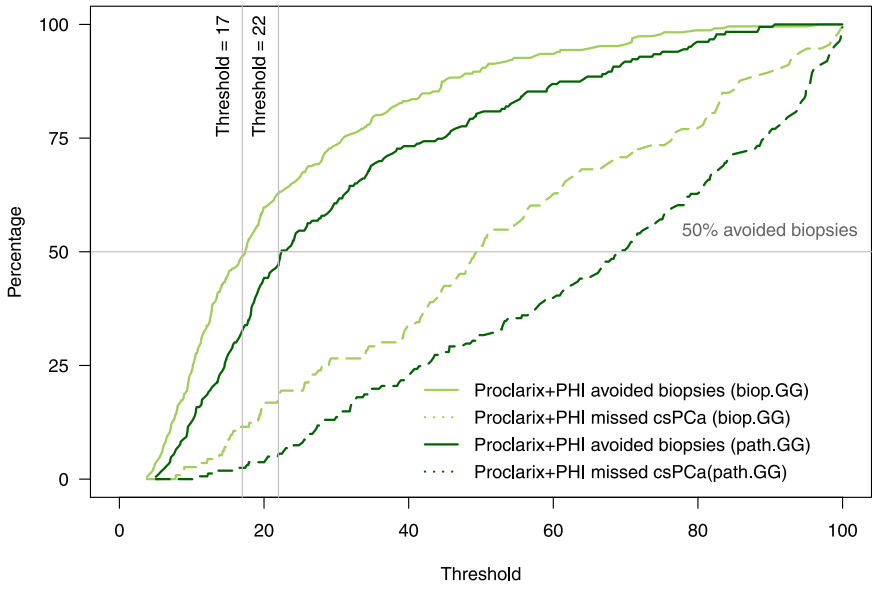


**FIGURE 3** Clinical utility curve analyses are shown for Proclarix + PHI model based on the biopsy grade group (light green lines) and pathological grade group (dark green lines). The figure shows the relationship between the rates of avoided biopsies (continuous lines) and the corresponding risk of missed csPCa (dotted lines). csPCa, clinically significant prostate cancer; PHI, prostate health index. [Color figure can be viewed at wileyonlinelibrary.com]

unnecessary biopsies, while missing less than 10% of patients diagnosed with csPCa (Figure 3).

## 4 | DISCUSSION

In this two-center retrospective study with 344 patients, we performed an evaluation of Proclarix and PHI for predicting csPCa, which was defined as GG ≥ 2 versus non-csPCa or negative biopsy as a control group. The GG was assessed in two different ways: either using only results from the biopsy (biopsy GG) or adjudicating biopsy results with RP findings (pathological GG). An accurate grading at biopsy is crucial for risk stratification and treatment decision making for the patients, and discrepancies between biopsy and pathological GG are common and remain an important issue.[15] In the present study, the prevalence of csPCa was underestimated using the GG determined at biopsy (34%) given the significantly higher rate of csPCa at final pathology following RP (47%). These findings have been previously described by Bullock et al.,[16] where the upgrading rate between biopsy and pathological GG was more common compared to downgrading. Furthermore, the overall csPCa rate based on biopsy GG of 47% in this population with a total PSA of 2–10 ng/ml was comparable with previously published studies.[17,18] Thus, we assume that the present cohort used in this study is representative of men undergoing PCa opportunistic screening.

When comparing Proclarix and PHI, regardless of how GG was assessed, AUCs were similar (values between 0.75 and 0.79, $p > 0.378$), and comparable to already reported values.[19] OR (1.04, 95%CI: 1.03–1.06) of PHI alone was similar to the one described previously.[20] Nevertheless, when applying specific cut-offs, the clinical utility of the score becomes more differentiated. On the one hand, using a cut-off of 10 for Proclarix could confirm the previously described clinical performance[5,8] of safely ruling out negative cases (NPV > 90%), reliably identifying csPCa (96% sensitivity) and avoiding roughly one-third of unneeded biopsies. PHI could—when using a cut-off of 27—avoid only a few biopsies (<14%) by missing only less than four out of 162 csPCa patients. According to PHI instruction for use—derived from the data used for the FDA application[21]—a value of 27 corresponds to the upper limit of the range, where patients having PSA values between 4 and 10 ng/ml and non-suspicious DRE have a 9.8% probability of PCa. As mentioned in 2014 by Wang et al. as well as by Bruzzese et al.,[22,23] the large variability of thresholds reported and recommended for PHI makes the decision making for the physician very challenging since there is actually no consensus regarding the most appropriate level for PHI. However, the PHI-test provider (Beckman Coulter) does not recommend a fixed cut-off, but rather the use of ranges corresponding to different PCa risk probabilities.[21] The recommended ranges for PHI values were determined using patients above 50 years old with PSA values of 4–10 ng/ml and negative DRE findings, very similar to the ones used for the clinical validation of Proclarix.[8] However, the ranges correspond to a risk of PCa and not necessarily of csPCa. Thus, 27 might not be the ideal cut-off when discriminating csPCa from iPCa or no PCa. Nevertheless, a PHI value of 27 was reported in several clinical studies either as cut-off or as upper limit of a low-risk range in relation to csPCa.[9,24–26]

PSA density is known to be a more sensitive and specific test than PSA in detecting insignificant and significant PCa in men with elevated PSA levels.[27] In this study, both Proclarix and PHI outperformed PSA density. Nevertheless, the use of PSA density remains useful to trigger further investigation, such as the use of Proclarix in men whose PSA may be elevated, but appropriate for the size of the prostate.

Although this study confirmed the clinical usefulness of Proclarix and PHI as a standalone test, the combination of Proclarix and PHI revealed the highest clinical performance. While both tests are sharing the results of total and free PSA, proPSA (PHI) and age, CTSD and THBS1 (Proclarix) revealed to be complementary and further

improved the clinical performance of the test. Thus, our hypothesis was confirmed that organ-specific kallikrein markers combined with more cancer-related markers CTSD and THBS1 originally discovered using a PTEN conditional knockout mouse model[28] do not only improve the clinical performance of the individual tests but achieve an acceptable level of confidence in safely ruling out insignificant cancer and benign disease while detecting aggressive cancer.

Additionally, it is noteworthy that PHI improves the performance of currently available risk calculators[29] and has already been incorporated in the ERSPC risk calculator. Other synergistic effects between two tests for PCa diagnosis have already been described[30,31] but are rather difficult to implement in clinical practice, as they very often require different types of samples, increasing the sample-logistic outlay. However, this is not the case for the combination of PHI and Proclarix, as both tests are serum-based, and the same sample can be used to measure both tests. Thus, implementation in clinical practice should be easily done.

Limitation to this study includes that this is a retrospective analysis of data combined from only two European centers. The use of biobanked samples for p2PSA measurement might impact PHI results due to limited protein stability.

## 5 | CONCLUSION

In conclusion, Proclarix and PHI accurately predicted csPCa with no significant differences when reporting AUC. Nevertheless, when using predefined cut-offs recommended by the manufacturers, the Proclarix-score (cut-off 10) outperformed PHI (cut-off 27) in terms of specificity and positive predictive value ($p < 0.002$) at similar sensitivities. Combining the Proclarix-score and PHI revealed the synergistic effect, showing the highest clinical net benefit. The combination would have avoided 56% of unneeded biopsies while accurately diagnosing csPCa with 91% sensitivity.

### ACKNOWLEDGMENT

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### CONFLICTS OF INTEREST

A. A. and R. S. received/held stock options and salaries and founder shares (R. S.) of Proteomedix. T. S. is an advisor to Proteomedix. T. S., A. A., and R. S. are inventors of the following patent application (WO2018011212) as well as R. S. on patent application (WO2009138392).

### DATA AVAILABILITY STATEMENT

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

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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