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## PHI for adverse pathology and PHI density for upgrading: two complementary predictors in localized prostate cancer

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**BACKGROUND:** The Prostate Health Index (PHI) has emerged as a promising biomarker for improving prostate cancer (PCa) detection. However, its role in predicting pathological aggressiveness at radical prostatectomy (RP) requires further elucidation. We aimed to assess the ability of PHI and PHI density (PHId) to predict adverse pathological features and ISUP upgrading in men undergoing robot-assisted RP.

**METHODS:** We conducted a prospective, single-center observational study including 130 patients who underwent RP. Total PSA, free PSA, and p2PSA were measured using the Access 2 platform, and PHI and PHId were calculated accordingly. Multivariable logistic regression models evaluated the association of PSA, PSA density, PHI, and PHId with two predefined endpoints: (i) adverse pathological features (pT  $\geq$  3 and/or pN1), and (ii) upgrading from biopsy to RP. Models were adjusted for DRE status, PI-RADS score, and biopsy ISUP grade. Discrimination was assessed using AUC and the DeLong test.

**RESULTS:** Median PSA, PHI, PSA density, and PHId were 6.21 ng/mL, 58, 0.15 ng/mL/cc, and 1.40, respectively. Adverse pathological features were identified in 39% of cases, and upgrading occurred in 59% (after exclusion of biopsy ISUP 5 cases). PHI emerged as the strongest independent predictor of adverse pathology (OR 1.04; 95% CI 1.02–1.07), yielding the highest model accuracy (AUC 0.86) and showing a sharp risk increase above PHI > 40. PHId was the only marker independently associated with upgrading (OR 1.54; 95% CI 1.10–2.29), achieving the best discrimination (AUC 0.83), with risk escalation particularly evident in patients with PI-RADS 4–5 lesions.

**CONCLUSIONS:** PHI and PHId provide complementary information in localized PCa. PHI accurately identifies patients at risk of adverse pathological extension, whereas PHId effectively captures the probability of grade reclassification at RP. Integrating these biomarkers into patient assessment may support precision-based decision-making and helping to minimize undertreatment.

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## INTRODUCTION

The introduction of multiparametric MRI (mpMRI) has improved the detection of clinically significant prostate cancer (PCa) while reducing the detection of clinically insignificant disease [1–3]. Nevertheless, insignificant diseases are still frequently diagnosed, representing around 8% of all PCa diagnosed [4, 5]. However, variability in PI-RADS interpretation among radiologists, together with limited access to 3-Tesla MRI scanners in many centers, still contributes to unnecessary prostate biopsies [6–10]. To limit unnecessary biopsies, associated with procedural complications, patient anxiety, and financial burden on national healthcare systems, new biomarkers have been investigated. The Prostate Health Index (PHI) is among the most promising tools in this setting [11–13]. PHI was initially introduced as a risk index for biopsy positivity [14]. In 2011, a

prospective, double-blind, multicenter case–control study of 892 men conducted by Catalona et al. demonstrated that, within the 2.0–10.0 ng/mL PSA range, PHI showed significantly greater specificity than PSA at 80–95% sensitivity for PCa detection. Rising PHI values were associated with a 4.7-fold increase in the risk of PCa and a 1.61-fold increase in the risk of significant disease on biopsy, supporting its role in reducing unnecessary biopsies with minimal loss of sensitivity [15]. In 2012, PHI received FDA approval for use in men aged  $\geq$  50 years with total PSA levels between 4.0 and 10.0 ng/mL and a negative digital rectal examination (DRE). Since then, substantial progress has been made in using PHI for disease characterization and treatment decision-making. Several prospective multicenter studies have confirmed that PHI consistently outperforms its individual components in detecting

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**Table 1.** Clinical and pathological characteristics of patients who underwent radical prostatectomy.

| Characteristic        | N = 130 <sup>a</sup> |
|-----------------------|----------------------|
| PSA, ng/ml            | 6.21 (4.72, 8.04)    |
| PHI                   | 58 (44, 74)          |
| PSA Density, ng/ml/cc | 0.15 (0.10, 0.24)    |
| PHI Density           | 1.40 (0.80, 2.62)    |
| Prostate Volume, cc   | 41 (28, 58)          |
| PI-RADS score         |                      |
| 2                     | 5 (3.8%)             |
| 3                     | 23 (18%)             |
| 4                     | 64 (49%)             |
| 5                     | 38 (29%)             |
| cT stage              |                      |
| T1c                   | 111 (85%)            |
| T2                    | 14 (11%)             |
| T3a                   | 4 (3.1%)             |
| T3b                   | 1 (0.8%)             |
| ISUP Grade at biopsy  |                      |
| 1                     | 52 (40%)             |
| 2                     | 26 (20%)             |
| 3                     | 17 (13%)             |
| 4                     | 31 (24%)             |
| 5                     | 4 (3%)               |
| ISUP Group at RP      |                      |
| 1                     | 19 (15%)             |
| 2                     | 10 (8%)              |
| 3                     | 16 (12%)             |
| 4                     | 66 (51%)             |
| 5                     | 19 (15%)             |
| pT stage              |                      |
| T2                    | 83 (64%)             |
| T3a                   | 34 (26%)             |
| T3b                   | 13 (10%)             |
| pN stage              |                      |
| 0                     | 62 (48%)             |
| 1                     | 6 (4.6%)             |
| x                     | 62 (48%)             |

<sup>a</sup>Median (IQR); n (%).

high-grade PCa, helping limit unnecessary biopsies and over-diagnosis while missing very few aggressive tumors [16–18]. Decision-making in PCa has consequently shifted from a PSA-only approach to multivariable models incorporating clinical risk indicators, with PHI proving valuable in nomograms for both initial and repeat biopsy to enhance risk stratification [19]. Importantly, economic analyses indicate that incorporating PHI into biopsy pathways for men with elevated PSA improves quality-adjusted survival and reduces costs, underscoring its cost-effectiveness [20]. Nevertheless, the potential utility of PHI in risk stratification of patients diagnosed with PCa has not been previously investigated. Accordingly, we aimed to evaluate the ability of PHI to predict adverse pathological features and ISUP upgrading in patients who underwent radical prostatectomy (RP).

## MATERIALS AND METHODS

### Study population

This was a prospective, observational, single-center study conducted in an RP cohort at the University Hospital “Federico II” of Naples between September 2022 and December 2024. All patients underwent mpMRI before prostate biopsy.

Prostate biopsies were performed after mpMRI using a combined approach, including both systematic sampling and MRI-targeted cores. Targeted biopsies were obtained using MRI/ultrasound fusion-guided targeting according to institutional practice. Systematic biopsies were performed using a standard 10–12 core template.

For each patient undergoing robot-assisted RP (RARP), serum samples and clinicopathological data were prospectively collected.

Eligible participants were men presenting with total PSA values between 2 and 10 ng/mL and either a negative or suspicious DRE. Exclusion criteria included: (i) a previous history of malignant disease; (ii) conditions known to influence PSA levels (recent surgical procedures, use of 5- $\alpha$ -reductase inhibitors, or acute urinary tract infection within the previous 3 months); and (iii) incomplete diagnostic information.

Overall, 130 patients fulfilled the enrollment criteria. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and approved by the local Ethics Committee (approval No. 118/20). Written informed consent was obtained from all participants. Preoperative prostate volume (PV) was assessed using mpMRI and calculated according to the standard ellipsoid formula.

Surgical specimens were reviewed by a dedicated uro-oncological pathologist with 15 years of experience and were classified according to the recommendations of the 2014 International Society of Urological Pathology (ISUP) Consensus Conference [21].

### Blood collection and PSA isoforms analysis

Peripheral blood was drawn immediately before RARP, processed within 3 h of collection, and serum aliquots were stored at  $-80^{\circ}\text{C}$ . In our study, blood sampling for PSA isoforms was performed immediately prior to radical prostatectomy, with a median interval of approximately 12 weeks (range 8–14) from biopsy.

PSA molecular forms were quantified on the Access 2 immunoassay platform (Dxl 800; Beckman Coulter) using Hybritech-calibrated methods to determine total PSA (tPSA), free PSA (fPSA), and p2PSA. The PHI was computed as  $p2\text{PSA}/f\text{PSA} \times \sqrt{t\text{PSA}}$ . PHI density (PHId) was derived by normalizing PHI to prostate volume ( $\text{PHId} = \text{PHI}/\text{PV}$ ), whereas PSA density PSAd was calculated as  $t\text{PSA}/\text{PV}$ .

### Outcome definitions

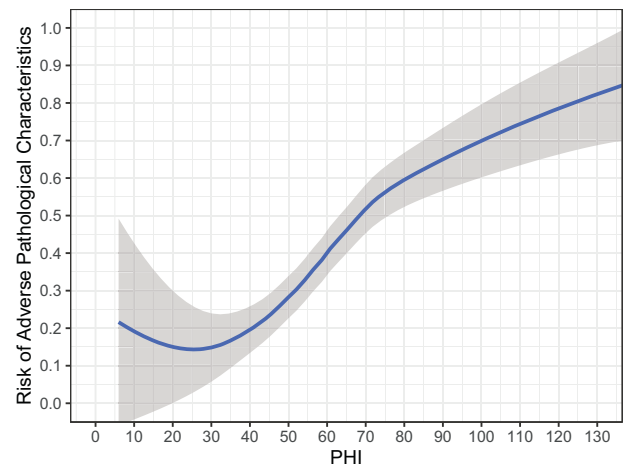
The outcomes of the study were upgrading and adverse pathological features. Upgrading was defined as an increase in the ISUP grade group from biopsy to RP in the main analyses. In additional analyses, we restricted the cohort to patients with ISUP grade 1 or 2 at biopsy and defined the outcome as upgrading to ISUP  $\geq 3$ , as this threshold may be more clinically relevant for patients who are candidates for active surveillance. Adverse pathological features were defined as the presence, at final pathology after RP, of extraprostatic tumor (pT  $\geq 3$ ) and/or lymph node involvement (pN1).

### Statistical analysis

Statistical analyses were conducted in several steps. No formal a priori sample size calculation was performed, as the study was exploratory and based on consecutive enrollment during the study period. First, patient characteristics were summarized using the median and interquartile range for continuous variables, and counts and percentages for categorical variables. Next, we evaluated the association and predictive accuracy of PHI and PHId for outcomes (any upgrading, upgrading from ISUP 1–2 to ISUP  $\geq 3$ , and adverse pathological features), and compared the results with those of two well-established markers, PSA and PSAd. For each outcome, we developed four separate multivariable logistic regression models, each incorporating one of the biomarkers (PSA, PSAd, PHI, and PHId) as the primary predictor. Models for any upgrading and adverse pathological features were adjusted for clinical variables, including DRE findings, PI-RADS score, and ISUP grade at biopsy. In the model for upgrading from ISUP 1–2 to ISUP  $\geq 3$ , DRE was not included as a covariate, since only one patient had a positive DRE in the analyzed subpopulation. Patients with ISUP grade 5 were excluded from the upgrading analysis, as further upgrading at radical prostatectomy is not applicable for this group.

**Table 2.** Multivariable models to evaluate the association between markers (PHI, PHI Density, PSA, PSA Density) and adverse pathological features (pT $\geq$ 3 and/or N1).

| Characteristic       | PHI             |                     |         | PHI density     |                     |         | PSA             |                     |         | PSA Density     |                     |         |
|----------------------|-----------------|---------------------|---------|-----------------|---------------------|---------|-----------------|---------------------|---------|-----------------|---------------------|---------|
|                      | OR <sup>a</sup> | 95% CI <sup>a</sup> | p value | OR <sup>a</sup> | 95% CI <sup>a</sup> | p value | OR <sup>a</sup> | 95% CI <sup>a</sup> | p value | OR <sup>a</sup> | 95% CI <sup>a</sup> | p value |
| <b>Marker</b>        | 1.04            | 1.02, 1.07          | <0.001  | 1.21            | 0.94, 1.56          | 0.14    | 1.28            | 1.04, 1.59          | 0.022   | 1.02            | 0.03, 29.9          | >0.9    |
| Positive DRE         | 23.8            | 3.84, 468           | 0.004   | 21.7            | 3.67, 418           | 0.005   | 30.4            | 4.39, 658           | 0.004   | 21.0            | 3.55, 405           | 0.005   |
| PI-RADS score        |                 |                     |         |                 |                     |         |                 |                     |         |                 |                     |         |
| ≤3                   | —               | —                   | —       | —               | —                   | —       | —               | —                   | —       | —               | —                   | —       |
| 4–5                  | 0.34            | 0.10, 1.18          | 0.089   | 0.65            | 0.21, 2.04          | 0.4     | 0.59            | 0.19, 1.85          | 0.3     | 0.80            | 0.26, 2.52          | 0.7     |
| ISUP Group at biopsy |                 |                     |         |                 |                     |         |                 |                     |         |                 |                     |         |
| 1                    | —               | —                   | —       | —               | —                   | —       | —               | —                   | —       | —               | —                   | —       |
| 2–3                  | 3.44            | 1.13, 11.5          | 0.034   | 2.75            | 1.03, 7.73          | 0.047   | 2.56            | 0.95, 7.23          | 0.068   | 2.82            | 1.07, 7.88          | 0.040   |
| 4–5                  | 4.70            | 1.22, 19.3          | 0.026   | 4.00            | 1.21, 13.6          | 0.023   | 4.40            | 1.32, 15.3          | 0.017   | 4.12            | 1.26, 13.9          | 0.020   |
| <b>AUC</b>           | 0.86            |                     |         | 0.78            |                     |         | 0.78            |                     |         | 0.75            |                     |         |

<sup>a</sup>OR odds ratio, CI confidence interval.**Fig. 1** Risk of adverse pathological features (pT $\geq$ 3 and/or N1) by PHI.

Similarly, in the analysis assessing the association of markers and upgrading from ISUP 1–2 to ISUP  $\geq$  3, only patients with ISUP  $\leq$  2 at biopsy were included. To assess model performance, we calculated the area under the receiver operating characteristic curve (AUC) for each model and compared them using the DeLong test. We also explored the relationship between PHI and the risk of adverse pathological features using locally estimated scatterplot smoothing (LOESS). Similarly, we plotted the risk of upgrading as a function of PHI for patients grouped according to mpMRI results (PI-RADS  $\leq$  3 vs PI-RADS 4–5). All tests were two-sided, with a significance level of 0.05. Analyses were performed using R version 4.1.0. No custom code central to the conclusions of this study was generated.

## RESULTS

Table 1 summarizes patient characteristics. Median PSA, PHI, PSAd, and PHI<sub>d</sub> were 6.21 ng/ml (interquartile range [IQR]: 4.72–8.04), 58 (IQR: 44–74), 0.15 (IQR: 0.10–0.24), and 1.40 (IQR: 0.80–2.62), respectively. Overall, 125 patients (96%) had a suspicious lesion on MRI (PI-RADS  $\geq$  3). Nineteen patients (15%) had a suspicious DRE. At biopsy, 40% of patients had ISUP 1, 20% ISUP 2, 13% ISUP 3, and 27% ISUP 4–5.

At final pathology, 15% of patients had ISUP 1, 8% ISUP 2, 12% ISUP 3, and 66% ISUP 4–5. Overall, 47 patients (36%) had extraprostatic disease (pT 3) at RP, and 6 patients (5%) had pN1.

### Adverse pathological features (pT $\geq$ 3 and/or pN1)

A total of 51 patients (39%) had adverse pathological features at final pathology. We assessed the association of each biomarker—PSA, PSAd, PHI, and PHI<sub>d</sub>—with adverse pathological features (Table 2). Only PHI (odds ratio [OR]: 1.04; 95% confidence interval [CI]: 1.02–1.07) and PSA (OR: 1.28; 95% CI: 1.04–1.59) were significantly associated with the outcome. The AUC was significantly higher for the model including PHI (0.86) compared with the others ( $\leq$  0.78) according to the DeLong test ( $p < 0.05$ ). Finally, we plotted the risk of adverse pathological features at final pathology against PHI (Fig. 1). The risk was approximately 20% for PHI values  $< 40$  but increased sharply for PHI values  $> 40$ .

### Upgrading

Table 3 summarizes the rate of upgrading for patients grouped according to ISUP grade at biopsy. Any upgrading from biopsy to RP occurred in 74 patients (59%), and upgrading of at least two grade groups occurred in 49 patients (39%). Overall, 33 of 52 patients (63%) with ISUP 1 at biopsy experienced upgrading at RP. Similarly, 49 patients (63%) with ISUP 1–2 at biopsy ( $n = 78$ ) were upgraded to ISUP  $\geq$  3 at RP. We assessed the association of each biomarker—PSA, PSAd, PHI, and PHI<sub>d</sub>—with upgrading. Four separate multivariable logistic regression models were developed,

**Table 3.** Rate of patients who experienced upgrading at radical prostatectomy for patients grouped according to ISUP Grade at biopsy.

| Characteristic           | Overall, <i>N</i> = 126 <sup>a</sup> | 1, <i>N</i> = 52 <sup>a</sup> | 2, <i>N</i> = 26 <sup>a</sup> | 3, <i>N</i> = 17 <sup>a</sup> | 4, <i>N</i> = 31 <sup>a</sup> |
|--------------------------|--------------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| <b>ISUP Group at RP</b>  |                                      |                               |                               |                               |                               |
| 1                        | 19 (15%)                             | 19 (37%)                      | 0 (0%)                        | 0 (0%)                        | 0 (0%)                        |
| 2                        | 10 (7.9%)                            | 6 (12%)                       | 4 (15%)                       | 0 (0%)                        | 0 (0%)                        |
| 3                        | 16 (13%)                             | 8 (15%)                       | 4 (15%)                       | 4 (24%)                       | 0 (0%)                        |
| 4                        | 66 (52%)                             | 16 (31%)                      | 16 (62%)                      | 9 (53%)                       | 25 (81%)                      |
| 5                        | 15 (12%)                             | 3 (5.8%)                      | 2 (7.7%)                      | 4 (24%)                       | 6 (19%)                       |
| <b>Overall Upgrading</b> | 74 (59%)                             | 33 (63%)                      | 22 (85%)                      | 13 (76%)                      | 6 (19%)                       |
| <b>≥2 Upgrading</b>      | 49 (39%)                             | 27 (52%)                      | 18 (69%)                      | 4 (24%)                       | 0 (0%)                        |

<sup>a</sup>*n* (%).

each incorporating one biomarker as the primary predictor, adjusted for clinical characteristics (DRE findings, PI-RADS score, ISUP group) (Table 4). Only PHId (OR: 1.54; 95% CI: 1.10–2.29) was significantly associated with upgrading. At the DeLong test, the model including PHId demonstrated the highest accuracy (0.83 vs  $\leq 0.81$ ,  $p < 0.05$ ). Fig. 2 illustrates the association between PHId and upgrading risk stratified by PI-RADS score. The risk of upgrading increased steadily (from 20% to 75%) with rising PHId values for patients with PI-RADS 4–5. Among patients with PI-RADS  $\leq 3$ , the risk of upgrading was approximately 40% for PHId values  $< 2.5$  but rose sharply for PHId values  $> 2.5$ . Finally, we repeated our analyses using only patients with ISUP 1 or 2 at biopsy ( $n$ : 78) and using as outcome an upgrading to an ISUP  $\geq 3$ . The results are reported in sTable 1, only PHI (OR: 1.04; 95% CI: 1.01, 1.07) and PHId (OR: 2.43; 95% CI: 1.38, 5.03) were significantly associated with the outcome. At the DeLong test, the model including PHId demonstrated the highest accuracy (0.81 vs  $\leq 0.78$ ,  $p < 0.05$ ).

## DISCUSSION

In this study, we evaluated the ability of PHI and PHId to predict adverse pathological features and ISUP upgrading in patients who underwent RP. Our results demonstrate a complementary role for these biomarkers. PHI emerged as the strongest independent predictor of adverse pathological features at RP, outperforming PSA, PSA<sub>d</sub>, and PHId in multivariable models. Specifically, PHI was significantly associated with pT  $\geq 3$  and/or pN1 disease, with a marked increase in risk observed above a threshold value of 40, underscoring its potential utility in preoperative risk stratification. Conversely, PHId was independently associated with ISUP upgrading, showing the highest predictive accuracy. Of particular interest is that the risk of upgrading increased sharply for PHId value  $> 2.5$  among patients with PI-RADS 3 lesions, underscoring its potential utility in preoperative risk stratification.

The upgrading rate observed in our cohort (59%) appears higher than that reported in many contemporary MRI-era series, where rates typically range between 20% and 40%. However, this finding should be interpreted in the context of the specific characteristics of our study population. First, our analysis was restricted to patients undergoing radical prostatectomy, thereby introducing an inherent selection bias toward individuals with clinically suspected higher-risk disease. Second, the vast majority of patients (96%) presented with MRI-visible lesions, with a predominance of PI-RADS 4–5 findings, reflecting a cohort enriched for biologically aggressive tumors. Third, despite the adoption of mpMRI-guided biopsy strategies, prostate biopsy remains subject to sampling limitations and intratumoral heterogeneity, which may lead to underestimation of tumor grade. Finally, the use of a sensitive definition of upgrading, based on any increase in ISUP grade, may have further contributed to the observed rate. Importantly, rather than representing a

methodological limitation, this context may highlight the clinical relevance of PHId as a tool capable of identifying patients at higher risk of pathological misclassification at biopsy and, consequently, more aggressive disease at final pathology.

Identifying PCa patients who are more likely to harbor adverse pathological features—namely, high-grade disease, extraprostatic extension, and lymph node involvement—is critical for appropriate patient selection, treatment planning, and clinical decision-making. Accurate preoperative risk stratification may guide the choice of surgical strategy and help balance oncologic control with functional preservation. For instance, this information is particularly relevant when selecting candidates for nerve-sparing RP, as well as when considering alternative management options such as active surveillance or focal therapies. MRI has been proposed as a staging tool to identify men at higher risk of adverse pathological characteristics [22, 23]. However, its relatively low sensitivity limits its standalone clinical utility [23]. Similarly, several prediction models integrating MRI findings with biopsy and clinical variables have been developed to estimate the risk of pathological upstaging and upgrading, but their performance remains only moderate [24–27]. In this context, our findings support the potential added value of incorporating PHI and PHId into future predictive tools aimed at improving the identification of patients with adverse pathological features.

In this context, the potential role of PHI and PHId is of particular interest, as they may assist urologists in identifying patients who, although apparently eligible for active surveillance according to standard clinical criteria, harbor biologically aggressive disease that may warrant early definitive treatment. The indolent clinical course observed in a large proportion of PCa cases underpins the widespread adoption of active surveillance as a strategy designed to defer or avoid radical treatment and associated morbidity in appropriately selected patients [28]. However, the success of active surveillance is critically dependent on accurate baseline risk stratification and on reliable tools capable of identifying disease progression early enough to justify therapeutic escalation. Traditionally, baseline risk stratification relies on PSA assessment, DRE, and prostate biopsies. While mpMRI has markedly improved the detection of clinically significant cancer and has become the reference imaging tool for active surveillance pathways, its performance remains limited by inter-reader variability, heterogeneity in acquisition protocols, and unequal access to high-quality scanners across centers [7]. In this context, our findings suggest the potential role of PHI and PHId as a practical, non-invasive tool to strengthen risk stratification. By helping identify men whose disease is more likely to harbor adverse pathology, PHI and PHId may support clinicians in distinguishing patients suitable for active surveillance from those who may benefit more from upfront definitive treatment.

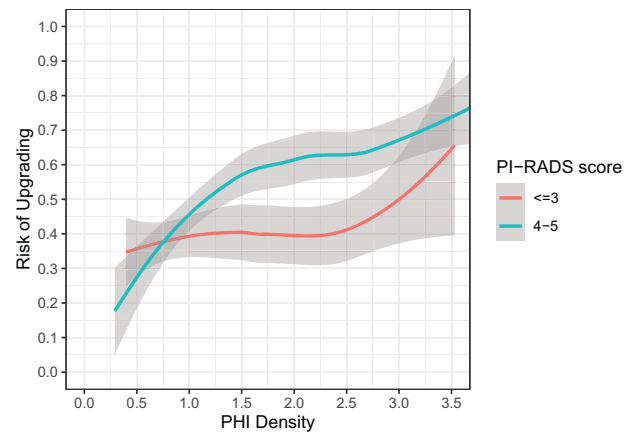
Our results suggest that PHI may contribute, together with other clinical and radiological variables, to identifying patients

**Table 4.** Multivariable models to evaluate the association between markers (PHI, PHI Density, PSA, PSA Density) and upgrading at radical prostatectomy.

| Characteristic                 | PHI             |                     |         | PHI density     |                     |         | PSA             |                     |         | PSA density     |                     |         |
|--------------------------------|-----------------|---------------------|---------|-----------------|---------------------|---------|-----------------|---------------------|---------|-----------------|---------------------|---------|
|                                | OR <sup>a</sup> | 95% CI <sup>a</sup> | p value | OR <sup>a</sup> | 95% CI <sup>a</sup> | p value | OR <sup>a</sup> | 95% CI <sup>a</sup> | p value | OR <sup>a</sup> | 95% CI <sup>a</sup> | p value |
| <b>Marker</b>                  |                 |                     |         |                 |                     |         |                 |                     |         |                 |                     |         |
| Positive DRE                   | 1.01            | 1.00, 1.03          | 0.068   | 1.54            | 1.10, 2.29          | 0.019   | 0.89            | 0.72, 1.10          | 0.3     | 22.4            | 0.50, 1,728         | 0.13    |
| PI-RADS score                  | 2.05            | 0.39, 13.0          | 0.4     | 2.26            | 0.44, 14.4          | 0.3     | 2.22            | 0.44, 13.3          | 0.3     | 2.23            | 0.47, 12.9          | 0.3     |
| ≤3                             | —               | —                   | —       | —               | —                   | —       | —               | —                   | —       | —               | —                   | —       |
| 4–5                            | 2.83            | 1.03, 7.90          | 0.044   | 2.40            | 0.85, 6.83          | 0.10    | 3.82            | 1.36, 11.2          | 0.012   | 2.63            | 0.93, 7.53          | 0.068   |
| <b>Gleason Group at biopsy</b> |                 |                     |         |                 |                     |         |                 |                     |         |                 |                     |         |
| 1                              | —               | —                   | —       | —               | —                   | —       | —               | —                   | —       | —               | —                   | —       |
| 2–3                            | 1.87            | 0.69, 5.31          | 0.2     | 1.88            | 0.68, 5.41          | 0.2     | 1.99            | 0.73, 5.72          | 0.2     | 1.84            | 0.67, 5.25          | 0.2     |
| 4                              | 0.05            | 0.01, 0.18          | <0.001  | 0.04            | 0.01, 0.17          | <0.001  | 0.06            | 0.01, 0.22          | <0.001  | 0.06            | 0.01, 0.21          | <0.001  |
| <b>AUC</b>                     | 0.81            |                     |         | 0.83            |                     |         | 0.79            |                     |         | 0.80            |                     |         |

Patients with ISUP group 5 at biopsy were excluded.

<sup>a</sup>OR odds ratio, CI confidence interval.

**Fig. 2** Risk of upgrading by PHI density. Patients are grouped according to PI-RADS score (red: PI-RADS ≤ 3; blue: PI-RADS 4–5).

who would benefit from intensified pretreatment staging, particularly with respect to lymph node or distant metastatic risk.

Finally, the importance of equity across clinical settings should also be considered. While mpMRI access remains variable across institutions and geographic regions, PHI represents an accessible, laboratory-based solution that could contribute to more uniform diagnostic pathways and may help mitigate the economic burden associated with the increasing incidence of prostate cancer, in line with international policy recommendations [29–31]. The integration of PHI and PHI<sub>d</sub> into multivariable nomograms or artificial intelligence -based decision-support tools may further enhance precision risk stratification, helping to bridge technological disparities and providing reproducible decision support at scale [32, 33].

PHI is an FDA-approved biomarker for detecting PCa in men with PSA values between 2 and 10 ng/mL. Multiple studies have shown that PHI outperforms PSA alone in cancer detection [34–38], particularly by discriminating clinically significant from indolent disease [39, 40]. Consistently, further evidence indicates that PHI is effective in identifying tumors with higher Gleason scores [41, 42]. In addition, previous studies have suggested a relationship between PHI and tumor burden, supporting its potential role as a surrogate marker of tumor volume and biological aggressiveness, even in the surgical setting [43].

Our study aligns with these observations, demonstrating that PHI exhibits strong predictive performance in anticipating adverse pathological features. Compared with other biomarkers, such as 4Kscore, IsoPSA, or genomic urine assays (e.g., PCA3 or SelectMDx), PHI has several practical advantages: it is FDA-approved, analytically standardized, widely available on routine immunoassay platforms, and cost-effective [29, 39, 41, 44]. Economic analyses performed in biopsy pathways have demonstrated improvements in quality-adjusted survival and meaningful reductions in diagnostic expenditures when PHI is integrated as a triage tool. These data echo broader health-policy priorities aimed at managing the anticipated global rise in PCa cases while limiting unnecessary imaging utilization and preserving healthcare resources [45].

Notwithstanding these strengths, our investigation has limitations that should temper interpretation. The sample size, although representative, remains relatively limited and monocentric, and validation in larger, ethnically diverse cohorts [46] is required to generalize cut-offs across populations. Although blood sampling was performed in proximity to surgery, we acknowledge that residual effects of prior prostate biopsy on PSA and its derivatives cannot be entirely excluded. Post-biopsy PSA fluctuations may persist for several weeks and could have introduced a degree of measurement variability, representing a potential source of non-

differential bias in our analyses. Another potential limitation relates to the assessment of lymph node status. Pelvic lymph node dissection (PLND) was performed according to a risk-adapted strategy based on preoperative nomograms, as recommended by the EAU guidelines, resulting in a proportion of pNx cases. As pN1 was included in the composite endpoint of adverse pathological features, this may have introduced a degree of verification bias. However, it should be noted that the rate of pN1 among patients not undergoing PLND according to the Briganti nomogram is very low and may reasonably be considered as pN0 [47]. Moreover, the majority of adverse pathological events in our cohort were driven by extraprostatic extension (pT  $\geq$ 3), which likely reduces the impact of incomplete nodal assessment on the overall findings. Additionally, the study was not longitudinally designed to assess clinical endpoints such as biochemical recurrence, metastasis, treatment-free survival, or patient-reported outcomes, variables that would more directly capture the impact of PHI-based decisions on long-term disease control and quality of life. Finally, we did not evaluate how PHI compares with genomic tissue assays or combined biomarker-mpMRI algorithms, both of which represent emerging standards in surveillance refinement [48].

Future research should therefore focus on the utility of PHI and PHId in identifying candidates for active surveillance, as well as in detecting patients on active surveillance who are at high risk of reclassification during follow-up. This should ideally be addressed through prospective, multicentre trials evaluating PHI-guided follow-up strategies, randomizing men to PHI-informed versus standard surveillance arms. Such trials should explore whether PHI-based decision thresholds translate into reductions in unnecessary repeat biopsies, improved metastasis-free survival, or better psychological outcomes. Moreover, incorporating PHI and PHId into machine-learning frameworks –together with PI-RADS score, tumor volume estimates, and digital pathology signatures– may enable predictive tools capable of tailoring surveillance intensity to individualized biological risk [32]. Conversely, the observed association between PHI and adverse pathological features suggests that future studies should also investigate the role of PHI in identifying patients at increased risk of lymph node involvement and distant metastases, who may benefit from intensified staging before definitive treatment.

In conclusion, our study reinforces the clinical utility of PHI and PHId as complementary predictors of PCa aggressiveness. PHI appears particularly valuable in identifying men at risk of adverse pathological features, while PHId sensitively captures grade upgrading potential. Integrating these biomarkers into active surveillance pathways has the potential to refine patient selection, reduce overtreatment, prioritize imaging and biopsy resources, and support individualized therapeutic decision-making. Larger prospective studies are warranted to consolidate these findings and to define the optimal framework for operationalizing PHI within precision-based prostate cancer management.

## DATA AVAILABILITY

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

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## AUTHOR CONTRIBUTIONS

DT and MFe conceived and designed the study. DT, MFi, CP, and RS performed the laboratory analyses and contributed to data acquisition. FP, GF, RB, FCh, MMA, MT, LMIJ, ES, VM, and AT contributed to the clinical assessment, patient enrollment, and collection of clinical and pathological data. FCr, CI, FDG, BB, AC, RA, and CdN contributed to the interpretation of the clinical findings and critically revised the manuscript for important intellectual content. FP and MFe performed the statistical analysis and interpreted the results. DT drafted the original manuscript and supervised the study. All authors reviewed, edited, and approved the final version of the manuscript.

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## COMPETING INTERESTS

Professor Terracciano reports receiving honoraria for scientific lectures, educational grants, and advisory board activities from Fujirebio, Novartis, and Roche, outside the submitted work. All other authors declare no conflicts of interest.

## ETHICAL STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and approved by the institutional Ethics Committee of the University of Naples Federico II (118/20, approved 5 July 2020).

## CONSENT

Participants provided written approved consent.

## ADDITIONAL INFORMATION

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