Improving the Prediction of Pathologic Outcomes in Patients Undergoing Radical Prostatectomy: The Value of Prostate Cancer Antigen 3 (PCA3), Prostate Health Index (Phi) and Sarcosine

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Abstract. *Background/Aim: Several efforts have been made to find biomarkers that could help clinicians to preoperatively determine prostate cancer (PCa) pathological characteristics and choose the best therapeutic approach, avoiding over-treatment. On this effort, prostate cancer antigen 3 (PCA3), prostate health index (phi) and sarcosine have been presented as promising tools. We evaluated the ability of these biomarkers to predict the pathologic PCa characteristics within a prospectively collected contemporary cohort of patients who underwent radical prostatectomy (RP) for clinically localized PCa at a single high-volume Institution. Materials and Methods: The prognostic performance of PCA3, phi and sarcosine were evaluated in 78 patients undergoing RP for biopsy-proven PCa. Receiver operating characteristic (ROC) curve analyses tested the accuracy (area under the curve (AUC)) in predicting PCa pathological characteristics. Decision curve analyses (DCA) were used to assess the clinical benefit of the three biomarkers. Results: We found that PCA3, phi and sarcosine levels were significantly higher in patients with tumor volume (TV) ≥0.5 ml, pathologic Gleason sum (GS) ≥7 and pT3 disease (all p-values ≤0.01). ROC curve analysis showed that phi is an accurate predictor of high-stage (AUC 0.85 [0.77-0.93]), high-grade (AUC 0.83 [0.73-0.93]) and high-volume disease (AUC 0.94 [0.88-0.99]). Sarcosine showed a comparable AUC (0.85 [0.76-0.94]) only for T3 stage prediction, whereas PCA3 score showed lower AUCs, ranging from 0.74 (for GS) to 0.86 (for TV). Conclusion: PCA3, phi and sarcosine are predictors of PCa characteristics at final pathology. Successful clinical translation of these findings would reduce the frequency of surveillance biopsies and may enhance acceptance of active surveillance (AS).*

Prostate-specific antigen (PSA) screening leads to an increasing number of men identified with low-stage and low-grade disease in the setting of prostate cancer (PCa). These subjects are good candidates for treatments other than radical prostatectomy (RP), such as active surveillance (AS) or focal therapy (1). The best treatment chosen should maximize oncologic and functional outcomes. Circulating and urinary biomarkers represent a promising approach to identify men with apparently low-risk biopsy pathology but who harbor potentially aggressive tumors unsuitable for active surveillance. Recent studies have shown that the Prostate Health Index (phi; [preoperative prostate-specific antigen isoform (p2PSA)/free PSA] x √ total PSA (tPSA)) improve the accuracy of tPSA and percentage of free PSA (%fPSA) in predicting the presence of PCa at prostate biopsy and it is also related to PCa aggressiveness at biopsy (2-7) and at RP (8, 9).

Conflicting results have been reported for predicting the pathologic PCa characteristics of prostate cancer antigen 3 (PCA3) (9-11).

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Sreekumar *et al*. (12) showed that sarcosine in prostate tissue is associated with prostate cancer progression. Since sarcosine was originally shown to be a mechanistic biomarker of proliferation and invasion (13), it could potentially serve as biomarker for progressive disease,

Currently, no evidence is available on the role of PCA3, phi and sarcosine in the prediction of PCa aggressiveness at final pathology after RP within a prospectively-collected contemporary cohort of patients.

The aim of this prospective observational study is to assess the accuracy of PCA3, phi and sarcosine in predicting pathological features in the same cohort of patients who underwent RP for clinically-localized PCa.

Materials and Methods

Study population. We evaluated 78 patients with biopsy-proven, clinically localized PCa, who were prospectively enrolled between January 2013 and December 2013 and underwent, within 3 months, laparoscopic or robot-assisted laparoscopic RP at one tertiary care institution (National Institute of Cancer, Naples, Italy). None of the study patients received neoadjuvant hormonal therapy (anti-androgens or luteinizing hormone-releasing hormone analogues or antagonists) and/or other hormonal preparations (*i.e*. 5-alpha reductase inhibitors) that could alter the PSA values. The local hospital ethics committee approved the study protocol (M2/33) and all participants signed written informed consent.

The primary end-point of the current study was to assess whether Phi, PCA3 and sarcosine significantly discriminate men with tumor volume (TV) ≥ 0.5ml, pathologic Gleason sum ≥7 and T stage ≥2 and might be used to stratify the risk of harboring clinically insignificant or more aggressive PCa at final pathology.

Measurement of biomarkers. Blood specimens were collected before initial prostate biopsy. Whole blood was allowed to clot before serum was separated by centrifugation. Serum aliquots were stored at -80°C until samples were processed according to Semjonow *et al*. (14). Specimens were analyzed in blinded fashion for PSA, fPSA and p2PSA by an Access 2 Immunoassay System analyzer (Beckman Coulter, Brea, CA, USA).

First catch urine samples were also collected before prostate biopsy and following an attentive digital rectal exam (DRE) (three strokes per lobe) and stored in a Progensa urine specimen transport kit as described by Groskopf *et al*. (15). Urine samples were processed and tested to quantify *PCA3* mRNA and *PSA* mRNA concentrations using the Progensa *PCA3* assay (Gen-probe, San Diego, CA, USA). The PCA3 score was calculated as *PCA3* mRNA/*PSA* mRNA ×1,000. Sarcosine was measured using the Sarcosine Assay Kit (Biovision, Mountain View, CA, USA) following the manufacturer's instructions.

Phi index and PCA3 score, for each single patient, were determined in the same laboratory (University of Naples, Naples, Italy), sarcosine was measured at the University of Bari, Italy. RP specimens were evaluated using serially 3-mm sectioned whole-mount specimens according to the Stanford protocol and primary and secondary GS were assigned by an experienced uropathologist at each center, blinded to the biomarkers value, according to the 2005 consensus conference of the International Society of Urological Pathology definitions. All tumor foci were identified and cumulative TV was assessed using computerized planimetry accounting for all tumor foci.

Statistical analysis. All statistical analyses were performed in R (R Development Core Team, 2012).

Median [min–max] values were used to describe continuous variables, whereas categorical variables were reported as number of occurrences and percentages. The Mann-Whytney and Chi-square test were used to assess differences among PCa patients. The predictive accuracy of the single markers was measured by the Area under the receiver operating characteristic (ROC) curve (area under the curve (AUC)). Differences in diagnostic performance were assessed using the De Long method. Because of the large number of the pairwise comparisons among markers and to control the family-wise error rate at level α=0.05, the significance of the DeLong test statistics was appraised by using the adaptive Bonferroni procedure (16). Finally, decision curve analysis (DCA) (17) was used to assess the net benefit (calculated by subtracting the proportions of false positives from the proportion of true positives, the former being weighted by the relative harms of false positives and false negatives results) of using PCA3, phi and sarcosine in guiding treatment decision making. Statistical significance was set at $p<0.05$ (unless in AUC pairwise comparisons as stated above).

Results

The demographic and clinical characteristics of the study population are listed in Table I. All patients had clinical stage T1-T2 with a preoperative PSA median value of 6.7 ng/ml. Biopsy GS ≤7 was found in 68 (87%) subjects. At final pathology, TV ≥0.5 ml was observed in 13 patients (16.7%), pathologic GS ≥7 was found in 48 patients (60.7%) and pT3 was diagnosed in 22 (28.2%) patients.

Figure 1 shows the comparison of biomarkers according to study end-points. In detail, PCA3, phi and sarcosine were significantly increased in subjects with TV ≥0.5 ml, pathological Gleason score ≥7 and pT3 stage (all *p*-values <0.01). Predictive accuracy was quantified by ROC curve analysis for each outcome of interest (Figure 2). The largest AUC's were obtained with phi for tumor volume (0.94; 95% confidence interval (CI)=0.88 to 0.99) and GS (0.94; 95% (CI)=0.88 to 0.99), whereas same AUCs values were found for phi (0.85; 95% (CI)=0.77 to 0.93) and sarcosine (0.85; 95% (CI)=0.76 to 0.94) for pathological stage. No significant differences in pairwise comparison of AUCs were observed, except for sarcosine *vs*. phi for TV outcome (p=0.004).

Results of DCA analysis are reported in Figure 3. Phi and PCA3 clearly result in greater net benefit compared to sarcosine in TV ≥0.5 ml and GS ≥7 probability, when it is plotted against various threshold probabilities. Conversely, sarcosine had an increased net benefit against PCA3 and phi for pT3 tumor, which endures for the range of threshold probabilities 25-50%.

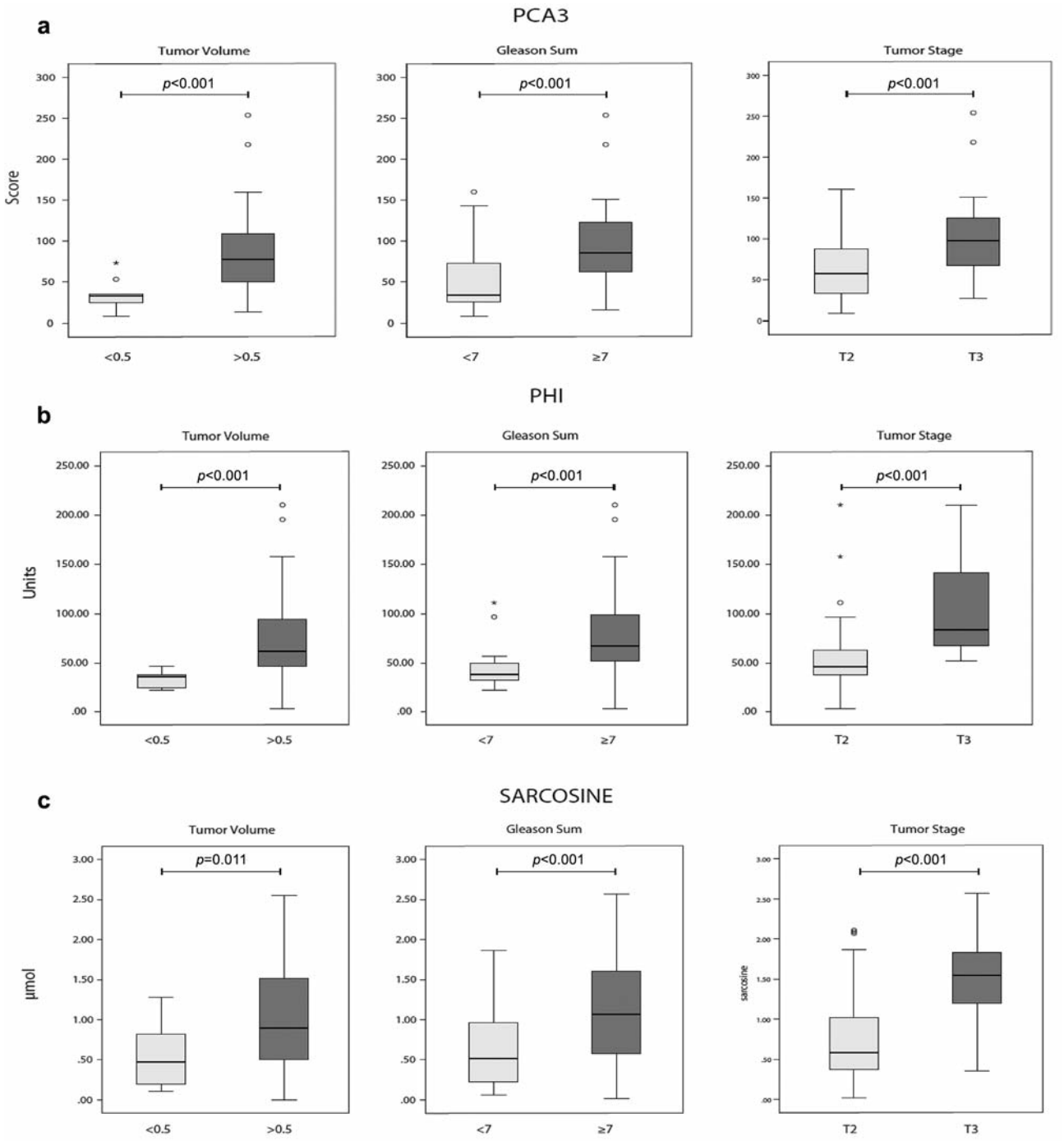

Figure 1. *Box plot showing the distribution of PCA3 values (a), phi values (b) and sarcosine (c), each relative to tumor volume, Gleason sum, tumor stage. Data are shown as median (horizontal line in the box) and Q1 and Q3 (borders of the box). Dots represent outlier values and asterisks represent extreme values. Q1, 25th percentile; Q3, 75th percentile; IQR (interquartile range), Q3-Q1.*

Discussion

The preoperative anticipation of histological prognostic features at RP would affect the therapeutic approaches to localized PCa, such as the decision for AS, preservation of neurovascular bundles and performing pelvic lymph node dissection.

Several patients with apparently low-risk PCa might harbor unfavorable disease due to inaccuracies in currently

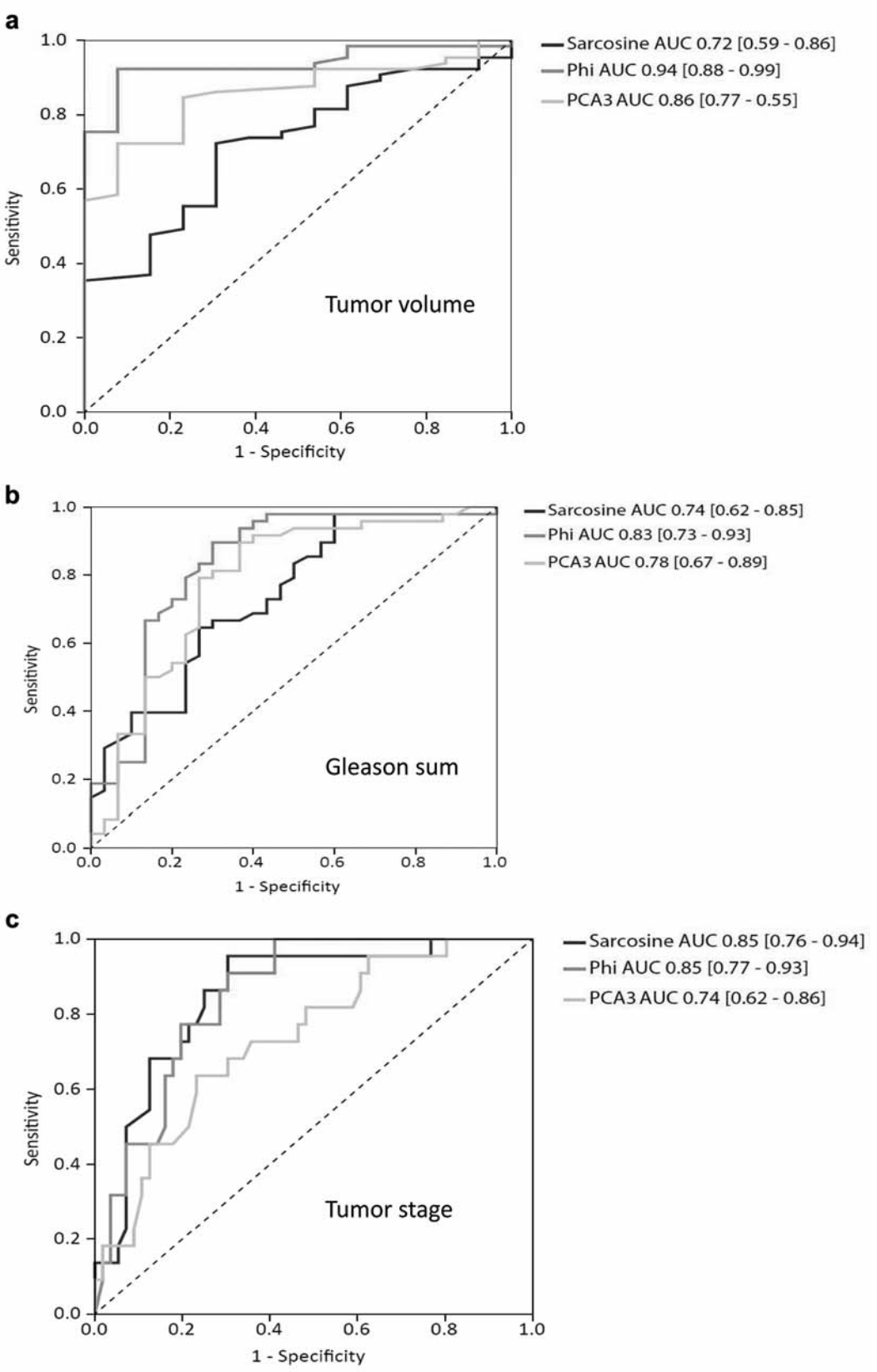

Figure 2. *Receiver operating characteristic (ROC) curve of all the analyzed markers as predictors of tumor volume (a), Gleason sum (b), tumor stage (c).*

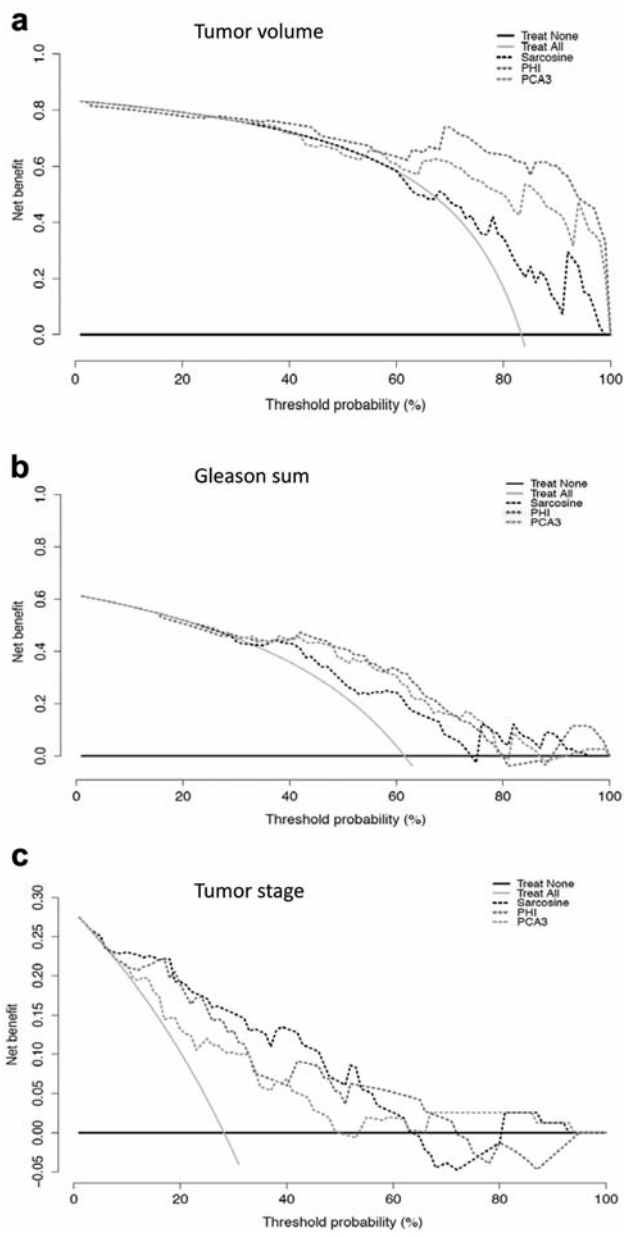

Figure 3. *Decision curve analysis of the effect of PCA3, phi and sarcosine on the detection of tumor volume ≥0.5 ml (a), Gleason sum ≥7 (b) and pT3 (c) at radical prostatectomy.*

Table I. *Clinical characteristics of the study population.*

Age	
Mean±Std. Dev.	64±5.2
Median [Range]	65 [49; 72]
BMI	
Mean±Std. Dev.	26.2±4.2
Median [Range]	26 [19.4; 36]
tPSA	
Mean±Std. Dev.	6.7±2.9
Median [Range]	6.13 [2.11; 17.86]
fPSA	
Mean±Std. Dev.	1±0.5
Median [Range]	0.88 [0.27; 3.3]
f/tPSA	
Mean±Std. Dev.	0.2±0.1
Median [Range]	0.16 [0.05; 0.9]
Phi	
Mean±Std. Dev.	69.9±45.3
Median [Range]	54.26 [3.05; 210.02]
PCA3	
Mean±Std. Dev.	75.9±47.1
Median [Range]	71.5 [8; 254]
Sarcosina	
Mean±Std. Dev.	1±0.6
Median [Range]	0.85 [0.02; 2.57]
Biopsy Gleason Sum	N (%)
≤6	53 (68.0)
7	15 (19.2)
≥8	10 (12.8)
Clinical Stage	N (%)
cT1c	71 (91)
cT2a	7 (9)
Prostatectomy Gleason Sum	N (%)
6	30 (38.5)
7	34 (43.6)
≥8	14 (18.0)
Pathological Stage	N (%)
pT2	56 (71.8)
pT3	22 (28.2)
Tumore Volume	
≥0.5	13 (16.7)
<0.5	65 (83.3)

BMI= Body mass index; tPSA= total PSA; fPSA= free PSA.

used tools. Therefore, several efforts have been made to find preoperative biomarkers that could help clinicians determine PCa pathological characteristics.

In the current study, we investigated the accuracy of PCA3, phi, and sarcosine in predicting PCa characteristics at final pathology in a same cohort of patients who underwent RP.

Although previous studies (8, 9, 10, 18, 19) have separately determined the accuracy of these markers in predicting pathological features of PCa at the time of RP, to the best of our knowledge, this is the first study to investigate these relationships in the same cohort of patients.

In this study, we showed that phi, PCA3 and sarcosine were independent predictors of TV ≥0.5 ml, GS ≥7 and pT3 stage. ROC curve analysis showed that phi, PCA3 and sarcosine have a good accuracy in the prediction of these three pathological outcomes. Of note, phi showed the largest AUCs and only for the prediction of TV there is a statistically significant difference between phi and sarcosine. A larger number of samples may probably allow reaching statistical significance. DCA analysis favored the use of phi

and PCA3 to predict TV and high GS for a wide range of threshold probabilities, whereas sarcosine to identify high stage tumor for a defined range of threshold probabilities lower than 50%.

Several studies have aimed to clarify, in separate study cohorts, the potential role of these new biomarkers in predicting pathological features of PCa at final pathology. The most extensively studied biomarker was PCA3. The majority of studies supported the hypothesis that PCA3 score was a significant predictor of low-volume disease (10, 11, 19-21), whereas several authors demonstrated limited ability of PCA3 in predicting aggressive disease, defined as GS sum ≥7 (10, 19, 22). According to Whitman *et al.* (11), PCA3 is an independent predictor of extra-capsular extension (ECE) on the RP specimen. Durand *et al.* (10) found a significant difference in PCA3 scores between the pT2 tumor group and the pT3/4 tumor group, probably due to large TV, strongly linked to ECE risk.

Recently, two different reports (8, 9) showed that phi is an accurate predictor of large TV, high-grade and high-stage PCa at RP.

Finally, Lucarelli *et al.* (18) showed that higher serum sarcosine levels were significantly associated with low- and intermediate-grade tumors in men with PSA <4 ng/ml. Conversely, tissue (23) and urinary (24) sarcosine content cannot be considered suitable predictors of tumor aggressiveness or biochemical recurrence.

In the present study, we provide evidence that urinary PCA3 score, phi and serum sarcosine had a good predictive value of histopathological findings. In particular, ROC curve analysis showed that phi is significantly more accurate than sarcosine in the prediction of TV. This is a relevant issue since smaller tumors are thought to be less aggressive and less frequently associated with progression (25).

Our DCA indicated that the clinical benefit in the prediction of different aspects of PCa aggressiveness is quite different for the three biomarkers. In fact, PCA3 and phi seem to provide a higher benefit to predict TV and GS, whereas sarcosine has an increased clinical benefit for high-stage cancer risk. This issue is of importance in order to improve the identification of cancers that require intervention, supporting clinicians in the choice of therapeutic strategy.

Even if these results are regarded as preliminary, PCA3, phi and sarcosine could have an important role in selecting men with insignificant PCa representing about one-third of new-diagnosed tumors (26). These patients may be candidates to prostate-sparing managements, such as active surveillance (AS) allowing to delay or avoid radical treatment and its related morbidity without compromising survival (27).

The strength of our study resides in a single-centre prospective cohort study in which, for the first time, the prognostic performance of the three biomarkers are contextually evaluated on RP histological findings.

Despite its strength, this study is limited by the relatively small size of our cohort. In addition, we did not evaluate the inclusion of PCA3, phi and sarcosine in predictive nomograms, which are often used for PCa prognosis, neither did we perform a comparison with the currently used tools. Consequently, further and larger studies are required to externally validate our findings and to compare or integrate these biomarkers with wide-used nomograms and risk calculators.

Conclusion

In the current study, we showed that, in a same cohort of patients who underwent RP, PCA3, phi and sarcosine were good predictors of large, high-grade and high-stage tumor. In clinical practice, these biomarkers could meaningfully be considered as important tools in patients' risk stratification and best treatment selection.

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