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ORIGINAL ARTICLE

Urinary PSA-ZINC biomarker outperforms standard of care in early detection of prostate cancer

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ABSTRACT

BACKGROUND: Urine is a promising biological fluid for prostate cancer (PCa) diagnostics due to its non-invasive collection and wide range of biomarkers. The aim of this study was to assess the role of urinary PSA (uPSA) and urinary Zinc (uZinc) as biomarkers for the diagnosis of PCa in combination with routine parameters of standard of care (SOC – blood PSA, abnormal DRE, age) and MRI in patients candidates for prostate biopsy.

METHODS: Urine samples after prostatic massages were collected from men with suspected PCa scheduled for prostate biopsy. Quantification of uPSA was performed by ECLIA platform and confirmed by ELISA assay, while uZinc measurement was evaluated by ICP-MS and confirmed by colorimetric in vitro assay. Six multivariate logistic regression analysis were performed to assess diagnostic performance of uPSA and uZinc (urine), SOC and MRI alone, and combination of MRI+SOC, MRI+urine and SOC+MRI+urine. The discriminative power of the logistic models was assessed by calculating the area under the receiver operating characteristic (ROC) curves (AUC).

RESULTS: Two hundred thirty-eight patients were included in the analysis; 145 of them were diagnosed with PCa. Urine test showed a better discrimination of HS from CP, in respect of uPSA and uZinc alone, both for PCa of any grade and Gleason Score ≥7 (4+3) (AUC 0.804 and 0.823 respectively). ROC curve combining SOC+MRI+urine showed an AUC=0.882, that is statistically different from SOC or MRI alone, or MRI+SOC (P=0.0001, P=0.0001, and P=0.008 respectively). PCa risk algorithm designed considering SOC+MRI+urine results in potential reduction of 57% of unnecessary biopsies compared to the current standard parameters.

CONCLUSIONS: The loss of uPSA and Zinc production and secretion during neoplastic transformation of the prostate could potentially represent a hallmark of PCa. Its combination with age, PSA and DRE, as well as with mpMRI could represent an interesting approach to improve the diagnostic accuracy of PCa.

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KEY WORDS: Diagnosis; Prostatic neoplasms; Mass screening; Urine; Biopsy; Magnetic resonance imaging.

The decline in prostate cancer (PCa) mortality over recent years can be attributed primarily to the widespread adoption of early screening examinations.[1] Nonetheless, the ongoing debate in the realm of PCa screening revolves around the delicate balance between identifying potentially life-threatening PCa cases that can benefit from therapy and avoiding unnecessary treatment for low-risk cancers that might lead to complications.[2]

The term "clinically significant" is widely used to differentiate PCa (csPCa) that may cause morbidity and/or death from potentially harmless tumor subtypes. This differentiation is critical because "non-clinically significant" PCa (ncsPCa) that cause no damage or symptoms are commonly found.Despite the concerns about over-diagnosis and over-treatment serum PSA test remains the only laboratory exam for this pathology.

Currently, multiparametric Magnetic Resonance Imaging (mpMRI) has been incorporated into European guidelines[3] as a recommended diagnostic tool to enhance the ability to detect csPCa,[4] while in the past decade, there has been extensive research into various serum and urine biomarkers.[5] Although several novel tests have become commercially available, none of them has been routinely adopted due to limited evidence supporting their benefits over the established standard of care (SOC) for the general population.[6]

During the process of neoplastic transformation, glandular cells undergo progressive alterations. This observation suggests that the transformation of prostate tissue can impact the composition of prostatic fluid. Based on this, the measurement of molecules normally and physiologically produced by the prostate in urine could provide useful information.[7]

It was observed that the amount of Zinc in the urine (uZinc) was lower in patients with cancer compared to those with a negative biopsy result, with a gradual decline as the disease advanced.[8] Similarly, the mean levels of PSA in urine (uPSA) in PCa were lower when compared to those in healthy individuals, with a declining trend associated with increasing tumor stage.[9]

In light of these findings, the aim of this study is to find out if the use of uPSA and uZinc as biomarkers in combination with routine parameters is able to better identify men with PCa among candidates for prostate biopsy.

Materials and methods

Study population and study design

Men candidate to first prostate biopsy were prospectively enrolled in the study. Prostate biopsy indication was decided according to European Guidelines[3] based on age, PSA levels, DRE findings and mpMRI suspicious for PCa.

Inclusion criteria were: age <75 years, PSA>4 ng/mL and unsuspected DRE; Suspected DRE; Suspected magnetic resonance imaging; PSA>20 ng/mL; No previous prostate biopsy in the last 6 months or diagnosis of prostate carcinoma.

The study was conducted according to the Declaration of Helsinki principles. Human investigations were performed after approval of the study by the Scientific Ethics Committee of San Luigi Gonzaga Hospital – AA.SS.LL. TO3 – TO4 – TO5 (Prot. No. 6387). Written informed consent was received from each participant before inclusion in the study and specimens were anonymized before analysis.

Urine samples were collected after a standardized DRE and before the biopsy. Histological specimens consisted of 6-20 core biopsy samples obtained with ultrasound guidance for both standard and fusion biopsies. Samples were divided into 3 groups, on the base of histological findings and Gleason Score (GS): healthy individuals; patients with GS≤7 (3+4) – ISUP<3; patients with GS≥7 (4+3) – ISUP≥3.

Sample collection, processing and analysis

For the sample collection, 45 mL of voided urine were collected after prostate massage to extract prostatic secretions, through three digital compressions in each lobe starting from the base, moving downwards to the middle and the apex in a timelapse of 30 seconds.

After a gentle shake of the sample, an aliquot of 15 mL was stored at -80 °C within 5 minutes from collection.

Urinary samples collected before prostate biop-

sy were tested for the presence of uPSA and uZinc.

Quantification of uPSA was performed by ELISA assay (R&D Systems, R&D Systems, Inc., Minneapolis, MN) after optimization on urine matrix following the manufacturer's instruction.[9] uZinc measurement was determined by inductively coupled plasma mass spectrometry (ICP-MS).

The concentration of both uPSA and uZinc has been normalized on the volume of urine collected.

Study endpoints and statistical analysis

We tested differences in uPSA, uZinc and routine parameters (blood PSA, age, DRE outcome, PiRADS) between healthy individuals and patients with prostate cancers of any grade.Pearson's correlation analysis was performed to determine whether there was correlation between urinary biomarkers, routine parameters and GS.

We performed univariate and multivariate logistic regression analysis to evaluate the uPSA and uZinc diagnostic performance of detecting all PCa or PCa with GS≤7(3+4) (ISUP<3) or GS≥7(4+3) (ISUP≥3). The discriminative power of the logistic models was assessed by calculating the area under the receiver operating characteristic (ROC) curves (AUC).

We compared first the diagnostic performance of three different multivariate logistic regression models including known risk factors for prostate cancer. The first model, denominated SOC model included serum PSA levels, age at biopsy and abnormal DRE. The second model (Urine model) included uPSA and uZinc levels. The third model (SOC+urine model) included both uPSA and uZinc levels as well as serum PSA, age and abnormal DRE.

Second, we generated four different multivariate logistic regression models by considering the PiRADS value (MRI), alone or in combination with the previous parameters to obtain MRI, MRI+SOC, MRI+urine, SOC+MRI+urine models.

Comparisons of AUCs provided by different models were determined using DeLong's method. In order to estimate potential optimism introduced by overfitting, the predictive models were internally validated through bootstrap method (1000 bootstrap samples). Statistical analyses were performed with MedCalc® Statistical Software version 19.8 (MedCalc Software Ltd., Ostend, Belgium).

TABLE I.—*Patient characteristics.*

Patients, N.	247
Evaluable samples, N. (%)	238 (96)
Age, years, mean (median; IQR)	69 (70; 64-75)
PSA, ng/mL, mean (median; IQR)	17.5 (7.4; 5.3-11)
DRE abnormal, N. (%)	74 (31)
PCa diagnosis, N. (%)	145 (61)
GS 6, N. (%)	9 (6.2)
GS 7 (3+4), N. (%)	57 (39.3)
GS 7 (4+3), N. (%)	40 (27.6)
GS 8, N. (%)	19 (13.1)
GS 9-10, N. (%)	20 (13.8)

PSA: prostate specific antigen; DRE: digital rectal examination; PCa: prostate cancer; GS: Gleason Score; IQR: InterQuartile Range.

Results

Patient characteristics

Among the N.=247 men initially assessed for eligibility, 9 urine samples were excluded from the study for difficulty with collection equipment. Ultimately, N.=238 men were included in this study.

The median age of the participants in the study was 70 years, with an interquartile range (IQR) of 64-75 years. The median PSA level was 7.4 ng/mL, with an IQR of 5.3-11 ng/mL. Approximately 31% of the men had abnormal digital rectal examination (DRE). Out of the total participants, 93 (39%) were found to be cancer-free (classified as healthy subjects), while 145 (61%) received a positive biopsy outcome. Among those with positive biopsy results, 9 (6.2%) had a GS of 6, 57 (39.3%) had a GS of 7 (3+4), 40 (27.6%) had a GS of 7(4+3), 19 (13.1%) had a GS of 8, and 20 (13.8%) had a GS of 9 or 10 (Table I).

Quantification of uPSA and uZinc

The ROUT method was developed as a method to identify outliers from nonlinear regression.[10] At first, we used it to identify uPSA value outliers in order to remove them (Q=0.1%): 1 for healthy, 5 for GS≤7 (3+4) and 3 for GS≥7 (4+3) (Figure 1A). The same for uZinc, for which out-

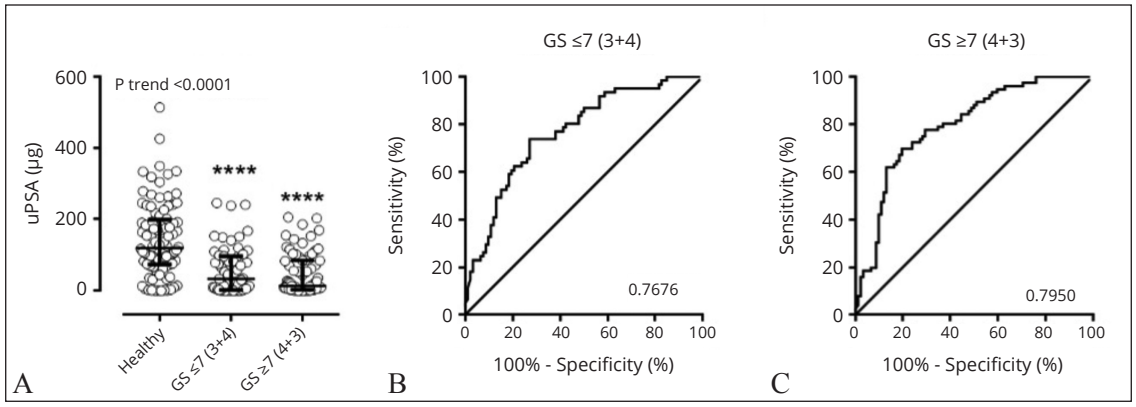

Figure 1.—Quantification of uPSA in different groups.

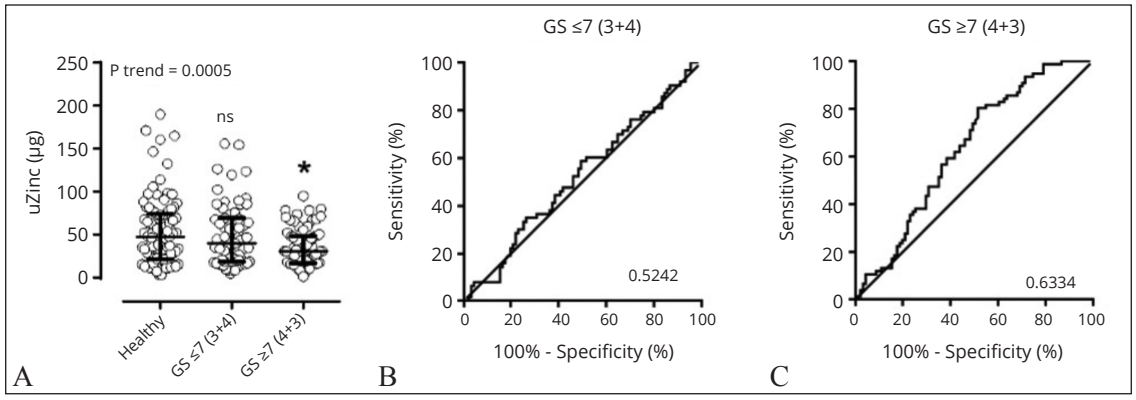

Figure 2.—Quantification of uZinc in different groups.

lier values were excluded: 2 for healthy, 2 for GS≤7 (3+4) and 3 for GS≥7 (4+3) (Figure 2A).

After outliers detection, 14 samples with uPSA and/or uZinc outlier value were excluded (N.=224).

TABLE II.—*Quantification of uPSA (A) and uZinc (B) in different groups.*

Diagnosis	Mean (µg)	Median (p25-p75)	P
(A) Quantification of uPSA in different groups.			
Healthy	142.4	119.7 (73.4-199.4)	ref
GS≤7 (3+4)	56.7	33.3 (1.9- 96.7)	0.0001
GS≥7 (4+3)	45.7	13.9 (3.3 – 84.8)	0.0001
P for trend<0.0001			
(B) Quantification of uZinc in different groups.			
Healthy	53.8	48 (21.8-74.9)	ref
GS≤7 (3+4)	49.9	40.7 (18.9– 69.6)	NS
GS≥7 (4+3)	35.1	30.9 (17.3 – 48.4)	0.0103
P for trend: 0.0005			

bPSA: blood prostate specific antigen; uZINC: urinary zinc; GS: Gleason Score; ref: Reference; NS: Not significant.

Quantification of uPSA revealed a mean concentration equal to 142.4 µg in healthy samples, 56.7 µg in GS≤7 (3+4) and 45.7 µg in GS≥7 (4+3) observing a gradual decrease in uPSA levels at increasing GS (Table II [A]). The mean levels of uPSA were significantly lower in patients with GS≤7 (3+4) and GS≥7 (4+3) compared to healthy subjects (P=0.0001) (Figure 1A, Table II [A]).

Subsequently, ROC curves were generated to assess the diagnostic utility of uPSA in GS≤7 (3+4) *vs.* healthy subjects and GS≥7 (4+3) *vs.* healthy subjects. The AUC for the ROC curve was statistically significant for both patient groups, with values of 0.768 and 0.795, respectively. This suggests that the assessment of uPSA effectively served as a reliable predictor for the presence of PCa of any grade (Figure 1B, C).

Quantification of uZinc revealed a mean con-

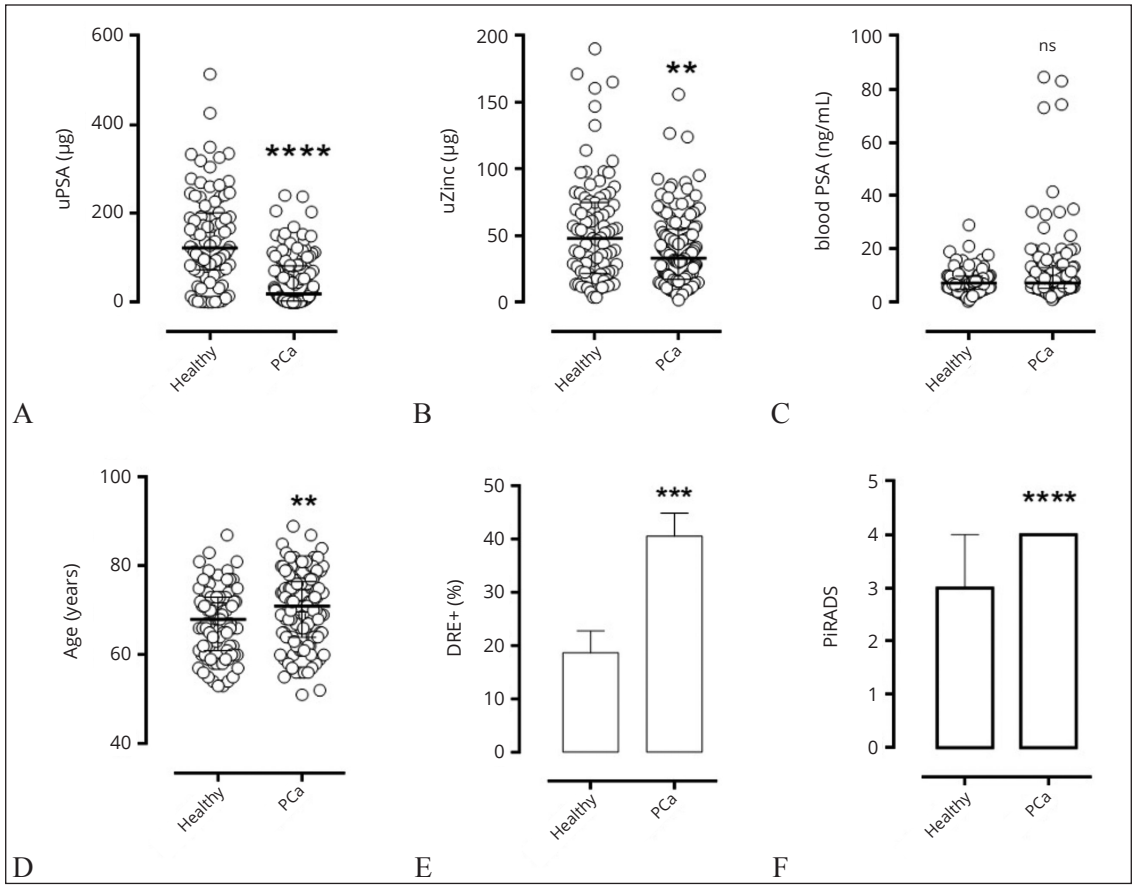

Figure 3.—Differences between healthy individuals and PCa patients for urine and standard parameters.

centration equal to 53.8 µg in healthy patients, 49.9 µg in GS≤7 (3+4) and 35.1 µg in GS≥7 (4+3) observing a gradual decrease in uZinc levels at increasing GS (Table II [B]). The mean levels of uZinc were significantly lower only in patients with GS≥7 (4+3) compared to healthy subjects (P=0.01). No differences were observed between patients with GS≤7 (3+4) and healthy subjects (Figure 2A, Table II [B]). uZinc displayed a mild diagnostic capability only in GS≥7 (4+3) patients (AUC=0.634), suggesting that uZinc loss could be a sign of advanced PCa (Figure 2B, C).

Our findings revealed statistically significant differences between healthy individuals and PCa patients in several parameters, including uPSA, uZinc, age, DRE, and PiRADS values (as shown in Figure 3, with P values of 0.0001, 0.0088, 0.0044, 0.0007, 0.0001, respectively), with the exception of blood PSA (bPSA).

There was no notable correlation discovered between urinary biomarkers and standard parameters, except for uPSA and PiRADS, which exhibited a significant correlation. Notably, a significant inverse correlation was detected between GS and both uPSA and uZinc (Supplementary Digital Material 1: Supplementary Table I).

These findings imply that a decrease in uPSA and uZinc levels may serve as an indicator of PCa progression when considered alongside routine indicators.

Diagnostic accuracy of uPSA and uZinc levels in PCa patients

To evaluate whether uPSA and uZinc can be considered biomarkers of PCa progression, we first evaluated their diagnostic capacity individually and then a model was defined considering the two

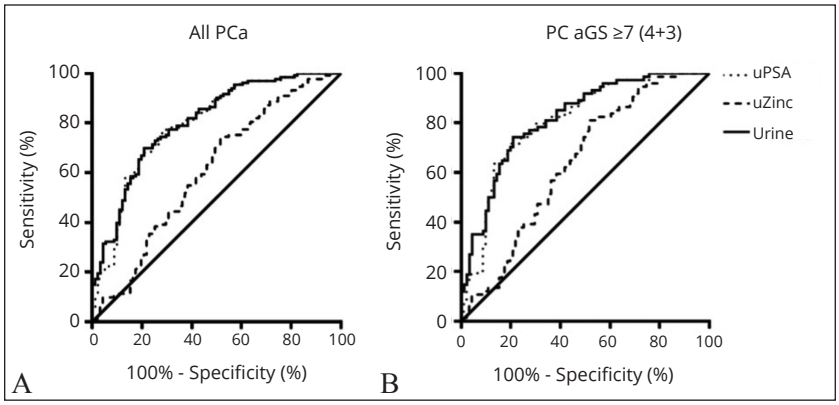

Figure 4.—Diagnostic performance of uPSA and uZinc alone or in combination.

molecules simultaneously to evaluate the possibility of representing a potential PCa signature.

We assessed the role of uPSA and uZinc, individually and in combination, as biomarkers for diagnosis of PCa of any grade or with a GS≥7 (4+3).

The analysis showed the AUC for uPSA and uZinc alone were 0.797 and 0.604 in All PCa and 0.807 and 0.638 in GS≥7 (4+3) respectively (Figure 4, Supplementary Digital Material 2: Supplementary Table II).

As shown in Supplementary Table II, the AUC of the combined detection of uPSA and uZinc (urine model) was higher for PCa of any grade and GS≥7 (4+3) (0.804 and 0.823 respectively) than the AUC of both biomarkers alone. In particular, the combination of uPSA and uZinc provides higher Specificity and Sensitivity compared to individual analyte both in PCa of any grade and in PCa GS≥7 (4+3).

Taken together, these results suggested that combined detection of uPSA and uZinc can provides better discrimination of healthy individuals from patients with prostate cancer.

Three logistic regression models were created and ORs calculated as shown in Table III. The routinary use of bPSA, Age and DRE (SOC model) is associated with PCa diagnosis with an OR of 2.8. It is interesting to notice that the introduction of urinary biomarkers (uPSA and uZinc) together (urine model) and their association with bPSA, age and DRE (SOC+Urine model) can increase the probability to find out PCa with OR of 7.4 and 11.1 respectively.

The predictive performance in detecting PCa for the three different models was evaluated by ROC curve analysis. Urine and SOC+Urine models provided an AUC of 0.804 and 0.843, respectively, that are statistically significantly different from SOC alone (P=0.0089 and P=0.0001, respectively); in addition, SOC+Urine model appears to be statistically significantly different from Urine model alone (P=0.023). Internal validation for the three models showed an optimism estimated of 0.001 (Figure 5, Supplementary Digital Material 3: Supplementary Table III). Both Urine and SOC+Urine models showed a

Table III.—*ORs for SOC, urine, SOC+urine models.*

Model	Odd Ratio	95% CI
ORs for SOC, urine, SOC+urine models		
SOC (PSA+DRE+Age)	2.813	1.562-5.065
Urine	7.439	3.981-13.899
SOC+Urine	11.063	5.782-21.167

SOC: standard of care; OR: odds ratio; CI: Confidence Interval; PSA: prostate specific antigen; DRE: digital rectal examination.

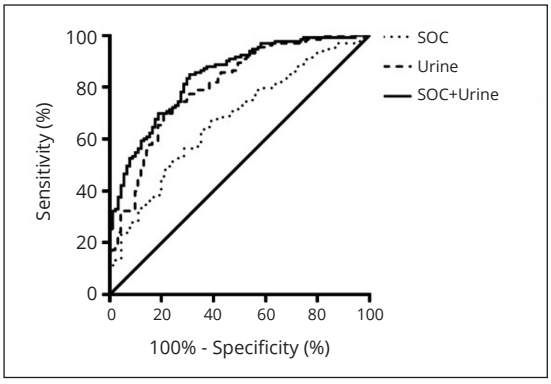

Figure 5.—Diagnostic performance of Standard and Urine parameters, alone or in combination.

specificity of 42.9% at 95% of Sensitivity, higher than SOC alone (13.2%). At this sensitivity, urine and the combination of SOC+urine showed higher positive predictive value (PPV), negative predictive value (NPV) and number need to predict (NNP), hence greater capacity in accurately classifying biopsies results compared to SOC (Supplementary Table III).

Combination of uPSA and uZinc with MRI results

The use of MRI-risk assessment is becoming the gold standard for identifying the need for biopsy in men suspected of having PCa in order to reducing the number of unnecessary procedures.

Among the 183 patients who underwent mpMRI, logistic regression models were created and ORs calculated as shown in Table IV. The routinary use of prostate MRI is associated with PCa diagnosis with an OR of 6.1, which increases at 6.6 in MRI+SOC, at 12.3 in MRI+urine and 13.6 in SOC+MRI+urine.

ROC curve analysis showed that MRI+urine and SOC+MRI+urine models provided an AUC of 0.868 and 0.882, respectively, that are statistically significantly different from MRI (P=0.0001 both) and from MRI+SOC (P=0.0081, 0.004, respectively) while no differences were evident between MRI and MRI+SOC (Figure 6, Supplementary Digital Material 4: Supplementary Table IV). Internal validation for the four models showed an optimism estimated of 0.001. MRI+urine and SOC+MRI+urine models displayed higher PPV, NPV and NNP compared to MRI alone and MRI+SOC (Supplementary Table IV).

TABLE IV.—*ORs for SOC, MRI, urine models and combinations.*

Model	OR	95% CI
ORs for SOC, MRI, Urine models and combinations.		
SOC	2.387	1.247-4.568
MRI	6.070	2.559-14.395
Urine	8.174	4.068-16.423
MRI+SOC	6.620	3.307-13.252
MRI+Urine	12.291	5.977-25.282
SOC+MRI+Urine	13.647	6.637-28.059

OR: odds ratio; MRI: magnetic resonance imaging; SOC: standard of care; CI: Confidence Interval.

PCa risk probability by using uPSA and uZinc biomarkers

In order to define the clinical utility of biomarkers for early detection of prostate cancer, the capability of potential reduction of biopsy on healthy individuals should be assessed. On the basis of multivariate regression models we tried to fit the probability of having cancer against the outcome of the biopsy. We selected three different cut-off probabilities corresponding to 97.5%, 95% and 90% of sensitivity.

For SOC+Urine model the cut-off were 0.25, 0.33 and 0.39, respectively.

For SOC+MRI+Urine model the cut-off were 0.27, 0.36 and 0.45, respectively.

The diagnostic performance of SOC+Urine and SOC+MRI+Urine models to have PCa with GS≤7(3+4) or GS≥7(4+3) was also evaluated.

For SOC+Urine a probability of>0.25 identified 93% (55/59) of GS≤7(3+4) and 97% (72/74) of GS≥7(4+3) cancers, while only 58% (53/91) of healthy individuals (Supplementary Digital Material 5: Supplementary Table V (a)). For SOC+MRI+urine model a probability of>0.27 identified 96% (52/54) of GS≤7(3+4) and 98%

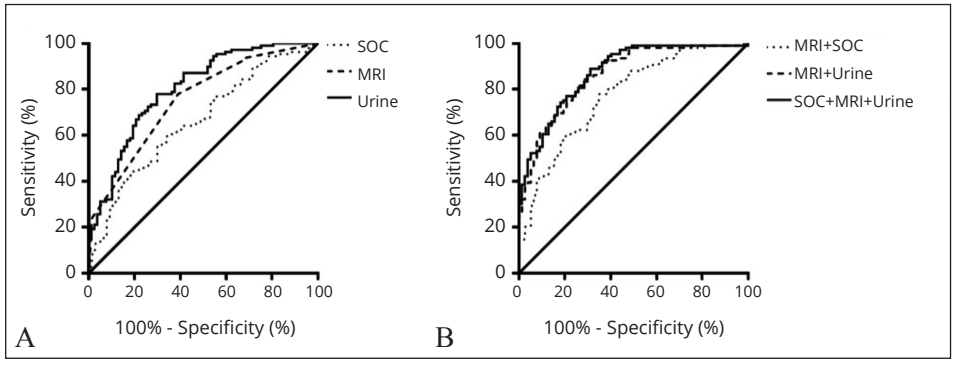

Figure 6.—Diagnostic performance of Standard, MRI and Urine parameters, alone or in combination.

(54/55) of GS≥7(4+3) cancers, while only 43% (33/77) of healthy individuals (Supplementary Digital Material 5: Supplementary Table V (b)).

The use of SOC+Urine and SOC+MRI+Urine model at 0.25 and 0.27 of probability, respectively displayed a potential reduction of 42% and 57% of unnecessary biopsies, respectively, by missing less than 3% of all cancers.

For higher probability, the higher potential saved biopsies is associated with the chance of missing more than 7%, of cancers.

Discussion

The actual advantages of PSA screening for PCa diagnosis and its influence on natural history of the disease remains a topic of debate across USA and Europe.[11-13]

Nowadays there is an urgent need in this field to find and confirm new biomarkers that can help spare patients from unnecessary biopsies and lower the chances of overdiagnosis and overtreatment for PCa.[7]

While several tests have displayed the potential to enhance the diagnostic and treatment processes, there is a notable absence of prospective studies confirming their impact on disease outcomes. Nevertheless, the most recent update of the European Association of Urology's (EAU-ESTRO-SIOG) guidelines did not recommend any of these tests to supplement the standard of care, considering only mpMRI as an "intermediate" diagnostic tool,[14] even with some controversies,[15, 16] to increase the diagnostic accuracy and potentially guide the prostate biopsy.[17, 18]

At the moment, there is a growing focus on exploring urine-based biomarkers for various urological cancers, making it a particularly appealing bio-fluid in the field of clinical proteomics.[19]

The measurement of uPSA represents an interesting parameter to obtain information about prostate physiology and pathology, as the presence of neoplastic transformation.[11] The detectable level of PSA in urine is directly linked to PSA expression in prostate tissue and is not associated with PSA levels in the bloodstream.

In a recent study, Occhipinti *et al.*[9] demonstrated that uPSA levels, lower in patients with PCa in comparison to healthy men, has the potential to distinguish clinically significant PCa from less aggressive forms.

In the same manner, previous studies demonstrated that the level of zinc in prostate tissue, prostatic secretion and urine is substantially lowered in the presence of neoplastic transformation compared to non-malignant pathologies and normal glands[8, 20] indicating that the analysis of uZinc can effectively distinguish between patients harboring PCa and those with other conditions, making it a promising candidate biomarker.

In the current study, levels of both uPSA and uZinc were evaluated in urine samples of patients undergoing first prostate biopsy for suspected PCa. We recorded that both uPSA and uZinc are lower in patients with PCa in comparison to healthy men, with a gradual decrease among increasing histologic grade. More specifically, uPSA levels were significantly different between healthy individuals, subjects with PCa GS≤7 (3+4) to subjects with PCa≥7 (4+3) ($P<0.001$), while uZinc levels were only significantly different in patients diagnosed with PCa≥7(4+3), suggesting the possibility to discern csPCa and ncsPCa. Performance of the combined analysis of uPSA plus uZinc (urine model) showed a higher diagnostic capability (AUC 0.804) compared to uPSA and uZinc alone for All PCa (AUC of 0.797 and 0.604, respectively). This advantage was found to be persistent even when analyzing diagnostic accuracy for csPCa, as the urine model (AUC 0.823) outperformed the uPSA and uZinc sampling alone (AUC 0.807 and 0.638, respectively).

Based on this consideration, attempting to assess the real clinical role of the Urine model along with the best timeframe for its execution within the PCa diagnostic algorithm, its diagnostic capability was compared with the fundamental assessments indicated by the guidelines in suspected PCa: the SOC (SOC model: DRE, bPSA, age) and the mpMRI. When comparing the AUC of the urine test with the SOC, urine model shows a higher diagnostic accuracy for PCa (AUC 0.804 *vs.* 0.677, respectively; $P=0.008$). Moreover, urinary test showed an additive value in combination with SOC, giving even better diagnostic results (AUC 0.843). These findings suggest its potential benefit at early stages of the diagnostic

pathway of PCa, paving the way for the design of future studies on its use as a screening tool; furthermore, its ability to effectively distinguish between csPCa and ncsPCa, strengthen the value of the the test.

Aiming to evaluate the role of the Urine test on the complete diagnostic pathway for PCa indicated by the European guidelines, we also analyzed its performance in patients undergone mpMRI before prostate biopsy. In this subcohort, the diagnostic accuracy of performing combined mpMRI and Urinary test (AUC 0.868) was significantly higher (P=0.004) than the diagnostic accuracy of performing SOC+MRI (AUC 0.778). Moreover, the combination of SOC+MRI+urine test further improved the accuracy of the model, reaching an AUC of 0.882. Considering this evidence, the combination of the Urine model, mpMRI, and the assessment of standard parameters works synergistically. The simultaneous evaluation of these factors has the potential to enhance accuracy in identifying suitable candidates for prostate biopsy, thereby decreasing the risks associated with false positive and false negative results. Nevertheless, in the last years, we have witnessed incremental availability of diagnostic tools for PCa involving both blood or urine biomarker testing and MRI strategies that can enhance clinical routines, enabling more effective early detection of PCa and offering valuable insights for biopsy decision-making.[21] Among those biomarkers Prostate Health Index (PHI),[22, 23] 4Kscore,[24] Select MDx,[25] PCA3[26, 27] and ConfirmMDx[28] showed promising results, yet the optimal sequence and timing remains to be determined and international guidelines do not recommend execution of any of these markers before the first biopsy, because of concerns about effectiveness in certain instances and costs.

The urine model could be competitive considering these aspects, being the urine sample easily obtainable. Moreover, uZinc measurement is currently employed in certain clinical biochemistry laboratories to identify instances of industrial zinc exposure, while uPSA measurement is conducted via ELISA assay, widely available technology in most of clinical biochemistry laboratories. In addition, this research group is putting effort into the creation of a device for the rapid home testing of uPSA and uZinc that could probably be performed at a negligible cost once development cost will be overcome.[29, 30]

Taken all these considerations together can be inferred to entail easily affordable costs, particularly since the available literature affirms that urine-based tests have proven to be more cost-effective than blood-based tests for PCa diagnosis.[31] Furthermore, given the high diagnostic accuracy demonstrated, especially when combined with SOC, the use of the urine test could lead to a reduction of the number of mpMRI performed in a selected patient population, especially when combining it with nomograms.[32, 33] This consideration is in line with findings of recent literature, which claim that using selected urine-based reflex tests to guide biopsy decisions is more cost effective than MRI in men with PSA ranging from 4 to 10 ng/mL.[34] Lastly, according to our simulation strategies of implementation of Urine model with SOC and MRI+SOC at 0.25 and 0.27 of probability, a potential reduction of 42% and 57% of unnecessary biopsies, respectively, was found. With these strategies less than 3% of all cancers were missed. Decreasing the number of unnecessary biopsies and therefore reducing the risk of complications, overdiagnosis and overtreatment has the potential to reduce both the economic and clinical burden of PCa diagnosis.

Limitations of the study

The current study is not devoid of limitation. The first limitation of our study is that not the whole patients underwent mpMRI for biopsy decision-making. Secondly, this study was conducted in a single referral center, limiting the effective representation of general daily practice. Thirdly, the heterogeneity in the quality of mp-MRI and the absence of centralized reading could potentially influence our findings. Fourthly, the broad diversity within the analyzed population, although reflective of real-life situations, may impact the validity of our results regarding various biopsy techniques. Lastly, another limitation arises from the inability to accurately estimate the real positive and negative predictive values of both targeted and systematic biopsies: in cases of negative biopsy, radical prostatectomy is not performed,

making it challenging to assess the actual prevalence of PCa in our cohort of patients.

Conclusions

The loss of PSA and Zinc production and secretion by the prostatic gland during neoplastic transformation could potentially represent a hallmark of PCa. Its combination with standard diagnostic parameters such as age, PSA and DRE, as well as with mpMRI could represent an interesting approach to improve the diagnostic accuracy of PCa.

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Conflicts of interest

Sergio Occhipinti is shareholder of NIB biotec s.r.l.

Authors' contributions

Daniele Amparore and Sabrina De Cillis contributed equally to this work; Sergio Occhipinti and Francesco Porpiglia contributed equally to the senior authorship. Daniele Amparore: study conceptualization, manuscript writing, supervision; Sabrina De Cillis: data interpretation, manuscript writing, supervision; Stefano Granato: data collecting, manuscript writing; Michele Ortenzi, Marcello Della Corte, Michele Sica, Alberto Piana: data collecting; Paolo Verri: English language revision; Stefano De Luca, study conceptualization, supervision; Matteo Manfredi, Cristian Fiori and Francesco Porpiglia: supervision; Giulio Mengozzi, Enrico Bergamaschi and Giuseppe Mariella: data analysis; Sergio Occhipinti: study conceptualization, data analysis, manuscript writing, supervision. All authors read and approved the final version of the manuscript.

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History

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SUPPLEMENTARY DIGITAL MATERIAL 1

Supplementary Table I.—Correlation matrix for urine and standard parameters.

Correlation matrix for urine and standard parameters							
	Age	**bPSA**	**DRE**	**PIRADS**	**uPSA**	**uZINC**	**GS**
Age	1	0,16*	0,15*	0,17*	-0,07	-0,11	0,23**
bPSA	0,16*	1	0,13*	0,09	-0,07	-0,05	0,19**
DRE	0,15*	0,13*	1	0,15*	-0,06	-0,12	0,36**
PIRADS	0,17*	0,09	0,15*	1	-0,24**	0,06	0,47**
uPSA	-0,07	-0,07	-0,06	-0,24**	1	0,35**	-0,47**
uZINC	-0,11	-0,05	-0,12	0,06	0,35**	1	-0,22**
GS	0,23*	0,19**	0,36**	0,47**	-0,47**	-0,22**	1

bPSA: blood prostate specific antigen; DRE: digital rectal examination; PIRADS: Prostate Imaging Reporting & Data System; uPSA: urine prostate specific antigen; uZINC: urinary zinc; GS: Gleason Score.

SUPPLEMENTARY DIGITAL MATERIAL 2

Supplementary Table II.—Combination of uPSA and uZinc in All PCa and in GS≥7 (4+3).

Combination of uPSA and uZinc in All PCa and in GS ≥ 7 (4+3).					
	Model	**AUC**	**SE**	**Specificity (95% Sens)**	**Sensitivity (95% Spec)**
All PCa	uPSA	0.797	0.031	42	21
	uZinc	0.604	0.039	16	10
	Urine	0.804	0.030	43	32
GS ≥ 7 4+3	uPSA	0.807	0.034	41	19
	uZinc	0.638	0.043	25	11
	Urine	0.823	0.032	43	35

uPSA: urine prostate specific antigen; uZINC: urinary zinc; PCa: prostate cancer; GS: Gleason Score; AUC: area under curve.

SUPPLEMENTARY DIGITAL MATERIAL 3

Supplementary Table III.—Comparison between SOC, urine, SOC+urine models.

Comparison between SOC, Urine, SOC+Urine models											
Model	**AUC**	**SE**	**95% CI**	**p**		**Optimism**	**Spec (95% Sens)**	**Sens (95% Spec)**	**PPV**	**NPV**	**NNP**
SOC	0.677	0.036	0.607-0.747	ref	-	0.001	13,2	23,3	61,5	63,2	3,9
Urine	0.804	0.030	0.745-0.862	0.0089	ref	0.001	42,9	30,3	70,6	84,8	1,8
SOC+Urine	0.843	0.026	0.792-0.893	0.0001	0.0231	0.001	42,9	41,7	70,6	84,8	1,8

SOC: standard of care; AUC: area under curve; CI: Confidence Interval; PPV: positive predictive value; NPV: negative predictive value; ref: reference.

SUPPLEMENTARY DIGITAL MATERIAL 4

Supplementary Table IV.—Comparison between MRI, SOC, Urine models and combinations.

Comparison between MRI, SOC, Urine models and combinations.											
Model	**AUC**	**SE**	**95% CI**	**p**		**Optimism**	**Spec (95% Sens)**	**Sens (95% Spec)**	**PPV**	**NPV**	**NNP**
SOC	0.659	0.04	0.586-0.727	ref	-	0.0001	18,2	13,9	62,9	82,3	2,9
MRI	0.757	0.032	0.688-0.817	0.0407	ref	0.0001	32,5	29,2	66,4	82,3	2,1
Urine	0.799	0.033	0.734-0.859	0.0069	ns	0.0001	44.2	25	71,3	85,8	1,7
MRI+SOC	0.778	0.034	0.711-0.835	0.0005	ns	0.0001	32,5	20,4	66,4	82,3	2,0
MRI+Urine	0.868	0.026	0.810-0.913	0.0001	0.0001	0.0001	53,3	39,8	74,8	87,9	1,6
SOC+MRI+Urine	0.882	0.024	0.826-0.924	0.0001	0.0001	0.0001	61	55,6	78,1	89,3	1,5

MRI: magnetic resonance imaging; SOC: standard of care; AUC: area under curve; CI: Confidence Interval; PPV: positive predictive value; NPV: negative predictive value; ref: reference.

SUPPLEMENTARY DIGITAL MATERIAL 5

Supplementary Table V.—Saved unnecessary biopsies in SOC+Urine model (a) and SOC+MRI+urine model (b).

Saved unnecessary biopsies in SOC+Urine model						
Cut-off (probability)	**All N (%)**	**Non-Cancer N (%)**	**GS ≤ 7 (3+4) N (%)**	**GS ≥ 7 (4+3) N (%)**	**Missed cancer N (%)**	**Saved unnecessary Biopsies N (%)**
0	224 (100)	91 (100)	59 (100)	74 (100)	0 (0)	0 (0)
0.25	180 (80)	53 (58)	56 (95)	72 (97)	5 (4)	38 (42)
0.33	172 (77)	50 (55)	53 (89)	70 (95)	10 (8)	41 (45)
0.39	152 (68)	41 (45)	43 (73)	68 (92)	18 (17)	50 (55)
Saved unnecessary biopsies in SOC+MRI+Urine model						
0	186 (100)	77 (100)	54 (100)	55 (100)	0 (0)	0 (0)
0.27	133 (72)	33 (43)	52 (96)	54 (98)	3 (3)	44 (57)
0.36	127 (68)	30 (39)	49 (91)	52 (95)	8 (7)	47 (61)
0.46	113 (61)	28 (36)	43 (80)	48 (87)	18 (17)	49 (64)

SOC: standard of care; GS: Gleason Score; MRI: magnetic resonance imaging.