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Clinical validation of the iXip index in avoiding unnecessary prostate biopsy: Results from a prospective multicenter study involving 426 patients

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ABSTRACT

Purpose: To assess the diagnostic accuracy of iXip, a novel biomarker for prostate cancer detection at initial biopsy based on an algorithm including patient age, prostate volume, PSA and PSA-IgM levels,.

Materials and methods: This was a prospective multicenter study involving 426 consecutive men undergoing initial prostate biopsy with at least 12 cores in a real-life clinical setting. Diagnostic accuracy of iXip for prostate cancer detection was calculated with AUC and compared to that of prostate volume, PSA and PSA-IgM levels. The correlation of iXip with tumor aggressiveness, defined as any cancer with Gleason score ≥7, was evaluated by Spearman ρ coefficient analysis.

Results: Prostate cancer was diagnosed in 193/426 patients (45%), of which 65 (35%) had Gleason score ≥7. iXip values were significantly higher in patients with cancer than in those without cancer (median value 55% vs. 39%, $p < 0.001$). iXip was the most accurate predictor of cancer (AUC=0.711), followed by prostate volume (AUC=0.660) and PSA level (AUC=0.543). By setting iXip cut-off at 20%, no patients with iXip values below the cut-off were diagnosed with cancer, resulting in a 5.6% (24/426) reduction of unnecessary prostate biopsies. A significant correlation between iXip values and Gleason score was observed ($\rho=0.347$; $p < 0.001$).

Conclusions: Our prospective multicenter study suggests that the novel biomarker iXip may be used with a 20% cut-off value in order to reduce the proportion of prostate biopsies by approximately 5%, without missing a single case of cancer. Moreover, higher iXip values are significantly correlated with tumor aggressiveness.

1. Introduction

Serum Prostate-Specific Antigen (PSA) has a low sensitivity and specificity for prostate cancer (PCa) detection, especially in the intermediate range of total PSA (tPSA) between 2.5 and 10 ng/mL [1]. Derivative parameters that have been proposed over the past decades have been disappointing, with the consequence that approximately 3 out of 4 men currently undergoing prostate biopsy (PB) do not have PCa, and are thus exposed to unnecessary and potentially morbid procedures [2].

Three recently developed tests, progensa (PCA3) [3], prostate health index (PHI, [−2]proPSA) [4], and the 4 K score [5] have been shown to improve the accuracy of tPSA and its derivatives for PCa detection, and have been proposed as complementary tools to tPSA to reduce the number of unnecessary PBs. These tests provide a risk estimation of PB-detectable PCa, but in all cases sensitivity does not reach 100% when a given cut-off value is set, implying that none of them allows the reduction of PBs number without missing real cases of PCa.

A novel biomarker for PCa detection was recently introduced based on the observation that, in men with PCa, serum PSA can be detected as PSA-IgM immune complexes, in which PSA is bound to IgMs [6]. This reflects an innate immunity phenomenon, which has been previously observed for other biomarkers associated with other malig-

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nancies, including squamous cell carcinoma antigen for hepatocellular carcinoma and carcinoembriogenic antigen for colon carcinoma [7,8].

A prostate biopsy has a slight risk of causing problems such as infection, bleeding into the urethra or bladder, bleeding from the rectum and allergic reaction to the anesthetic medicines used during the biopsy. These are some reasons to research novel non or minimal invasive methods for biopsy reduction.

To investigate the application of PSA-IgM immune complexes in the clinical practice, we have developed an algorithm that combines PSA-IgM levels with patient age, prostate volume and tPSA to obtain a predictive index, called iXip [9]. Unlike other biomarker algorithms for PCa detection, where the biological rationale is unclear, iXip was developed to correlate the levels of the novel biomarker PSA-IgM with established variables that are biologically associated with PCa. Of note, the algorithm has been developed in order to optimize the receiver operating characteristic (ROC) curve at its ends, rather than to obtain the best possible curve based on the highest area under the ROC curve (AUC) value. This was done to get > 10% sensitivity at 100% specificity and > 10% specificity at 100% sensitivity. The algorithm has been tested in an exploratory, proof-of-principle study in 160 men undergoing initial PB, who could be stratified by their risk of having biopsy-detectable PCa based on their iXip value. In this study, no patient with a ≤20% iXip value had PCa at PB, thus suggesting that iXip may be used to reduce the number of PBs without missing any PCa case.

The present study had two objectives: 1) to prospectively validate the diagnostic accuracy of iXip (previously elaborated and applied with no modification) for PCa detection in a large, external, multicenter cohort of men undergoing initial extended PB in a real-life clinical setting; 2) to evaluate the association of iXip with PCa aggressiveness at PB.

2. Patients and methods

2.1. Study design and participants

This was a prospective multicenter observational study of diagnostic accuracy approved by the Institutional Review Board for Human Subjects Research of the University of Padua (N. 0050868) and designed, conducted and reported according to the Standards for the Reporting of Diagnostic Accuracy Studies guidelines [10].

Between March 2010 and December 2011, all consecutive patients referred for PB to one of five urology departments located in North-Eastern Italy (Belluno, Brescia, Camposampiero, Padua and Pordenone) were screened for possible involvement in the present study. Inclusion criteria were: age > 18 years, abnormal digital rectal examination, and serum tPSA level > 4 ng/mL or > 2.5 ng/mL in case of familial history. Exclusion criteria were: previous PB, previous prostate surgery, previous or concomitant malignancies, active infections, autoimmune diseases, and medication with steroids, immunosuppressive drugs and/or 5α-reductase inhibitors. Of 500 screened patients, 426 were eventually enrolled in the study after providing written informed consent (Fig. 1).

2.2. Study protocol

Before any prostatic manipulation, a serum sample was collected. tPSA and free PSA (fPSA) levels were determined using Hybritech Access test on UniCelDxI800 (Beckman Coulter, Brea, CA, USA). PSA-IgM levels were measured in duplicate using Prostate-IC ELISA kit (Code XG007, Xeptagen SpA, Venice, Italy) with a < 15% variation coefficient. Prostate-IC was performed on DSX Automated ELISA System (Dynex Technologies Inc., Chantilly, VA, USA), a computer-controlled microplate processing system that fully automates ELISA assays. The iXip index was calculated as previously reported [9].

All patients underwent a systematic TRUS-guided PB with an extended scheme consisting of at least 12 cores taken from the peripheral zone (apex, midgland and base) with additional cores taken when necessary or in case of increased prostate volume. Immediately before sampling, prostate volume was measured with TRUS using the ellipsoidal prolate formula. Specimens were collected in single-core containers, and centrally evaluated by a single experienced genitourinary pathologist blinded to all clinical data. PCa was identified and graded according to the 2005 ISUP modified Gleason grading system [11]. Patients diagnosed with high-grade intraepithelial neoplasm or atypical small acinar proliferation were considered negative for PCa.

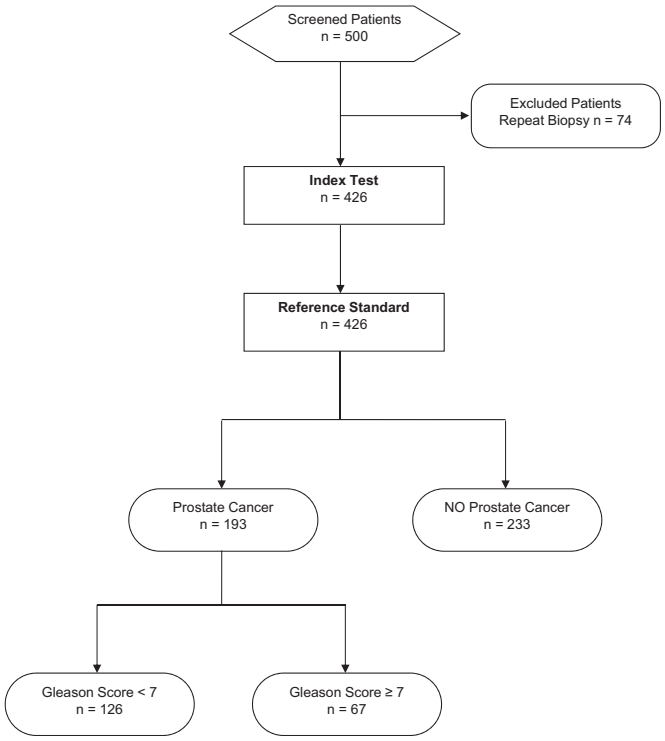

Fig. 1. Standards for the Reporting of Diagnostic Accuracy Studies flow diagram.

2.3. Study endpoints

The primary endpoint was the diagnostic accuracy of iXip (index test) compared to tPSA (reference test) for PCa detection at initial extended PB. The proportion of PBs that could be spared if the index test had to be used in the decisional PB pathway was also calculated. Secondary endpoint was the correlation between iXip and PCa aggressiveness, which we defined as any Gleason score (GS)≥7 cancer, in line with previous similar diagnostic studies [12,13].

2.4. Statistical analyses

Given a PCa rate at biopsy of 0.45, 1-β > 95% and α=5%, sample size was 300 patients. The Kolmogorov-Smirnov test was used to assess the normality of continuous variables. Student t test, Wilcoxon rank-sum or χ^2 test with Yates continuity were used for comparisons of normally and non-normally distributed variables as appropriate. Correlation was assessed by Spearman's rank coefficient analysis.

Diagnostic accuracy for each marker was quantified with AUC. The gain in diagnostic accuracy was calculated, and AUCs were compared using the Hanley and McNeil method [14].

Multivariable logistic regression models to predict PB-detectable PCa were fit including tPSA and prostate volume as explanatory variables.

For all comparisons, a two-sided p value < 0.05 was accepted as significant. All analyses were performed using R v.3.0.1 (The R Foundation for Statistical Computing, Vienna, Austria) and SPSS

Table 1
Descriptive characteristics of the study population.

	Absence of PCa	Presence of PCa	Overall	p-Value
Patients (%)	233 (54.7%)	193 (45.3%)	426 (100%)	NA
Age[a] (range) [yr]	65.0 ± 6.9	67.4 ± 8.0	66.1 ± 7.5	< 0.001*
Patients with tPSA levels in the range 4÷10 ng/mL	158 (37.1%)	112 (26.3%)	270 (63.4%)	< 0.05°
Patients with abnormal DRE (%)	56 (13.1%)	92 (21.6%)	148 (34.7%)	< 0.001°
Patients with tPSA levels in the range 4÷10 ng/mL and abnormal DRE (%)	27 (6.3%)	40 (9.4%)	67 (15.7%)	< 0.05°
Gleason score at prostate biopsy (%)				
< 7	NA	126 (29.6%)	NA	NA
≥ 7	NA	67 (15.7%)	NA	NA
Prostate volume (range)[b] [mL]	47.0 (33.1÷70.0)	35.0 (27.0÷48.0)	40.0 (30.0÷60.0)	< 0.001#
tPSA (range)[b] [ng/mL]	5.8 (4.6÷8.5)	6.0 (4.5÷10.0)	5.9 (4.5÷8.9)	0.130#
fPSA (range)[b] [ng/mL]	0.81 (0.46÷1.25)	0.55 (0.35÷0.88)	0.67 (0.39÷1.10)	< 0.01#
%fPSA (range)[b] [%]	13.0 (9.6÷18.7)	11.0 (7.25÷15.9)	12.4 (8.3÷17.0)	< 0.05#
PSA-IgM (range)[b] [AU/mL]	78.0 (43.6÷152.9)	76.7 (36.0÷145.1)	77.2 (39.9÷149.0)	0.613#
iXip (range)[b] [%]	39.2 (27.2÷54.7)	55.2 (41.3÷66.1)	45.5 (31.8÷62.0)	< 0.001#
iXip (range)[b] [%]				
Gleason score (%) < 7	39.2 (27.2÷54.7)	51.3 (39.1÷65.2)	43.0 (30.2÷59.4)	< 0.001#
iXip (range)[b] [%]				
Gleason score (%) ≥7	39.2 (27.2÷54.7)	59.6 (48.1÷67.6)	43.9 (29.2÷60.2)	< 0.001#

DRE=digital rectal examination; fPSA=free PSA; NA=not applicable; PCa=prostate cancer; PSA=prostate-specific antigen; tPSA=total PSA; %fPSA=percentage of free PSA to total PSA.
[a] data are expressed as mean and standard deviation.
[b] data are expressed as median and interquartile range.
[#] Wilcoxon rank sum (Mann–Whitney) test.
[*] Student *t* test.
[°] x2 test with Yates continuity correction.

v.16.0.1 (IBM Corp., Armonk, NY, USA) software.

3. Results

Table 1 summarizes patient characteristics. PCa was detected in 193 of 426 patients (45.3%). Of the 193 patients with PCa at PB, 126 (65%) had a GS < 7 and 67 (35%) had a GS≥7 cancer. tPSA values did not significantly differ between men with or without PCa. Prostate volume was significantly higher in patients without PCa compared to their counterparts with PCa. Conversely, patients without PCa had significantly lower iXip values than patients with PCa.

iXip was the most accurate predictor of PCa (AUC=0.694) compared to prostate volume (AUC=0.660), tPSA (AUC=0.543) and fPSA (AUC=0.580) levels (Table 2 and Fig. 2).

As shown in Table 2, the cut-off for each marker was adjusted in order to obtain an 11.5% specificity; then, positive (PPV) and negative (NPV) predictive values were calculated. In more than 1:10 men without PB-detectable PCa, iXip was able to properly identify the unnecessary biopsies (100% NPV). All other markers had a NPV < 90% and did not correctly distinguish between patients with or without PB-detectable PCa.

According to the iXip values obtained, patients were arbitrarily stratified into four different risk groups with no, low, intermediate, or high likelihood of PB-detectable PCa (Table 3). At a 20% iXip cut-off (no-risk group), sensitivity was 100% and specificity 11.5%, meaning that all patients with iXip values equal to or lower than 20% had no PB-detectable PCa. None of the other variables achieved this condition. At a 30% iXip cut-off (low-risk group), there was a 2.8% likelihood of detecting PCa. Avoiding PB in these patients could have spared 92 (21.6%) PBs at the price of missing 12 (6.2%) cases of PCa. However, in this risk category, only a minority (3/12) of patients had a GS ≥7 cancer (Table 3). For intermediate-risk category, the reduction of unnecessary PBs would have been higher (57%), but 21% of patients would have had their PCa missed.

Table 2
Diagnostic performance of tumor markers, diagnostic parameters and multivariable models (prevalence 45%).

Variable	AUC	Sensitivity (Specificity 11.5%)	NPV	PPV
tPSA	0.543	88.1%	52.7%	45.1%
%fPSA	0.603	94.3%	70.0%	46.7%
Prostate volume	0.682	97.4%	83.6%	47.5%
PSA-IgM	0.514	86.0%	48.7%	44.5%
Base model[a]	0.723	97.4%	83.8%	47.6%
Base model[a]+Age	0.755	96.4%	78.7%	47.3%
iXip	0.711	100.0%	100.0%	48.2%

AUC=area under ROC curve; NPV=negative predictive value; PPV=positive predictive value; tPSA=total PSA; %fPSA=percentage of free PSA to total PSA.
[a] Base Model=tPSA+prostate volume.

Patients with GS ≥7 cancers showed significantly higher iXip values than those with GS < 7. In patients with iXip values > 50%, the majority of GS 8 (10/15, 66%) and GS 9 (8/9, 89%) cancers could be found. Also, iXip was a more accurate predictor of high-grade cancer (AUC=0.767) than of any-grade cancer (AUC=0.694) (Table 4 and Fig. 2). Determination of sensitivity and specificity at different iXip cut-off values in whole population and stratified by GS confirmed the correlation between iXip values and PCa aggressiveness. In fact, at different iXip cut-off values and fixed specificities, the sensitivity is always considerably higher in cancer patients with GS ≥7(Table 5).

No significant difference in iXip performance was observed between the whole cohort and patients with tPSA levels lower and greater than 10 ng/mL (AUC comparisons, p=0.428 and 0.470, respectively).

Spearman ρ coefficient analysis demonstrated a significant correlation between iXip and GS (ρ=0.347, p < 0.001).

4. Discussion

In a large, multicenter, prospective and external cohort of men submitted to first extended PB, we demonstrated that iXip was an accurate predictor of PCa and was able to stratify the risk of PB-detectable PCa, with lower values indicating a lower risk of detecting PCa. In particular, the previously identified iXip value of 20% was confirmed to be a relevant cut-off, below which no single case of PCa was diagnosed. The main clinical implication of our study is that the novel biomarker iXip can safely reduce the number of unnecessary PBs.

To the best of our knowledge, this is an unprecedented finding. In fact, for none of the available diagnostic biomarkers used for PCa

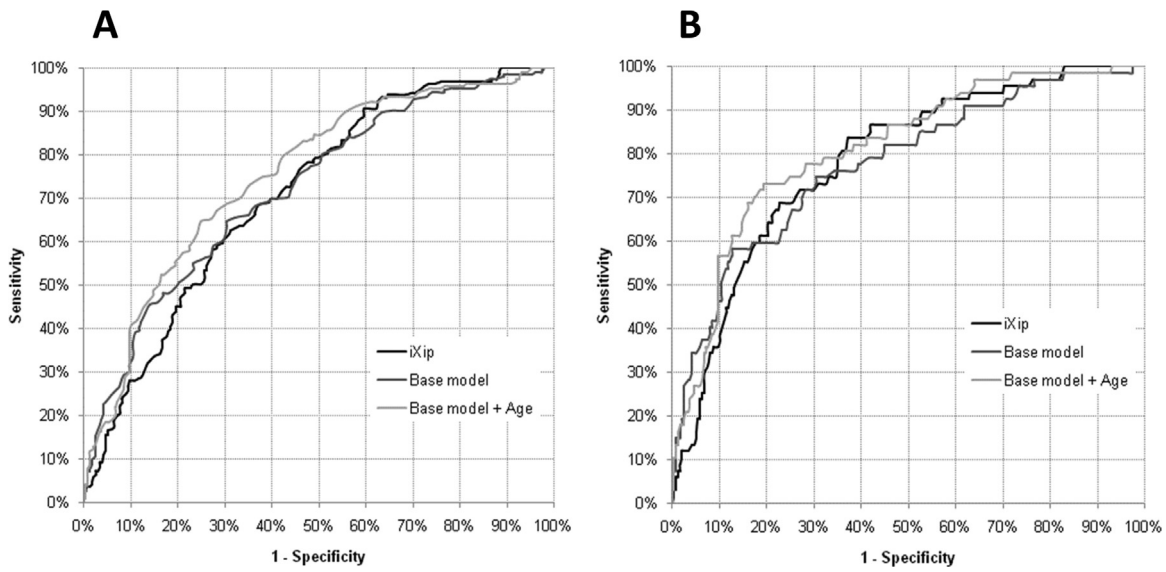

Fig. 2. A) Receiver operating characteristic curves depicting the accuracy of individual predictors of any prostate cancer. B) Receiver operating characteristic curves depicting the accuracy of individual predictors of Gleason score ≥7 prostate cancer.

Table 3
Stratification of iXip values into risk categories with corresponding proportion of avoidable prostate biopsies.

Risk iXip cutoff	No 20%	Low 30%	Intermediate 50%	High 80%
Sensitivity	100%	93.8	59.6%	2.1%
Specificity	10.3%	34.3%	70.8%	100%
Patients without PCa	24/233	80/233	165/233	233/233
Reduction of PCa-negative biopsy	**10.3%**	**34.3%**	**70.8%**	**100.0%**
Spared/missed ratio	**– (24/0)**	**7.7 (92/12)**	**3.11 (243/78)**	**2.23 (422/189)**
Patients with PCa	0/193	12/193	78/193	189/193
Gleason score < 7 (%)	0 (0.0%)	9 (75.0%)	60 (76.9%)	123 (65.0%)
Gleason score=7 (%)	0 (0.0%)	2 (16.7%)	12 (15.4%)	42 (22.2%)
Gleason score=3+4 (%)	0 (0.0%)	2 (16.7%)	5 (6.4%)	24 (12.7%)
Gleason score=4+3 (%)	0 (0.0%)	0 (0.0%)	7 (9.0%)	18 (9.5%)
Gleason score=8 (%)	0 (0.0%)	1 (8.3%)	5 (6.4%)	15 (7.9%)
Gleason score=9 (%)	0 (0.0%)	0 (0.0%)	1 (1.3%)	9 (4.9%)
Reduction of PCa-positive biopsy	**0.0%**	**6.2%**	**40.4%**	**97.9%**

PCa=prostate cancer.

Table 4
iXip values in patients without (controls) and with (cases) prostate cancer at biopsy, the latter stratified by Gleason score < 7 and ≥7.

Subjects	n	iXip mean ± SD	iXip median (min÷max)	W p-value[a]	AUC	R p-value[b]
Controls	233	40.5 ± 17.5%	39.2% (0.0÷78.0%)	–	–	–
Cases	193	53.7 ± 15.6%	55.2% (20.8÷90.0%)	< 0.001	0.711	–
Cases Gleason score < 7	126	51.9 ± 16.5%	51.3% (20.8÷90.0%)	< 0.001	0.682	0.036
Cases Gleason score ≥7	67	56.9 ± 13.4%	59.6% (21.1÷81.9%)	< 0.001	0.767	

AUC=Area under ROC curve between different case subpopulation and control subjects; SD=standard deviation.
[a] W p-value=Wilcoxon rank sum (Mann–Whitney) test between different case subpopulation and control subjects.
[b] R p-value=ROC-AUCs comparison between control subjects and population with Gleason score < 7 and ≥7.

detection, including the newest ones, PCA3 and PHI, a cut-off value has been identified that gives 100% sensitivity or specificity. Both PCA3 and PHI have been shown to improve discrimination between men with and without PCa, mainly in the tPSA range 2.5–10 ng/mL, and between cases of clinically significant and insignificant disease [12,13,15]. As for PCA3, the likelihood of positive PB is 17% for a score < 35, 43% for a score > 35, and 69% for a score > 100 [15]. Even in the lowest score category, there is a non-negligible likelihood of finding PCa. Moreover, the EGAPP Working Group judged the performance of PCA3 testing inadequate to inform doctors as when to repeat the biopsy for prostate cancer in previously negative patients, or when to conduct initial biopsies in at-risk men [16].

As for PCa risk determined with PHI, in the range 0–20.9 there is a low risk (8,4% of probability), and in the range 21–39.9 there is a moderate risk (21% of probability), while with a PHI > 40 there is a high risk (44% of probability) [13]. Thus, even in the lowest PHI range, there still is a consistent probability to miss PCa-positive cases; under the best conditions, for 100 spared biopsies (15.5%), 26 PCa-positive cases (9.8%) are lost, giving a 3.8 spared/missed ratio [13]. In our study, by using iXip with a 30% cut-off, 92 biopsies could be spared (21.6%), with only 12 cases (6.2%) lost, giving a 7.7 spared/missed ratio. Although higher PHI index is associated with greater risk of having prostate cancer, this biomarker is not able to spare unnecessary negative biopsies without loss of PCa-positive cases.

The proportion of patients with iXip value below the 20% cut-off, who may be safely spared PB, represents 5.6% (24/426) of the entire cohort. It may be argued that this rate is low. However, due to the accumulating bulk of literature reporting on increasing rate of infectious and potentially lethal complications following PB [17–19], we believe this rate is clinically significant. In addition, reducing even a small proportion of PBs translates into a significant cost reduction, considering the millions of procedures that are routinely performed worldwide every year.

This study also confirms a previously found correlation between iXip values and PCa aggressiveness, defined at PB as GS ≥7 cancers. Higher iXip values were significantly associated with higher-grade cancers. Future research should focus on the identification of a cut-off

Table 5
Sensitivity and specificity of iXip on whole population and stratified by Gleason score based on different value of cut-off.

Cut-off	Gleason	Sensitivity	Specificity
0.20	ALL	100.0%	10.3%
	< 7		
	≥7		
0.30	ALL	93.8%	34.3%
	< 7	92.9%	
	≥7	95.5%	
0.35	ALL	84.5%	43.3%
	< 7	81.0%	
	≥7	91.0%	
0.40	ALL	78.8%	51.1%
	< 7	74.6%	
	≥7	86.6%	
0.45	ALL	68.4%	62.7%
	< 7	60.3%	
	≥7	83.6%	
0.50	ALL	59.6%	70.8%
	< 7	52.4%	
	≥7	73.1%	

value for iXip above which only clinically significant disease can be detected. In our study, increasing the iXip cut-off to 30% resulted in an increased number of avoidable biopsies (21.6%), but with 6.2% of PCa cases being missed. Most of these cases were GS < 7 cancers. We acknowledge that the definition of tumor aggressiveness by GS ≥7 is prone to criticism, however it was used by two recent similar diagnostic studies [12,13]. iXip, as well as other novel markers, should be correlated with definitive pathology after radical prostatectomy, and, ideally, with oncological outcome after treatment.

Admittedly, the absolute number of patients in the two iXip categories ≤20% (n=24) and 20%-30% (n=92) was low, therefore we cannot draw definitive conclusions. Additional studies with larger cohorts are required to better characterize patients with iXip values in these ranges.

A major concern related to PCa screening and early detection is overdiagnosis and overtreatment of indolent disease. Strategies to reduce overdiagnosis are necessary, as are strategies to differentiate indolent from aggressive cancers. One solution could be performing PBs only in men with a clinically significant and potentially lethal cancer. Our findings suggest that the use of iXip could not only safely avoid unnecessary PBs, but could also maximize the detection of aggressive cancers.

Our study has several strengths. First, this is one of the few diagnostic studies testing novel biomarkers which prospectively enrolled a large cohort of contemporary PB candidates (n=426) from multiple clinical centers, using the same operative procedures with regard to serum sample collection and storage, centralized PSA-IgM determination, standardized PB protocol and centralized specimen analysis by a single experienced and blinded genitourinary pathologist. Second, the large sample size allowed studying the correlation between iXip and PCa aggressiveness. Third, although the base models have been calculated on the same cohort, they do not meet the requirements obtained with iXip to correctly identify men without PB-detectable PCa. Unlike the base models that are cohort-dependent, iXip was calculated on a previous independent dataset, and applied and validated on the current cohort with no modification.

We acknowledge the following limitations to our analysis. First, iXip requires prostate volume to be measured with an invasive exam (i.e. TRUS), which might limit patient acceptance to testing. Second, it might well be that adding further variables in our algorithm would result in a further optimization of the diagnostic performance, which would translate in a further reduction in the number of unnecessary PBs. Third, this marker was used in a for-cause cohort of men already selected for prostate biopsy and may perform differently in a screening setting where prevalence of prostate cancer is lower. Fourth, the diagnostic performance of iXip has to be compared with that of other novel biomarkers, such as PCA3 and PHI, in formal head-to-head comparative studies.

5. Conclusions

Our prospective, multicenter study suggests that the novel biomarker iXip may be used with a 20% cut-off value in order to reduce the proportion of PBs by approximately 5% without missing a single case of PCa. Moreover, higher iXip values are significantly associated with PCa aggressiveness, defined as any GS ≥7 cancer at PB.

The implementation of iXip in the diagnostic pathway of early PCa detection might result in a reduction of the proportion of unnecessary PBs and in the identification of patients with clinically significant PCa, allowing an overall decrease in overdiagnosis and overtreatment. Additional, large-scale studies are warranted to corroborate our findings.

Conflict of interest

The authors declare they have no conflict of interest related to this manuscript.

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