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Article

Soluble PD-L1 and PD-1 Significantly Improve the Accuracy of a Diagnostic Model of mRNA Transcripts in the Diagnosis of Prostate Cancer

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Simple Summary: Prostate cancer is a highly heterogeneous disease, requiring novel and easily applicable biomarkers for accurate diagnosis and management. This study aimed to evaluate the role of soluble PD-L1 and PD-1 in combination with urinary mRNA expression of *PCA3*, *PSMA*, and *AR* genes in diagnosing clinically significant prostate cancer. Our findings highlight the potential of integrating all these liquid-based biomarkers together, particularly the promising combination of soluble PD-L1, PD-1 and *AR*, which demonstrates the best distinction of clinically significant prostate cancer (AUC of 0.97, an accuracy of 0.95, a sensitivity of 100%, a specificity of 95%) and overall enabling a multifactorial approach that significantly enhances the accuracy of prostate cancer diagnostics and is both easily applicable and cost-effective.

Abstract: Objectives: To assess the role of soluble PD-L1 and PD-1 in combination with mRNA transcripts of *PCA3*, *PSMA*, *AR* genes in diagnostics of clinically significant prostate cancer (PCa). **Methods:** For 68 PCa patients, plasma sPD-L1 and sPD-1 were measured by ELISA method and the urinary *PSMA*, *PCA3* and *AR* were tested using RT-qPCR. **Results:** *PSMA* and *AR* were identified as the most reliable biomarkers for predicting clinically significant prostate cancer, with AUCs of 0.81 and 0.78, respectively. Additionally, the expression levels of *PSMA* and *PCA3* were associated with the pT3 stage. Despite the diagnostic potential of mRNA transcripts alone, the addition of sPD-L1 and sPD-1 significantly enhanced diagnostic accuracy. Combination doubled the specificity of *PCA3* from 44.1% to 88.0%, compared to *PCA3* alone. sPD-L1, sPD-1 and *AR* resulted in the best distinction between clinically significant and insignificant prostate cancer, achieving an AUC of 0.97, an accuracy of 0.95, a sensitivity of 100%, a specificity of 95%, revealing the potential of *AR* in distinguishing clinically significant PCa, more effectively than *PSMA* and *PCA3*. Our findings highlight the significant potential of sPD-1 in enhancing diagnostic accuracy when added to a triple gene panel, achieving an AUC of 0.90 compared to an AUC of 0.78 without sPD-1. This underscores the value of sPD-1, which has been less explored than sPD-L1 in cancer diagnostics. **Conclusions:** sPD-L1 and sPD-1 has a potential to significantly enhance the accuracy of PCa diagnostics when added to the diagnostic panel, alongside *PSMA*, *PCA3*, and *AR* mRNA transcripts.

Keywords: prostate cancer; sPD-L1; sPD-1; androgen receptor; mRNA transcripts

1. Introduction

Prostate cancer (PCa) continues to rank as the second most prevalent cancer in men globally [1,2] comprising roughly 15% of all cancer diagnoses worldwide. Forecasts indicate that the annual number of new prostate cancer cases is expected to increase from 1.4 million in 2020 to 2.9 million by 2040, based on the analysis of global demographic shifts and the rising rates of life expectancy [3]. Considering that PCa is characterized as heterogeneous disease [4] and variety of risk factors are involved in prostate cancer progression such as environmental as well as genetic and molecular

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factors [5]. There is a pressing need for the development of innovative and efficient diagnostic tools to enhance prostate cancer detection, with a focus on personalized medicine approaches. Ongoing research studies are focused on developing noninvasive methods for clinically significant PCa diagnosis, such as liquid biopsy assays. PSMA (Prostate-Specific Membrane Antigen), PCA3 (Prostate-Specific Membrane Antigen), and AR (Androgen Receptor) are involved in the development of PCa and are variously used in its diagnostics [6–8] along with soluble PD-L1 and PD-1 (sPD-L1 and sPD-1) which demonstrated prognostic significance in our previous research [9], are obtained by utilizing minimally invasive methods. sPD-L1 and sPD-1, which originate from their membrane-bound counterparts, − PD-L1 and PD-1, have gained significant attention in recent research due to their potential as prognostic and predictive markers in different cancer types [10–12]. Meanwhile PSMA is a cell surface protein highly expressed in prostate cancer cells and PSMA based imaging, such as PET/CT, has shown high sensitivity and specificity in detecting prostate cancer lesions, particularly in cases of biochemical recurrence and metastatic disease [13,14]. The AR signaling pathway plays a central role in the growth of prostate cancer and AR expression has been detected in nearly all cases of primary and metastatic prostate cancer, irrespective of their stage or grade [15,16]. PCA3 is overexpressed in PCa [17] and provides greater diagnostic specificity and sensitivity than the main PCa serum biomarker PSA (prostate specific antigen) [18]. Combining these genes and circulating soluble molecules may enhance the accuracy of detection and characterization of PCa. By integrating information from these different markers, a more comprehensive molecular and immune profile of the patient's prostate cancer could be obtained, which can aid in diagnosis, risk stratification, treatment selection, and monitoring of disease progression.

2. Materials and Methods

2.1. Characteristics of PCa Population

In a cohort of PCa patients evaluated for soluble PD-L1 and PD-1 levels in our previous research [9], gene expression was additionally examined in 72 cases to further assess their diagnostic value in this study. 4 cases were removed due to outlier values in gene expression, thus the study included 68 PCa patients. The PCa cases were divided into clinically significant and not clinically significant PCa groups where clinical significance was defined as cases with International Society of Urological Pathology (ISUP) ≥ 3. These patients are deemed to have unfavorable PCa risk. The clinical characteristics of the PCa group are provided in Table 1.

The inclusion and exclusion criteria are well described in the paper of Bosas, clearly outlining the participant selection process [19].

Table 1. Clinical characteristics of the PCa group.

Clinical characteristic	Clinically not significant PCa	Clinically significant PCa	All cases	p
n =	59	9	68	-
Mean Age (min-max)	68.0 (56-82)	69.3 (62-76)	68.2 (56-82)	0.46
Median PSA (pre-op) (IQR)	6.00 (3.85)	8.86 (7.117)	6.23 (4.10)	0.09
ISUP grade:				
ISUP 1	18	-	18	
ISUP 2	41	-	41	<0.001
ISUP 3	-	9	9	
Stage:				
pT2	47	2	49	0.001
pT3	12	7	19i	

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2.2. Blood Sampling

The blood sampling of sPD-L1 and sPD-1 was thoroughly detailed in our previous paper [9].

2.3. Urine Sampling

The urine sampling is described in detail in previous research [20].

2.4. Analysis of Soluble PD-L1 and PD-1

A commercially available ELISA kits for PD-L1 and PD-1 were used to measure the soluble forms of both proteins in plasma, following the manufacturer's instructions (Invitrogen, Thermo Fisher Sci-entific, Vienna, Austria). sPD-L1 and sPD-1 control samples were included in each kit at known concentrations. The optical density was measured using plate reader BioTek Elx800 TM (BIO-Tek Instruments, Inc., Vermont, USA) at 450 nm. Two duplicates of each sample were measured. Blanks and standards were assayed as directed by manufacturer.

2.5. Analysis of mRNA Expression of PCA3, PSMA and AR Genes

Total RNA form washed urine sediment samples extracted using the TRIzol Reagent (Invitrogen, Thermo Fisher Scientific (TFS), Carsbad, CA, USA) following the manufacturer's protocol. The RNA concentration and purity assessed using Nanodrop 2000 spectrophotometer (Thermo Scien-tific, Wilmington, DE, USA). The RNA samples stored at -80°C until copy DNA (cDNA) synthesis step. Two-Step RT-qPCR was used to assay AR, PSMA, and PCA3 mRNA relative quantities in the urine sediment samples. The Maxima First Stand cDNA Synthesis Kit for RT-qPCR with dsDNase (TFS, Vilnius, Lithuania) and the Maxima SYBR Green qPCR Master Mix (2X), with separate ROX vial (TFS, Vilnius, Lithuania) was used for the two-step RT-qPCR following the manufactur-er's protocols. The qPCR reactions performed on QuantStudio 5 Real-Time PCR System (Applied Biosystems, TFS, Singapore). RT-qPCR data pre-processing performed on QuantStudio Design & Analysis software v1.4.3 (Applied biosystems, TFS). The quantification cycle (Ct) values reported using the automatic threshold baseline. Ct values <35 cycle was removed from subsequent analysis. For each sample, melt-curve analysis was performed to evaluate the amplicon size. The initial Ct values normalized to the HPRT1 gene expression using log22-ΔCt and then divided by the PSA gene expression, these normalized relative expression values were used in further statistical data analysis.

2.6. Statistical Analysis

Statistical analysis and data visualization performed on Python 3.11.5 (Python Software Foundation) and Rx4.3.1 [21,22] software. Data normalcy determined using Shapiro-Wilk W test. Cases exceed ing three interquartile ranges deemed outliers and removed from all statistical analysis. Associations between two independent samples tested using Welch's t test or Mann-Whitney U test as appropriate. Receiver operating characteristic curve (ROC) analysis [23] together with logistic regression was utilised to measure biomarker and feature combination accuracy to predict clinically significant PCa. Results considered significant when the $p \leq 0.05$.

3. Results

3.1. Biomarker Association with Prostate Cancer Clinical Features

Analysis of relative *AR*, *PCA3*, and *PSMA* mRNA expression in urine discovered significant increase in *PSMA* expression in clinically significant PCa when compared with clinically insignificant PCa cases ($p = 0.039$) (Figures 1 and S1A) as well as significant associations between pT3 and increase of *PCA3* and *PSMA* relative expression ($p = 0.031$ and $p < 0.001$ respectively) (Figure S2A) and *PSMA* expression and tumor grade (grade 1 *vs* grade 3 $p = 0.005$, grade 1 *vs* grade 2 $p = 0.011$) (Figure S3A).

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Soluble PD-1 and PD-L1 revealed sPD-L1 association with clinically significant PCa (sPDL1 p = 0.033) (Figures 1 and S1B), increased stage (sPDL1 p = 0.031) (Figure S2B), and grade 3 PCa (grade 2 vs grade 3 sPDL1 = 0.026) (Figure S3B), while sPD-1 showed no differences in any of the clinical features examined.

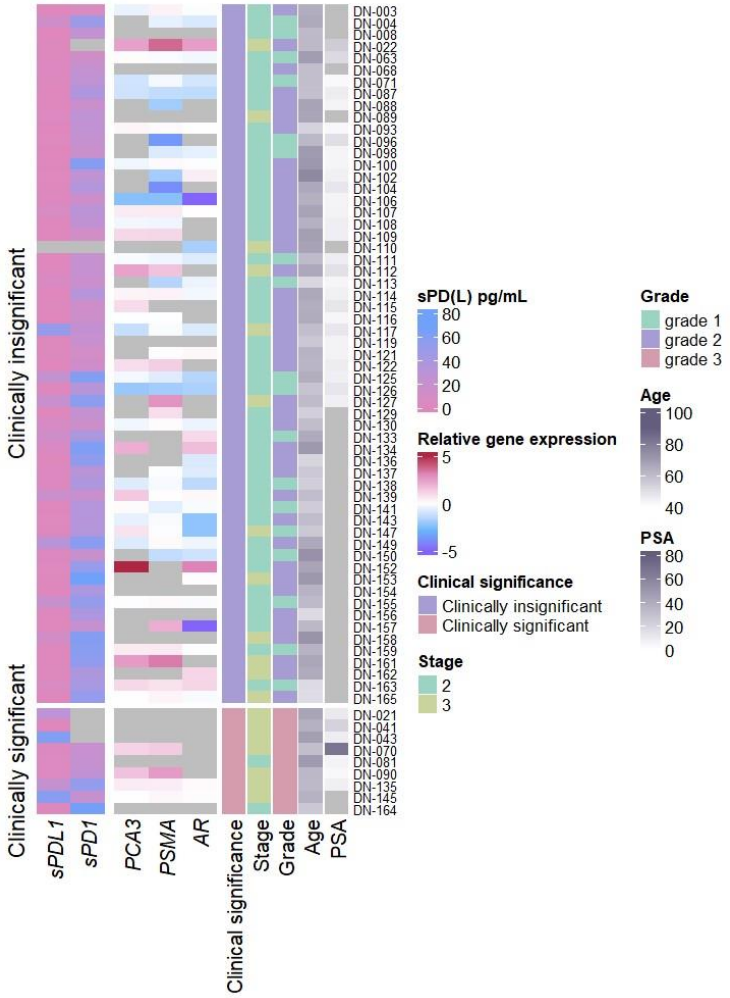

Figure 1. Heatmap depicting sPD-L1/sPD-1 biomarker concentrations in plasma and gene expression in urine sediment samples from prostate cancer patients together with clinical features. Clinically significant cases ISUP ≥ 3, Stage – pathological stage pT2 or pT3. Grey color depicts no available data.

No significant association between relative *AR*, *PCA3*, and *PSMA* mRNA expression and either the plasma biomarkers (sPD-L1 or sPD-1) or other clinical features (age, serum PSA concentration or immune cell count) was discovered (data not shown).

3.2. Prediction of Clinically Significant PCa Using Liquid Biopsy Biomarkers

ROC analysis revealed *PSMA* as the best single gene expression biomarker predictor of clinically significant PCa (AUC = 0.81) (Figure 2). Overall, single urinary transcript biomarkers showed perfect sensitivity with *AR* boasting highest sensitivity (0.78). On the other hand, sPD-L1 showed best single biomarker specificity (0.97), but lowest sensitivity (0.44).

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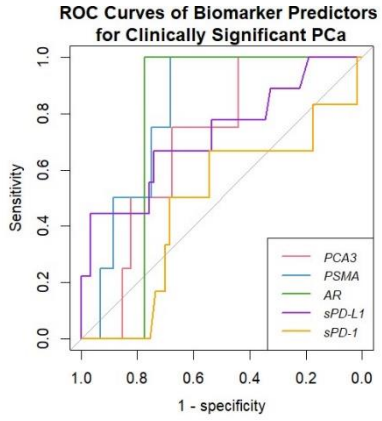


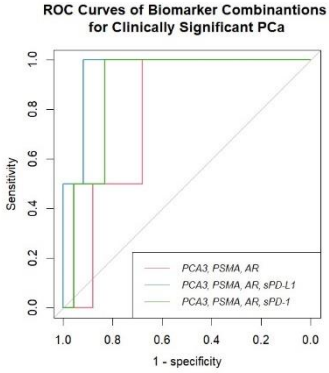
ROC metrics

Single biomarker predictions of clinically significant PCa

Predictor	AUC	threshold	accuracy	sensitivity	specificity	precision	npv	tpr	fpr
PCA3	0.699	−0.066	0.500	1.000	0.441	0.174	1.000	1.000	0.559
PSMA	0.812	0.181	0.708	1.000	0.682	0.222	1.000	1.000	0.318
AR	0.775	0.088	0.786	1.000	0.775	0.182	1.000	1.000	0.225
sPD-L1	0.723	22.605	0.896	0.444	0.966	0.667	0.918	0.444	0.034
sPD-1	0.478	25.725	0.556	0.667	0.544	0.133	0.939	0.667	0.456

Figure 2. ROC analysis of biomarker prediction of clinically significant PCa. npv – negative predictive value, tpr – true positive rate, fpr – false positive rate.

Regarding the combination of urine and plasma biomarkers together, an increase in the AUC values was noticed. While combining the three mRNA expression did not increase the prediction of clinically significant PCa (AUC 0.78 *vs* AUC 0.81 of *PSMA* biomarker), a combination of gene expression and sPD-1 and sPD-L1 increases AUC and overall specificity and accuracy of clinically significant PCa prediction (AUC 0.96 for three gene signature + sPD-L1) (Figure 3).



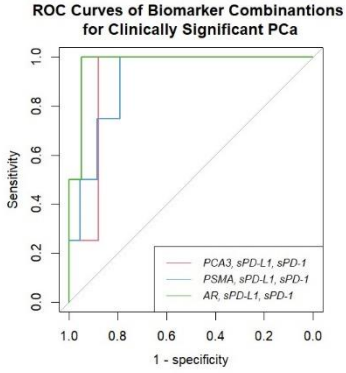
ROC metrics

Gene expression + sPD-L1 or sPD-1

Predictor	AUC	threshold	accuracy	sensitivity	specificity	precision	npv	tpr	fpr
PCA3, PSMA, AR	0.780	0.083	0.704	1.000	0.680	0.200	1.000	1.000	0.320
PCA3, PSMA, AR, sPD-L1	0.960	0.119	0.926	1.000	0.920	0.500	1.000	1.000	0.080
PCA3, PSMA, AR, sPD-1	0.896	0.120	0.846	1.000	0.833	0.333	1.000	1.000	0.167

Figure 3. ROC analysis of biomarker combinations for prediction of clinically significant PCa. Npv – negative predictive value, tpr – true positive rate, fpr – false positive rate.

Of note, the addition of plasma sPD-L1 and sPD-1 to *PCA3* or *AR* gene expression biomarkers combination increased the prediction specificity, when compared to other biomarker combinations (Figure 4). The combination of *AR* and 2 plasma biomarkers showed overall the best separation of clinically significant PCa cases from the clinically insignificant PCa (AUC = 0.97) out of all biomarkers and biomarker combinations examined.



ROC metrics

Gene expression + sPD-1 + sPD-L1

Predictor	AUC	threshold	accuracy	sensitivity	specificity	precision	npv	tpr	fpr
PCA3, sPD-1, sPD-L1	0.909	0.106	0.892	1.000	0.879	0.500	1.000	1.000	0.121
PSMA, sPD-1, sPD-L1	0.907	0.066	0.809	1.000	0.791	0.308	1.000	1.000	0.209
AR, sPD-1, sPD-L1	0.974	0.125	0.950	1.000	0.947	0.500	1.000	1.000	0.053

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Figure 4. ROC analysis of single gene expression and serum biomarker combinations for prediction of clinically significant PCa. Npv – negative predictive value, tpr – true positive rate, fpr – false positive rate.

Overall, while *PSMA* exhibited the strongest clinical significance as a standalone biomarker, *AR* demonstrated comparatively modest results of an AUC. However, according to ROC analysis, mRNA of *AR* from urine in addition of two plasma biomarkers, sPD-L1 and sPD-1, achieved the best separation between clinically significant and clinically insignificant PCa cases, with an AUC of 0.97, accuracy of 0.95, sensitivity of 100% and specificity of 95%. Notably, the inclusion of two soluble biomarkers significantly enhanced diagnostic accuracy from 0.79 to 0.95, specifically, the AUC for *AR* rose from 0.78 to 0.97, and for *PCA3*, it increased from 0.70 to 0.91. Additionally, it enhanced specificity, increasing it from 77.5% to 95.0% for *AR* and doubled it from 44.1% to 88.0% for *PCA3*, compared to the use of a single genetic biomarker. sPD-L1 and sPD-1 had the least impact on enhancing the predictive value of *PSMA*, increasing its AUC of 0.81 to 0.91 and specificity from 68.0 to 79.0 (Figures 2 and 4). The panel comprising three mRNA transcripts along with sPD-L1 offers a similar enhancement in diagnostic properties compared to combining *AR* with two plasma biomarkers with an AUC of 0.96 and 0.97, accuracy of 0.93 and 0.95, specificity of 0.92 and 0.95, respectively (Figures 3 and 4). The combination of sPD-L1 and sPD-1 yields an AUC of 0.72, with an accuracy of 0.52, sensitivity of 100% and specificity of 0.47 (data not shown).

4. Discussion

4.1. Significance of sPD-L1 and sPD-1 along with mRNA of PSMA, PCA3 and AR Genes in PCa

In the context of the intensive investigations for convenient biomarkers, a novel multifactorial approach that combines urine and blood biomarkers encompassing various aspects of the disease not only enhances detection but also offers a comprehensive assessment of prostate cancer. This approach highlights the potential of non-invasive liquid biopsies in improving the diagnosis and management of PCa. Building on our previous research which identified plasma sPD-L1/sPD-1 as a potential biomarker of PCa [9], we investigated gene expression in the urine samples of the same patients. As it is shown in Figures 1 and S1B–S3B, sPD-L1 can differentiate between clinically significant and non-significant prostate cancer ($p = 0{,}033$) and is associated with higher tumor stages ($p = 0.031$) and ISUP grading ($p = 0.026$) in PCa. Similarly, elevated sPD-L1 levels are consistently linked to larger tumors, advanced stages, and metastasis across different cancers [24,26].

In our study significant associations were identified between *PSMA* expression and clinically significant prostate cancer ($p = 0.039$) (Figures 1 and S1A), as well as among the three genes examined, *PSMA* emerged as the most reliable single biomarker for predicting clinically significant PCa with an AUC of 0.81 (Figure 2). Similarly, Rigau reported *PSMA* (AUC 0.74) outperformed *PSGR* (AUC 0.66) and *PCA3* (AUC 0.61) in predicting PCa within the PSA "gray zone" of 4–10 ng/ml [27]. Furthermore, we found that the expression levels of both *PSMA* and *PCA3* were associated with the pT3 stage ($p < 0.05$) (Figures 1 and S2A), while *PSMA* also was linked to ISUP grading (Figures 1 and S3A) indicating their potential as biomarkers for disease severity and progression. Despite *AR* not demonstrating any association with cancer advancement, in single-biomarker assessment it's AUC was slightly lower compared to *PSMA*, however it exhibited higher diagnostic accuracy than all the three urine biomarkers combined (0.70 *vs* 0.79) (Figures 2 and 3). Comparative analysis to other studies also suggests the involvement of *PSMA*, *PCA3*, and *AR* genes into prognosis and prediction of PCa. Blood PSMA-based biomarkers have been linked to malignancy risk [28] and predicted worse survival rates in metastatic PCa [29]. Higher PSMA expression correlated with advanced tumor stages and grades in biopsies and prostatectomy specimens [30]. Urine exosomal PSMA showed high diagnostic accuracy for significant PCa, correlating strongly with Gleason scores [31]. Similarly, *PCA3* scores have been associated with tumor aggressiveness [32], higher Gleason scores [18,33] and advanced clinical stages [33]. Moreover, various non-coding RNAs have been shown to influence prostate

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cancer progression by modulating *AR* signaling, highlighting their potential as biomarkers and therapeutic targets [34].

Although mentioned studies have shown that monitoring an RNA transcript from *PSMA*, *PCA3*, and *AR* genes can be beneficial for prostate cancer diagnosis, however relying on disease specific markers may not fully reflect the disease's complexity and heterogenous nature.

4.2. Combinations of Plasma sPD-L1/sPD-1 with mRNA of PSMA, PCA3 and AR Genes in PCa

To improve PCa diagnosis the combination of several different markers has been shown promising. While in our study the combination of all three mRNA expressions did not enhance the prediction of clinically significant prostate cancer (AUC 0.78) compared to the *PSMA* and *AR* biomarkers alone (AUC 0.81 and 0.78 respectively) (Figures 2 and 3). The addition of sPD-L1 to a triple gene expression panel has significantly enhanced the model's performance, resulting in diagnostic accuracy of 0.93 and in an AUC of 0.96, and rise of specificity from 68% to 92%, as illustrated in Figure 3. The composition of three genes along with sPD-1 also increased diagnostic accuracy from 0.70 to 0.85 for predicting clinically significant PCa and reflected in an AUC (0.90 *vs* 0.78) (Figure 3). Such multiaspected approach of combining mRNA of *PCA3*/*PSMA*/*AR* gene expression with sPD-L1/sPD-1 offers a comprehensive coverage, including tumor biology, immune response, and heterogeneity of prostate cancer. sPD-L1 has emerged as a promising biomarker for various cancers, including gastric [24] and lung cancers [35]. These findings highlight sPD-L1's broader applicability across cancers, making it valuable for diagnostics and treatment monitoring, due to its direct involvement in immune suppression [36], correlation with tumor burden, aggressiveness [37] and consistent association with clinical outcomes [11,38,39]. In contrast, sPD-1 primarily reflects immune activation, however high pretherapeutic sPD-1 levels suggest worse prognosis [12,40]. Previous studies have described correlations rather than combinations involving sPD-L1 and sPD-1. sPD-L1 is linked to neutrophil to lymphocyte ratio in advanced cancers [39]. Higher levels linked to low hemoglobin and albumin and elevated C-reactive protein in gastric cancer [41]. In pancreatic cancer, combining sPD-L1/PD-L2/B7-H5/CA19-9 improves diagnostic sensitivity, though sPD-1 did not add significance [42]. In PD-1 blockade therapy, sPD-1 and sPD-L1 levels together indicate treatment outcomes [43,44].

To the best of our knowledge, our study is the first successfully combining sPD-1 with a non sPD-L1 biomarker across multiple cancers, demonstrating that incorporating sPD-1 with mRNA transcripts improves diagnostic accuracy for clinically significant prostate cancer (Figure 3). Remarkably how non-specific PCa biomarkers like sPD-L1 and sPD-1, can enhance the diagnostic accuracy of PCa-specific biomarkers (Figures 2–4). This improvement highlights sPD-L1 and sPD-1 role in tumor development and suggests that combined biomarkers could refine diagnostic panels by capturing the complexity of disease progression for better prognostic assessment.

Interpreting our best biomarkers combination (*AR*, sPD-L1, sPD-1), – mRNA of urinary *AR* provides insights into the androgen receptor pathway, which is implicated in PCa development and progression [45,46]. Meanwhile, plasma sPD-L1 and PD-1 levels potentially reflect the tumor's immune microenvironment [12,40]. The combination of *AR, PSMA, PCA3* transcripts plus sPD-L1 shows comparable diagnostic properties and is likely more comprehensive for PCa assessment, as it incorporates multiple prostate cancer-specific markers and offers detailed insights into the cancer's characteristics. However, AR paired with sPD-L1 and sPD-1 require fewer biomarkers and offer slightly improved accuracy overall (Figures 3 and 4). Furthermore, androgen receptor signaling has been found to affect the expression of PD-L1 in prostate cancer, with AR activation linked to higher PD-L1 levels [47,48]. Additionally, scores for AR activity were significantly positively correlated with PD-1 methylation, resulting in an association with significantly reduced BCR (biochemical recurrence) - free survival after radical prostatectomy [49], suggesting an AR influence on the PD-L1/PD-1 axis. Further studies are warranted to explore potential associations between AR, sPD-L1 and sPD-1, particularly considering the economic advantages and convenience of implementing such a diagnostic panel.

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Integrating blood and urine biomarkers together significantly improves PCa detection and are supported by commercially available tests. SelectMDx Urine Test, including *DLX1*, *HOXC6*, *KLK3(PSA)* and other parameters achieved an AUC of 0.85 with 93% sensitivity and 47% specificity [50]. While the Michigan Prostate Score (MiPS), consisting of urine mRNA of *T2-ERG* and *PCA3*, and serum PSA also outperformed regular PSA test [51]. Additionally, scientific studies confirm the effectiveness of combining biomarkers obtained from different body fluids. Urinary exosomal *PCA3* and *PSMA* with serum PSA and PI-RADS achieved higher AUC than PSA alone [52], as well as urinary *PCA3* enhanced diagnostic performance of PSA in high-risk populations [53].

5. Conclusions

Urine and plasma are easily accessible biofluids, allowing for less invasive and repeatable sampling, longitudinal monitoring and potentially reducing unnecessary biopsies [54]. Our results demonstrate that the inclusion of sPD-L1 and sPD-1 in a diagnostic panel, together with *PSMA*, *PCA3*, and *AR* mRNA transcripts, has the potential significantly to improve the accuracy and specificity of PCa diagnostics. Future efforts should focus on refining multi-biomarker panels for greater diagnostic accuracy and developing multifactorial approaches for more personalized prostate cancer management.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Relative expression values of *AR*, *PCA3*, and *PSMA* mRNAs as well as sPD-L1 and sPD-1 in clinicly significant and insignificant PCa cases. Figure S2: Relative expression values of *AR*, *PCA3*, and *PSMA* mRNAs as well as sPD-L1 and sPD-1 in association with tumor stage. Figure S3: Relative expression values of *AR*, *PCA3*, and *PSMA* mRNAs as well as sPD-L1 and sPD-1 in association with ISUP grading.

Author Contributions: Conceptualization: MZ,IV,RS,VP; Methodology: MZ,IV,ZS,ND,AM; Validation: MZ,IV,RS,VP; Formal analysis: IV,ZS, MZ; Investigation: MZ,IV,ZS,PB,ND,AM; Data curation: MZ,IV,ZS,PB,ND,AM; Writing – original draft preparation: MZ,IV; Writing – review and editing RS,VP; Visualization MZ,IV,RS,VP; Supervision:RS,VP; Project administration:VP; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Regional Review Board (Vilnius, Lithuania, 158200-17-928-442). All research methods were carried out in accordance with the relevant Lithuanian national guidelines and regulations.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to protection of participants privacy.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

PCa - prostate cancer; *PSMA* - prostate-specific membrane antigen; *PCA3* - prostate-specific membrane antigen; *AR* - androgen receptor; sPD-L1 soluble PD- L1, sPD-1 – soluble PD-1; PSA - prostate specific antigen; ISUP - International Society of Urological Pathology; ELISA - Enzyme-Linked Immunosorbent Assay; ROC – Receiver Operating Characteristic; AUC – Area Under Curve.

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