ORIGINAL RESEARCH

The Diagnostic Value of Serum TGF-β1, p2PSA Combined with PSA in Prostate Cancer

Xi Guo, MD; Xiumei Sun, MM; Ping Ye, BM

ABSTRACT

Objective • To investigate the diagnostic value of transforming growth factor-β1 (TGF-β1), prostate-specific antigen isomer 2 (p2PSA) combined with a prostate-specific antigen (PSA) in prostate cancer (PCa).

Methods • From October 1, 2019 to September 1, 2022 we enrolled a total of 90 patients with PCa90 patients with PCa in the urology department of our hospital were selected as the PCa group, 90 patients with benign prostatic hyperplasia (BPH) were selected as the BPH group, and 90 healthy people were selected as a healthy control group. The levels of TGF-β1, p2PSA and PSA in serum were detected, and the differences in TGF-β1, p2PSA and PSA levels among the three groups and PCa patients with different pathological parameters were compared. Univariate and Logistic regression analyses were used to analyze the independent risk factors affecting the occurrence of PCa. With pathological results as the 'gold standard', the diagnostic efficacy of TGF-β1, p2PSA and PSA alone and their combination for PCa was analyzed by the receiver operating characteristic (ROC) curve.

Results • The levels of serum PSA, p2PSA, and TGF-β1 in the PCa group were higher than those in the BPH group and control group ($P < .001$), and those in BPH group were higher than those in the control group ($P < .001$). The serum indexes of PCa group increased with the increase of Glerson grade and TNM stage ($P < .001$). The serum indexes of patients with lymph and bone metastasis were significantly higher than those without lymph and bone metastasis ($P < .001$). Logistic regression analysis showed that PSA, p2PSA and TGF-β1 were independent risk factors for PCa ($P < .001$). The area under the ROC curve (AUC) of PSA, p2PSA, TGF-β1 and combined detection were 0.738, 0.862, 0.821 and 0.932, respectively. The AUC of combined detection was greater than that of single detection ($P < .001$).

Conclusion • The expression levels of serum TGF-β1, p2PSA and PSA are related to PCa and are independent risk factors for PCa. The combined detection of the three groups can improve the diagnostic efficacy of PCa. Combined testing improves diagnostic accuracy for prostate cancer, allows for early intervention, and improves patient survival and confidence in treatment options. This will significantly improve the clinical management of prostate cancer. Future studies could explore other biomarkers or molecular indicators to further improve the accuracy of diagnosis and grading of prostate cancer. Additionally, differences between different populations and subtypes can be studied to better understand the heterogeneity of prostate cancer. (*Altern Ther Health Med.* 2024;30(7):184-191).

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INTRODUCTION

Prostate cancer (PCa) is a malignant tumor of the genitourinary system that presents a growing and significant threat to men's health, with its incidence steadily increasing each year.[1] According to the latest statistics, the incidence of prostate cancer has been rising. This highlights the urgency of investigating how to improve early diagnosis and treatment of prostate cancer to improve patient survival and quality of life.Early detection and accurate diagnosis of PCa are paramount in improving patient outcomes and reducing mortality rates. One of the primary tools used for clinical screening of PCa is the measurement of prostate-specific antigen (PSA) levels in the blood.[2] However, the PSA test,

while widely used, has limitations, particularly in terms of specificity. This limitation has raised concerns about potential overdiagnosis, leading to unnecessary treatments and anxiety for patients. A specific challenge arises when dealing with patients whose PSA levels fall within the range of 4-10 μg/L, as the PSA positivity rate in this range does not exceed 30%.[3] Moreover, distinguishing between PCa and benign prostatic hyperplasia (BPH), a non-cancerous enlargement of the prostate, can be particularly challenging using PSA levels alone. This diagnostic uncertainty can lead to delays in appropriate treatment or the unnecessary biopsy of benign conditions. The primary objective of this study is to investigate the diagnostic value of TGF-β1, p2PSA, and PSA in distinguishing between prostate cancer (PCa) and benign prostatic hyperplasia (BPH).

To address these limitations and enhance the accuracy of PCa diagnosis, researchers have turned their attention to novel biomarkers, such as prostate-specific antigen isoform [-2] (p2PSA).[4] p2PSA is a truncated form of the PSA precursor, and previous studies have indicated its significance in PCa diagnosis. Additionally, there is growing interest in the role of transforming growth factor-β1 (TGF-β1), a growth factor associated with critical cellular processes, including cell growth, differentiation, proliferation, and immune function. Studies have shown that TGF-β1 is highly expressed in PCa, and its levels increase more significantly with disease progression.[5]

This study is unique in that it explores the potential value of combined detection of multiple biomarkers to improve early diagnosis of prostate cancer. Furthermore, our study attempts to fill a knowledge gap in the current literature on how to combine these markers to improve the diagnostic accuracy of prostate cancer.Given these insights, this study aimed to investigate and analyze the correlation of TGF-β1, p2PSA, and PSA levels with both PCa and BPH. By doing so, it sought to shed light on the potential diagnostic value of each of these markers in distinguishing between PCa and BPH, addressing the pressing need for more specific and reliable diagnostic tools in the management of prostate health. Next, this study describes our research methods in detail in order to illustrate how we explored this important question.

MATERIALS AND METHODS

Research Subjects

Retrospective analysis was made on the clinical data of patients who underwent PSA screening in the Urology Department of our hospital from October 2019 to September 2022. According to the pathological diagnosis results, 90 cases diagnosed with PCa were selected and divided into the PCa group, while 90 cases diagnosed with BPH were divided into the BPH group.

Inclusion criteria: (1) Meets the diagnostic criteria of PCa or BPH[4] and is diagnosed for the first time; (2) There is no clear history of anti-tumor treatment in the past; (3) Age ≥ 18 years old; (4) The clinical data is complete. Exclusion criteria: (1) Patients with malignant tumors or metastatic PCa in other parts; (2) Patients with reproductive Urinary system infection; (3) Merge with other prostate diseases; (4) Patients with concomitant immune function disorders and coagulation disorders. Another 90 healthy individuals who underwent physical examination during the same period were selected as the control group.

Testing Methods

All patients underwent Prostate-Specific Antigen (PSA), Prostate-Specific Antigen(p2PSA) and Transforming Growth Factor Beta 1(TGF-β1) testing. No sexual intercourse was performed 48h before the test. No rectal examination, prostate massage or urethral instrumentation was performed. To ensure the standardization and accuracy of the test methods, certain precautions were taken. Firstly, patients were required to abstain from sexual intercourse for 48 hours prior to testing. This precaution is based on the understanding that sexual activity can lead to transient elevations in prostate-specific antigen (PSA) levels in the bloodstream, which typically return to baseline within a few days. By implementing this 48-hour abstinence period, the study aimed to obtain consistent and accurate baseline PSA measurements, minimizing the potential for artificially elevated PSA levels that could lead to misinterpretation. Secondly, the study excluded rectal examinations, prostate massages, and urinary catheterization prior to testing. These procedures have the potential to introduce variability and interference in the test results. Rectal examinations and prostate massages can stimulate the prostate gland, causing temporary fluctuations in PSA levels.

Similarly, urinary catheterization can disrupt the normal physiology of the prostate and urinary tract, potentially affecting PSA levels. By avoiding these procedures before testing, the study sought to eliminate confounding factors that might compromise the accuracy and reliability of PSA, p2PSA, and TGF-β1 measurements. This rigorous approach ensures that the observed changes in these biomarkers accurately reflect the patient's actual physiological state, enhancing the precision and clinical relevance of the study's findings.

5 mL of fasting venous blood was collected early in the morning, and the serum was centrifuged at 3000 r/min to obtain the raw layer for testing. Serum PSA was measured using a Roche Cobas e-601 electrochemiluminescence immunoassay and matching reagents. Serum p2PSA was measured using an ACCESS2 fully automated immunoassay and accompanying reagents; serum TGF-β1 was measured using an enzyme-linked immunosorbent assay with a fully automated ELISA.

Pathological biopsies in prostate cancer diagnosis are greatly facilitated by the expertise of ultrasound physicians and experienced urology attending physicians. These medical professionals harness the power of advanced imaging techniques such as ultrasound and MRI to guide the biopsy process effectively. A sonographer is responsible for using ultrasound imaging to guide the prostate biopsy process. They use ultrasound equipment to view the patient's prostate in real time, ensuring that the biopsy needle accurately samples suspected abnormal areas. This helps improve the

accuracy of the biopsy and reduces the risk of complications. Ultrasound and MRI are indispensable tools in guiding prostate biopsies. Ultrasound involves the insertion of a probe into the rectum, producing real-time images of the prostate gland. These images provide valuable insights into the size, shape, and location of potential abnormalities within the prostate. Physicians rely on this visual information to precisely position the biopsy needle, ensuring the collection of samples from the most relevant areas. This targeted approach significantly enhances the chances of obtaining meaningful biopsy specimens. MRI, on the other hand, offers detailed anatomical and functional information, revealing any irregularities, including tumor lesions, with high clarity. Radiologists identify suspicious areas on MRI images, which urology attending physicians use to plan biopsy procedures. MRI-guided biopsies are particularly effective for targeting areas that may not be easily visible with ultrasound alone, guaranteeing the accurate sampling of even small or deep-seated lesions. After a puncture, the pathologist (associate Chief physician) will analyze the pathological results of the biopsy tissue, and give Gleason score to the results.[6] Among them, a score of ≤ 4 is rated as highly differentiated, 5-7 is rated as moderately differentiated, and 8-10 is rated as poorly differentiated.

The Gleason score is a method used to evaluate prostate cancer tissue biopsy specimens to determine how malignant the cancer is. It consists of 3 different grades, namely 3 points, 4 points and 5 points. The specific meanings are as follows: Gleason score 3+3=6: This is the lowest grade of prostate cancer, indicating that the cancer cells are milder and less aggressive. Invasive. Typically, this cancer grows slowly and has a higher cure rate. Gleason score 3+4=7: This is a moderately malignant prostate cancer. Some cancer cells show a certain degree of invasiveness, but there are still good treatment prospects. Gleason score 4+3=7: This is also a 7 rating, but it has a slightly different meaning than the previous case. Here, more cancer cells appeared to be more aggressive, but still less aggressive than higher-grade prostate cancer. Gleason score 4+4=8 or higher: These scores indicate highly malignant prostate cancer, in which the cancer cells are more aggressive and difficult to treat.

Statistical analysis

Statistical analyses were performed using SPSS 24.0 (IBM, Armonk, NY, USA). Continuous data are presented as mean ($\bar{x}$) ± standard deviation (s) and were compared using analysis of variance (ANOVA) for inter-group comparisons. SPSS was chosen for data analysis because it is a powerful statistical analysis tool that is particularly suitable for processing medical and biological data. We used specific statistical tests and methods to fully assess the differences and correlations in our data and ensure that our findings were trustworthy.The counting data is represented by [n(%)], and the comparison between groups is conducted using χ^2; Single factor and logistic regression analysis were used to identify independent risk factors affecting the occurrence of PCa. Using pathological results as the gold standard, evaluate the diagnostic value of individual and combined detection of each indicator; Draw the receiver operating characteristic (ROC) curve, and compare the area under the curve (AUC) using rank sum test; The difference was statistically significant with $P < .05$.

Pathology results are used as the gold standard because it provides a detailed assessment at the histological and cytological levels that can unambiguously determine whether malignant changes are present in the prostate tissue. This evaluation, which typically involves biopsy and histological analysis under a microscope, provides a highly precise diagnosis and is considered the gold standard for prostate cancer diagnosis. Other methods such as imaging, although useful, often require further confirmation, and pathology results often provide the most reliable diagnostic information.

RESULTS

Comparison of General Information of the Three Groups

In this comparative study involving three groups, namely the Prostate Cancer (PCa) group, Benign Prostatic Hyperplasia (BPH) group, and Control group, several general characteristics were evaluated. The mean age of the PCa group was 62.24 years (±8.64 years), while their average Body Mass Index (BMI) was 23.42 kg/m^2 (±2.34 kg/m^2). Notably, 34.44% of individuals in the PCa group had a history of hypertension, 27.78% had a history of diabetes, and 18.89% had dyslipidemia. In contrast, the BPH group had a slightly higher mean age of 63.15 years (±8.81 years) and a similar BMI of 23.19 kg/m^2 (±2.28 kg/m^2). They exhibited a history of hypertension in 31.11% of cases, diabetes in 22.22%, and dyslipidemia in 16.67%. Lastly, the Control group had a mean age of 61.12 years (±7.98 years) and a BMI of 23.31 kg/m^2 (±2.31 kg/m^2), with 25.56% having a history of hypertension, 24.44% having a history of diabetes, and 12.22% having dyslipidemia. Statistical analysis (F/χ2 and P values) indicated that there were no significant differences between the groups in terms of age, BMI, and history of hypertension, diabetes, or dyslipidemia ($P > .05$). See Table 1, Figure 1.

Comparison of Serum PSA, p2PSA and TGF-β1 Levels in the Three Groups

Table 2 presents a comparison of serum PSA (Prostate-Specific Antigen), p2PSA (pro-PSA), and TGF-β1 (Transforming Growth Factor-beta 1) levels across three groups: the Prostate Cancer (PCa) group, Benign Prostatic Hyperplasia (BPH) group, and Control group. In the PCa group, the mean PSA level was 16.53 μg/L (±4.94), p2PSA was 16.48 ng/L (±4.87), and TGF-β1 was 12.16 ng/mL (±3.99). Notably, the BPH group exhibited significantly lower levels in all three markers: PSA (10.42 μg/L ±3.47), p2PSA (7.36 ng/L ±2.43), and TGF-β1 (5.97 ng/mL ±1.93). Similarly, the Control group had lower levels compared to the PCa group: PSA (2.76 μg/L ±0.84), p2PSA (2.82 ng/L ±0.93), and TGF-β1 (4.16 ng/mL ±1.34). Statistical analysis revealed significant differences among the groups for all three markers

Table 1. Comparison of general information of the three groups [$\bar{x} \pm s$, n(%)]

Group	Age (years)	Body Mass Index (kg/m²)	History of illness		
			History of hypertension	History of diabetes	Dyslipidemia
PCa group (n = 90)	62.24±8.64	23.42±2.34	31 (34.44)	25 (27.78)	17 (18.89)
BPH group (n = 90)	63.15±8.81	23.19±2.28	28 (31.11)	20 (22.22)	15 (16.67)
Control group (n = 90)	61.12±7.98	23.31±2.31	23 (25.56)	22 (24.44)	11 (12.22)
F/χ^2	1.426	1.068	1.716	0.754	1.549
P value	.133	.281	.424	.686	.461

Figure 1. Previous medical history statistics

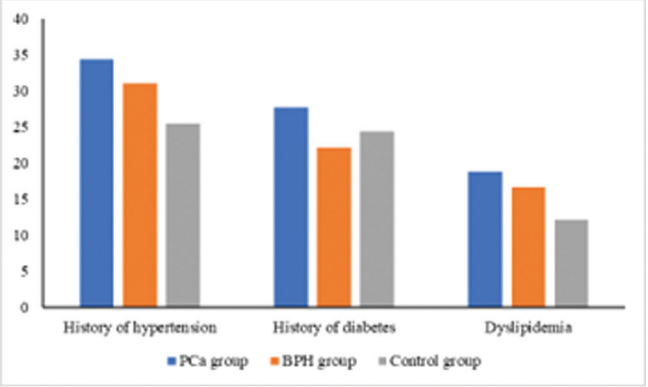
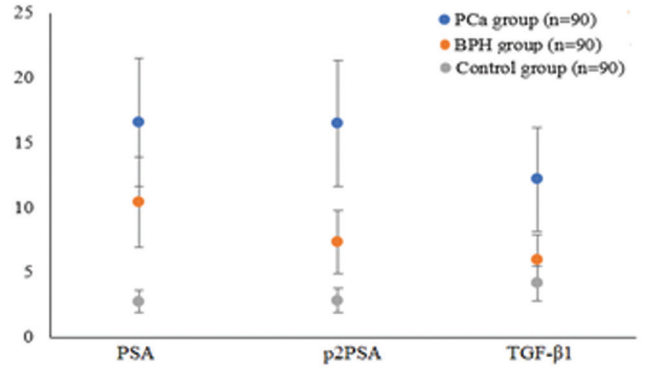


Table 2. Comparison of serum PSA, p2PSA and TGF-β1 levels in the three groups ($\bar{x} \pm s$)

Group	PSA (μg/L)	p2PSA (ng/L)	TGF-β1 (ng/mL)
PCa group (n = 90)	16.53±4.94	16.48±4.87	12.16±3.99
BPH group (n = 90)	10.42±3.47[a]	7.36±2.43[a]	5.97±1.93[a]
Control group (n = 90)	2.76±0.84[ab]	2.82±0.93[ab]	4.16±1.34[ab]
F	34.581	23.588	27.511
P value	<.001	<.001	<.001

[a]*P* < .05 compared to PCa group
[b]*P* < .05 compared to BPH group

Figure 2. Comparison of serum PSA, p2PSA and TGF-β1 levels in the three groups



(F-values: 34.581 for PSA, 23.588 for p2PSA, and 27.511 for TGF-β1; *P* < .001). Moreover, post-hoc tests indicated that both the BPH and Control groups had significantly lower levels (*P* < .05) compared to the PCa group, with the Control group also differing significantly from the BPH group. See Table 2, Figure 2.

When we observed that higher PSA, p2PSA, and TGF-β1 levels were associated with an increased likelihood of prostate cancer, this hinted at the importance of these biomarkers in the early detection of prostate cancer. High levels may reflect abnormal prostate biological processes, including hyperplasia and inflammation. Through these markers, we may be able to identify patients at risk for prostate cancer earlier, hopefully improving the effectiveness of early screening and intervention and reducing unnecessary pathological biopsies for patients. This will have a profound impact on the early diagnosis and treatment of prostate cancer, improving the chances of successful treatment.

Table 3. Comparison of Serum PSA, p2PSA and TGF-β1 Levels in PCa Patients with Different Pathological Parameters

Pathological parameters	PSA (μg/L)	p2PSA (ng/L)	TGF-β1 (ng/mL)
Glerson Rating			
Highly differentiated (n=23)	14.42±3.68	13.79±3.84	9.64±2.88
Medium/low differentiation (n=67)	27.11±6.34	28.03±6.78	14.16±3.82
t	9.068	9.538	5.184
P value	.000	.000	.000
TNM Stages			
Stage I-II (n=52)	12.39±3.73	13.05±3.91	8.18±2.75
Stage III-IV (n=38)	26.18±6.15	27.42±6.28	15.32±4.61
t	13.199	13.349	9.167
P value	.000	.000	.000
Lymph node metastasis			
None (n=49)	13.82±4.07	14.04±4.31	8.37±2.82
Yes (n=41)	28.79±6.38	28.87±6.44	15.68±4.72
t	13.478	13.015	9.081
P value	.000	.000	.000
Bone metastases			
None (n=79)	14.42±4.17	14.83±4.25	8.89±2.81
Yes (n=11)	27.94±6.82	28.16±7.08	16.07±5.10
t	9.234	8.891	7.071
P value	.000	.000	.000

Comparison of Serum PSA, p2PSA and TGF-β1 Levels in PCa Patients with Different Pathological Parameters

Table 3 provides a comprehensive comparison of serum PSA (Prostate-Specific Antigen), p2PSA (pro-PSA), and TGF-β1 (Transforming Growth Factor-beta 1) levels among Prostate Cancer (PCa) patients with different pathological parameters. When examining Gleason Rating, highly differentiated PCa cases (n=23) displayed lower mean PSA (14.42 μg/L ±3.68), p2PSA (13.79 ng/L ±3.84), and TGF-β1 (9.64 ng/mL ±2.88) levels than those with medium/low differentiation (n=67), who exhibited higher values (PSA: 27.11 μg/L ±6.34, p2PSA: 28.03 ng/L ±6.78, TGF-β1: 14.16 ng/mL ±3.82). Significant differences were observed in all three markers (*P* < .001).

Similarly, when assessing TNM Stages, patients in Stage I-II (n=52) had lower levels of PSA (12.39 μg/L ±3.73), p2PSA (13.05 ng/L ±3.91), and TGF-β1 (8.18 ng/mL ±2.75) compared to those in Stage III-IV (n=38) with higher levels (PSA: 26.18 μg/L ±6.15, p2PSA: 27.42 ng/L ±6.28, TGF-β1: 15.32 ng/mL ±4.61). These differences were statistically significant (*P* < .001).

Moreover, when evaluating Lymph Node Metastasis and Bone Metastases, patients without lymph node metastasis or bone metastases had lower levels of PSA, p2PSA, and TGF-β1 compared to those with such metastases, and all differences were statistically significant (*P* < .001). These findings collectively suggest that serum levels of PSA, p2PSA, and TGF-β1 are closely associated with different pathological parameters in PCa patients, providing valuable insights for clinical assessment and management. See Table 3.

Logistic Regression Analysis

Table 4 presents the results of logistic regression analysis evaluating the impact of serum biomarkers, namely PSA (Prostate-Specific Antigen), p2PSA (pro-PSA), and TGF-β1 (Transforming Growth Factor-beta 1), on the likelihood of Prostate Cancer (PCa). The analysis revealed significant associations between these biomarkers and PCa. Specifically, PSA exhibited a β coefficient of 1.124 (SE = 0.342), with a Wald statistic of 10.801 and $P = .001$. This implies that for every unit increase in PSA, the odds of having PCa increased by a factor of 3.077, as indicated by the odds ratio (OR) of 3.077, with a 95% confidence interval (CI) ranging from 1.574 to 6.015. Similarly, p2PSA showed a β coefficient of 1.217 (SE = 0.451), a Wald statistic of 7.282, and a P-value of 0.007. This suggests that for each unit increase in p2PSA, the odds of having PCa increased by a factor of 3.377 (OR = 3.377), with a 95% CI spanning from 1.395 to 8.174. Additionally, TGF-β1 demonstrated a β coefficient of 1.086 (SE = 0.373), a Wald statistic of 8.477, and a $P = .003$. For every unit increase in PSA, the odds of having PCa increased by a factor of 3.077, as indicated by the odds ratio (OR) of 3.077, with a 95% confidence interval (CI) ranging from 1.574 to 6.015. This indicates that for every unit increase in TGF-β1, the odds of having PCa increased by a factor of 2.962 (OR = 2.962), with a 95% CI ranging from 1.426 to 6.154.

In summary, the logistic regression analysis highlights that higher levels of PSA, p2PSA, and TGF-β1 are significantly associated with an increased likelihood of Prostate Cancer, underscoring their potential as valuable biomarkers for PCa diagnosis and risk assessment. See Table 4.

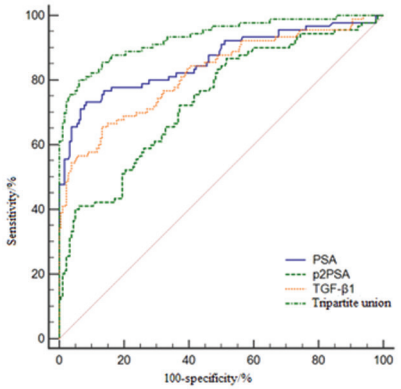
Table 4. Logistic regression analysis of serum TGF-β1, p2PSA and PSA on PCa

Variable	β	SE	Wald	*P* value	OR	95%CI
PSA	1.124	0.342	10.801	.001	3.077	1.574~6.015
p2PSA	1.217	0.451	7.282	.007	3.377	1.395~8.174
TGF-β1	1.086	0.373	8.477	.003	2.962	1.426~6.154

Table 5. Diagnostic efficacy of serum TGF-β1, p2PSA and PSA alone and in combination for PCa

Indicators	Truncation value	AUC	SE	*P* value	95%CI	Sensitivity	Specificity
PSA	7.289	0.738	0.033	<.001	0.682~0.790	86.7	48.3
p2PSA	13.646	0.862	0.027	<.001	0.815~0.901	73.33	91.67
TGF-β1	11.048	0.821	0.029	<.001	0.770~0.865	65.6	86.7
Combined testing	-	0.932	0.018	<.001	0.895~0.956	80.0	93.9

Figure 3. ROC curve analysis of serum TGF-β1, p2PSA and PSA alone and in combination for PCa.



Diagnostic Efficacy of Serum TGF-β1, p2PSA and PSA Alone and in Combination for PCa

Table 5 summarizes the diagnostic efficacy of serum biomarkers, including PSA (Prostate-Specific Antigen), p2PSA (pro-PSA), and TGF-β1 (Transforming Growth Factor-beta 1), both individually and in combination, for the detection of Prostate Cancer (PCa). Individually, PSA demonstrated an area under the curve (AUC) of 0.738 with a standard error (SE) of 0.033, indicating good discrimination for PCa. The optimal truncation value for PSA was 7.289. It achieved a sensitivity of 86.7% and a specificity of 48.3%.p2PSA displayed a higher AUC of 0.862 (SE = 0.027) with a truncation value of 13.646. It had a sensitivity of 73.33% and specificity of 91.67%, signifying superior diagnostic performance compared to PSA. TGF-β1, with an AUC of 0.821 (SE = 0.029) and a truncation value of 11.048, exhibited good diagnostic accuracy. Its sensitivity was 65.6%, and its specificity was 86.7%. When combining all three biomarkers, the AUC significantly increased to 0.932 (SE = 0.018), indicating excellent diagnostic discrimination for PCa. This combined testing approach achieved a sensitivity of 80.0% and an impressive specificity of 93.9%.

These diagnostic performance indicators provide critical information on the accuracy and feasibility of biomarkers. AUC (area under the curve) is often used to evaluate the discriminating ability of a biomarker. AUC values range between 0.5 and 1, with 1 indicating perfect accuracy and 0.5 indicating purely random accuracy. Therefore, a higher AUC value indicates that the biomarker has a stronger and more reliable diagnostic ability.

Sensitivity and specificity reflect the diagnostic effectiveness of a biomarker. High sensitivity means that the biomarker correctly identifies cases that actually have prostate cancer, while high specificity means that the biomarker correctly excludes cases that do not have prostate cancer. Therefore, in practice, higher sensitivity and specificity mean that biomarkers are more valuable in screening and diagnosis, and can accurately identify patients' prostate cancer risk, reducing the rate of misdiagnosis and missed diagnosis.

Taken together, higher AUC, sensitivity, and specificity values indicate that the biomarker is more reliable in the diagnosis of prostate cancer, which will provide more information for clinicians and contribute to more accurate assessment. A patient's prostate cancer risk to guide further evaluation and treatment decisions.

In summary, the results suggest that when used in combination, PSA, p2PSA, and TGF-β1 provide a powerful diagnostic tool for the detection of Prostate Cancer, offering high sensitivity and specificity, which could be valuable for clinical diagnosis and risk assessment. See Table 5 and Figure 3.

DISCUSSION

According to the latest cancer data,[7] PCa ranks 2nd and 3rd in incidence and mortality of all malignant tumors in

men, and the trend is increasing year by year. However, the onset of PCa is insidious, and there are often no characteristic manifestations in the early stage, and most patients are diagnosed in the middle and late stages, and the best time for treatment has been missed. PSA was the first indicator used for early screening of PCa, but in addition to PCa, other causes of elevated PSA include BPH, prostatitis and other benign prostate diseases and prostate instrumentation.[8] Therefore, PSA alone is not ideal as an early screening indicator for PCa. In this study, serum PSA levels were higher in the PCa group than in both the BPH and control groups and were closely associated with Glerson's grading, TNM stage, lymphatic metastases and bone metastases; the ROC curve showed that the sensitivity and specificity of PSA for PCa diagnosis were not high. The ROC curve showed that the sensitivity and specificity of PSA for the diagnosis of PCa were not high. It is important to find more accurate tumor markers or combined diagnostic methods to improve the accuracy of PCa diagnosis.

These findings have important clinical relevance and may have a positive impact on the diagnosis and treatment of prostate cancer. First, high PSA, p2PSA, and TGF-β1 levels are associated with increased risk of prostate cancer, suggesting that these biomarkers could be used for early screening to help identify patients at risk for prostate cancer. This helps doctors decide more accurately whether to perform further examinations, reduces unnecessary biological specimen collection and pathological examinations, and reduces costs and patient discomfort. Secondly, by combining PSA, p2PSA and TGF-β1, the diagnostic accuracy of prostate cancer can be improved. This means doctors can more reliably distinguish prostate cancer from other prostate diseases, avoiding misdiagnosis. This is crucial for treatment decisions as it ensures that only patients who truly have prostate cancer receive treatment, thus avoiding unnecessary interventions and potential treatment side effects. Ultimately, these findings also provide more information for personalized treatment of prostate cancer. By more accurately assessing the risk and type of prostate cancer, doctors can choose more appropriate treatment options, thereby increasing the effectiveness of treatment and reducing side effects and complications for patients.

p2PSA is a homologous isomer of PSA, which is considered to be of high value in the prediction of PCa diagnosis.[9] In this study, the serum p2PSA level in the PCa group was higher than that in the BPH group and the control group and was closely related to Glerson's grading, TNM stage, lymphatic metastasis and bone metastasis; the ROC curve showed that the diagnostic value of p2PSA for PCa diagnosis was higher than that of PSA. thus indicating that the predictive value of p2PSA for PCa is higher than that of the traditional PSA index. Filella et al.[10] confirmed that p2PSA could improve the diagnostic accuracy of PCa and BPH when PSA was in the range of 4-20 ng/mL. Another study confirmed[11] that p2PSA and its derivatives are highly specific screening indicators for PCa and have high value for PCa diagnosis. p2PSA is a truncated form of PSA precursor (proPSA). proPSA is hydrolyzed by proteases into truncated forms of varying lengths, of which p2PSA is the slowest to cleave and the most stable in nature.[12] Therefore, its specificity and accuracy for PCa diagnosis are higher compared to conventional PSA.

Recent studies have shown[13-15] that there is a close association between TGF-β1 and a variety of tumourigenesis, development and prognosis. In this study, the serum TGF-β1 level in the PCa group was higher than that in the BPH group and the control group, and it showed an increasing trend with the increase of pathological grade and clinical stage, and the TGF-β1 level in patients with lymphatic and bone metastases was significantly higher than that in patients without lymphatic and bone metastases; the ROC curve showed that the diagnostic value of TGF-β1 for PCa diagnosis was higher than that of PSA. The significant increase in serum TGF-β1 level suggested the possibility of PCa metastasis. Fossati[16] showed that the positive expression rate of TGF-β1 in PCa and BPH tissues was 70.00% and 24.00%, respectively, and TGF-β1 has a high value for the diagnosis of PCa, which is consistent with the results of this study. Previous studies also showed that,[17] and detecting serum TGF- β 1 levels in PCa patients is helpful in the differential diagnosis of bone metastasis. TGF-β1 is a multifunctional cytokine that is widely distributed in epithelial, endothelial and neural sites and plays an important regulatory role in the cellular life cycle. In normal cells, TGF-β1 is involved in cell proliferation, differentiation and maturation; while in tumor cells, TGF-β1 is involved in the growth and multiplication of tumor cells and promotes their invasion and metastasis. Meanwhile, during the progression of PCa, TGF-β1 can stimulate neovascularization and promote vascular smooth muscle differentiation,[18] which in turn stabilizes neovascularization and improves the good environment and nutritional basis for tumor growth. TGF-β1 exerts potent immunosuppressive effects through diverse mechanisms impacting key components of the immune system.[19] It significantly inhibits the activity of T lymphocytes, including cytotoxic T cells (CD8+ T cells) and helper T cells (CD4+ T cells), impairing their ability to mount effective anti-tumor immune responses.[16] Additionally, TGF-β1 dampens the cytotoxic activity of natural killer (NK) cells, diminishing their capacity to detect and eliminate cancer cells. Furthermore, TGF-β1 influences monocyte differentiation into immunosuppressive M2-type macrophages, promoting tumor growth while suppressing anti-tumor immune responses.[16] Dendritic cells, critical for initiating immune responses, also suffer from impaired maturation and antigen-presentation in the presence of TGF-β1, hindering their ability to activate T cells effectively.[17] Collectively, these immunosuppressive actions create an unfavorable microenvironment within tumors, where immune cells are less active or inhibited, enabling cancer cells to evade immune surveillance and proliferate.[18] Understanding these mechanisms is pivotal for developing targeted therapies that

counteract TGF-β1's immunosuppressive effects, potentially enhancing anti-tumor immune responses in conditions like prostate cancer.[19]

The combined assessment of PSA, p2PSA, and TGF-β1 in diagnosing Prostate Cancer (PCa) offers a comprehensive evaluation of the disease by capitalizing on their distinct roles. PSA serves as an initial screening tool, although it lacks specificity, as it can be elevated in non-cancerous conditions like Benign Prostatic Hyperplasia (BPH). In contrast, p2PSA, a more stable isoform of PSA, enhances specificity, particularly in the "gray zone" of PSA levels (4-20 ng/mL), where distinguishing PCa from BPH is challenging. TGF-β1 reflects PCa aggressiveness and potential metastasis. This combination approach delivers several advantages: it heightens sensitivity for PCa detection, even when PSA levels alone may not raise concerns; it bolsters specificity by incorporating p2PSA and TGF-β1, reducing false positives and unnecessary invasive procedures; and it aids in assessing the cancer's aggressiveness, thus informing treatment strategies. PSA, p2PSA and TGF-β1 are all valuable for the early screening and diagnosis of PCa. In this study, the diagnostic efficacy of the three tests alone and in combination with PCa was assessed by ROC curves. The results showed that all three tests alone and in combination had good AUCs for PCa, and the diagnostic value was higher when the tests were combined. This suggests that the combination of the three tests can significantly improve the diagnostic efficacy of PCa, reduce the possibility of missed diagnosis or misdiagnosis, and reduce unnecessary puncture biopsies, which is related to the fact that the combination of the indicators can provide a comprehensive evaluation of PCa from multiple perspectives.

Combining PSA, p2PSA, and TGF-β1 has significant advantages as it allows for the complementary effects of these biomarkers, thereby improving the diagnostic accuracy of prostate cancer. This combined use allows for a more comprehensive assessment of prostate cancer risk and presence, providing healthcare professionals with more information to better guide clinical decisions. First, PSA (prostate-specific antigen) is the standard biomarker for prostate cancer screening, but it can be interfered with by prostatitis and hyperplasia, leading to false-positive results. p2PSA is a derivative that improves the specificity of prostate cancer detection. When used with PSA, it can reduce the occurrence of false-positive results, thereby improving the accuracy of prostate cancer screening. Second, TGF-β1 (transforming growth factor β1) elevation in prostate cancer is associated with disease progression and invasion. Combining TGF-β1 with PSA and p2PSA can provide more information about the condition of prostate cancer. This combined use helps more accurately identify those patients who require further testing and treatment, reducing the risk of overdiagnosis and intervention. Ultimately, this combined approach provides a more comprehensive assessment of prostate cancer, allowing healthcare professionals to better differentiate prostate cancer from other prostate diseases to better guide treatment decisions. Therefore, it has potential importance in clinical practice to help improve early screening and diagnostic accuracy of prostate cancer.

This study's findings, especially regarding TGF-β1, hold profound significance for early prostate cancer (PCa) screening. Elevated serum TGF-β1 levels in PCa patients, distinguishing them from benign prostatic hyperplasia (BPH), offer promise for enhanced early detection. TGF-β1's correlation with disease grade, clinical stage, and metastatic potential underscores its potential as a robust biomarker. Its superiority to PSA, as demonstrated in this study, suggests its inclusion in routine PCa screening protocols, reducing overdiagnosis risks. Additionally, insights into TGF-β1's multifaceted role in tumor progression open doors for novel therapies and improved PCa management. These findings equip healthcare professionals with valuable tools for more accurate PCa diagnosis and intervention, ultimately improving patient outcomes. However, this study has some limitations. Firstly, this study is a single-center study with a small sample size; secondly, there are many factors affecting PCa, and whether the above indicators can reflect the condition of PCa patients in a timely and accurate manner needs to be analyzed in a large sample, before and after treatment control and more abundant tests.

Our findings have a wide range of potential clinical applications. First, these findings may provide opportunities for the development of new screening and diagnostic tests for prostate cancer. The combined use of biomarkers such as PSA, p2PSA and TGF-β1 can increase the detection specificity of prostate cancer and reduce false-positive results, thereby improving the accuracy of screening. This may facilitate early diagnosis and provide earlier opportunities for treatment, thereby improving patient outcomes. Additionally, our study may have implications for prostate cancer screening guidelines. Existing screening guidelines are often based on PSA as a single marker. However, our findings suggest that the combined use of multiple biomarkers may be more beneficial for early diagnosis of prostate cancer. Therefore, future screening guidelines may consider including these biomarkers to improve screening accuracy. Another potential clinical application is in the management of prostate cancer patients. The combined use of these biomarkers can provide healthcare professionals with a more comprehensive picture of a patient's condition, helping to determine a patient's risk and severity of illness. This will help better guide treatment decisions, including options such as surgery, radiation therapy, and hormone therapy.

This is a future direction for some research to further explore this area. First, although our study highlights the importance of three biomarkers: PSA, p2PSA, and TGF-β1, the field of prostate cancer biomarkers remains to be explored in depth. Other potential biomarkers, especially for early diagnosis, may require more research. Future studies could look for new biomarkers or explore the combination of these markers with other factors to further improve the accuracy of early diagnosis of prostate cancer.

Regarding sample size and generalizability, we acknowledge the limitations of this study, including the

relatively small sample size and single-center design. This may affect the external validity and generalizability of the study results. Therefore, future studies are needed to validate our results with larger multicenter studies to ensure their applicability in a wider population.

Regarding publication implications, our study may have a positive impact on the field of prostate cancer screening and diagnosis. Especially with regard to screening and early diagnosis, our findings may help guide future revisions of clinical guidelines to better include the use of these biomarkers. Additionally, our study may inspire other researchers to delve deeper into biomarkers for prostate cancer diagnosis and conduct additional studies to validate and refine these findings.

In terms of clinical guidelines, our findings may help drive updates to clinical guidelines for prostate cancer screening and diagnosis. This will depend on the results of more large-scale, multicenter studies, as well as further evaluation by relevant professional organizations. In the future, the use of these biomarkers may be more widely incorporated into clinical practice in prostate cancer to improve the accuracy of early diagnosis and patient prognosis.

ETHICAL COMPLIANCE

This study was approved by the ethics committee of Ganzhou People's Hospital.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to report relevant to this article.

AUTHOR CONTRIBUTIONS

XG, XS and PY designed the study and performed the experiments; XG and XS collected the data, PY analyzed the data, XG, XS and PY prepared the manuscript. All authors read and approved the final manuscript. XG and XS contributed equally to this work.

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