



Clinical value of prostate-specific antigen combined with tumor abnormal protein (TAP) in the diagnosis of prostate puncture

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Background: In this study, we aimed to test the clinical value of total prostate-specific antigen (T-PSA), free prostate-specific antigen (F-PSA), and T-PSA combined with tumor abnormal glycoprotein (TAP) for early diagnosis of prostate cancer.

Methods: The levels of serum T-PSA and F-PSA were measured in 105 malignant prostate tumors and 97 benign prostate tissues using chemiluminescence immunoassay. The concentration of TAP in the serum of patients was tested by the agglutination method. Differences in PSA levels and F-PSA/T-PSA ratio in two patient groups were analyzed by t-test. TAP concentrations were compared using Chi-square test. The sensitivity, specificity, and accuracy of PSA combined with TAP for the diagnosis of prostate cancer were analyzed.

Results: The serum PSA level in patients with malignant tumors were higher than that in patients with benign tumors ($P < 0.0001$). TAP positivity in patients with prostate cancer was higher than that in patients with benign tumors ($P < 0.0001$). The number of cases with positive and weakly positive TAP values in three intervals of T-PSA concentrations was higher in patients with malignant tumors, while the number of negative cases was higher in patients with benign tumors ($P < 0.05$). The sensitivity, specificity, and accuracy of T-PSA combined with TAP in the diagnosis of prostate cancer were 97.14%, 67.01%, and 81.68% respectively, which was higher compared to the other measured markers.

Conclusions: The results of our study indicate that PSA combined with TAP is a sensitive, specific, and accurate diagnostic marker of prostate cancer. Therefore, it has potential clinical relevance for the early screening of prostate cancer.

Keywords: Prostate-specific antigen (PSA); tumor abnormal glycoprotein; prostate cancer; diagnostic value

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Introduction

Prostate cancer (PCa) is the second most common neoplasia among men and the sixth leading cause of cancer-specific death worldwide (1). In 2014, there were an estimated 238,590 new cases of prostate cancer and 29,480 deaths

due to prostate cancer in the United States alone (2). It is also the most commonly diagnosed malignancy in China, at least in part due to advances in prostate cancer diagnostics and an increasing awareness of citizens concerning their own health. The prognosis of prostate cancer is directly

related to the time of the diagnosis. The serum level of prostate-specific antigen (PSA) is a widely used prognostic biomarker of prostate cancer progression (3). However, its diagnostic value is limited. Therefore, novel techniques and biomarkers for early diagnostics of prostate cancer are of particular importance. Currently, the diagnostics of prostate cancer relies mainly on prostate puncture biopsy and magnetic resonance imaging (MRI). Because there are no obvious clinical manifestations in patients with early prostate cancer, the application of these methods for the diagnostics of early prostate cancer is limited, while the detection of tumor markers (TM) is a simple, reproducible, and conducive method of disease monitoring. The detection of TM in the serum or other body fluids is the perspective method for early cancer detection and prediction of disease outcome.

Although measurement of PSA blood levels was approved by the US Food and Drug Administration for the early detection of prostate cancer in 1995, the use of PSA in prostate cancer diagnostics remained controversial despite its widespread application by clinicians in the United States. Although PSA is a valuable diagnostic indicator, the use of PSA alone as a diagnostic biomarker often results in overdiagnosis. Consequently, many patients undergo prostate biopsy based solely on PSA levels, which may increase the risk of bleeding and infection (4).

TAP (tumor abnormal protein) is an abnormal glycoprotein and calcium-histone protein complex produced by cancer cell metabolism and is a common indicator of the abnormal proliferation of cancer cells. Numerous studies have shown that TAP is closely associated with the occurrence, progression, and prognosis of malignant tumors (5).

Several reports have shown that some, if not all, abnormal glycosylation is the result of initial carcinogenic transformation and is a critical event in the induction of cancer invasion and metastasis. When abnormal glycosylation occurs, various glycoproteins with abnormal polysaccharide structure are produced on the cell surface. The amount of TAP in circulation can be tested using specific lectins that induce coagulation of glycoproteins to form specific crystalline aggregates. These aggregates can be counted using the TAP image analysis system or a microscope. Importantly, the amount of TAP aggregates in the blood is a useful parameter for early detection and accurate cancer diagnosis (6).

In this study, we investigated the clinical value of PSA, free PSA (FPSA), FPSA/PSA ratio, and PSA in combination with TAP, as tools for prostate cancer diagnostics.

Methods

Patients and specimen selection

Tissue specimens from 202 patients who underwent prostate puncture biopsy were obtained from the archives of the department of pathology at the First Affiliated Hospital of An Hui's Medical University between April 2016 and November 2017. All cases were confirmed upon histopathological examination. The median age of patients was 59.5 years (range, 34–85 years). All the patients were monitored inside the hospital inpatient department and diagnosed by transrectal prostate puncture followed by histopathological analysis. Fasting venous blood for the detection of PSA, FPSA, and TAP levels was collected in the morning. The study was approved by the local ethics committee, and written informed consent was obtained from all patients before their enrolment in the study. The ethics approval number is Quick-PJ 2019-03-18.

The following exclusion criteria were applied:

- (I) Cancer invading organs other than the prostate;
- (II) The presence of other tumors, severe cardiovascular and cerebrovascular diseases, mental illness, thyroid disease, moderate to severe anemia, and severe liver and kidney function abnormalities;
- (III) TAP positive interference factor: progressive rheumatoid arthritis or rheumatism, abnormally increased glycated hemoglobin, unhealed fractures, long-term artificial metal implants, autoimmune diseases, etc.;
- (IV) Cases in which clinical data are missing, incomplete, or cannot be statistically analyzed.

Detection methods

PSA, FPSA, and FPSA/TPSA ratio measurement

Fasting venous blood (2 mL) was taken in the morning before DRE, prostate puncture, and transurethral instrumental examination. Serum was collected and subjected to chemical immunoassay. In patients with indwelling catheterization, the blood sample was taken 48 h after catheterization. The normal reference value for TPSA was 0–4 ng/mL and FPSA/TPSA ratio >0.16.

TAP detection procedure

Our experimental methods have been used by other researchers (5). The peripheral blood collected from participants was first dropped on glass slides and then

prepared for a blood smear. The TAP detection reagent (Ruisheng Medical Technology, Zhejiang, China) was dropped on the surface of the blood smear after it dried naturally. After 1.5 to 2 hours, an agglutination reagent was dropped on the blood smear. The agglomerated particles were observed by using a TAP integration reading microscope, the area of condensed particles was measured, and the values of TAP were recorded.

The results were interpreted as follows: (I) TAP positive (large aggregates): a crystal-like condensate with a polygonal, elliptical, or irregular circular shape and a complete edge was observed, and a crystal-like aggregate particle area $\geq 225 \mu\text{m}^2$ or having three or more condensates with an area of $121\text{--}225 \mu\text{m}^2$, were observed in the specimen; (II) TAP weakly positive (smaller condensate): a crystal-like polygonal, elliptical, or irregularly rounded aggregate with a relatively complete edge was observed, and a crystal-like aggregate particle area in the range of $121\text{--}225 \mu\text{m}^2$ or having three or more condensates with an area of $81\text{--}121 \mu\text{m}^2$, were observed in the specimen; (III) TAP negative (no detectable condensate): no crystal-like aggregates or any loose, sand-like particles, as well as snowflake-like, tree-like, or dendrite-like dark brown small particles, were observed, and condensates with an area of $<81 \mu\text{m}^2$ or two or fewer condensates with an area of $81\text{--}121 \mu\text{m}^2$ were observed.

Prostate puncture and biopsy

Prostate punctures were performed, and biopsy samples were collected from patients with suspected prostate cancer [including abnormal prostate MRI results, total PSA (TPSA) levels $\geq 10 \text{ ng/mL}$ or in case of TPSA levels in the range $4\text{--}10 \text{ ng/mL}$ with concomitant FPSA/TPSA (F/T) ratio <0.16]. The prostate was punctured 12 times using a puncture needle in the following zones: the median line at the side of the gland, the middle and the tip, and the outer circumference of the prostate. The biopsy specimens were fixed with neutral formaldehyde and sent for pathological examination.

Statistical analysis

All data were analyzed using GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA), and $P < 0.05$ was considered to be statistically significant. Median [Interquartile Range] was used to describe the age in all participants. The basic characteristics of patients in the T-PSA, F-PSA, and F/T groups were compared using the *t*-test. Chi-square test was performed to compare TAP

values between the groups.

Results

General characteristics of the patients

Out of 202 patients that underwent prostate puncture, 105 patients were diagnosed with cancer, while 97 patients had benign tumors. The age of all 202 patients was in the range of 34–85 years old. In patients with prostate cancer, the age was 71.84 ± 7.9 years old, whereas in patients with benign tumors, the age was 68.61 ± 8.19 years old.

TPSA, FPSA, and F/T ratio values in the patient groups

The levels of T-PSA and F-PSA in the prostate cancer group were significantly higher than those in the benign group, and the difference was statistically significant ($P < 0.0001$, Table 1). In addition, the F/T ratio was lower in the prostate cancer group, although this tendency did not reach statistical significance presumably due to insufficient sample size ($P = 0.0698$, Table 1). However, the obtained results suggested the potential clinical significance of F/T ratio for prostate cancer diagnostics and should be further evaluated.

Comparison of TAP positivity in the range of T-PSA concentrations (<4 , $4\text{--}10$, and $>10 \text{ ng/mL}$)

The number of cases with positive or weakly positive TAP values was higher in patients with malignant tumors in all three intervals of T-PSA concentrations, and the differences between the groups were statistically significant (Figure 1). The difference between the number of TAP positive and TAP negative cases was the highest in patients with T-PSA concentration $<4 \text{ ng/mL}$, whereas the smallest difference in the number of TAP positive and TAP negative cases was in the benign group with T-PSA $>10 \text{ ng/mL}$ (Figure 1A,C).

Comparison of TAP, T-PSA, T-PSA combined with TAP as diagnostic markers in prostate cancer

The sensitivity of T-PSA combined with TAP as a diagnostic marker of prostate cancer was 97.14%, the specificity was 67.01%, and the accuracy was 81.68% (Table 2), which were higher compared to other markers. In summary, T-PSA combined with TAP was a more sensitive, more specific, and more accurate parameter for the diagnosis of prostate cancer compared to TAP alone, T-PSA

Table 1 Comparison of age, T-PSA and F-PSA concentrations, and F/T ratio, TAP and Gleason score between groups of patients with prostate cancer and benign tumors

Variable	Malignant (n=105)	Benign (n=97)	P
Age	71.84±7.9	68.61±8.19	0.0049
T-PSA	42.48±4.55	15.17±1.301	<0.0001
F-PSA	6.871±0.9231	2.96±0.3629	<0.0001
F/T	0.1224±0.01033	0.1499±0.01102	0.0698
TAP, case (%)			<0.0001
1	22 (20.95)	60 (61.86)	
2	42 (40.00)	37 (38.14)	
3	41 (39.05)	0	
Gleason (cases)			-
≤7	51	-	
8–10	54	-	

T-PSA, total prostate specific antigen; F-PSA, free prostate specific antigen; TAP, tumor abnormal glycoprotein.

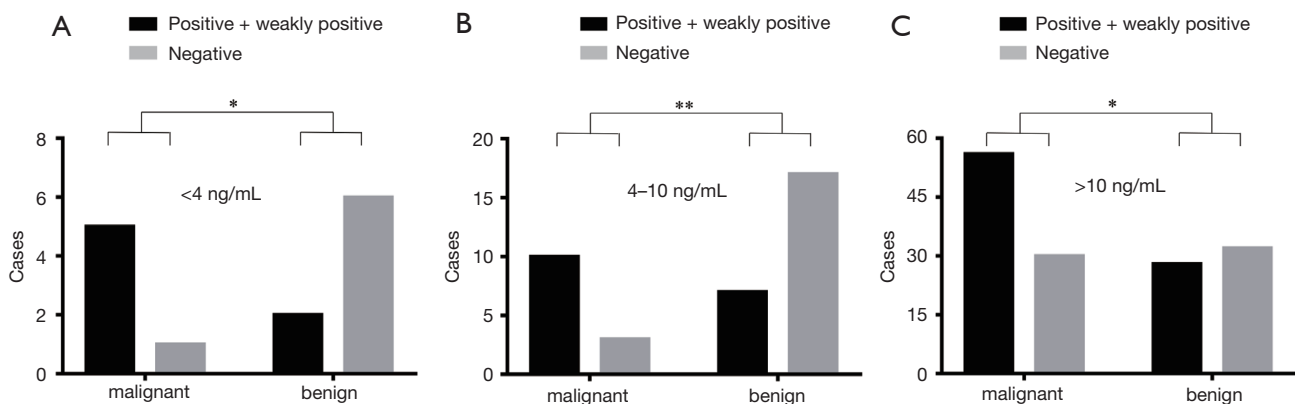


Figure 1 The number of TAP positive and negative cases in patients with prostate cancer and benign tumors in the range of T-PSA concentrations (<4, 4–10, and >10 ng/mL).*, P<0.05; **, P<0.01. T-PSA, total prostate specific antigen; TAP, tumor abnormal glycoprotein.

alone, and the T-PSA and F/T ratio combination.

Discussion

The current study shows that the assessment of T-PSA concentration combined with TAP measurement has an advantage as a diagnostic marker of prostate cancer in terms of sensitivity, specificity, and accuracy compared to other evaluated markers and combinations. At present, tumor biomarkers play an essential role as a supportive tool for cancer diagnostics and staging. They can be used not only for the preoperative staging of tumors but

also for evaluating the effectiveness of treatment during postoperative monitoring, as well as for the early detection of disease recurrence (7). Since the evaluation of serum PSA levels is usually insufficient for reliable cancer diagnostics, additional modifications have been introduced to improve the specificity of PSA as a tumor biomarker. These improvements include determination of PSA velocity, PSA density (PSAD), and the assessment of molecular forms of PSA (free vs. bound) (8-11). The assessment of PSA physical properties and molecular forms is a standard approach for the clinical evaluation of prostate cancer (12-14). Indeed, regular repetition of the PSA test in patients with

Table 2 Comparison of TAP, T-PSA, F/T, T-PSA combined with TAP and T-PSA combined with F/T ratio as diagnostic markers in prostate cancer

Tumor markers	Malignant	Benign	Sensitivity (%)	Specificity (%)	Accuracy (%)	Youden Index
T-PSA			94.29 (99/105)	7.22 (7/97)	52.48 (106/202)	0.015
Positive	99	90				
Negative	6	7				
TAP			79.05 (83/105)	61.86 (60/97)	70.79 (143/202)	0.4093
Positive	83	37				
Negative	22	60				
F/T			66.67 (70/105)	36.08 (35/97)	51.98 (105/202)	0.0275
Positive	70	62				
Negative	35	35				
T-PSA + TAP			97.14 (102/105)	67.01 (65/97)	81.68 (165/202)	0.6224
Positive	102	32				
Negative	3	65				
T-PSA + F/T			96.19 (101/105)	37.11 (36/97)	67.82 (137/202)	0.333
Positive	101	61				
Negative	4	36				

T-PSA, total prostate specific antigen; F-PSA, free prostate specific antigen; TAP, tumor abnormal glycoprotein.

elevated PSA levels reduces the need for prostate biopsy and decreases the risk of over-diagnosis. Thus, men with elevated PSA levels should undergo repeated PSA tests prior to prostate biopsy (15).

Diagnostic approaches for prostate cancer detection are developing, and modern imaging techniques (for example, multiparametric magnetic resonance imaging) enable better detection of prostate cancer, especially when combined with the assessment of various biomarkers in the blood (e.g., genomic markers) (16,17). Thus, the PSA test, which is performed solely with the support of transrectal ultrasound-guided biopsy, may no longer be the optimal approach for the early detection of prostate cancer.

Furthermore, although positron emission tomography-CT is a powerful tool to predict the presence of local lymph node and distant metastases (18), this method is unacceptable for most of the patients because of over-exposure to X-rays.

Glycosylation is one of the posttranslational protein modifications required for the regulation of cellular function. Abnormal protein glycosylation has been identified in almost all types of cancer and correlated with tumor progression, metastasis, and

patient survival. For example, it was shown that the detection of abnormally glycosylated protein (TAP) in combination with other diagnostic tools in gastric cancer might be used to screen high-risk populations (19). Furthermore, Korkolopoulou *et al.* showed that TAP can be used as a diagnostic marker in lung cancer (20).

The exposure to carcinogenic factors promotes the activation of proto-oncogenes, leading to the development of cancer. Genetic mutations result in the alteration of protein function and the production of abnormal proteins and glycoproteins. One of these is calcium-histone (21), the protein involved in DNA packaging in the nucleus. Under the influence of certain carcinogens, calcium-histone can be separated from the DNA and released into circulation. As a result, exposed DNA can be easily damaged, which results in malignant transformation. Other types of proteins with altered structure are glycoproteins with abnormal sugar chains that are present in the cell membrane (22). Compared to normal glycoproteins, these glycoproteins contain longer sugar chains with more complex branched structures and abnormal molecular mass (23). TAP represents a complex of abnormal glycoproteins, the expression of which increases during malignant cell

transformation. Therefore, the assessment of TAP positivity can be used to indirectly reflect the number of transformed cells and the extent of the malignant process. During tumor cell growth, a large amount of TAP is released and can be detected in the peripheral blood. The TAP test utilizes clotting agents that induce aggregation of abnormal tumor proteins and the formation of specific crystalline particles. However, these particles will not be formed in the absence of TAP in blood samples. The TAP detection technique is a multistep coupling condensation reaction. In the first stage of the reaction, various coagulants are applied to form a primary condensate containing abnormal glycoproteins. After that, the same or different condensate is formed by calcium-histones, which is observed during the examination of aggregated TAP particles.

In this study, we have found that the concentrations of T-PSA and F-PSA in the group of patients with prostate cancer were significantly higher than those in the benign group. However, the difference in F/T ratio between the groups was small. Of note, the TAP positivity was higher in the malignant group, and the difference was statistically significant. Also, we found a higher number of TAP positive cases in prostate cancer patients compared to patients with benign tumors in three intervals of T-PSA concentrations, indicating the potential diagnostic value of TAP.

The clinical symptoms of prostate cancer are diverse and often difficult to distinguish from benign prostatic hyperplasia, which makes it difficult for early clinical diagnosis. Therefore, this study also compared the sensitivity, specificity, and accuracy of tumor markers including T-PSA, TAP, F/T and T-PSA + TAP, and T-PSA + F/T. The results showed that the combination of T-PSA + TAP was a more sensitive, more specific, and more accurate indicator of prostate cancer than the other measured parameters, indicating its potential significance for the early diagnosis of prostate cancer, which reduces the likelihood of a missed diagnosis to the maximum extent. This provides a novel scientific basis for the screening and detection of early prostate cancer.

Currently, there are a limited number of studies focused on the role of abnormal glycoproteins in cancer diagnostics. As a new, improved method of tumor detection with potential clinical significance, TAP assessment has recently attracted more attention in the scientific society. Our study shows that TAP assessment can be used as a tool for prostate cancer diagnostics. For patients with clinical signs and symptoms specifically, the TAP test combined with the routine tumor marker detection may greatly improve the

accuracy of a cancer diagnosis. However, further studies on extended patient cohorts are needed to confirm the clinical relevance of TAP as a diagnostic marker for prostate cancer.

Conclusions

An analysis and comparison of serum PSA levels in prostate cancer patients and patients with benign tumors showed that F-PSA and T-PSA levels were higher in prostate cancer patients, while F/T ratio was higher in patients with benign tumors. Also, TAP positivity was higher in prostate cancer patients compared to patients with benign tumors. These findings suggest that elevated TAP levels have a positive association with prostate cancer, while the ratio of F-PSA to T-PSA is inversely associated with prostate cancer. A comparison of the sensitivity, specificity, and accuracy of several tumor markers revealed that the measurement of T-PSA combined with TAP was of clinical value for the early screening of prostate cancer.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2019.11.05>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of The First Affiliated Hospital of Anhui Medical University (Hefei China), and informed consent was signed by the patients or guardians. The ethics approval number is Quick-PJ 2019-03-18.

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