

## Original Article

# Tumor abnormal protein (TAP) examination contributes to primary diagnosis of bladder cancer

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**Abstract:** Objective: This study aims to investigate the application value of tumor abnormal protein (TAP) examination in the diagnosis of urothelial carcinoma of the bladder. Method: Abnormal sugar chain glycoproteins in the peripheral blood of 87 patients with urothelial carcinoma of the bladder were detected, and compared with non-tumor patients accompanied by hematuria. Result: TAP examination showed that the positive rate of the abnormal sugar chain glycoprotein in the peripheral blood of the 87 patients with urothelial carcinoma of the bladder was 78.16%, whereas that of the non-tumor patients was 10.81%. The former is significantly higher than the latter ( $P < 0.01$ ). Conclusion: TAP examination can be used to detect urothelial carcinoma of the bladder, and would be helpful in the diagnosis of urothelial carcinoma of the bladder by combining the clinical signs and symptoms.

**Keywords:** Bladder, glycoprotein, tumor abnormal protein, urothelial carcinoma

## Introduction

Urothelial carcinoma of the bladder is one of the most common urinary tract malignant tumors, with complicated biological behavior and high rate of relapse, accounting for approximately 90% of bladder cancers [1, 2]. Given the lack of reliable early diagnostic markers, bladder cancer usually has poor prognosis, and gives rise to serious threat to human health. Therefore, revealing the pathogenesis of bladder cancer and finding an effective tumor marker is necessary. At present, microscopic examination of the bladder biopsy, supplemented by urine cytology, is the gold standard for bladder cancer diagnosis. However, these methods have their own limitation. Urine-exfoliated cell examination lacks sensitivity to low grade tumors. Cystoscopic examination inevitably causes pain because it is invasive, with the risk of infection and bleeding [3]. Hence, finding a no-invasive method for early diagnosis and prognosis evaluation of bladder cancer is important. In recent years, an increasing number of bladder cancer markers have been discovered in urine, for example, the bladder cancer specific nuclear matrix protein-4, urinary

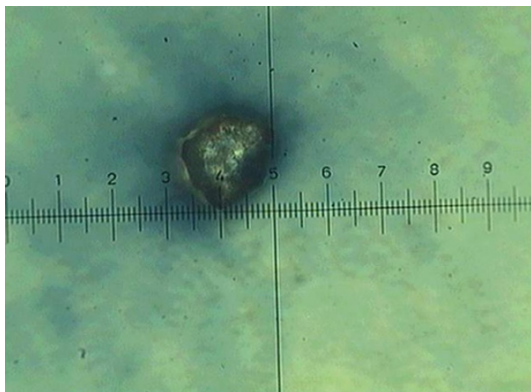
bladder cancer antigen, and urinary nuclear matrix protein 22 [4-6]. Changes of tumor abnormal protein (TAP) in the peripheral blood can occur in the early stage of tumor, which can be completely shown in a tumor abnormal protein examination system. This study adopts TAP examination to detect abnormal sugar chain glycoproteins in the peripheral blood of bladder cancer patients and non-tumor patients, and investigate its value in bladder cancer diagnosis.

## Materials and methods

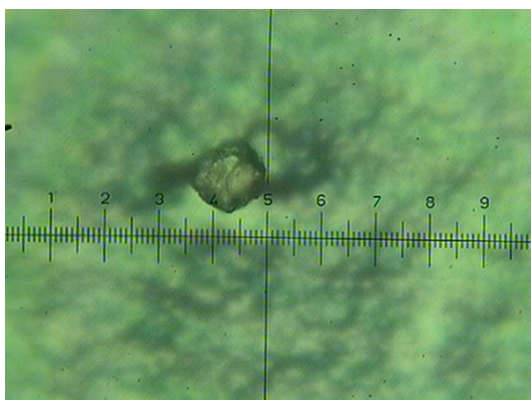
### General information

A total of 87 patients (60 males and 27 females; aged 39 to 86 years, with an average of  $65.4 \pm 8.55$  years) with urothelial carcinoma of the bladder were admitted in the General Hospital of Jinan Military Command from January 2013 to April 2015. Patients with immunodeficiency, hepatitis, diabetes, tuberculosis, and other diseases were excluded. The clinical symptoms of the patients mainly include visible hematuria or bladder occupied lesions shown in ultrasonic detection. All patients were

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**Figure 1.** TAP positive/larger condensates: a single condensate with an area of  $335 \mu\text{m}^2$ .



**Figure 2.** TAP positive/smaller condensates: a single condensate with an area of  $172 \mu\text{m}^2$ .

diagnosed to have urothelial carcinoma of the bladder through pathological biopsy, with normal liver and kidney functions and blood routine. Patients in the control group included 74 non-tumor cases with visible hematuria. All patients signed an informed consent, and this study was approved by the Ethics Committee of General Hospital of Jinan Military Command.

### *Reagent and instrument*

TAP multi-purpose diagnostic instrument and TAP coagulant were obtained from Zhejiang Ruisheng Medical Technology Co. Ltd.

### *Method*

*Slice preparation:* One droplet of peripheral blood was collected, pushed on three blood smears with even thickness on table, and then naturally dried.



**Figure 3.** TAP negative: no condensate.

*Staining:* The dried blood smears were transferred to a purified operating table. After 10 minutes, a dropper was used to vertically add the coagulant on the blood smears, with three droplets for each smear, to form round patches after 1.5-2 h.

*Microscopic examination:* A  $4\times$  flat-field achromatic objective lens was used to observe the three spots on the blood smears to find the specific form of condensate.

### *Result determination*

*TAP positive/larger condensates:* Condensates of abnormal sugar chain glycoproteins having a single condensate with an area of  $\geq 225 \mu\text{m}^2$  (**Figure 1**) or having three or more condensates with an area of  $121\text{-}225 \mu\text{m}^2$ , were observed in the specimen.

*TAP positive/smaller condensates:* Condensates of abnormal sugar chain glycoproteins having two condensates with an area of  $121\text{-}225 \mu\text{m}^2$  (**Figure 2**) or having three or more condensates with an area of  $81\text{-}121 \mu\text{m}^2$ , were observed in the specimen.

*TAP negative/no obvious condensates:* No condensate of abnormal sugar chain glycoproteins, condensates with an area of  $<81 \mu\text{m}^2$  (**Figure 3**), or two or less condensates with an area of  $81\text{-}121 \mu\text{m}^2$  were observed.

### *Statistical method*

SPSS17.0 software was used for statistical analyses.  $\chi^2$  test was employed for comparison between groups. A  $P < 0.05$  indicates statistical difference.

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**Table 1.** Comparison of TAP examination results between urothelial carcinoma of the bladder and non-tumor groups

	Cases	Positive cases	Negative cases	Positive rate	P value
Bladder cancer	87	68	19	78.16%	0.000
Non-tumor lesions	74	8	66	10.81%	

### Result

Among the 87 urothelial carcinoma of the bladder patients, 68 were positive by TAP examination. Only eight patients were positive among the 74 non-tumor patients. The difference in the positive result of TAP examination between the urothelial carcinoma of the bladder group and the non-tumor group exhibited a statistical significance ( $\chi^2=72.781$ ,  $P<0.01$ ; **Table 1**); that is, the positive rate of the patients with urothelial carcinoma of the bladder is significantly higher than that of the non-tumor patients. Therefore, TAP can be used to detect urothelial carcinoma of the bladder and would be helpful in the diagnosis of urothelial carcinoma by combining the clinical signs and symptoms.

### Discussion

The stimulation of the carcinogenic factors promotes the gradual variation of proto-oncogenes and anti-oncogenes, which leads to the occurrence of cancer. Genetic mutation results in the changes of functional protein, which lead to the production of abnormal sugar chain glycoproteins. One is calcium-histone, which is the peripheral substance of DNA in the nucleus [7]. When carcinogenic factors affect the nucleus, calcium-histone is separated from the DNA chain and released in the blood; hence, bare DNA is easily damaged, leading to the occurrence of cancer cells. The other is glycoproteins with abnormal sugar chains, which exist in the cell membrane. It is a glycoprotein with abnormal function produced by the mutant gene [8]. Compared with the normal glycoprotein, these glycoproteins show a longer sugar chain and more complicated branching structure, with abnormal quality and quantity [9]. TAP is the complex of abnormal glycoproteins, calcium-histone and the common material that genes express after cells become cancerous. Thus, TAP can be used to indirectly reflect the quantity and degree of cancerous cells. When the

tumor cells grow to a certain amount, a large number of these substances are discharged to the blood, and can be detected in the peripheral blood. TAP examination is a specific identification technology with the help of coagulant that contributes to aggregation of tumor abnormal protein and formation of specific crystalline particles. However, human blood specimen without TAP substance cannot undergo crystallization. The TAP examination technology is a multistage coupling condensation reaction. First, various coagulants are used with various abnormal sugar chain glycoproteins to form primary condensates. Then, the same or various primary condensates agglomerate to form the secondary condensates bridged through calcium-histone, which are the condensed particles observed in TAP examination.

Several studies have shown that the occurrence of abnormal sugar chain glycoproteins is closely related to tumor [10]. Many abnormal sugar chain structures were found in malignant tumor, for example, alpha fetoprotein, carcino-embryonic antigen, carbohydrate antigen, transferrin, alkaline phosphatase,  $\gamma$ -glutamyl transferase, human chorionic gonadotropin, T antigen,  $\alpha$ 1-antitrypsin, and prostatic acid phosphatase. The amount of TAP in the blood increases with the development of tumor, which is an important clue for the early detection of cancer. TAP detection is a combination detection of tens of tumor markers (abnormal sugar chain glycoprotein) in the same reaction system, such as AFP, CEA, and CA series. This method can detect more tumor markers, including some markers that could not be separately detect. This method is sensitive to common tumors; hence, it can effectively reduce the miss rate.

This study shows that the sensitivity of TAP examination to urothelial carcinoma of the bladder is 78.16%, with a specificity of 89.19%. The positive rate of bladder cancer patients is significantly different from that of non-tumor patients; the difference has statistical significance. TAP examination has been demonstrated to help screen out bladder cancer patients and has certain significance to the diagnosis of bladder cancer in combination with the clinical symptoms of patients.

Among the 87 cases of urothelial carcinoma of the bladder, 19 are negative in TAP examination. The reasons why TAP detection for tumor patients is normal are as follows: (1) the growth of tumor cells is inhibited during surgical operation and radiation and chemotherapy, leading to a decrease in the secretion of abnormal proteins; (2) the level of proteins in the body of the patients in the terminal phase is low, causing the secretion of abnormal sugar chain glycoproteins to be lower than the detectable amount; and (3) the cancer cells in the body are relatively stationary, thus the proliferation of tumor cells is not evident and inactive, with low secretion of abnormal sugar chain glycoproteins.

Among the 74 non-tumor patients in this study, eight cases are positive in TAP examination, which may be attributed to other diseases that result in the expression of abnormal sugar chain glycoproteins or early precancerous lesions and hyperplasia. These patients should be periodically reviewed. The positive patients are suggested to adopt preventive interruption therapy. A number of studies have shown a significantly higher positive rate in the digestive system of the higher-risk population than that of the normal population [11]. Moreover, in 18 months of follow-up, 3.6% of the patients showed a malignant tumor [11]. Therefore, TAP detection can be used for early detection of tumors. In the early asymptomatic stage when the tumor cannot be found by a physical method, the expression of abnormal sugar chain glycoproteins in the peripheral blood is sufficient for detection.

At present, studies on abnormal sugar chain glycoproteins are limited. TAP examination has attracted increasing scholars' attention as a new and better tumor detection method with potential clinical application value. This paper shows that TAP examination can be regarded as a tool for the diagnosis of bladder cancer. Clinically, urothelial carcinoma of the bladder does not have specific tumor markers. For the patients with clinical signs and symptoms, combined with routine tumor marker detection, TAP examination can greatly improve the accuracy and is helpful in the diagnosis of the tumor.

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### Disclosure of conflict of interest

None.

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