Prognostic value of serum tumor abnormal protein in gastric cancer patients

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Abstract. Aberrant glycosylation of protein occurs in nearly all types of cancers and has been confirmed to be associated with tumor progression, metastasis and the survival rate of patients. The present study aimed to explore the prognostic value of tumor abnormal protein (TAP) in gastric cancer patients. TAP was detected in the blood of 42 gastric cancer patients and 56 healthy volunteers by using the TAP testing kit. Univariate and multivariate Cox regression analysis were performed to evaluate the prognostic value of TAP. In total, 64.3% of gastric cancer patients were positive for TAP, and TAP was significantly correlated with poor prognosis [progression-free survival (PFS), 4.2 vs. 12.6 months; P=0.043]. TAP [hazard ratio (HR), 64.487; P<0.01), differentiation (HR, 17.279; P<0.01) and TNM stage (HR, 45.480; P<0.01) were found to be independent predictive factors for PFS. Furthermore, Kaplan-Meier curves indicated that TAP is associated with a reduced PFS in gastric cancer patients. The results of the present study therefore indicated that the TAP test has significant prognostic value for gastric cancer patients.

Introduction

Gastric cancer is the second leading cause of cancer-associated mortality worldwide with a five-year survival rate of <30% (1,2). For patients with localized gastric cancer, surgery remains the basic treatment (3). However, the majority of patients are diagnosed at an advanced stage, at which radical surgery is no longer possible, and the outcome of available treatments remains unsatisfactory. Palliative chemotherapy is widely applied for the treatment of advanced gastric cancer patients and has become a standard clinical practice (4).

Glycosylation is one of the biochemical mechanisms which regulate cellular functions. Aberrant glycosylation of numerous proteins has been identified in nearly all types of cancers and has been confirmed to be associated with tumor progression, metastasis and the survival rate of patients (5-8). Most clinical tumor markers are glycoproteins; however, detection of specific cancer-associated alterations in glycan structures, such as alpha-fetoprotein-(AFP)L3 (9,10), may improve their specificity; furthermore, novel biomarkers are currently being discovered (11).

Tumor abnormal protein (TAP) is a collective term for glycoproteins produced during the development of a variety of malignant tumors as their common feature. Meezan *et al* (12) first demonstrated that cancer-associated glycans differ from glycans on healthy cells. Numerous tumor-associated glycans are present at low levels in normal tissues and at elevated levels on tumors (13). When TAP levels reach a certain threshold, they can be detected in the peripheral blood.

The present study was performed to assess the prognostic value of TAP in gastric cancer patients.

Materials and methods

Patients. A total of 42 patients with histological diagnosis of gastric cancer who were treated at the Affiliated Changzhou Tumor Hospital of Soochow University (Changzhou, China) between January and June 2014 were enrolled in the present study. Written informed consent was obtained from all the patients. All procedures were performed according to the guidelines of the local Ethics Committee. All patients selected for the present study had been diagnosed with gastric cancer by histopathology and had not received any pre-operative adjuvant chemotherapy, radiotherapy or targeted therapy. Patients presenting with other types of malignant tumor alongside gastric cancer, rheumatoid arthritis or active tuberculosis, as well as immunocompromised, pregnant and diabetic patients, were excluded from the study. The clinical data of the patients are shown in Table I. In addition, 56 healthy volunteers were recruited for the present study. All the patients were followed up until July, 2015.

Detection of TAP. TAP was detected using a TAP testing kit and examination system (Zhejiang Ruisheng Medical Technology, Ltd., Cixi, China). Peripheral blood (25 μl) was collected from the fingertip of each patient and blood smears of uniform thickness were prepared, followed by drying at ambient temperature in a horizontal position for 10 min.

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Coagulation auxiliaries were then added to the blood smears and after 1.5-2 h, condensed particles had formed. The shape of these particles, which is indicative of the TAP status of the sample, was then examined under a TAP detection image analyzer. Samples were identified to be TAP-positive when condensed particles had formed fulfilling the following criteria: A diameter of >38 μm, with marginal integrity, refraction of the oval, irregular or polygonal shape, a lightly stained area in the center and accumulation of small fragments in the immediate surrounding area of the particle. Samples were confirmed as TAP-negative when dendritic-shaped or no particles were observed.

Statistical analysis. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL, USA). Data were compared by using the χ^2 test. Progression-free survival was defined as the period from the time of diagnosis to disease progression or succumbing to the disease. The Kaplan-Meier curve was used to describe progression-free survival (PFS) and differences between groups were compared by using the log-rank test. Univariate analysis comprised gender (male vs. female), age (<60 vs. ≥60 years), differentiation (well vs. poor), tumor-node-metastasis (TNM) stage (I-III vs. IV) and TAP status (positive vs. negative). Factors with statistical significance or a tendency towards significance (P<0.2) at univariate analysis were subjected to multivariate Cox regression analysis. For all comparisons, P<0.05 was considered to indicate a statistically significant difference.

Table I. Clinical characteristics of the study population (n=42).

Characteristics	Patient number	%
Age, years [median (range)]	66 (33-78)	
Gender (female/male)	12/30	28.6/71.4
Localization		
Cardia	21	50.0
Body	11	26.2
Antrum	10	23.8
Tumor size, cm		
<5	15	35.7
≥5	27	64.3
Invasion depth		
T1-T3	8	19.0
T4	34	81.0
Differentiation		
Well	14	33.3
Poor	28	66.7
TNM stage		
I-II	14	33.3
III	7	16.7
IV	21	50.0

TNM, tumor-node-metastasis.

Results

A total of 42 gastric cancer patients (30 males and 12 females; age range, 33-78 years) were enrolled in the present study, whose clinical characteristics are listed in Table I. TAP was detected in 27 gastric cancer patients, accounting for a TAP-positive rate of 64.3%, which was significantly higher than that in healthy volunteers (16.1%; P<0.01) (Table II). As shown in Table III, the prevalence of TAP positivity was not associated with gender, tumor location, tumor size, depth of invasion and the presence of lymph-node metastasis (P>0.05), while it was significantly associated with the age, TNM stage and degree of differentiation (P=0.008, 0.034 and 0.040, respectively). The median PFS of TAP-positive patients was 4.2 months, which was significantly lower than that for TAP-negative patients (12.6 months; P=0.043). According to multivariate Cox regression analysis, the TAP status (P<0.001; hazard ratio (HR), 64.487; 95% confidence interval (CI), 11.905-349.315), degree of differentiation (P<0.001; HR, 17.279; 95% CI, 4.504-66.296) and TNM stage (P<0.001; HR, 45.480; 95% CI, 9.370-220.758) were independent predictive factors for reduced PFS (Table IV and Fig. 1).

Discussion

As at present, the majority of patients with gastric cancer succumb to the disease due to metastasis and recurrence, early diagnosis and real-time monitoring of metastasis are important to improve the survival time of the patients. Although computed tomography (CT) and ultrasonography may be used to identify metastasis in gastric cancer patients, they have limitations and may not be sufficiently accurate (14,15). While the predictive value of positron emission tomography-CT is high with regard to local lymph node metastasis and distant metastasis (16), this method is not affordable for the majority of patients. Glycosylation has been indicated to be an important factor at early stages of cancer development (5). The present study demonstrated that 12 of the 14 early-stage gastric cancer patients (85.7%) were TAP-positive, supporting this role of TAP in the early stage of gastric cancer. Furthermore, the present study indicated the aptness of TAP detection for early diagnosis of gastric cancer and may be applied for screening of high-risk populations in combination with other diagnostic tools. He *et al* (17) revealed that TAP may be used as an indicator for the diagnosis of lung cancer and for evaluating the progress of lung cancer patients.

Serum tumor markers are characteristic substances present in malignant cells or produced by abnormal malignant cells. Several of the most frequently used tumor markers, including carbohydrate antigen (CA)724, CA199, carcinoembryonic antigen (CEA) and CA242, have been confirmed to jointly provide information aiding in the diagnosis, classification, prognosis and treatment selection in gastric cancer (18-21), while the value of information provided by of any of them alone is low. Jing *et al* (22) found that the sensitivity of CA724, CA199, CEA and CA242 was only 25.4, 36.2, 26.8 and 42.9%, respectively. Therefore, it is desirable to identify novel biomarkers with high sensitivity and specificity. The TAP testing kit manufactured by Zhejiang Ruisheng Medical Technology, Ltd. contains a combination of lectins, which can recognize and bind to specific

Table II. TAP detection in gastric cancer patients and healthy volunteers.

Groups	TAP-positive	TAP-negative	Positive rate (%)	χ^2	P-value
Gastric cancer patients	27	15	64.3	24.006	<0.001
Healthy volunteers	9	47	16.1		

TAP, tumor abnormal protein.

Table III. Association between TAP and clinicopathological characteristics of gastric cancer patients.

Characteristics	Number	TAP-positive	TAP-negative	Positive rate (%)	χ^2	P-value
Gender					0.259	0.611
Male	30	20	10	66.7		
Female	12	7	5	58.3		
Age, years					7.010	0.008
<60	12	4	8	33.3		
≥60	30	23	7	76.7		
Localization					0.196	0.907
Cardia	21	13	8	61.9		
Body	11	7	4	63.6		
Antrum	10	7	3	70.0		
Tumor size, cm					0.058	0.810
<5	15	10	5	66.7		
≥5	27	17	10	63.0		
Invasion depth					0.494	0.482
T1-T3	8	6	2	75.0		
T4	34	21	13	61.8		
Differentiation					4.200	0.040
Well	14	12	2	85.7		
Poor	28	15	13	53.6		
TNM stage					6.741	0.034
I-II	14	12	2	85.7		
III	7	2	5	28.6		
IV	21	13	8	61.9		
LNM					3.780	0.052
Positive	32	18	14	56.3		
Negative	10	9	1	90.0		

TNM, tumor-node-metastasis; LNM, lymph node metastasis; TAP, tumor abnormal protein.

sugar molecules with high specificity, thus interlinking a variety of abnormal glycoproteins via their sugar chains to form characteristically shaped crystalloid, which can then be observed using the TAP detection image analyzer. The TAP detection system allows for combined detection of several tumor markers in the same system, therefore improving the sensitivity and specificity of detection. The overall sensitivity and specificity of TAP detection in various types of cancer patients are 85.8 and 80.2%, respectively (23). Jin *et al* (24) reported that the sensitivity and specificity of TAP detection were 87.8 and 87.2%, respectively, for patients with malignant tumors of the digestive system. In the present study, 64.3% of gastric cancer patients were determined to be TAP-positive, which was significantly higher than the percentage of TAP-positive healthy volunteers (16.1%; P<0.01), indicating that TAP has a higher sensitivity for the detection of gastric cancer, which may be of significant diagnostic value. Numerous factors have been confirmed to be associated with the prognosis of gastric cancer patients, such as the pathological stage and the tumor differentiation. Certain studies have shown a correlation between changes in glycosylation and poor prognosis (25,26). The present study showed that TAP, differentiation and TNM stage were independent

Table IV. Univariate analysis and multivariate Cox regression analysis for PFS for all patients.

Characteristics	Univariate analysis		Multivariate Cox regression analysis		
	Median PFS (months)	Log-rank P-value	HR	95% CI	P-value
All patients	8.3				
Age, years					
<60	10.4	0.858	1.044	0.995-1.095	0.082
≥60	5.8				
Gender					
Male	8.3	0.558	1.059	0.461-2.433	0.892
Female	4.9				
TAP					
Positive	4.2	0.043	64.487	11.905-349.315	<0.001
Negative	12.6				
Differentiation					
Well	12.6	0.009	17.279	4.504-66.296	<0.001
Poor	4.9				
TNM stage					
I-III	14.0	<0.001	45.480	9.370-220.758	<0.001
IV	3.5				

HR, hazard ratio; CI, confidence interval; TAP, tumor abnormal protein; PFS, progression-free survival; TNM, tumor-node-metastasis.

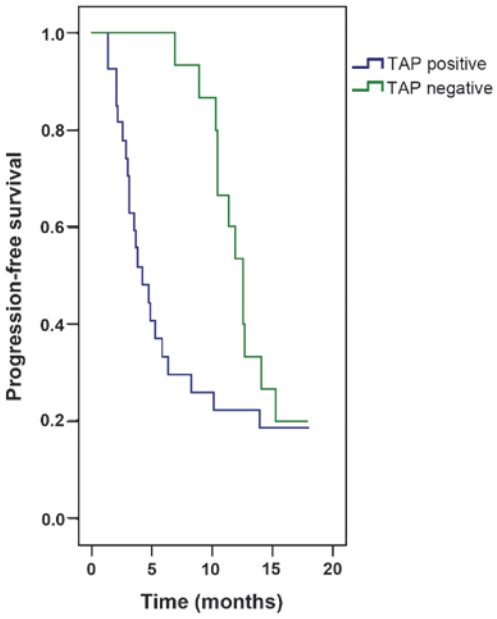

Figure 1. Kaplan-Meier progression-free survival curves for TAP-positive and TAP-negative patients with gastric cancer. TAP, tumor abnormal protein.

predictive factors for reduced PFS in gastric cancer patients by multivariate Cox regression analysis (HR, 64.487, P<0.01; HR, 17.279, P<0.01; HR: 45.480, P<0.01, respectively). The median PFS of TAP-positive patients was significantly correlated to a worse prognosis as compared to TAP-negative patients (PFS, 4.2 vs. 12.6 months; P=0.043) according to Kaplan-Meier survival curve analysis. Therefore, TAP was indicated to be a risk factor in patients with gastric cancer. Therapeutic interventions for TAP-positive patients are of great practical significance for cancer prevention and treatment. Certain anti-cancer vaccines based on glycans have produced promising results (27,28).

The present study further showed that the TAP-positive rate in gastric cancer patients aged ≥60 years (76.7%) was significantly higher than that of patients aged <60 years (33.3%; P=0.008). χ^2 analysis showed that TAP was positively correlated with patient age, which was consistent with the findings of Shao *et al* (29), suggesting that screening of elderly patients for TAP should be introduced in the clinical setting. Moreover, significant differences in the TAP-positive rate were identified between different TNM stages and grades of differentiation (P=0.034 and 0.040, respectively). Of note, TAP positivity was most prevalent among patients with well-differentiated tumors and during the early stages of gastric cancer; this finding may be attributed to the decreased metabolic activity of the cancer cells during gastric cancer progression, leading to decreased secretion of glycoproteins, so that TAP is no longer detectable in advanced-stage patients. Finally, it was revealed that the TAP status was not correlated with gender, tumor location, tumor size, depth of invasion, or the presence of lymph node metastasis (P>0.05).

It is worth mentioning that the present study had certain limitations. The study cohort was relatively small and the time to follow-up was short; therefore, the present study only serves as a preliminary assessment of the correlation between TAP and the PFS of gastric cancer patients. A further large-scale, prospective, multicenter study is therefore required to confirm the results of the present study. Wu *et al* (30) reported that TAP

detection can be utilized as a novel method for evaluating the efficacy of chemotherapy in patients with metastatic colorectal cancer. Similarly, Liu *et al* (31) found that the TAP status was a factor for the prediction of the efficacy of chemotherapy in gastric cancer patients with a higher efficiency than that of conventional tumor markers. The TAP test therefore holds promise in the monitoring of cancer patient responses to chemotherapy, which requires further elucidation for implementation in the clinic.

In conclusion, the present study indicated that the TAP status is an independent predictive marker for PFS in patients with gastric cancer. Furthermore, the TAP status was significantly correlated with the tumor stage, patient age and tumor differentiation. These findings, combined with those of previous studies, indicated that TAP detection represents a promising diagnostic and prognostic tool for gastric cancer and may also be utilized for monitoring the response of patients to chemotherapy response.

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