Quantification of Tumor Abnormal Proteins in the Diagnosis and Postoperative Prognostic Evaluation of Gastric Cancer

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ABSTRACT

BACKGROUND: Abnormal glycosylation of proteins has been identified in almost all types of cancers and is closely related to the cancer progression, metastasis, and survival of cancer patients. This study was to explore the values of serum tumor abnormal protein (TAP), an abnormal glycochain protein, in the diagnosis and prognosis of gastric cancer (GC).

METHODS: A total of 335 GC patients were included as the study group, and another 335 subjects served as the control group. Tumor abnormal protein expression was compared between the 2 groups. Correlation analysis was used to assess the correlations of TAP with clinicopathological factors. Gastric cancer patients were divided into training set and test set at a ratio of 2:1. Univariate and multivariate Cox regression analyses in training set were used to evaluate the prognostic significance of TAP in GC patients and explore the independent risk factors for overall survival (OS) and disease-free survival (DFS) to establish a prognostic model, followed by testing of the model. According to the median of TAP, 335 GC patients were divided into 2 groups to plot the survival curves of OS and DFS.

RESULTS: Tumor abnormal protein expression in the study group was significantly higher than in the control group. Taking the best cut-off value of TAP (110.128 μm²) as the diagnostic criteria for GC, the sensitivity and specificity of TAP were 83.58% and 97.61%, respectively, and the area under the receiver operating characteristics (ROC) curve was 0.935, which was not inferior to computed tomography (CT). Tumor abnormal protein expression was an independent risk factor for OS and DFS. The prognostic predictive value of TAP was better than that of pathological stage in GC patients. The model with TAP was effective in predicting prognosis.

CONCLUSION: Tumor abnormal protein is an effective indicator for early screening and prognostic evaluation of GC and can also assist the clinical diagnosis and treatment of GC.

KEYWORDS: Tumor abnormal protein, gastric cancer, pathology, predictive diagnosis, evaluation of therapeutic effect

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Introduction

Gastric cancer (GC) is an epithelial malignant tumor that originates from the stomach.1 In China, GC is the second most common cancer and the third leading cause of death.2 There are about 1.2 million new cases of GC each year worldwide, and about 40% of new cases are found in China.2 Due to the insidious onset of GC and lack of specific clinical manifestations, most patients are diagnosed with GC at advanced stage, thus causing the 5-year overall survival (OS) rate lower than 50%.3 Therefore, the early diagnosis and prognostic evaluation of GC are very important.4 The traditional markers used in the diagnosis and therapeutic efficacy assessment of GC (such as carcinoembryonic antigen [CEA], glycochain 19–9 [CA19–9], glycochain protein 72–4 [CA72–4], and glycochain protein 125 [CA125]) usually have a poor sensitivity.5 Although pathological examination after biopsy by gastroscopy is the gold standard for the diagnosis of GC, gastroscopy is an invasive examination that can cause pain and discomfort to patients. Therefore, increasing studies have been conducted to investigate the non-invasive and effective biomarkers for the early diagnosis and prognostic evaluation of GC.
In the malignant transformation of normal cells, the inactivation of glycosylation modifying enzymes may cause the production of abnormal proteins. Tumor abnormal glycoprotein, also known as tumor abnormal protein (TAP), is a covalent complex formed by the abnormal proteins and glycochains.\textsuperscript{6} Tumor abnormal protein can be released into the blood and identified by detecting fingertip blood. The TAP detection usually employs multistage coupling technology for the detection of abnormal glycochains. That is, different glycochains are specifically recognized through different lectins and then the structure of abnormal glycochains in the tumor-related glycoproteins is detected for the identification of cancer cells.\textsuperscript{7} Owing to the convenience and rapidity, TAP detection has been gradually applied in the clinical screening of a variety of cancers.\textsuperscript{8,9} However, few studies have been conducted to investigate its use in the GC. In this study, TAP was detected in non-GC subjects and GC patients, aiming to evaluate the diagnostic value of TAP in GC. Furthermore, the survival rate was compared between GC patients with normal and high TAP expression, aiming to assess the prognostic value of TAP in GC patients.

Materials and Methods

General characteristics

This was a retrospective study. Totally, 335 patients who were pathologically diagnosed with GC after surgery in the Department of General Surgery, the Affiliated Suzhou Hospital of Nanjing Medical University (China) between January 2016 and December 2018 were recruited into the study group; 335 subjects with normal findings on computed tomography (CT) and gastroscopy were recruited from 1897 subjects in the same period as the control group. The study was approved by the Ethics Committee of the Affiliated Suzhou Hospital of Nanjing Medical University (approval id. KL901216). The exclusion criteria were as follows: (1) patients were unable to undergo surgery due to dysfunction of important organs or coagulation dysfunction; (2) patients had gastric stromal tumors, other tumors at the stomach junction, gastric benign tumors, or long-term gastritis; (3) patients had immunodeficiency or tumors of other organs; (4) patients were diagnosed with diabetes, rheumatic disease, rheumatoid, other autoimmune diseases, or nonhealing fracture; and (5) patients had active hepatitis or tuberculosis. These factors may affect the surgery or TAP expression.\textsuperscript{10,11}

Observations

Following information was recorded: age, sex, body weight, OS, disease-free survival (DFS), CEA, CA19-9, CA72-4, CA125, tumor volume, pathological stage (tumor node metastasis [TNM] stage; histological grade; vascular invasion and neural invasion), immunohistochemical findings (TP53, CDH1, and Ki-67), and TAP expression.

Detection of TAP

(a) Reagents: The TAP detection kit (Aggregation Method) was purchased from Zhejiang Ruisheng Medical Technology, Ltd., Cixi, China.

(b) Slide preparation and staining: Fresh blood was collected from the fingertips of each subject onto the slide, which was allowed to air-dry at room temperature. The TAP detection reagent was added onto each slide (3 drops/slide; about 50 μL/drop) at 3 sites, followed by incubation for about 2 hours.

(c) Interpretation of results: Under a microscope, the TAP expression was analyzed. In brief, the 3 spots on the slide were scanned under the microscope, and specific aggregates were identified.

Comparison of TAP expression between GC patients and non-GC subjects

The best cut-off value of TAP was calculated for GC patients and controls. The effects of TAP on the sensitivity, specificity, missed diagnostic rate, misdiagnostic rate, Youden index, positive predictive value, negative predictive value, overall compliance rate, and area under the receiver operating characteristics curve (AUC) for GC diagnosis were further evaluated. In addition, TAP was compared with CEA, CA19-9, CA72-4, CA125, CT findings, and findings from the first gastroscopy in the diagnosis of GC. The receiver operating characteristics (ROC) curve was used to evaluate the diagnostic value of different indicators for GC.

Correlations of clinicopathological characteristics with TAP expression

The correlation analysis was used to analyze the correlations of TAP expression with clinicopathological characteristics. Pearson's correlation analysis was used to analyze continuous variables that were normally distributed and had a linear relationship; when the data did not obey a bivariate normal distribution, Spearman's correlation analysis was used; when the data were 2 ordered categorical variables, Kendall's tau-\textit{b} correlation analysis was used.

Risk factors for OS and DFS

A total of 335 GC patients were divided into training set and test set at a ratio of 2:1. In the training set, univariate and multivariate Cox regression analyses were used to evaluate the risk factors of OS and DFS and to determine the independent risk factors for the prognosis of GC. The reliability of risk factors from Cox regression was verified by Lasso regression (10-fold cross-validation), and the risk score for each risk factor was calculated. The independent risk factors of GC prognosis were used as dependent variables to establish a prognostic prediction
Results

TAP expression in GC patients and controls

The TAP expression in the GC patients was significantly higher than in the controls [133.54 (121.55, 145.25) μm² vs 90.03 (79.25, 98.99) μm²] (P < .001; Table 1, Figure 2).

Diagnostic value of TAP expression in GC

Taking the best cut-off value of TAP [110.128 μm²] as the diagnostic criterion for GC, the sensitivity and specificity of TAP were 83.58% and 97.61%; the missed diagnosis rate and the misdiagnosis rate were 16.42% and 2.39%, respectively; the Youden Index was 0.8120; the positive predictive value was 3.38%; and the negative predictive value was 99.99%; the overall coincidence rate was 90.60% (Table 2). The AUC for TAP was 0.935 in the diagnosis of GC. The sensitivity and specificity of GC patients positive to TAP were significantly higher than those of patients positive to CEA, CA19-9, CA72-4, and CA125. The AUC of TAP was not inferior to CT (0.935 vs 0.933) (Figure 3A). The sensitivity of combined TAP and CT in the diagnosis of GC significantly increased, reaching 91.34% (Figure 3B). The sensitivity and specificity of combined TAP, CT, and first gastroscopy reached as high as 100% (Table 3; Figure 3C).

Relationships of TAP expression with clinicopathological factors in GC patients

The clinical characteristics of 335 GC patients are shown in Table 4. The correlation analysis showed TAP expression in the GC patients was positively related to the tumor volume, histological grade, pathologic stage, vascular invasion, Lauren type, Borrmann type, TP53 mutation, CDH1 mutation, Ki67, CEA, and CA724; however, TAP expression was negatively correlated with OS and DFS (Table 5, Figure 4).

Prognostic value of TAP expression in GC patients after surgery

The clinical characteristics of GC patients in training set and test set are shown in Table 4. The univariate and multivariate Cox regression analyses were employed to investigate the prognostic factors related to the OS and DFS of GC patients after surgery. Results showed that TAP and pathological stage were the risk factors for OS and DFS (Tables 6 and 7). The reliability of the risk factors obtained by Cox regression analysis was verified by Lasso regression. Lasso regression coefficients and Lasso regression variable trajectories are shown in Figure 5. The risk scores for each risk factor of OS and DFS are shown in Figure 6. According to the quartiles of TAP value (121.53, 133.54, and 145.27), the TAP value of GC patients was divided into 4 intervals: ≤121.53, >121.53, ≤133.54, >133.54, and ≤145.27, >145.27, which were defined as 1, 2, 3, and 4. The prognostic prediction model established by TAP and pathological stage is shown in Figure 7A and B. The model was effective in predicting the prognosis of GC patients at the third and fourth year. The fit of the model was good (Figure 7C and D).

Moreover, the ROC curves of TAP and pathological stage as the risk factors of OS and DFS were delineated (Figure 8). The AUCs of OS about TAP and pathological stage were 0.966 and 0.779, respectively (Figure 8A). Similarly, the AUC of DFS about TAP and pathological stage were 0.942 and 0.776, respectively (Figure 8B). The time-dependent prognostic ROC curves of OS and DFS are shown in Figure 8C and D.

Finally, Kaplan–Meier analysis showed that the increased TAP expression was related to the reductions of OS and DFS in the GC patients (Figure 9).
Figure 1. Schematic of the study design.
CT indicates computed tomography; TAP, tumor abnormal protein.
These indicated that TAP was the most relevant independent risk factor of OS and DFS.

**Discussion**

Tumor abnormal protein is a general term for glycoproteins produced on the surface of tumor cells during the occurrence and development of a variety of malignant tumors, such as the 3-antenna with core Fuc produced by abnormal glycosylation of alpha-fetoprotein (AFP) or transferrin, and the multiantenna and biantenna produced by abnormal glycosylation of CEA or human chorionic gonadotropin (HCG) protein. When the tumor cell nucleus develops abnormal division or the nuclear differentiation and maturation are impaired, the abnormal fragmentation of tumor cell membrane may significantly promote the release of TAP. The released TAP can activate the signal transduction pathways in tumor cells and stimulate the abnormal transcription and proliferation.

Our results were consistent with previous findings that the GC patients have a high TAP expression. This indicates that TAP can be used for the early screening of GC.

In the detection of traditional tumor markers, immunological techniques, including enzyme-linked immunoassay, radioimmunoassay, and chemiluminescence, are frequently employed to identify the "proteins" in the glycoproteins, which may assist the diagnosis of cancers. Of note, a study has shown that the conventional tumor markers, such as CEA, CA19-9, CA125, and CA72-4, have low sensitivity and specificity in the GC patients. In addition, the tumor markers will increase significantly only at the late stage of the tumor. For patients with resectable tumors, the tumor markers are generally in the normal range. Therefore, traditional tumor markers are not reliable for tumor diagnosis, which was also confirmed in our study. The TAP detection system contains 10 types of lectins, including wheat germ agglutinin (WGA), cassava agglutinin (ConA), and Datura agglutinin (DSA). Lectins are a class of glucose-binding proteins that can specifically recognize and bind to specific glycosyl sequences in the monosaccharides or oligosaccharides with a specific structure. They are useful tools for detecting abnormal glycochain structures in tumor-associated glycoproteins. Different lectins can bind different glycochains. Therefore, the TAP system can specifically recognize and bind to 19 kinds of glycoproteins with abnormal glycochains at one time, which greatly improves the sensitivity and specificity of tumor-assisted diagnosis (Supplemental Table S2). This study also confirmed that the sensitivity and specificity of TAP expression for the diagnosis of GC were not inferior to the findings from CT, and the sensitivity and specificity of

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**Table 1. Clinicopathological characteristics of subjects in the control group and GC group.**

| CHARACTERISTICS | CONTROL GROUP | GC GROUP | P     |
|----------------|---------------|----------|-------|
| n              | 335           | 335      |       |
| Sex, n (%)     |               |          |       |
| female         | 81 (12.1%)    | 98 (14.6%)| .162  |
| male           | 254 (37.9%)   | 237 (35.4%)|       |
| Age (year)     | 65 (56, 74)   | 67 (60, 73) | .314  |
| TAP (μm²)      | 90.03 (79.25, 98.99) | 133.54 (121.55, 145.25) | <.001 |
| CEA (ng/mL)    | 2.39 (1.23, 3.65) | 2.43 (1.24, 3.98) | .044  |
| CA199 (U/mL)   | 8.8 (8.8, 13.8) | 17.4 (9.3, 21.5) | <.001 |
| CA724 (U/mL)   | 2.86 (1.61, 4.76) | 2.86 (1.61, 4.76) | .493  |
| CA125 (U/mL)   | 13.8 (6.98, 19.2) | 13.8 (6.98, 19.2) | .996  |

Abbreviations: CEA, carcinoembryonic antigen; GC, gastric cancer; TAP, tumor abnormal protein. Bold values imply a statistically significant difference between the two groups.
Table 2. Role of TAP expression in the early detection and diagnosis of GC.

| INDICATIONS                  | CANCEROUS LESIONS | NONCANCEROUS LESIONS |
|------------------------------|-------------------|----------------------|
| Positive TAP expression      | 280(a)            | 8(b)                 |
| Negative TAP expression      | 55(c)             | 327(d)               |
| Sensitivity                  | \(a/(a + c) \times 100\%) = 83.58\% |
| Specificity                  | \(d/(b + d) \times 100\%) = 97.61\% |
| Missed diagnostic rate       | \(c/(a + c) \times 100\%) = 16.42\% |
| Misdiagnostic rate           | \(b/(b + d) \times 100\%) = 2.39\% |
| Youden Index                 | \(a/(a + c) + d/(b + d) - 1 = 0.8120\) |
| Positive predictive value    | \(P \times \text{Sensitivity} \times [1 - \text{Specificity}] \times 100\% = 3.38\% |
| Negative predictive value    | \((1 - P) \times \text{Specificity} \times [1 - \text{Sensitivity}] \times P = 99.99\% |
| Overall compliance rate      | \((a + d)/(a + b + c + d) \times 100\% = 90.60\% |

Abbreviations: GC, gastric cancer; P, prevalence; TAP, tumor abnormal protein.

Table 3. Performance of CEA, CA19-9, CA72-4, CA125, CT, first gastroscopy, and TAP in the diagnosis of GC.

| TUMOR MARK     | SENSITIVITY (%) | SPECIFICITY (%) | AREA UNDER THE ROC CURVE |
|----------------|-----------------|-----------------|--------------------------|
| CEA            | 26.27           | 96.72           | 0.545                    |
| CA19-9         | 62.99           | 84.78           | 0.683                    |
| CA72-4         | 18.21           | 94.93           | 0.515                    |
| CA125          | 1.49            | 100             | 0.500                    |
| TAP            | 85.58           | 97.61           | 0.935                    |
| CT             | 86.57           | 100             | 0.933                    |
| First gastroscopy | 99.4          | 100             | 0.997                    |
| TAP & CT       | 91.34           | 97.61           | 0.960                    |
| TAP & CT & first gastroscopy | 100            | 100             | 1.000                    |

Abbreviations: CEA carcinoembryonic antigen; CT, computerized tomography; GC, gastric cancer; ROC, receiver operating characteristic; TAP, tumor abnormal protein.
Table 4. Biochemical and clinicopathological characteristics of GC patients.

| CHARACTERISTIC                        | OVERALL | PROGNOSTIC MODEL | P  |
|--------------------------------------|---------|------------------|----|
|                                      | n       | TRAINING SET     | TEST SET |    |
| n                                    | 335     | 223              | 112      |    |
| Sex, n (%)                           |         |                  | 1.000    |    |
| Female                               | 98 (29.3%) | 65 (19.4%)       | 33 (9.9%) |    |
| Male                                 | 237 (70.7%) | 158 (47.2%)       | 79 (23.6%) |    |
| Age (year)                           | 67 (60, 73) | 67 (60, 74)       | 66 (61, 72) | .739 |
| Body weight (kg)                     | 68 (60, 74) | 68 (61, 74)       | 67.5 (57, 74) | .717 |
| Tumor volume (cm³)                   | 13.8 (8.3, 28.5) | 13.6 (7.8, 27.95) | 15.25 (8.5, 28.57) | .445 |
| Histological grade, n (%)            |         |                  | .520     |    |
| 1                                    | 45 (13.4%) | 33 (9.9%)       | 12 (3.6%) |    |
| 2                                    | 20 (6%) | 12 (3.6%)       | 8 (2.4%) |    |
| 3                                    | 64 (19.1%) | 45 (13.4%)       | 19 (5.7%) |    |
| 4                                    | 92 (27.5%) | 63 (18.8%)       | 29 (8.7%) |    |
| 5                                    | 114 (34%) | 70 (20.9%)       | 44 (13.1%) |    |
| Pathologic stage, n (%)              |         |                  | .974     |    |
| I                                    | 39 (11.6%) | 26 (7.8%)       | 13 (3.9%) |    |
| II                                   | 110 (32.8%) | 74 (22.1%)       | 36 (10.7%) |    |
| III                                  | 184 (54.9%) | 121 (36.1%)       | 63 (18.8%) |    |
| IV                                   | 2 (0.6%) | 2 (0.6%)       | 0 (0%) |    |
| Vascular invasion, n (%)             |         |                  | .276     |    |
| No                                   | 183 (54.6%) | 127 (37.9%)       | 56 (16.7%) |    |
| Yes                                  | 152 (45.4%) | 96 (28.7%)       | 56 (16.7%) |    |
| Neural invasion, n (%)               |         |                  | 1.000    |    |
| No                                   | 185 (55.2%) | 123 (36.7%)       | 62 (18.5%) |    |
| Yes                                  | 150 (44.8%) | 100 (29.9%)       | 50 (14.9%) |    |
| Lauren type, n (%)                   |         |                  | .717     |    |
| 1                                    | 156 (46.8%) | 106 (31.8%)       | 50 (15%) |    |
| 2                                    | 95 (28.5%) | 61 (18.3%)       | 34 (10.2%) |    |
| 3                                    | 75 (22.5%) | 49 (14.7%)       | 26 (7.8%) |    |
| 4                                    | 7 (2.1%) | 6 (1.8%)       | 1 (0.3%) |    |
| Borrmann type, n (%)                 |         |                  | .278     |    |
| 1                                    | 35 (10.5%) | 27 (8.1%)       | 8 (2.4%) |    |
| 2                                    | 107 (32.1%) | 75 (22.5%)       | 32 (9.6%) |    |
| 3                                    | 111 (33.3%) | 68 (20.4%)       | 43 (12.9%) |    |
| 4                                    | 80 (24%) | 52 (15.6%)       | 28 (8.4%) |    |
| TP53 mutation, n (%)                 |         |                  | .315     |    |
| No                                   | 170 (50.7%) | 118 (35.2%)       | 52 (15.5%) |    |
| Yes                                  | 165 (49.3%) | 105 (31.3%)       | 60 (17.9%) |    |

(Continued)
Table 5. Correlation analysis between TAP and clinicopathologic characteristics in the GC patients.

| VARIABLES                  | TAP EXPRESSION LEVEL | P   |
|----------------------------|----------------------|-----|
| Sex                        | 0.004                | .936|
| Age                        | 0.001                | .987|
| Body weight                | −0.035               | .522|
| Tumor volume               | 0.175                | .001|
| Histological grade         | 0.188                | .001|
| Pathologic stage           | 0.502                | <.001|
| Vascular invasion          | 0.102                | .023|
| Neural invasion            | 0.073                | .104|
| Lauren type                | 0.357                | <.001|
| Borrmann type              | 0.310                | <.001|
| TP53 mutation              | 0.198                | <.001|
| CDH1 mutation              | 0.228                | <.001|
| Ki67                       | 0.266                | <.001|
| CEA                        | 0.289                | <.001|
| CA199                      | 0.089                | .103|
| CA724                      | 0.220                | <.001|
| CA125                      | 0.115                | .036|
| OS                         | −0.645               | <.001|
| DFS                        | −0.707               | <.001|

Abbreviations: CEA, carcinoembryonic antigen; DFS, disease-free survival; GC, gastric cancer; OS, overall survival; TAP, tumor abnormal protein.
Figure 4. Correlations between TAP and clinicopathologic characteristics in the GC patients. CEA indicates carcinoembryonic antigen; DFS, disease-free survival; GC, gastric cancer; OS, overall survival; TAP, tumor abnormal protein.

Table 6. Univariate and multivariate COX regression analyses of prognostic factors related to OS in GC patients.

| CHARACTERISTICS | TOTAL (N) | UNIVARIATE ANALYSIS | MULTIVARIATE ANALYSIS |
|-----------------|-----------|----------------------|-----------------------|
|                 |           | HAZARD RATIO (95% CI) | P VALUE   | HAZARD RATIO (95% CI) | P VALUE |
| Tumor volume    | 216       | 1.021 (1.009-1.033)   | <.001     | 0.994 (0.978-1.010)   | .438    |
| Histological grade | 216   | 1.315 (1.116-1.549)   | .001      | 0.978 (0.825-1.159)   | .794    |
| Pathologic stage | 216      | 7.391 (4.484-12.183)  | <.001     | 2.260 (1.344-3.800)   | .002    |
| Vascular invasion | 216    | 1.200 (0.808-1.781)   | .367      |                       |         |
| Neural invasion  | 216       | 1.419 (0.957-2.104)   | .082      |                       |         |
| Laurén type     | 215       | 1.587 (1.269-1.986)   | <.001     | 0.831 (0.625-1.104)   | .201    |
| Borrmann type   | 215       | 1.553 (1.259-1.914)   | <.001     | 1.030 (0.808-1.314)   | .809    |
| Ki67            | 216       | 1.019 (1.010-1.029)   | <.001     | 0.992 (0.981-1.003)   | .160    |
| TP53 mutation   | 216       | 2.483 (1.646-3.747)   | <.001     | 1.040 (0.636-1.703)   | .875    |
| CDH1 mutation   | 216       | 2.624 (1.735-3.969)   | <.001     | 1.268 (0.791-2.033)   | .325    |
| TAP             | 216       | 1.107 (1.086-1.129)   | <.001     | 1.099 (1.075-1.123)   | <.001   |

Abbreviations: CI, confidence interval; GC, gastric cancer; OS, overall survival; TAP, tumor abnormal protein. Bold values imply a statistically significant correlation between the variables and overall survival.
Table 7. Univariate and multivariate COX regression analyses of prognostic factors related to DFS in the GC patients.

| CHARACTERISTICS      | TOTAL (N) | UNIVARIATE ANALYSIS | MULTIVARIATE ANALYSIS |
|----------------------|-----------|----------------------|-----------------------|
|                      |           | HAZARD RATIO (95% CI) | P VALUE | HAZARD RATIO (95% CI) | P VALUE |
| Tumor volume         | 216       | 1.019 (1.009-1.030)   | <.001    | 0.994 (0.981-1.008)   | .391    |
| Histological grade   | 216       | 1.244 (1.083-1.429)   | .002     | 0.959 (0.830-1.108)   | .571    |
| Pathologic stage     | 216       | 4.715 (3.234-6.873)   | <.001    | 1.878 (1.251-2.820)   | .002    |
| Vascular invasion    | 216       | 1.393 (0.980-1.981)   | .065     |                      |         |
| Neural invasion      | 216       | 1.431 (1.007-2.032)   | .045     | 1.066 (0.732-1.552)   | .739    |
| Laurén type          | 215       | 1.553 (1.270-1.899)   | <.001    | 0.887 (0.695-1.131)   | .333    |
| Borrmann type        | 215       | 1.535 (1.278-1.843)   | <.001    | 1.059 (0.866-1.296)   | .577    |
| Ki67                 | 216       | 1.018 (1.010-1.027)   | <.001    | 0.992 (0.983-1.002)   | .121    |
| TP53 mutation        | 216       | 2.481 (1.727-3.565)   | <.001    | 1.375 (0.896-2.109)   | .145    |
| CDH1 mutation        | 216       | 2.539 (1.763-3.657)   | <.001    | 1.394 (0.923-2.107)   | .115    |
| TAP                  | 216       | 1.086 (1.072-1.100)   | <.001    | 1.078 (1.061-1.094)   | <.001   |

Abbreviations: CI, confidence interval; DFS, disease-free survival; GC, gastric cancer; TAP, tumor abnormal protein. Bold values imply a statistically significant correlation between the variables and disease-free survival.

Figure 5. Coefficients of Lasso regression about the risk factors of OS (A) and DFS (B) and variable trajectories of Lasso regression about the risk factors of OS (C) and DFS (D).

DFS indicates disease-free survival; OS, overall survival.
Figure 6. The risk scores of risk factors of overall survival (A) and disease-free survival (B). TAP indicates tumor abnormal protein.

Figure 7. Prognostic prediction models of overall survival (A) and disease-free survival (B) and calibration curve of overall survival (C) and disease-free survival (D). TAP indicates tumor abnormal protein.
combined TAP and CT were above 90%, and the sensitivity and specificity of combined TAP, CT, and first gastroscopy were as high as 100%. Tumor abnormal protein can be used to monitor the metabolism of tumor cells as well as recurrence and metastasis of cancers, which can be performed before imaging examinations. Therefore, the introduction of TAP

**Figure 8.** Prognostic ROC curves of overall survival (A) and disease-free survival (B) about TAP and pathological stage and time-dependent prognostic ROC curves of overall survival (C) and disease-free survival (D) about TAP. FPR indicates false-positive rate; ROC, receiver operating characteristic; TAP, tumor abnormal protein.

**Figure 9.** Kaplan–Meier analysis of overall survival (A) and disease-free survival (B) in 335 gastric cancer patients stratified by TAP expression. TAP indicates tumor abnormal protein. HR, Hazard Ratio.
E-cadherin can weaken the intercellular adhesion, promote the feature of epithelial-mesenchymal transition. The loss of and the abnormal E-cadherin expression has been found as a mutation of related genes and the alteration of related pathways, which are involved in the occurrence and development of GC.

Our study indicated that TAP expression in the GC patients was positively related to the tumor volume, histological grade, pathologic stage, vascular invasion, Lauren type, Borrmann type, CEA and CA724; however, TAP expression was negatively correlated with OS and DFS. Deterioration of tumor-related clinical characteristics indicates the elevation of tumor burden and the active metabolism in tumor cells. Therefore, abnormal glycoproteins secreted by tumor cells increase, which is manifested by the increased TAP in the peripheral blood. This indicates that the increased TAP is related to the poor prognosis of GC patients after surgery, which is consistent with previously reported.

In addition, the univariate and multivariate COX regression analyses were employed to investigate the risk factors of OS and DFS in the GC patients. Results showed that TAP was an independent risk factor of OS and DFS. The Kaplan–Meier analysis further confirmed that the increased TAP was related to the reductions of OS and DFS in the GC patients. The ROC curve showed the prognostic value of TAP was better than that of pathological stage in the GC patients. The model established with TAP and pathological stage was effective in predicting the prognosis of GC patients at the third and fourth year. Therefore, TAP is a good prognostic factor for GC patients, and the survival time of GC patients can be evaluated through related models.

Furthermore, there is evidence showing that TAP is related to the mutation of Ki-67, TP53, and CDH1 genes, which has also been observed in our study. Wild-type TP53 is an important tumor suppressor gene and mainly functions to regulate cell cycle, repair damaged DNA, inhibit angiogenesis in the cancer, and induce cell apoptosis. It plays a key role in maintaining genetic stability. Studies have indicated that the glycosylation of certain genes can resuscitate wild-type p53 expression and p53-dependent apoptosis in mutant p53 tumors. In addition, p53 is a potent inhibitor of glycosylation. Therefore, it is speculated that there is an interaction between TP53 mutation and TAP production. Ki-67 is closely related to the cell cycle. Ki-67 is not expressed in the quiescent cells (G0 phase), but it is expressed in other phases of cell cycle (G1, S, G2, and M phases). Ki-67 has been used as a marker of nuclear proliferation and is positively related to tumor malignancy, tumor cell proliferation, and tumor invasiveness. E-cadherin is a transmembrane glycoprotein in human and animal epithelial cells. It can promote the integrity of cell structure and is involved in the embryonic development. It is also an essential component for cell adhesion and maintenance of epithelial integrity. The downstream signaling pathways of E-cadherin include Hippo, Wnt, transforming growth factor-β, and nuclear factor-κB, and the abnormal E-cadherin expression has been found as a feature of epithelial-mesenchymal transition. The loss of E-cadherin can weaken the intercellular adhesion, promote the cell migration, and disorder the cell polarity, which are conducive to tumor occurrence, invasion, and metastasis. Studies have found that the proliferation, immune response, metabolism, and invasion of GC are related to the O-glycosylation, N-glycosylation of related proteins and the production of abnormal glycochains. Moreover, the degree of N-glycan branching of α5 integrin and the aberrant expression in β1,6-N-acetylglucosamine-branched N-glycans structures of the β1 integrin subunit have been shown to positively regulate epidermal growth factor receptor signaling pathway. Therefore, the TAP expression may be correlated with the mutation of related genes and the alteration of related pathways, which are involved in the occurrence and development of GC.

However, there were several limitations in this study. First, this was a retrospective study, which affected the validity of detections. Second, the sample size was small, which may bias the results. Third, there were differences in the treatment of patients after surgery, which may also bias the results in this study. Moreover, our results showed that TAP may be correlated with mutations of related genes and alteration of related pathways. In our study, only a small number of patients received the detection of mismatch repair genes and microsatellite instability, and thus the mechanisms can not be well explored. Therefore, more clinical studies with large sample size and more detailed clinical information and hierarchical analysis are needed to confirm our findings. In addition, the relationships of TAP and related pathways with the occurrence and development of GC are warranted to be validated in vivo and in vitro.

**Conclusions**

Tumor abnormal protein is an effective factor for the early screening and prognostic evaluation of GC. In addition, TAP detection is simple and relatively noninvasive and therefore is clinically practical. We recommend that TAP can be detected before precede CT and gastroscopy for the preliminary assessment of tumor occurrence and development.

**Author Contributions**

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