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ORIGINAL ARTICLE

Prognostic role of plasma levels of γ-glutamyl transpeptidase in patients with advanced gastric cancer treated with anti-PD-1 immunotherapy*

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Abstract

Objective Antibodies targeting programmed cell death protein 1 (PD-1) have become the mainstay of treatment for chemotherapy-refractory gastric cancer, characterized by high levels of programmed cell death ligand-1 (PDL-1) expression. However, the routine clinical implementation of PDL-1 testing is currently limited by the lack of robust detection methods. In this regard, the role of plasma γ-glutamyl transpeptidase (GGT), an N-terminal nucleophilic hydrolase, as an independent predictor of the efficacy of anti-PD-1 therapy remains unknown. In this study, we aimed to assessed the prognostic role of changes in plasma GGT levels (6 weeks vs. baseline) in patients with advanced gastric cancer treated with anti-PD-1 immunotherapy.

Methods We retrospectively analyzed data from 57 patients with gastric cancer treated with anti-PD-1 antibodies (camrelizumab, sintilimab, nivolumab, tislelizumab, and toripalimab) at the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, from July 2018 to February 2021.

Results We found that after 6 weeks of treatment, there were significant differences between responders and non-responders with respect to plasma GGT levels ($P < 0.001$). Multivariate logistic regression analysis revealed that the continuous value of the 6-week difference in GGT levels (OR = 1.437, 95% CI = 1.116–1.849, $P = 0.005$) and 6-week difference in GGT ≥ 0 or < 0 (OR = 53.675, 95% CI = 6.379–451.669, $P < 0.001$) were independent predictors of disease control. Survival analysis indicated that a reduction in plasma GGT6 levels during treatment was significantly associated with a favorable progression-free survival (PFS) and overall survival ($P < 0.001$). Consistently, univariate and multivariate Cox regression analyses revealed that a reduction in plasma GGT6 levels during treatment was an independent predictor of PFS (HR = 1.033, 95% CI = 1.013–1.053, $P = 0.001$).

Conclusion Alterations in plasma GGT levels during treatment can be used as a predictor of disease progression and survival in patients with advanced gastric cancer undergoing treatment with anti-PD-1 antibodies.

Key words: gastric cancer; programmed cell death receptor 1; γ-glutamyl transpeptidase (GGT); prognosis

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Targeting immune checkpoints using immune checkpoint inhibitors has become an important treatment method for intractable gastric cancer. In Asian patients with gastric cancer, the anti-programmed cell death protein 1 (PD-1) monoclonal antibody nivolumab has been demonstrated to improve overall survival (OS) rate compared with a placebo treatment [1]. Responses to anti-PD-1 antibodies have been associated with the levels of programmed cell death ligand-1 (PDL-1) expression, with high levels of PDL-1 expression being established to be a predictor of the response to PD1 blockade [2]. Accordingly, plasma high levels of PDL-1 can serve as a prognostic factor predicting good prognosis. However, some patients with PDL-1-positive tumors show early progression in response to anti-PD-1 monotherapy [3]. Furthermore, using PDL-1 expression as a biomarker requires clinical samples and a robust PDL-1 detection method [4], the current lack of which is limiting the utility of PDL-1 as a predictive biomarker. Comparatively, serological biomarkers are readily evaluated and may be useful independent predictors of the response to anti-PD-1 therapy.

Serum γ-glutamyl transpeptidase (GGT) is a cell surface N-terminal nucleophilic hydrolase involved in intracellular oxygen homeostasis. Increased levels of GGT are considered to be indicative of oxidative stress [5], and GGT has also been proposed as a potential prognostic marker for cancer. Indeed, serum levels of GGT have been shown to be associated with the risk of gastric cancer [6], cancer progression, and drug resistance [7]. However, in 65 patients with gastric cancer, Wang *et al* [8] found serum GGT to be a poor prognostic factor for this disease. Nonetheless, the predictive value of GGT levels in patients receiving anti-PD-1 therapy remains to be determined. In this study, we investigated the utility of plasma GGT levels with respect to predicting the response to anti-PD-1 therapy in patients with gastric cancer.

Patients and methods

Patients

For the purposes of this study, we assessed the data obtained for 57 patients (34 men and 23 women) with gastric cancer who had undergone treatment with anti-PD-1 antibodies (camrelizumab, sintilimab, nivolumab, tislelizumab, and toripalimab) at the Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, from July 2018 to February 2021. Inclusion criteria for patient enrolment were as follows: (1) diagnosis of primary gastric cancer after pathological biopsy, (2) metastatic gastric cancer treated with at least two cycles of PD-1 blockade, and (3) complete clinicopathological and follow-up data. The exclusion criteria were as follows: (1) diagnosis of other tumors or recurrent gastric cancer, (2) liver or kidney dysfunction, and (3) tumor HER2 negativity determined by genetic testing. The study was approved by the Hospital Ethics Review Committee. All study procedures complied with the ethical standards of the Ethics Review Committee and the ethical requirements of the Declaration of Helsinki. To obtain the consent of patients, we adopted an opt-out approach in this retrospective study.

Data collection

We collected patients' baseline characteristics and clinical data, including age, gender, treatment with radiotherapy or chemotherapy, metastatic status (distant organ metastasis, peritoneal metastasis, distant organ and peritoneal metastasis), lines of therapy (first-line, second-line, or third-line and above), and GGT levels. Clinical response was determined according to the Response Evaluation Criteria in Solid Tumors (RECIST) as a complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). CR, PR, and SD were considered as a response, and PD was considered non-response. Plasma GGT levels were evaluated on the first day of anti-PD-1 treatment (baseline) and after 6 weeks of treatment. The difference between these two points was also calculated.

Data analysis

Data are expressed as the median (range), mean ± standard deviation, or numerical (percentage) values. Continuous variables were compared using a *t*-test, and categorical variables were analyzed using Pearson's chi-squared test or Fisher's exact test. Numerical variables that did not meet the conditions of normality and homogeneity of variance were analyzed using a rank-sum test. Univariate and multivariate Cox logistic regression analyses were conducted to identify risk factors associated with disease progression. Two-sided P-values ≤ 0.05 were considered to indicate statistical significance. Survival analysis was performed using the Kaplan-Meier method and the log-rank test. SPSS 26.0 software (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA) was used for all statistical analyses.

Results

Patient characteristics and tumor response

The baseline characteristics of the 57 patients included in this study are summarized in Table 1. The median age of the patients was 56 years (range, 23–76). Among the 57 patients, 34 (59.6%) were men. Twenty-eight (49.1%) patients received surgery, 49 (85.9%) received chemotherapy, 19 (33.3%) received first-line PD-1 blockade, 20 (35.1%) received second-line PD-1 blockade,

Table 1 Patient characteristics [*n* (%)]

Variable	*n* = 57
Age: Median (range, years)	56 (23–76)
Sex: Male/female	34/23
Metastasis: Organ/peritoneum/organ and peritoneum	31/9/17
Surgery: Yes/no	28/29
Chemotherapy: Yes/no	49/8
Lines of anti-PD-1 therapy: 1/2/≥ 3	19/20/18
Objective tumor response	
Complete response	0 (0%)
Partial response	10 (14.0%)
Stable disease	13 (17.5%)
Progressive disease	34 (59.6%)

and 18 (31.6%) received third-line PD-1 blockade or above. None of the patients had CR, eight (14.0%) had PR, 10 (17.5%) had SD, and 34 (59.6%) had PD.

Differences in clinicopathological characteristics between responders and non-responders

Differences in the clinicopathological characteristics of responders and non-responders are shown in Table 2. Responders were found to be characterized by a reduction in GGT levels from baseline, whereas non-responders showed an increase in GGT levels during treatment, and the difference in the change in GGT levels between responders and non-responders was found to be statistically significant ($P < 0.001$).

Factors predicting the response to PD-1 blockade

To identify factors predicting a treatment response, we performed univariate and multivariate logistic regression analyses (Table 3). Univariate analysis revealed the following to be significant predictors of the response to PD-1 blockade: peritoneal metastasis [odds ratio (OR) = 0.201, 95% confidence interval (CI) = 0.048–0.841, P = 0.028], continuous 6-week difference in GGT (change in GGT levels from baseline, as a continuous variable) (OR = 1.365, 95% CI = 1.122–1.661, P = 0.002), and 6-week difference in GGT < 0 (patients with reduced GGT levels during treatment) (OR = 23.619, 95% CI = 5.371–103.858, $P < 0.001$). Multivariate regression analysis revealed that the continuous value of the 6-week difference in GGT (OR = 1.437, 95% CI = 1.116–1.849, P = 0.005) and the 6-week difference in GGT ≥ 0 or < 0 (OR = 53.675, 95% CI = 6.379–451.669, $P < 0.001$) were independent factors predicting the response to anti-PD-1 treatment.

Table 3 Factors predicting the response to PD-1 blockad

Variable	Odds ratio	95% CI	*P*-value
Univariate analysis			
Age	0.992	0.948–1.038	0.726
Sex: Male	1.089	0.370–3.208	0.877
Metastasis			
Peritoneum	0.201	0.048–0.841	0.028*
Distant organs and peritoneum	0.268	0.044–1.640	0.154
Surgery: Yes	0.917	0.318–2.643	0.872
Chemotherapy: Yes	1.149	0.246–5.365	0.859
Lines of therapy			
2	0.364	0.095–1.386	0.138
≥ 3	1.167	0.297–4.588	0.825
GGT			
Baseline	1.002	0.995–1.008	0.640
6 weeks	1.010	0.993–1.027	0.255
6-week difference (continuous)	1.365	1.122–1.661	0.002*
6-week difference (< 0)	23.619	5.371–103.858	0.000*
Multivariate analysis: GGT			
6-week difference (continuous)	1.437	1.116–1.849	0.005*
6-week difference (< 0)	53.675	6.379–451.669	0.000*

Note: * $P < 0.05$

Survival analysis

Kaplan-Meier analysis revealed that progression-free survival (PFS) was significantly higher in patients with increased GGT levels during treatment than in those with reduced GGT levels during treatment ($P < 0.001$; Fig. 1). The mean PFS time of patients in the GGT group with a 6-week difference < 0 and a 6-week difference ≥ 0 was

Table 2 Differences in clinicopathological characteristics between responders and non-responders (*n*)

Variable	Responders (*n* = 23)	Non-responders (*n* = 34)	*P*-value
Age: Mean (years, SD)	55.52 (10.58)	54.41 (12.75)	0.732 [a]
Sex: Male/female	14/9	20/14	1.000 [b]
Metastasis: Organ/peritoneum/both	16/4/3	15/5/4	0.079 [c]
Surgery: Yes/no	11/12	17/17	1.000
Chemotherapy: Yes/no	3/20	5/29	0.590 [c]
Lines of therapy: 1/2/≥ 3	11/6/6	8/14/12	0.158 [b]
GGT (median, IQR)			
Baseline	28.00 (17.00, 36.00)	21.50 (14.00, 47.50)	0.425 [d]
6 weeks	24.00 (17.00, 34.00)	28.50 (17.75, 72.50)	0.286 [d]
6-week difference	–2 (–6.00, 0.00)	5.50 (2.00, 20.00)	< 0.001*

Note: * $P < 0.05$; [a] *t*-test; [b] Chi-square test; [c] Fisher exact test; [d] Rank-sum test

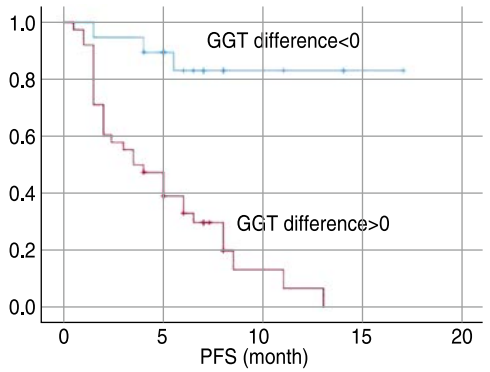

Fig. 1 Kaplan-Meier survival curves for progression-free survival stratified by γ-glutamyl transpeptidase (GGT)

14.765 and 4.954 months, respectively.

Univariate and multivariate Cox regression analysis of progression-free survival

Cox regression analysis was performed to assess the relationship between alterations in GGT levels during treatment and PFS (Table 4). Univariate (HR = 8.916, 95% CI = 2.693–29.516, $P < 0.001$) and multivariate (HR = 1.033, 95% CI = 1.013–1.053, $P = 0.001$) regression analysis revealed a 6-week difference in GGT < 0 to be an independent predictor of PFS.

Table 4 Univariate and multivariate Cox analyses of progression-free survival

Variable	Hazard ratio	95% CI	*P*-value
Univariate analysis			
Age	0.990	0.960–1.021	0.514
Sex	0.924	0.465–1.835	0.882
Metastasis			
Peritoneum	0.448	0.214–0.938	0.033
Distant organs and peritoneum	0.639	0.228–1.794	0.395
Surgery	0.839	0.427–1.649	0.611
Chemotherapy	0.978	0.377–2.536	0.963
Lines of therapy			
2	0.553	0.222–1.376	0.203
≥ 3	1.301	0.593–2.857	0.511
GGT baseline	1.001	0.999–1.004	0.325
GGT 6-week difference (< 0)	8.916	2.693–29.516	0.000*
Multivariate analysis			
GGT 6-week difference (< 0)	1.033	1.013–1.053	0.001*

Note: *$P < 0.05$

Discussion

Gastric cancer is among the major causes of cancer-related death worldwide. Although patients with early-stage gastric cancer can be treated with surgery, mortality rates remain high. Given the lack of effective screening strategies, most Chinese patients with gastric cancer are diagnosed at an advanced stage of disease progression [9]; thus, treatment options for these patients remain limited, and the prognosis is relatively poor. The key roles of immune checkpoints, including PD-1 and PDL-1, as immune escape mechanisms have been extensively demonstrated, and anti-PD-1/PD-L1 antibodies are used to treat a number of cancers, notably renal and lung cancers [10]. PD-1 blockade has also been shown to have a good safety profile and high efficacy in patients with gastric cancer [1]. Nevertheless, despite the promising prognostic value of PDL-1 levels in gastric cancer, its clinical application remains limited. As an alternative, in this study, we evaluated the prognostic utility of GGT levels in patients with advanced gastric cancer treated with PD-1 blockade. We found that alterations in GGT levels during treatment could be used to predict the response to anti-PD-1 therapy. Notably, an increase in GGT levels during treatment was found to be associated with disease progression and poor survival in patients undergoing anti-PD-1 therapy. To the best of our knowledge, this is the first study to demonstrate the prognostic role of GGT levels during treatment in patients with advanced gastric cancer treated with anti-PD-1 antibodies.

In humans, plasma GGT is predominantly derived from the liver and is accordingly used as an indicator of liver dysfunction and obstructive liver disease. Given that sex, diet, liver dysfunction, and kidney dysfunction are key factors affecting plasmas levels of GGT, patients with hepatic and renal dysfunction were excluded from this study. Interestingly, we found that metastasis in distant organs, including the liver, is unassociated with a response to anti-PD-1 therapy in patients with gastric cancer. The findings of a previous meta-analysis have indicated that baseline GGT levels are correlated with the overall cancer risk (RR = 1.32, 95% CI = 1.15–1.52), as well as with the risk of digestive cancer (RR = 1.94, 95% CI = 1.35–2.79) [11]. In addition, high GGT levels have been found to be associated with a poor prognosis in patients with liver, breast, renal cell, and endometrial cancers [12]. However, the role of GGT in carcinogenesis is yet to be sufficiently clarified. Corti *et al* [13] found that high GGT levels can lead to enhanced iron intake, the generation of hydroxyl radicals, and DNA damage. In turn, genomic instability and gene mutations can suppress immune responses and promote the proliferation of cancer cells, thereby leading to disease progression [14]. Moreover, GGT has been observed to promote the hydrolysis and peptide transfer of extracellular glutathione, glutathione degradation, oxidative stress, and reactive oxygen species generation, thereby further enhancing cancer cell proliferation and drug resistance [15–16]. To some extent, these mechanisms can provide an explanation to account for the poor prognosis of patients with gastric cancer and increased

GGT levels during anti-PD-1 treatment.

It has previously been shown that GGT levels are significantly correlated with OS in patients with advanced gastric cancer (HR = 1.006, 95% CI = 1.003–1.009, $P < 0.001$) [17]. To assess the relationship between the dynamic changes in GGT levels and prognosis in patients treated with anti-PD-1 antibodies, we conducted survival and multivariate Cox regression analyses. We accordingly established that an increase in GGT levels during treatment was associated with a favorable prognosis and improved survival. Importantly, multivariate Cox regression analysis identified an increase in GGT levels during treatment as an independent predictor of PFS, thereby indicating that alterations in the levels of GGT during PD-1 blockade can be used to predict treatment response and prognosis in patients with advanced gastric cancer.

In the cohort assessed in this study, we detected no significant difference between the baseline and 6-week values of GGT among responders and non-responders. Contrastingly, alterations in GGT levels during treatment were found to differ significantly between these two groups ($P < 0.001$). In the responders, we noted a reduction in GGT levels during treatment, whereas in the non-responders, there were increases in the levels of GGT during immunotherapy. Consistently, the findings of multivariate logistic regression analysis indicated that a reduction in GGT levels during treatment can serve as an independent predictor of disease control. These findings accordingly indicate that the direction of alterations in GGT levels during treatment can be used to predict the response to PD-1 blockade in patients with advanced gastric cancer.

In addition to plasma GGT levels, we also identified peritoneal metastasis as being associated with a treatment response in patients with gastric cancer, which is consistent with findings of previous clinical studies in which patients with gastric cancer received systemic chemotherapy [18]. These findings would thus tend to indicate that metastatic tumor cells in the peritoneum may act as a barrier that prevents drugs from reaching their therapeutic targets.

Despite our promising findings, this study does have an important limitation, in that it was a single-center retrospective study with a small cohort size. Consequently, our results need to be further confirmed by large-scale multi-center prospective trials. In addition, there was a lack of uniformity in the lines of treatment among patients, and thus whether GGT is a prognostic factor of anti-PD-1 antibody anti-tumor effect in patients receiving the same therapeutic line again needs to be further confirmed. Moreover, the efficacy of other clinicopathological variables in predicting an anti-PD-1 treatment response merits further investigation.

In conclusion, our findings provide convincing evidence that alterations in plasma GGT levels during anti-PD-1 therapy independently predict disease progression and poor survival in patients with advanced gastric cancer. These findings also indicate that plasma GGT levels can be used as a marker to predict the response to anti-PD-1 immunotherapy in these patients.

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Conflicts of interest

All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

Author contributions

All authors contributed to data acquisition, data interpretation, and reviewed and approved the final version of this manuscript.

Data availability statement

All data generated or analyzed during this study are included in this published article (and the accompanying supplementary information files).

Ethical approval

Not applicable.

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