ORIGINAL RESEARCH

Clinical significance of serum total oxidant/ antioxidant status in patients with operable and advanced gastric cancer

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Purpose: Oxidative stress was significantly associated with the development of malignancies. The purpose of this study was to evaluate the significance of serum total oxidant/antioxidant status in operable advanced gastric cancer patients.

Materials and methods: A total of 284 patients who underwent curative resection for primary stage III gastric cancer were enrolled. Total oxidant status, total antioxidant status, and oxidative stress index (OSI) were evaluated within 24 hours before surgery, and compared with 120 healthy donors. The correlation between the OSI and survival outcome was analyzed by the Kaplan–Meier method with log-rank test and Cox's regression methods, respectively.

Results: Mean OSI of gastric cancer patients was higher than healthy controls (1.41 ± 0.96 vs 0.78 ± 0.42, $P<0.001$). All patients were stratified into two groups using the optimal cutoff value (1.42) of OSI using a sensitivity of 94.1% and a specificity of 64.0% as optimal conditions from receiver operating curve analysis. Patients with an OSI ≥1.42 had poorer mean overall survival (45.6 vs 29.8 months, $P=0.022$) and mean recurrence-free survival (43.3 vs 28.1 months, $P=0.011$) than patients with an OSI <1.42 in univariate analysis, and OSI was also confirmed as an independent predictor for survival for gastric cancer in multivariate analysis (hazard ratio, 0.541; 95% CI: 0.127–1.102; $P=0.01$).

Conclusion: Preoperative OSI can be considered as an independent prognostic factor for operable and advanced gastric cancer.

Keywords: oxidative stress index, survival, gastric cancer

Introduction

Gastric cancer is one of most prevalent cancers worldwide, and also accounts for the second most frequent malignancy and the third leading cause of cancer-related death in People's Republic of China.[1,2] Despite the great improvement in early diagnosis and comprehensive treatments, the long-term outcomes of gastric cancer patients are far from satisfying, with a 5-year survival rate of 20%–30%.[3–5] Surgery remains the only curative treatment for localized gastric cancer.[6] However, 50%–90% of the patients with advanced and localized gastric cancer still had poor postoperative prognosis due to local recurrence or distant metastasis.[7–9] For such a patient population, preoperative risk stratification may be useful to guide comprehensive treatments and improve the short- and long-term clinical outcome. Some prognostic biomarkers which can be obtained easily and inexpensively in clinical practice have attracted the attention of many physicians, and an increasing number of studies have been conducted.[7,10,11] It has been revealed that oxidative stress plays a crucial role in promoting tumor proliferation, invasion, and metastases.[12,13] However, prospective studies investigating

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the association between development of gastric cancer and parameters of oxidative stress are scarce.

Oxidative stress participates in macromolecular oxidative damage, results in tissue protein denaturation, DNA damage, and lipid peroxidation, and interferes with normal metabolic activity, thus inducing the occurrence and/or development of cancer.[14,15] Reactive oxygen species (ROS), as the main active oxides mediating oxidative stress, are involved in the occurrence and development of cancer through induction of DNA damage and genetic mutations, inhibition of apoptosis, and promotion of the proliferation, invasion, and metastasis of tumor cells.[16,17] Finding biomarkers inflecting the oxidative stress status may be helpful for prognosis evaluation and risk stratification in gastric cancer patients.

Therefore, the total oxidant status (TOS) is usually used to evaluate the overall oxidation state of the body, while the total antioxidant status (TAS) is applied to measure the overall antioxidant status.[18,19] Furthermore, the oxidative stress index (OSI), the ratio of TOS to TAS, is considered as a more precise biomarker reflecting oxidative stress. OSI can reflect imbalance between oxidation and antioxidants through comprehensive measurement of TAS and TOS.[20] However, there was no data regarding the evaluation of TOS, TAS, and OSI in patients with gastric cancer. In this study, we tried to evaluate preoperative serum total oxidant/antioxidant status in gastric cancer patients though TOS, TAS, and OSI measurements, and analyze the clinical significance of such an indicator in gastric cancer patients who underwent curative resection.

Materials and methods
Patients
Data of 284 consecutive patients who underwent curative resection for histologically confirmed stage III gastric cancer between June 1, 2010, and June 1, 2016, at the Department of Gastroenterology in the First Affiliated Hospital of Xin-Xiang Medical University were prospectively collected in a preplanned database. According to the planed inclusion criteria, only histologically and clinically confirmed stage III gastric cancer patients, patients aged >18 years, and those with a life expectancy more than 6 months were enrolled. Patients with preoperative severe comorbidity, such as ischemic diseases, severe cardiac or neurological disorders, or other kind of malignant disease, chronic inflammatory disease, acute and chronic infective disease, and immunosuppressive disorders, were excluded from the study. Likewise, patients who received preoperative adjuvant treatments and chemotherapy also were excluded. According to the 2015 NCCN Clinical

Practice Guidelines in Oncology, all gastric cancer patients received a standard curative resection, and then were treated with postoperative standard chemotherapeutic regimens based on histopathological and clinical characteristics of tumor, including epirubicin, cisplatin, and 5-fluorouracil or docetaxel, cisplatin, and 5-fluorouracil. A control group that consisted of 112 age- and sex-matched healthy volunteers was enrolled. Written informed consent was obtained from individual patients and subjects. This study was approved by the Ethics Committee of the First Affiliated Hospital of Xin-Xiang Medical University.

Clinical assessment
Demographic and clinicopathological characteristics of all the patients were collected by one physician, and further checked by another attending surgeon; the data included gender, age, tumor site and size, tumor invasion depth, lymph node status, TNM stage, and pathological differentiation. The histopathological and clinical staging evaluation of tumor was based on postoperative histopathological examination of specimens and clinical assessment, according to the seventh edition American Joint Committee on Cancer TNM classification. Outcome was evaluated as overall survival (OS) and recurrence-free survival (RFS). OS was accurately defined as the duration from date of surgery to death, whereas RFS was calculated as the time from surgery to tumor recurrence. All patients were followed up at regular intervals through outpatient visits with physical examination and laboratory and radiographic studies every 3 months for the first 2 years, every 6 months for the next 3 years, and once annually thereafter. Gastroscopy, and enhanced abdominal computed tomography or magnetic resonance imaging scans were performed generally every 12 months. Clinical follow-up lasted from the date of surgery to either the time of death or February 2017.

Measurement of TOS, TAS, and OSI
The TOS and TAS level were measured according to Erel's methods.[18,19] Briefly, approximately 5 mL peripheral venous blood samples of gastric cancer patients and healthy controls were obtained and stored in a blood collection tube (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) on the day before the surgery. All study participants fasted for at least 12 hours before blood collection. After centrifugation, the sera were decanted and measured immediately using commercially available test kits (Rel Assay Diagnostics kit; Mega Tip, Gaziantep, Turkey) according to the manufacturer's instructions and using their reagents and

equipment. The results of the TAS are expressed as mmol Trolox Eq/L whereas the results of the TOS are expressed as µmol H_2O_2 Eq/L. OSI values were calculated as follows: OSI (arbitrary unit) = TOS (µmol H_2O_2 Eq/L)/TAS (µmol Trolox Eq/L) ×100.

Statistical analysis

All statistical analyses were carried out with SPSS version 20.0 (IBM Corporation, Armonk, NY, USA). All tests were two-sided, and a *P*-value <0.05 was considered statistically significant. Data for categorical variables were expressed as percentage and for continuous variables as mean ± SD. The optimal cutoff value of the OSI predicting survival was determined based on receiver operating characteristic (ROC) curve analysis. The χ^2 test or Fisher's exact test was used to analyze categorical variables, whereas continuous variables in normal distribution were analyzed by independent Student's *t*-test, or by Mann–Whitney *U*-test for abnormal distribution data. The OS and RFS and survival curve were based on Kaplan–Meier analyses by using the log-rank test. The Cox regression model was used to assess the hazard ratio (HR) and multivariate analysis.

Results

Patient

All baseline demographic and clinicopathologic data of the 284 patients with stage III gastric cancer are summarized in Table 1. Among gastric cancer patients, the average age was 58.6±7.6 years, and 196 (69.0%) patients were males. The most frequent site of tumor formation was the stomach body, which accounted for 140 of 274 patients (51.1%). There were 95 patients identified with T2 tumor stage, and 189 patients with T3 or T4 tumor stage. Histopathological examination revealed that 164 patients presented with well/moderately differentiated adenocarcinomas, while 120 patients had poorly differentiated tumors. The number of TNM stage IIIA, stage IIIB, and stage IIIC patients was 32 (11.7%), 94 (34.3%), and 148 (54.0%), respectively. Moreover, there was no statistically significant association between the OSI and clinicopathologic characteristics including age and gender of patients, tumor size, histological differentiation type, T stage, lymph node involvement, and recurrent type, except the TAS (Table 1).

Serum TOS, TAS, and OSI levels in patients with gastric cancer

The mean TOS level from 274 patients with stage III gastric cancer was 23.16±13.76, which was significantly higher than

Table 1 Correlation between the OSI and clinicopathologic characteristics in patients with gastric cancer

| Characteristic	OSI		*P*-value
	≥1.42 (n=193)	<1.42 (n=91)	
Age (years)			0.971
≥60	95	45	
<60	98	46	
Gender			0.546
Male	131	65	
Female	62	26	
BMI (kg/m²)	23.2±6.1	23.6±7.8	0.638
CRP (mg/L)	112.4±84.5	128.5±78.4	0.136
TAS (mmol Trolox Eq/L)	24.6±16.2	20.4±12.2	0.029
TOS (µmol H_2O_2 Eq/L)	1.4±0.6	1.5±0.8	0.242
Tumor site			0.420
Gastric cardia	42	25	
Gastric body	100	40	
Gastric antrum	51	26	
Tumor size (cm)			0.957
≥5	60	28	
<5	133	63	
Tumor invasion depth			0.701
T2	62	33	
T3 + T4	131	58	
Lymph node involvement			0.643
N0	99	44	
N1	94	47	
Pathological differentiation			0.690
Well/moderate	113	51	
Poor	80	40	
Recurrence type			0.334
Local relapse	65	36	
Metastasis	128	55	

Abbreviations: BMI, body mass index; CRP, C-reactive protein; OSI, oxidative stress index; TAS, total antioxidant status; TOS, total oxidant status.

that of healthy controls (n=120; 14.23±10.08; *P*<0.001). However, the mean TAS of gastric cancer patients was lower than healthy controls (1.52±0.66 vs 1.78±1.31, *P*=0.01). The mean OSI of gastric cancer patients was also higher than that of controls (1.41±0.96 vs 0.78±0.42, *P*<0.001), as can be seen from Figure 1.

Prognostic significance of OSI for gastric cancer

The cutoff value for the OSI for predicting survival of gastric cancer patients in the ROC analysis was 1.42, with a sensitivity of 94.1% and a specificity of 64.0%. The area under curve was 0.793 with a 95% confidence interval (95% CI) between 0.676 and 0.910, *P*<0.001 (Figure 2).

The median follow-up duration was 25.6 months (6.5–76.5 months). Finally, 173 (63.1%) out of all 274 patients had died, including 44 (51.2%) in 86 patients
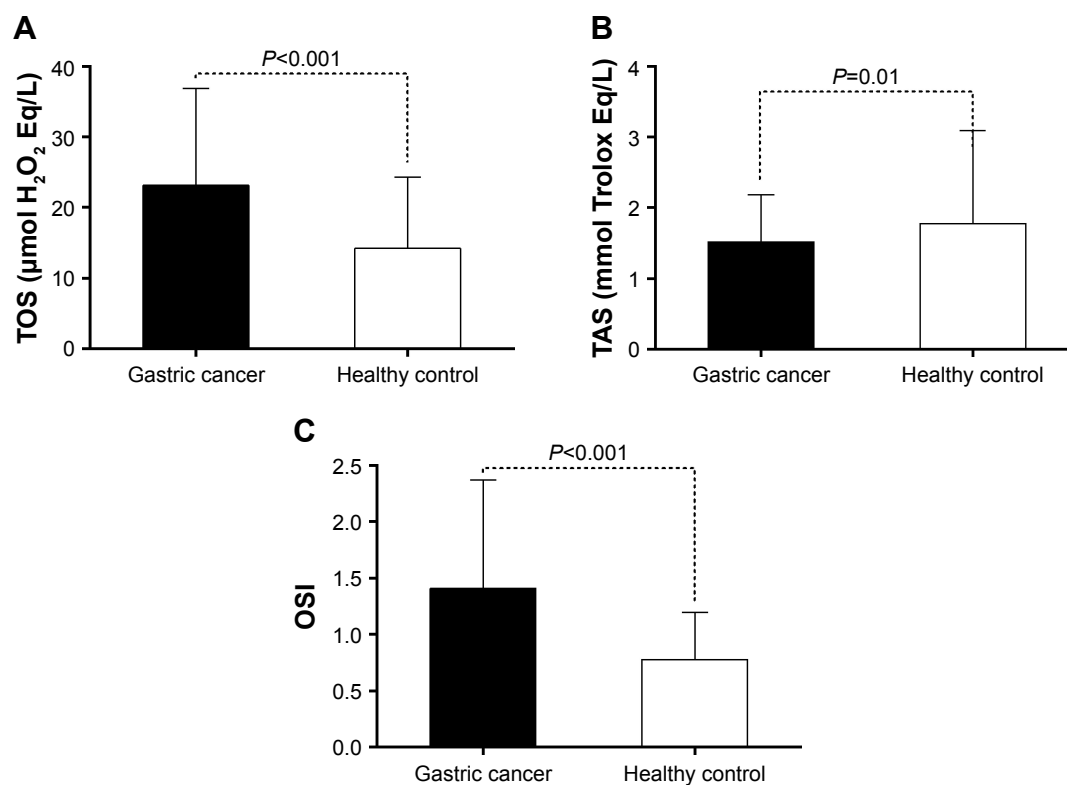
Figure 1 Evaluation of TOS/TAS status in patients with advanced gastric cancer.
Notes: (**A**) Shows the results of the difference of TOS between patients with gastric cancer (n=284) and healthy controls (n=120) (mean TOS in gastric cancer, 23.16±13.76; in healthy subjects, 14.23±10.08, $P<0.001$). (**B**) Shows the mean TAS of gastric cancer patients, which was lower than healthy controls (1.52±0.66 vs 1.78±1.31, $P=0.01$). (**C**) Shows that mean OSI of gastric cancer patients was also higher than that of controls (1.41±0.96 vs 0.78±0.42, $P<0.001$).
Abbreviations: OSI, oxidative stress index; TAS, total antioxidant status; TOS, total oxidant status.
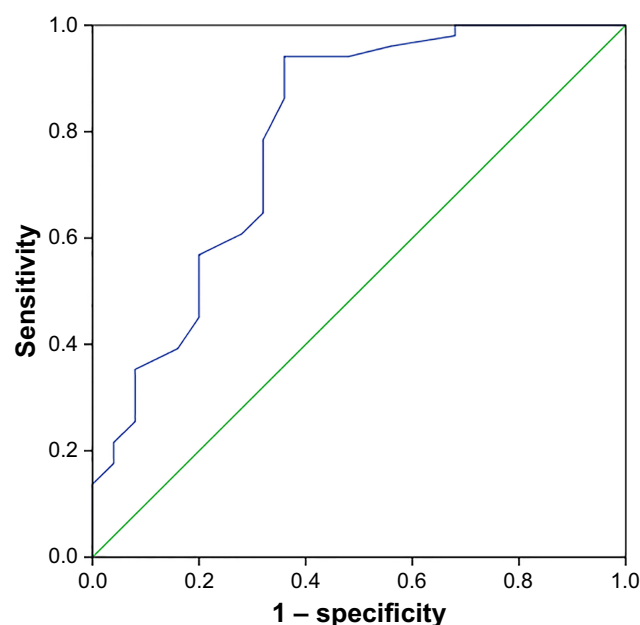
Figure 2 ROC for OSI predicting survival of gastric cancer.
Note: The cutoff value of OSI was 1.42, the AUC was 0.7394 with a 95% CI for the area between 0.672 and 0.916, $P<0.001$.
**Abbreviations:** AUC, area under the curve; ROC, receiver operating characteristic curve; OSI, oxidative stress index.

with an OSI <1.42 and 129 (68.6%) out of 188 patients with an OSI ≥1.42, respectively. One hundred and thirty-four (71.3%) in group of 188 patients with an OSI ≥1.42 had tumor relapse, while 47 (54.7%) in 86 patients with an OSI <1.42 suffered recurrence. Kaplan–Meier univariate survival analysis showed lymph node involvement, histological differentiation type, and preoperative OSI were related to postoperative survival of stage III gastric cancer patients. Among these prognostic factors, the mean OS of patients with a preoperative OSI <1.42 was 45.6 months, which was significantly higher than the 29.84 months of those with an OSI ≥1.42 ($P=0.022$) (Table 2, Figure 3). Furthermore, we obtained a similar result in the multivariate analysis for OS (HR, 0.541; 95% CI: 0.127–1.102; $P=0.01$) (Table 2). Otherwise, in univariate analysis of RFS, patients with a high OSI also had shorter mean RFS than patients with a low OSI (43.3 vs 28.1 months, $P=0.011$), and the OSI was also confirmed as a significant independent predictor for RFS in multivariate analysis (HR, 0.871; 95% CI: 0.294–1.662; $P=0.023$) (Table 3, Figure 3).

Table 2 Univariate and multivariate analyses of the prognostic factors of OS for stage III gastric cancer

Characteristics	Univariate			Multivariate		
	N	MS (months)	P-value	OR	95% CI	P-value
Age (years)			0.312			
≥60	140	32.8				
<60	144	31.7				
Gender			0.942			
Male	196	26.7				
Female	88	30.4				
Tumor site			0.831			
Gastric cardia	67	29.2				
Gastric body	140	27.2				
Gastric antrum	77	28.3				
Tumor size (cm)			0.082			
≥5	88	27.5				
<5	196	36.2				
Tumor invasion depth			0.351			
T2	95	32.5				
T3 + T4	189	22.7				
Lymph node involvement			0.011	2.141	0.723–3.214	0.012
N0	143	38.3				
N1	141	24				
Pathological differentiation			0.032	1.371	0.821–2.765	0.021
Well/moderate	164	32.3				
Poor	120	24.2				
OSI			0.022	0.541	0.127–1.102	0.010
<1.42	91	45.6				
≥1.42	193	29.8				

Abbreviations: CI, confidence interval; MS, median survival; OR, odds ratio; OS, overall survival; OSI, oxidative stress index.
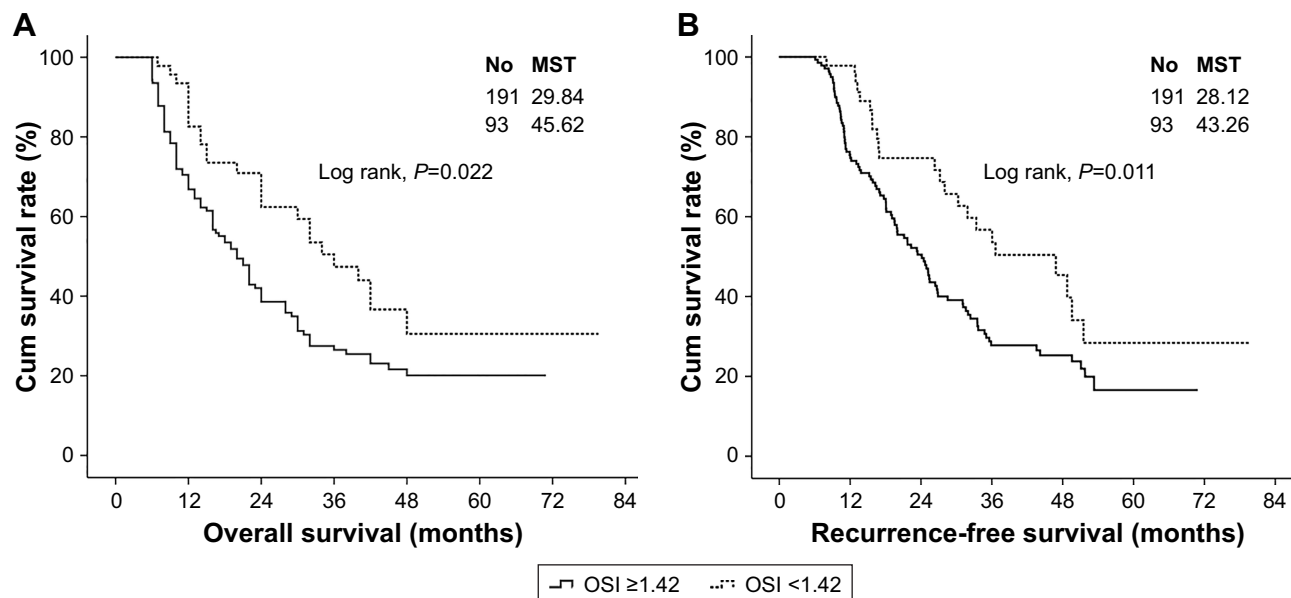
Figure 3 Prognostic significance of serum OSI for stage III gastric cancer.
Notes: (**A**) Left panel shows the association between OSI and OS (mean OS in OSI <1.42, 45.62 months; in OSI ≥1.42, 29.84 months, P=0.022). (**B**) Right panel shows the association between OSI and RFS (mean RFS in OSI <1.42, 43.26 months; in OSI ≥1.42, 28.12 months, P=0.011).
Abbreviations: OS, overall survival; OSI, oxidative stress index; RFS, recurrence-free survival; MST, mean survival time; Cum, cumulative.

Table 3 Univariate and multivariate analyses of the prognostic factors of RFS for stage III gastric cancer

Characteristics	Univariate			Multivariate		
	N	MS (months)	P-value	OR	95% CI	P-value
Age (years)			0.731			
≥60	140	26.8				
<60	144	26.4				
Gender			0.923			
Male	196	24.5				
Female	88	28.4				
Tumor site			0.312			
Gastric cardia	67	26.2				
Gastric body	140	26.1				
Gastric antrum	77	25.2				
Tumor size (cm)			0.232			
≥5	88	26.3				
<5	196	27.8				
Tumor invasion depth			0.451			
T2	95	27.4				
T3 + T4	189	24.5				
Lymph node involvement			0.032	1.731	0.421–2.212	0.010
N0	143	36.2				
N1	141	21.3				
Pathological differentiation			0.012			
Well/moderate	164	31.1				
Poor	120	21.1				
OSI			0.011	0.871	0.294–1.662	0.023
<1.42	91	43.3				
≥1.42	193	28.1				

Abbreviations: CI, confidence interval; MS, median survival; RFS, recurrent-free survival; OR, odds ratio; OSI, oxidative stress index.

A multivariate analysis included age and gender of patients, tumor size, lymph node involvement, histological differentiation type, and preoperative OSI into the Cox regression model to determine independent prognostic biomarkers for stage III gastric cancer. The result showed that OSI (HR, 0.541; 95% CI: 0.127–1.102; P=0.01), lymph node involvement (HR, 2.141; 95% CI: 0.723–3.214; P=0.012) and pathological differentiation (HR, 1.371; 95% CI: 0.821–2.765; P=0.021) were the independent prognostic factors for OS (Table 2). Furthermore, OSI (HR, 0.871; 95% CI: 0.294–1.662; P=0.023) and lymph node involvement (HR, 1.731; 95% CI: 0.421–2.212; P=0.002) were the independent prognostic factors for RFS (Table 3).

Correlation of OSI with prognosis of stage IIIA, IIIB, and IIIC gastric cancer patients

In setting of fewer stage IIIA cases, we combine patients with stage IIIA and IIIB into one group (n=126), with the other group consisting of stage IIIC patients (n=148). For stage IIIA and IIIB group, the mean OS of patients with an OSI ≥1.42 was significantly poorer than that of patients with

an OSI <1.42 (41.0 vs 36.6 months, P=0.039), and the RFS analysis showed nonsignificant results (41.0 vs 34.4 months, P=0.296). For stage IIIC patients, patients with an OSI <1.42 had better mean OS (42.4 vs 24.7 months, P=0.004) and mean RFS (38.3 vs 21.9 months, P=0.008), compared to patients with an OSI ≥1.42 (Figure 4A–D).

Discussion

In this study, we prospectively evaluated the preoperative OSI, TAS, and TOS of patients with stage III gastric cancer and found that gastric cancer patients had a significant higher TOS and OSI level and lower TAS level than healthy controls, and further found that OSI was a useful and independent predictive biomarker for survival in patients with advanced gastric cancer by univariate and multivariate analysis. Therefore, we confirmed that the OSI can be evaluated for optimal risk stratification of individual patients with gastric cancer and can serve as a biomarker for predicting postoperative prognosis of patients with advanced gastric cancer.

Oxidative stress has important roles in the occurrence, development, and metastasis of cancer.[21–23] Oxidative stress induces production of ROS, and then results in protein
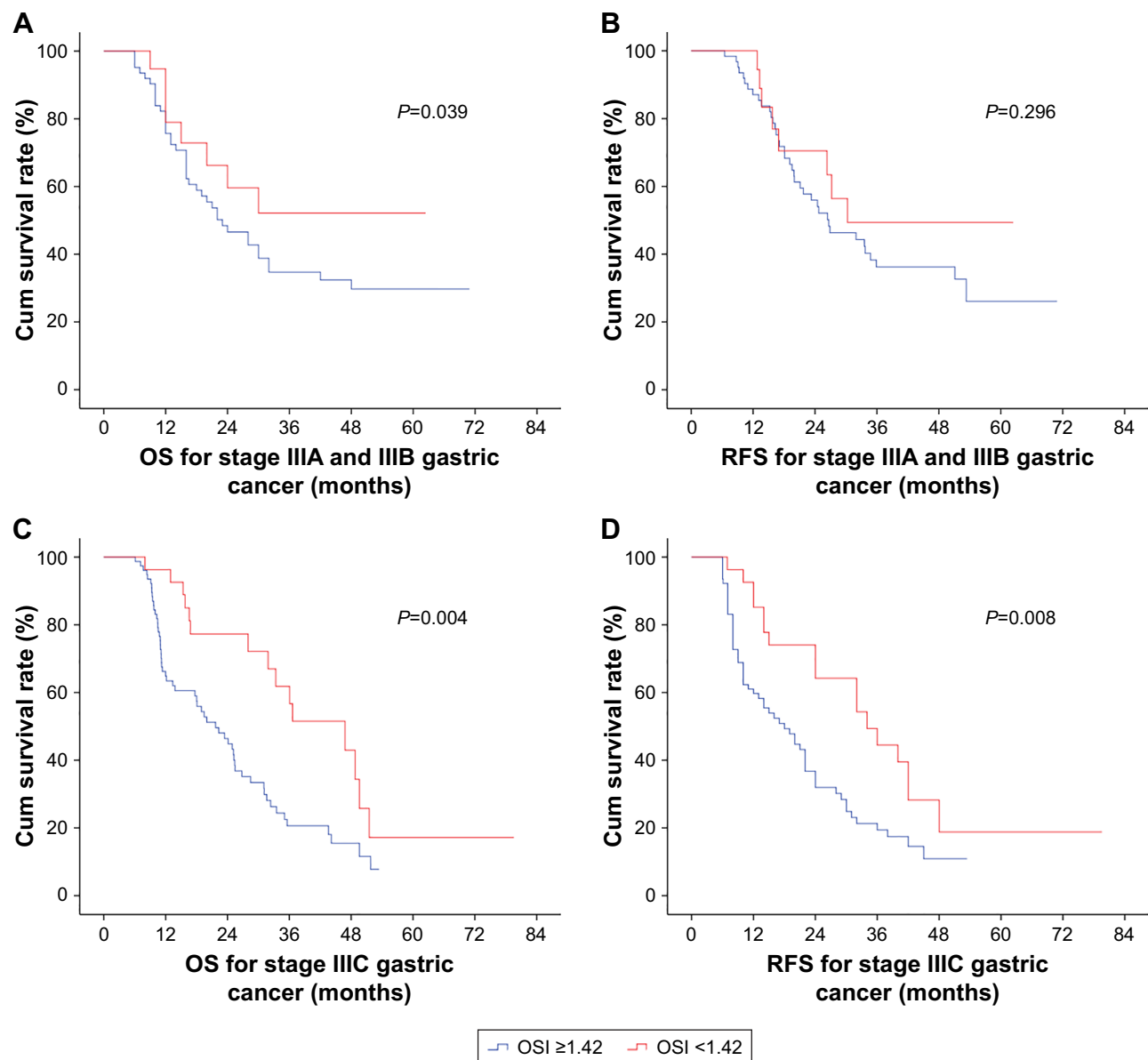
Figure 4 Survivability based on TNM stage.
Notes: Panel (**A**) and panel (**B**) show that there was a statistically significant correlation between OSI and OS, but not RFS of stage IIIA and IIIB gastric cancer patients. Panels (**C** and **D**) shows there was a significant association between OSI and OS and RFS of stage IIIC cancer patients.
Abbreviations: OS, overall survival; OSI, oxidative stress index; RFS, recurrence-free survival; Cum, cumulative.

denaturation or DNA damage, thus inhibiting normal physiological functions of various intracellular components, and finally leading to cancer development. In detail, ROS can oxidize guanine in DNA and RNA to form 8-hydroxyguanine (8-OHG),[24] which potentially introduces missense mutations.[25] Moreover, prior studies have found that there was a strong correlation between the formation of 8-OHG and carcinogenesis.[26,27] Otherwise, ROS widely participates in human tumorigenesis by affecting the major intracellular signaling pathways, such as MAP kinase and PI3K/Akt pathways.[28,29] It has been revealed that several biomarkers directly reflecting oxidative stress status were

significantly associated with cancer, such as TOS, TAS, OSI, and ischemia-modified albumin.[30–33] In this study, we confirmed that TOS and OSI levels in gastric cancer patients were higher than healthy subjects, further suggesting the important roles of oxidative stress in the development of gastric cancer.

Oxidative stress can promote several pathways that enable tumor progression and aggressiveness by regulation of proliferation, apoptosis, and invasion of tumor cells.[34,35] Furthermore, the severity of tissue inflammation and oxidative stress is directly associated with the prognosis of cancer.[36] Thus, OSI, as a biomarker directly reflecting imbalance

between oxidation and antioxidant oxidative stress has been confirmed as a potential prognostic biomarker in other diseases involved with oxidative stress.[37,38] In current study, we first explored prognostic significance of preoperative OSI in patients with stage III gastric cancer and found that OSI was significantly associated with OS and RFS of patients with gastric cancer.

Furthermore, in this study, we first confirmed that the OSI was an independent biomarker for prognosis in gastric cancer patients compared with other classical predictors including lymph node involvement and pathological differentiation. At the end of study, mortality rate was higher for patients with high OSI than those with lower levels, so physicians should be aware of the risk of patients with a high OSI and provide more proactive intervention to decrease OSI. However, OSI is not a direct contributor to poor outcomes, but a marker of severe oxidative stress. Targeting the original abnormal process might be a reasonable strategy to decrease OSI, such as mitochondrial targeted therapy.[39]

Several limitations may influence the interpretation of the results of this study. One limitation is that it is a single center study. A large-scale, multicenter, prospective study should be conducted to confirm long-term results and obtain more definite evidence. Furthermore, we analyzed the cutoff levels of the OSI though ROC curve in a small cohort, which may imply some overestimation.[40] Thus, the results of this study may not be comparable with those of other studies. A meta-analysis including various OSI validation studies may be required to confirm more definite cutoff values for the OSI.

Conclusion

Our study confirmed that patients with advanced gastric cancer have higher TOS and OSI. More importantly, we found that preoperative OSI was an independent prognostic factor for operable and advanced gastric cancer.

Disclosure

The authors report no conflicts of interest in this work.

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