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Original Article

A tumor marker panel scoring system to improve clinical stage in gastric cancer

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Abstract: Objective: To investigate the correlation among tumor markers test/reference cutoff ratio score (TRRS), disease progression and clinical stages in gastric cancer (GC). Methods: 708 patients hospitalized because of GC for the first time with preoperative tumor makers [carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, and CA125] measured between January 2005 and December 2014 at Zhongnan Hospital of Wuhan University were enrolled. We analyzed the relationship among tumor makers, disease progression and clinical stages, retrospectively. Results: CEA, CA19-9 and CA125 test/reference cutoff ratio (TRR) levels were increased significantly according to the clinical stages, TRRS were significantly correlated with the depth of tumor invasion, lymph node metastasis, distant metastasis and clinical stage (All $P < 0.001$). A tumor marker panel scoring system (TMSS) was devised as we defined Negative TMSS (TMSS = 0), Low TMSS ($1 \leq TMSS \leq 3$), Middle TMSS ($4 \leq TMSS \leq 6$), High TMSS ($7 \leq TMSS \leq 9$) based on the calculated TRR score. TMSS was significantly correlated with clinical stage. The later the TNM stage was, the higher the percentage of Middle and High TMSS will became. The distribution of TMSS in GC patients with High TMSS was found in advanced stages (stage III and IV). Conclusions: CEA, CA19-9 and CA125 TRRS are useful indicators for disease progression in GC patients hospitalized for the first time, the TMSS could be a useful tool for the prediction of clinical stage, especially for the advanced stage.

Keywords: Gastric cancer, tumor marker, TMSS, clinical stage

Introduction

Gastric cancer (GC) is one of the most common malignancies and a major health problem worldwide, affecting about one million people per year [1]. China has the highest incidence and mortality rate of GC [2]; and approximately 84% of GC patients in China will have advanced disease [3], and the 5-year survival rate is only 27.4% [4]. Though much progress has been achieved in the treatment of GC, personalized strategies for GC patients according to the clinical stages are important for the improvement of prognosis. Accurate assessment of TNM stage is the most significant element in determining appropriate treatment plans and one of the strongest predictors of recurrence and survival in patients with GC.

Tumor markers (TMs) are substances produced by tumor cells or secreted by the tissue as a response to the tumor. TMs detecting is recommended for the screening, diagnosis, and classification of tumors, as well as in the prognostic assessment and monitoring of recurrence and metastasis in GC cases [5-7]. Our previous studies have shown that carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9 and CA125 are the most important serum TMs that have been evaluated for GC [8-11].

Many studies have reached a consensus that high level of specific TMs that several times above the upper cutoff value predicts poor prognosis. For example, Takahashi *et al.* [12] reported that cancer recurrence was more frequent in patients with high preoperative CEA and CA19-9 levels, especially when the ratios were higher than two fold the upper normal limit. Kim *et al.* [13] and Ishigami *et al.* [14] reported that patients with CEA levels two times

Table 1. Major clinico-pathologic characteristics of 708 GC patients

Variables	N (%)
Gender	
Male	450 (63.6)
Female	258 (36.4)
Age (yr) range (median)	18-89 (58)
≤ 60	408 (57.6)
> 60	300 (42.4)
Pathological type	
Adeno WD/ID	157 (22.2)
Adeno PD/UN	445 (62.8)
Signet ring/Mucinous Ca	92 (13.0)
Other types*	14 (2.0)
Tumor invasion	
T1	84 (11.9)
T2	89 (12.6)
T3	57 (8.0)
T4	478 (67.5)
Lymph node metastasis	
N0	234 (33.0)
N1	109 (15.4)
N2	138 (19.5)
N3	227 (32.1)
Distant metastasis	
M0	548 (77.4)
M1	160 (22.6)
Clinical stage	
Stage I	125 (17.6)
Stage II	123 (17.4)
Stage III	300 (42.4)
Stage IV	160 (22.6)
Tumor marker	
CEA (+)	136 (19.2)
CA199 (+)	193 (27.3)
CA125 (+)	138 (19.5)

Abbreviations: GC, gastric cancer; Adeno WD/ID, well differentiated or intermediately differentiated adenocarcinoma; Adeno PD/UN, poorly differentiated or undifferentiated adenocarcinoma; Signet ring/Mucinous Ca, Signet ring cell carcinoma or mucinous adenocarcinoma. CEA (+): CEA > 5 ng/ml; CA199 (+): CA199 > 37 U/ml; CA125 (+): CA125 > 35 U/ml. *Other types included squamous cell carcinoma 7 cases, adenosquamous carcinoma 2 cases, and unclassified carcinoma 5 cases.

higher than normal limits had a more prominent serosal invasion, much more lymph node involvement, more advanced stage and poorer differentiation. A study by Lee *et al.* [15] showed poorer prognosis in patients with CEA levels greater than twice the normal limit, and CA19-9 levels greater than three times. Zhang *et al.* [9] indicated that the survival of CA19-9 > 5 times of the upper limit group was significant shorter than the normal CA19-9 value group. While Sisik *et al.* [16] have demonstrated that CEA greater than six times the cutoff value, and CA19-9 greater than three times the cutoff value was concluded to be an indicator of advanced stage. These studies suggest that the times of upper normal limit of TMs can be an indicator of GC stage, but the exact quantitative relationship between TM elevation times and the accuracy of the TNM stage remains unclear.

Based on the above findings, this study aimed to analyze the TMs test/reference cutoff ratio (TRR), to develop a tumor marker panel scoring system (TMSS), and to investigate its performance in accurate preoperative clinical stage assessment for GC patients.

Material and methods

Patients

708 newly diagnosed GC patients admitted to Zhongnan Hospital of Wuhan University from January 2005 to December 2014 with complete medical records and no preoperative treatment were enrolled. All patients were histologically confirmed with GC. Patients who showed any of the following conditions were excluded: having synchronous multiple primary malignancy besides GC, insufficient TMs (not all the three TMs were detected). The clinicopathologic features of these patients including age, sex, differentiation, depth of wall invasion, lymph node metastasis, distant metastasis, and TNM (Tumor-Node-Metastasis) stage were reviewed. The pathological type is based on the World Health Organization (WHO) histopathology classification. Patients were staged according to the criteria of the AJCC (American Joint Committee on Cancer) 7th edition [17].

Serum tumor marker detection and data analysis

Three milliliters of fasting blood was obtained from each patient in the next morning following admission and serum was separated, CEA, CA199 and CA125 were assayed by chemiluminescence immunoassay at Department of

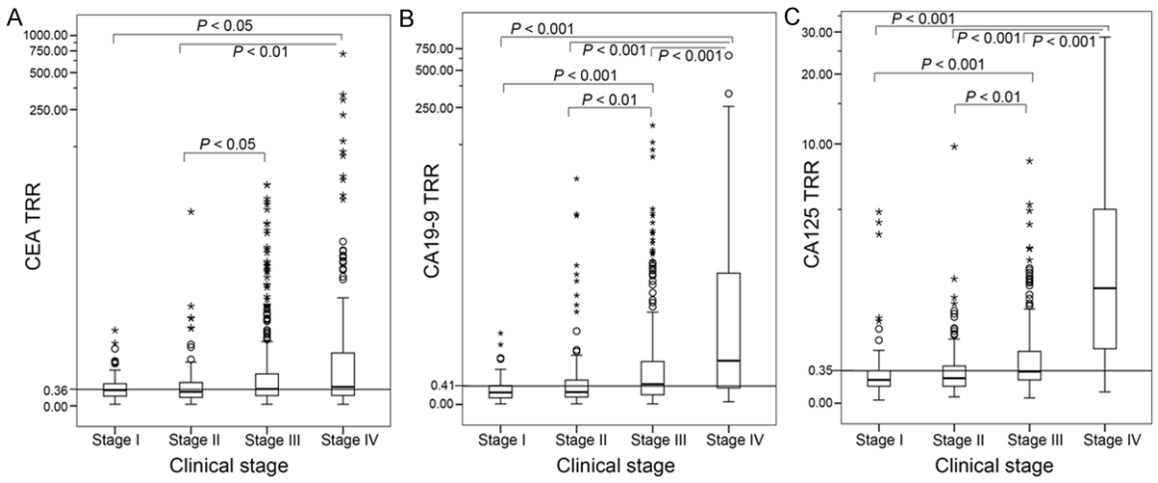

Figure 1. Tukey's boxplot showing positive correlation between CEA (A), CA19-9 (B) and CA125 (C) TRR levels and clinical stages. Nonparametric multiple comparison tests (Kruskal-Wallis test) indicated these TRR levels were increased significantly according to the clinical stages. Horizontal line median value, columns interquartile ranges, whiskers from the ends of the columns to the outermost data points within the upper quartile + [1.5 × (interquartile range)] and lower quartile - [1.5 × (interquartile range)].

Laboratory Medicine of Zhongnan Hospital of Wuhan University. The data were collected. The normal reference values of the TMs were as follows: CEA ≤ 5 ng/ml, CA19-9 ≤ 37 U/ml, CA125 ≤ 35 U/ml. We took the normal reference of TM as a cut-off value. According to the reference cutoff value (5 ng/mL for CEA, 37 U/mL for CA19-9, and 35 U/mL for CA125), the TRR was defined as the test value divided by the reference cut off value of each TM (CEA TRR = test value/5, CA19-9 TRR = test value/37, CA125 TRR = test value/35). Based on the related references [9, 12-16] which suggest different TM TRR levels may be an indicator of GC stage, TRR was divided into four categories: TRR < 1.00, 1.00 < TRR ≤ 3.00, 3.00 < TRR ≤ 6.00 and TRR > 6.00. Each category got a score which was classified as TRR score 0 (TRRS0), TRR score 1 (TRRS1), TRR score 2 (TRRS2) and TRR score 3 (TRRS3), respectively. The TRR scores of three TMs were analyzed and special attention was focused on the relationship between each TM TRRS and clinical outcomes of GC, in order to investigate the exact quantitative relationship between TM elevation times and the accuracy of the TNM stage.

Statistical analysis

Data were analyzed with SPSS 20.0 (SPSS Science Inc., Chicago, IL, USA) and Prism 5.0 (GraphPad Software, La Jolla, CA, USA). Nonparametric Kruskal-Wallis test or Mann-Whitney U test was used for the comparison of TMs between different groups, if appropriate. Spearman correlation analysis was used to test the relationship between TM levels and clinical characteristics. Differences with *P* values less than 0.05 ($P < 0.05$) were considered statistically significant.

Results

Clinic-pathological characteristics

Of the 708 patients, 450 patients were male and 258 patients were female with a median age of 58 (range: 18-89) years. Poorly differentiated or undifferentiated tumors were observed in 445 patients (62.8%), and moderately and well differentiated tumors were in 157 (22.2%). Over 65% of the GC patients were of clinical stage III and beyond: 125 (17.6%) Stage I, 123 (17.4%) Stage II, 300 (42.4%) Stage III, and 160 (22.6%) Stage IV. In terms of TM levels, CEA was elevated in 136 patients (19.2%), CA19-9 was elevated in 193 patients (27.3%) and CA125 was elevated in 138 patients (19.5%). Major clinic-pathological characteristics of these patients were summarized in **Table 1**.

Elevation rates of serum TMs and TRR

The pretreatment levels of CEA, CA19-9 and CA125 were above the cut-off levels in 19.2%,

Table 2. Relationships between tumor marker TRRS and GC progression

Tumor marker TRRS		Tumor invasion n (%)				P* R#	Lymph node metastasis n (%)				P* R#	Distant metastasis n (%)		P& R#
		T1	T2	T3	T4		N0	N1	N2	N3		M0	M1	
CEA	TRRS0	80	80	36	376	< 0.001	211	91	104	166	< 0.001	460	112	< 0.001
		95.2%	89.9%	63.2%	78.7%	0.109	90.2%	83.5%	75.4%	73.1%	0.193	83.9%	70.0%	0.154
	TRRS1	3	4	6	45		14	9	16	19		39	19	
		3.6%	4.5%	10.5%	9.4%		6.0%	8.3%	11.6%	8.4%		7.1%	11.9%	
	TRRS2	0	3	4	20		5	2	8	12		22	5	
		0.0%	3.4%	7.0%	4.2%		2.1%	1.8%	5.8%	5.3%		4.0%	3.1%	
	TRRS3	0	2	11	37		4	7	10	30		27	24	
		0.0%	2.2%	19.3%	7.7%		1.7%	6.4%	7.2%	13.2%		4.9%	15.0%	
CA19-9	TRRS0	83	75	41	316	< 0.001	207	87	91	130	< 0.001	442	73	< 0.001
		98.8%	84.3%	71.9%	66.1%	0.241	88.5%	79.8%	65.9%	57.3%	0.302	80.7%	45.6%	0.344
	TRRS1	1	10	4	59		15	9	19	31		50	24	
		1.2%	11.2%	7.0%	12.3%		6.4%	8.3%	13.8%	13.7%		9.1%	15.0%	
	TRRS2	0	0	5	28		4	3	8	18		16	17	
		0.0%	0.0%	8.8%	5.9%		1.7%	2.8%	5.8%	7.9%		2.9%	10.6%	
	TRRS3	0	4	7	75		8	10	20	48		40	46	
		0.0%	4.5%	12.3%	15.7%		3.4%	9.2%	14.5%	21.1%		7.3%	28.8%	
CA125	TRRS0	82	80	38	370	< 0.001	222	90	110	148	< 0.001	506	64	< 0.001
		97.6%	89.9%	66.7%	77.4%	0.146	94.9%	82.6%	79.7%	65.2%	0.307	92.3%	40.0%	0.572
	TRRS1	1	5	10	48		8	11	14	31		32	32	
		1.2%	5.6%	17.5%	10.0%		3.4%	10.1%	10.1%	13.7%		5.8%	20.0%	
	TRRS2	1	2	5	33		4	4	8	25		8	33	
		1.2%	2.2%	8.8%	6.9%		1.7%	3.7%	5.8%	11.0%		1.5%	20.6%	
	TRRS3	0	2	4	27		0	4	6	23		2	31	
		0.0%	2.2%	7.0%	5.6%		0.0%	3.7%	4.3%	10.1%		0.4%	19.4%	

Abbreviations: GC, gastric cancer; TRRS, test/reference cutoff ratio score. For CEA TRRS, statistical significance was found: T1 vs. T3 P < 0. 001, T1 vs. T4 P < 0. 01, T2 vs. T3 P < 0.001, T3 vs. T4 P < 0.05; N0 vs. N2 P < 0.01, N0 vs. N3 P < 0.001; M0 vs. M1 P < 0.001. For CA19-9 TRRS, statistical significance was found: T1 vs. T3 P < 0. 01, T1 vs. T4 P < 0.001, T2 vs. T4 P < 0. 01; N0 vs. N2 P < 0.001, N0 vs. N3 P < 0.001; N1 vs. N3 P < 0.001; M0 vs. M1 P < 0.001. For CA125 TRRS, statistical significance was found: T1 vs. T3 P < 0. 001, T1 vs. T4 P < 0.001; T2 vs. T3 P < 0.01, T2 vs. T4 P < 0.05; N0 vs. N1 P < 0.05, N0 vs. N2 P < 0. 01, N0 vs. N3 P < 0.001; N1 vs. N3 P < 0. 01; N2 vs. N3 P < 0.01; M0 vs. M1 P < 0.001. *Kruskal-Wallis test; &Mann-Whitney U test; #Spearman correlation analysis.

27.3% and 19.5% of cases, respectively. There was a tendency toward increasing TM levels with advancing cancer stage as CEA being elevated 5.6% in stage I, 10.6% in stage II, 22.7% in stage III and 30.0% in stage IV. For CA19-9, a similar increasing pattern was found with 3.2% elevated in stage I, 15.4% in stage II, 27.7% in stage III, and 54.4% in stage IV cancers. CA125 exceeded our defined threshold in 4.0% of stage I, 5.7% of stage II, 10.6% of stage III, and 60.0% of stage IV cancers. The serum CEA, CA19-9 and CA125 TRR's quartile range increased as the clinical stages increased, the distribution of TRR was right skewed. Moreover, with the increasing of clinical stages, the value and the numbers of outliers increased. Nonparametric multiple comparison tests (Kruskal-Wallis test) indicated serum CEA, CA19-9 and CA125 TRR levels were increased significantly according to the clinical stages (**Figure 1**).

Association between tumor marker TRRS and GC progression

Local tumor invasion depth, lymph node status and distant metastasis reflect GC progression. The proportion of CEA TRRS1, TRRS2 and TRRS3 were significantly higher in the patients with deeper tumor invasion, more lymph node involvement and distant metastasis (All $P < 0.001$), CEA TRRS had positive correlation to tumor invasion depth, lymph node status and distant metastasis (**Table 2**), so were CA19-9 TRRS and CA125 TRRS. But there was no significant difference in the elevation rates of serum TMs among GC patients with different differentiations (data not shown).

Significance of TMSS in clinical stage of GC patients

CEA, CA19-9 and CA125 TRRS and clinical stage had significant positive correlation (**Table**

Table 3. Relationships between TRRS and each clinical stage

Clinical stage		CEA TRRS				CA19-9 TRRS				CA125 TRRS			
		0	1	2	3	0	1	2	3	0	1	2	3
Stage I	n	118	6	1	0	121	4	0	0	120	2	3	0
	(%)	94.4%	4.8%	0.8%	0.0%	96.8%	3.2%	0.0%	0.0%	96.0%	1.6%	2.4%	0.0%
Stage II	n	110	7	5	1	104	10	2	7	116	6	0	1
	(%)	89.4%	5.7%	4.1%	0.8%	84.6%	8.1%	1.6%	5.7%	94.3%	4.9%	0.0%	0.8%
Stage III	n	232	26	16	26	217	36	14	33	270	24	5	1
	(%)	77.3%	8.7%	5.3%	8.7%	72.3%	12.0%	4.7%	11.0%	90.0%	8.0%	1.7%	0.3%
Stage IV	n	112	19	5	24	73	24	17	46	64	32	33	31
	(%)	70.0%	11.9%	3.1%	15.0%	45.6%	15.0%	10.6%	28.8%	40.0%	20.0%	20.6%	19.4%
P*		< 0.001				< 0.001				<0.001			
R#		0.229				0.388				0.470			

Abbreviations: TRRS, test/reference cutoff ratio score. For CEA TRRS, statistical significance were found: Stage I vs. Stage III P < 0.001, Stage I vs. Stage IV P < 0.001; Stage II vs. Stage III P < 0.01, Stage II vs. Stage IV P < 0.001; For CA19-9 TRRS, statistical significance were found: Stage I vs. Stage III P < 0.001, Stage I vs. Stage IV P < 0.001; Stage II vs. Stage IV P < 0.001; Stage III vs. Stage IV P < 0.001; For CA125 TRRS, statistical significance were found: Stage I vs. Stage IV P < 0.001; Stage II vs. Stage IV P < 0.001; Stage III vs. Stage IV P < 0.001. *Kruskal-Wallis test; #Spearman correlation analysis.

3), the more advanced the TNM stage was, the higher the proportion of TRRS1, TRRS2 and TRRS3 (All $P < 0.001$) became. To assess the ability of TRRS changes in predicting GC clinical stage, a tumor marker panel scoring system (TMSS) was developed, assigning TRRS0 as score 0, TRRS1 as score 1, TRRS2 as score 2 and TRRS3 as score 3. The TMSS was the overall score calculated from the sum of the three TM scores. Next the TMSS (the sum of the scores) was divided into four categories: Negative TMSS (TMSS = 0, n = 401) as none TM was elevated, Low TMSS ($1 \leq TMSS \leq 3$, n = 211) as at least one TM was elevated, Middle TMSS ($4 \leq TMSS \leq 6$, n = 81) as at least two TMs were elevated, and High TMSS ($7 \leq TMSS \leq 9$, n = 15) as all three TMs were elevated. The results showed the TMSS of Stage I were all ≤ 3, and the TMSS of Stage II were all ≤ 6 (**Figure 2A**), the later the TNM stage was, the higher the TMSS value became. There was a statistically significant relationship between TMSS and clinical stage ($P < 0.001$, correlation coefficient r = 0.520; **Figure 2B**). Regarding the distribution of TMSS across clinical stage, it was found that the later the TNM stage was, the higher the proportion of Middle TMSS and High TMSS became (**Figure 2C**). Furthermore, patients with High TMSS were all in advanced stages (stage III and IV) (**Figure 2D**).

Discussion

TMs are antigens and biologically active substances produced by tumor cells when oncogene expression is abnormal. They reflect the changes in related gene expression during the process of tumor progression as normal tissue and benign lesions produce low levels or none of these markers. The detection of TMs has become a routine in GC. The commonly researched TMs in GC are CEA, CA19-9 and CA125. CEA is a cell surface glycoprotein that is first described in 1965 by Gold and Freedmann as an antigen expressed by gastrointestinal carcinoma [18]. As an incomplete glycolipid antigen of the Lewis blood group, CA19-9 predominantly increases in gastric, colorectal, ovarian, liver and bile duct cancer [19]. CA125 is a heterogeneous cell membrane glycoprotein related with malignant conditions such as ovarian, uterine, lung, and pancreatic cancer [20]. Different studies have reported that the elevation rates of these TMs vary widely, for example 14%-35% for CEA [11, 14, 21-23], 8.7%-50% for CA19-9 [11, 14, 21-23], and 5.7%-31.8% for CA125 [11, 21, 22]. In our study, the elevation rate for serum CEA, CA19-9 and CA125 of GC patients was 19.2%, 27.3% and 19.5%, respectively, which was in accordance with precursors work. The elevation rate of TMs in advanced GC was much higher than that of the early stage, suggesting a coherence between TMs elevation rates and GC progression. Thus we defined test/reference cutoff ratio (TRR) as the ratio of multiples for upper normal limit of each TM. Nonparametric multiple comparison tests (Kruskal-Wallis test) indicated serum CEA, CA19-9 and CA125 TRR levels were increased signifi-

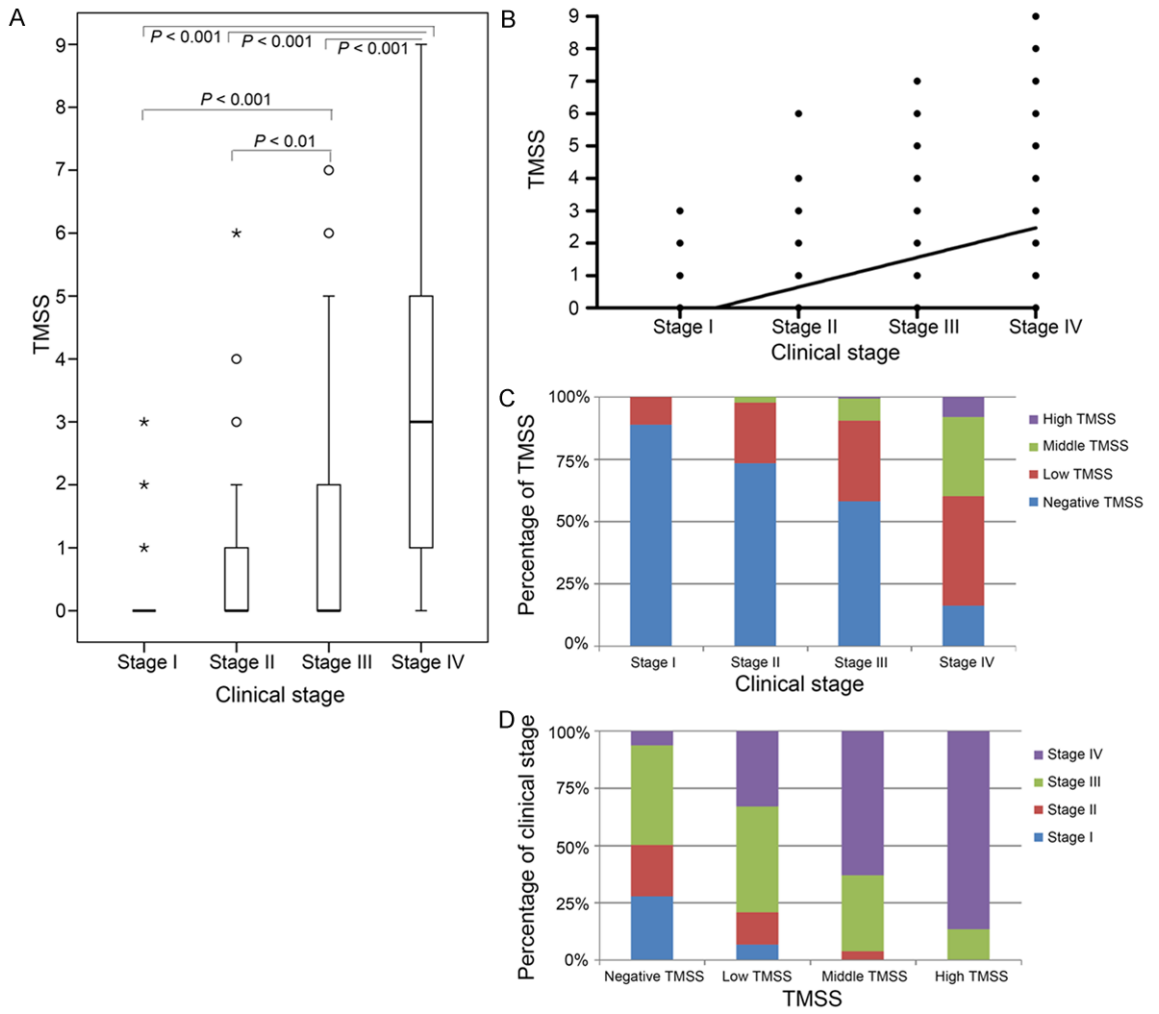

Figure 2. Relationships between TMSS and each clinical stage. A. Tukey's boxplot showing relationships between TMSS levels and clinical stages. Nonparametric multiple comparison tests (Kruskal-Wallis test) indicated TMSS levels were increased significantly according to the clinical stages. Whiskers, and columns, as in **Figure 1**. B. Positive correlation between TMSS levels and clinical stages in GC patients. $P < 0.001$, correlation coefficient $r = 0.520$. C. Distribution of TMSS across clinical stage. The later the TNM stage was, the higher the proportion of Middle TMSS and High TMSS became. D. Distribution of clinical stage across TMSS. The GC patients with High TMSS were all in advanced stages (stage III and IV).

cantly according to the clinical stages (**Figure 1**). According to our results and related references [9, 12-16], TRR was divided into four categories and each category was given a TRR score (TRRS). Subsequently, three TM TRRS were analyzed and special attention was focused on the relationship between each TM TRRS and clinical outcomes of GC patients.

For long a strong association has been established between TM levels and tumor stage, the depth of invasion, lymph node involvement and the presence of distant metastasis. For example, it has been shown that CEA strongly correlates with serosal invasion, lymph node involvement, the presence of distant metastasis and advanced stage [24-27]. Other studies have revealed that elevated CA19-9 is associated with tumor depth, nodal involvement, metastasis and stage [27-30]. Meanwhile CA125 was reported to have relationships with peritoneal dissemination and lymph node involvement [22, 31, 32]. However, the association is still controversial. Duraker *et al.* [33] reported that no relationship was found between CEA positivity and hepatic and peritoneal metastasis, and no correlation existed between CA19-9 positivity and lymph node, hepatic and peritoneal metastasis. Another study conducted by Gwak and coworkers found that there was no signifi-

cant association between CEA and nodal involvement, depth of invasion and stage, and no statistically significant relationship between CA19-9 and depth of invasion, nodal involvement and staging except for metastasis [34]. Overall, the causes for such inconsistency may be due to the differences in sample size, the detection technique, cut-off values, and the variety of included patients in these studies. For instance, some studies enrolled resectable GC patients, while the others selected GC patients with peritoneal metastasis. In the present study, we found a positive relationship between CEA, CA19-9 and CA125 TRRS and tumor invasion depth, lymph node status, distant metastasis, and clinical stage (All $P < 0.001$; **Tables 2**, **3**). Furthermore, for CEA TRRS, the difference described above was mainly existed in T1 and T3, T4; T3 and T2, T4; N0 and N2, N3; stage I and stage III, stage IV; stage II and stage III, stage IV. For CA19-9 TRRS, the difference was found in T1 and T3, T4; T2 and T4; N0 and N2, N3; N1 and N3; stage I and stage III, stage IV; stage II, stage III and stage IV. For CA125 TRRS, it was mainly in T1 and T3, T4; T2 and T3, T4; N0 and N1, N2, N3; N1, N2 and N3; stage I, stage II, stage III and stage IV. These findings suggested that numerous information could be explored from TM TRRS system to indicate the clinical stage of GC patients, thus providing diagnostic basis for clinical individualized treatment strategies.

Currently, TNM stage is widely used in practice to guide clinicians in prognostication and treatment planning for GC. However, it is difficult to accurately estimate N stage before surgery, thus resulting in difficulties in confirming the TNM stage of GC patients. Therefore, it becomes an important concern for routine clinical practice as how to evaluate the clinical stage accurately for GC patients to guide the initial treatment. Previous researches reported that the GC stage could affect serum TM levels [21, 26, 33]. Our results also showed a significant positive correlation between TMs TRRS and clinical stage (**Table 3**). The more advanced the TNM stage was, the higher the proportion of TRRS1, TRRS2 and TRRS3 (All $P < 0.001$) became. To assess the ability of TRRS changes in predicting GC clinical stage, a tumor marker panel scoring system (TMSS) was developed and recommended by this study. It was interesting that GC patients with High TMSS were all in advanced stages (stage III and IV) (**Figure 2D**). TMs are not causes for cancer but rather proteins shed by tumor cells, making it a useful indicator of clinical stages. Tumor cells expressing intercellular adhesion molecules, like CEA and CA19-9, may have a greater invasive potential [23, 28, 29, 35]. CEA may be involved in tumor cell adhesion onto liver parenchyma, which might explain the correlation between CEA levels and hepatic metastasis [35]. CA19-9 expressed by cancer cells mainly binds to the endothelial cell surface receptors P-selectin and E-selectin, which suggests its significance in the process of hematogenous metastasis [35]. Indeed, it has been shown that CA125 on the cancer cell surface membrane promotes peritoneal metastasis by initiating cells attachment to the mesothelial cells via binding to their cell surface molecule mesothelin. Therefore, elevated CA125 may be a result of cancer progression [36-38]. In conclusion, TMs can be produced directly by tumor or non-tumor cells as a response to the tumor genesis, so higher level of TM reflect higher tumor burdens. This theoretical viewpoint offers a new insight to understand why patients with High TMSS have tumors with advanced stages (stage III and IV).

This study also has some limitations. First, we did not evaluate the TMSS levels of serum TMs in predicting survival rates, and the correlation between TMSS and survival was uncertain. In addition, our study did not include preoperative CA72-4 and CA242 that have been shown to be important TMs for GC [28, 32, 39, 40]. As a result, it is not clear whether our proposed scoring system could achieve more compelling results with more TMs added to the research list. What's more, uncontrollable confounding factors, such as GC patient selection and potential laboratory errors in TMs examination, may have produced bias. This study is a retrospective study and further prospective studies should be conducted to validate the findings of the TMSS.

Conclusion

Our findings suggest that the preoperative status of serum CEA, CA19-9 and CA125 correlates with tumor stage, the depth of invasion, lymph node involvement and the presence of distant metastasis. CEA, CA19-9, CA125 TRRS are useful indicators for disease progression in GC patients hospitalized for the first time, and

the TMSS could be a useful tool for predicting clinical stage, especially for the advanced stage. Patients with high TMSS are prone to be in advanced stages (stage III and IV).

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Disclosure of conflict of interest

None.

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