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Original Article

Effectiveness of managing suspected metastasis using plasma D-dimer testing in gastric cancer patients

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Abstract: Tumor metastasis is closely related to the coagulation system. Tumor metastasis and hypercoagulability promote each other through multiple mechanisms. However, whether coagulation indicators can reflect tumor metastasis remains to be explored. Clinical characteristics of a total of 3447 patients from three tertiary referral centers were collected. Then the diagnostic efficacy of FDP, D-dimer and GC tumor markers [Carcinoembryonic antigen (CEA), Carbohydrate antigen 19-9 (CA19-9) and Carbohydrate antigen 72-4 (CA72-4)] for GC metastases was evaluated by the receiver operating characteristic curve (ROC) analyses. Then we conducted a joint ROC curve analysis. The effects of coagulation parameters and tumor markers on gastric cancer metastasis were assessed using multiple logistic regression analysis. 2049 patients were diagnosed with primary GC, 1398 patients with metastatic GC. Based on comparison of AUC, FDP (cutoff, 1.915) had significantly higher diagnostic efficacy than fibrinogen (P<0.001), CEA (P<0.001), CA199 (P<0.001) and CA724 (P<0.001). No significant difference was observed between D-dimer (cutoff, 0.905) and FDP (P=0.158). The AUC of tumor markers combined with coagulation indexes was higher than that without combination (P<0.001). In multiple logistic regression analysis, age, smoking, D-dimer, FDP, CEA, CA19-9, CA72-4 were found to be significantly associated with GC metastasis (all P<0.001, except for smoking P=0.004). We conclude that plasma FDP and D-dimer may be novel clinical biomarkers for screening metastases of GC.

Keywords: Gastric cancer, D-dimer, FDP, tumor marker, diagnostic efficacy

Introduction

GC is a well-known global health threat. It is estimated that with more than 1 million new cases each year and GC is the fifth most diagnosed malignancy worldwide [1]. GC is the second leading cause of cancer-related death in China [2]. Despite the morbidity and mortality of GC have gradually decreased over the past few decades, the five-year survival rate for GC remains worrying. This is largely because asymptomatic early GC and early GC metastasis cannot be screened effectively.

As conventional GC biomarkers, CEA, CA19-9, and CA72-4 are frequently used in GC diagnosis, staging assessment, prognosis prediction, therapeutic monitoring and recurrences detection [3]. However, the sensitivity and specificity of these three markers are not sufficient for predicting peritoneal metastasis [4]. Fibrin degradation products (FDP) and D-dimer both are fibrin degradation products, and both of them are meaningful for screening or diagnose of coagulopathies and thrombotic disease. Kanda, M. et al. found a positive correlation between coagulation score and the incidence of postoperative complications [5]. Many tumors are often in a hypercoagulable state. This cancer-related coagulation abnormalities is known as Trousseau's syndrome which is closely related to thrombosis and tumor progression [6]. Exactly, an increasing number of studies have focused on the pathological mechanism of tumor progression based on abnormal coagulation. In lung cancer, activation of the coagulation system has more effect on the metastases than on the primary tumor [7]. Elevated D-dimer levels on postoperative day 7 may predict long-term tumor prognosis in patients undergoing gastrectomy for locally advanced gastric cancer [8]. Furthermore, coagulation abnormalities

are also closely related to breast [9, 10], esophageal [11], colorectal cancer [12]. Tumor-associated hypercoagulation is also conducive to tumor metastasis. Activation of the platelet and coagulation system protects tumor cells from immune clearance and promotes their adhesion to the endothelium, promoting tumor blood metastasis [13]. Consistently, our previous study found that plasma D-dimer was closely related to asymptomatic hematogenous metastasis of GC [14]. However, few literatures systematically explore sensitivity and specificity of coagulation indicators for metastases screening. In this context, our research aims to explore whether plasma D-dimer and FDP levels can be used as novel biomarkers for screening GC metastasis.

In this study we determined whether plasma D-dimer and FDP levels can better predict GC metastasis than conventional GC tumor markers (CEA, CA19-9 and CA72-4). Plasma clotting indicators were collected, the ROC analysis and comparison of AUC was performed to evaluate the diagnostic efficacy of D-dimer, FDP and GC markers.

Materials and methods

Patients

We retrospectively analyzed 3447 GC cases from three tertiary medical centers, 2709 (1544 without metastasis, 1165 with metastasis) at First Affiliated Hospital of Xi'an Jiaotong University (FAH), Xi'an, China, form January 2010 to December 2018; 407 (264 without metastasis, 143 with metastasis) at Shaanxi Provincial Cancer Hospital (SPCH), Xi'an, China, from July 2014 to September 2018; 331 (252 without metastasis, 79 with metastasis) at Shaanxi Provincial People's Hospital (SPPH), Xi'an, China, from December 2016 to November 2019. Inclusion criteria: (1) newly diagnosed with GC, (2) all GC patients had pre-treatment GC markers and coagulation test, (3) age ≥18 years. Exclusion criteria: (1) Concurrent or secondary to other tumors, (2) had history of thrombotic disease, receive anticoagulant therapy or antiplatelet therapy, (3) acute infection or disseminated intravascular coagulation, (4) pregnancy or lactation. Both coagulation indicators and tumor markers were those closest to the time of treatment. This study was approved by the Ethics Committee of First Affiliated Hospital of Xi'an Jiaotong University and funded by the National Natural Science Foundation of China (NO.81501826).

Coagulation index assay

Venous blood was collected in sodium citrate tubes. The levels of FIB, FDP and D-dimer were analyzed by latex-enhanced immunoturbidimetric assay. All the samples were collected when they first get pathological diagnosis before any treatments. The normal level of FIB, D-Dimer and FDP in human plasma is less than 4.0 g/l, 1.0 mg/l and 5.0 mg/l, respectively.

Evaluation of baseline characteristics

We collected gender, age at first diagnosis, laboratory test before any treatment [including prothrombin time (PT), prothrombin activity (PTA), prothrombin ratio (PTR), activated partial prothrombin time (APTT), international normalized ratio (INR), thrombin time (TT), fibrinogen (FIB), D-dimer, FDP, CA19-9 and CA72-4].

Statistical analysis

Taking into account the selectivity and geographical influence of patients' access to hospitals, we used *Z-score* method to standardize data and conducted a multilevel analysis to evaluate residual between hospitals. Briefly, we specified hospitals as level 2 and patients as level 1 and constructed a two-level intercept-only model in which there was intercept only without other explaining variates. The model equation is $y_{ij}=\beta_{0ij}*x_0$ in which $\beta_{0ij}=\beta_0+u_{0j}+e_{0ij}$. Where x_0 is a constant of 1, β_0 is a constant, u_{0j}, the departure of the j-th hospital's intercept from the overall value, is a level 2 residual which is the same for all patients in hospital j, e_{0ij} is the departure of the i-th patient's actual laboratory result from the overall value, is a level 1 residual in hospital j. In this equation, both u_{0j} and e_{0ij} are random quantities, whose means are equal to zero; they form the random part of the model. We assume that they follow a normal distribution with variances, σ_u^2 and σ_e^2 respectively.

Cases were divided into two groups, with GC metastases or not. Count data were presented as frequencies and percentages and compared using the χ^2 test or Fisher exact test. Continuous variables that are not normally dis-

Table 1. Demographic and baseline characteristics of patients (n=3447)

Characteristic	Overall (N=3447)	Non-metastasis (N=2049)	Metastasis (N=1398)	*P* value
Gender, no. (%)				<0.001
Male	2502 (72.6)	1556 (75.9)	946 (67.7)	
Female	945 (27.4)	493 (24.1)	452 (32.3)	
Age, years	61 (53-68)	61 (54-68)	60 (52-68)	0.019
Smoking	348 (10.1)	171 (8.3)	177 (12.7)	<0.001
AI	145 (4.2)	89 (4.3)	56 (4)	0.628
Hyperlipidemia	49 (1.4)	34 (1.7)	15 (1.1)	0.153
Diabetes	82 (2.4)	40 (2)	42 (3)	0.047
PT, second	13.2 (12.7-13.83)	13.1 (12.6-13.7)	13.4 (12.8-14.1)	<0.001
PTA, %	91.15 (79.9-102.85)	94 (83.1-104.7)	88.2 (76.88-99.5)	<0.001
PTR	1.04 (0.99-1.09)	1.02 (0.98-1.07)	1.05 (1.01-1.11)	<0.001
INR	1.04 (0.99-1.09)	1.03 (0.98-1.08)	1.05 (1-1.12)	<0.001
APTT, second	34.9 (32-28)	34.3 (31.6-37.1)	35.8 (33-39)	<0.001
TT, second	16.2 (15.5-16.9)	16.3 (15.6-17)	16.1 (15.4-16.9)	<0.001
FIB, g/l	3.44 (2.82-4.22)	3.27 (2.68-3.95)	3.77 (3.05-4.59)	<0.001
D-dimer, mg/l	0.71 (0.35-1.8)	0.5 (0.2-0.9)	1.7 (0.8-3.7)	<0.001
FDP, mg/l	1.7 (1-4.1)	1.2 (0.7-2.05)	4 (1.87-9.2)	<0.001
CEA, ng/ml	3.1 (1.67-9.67)	2.51 (1.48-5.06)	4.95 (2.14-23.53)	<0.001
CA199, U/ml	13.18 (6.92-36.95)	10.72 (6.1-20.8)	21.65 (8.98-134.88)	<0.001
CA724, U/ml	2.84 (1.31-8.91)	2.12 (1.19-5.74)	5.24 (1.84-21.31)	<0.001
Platelet, 10^9/l	208 (159-266)	209 (162-263)	206 (154-269)	0.298

Values in parentheses are percent or interquartile ranges. AI, Aseptic inflammation.

tributed are represented by median and interquartile range (IQR) and compared with log-rank tests, while continuous normal variables were presented as mean ± standard deviation and compared using Student's *t-tests*. AUC were compared using DeLong's test. Multiple logistic regression was performed to assess the relationship between explanatory variables and GC metastasis.

Statistical analysis and plotting were performed with SPSS Statistics (version 20.0, IL, USA). DeLong's test was performed using MedCalc (version 19.4.1, USA). Multilevel analysis was conducted using MLwin (version 2.36, UK). 2-sided *P*<0.05 were considered statistical significantly.

Results

Plasma D-dimer and FDP levels were markedly elevated in metastatic GC

We collected data from 3,557 GC patients in three tertiary referral centers and 3447 cases were included in the analysis. Other patients without D-dimer data were excluded. Results of the two-level intercept-only model revealed that level 2 (hospital) residual for any one of target indicators was too small to be considered (Figure S1 in the supplement). Patient baseline data and laboratory tests are listed in **Table 1**. The median age was 61 (IQR, 53-68). Male were the majority (2502, 72.6%). We divided patients into two groups according to whether the tumor has metastasized. There were 1398 (40.6%) cases in the metastasis group.

As shown in **Table 1**, metastasis group had a greater proportion of female (32.4% vs. 24.1%, P<0.001), younger age (median, 60 vs. 61, P=0.019), more smokers (12.7% vs. 8.3%, P< 0.001), more diabetics (3% vs. 2%, P=0.047), higher PT (median, 13.4 vs. 13.1, P<0.001), lower PTA (median, 88.2 vs. 94, P<0.001), higher PTR (median, 1.05 vs. 1.02, P<0.001), higher INR (median, 1.05 vs. 1.03, P<0.001), higher APTT (median, 35.8 vs. 34.3, P<0.001), lower TT (median, 16.1 vs. 16.3, P<0.001), higher FIB (median, 3.77 vs. 3.27, P<0.001), higher D-dimer (median, 1.7 vs. 0.5, P<0.001), higher FDP (median, 4 vs. 1.2, P<0.001), higher CEA (median, 4.95 vs. 2.51, P<0.001), higher CA19-

Table 2. AUC and the diagnostic indicators for GC metastases (n=3447)

	AUC	95% CI	P[a]	Cut-point	Cut-point#	Sen	Spe	Youden index	P[b]
FDP	0.791	0.775-0.808	<0.001	-0.2186	1.915	0.73	0.727	0.457	0.982
D-dimer	0.774	0.757-0.792	<0.001	-0.2033	0.905	0.682	0.763	0.445	0.003
FIB	0.634	0.614-0.655	<0.001	-0.2465	3.635	0.549	0.657	0.206	<0.001
CEA	0.643	0.617-0.669	<0.001	-0.0809	4.34	0.482	0.761	0.243	<0.001
CA199	0.65	0.624-0.676	<0.001	-0.1852	18.685	0.497	0.745	0.242	<0.001
CA724	0.657	0.632-0.683	<0.001	-0.2104	6.96	0.446	0.799	0.245	<0.001
PRE1	0.721	0.697-0.744	<0.001	0.3494	0.342	0.629	0.721	0.35	<0.001
PRE2	0.799	0.779-0.82	<0.001	0.3304	0.327	0.735	0.742	0.477	Ref[c]

AUC, area under the curve; CI, confidence interval; P[a], *P* value for ROC curve of corresponding indicator; Cut-point, cutoff value at the maximum of Youden index; Cut-point#, the cut-off point obtained from the standardized cut-off point; Sen, sensitivity; Spe, specificity; P[b], *P* value for comparison of AUC of PRE2 with other indicators; PRE1, prediction probability obtained by binary logistic regression combined with GC markers (CEA, CA199, CA724); PRE2, prediction probability obtained by binary logistic regression combined with GC markers, D-dimer and FDP; Ref[c], Reference.

9 (median, 21.65 vs. 10.72, P<0.001) and higher CA72-4 (median, 5.24 vs. 2.12, P<0.001) than non-metastasis group. There was no difference in aseptic inflammation (P=0.628), hyperlipidemia (P=0.153) and platelet levels (P=0.298) between the two groups.

Plasma D-dimer and FDP levels had higher diagnostic efficacy than conventional markers for GC metastasis

As plasma D-dimer and FDP levels of metastasis group significantly higher than non-metastasis group, we hypothesized that plasma D-dimer and FDP could be used as auxiliary screening indicators for tumor metastasis. Based on ROC analysis and AUC comparison, the AUC (95% CI) of D-dimer (0.774, 0.757-0.792), FDP (0.791, 0.775-0.808), FIB (0.634, 0.614-0.655), CEA (0.643, 0.617-0.669), CA19-9 (0.65, 0.624-0.676), and CA72-4 (0.657, 0.632-0.683) were calculated. The AUC of FDP was significantly higher than FIB (*P*<0.001), CEA (*P*<0.001), CA19-9 (*P*<0.001) and CA72-4 (*P*<0.001) except for D-dimer (*P*=0.158) (data not shown). The optimal cut-point, sensitivity and specificity were obtained at the maximum of the Youden index. The sensitivity and specificity of FDP (cut-point, 1.915) were 73% and 72.7%, D-dimer (cut-point, 0.905) were 68.2% and 76.3%, CEA (cut-point, 4.34) were 48.2% and 76.1%, CA19-9 (cut-point, 18.685) were 49.7% and 74.5%, CA72-4 (cut-point, 6.96) were 44.6% and 79.9%, respectively. The predicted probability of combining GC markers (PRE1) and the predicted probability of combining D-dimer, FDP and GC markers (PRE2) was obtained by binary logistic regression. Multivariate combined ROC analysis also verified that PRE2 was much more effective than PRE1. The AUC of PRE2 (AUC=0.799, 95% CI: 0.779-0.82) was higher than that PRE1 (AUC=0.721, 95% CI: 0.697-0.744) (P<0.001), with 73.5% sensitivity and 74.2% specificity for the former and 62.9% sensitivity and 72.1 specificity for the latter. Notably, the AUC of FDP is comparable to that of PRE2 (P=0.982) (**Table 2**).

To assess whether D-dimer and FDP still have diagnostic advantage for single site metastasis or peritoneal metastasis, ROC analysis and comparison of AUC were performed using data stratified by different metastasis sites. For peritoneal metastasis (n=319), AUC (95% CI) of D-dimer was 0.808 (0.77-0.846), FDP was 0.84 (0.805-0.875), FIB was 0.685 (0.639-0.732), CEA was 0.55 (0.499-0.601), CA19-9 was 0.622 (0.569-0.675) and CA72-4 was 0.694 (0.648-0.74), respectively (Table S1). Diagnostic efficacy of D-dimer was comparative to FDP (P=0.289), while FDP had higher diagnostic performance than FIB (P<0.001), CEA (P<0.001), CA19-9 (P<0.001) and CA72-4 (P<0.001) (**Figure 1C**, **1D**). For osseous metastasis (n=184), AUC (95% CI) of D-dimer was 0.784 (0.746-0.823), FDP was 0.795 (0.758-0.832), FIB was 0.612 (0.566-0.659), CEA was 0.649 (0.593-0.705), CA19-9 was 0.556 (0.499-0.613) and CA72-4 was 0.582 (0.52-0.643), respectively (Table S2). Similar to peritoneal metastasis, diagnostic efficacy of D-dimer and FDP were significantly higher than FIB and GC tumor markers (**Figure 1E**, **1F**). As

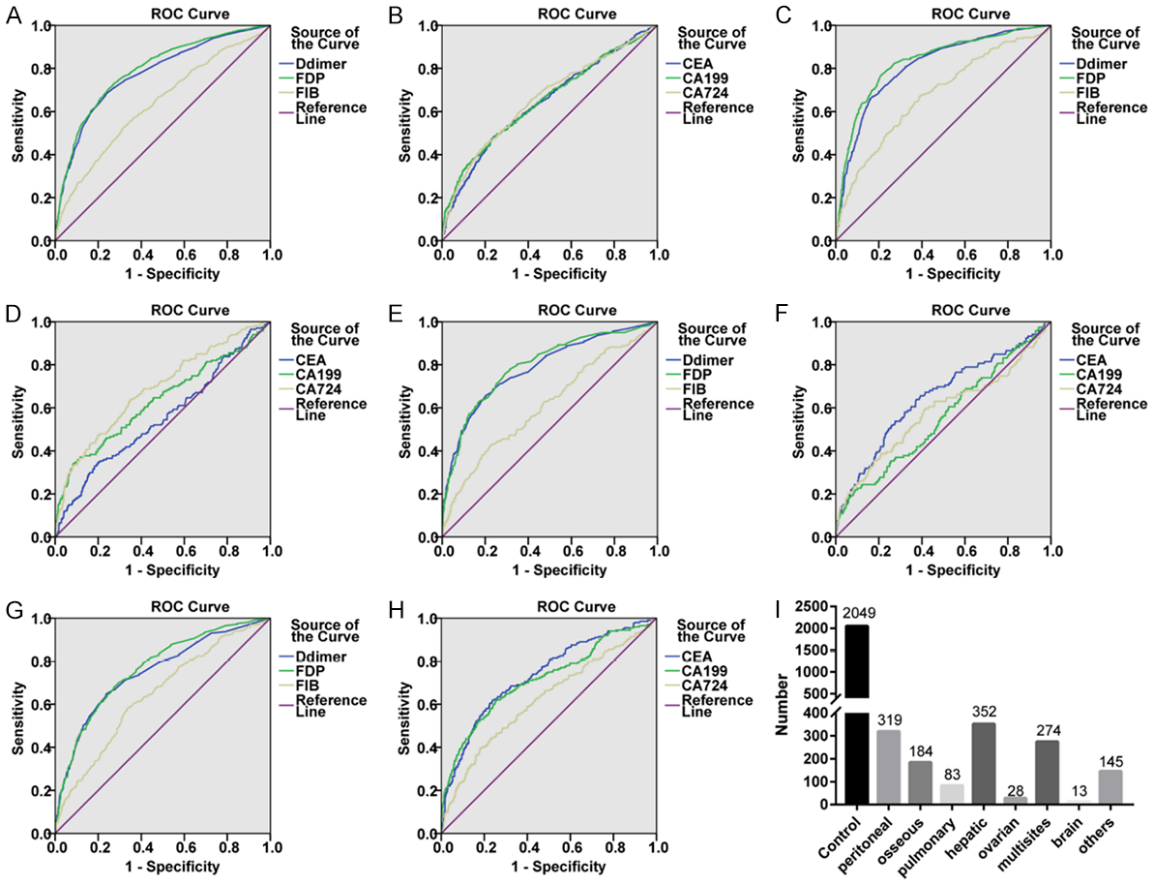

Figure 1. ROC analysis for the prediction of GC metastasis. AUC indicates the diagnostic power of FDP, D-dimer, FIB levels for all cases (A), peritoneal metastasis (C), osseous metastasis (E) and hepatic metastasis (G); AUC indicates the diagnostic power of CEA, CA19-9, CA72-4 for all cases (B), peritoneal metastasis (D), osseous metastasis (F) and hepatic metastasis (H). Number of cases of control group and different sites of metastasis (I).

for hepatic metastasis (n=352), even though FDP had higher AUC [0.766, 95% CI, (0.737-0.794)] than D-dimer [AUC (95% CI), 0.748 (0.717-0.779)] (P=0.412), CEA [AUC (95% CI), 0.735 (0.695-0.774)] (P=0.215) and CA19-9 [AUC (95% CI), 0.715 (0.672-0.758)] (P=0.055), no statistical significance was observed but not FIB (P<0.001) and CA72-4 (P<0.001) (Table S3; **Figure 1G**, **1H**). We did not analyze other site such as pulmonary metastasis (n=83) and ovarian metastasis (n=28) et al. on account of small sample size (**Figure 1I**). As GC mostly metastasize to peritoneal, hepatic and osseous, D-dimer and FDP seem to be novel screening markers for GC metastases in most cases.

In the high D-dimer group using the cutoff value of ROC curves, elder age was significant. Patients with higher FDP were older (**Table 3**). Multiple logistic regression analysis showed that younger age (OR 0.533, 95% CI 0.423-0.673, P<0.001), higher D-dimer (OR 2.756, 95% CI 2.038-3.727, P<0.001), higher FDP (OR 3.304, 95% CI 2.445-4.464, P<0.001), higher CEA (OR 1.815, 95% CI 1.428-2.308, P<0.001), higher CA199 (OR 1.709, 95% CI 1.349-2.164, P<0.001), higher CA724 (OR 1.877, 95% CI 1.472-2.392, P<0.001) and smoking (OR 1.713, 95% CI 1.189-2.469, P=0.004) were independent risk factors for GC metastasis (**Table 4**).

Discussion

In our present study, we have investigated the relationship between plasma D-dimer, FDP and GC metastasis. To reveal their predictive value more refined, we collected clinical data from multiple centers. Our current study indicated that plasma D-dimer and FDP levels may predict GC metastasis more effectively, including peritoneal, hepatic metastasis and osseous

Table 3. Patient characteristics according to D-dimer and FDP

	D-dimer		*P* value	FDP[1]		*P* value
	Low (N=1998)	High (N=1449)		Low (N=1773)	High (N=1486)	
Age, median	60 (53-67)	62 (54-69)	<0.001	60 (53-66)	62 (54-69)	<0.001
Smoking			0.092			0.077
no	1811	1288		1606	1318	
yes	187	161		167	168	
AI			0.737			0.458
no	1912	1390		1692	1426	
yes	86	59		81	60	
Hyperlipidemia			0.18			0.537
no	1965	1433		1749	1426	
yes	33	16		24	24	
Diabetes			0.211			0.358
no	1956	1409		1733	1445	
yes	42	40		40	41	

[1]188 cases censored. Values in parentheses are interquartile ranges.

Table 4. Univariate and multivariate analyses of variables for GC metastasis

	Univariate analysis			Multivariate analysis		
	Odds ratio	95% CI	*P* value	Odds ratio	95% CI	*P* value
Age, years						
<60 vs. ≥60	0.821	0.716-0.941	0.005	0.533	0.423-0.673	<0.001
D-dimer						
Low vs. high	7.85	6.73-9.156	<0.001	2.756	2.038-3.727	<0.001
FDP						
Low vs. high	7.525	6.419-8.821	<0.001	3.304	2.445-4.464	<0.001
CEA						
Low vs. high	3.076	2.614-3.619	<0.001	1.815	1.428-2.308	<0.001
CA199						
Low vs. high	3.095	2.622-3.653	<0.001	1.709	1.349-2.164	<0.001
CA724						
Low vs. high	3.211	2.62-3.935	<0.001	1.877	1.472-2.392	<0.001
Smoking						
No vs. yes	1.592	1.275-1.988	<0.001	1.713	1.189-2.469	0.004
AI						
No vs. yes	0.919	0.653-1.293	0.628			
Hyperlipidemia						
No vs. yes	0.643	0.349-1.185	0.157			
Diabetes						
No vs. yes	1.556	1.003-2.412	0.048	1.689	0.886-3.22	0.111

metastasis, than CEA, CA19-9 and CA72-4. Due to low cost, non-invasive and high diagnostic efficiency, FDP and D-dimer can be used as novel GC markers to assist clinicians in the assessment and diagnosis of GC metastasis.

As resistant to conventional therapy, metastasis contribute the major cause of death from cancer. However, most patients are firstly diagnosed with metastasis [15]. Approximately half of GC patients suffer from tumor recurrence or metastasis after curative resection [16]. Biomarkers of GC are generally divided into two categories, namely, classical (CEA, CA19-9, CA72-4, CA12-5, CA 50, Mg7Ag, Pepsinogens) and novel biomarkers [Calcium/calmodulin-

dependent protein kinase kinase 2 (CAMKK2), Human epidermal growth factor receptor 2 (HER2), Stem cells, P28GANK, microRNA and DNA hypomethylation] [17]. Tumor markers are not useful for early cancer but for detecting recurrence and distant metastasis, predicting patient survival. In fact, early GC is usually asymptomatic. At present, the commonly used indicators in clinical detection of early metastasis include CEA, CA19-9, CA72-4 and CA125 [18]. Monitoring the combinations of CEA, CA19-9, and CA72-4 before surgery or chemotherapy are the most effective ways for detection of recurrence or evaluation of the response [19]. It is worth noting that elevated D-dimer may indicate the presence of early tumors. Patients with unprovoked venous thromboembolism with D-dimer levels >4 mg/L have a higher risk of potential cancer. Additionally, 67% of these patients are diagnosed with metastatic cancer [20]. Most cancer patients have a tumor-specific D-dimer reference range. D-dimer ranges (median, 5th, 95th) of GC patients (mg/L) were 0.65, 0.22, 5.03. It was significantly higher than that of healthy controls (0.18, 0.07, 0.57) [21]. In our current study, the D-dimer levels (median, IQR) of metastatic group (1.7, 0.8-3.7 mg/L) was significantly higher than primary tumors group (0.5, 0.2-0.9 mg/L) (P<0.001). It's not hard to find that D-dimer levels in healthy people, patients with primary tumors and patients with metastatic tumors gradually increases step by step. That is to say, unexplained elevated D-dimer in healthy people may have underlying tumor diseases, and patients with primary GC with D-dimer higher than the tumor-specific reference range (0.5, 0.2-0.9 mg/L) may have occult tumor metastasis. Previous studies have shown that plasma D-dimer levels associate with distant metastasis of multiple tumors, including colorectal [12], breast, pancreatic and GC [22-24]. D-dimer levels were significantly elevated in patients with peritoneal dissemination compared with those without [25]. But few studies systematically analyze the performance of D-dimer for screening GC metastasis. Our result suggested that plasma FDP and D-dimer levels were much higher in patients with metastatic GC than those without. Other coagulation indicators such as FIB were also significantly elevated. Among coagulation indicators (PT, PTA, PTR, INR, APTT, TT, FIB, D-dimer, FDP) D-dimer and FDP were much more efficient for diagnosing GC metastasis (**Figure 1A**, **1B**). By comparing diagnostic efficacy of D-dimer, FDP, FIB and GC markers for GC metastasis using ROC analysis and AUC analysis, we were surprised to find that FDP had higher performance than D-dimer but not significantly, but dramatically higher diagnostic efficacy than FIB, CEA, CA19-9 and CA72-4 (**Table 2**). To be specific, the sensitivity and specificity of FDP (cut-point, 1.915) were 73% and 72.7%, D-dimer (cut-point, 0.905) were 68.2% and 76.3%, CEA (cut-point, 4.34) were 48.2% and 76.1%, CA19-9 (cut-point, 18.685) were 49.7% and 74.5%, CA72-4 (cut-point, 6.96) were 44.6% and 79.9%, respectively. ROC analysis combined with GC markers and coagulation indicators further illustrates their superior diagnostic efficacy.

Subgroup analysis was performed to explore the connection between FDP and tumor metastasis. we found that FDP was still the most efficient indicator for predicting peritoneal metastasis, hepatic metastasis or osseous metastasis of GC, followed by D-dimer. AUC of FDP was higher than D-dimer in all cases in our study but not significantly. FDP had remarkably superiority serving as clinical GC tumor markers in comparison with traditional ones (CEA, CA19-9, CA72-4) except for CEA (P=0.215) and CA19-9 (P=0.055) in the case of hepatic metastasis in which even though without statistical significance, AUC of FDP was much higher than D-dimer, CEA and CA19-9. As stated, the optimal cut-points of D-dimer and FDP were different from their reference values when serving as markers for coagulation disorders. Furthermore, few literatures illustrate the screening function of FDP for GC metastasis, let alone subgroup analysis according to metastatic sites. Our findings may provide reference for clinicians diagnosing metastatic GC.

In recent years, the causal relationship between tumor metastasis and hypercoagulability is not clear. Cancer cells can activate the hemostatic system through the expression of procoagulant proteins, the exposure of procoagulant lipids, the release of inflammatory cytokines and microparticles, and the adhesion with host vascular cells [26]. For example, tissue factor (TF) is a transmembrane glycoprotein and TF-factor VIIa complex is the main activator of blood coagulation. Tumors express TF and release

TF-positive extracellular vesicles (EVs) into the circulation to promote the activation of coagulation [27]. On the other hand, different components of the hemostatic system, including thrombin, TF and FVIIa, FXa, fibrinogen and vascular cells promote tumor angiogenesis and metastasis. Fibrin deposition can be used as a new blood vessel scaffold to promote angiogenesis, bind and isolate growth factors (bFGF, VEGF and insulin-like growth factor-1) to protect them from proteolytic degradation, stabilize tumor cell endothelial adhesion and promote metastasis. Tumor cells can activate platelets by releasing aggregation-promoting substances to form tumor cell-platelet thrombus, and support the formation of metastasis by preventing the interaction between tumor and innate immune cells [28]. Thrombin interacts with protease-activated receptors expressed by tumor cells and endothelial cells. PAR-1 cleaved by thrombin stimulates the release of growth factors, chemokines and extracellular proteins which promotes tumor cell proliferation and migration [29]. To sum up, tumor metastasis and coagulation activation are mutually promoting relations. It seems that monitoring coagulation indicators may indicate potential metastasis. Consistently, we found FIB, FDP, D-dimer were closely related to GC metastasis. Their diagnostic efficacy for metastasis is comparable to classic tumor markers.

Cancer patients are at increased risk of venous thromboembolism [30]. The incidence rate of first venous thromboembolism (VTE) in patients with active cancer was 5.8 (95% CI 5.7-6.0) per 100 person-years. The total recurrence rate was 9.6 per 100 person-years (95% CI 8.8-10.4), reaching a peak of 22.1 in the first 6 months [31]. The fact that increased D-dimer levels are associated with advanced age, after surgery, during pregnancy and the puerperium, with cancer and chronic inflammatory conditions, with acute thrombosis and with many other disorders makes it more sensitive and less specific [32]. As cardiovascular risk factors, diabetes, hyperlipidemia, or smoking can increase circulating tissue factor levels and blood clotting activity [33-35]. Increasing evidence suggests that inflammation leads to activation of coagulation and that coagulation also significantly affects inflammatory activity [36]. In this study, we excluded acute inflammation and analyzed aseptic inflammation. Even if the relative pathways are not completely clear, aseptic inflammation is caused by the rupture of cell plasma membrane liberating intracellular substances [37]. However, moderate local aseptic inflammation does not cause significant changes in plasma D-dimer [38]. Strictly speaking, tumors and diabetes are also aseptic inflammations. Considering their effect on the coagulation system, we analyzed them individually. Through multiple logistic regression analysis, we found that both D-dimer and FDP were independent risk factors for GC metastasis. Among the above factors interacting with the coagulation system, smoking and diabetes also increase the risk of GC metastasis.

Through AUC analysis, we got a series of optimal cut-off values and corresponding sensitivity and specificity. For any kind of GC metastasis (peritoneal, single organ, or multisite metastases), the cut-off values of D-dimer, FDP, FIB, CEA, CA19-9 and CA72-4 at the maximum of Youden index were 0.905, 1.915, 3.635, 4.34, 18.685 and 6.96, respectively. This inspired us that for screening GC metastasis the cut-points of D-dimer and FDP were different from that for indicating coagulation disorders. After excluding other factors that contribute to increased D-dimer and FDP levels, clinician should beware of GC metastasis when inspection results exceeding these values.

Data missing about other tumor markers like CA12-5, alpha-fetoprotein (AFP) etc. were limitations of our study. This is because the higher price prevents more GC markers from being routinely checked. All cases coming from three tertiary referral centers in northwest China and small simple size from SPPH and SPCH may influence the population uniformity and universality of our study. We did not collect enough cases for other site of GC metastases (brain, ovarian, pulmonary *etc.*). Further prospective studies will help verify our conclusions. Research about whether FDP has predictive value for metastasis in other kind of cancers would be meaningful.

In summary, plasma D-dimer and FDP levels have higher diagnostic efficacy than traditional GC biomarkers namely CEA, CA19-9, CA72-4. Classical GC tumor markers combined with coagulation indicators would be more effective for early detection of GC metastasis.

Disclosure of conflict of interest

None.

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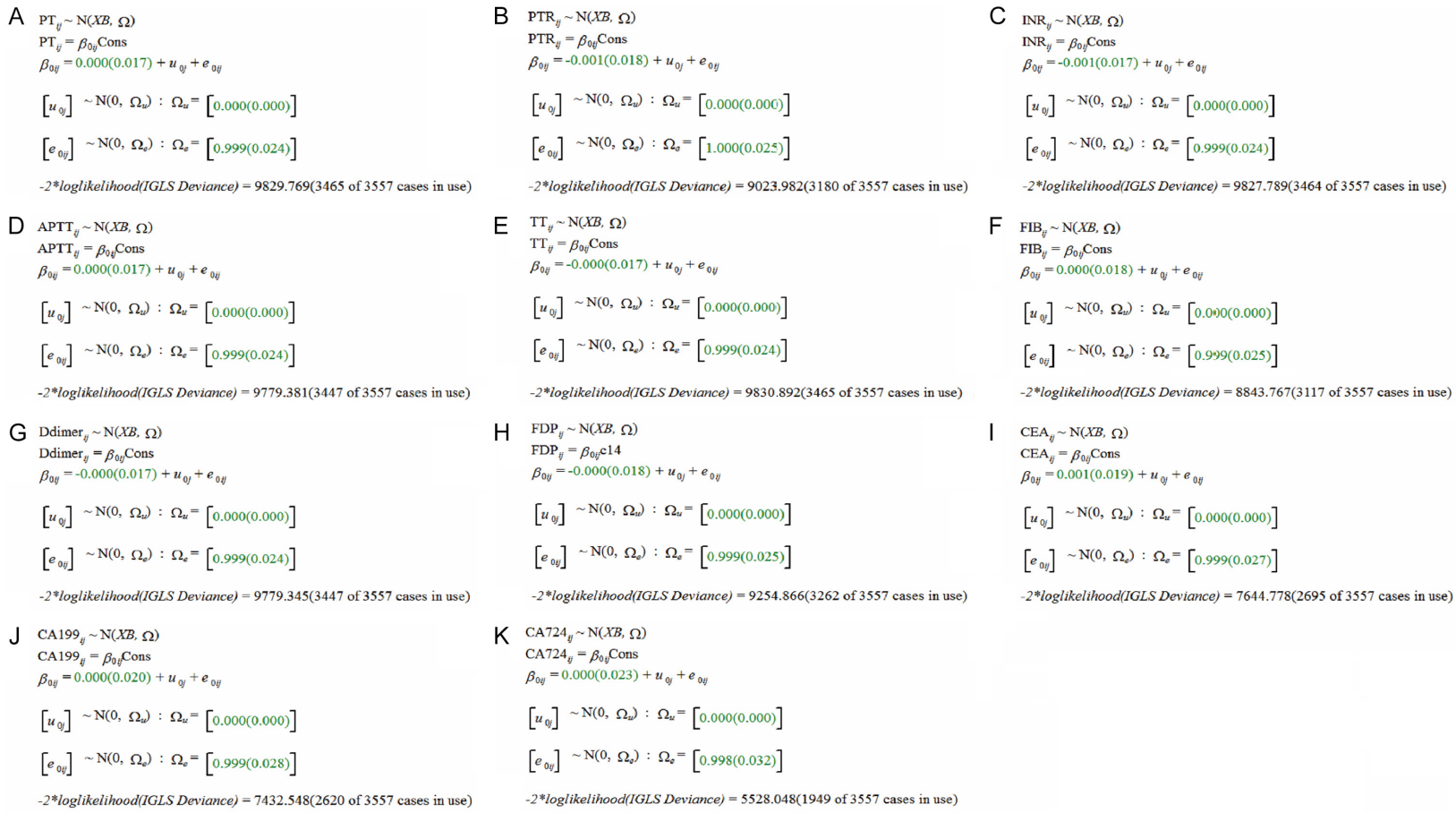

Figure S1. Two-level intercept-only model equations for indicators. (A) for PT, (B) for PTR, (C) for INR, (D) for APTT, (E) for TT, (F) for FIB, (G) for D-dimer, (H) for FDP, (I) for CEA, (J) for CA199 and (K) for CA724.

Table S1. AUC and the diagnostic indicators for peritoneal metastasis

	AUC	95% CI	P[a]	Cut-point	Sen	Spe	Youden index	P[b]
FDP	0.84	0.805-0.875	<0.001	2.35	0.768	0.79	0.558	Ref[c]
D-dimer	0.808	0.77-0.846	<0.001	1.225	0.659	0.84	0.499	0.289
FIB	0.685	0.639-0.732	<0.001	3.515	0.67	0.388	0.058	<0.001
CEA	0.55	0.499-0.601	0.024	5.085	0.351	0.798	0.149	<0.001
CA199	0.622	0.569-0.675	<0.001	48.84	0.374	0.886	0.26	<0.001
CA724	0.694	0.648-0.74	<0.001	3.41	0.643	0.647	0.29	<0.001

P[a], *P* value for ROC curve of corresponding indicator; Cut-point, cutoff value at the maximum of Youden index; Sen, sensitivity; Spe, specificity; P[b], *P* value for comparison of AUC of FDP with other indicators; Ref[c], Reference.

Table S2. AUC and the diagnostic indicators for osseous metastasis

	AUC	95% CI	P[a]	Cut-point	Sen	Spe	Youden index	P[b]
FDP	0.795	0.758-0.832	<0.001	1.875	0.758	0.708	0.466	Ref[c]
D-dimer	0.784	0.746-0.823	<0.001	1.035	0.657	0.798	0.455	0.69
FIB	0.612	0.566-0.659	<0.001	4.035	0.427	0.774	0.201	<0.001
CEA	0.649	0.593-0.705	<0.001	3.015	0.639	0.626	0.265	<0.001
CA199	0.556	0.499-0.613	0.043	66.595	0.218	0.91	0.128	<0.001
CA724	0.582	0.52-0.643	0.003	2.75	0.597	0.585	0.182	<0.001

P[a], *P* value for ROC curve of corresponding indicator; Cut-point, cutoff value at the maximum of Youden index; Sen, sensitivity; Spe, specificity; P[b], *P* value for comparison of AUC of FDP with other indicators; Ref[c], Reference.

Table S3. AUC and the diagnostic indicators for hepatic metastasis

	AUC	95% CI	P[a]	Cut-point	Sen	Spe	Youden index	P[b]
FDP	0.787	0.751-0.823	<0.001	1.915	0.679	0.727	0.406	Ref[c]
D-dimer	0.76	0.727-0.804	<0.001	0.905	0.648	0.763	0.411	0.412
FIB	0.655	0.612-0.698	<0.001	3.655	0.582	0.663	0.245	<0.001
CEA	0.732	0.691-0.772	<0.001	4.34	0.62	0.761	0.381	0.215
CA199	0.703	0.658-0.748	<0.001	18.685	0.62	0.745	0.365	0.055
CA724	0.623	0.576-0.698	<0.001	7.76	0.395	0.82	0.215	<0.001

P[a], *P* value for ROC curve of corresponding indicator; Cut-point, cutoff value at the maximum of Youden index; Sen, sensitivity; Spe, specificity; P[b], *P* value for comparison of AUC of FDP with other indicators; Ref[c], Reference.