Clinical application of serum CST4 combined with tumor markers in the diagnosis of digestive system malignant tumors

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Abstract. The aim of the present study was to evaluate the diagnostic value of plasma human cystatin-S (CST4) in patients with digestive system malignant tumors. CST4 and tumor markers, such as α-fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen (CA)199, CA125, CA153 and CA724, were detected in blood samples from 100 patients with a digestive system malignant tumor and 100 patients with benign digestive system diseases. The tumor markers AFP, CEA, CA199, CA125, CA153 and CA724 were detected using an electrochemiluminescence immunoassay, and CST4 levels were detected using a human CST4 ELISA kit. The results demonstrated that the sensitivities of AFP and CA153 (both 5.00%) were significantly lower than that of CST4 (38.00%) in the diagnosis of digestive system malignancy (P<0.001), and CA724 (18.00%) was also less sensitive than CST4 (P<0.05). The sensitivities of CA199 (26.00%), CEA (31.00%) and CA125 (25.00%) were similar to that of CST4 (P>0.05). There was no significant difference in the CEA, CA125, CA724 and CST4 specificities (P>0.05), which were 91.00, 95.00, 94.00 and 83.00%, respectively. The specificities of AFP (99.00%), CA199 (98.00%) and CA153 (100.00%) were significantly higher than that of CST4 (P<0.01). By constructing a receiver operating characteristic curve and comparing the area under the curve as well as sensitivity, the findings of the present study demonstrated that combining CST4 with AFP, CEA, CA199, CA125, CA153 and CA724 can significantly enhance the diagnostic sensitivity for malignancies of the digestive system. However, the introduction of CST4 into the traditional diagnostic groups (CEA + AFP, CA199 + CA125 + CA153 + CA724 and AFP + CEA + CA199 + CA125 + CA153 + CA724) resulted in an increased sensitivity and loss of specificity, thereby not offering significant advantages in terms of comprehensive diagnostic efficiency compared with the traditional diagnostic groups. In conclusion, CST4 detection may be a promising diagnostic tool. Nonetheless, the potential false positive results in tumor diagnosis should be taken into consideration when developing new diagnostic groups involving CST4.

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Introduction

According to the World Health Organization classification of tumors of the digestive system (fifth edition) published in 2019, malignant tumors of the digestive system include colorectal, gastric, esophageal, pancreatic and liver cancer, cholangiocarcinoma, lymphoma and mesenchymal tumors (1). The results of a survey of cancer cases and deaths in China in 2022 conducted by the National Cancer Center of the Chinese Academy of Medical Sciences and Peking Union Medical College indicated that lung, colorectal, stomach, liver and breast cancer were the top five causes of cancer-associated mortality among Chinese residents (2). The incidence and mortality rates of digestive system tumors remains high, with an estimated 4.8 million new cases and 3.4 million related deaths worldwide in 2019, accounting for 26% of the global cancer incidence and 35% of all cancer-related deaths. Therefore, digestive system tumors still pose a major challenge to global public health (3). Most malignant tumors of the digestive system still lack effective targeted drugs, and conventional radiotherapy and chemotherapy have limited therapeutic effects (4). Therefore, early detection and radical surgery remain the main routes to reduce distant metastases and disease mortality. At present, the mainstream diagnostic methods are abdominal high-resolution CT (5), abdominal B ultrasound (6), fibrogastroscopy and fibrocolonoscopy (7). Therefore, biomarkers with high sensitivity and specificity are required for early disease detection and intervention. In addition, serological tumor markers, a non-invasive diagnostic method, are widely applicable and relatively safe, and have an important role in the screening of malignant tumors of the digestive system (8).

Cysteine proteases can regulate physiological processes by controlling the hydrolysis of target proteins. The activity of cysteine proteases is strictly regulated at various levels, including genetic and epigenetic factors that control gene expression and protein biosynthesis, post-translational

modifications that affect protein transport and proenzyme activation (9). If the protease-inhibitor-substrate balance is disrupted, it may lead to changes in protease signaling and result in the occurrence and development of diseases, such as various inflammatory conditions, neurodegenerative and cardiovascular diseases, viral infections, atherosclerosis and osteoporosis (10). The development of cancer is a multi-step process, where different procedures are reflected by distinct genetic changes, which gradually transform normal cells into highly malignant cells. For example, mutations in the catalytic subunit of the PI3-kinase subtype within tumor cells can excessively activate the PI3-kinase signal transduction pathway, while carcinogenic mutations in Ras genes can impair Ras GTPase activity, thereby compromising the negative feedback mechanism of signal transduction. The mutation of different genes can further exacerbate the malignant proliferation of cells. These processes mainly include maintaining proliferation signals, escaping growth inhibitors, resisting cell apoptosis, inducing angiogenesis and migration and activating invasion and metastasis pathways (11). Cysteine proteases, as pro-invasive enzymes, interfere with cytokine/chemokine signaling, regulate cell adhesion, migration and endocytosis, participate in the antitumor immune response and cell apoptosis and promote cell invasion, angiogenesis and metastasis through their effects on extracellular matrix (12). Cysteine protease inhibitors are reversible or irreversible inhibitors that widely exist in living organisms, and can limit the excessive activity of cysteine proteases, which can indirectly reflect the equilibrium state between the two molecules and influence the proliferation of tumor cells in patients (13).

Cystatin is a protease inhibitor that is found in a number of human cells and tissues. There are 12 different types of cystatin, which are divided into types I, II and III based on the differences in their *in vivo* distribution (14). The distribution of type I is intracellular, while type II is extracellular, and type III primarily consists of intravascular inhibitors (14). Cystatin type II is composed of non-glycosylated proteins, including cystatin C, D, E/M, F, G, S, SN and SA (14). Cystatin-S (CST4), a member of the cystatin superfamily, is also known as cystatin SA-III. CST4 can specifically bind to cysteine protease to regulate its activity (15). It has been shown that CST4 enhances the invasiveness of gastric cancer and promotes its progression by regulating extracellular leucine rich repeat and fibronectin type III domain containing 2 signaling (16). Furthermore, it has been demonstrated that high CST4 expression in ovarian cancer is closely related to a poor prognosis (17). A recent study has also shown that both serum CST4 and DR-70 have diagnostic value in patients with early colorectal cancer, and the combined detection of CST4 and DR-70 is used to further improve the early diagnosis of this disease (18). However, the clinical value of CST4 combined with related tumor markers in the diagnosis of digestive system malignancies has not been elucidated in the current literature. In addition, Yang *et al* (19) found through cancer gene mapping that CST4 was highly expressed in esophageal squamous cell carcinoma, and survival analysis showed that patients with high CST4 expression had a poor overall survival. A previous study has also shown that CST4 can promote the occurrence of bone metastasis in combination with two plasminogen activators (the tissue-type and urokinase-type plasminogen activators) *in vivo* (20). In conclusion, CST4 still shows promise as a general tumor indicator, but clinical data from more tumor types are required.

α-Fetoprotein (AFP), an indicator used early in the detection of malignant digestive system tumors, is now mostly used in the early screening of liver cancer. Although the diagnostic efficacy of AFP alone is poor, AFP can have better accuracy when combined with other indicators (21). Carcinoembryonic antigen (CEA), an indicator used early in the screening of malignant gastrointestinal tumors, is not only widely used in the diagnosis and prognosis management of colorectal cancer (22,23), but it also has certain application prospects in the monitoring of postoperative recurrence of gastric cancer when combined with related serological indicators (24). Carbohydrate antigen (CA)199, CA125, CA153 and CA724 are tumor markers widely used in the clinical diagnosis of malignant digestive system tumors and have good applications in the early diagnosis and prognosis evaluation of colorectal (25), gastric (26) and pancreatic (27) cancer. Although the mainstream auxiliary diagnostic markers of digestive system malignancies, such as CEA, AFP and serum oncology markers, are still the commonly used indicators of digestive malignant disease, a novel type of oncology indicator with higher sensitivity and specificity is still required.

Therefore, the aim of the present study was to evaluate the serum CST4 level and its positivity rate in patients with malignant and benign digestive system diseases, to analyze the sensitivity and specificity of CST4 in the diagnosis of malignant digestive system tumors and examine whether there are differences in the diagnostic efficacy of CST4 compared with other tumor markers.

Materials and methods

Patients. A combined total of 200 in-patients and out-patients who visited the Department of Gastrointestinal Surgery, Gastroenterology and Oncology at the affiliated Chaohu Hospital of Anhui Medical University (ChaoHu, China) between June 2022 and March 2023 were included in the present retrospective study. These 200 patients included 100 patients with malignant digestive system tumors (the observation group) and 100 patients with benign diseases (the control group). In the observation group, 26 patients underwent chemotherapy, 2 patients received immunotherapy, 1 patient received targeted therapy, and 1 patient underwent radiation therapy. Additionally, a total of 32 patients underwent two or more combination therapies. Furthermore, it should be noted that adjuvant therapy was not administered to a total of 38 patients. The control group consisted of patients who were selected from the ward or outpatient department and all of them had digestive diseases, excluding individuals without any underlying health conditions. The inclusion criteria for patients in the observation group were as follows: i) Patients are 18 years of age or older; ii) patients have complete medical records at the hospital; and iii) patients with a clinical, imaging and pathological diagnosis of a digestive system malignant tumor. The exclusion criteria for the observation group were as follows: i) Patients are <18 years old or have incomplete medical records; ii) confirmation of non-digestive system malignant tumor through clinical diagnosis and pathology, with exclusion

of patients with metastasis of non-digestive system malignant tumors to the digestive system; and iii) confirmation of double primary malignancies through clinical diagnosis, imaging study and pathology, but inclusion if all sources of malignancy are from the digestive system and exclusion if the sources are different. The inclusion criteria for the control group were as follows: i) Patients are 18 years of age or older; ii) patients have complete medical records at the hospital; and iii) patients with a clinical, imaging and pathological diagnosis of a benign condition. The exclusion criteria for the control group were as follows: i) Patients are <18 years old or have incomplete medical records; and ii) patients with a history of malignancy. Ethics approval was granted by the Ethics Committee of The Affiliated Chaohu Hospital of Anhui Medical University (approval no. KYXM-202201-058; Chaohu, China).

Data collection. The relevant information was retrieved from the electronic database and medical record system of The Affiliated Chaohu Hospital of Anhui Medical University. The patient information collected includes: i) Records of admission or outpatient visits; ii) records of disease course; iii) records of laboratory examinations; iv) records of imaging examinations; v) reports of pathology findings; and vi) discharge summaries. The key variables included in the analysis encompassed age, sex, history of hypertension and diabetes, diagnosis, pathology, distant metastasis and levels of CST4, AFP, CEA, CA199, CA125, CA153 and CA724.

Detection method. The kits and analytical methods were utilized in accordance with the instructions provided by the manufacturer. CST4 levels were detected using a human CST4 ELISA kit (cat. no. 20173403280; Shanghai Liangrun Biomedical Technology Co., Ltd.). The cut-off value used to indicate positive cancer results was 101.0 U/ml. The tumor markers AFP, CEA, CA199, CA125, CA153 and CA724 were detected through electrochemiluminescence immunoassays using the Cobas® e801 analytical unit (Roche Diagnostics). AFP levels were detected using Elecsys AFP kit (cat. no. 07026706190); CEA levels were detected using Elecsys CEA kit (cat. no. 07027079190); CA199 levels were detected using Elecsys CA199 kit (cat. no. 07027028190); CA125 levels were detected using Elecsys CA125 II kit (cat. no. 07026986190); CA153 levels were detected using Elecsys CA125 II kit (cat. no. 07027001190); and CA724 levels were detected using Elecsys CA724 kit (cat. no. 07324910190) (all from Roche Diagnostics). The cut-off values used for AFP, CEA, CA199, CA125, CA153 and CA724 to indicate positive cancer results were 10.0 ng/ml, 5.0 ng/ml, 39.0 U/ml, 35.0 U/ml, 31.3 U/ml and 8.2 U/ml, respectively.

Statistical analysis. All data in the present study were analyzed using SPSS 29.0 (IBM Corp.), JMP 16.2.0 (SAS Institute, Inc.) and GraphPad Prism 8.0 (Dotmatics). Data normality was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Data conforming to a normal distribution are presented as the mean ± standard deviation. Data that did not follow a normal distribution are presented as the median and interquartile range (IQR). Given the presence of multiple groups of tumor indicators, their specific distributions are provided in Table SI. Mann-Whitney U test was employed to compare two groups of data. For multiple datasets, Kruskal-Wallis followed by the Steel-Dwass post hoc test was used. The McNemar test followed by Bonferroni correction was used to compare the rates of diagnostic methods. The McNemar test requires the addition of 0.5 to each cell count if both cells have a value of 0, ensuring compatibility with the SPSS 29.0 software package for accurate result output. The χ^2 test and Fisher's exact test were used to compare qualitative data. The comprehensive diagnostic efficiency was examined using the area under the receiver operating characteristic (ROC) curve, and the Delong test followed by Bonferroni correction was performed to compare the areas under the curve (AUCs). The probability value of combined diagnosis was fitted using binary logistic regression and then the ROC curves were plotted. After counting the number of true positives (TP), false positives (FP), true negatives (TN) and false negatives (FN) of each indicator, the following formula was used to calculate the relevant indicators: Sensitivity=TP/(TP + FN); Specificity=TN/(FP + TN); Positive predictive value=TP/(TP + FP); Negative predictive value=TN/(FN + TN); +LR=Sensitivity/(1-Specificity); -LR=(1-Sensitivity)/Specificity; Accuracy=(TP + TN)/(TP + FP + TN + FN). All statistical analyses were two-tailed, and $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patient clinical data. A total of 100 patients with digestive malignancies and 100 patients with benign diseases were included in the present study. Patients in the observation group were aged 43-86 years [median (IQR), 70.00 (60.00, 75.75) years] and included 71 men and 29 women. The observation group included 15 patients with esophageal malignancies, 40 patients with stomach malignancies, 12 patients with pancreatic malignancies, 16 patients with colon malignancies and 17 patients with rectal malignancies. Patients in the control group were aged 18-101 years (mean ± standard deviation, 63.00±14.61 years) and included 58 men and 42 women. The control group consisted of 45 patients with gastrointestinal polyps and 55 patients with non-gastrointestinal polyps, presenting with gastritis, gastric ulcer, constipation, diarrhea, colitis, abnormal appetite and other related conditions. The clinical data of these patients are presented in Table I. There was no significant difference in sex distribution between the observation and control groups ($P>0.05$), but there was a significant difference in the age of the two groups. The number of elderly patients (aged ≥60 years) in the observation group was higher than that in the control group ($P<0.01$), indicating that the risk of digestive malignancies increased with age. There was no significant difference in the number of patients with hypertension and diabetes between the observation and control groups ($P>0.05$), indicating that these conditions were not associated with digestive malignancies.

Comparison of the serum levels of CST4 and related tumor markers. To facilitate a more comprehensive evaluation of the serum distribution of seven indicators, the control group was further divided into two subgroups, one comprising patients with gastrointestinal polyps and the other consisting of patients with non-gastrointestinal polyps. The results demonstrated that

Table I. Clinical data of patients with digestive system malignant tumors and benign diseases.

Characteristic	Digestive malignant tumors, n (%)	Digestive benign diseases, n (%)	χ^2	P-value	OR (95% CI)
Sex					
Male	71 (71.00)	58 (58.00)	3.690	0.055	1.224 (0.994-1.508)
Female	29 (29.00)	42 (42.00)			
Age, years					
≥60	76 (76.00)	56 (56.00)	8.913	0.003	1.357 (1.105-1.667)
<60	24 (24.00)	44 (44.00)			
Hypertension					
Yes	26 (26.00)	28 (28.00)	0.101	0.750	0.929 (0.588-1.465)
No	74 (74.00)	72 (72.00)			
Diabetes					
Yes	11 (11.00)	11 (11.00)	0.000	1.000	1.000 (0.455-2.200)
No	89 (89.00)	89 (89.00)			

OR, odds ratio.

there were no significant differences in the serum levels of CST4, CEA, CA199, CA125, CA153 and CA724 between patients with gastrointestinal polyps and patients with non-gastrointestinal polyps (P>0.05; Fig. 1A and C-G). The levels of AFP were significantly different between patients with gastrointestinal polyps and patients with non-gastrointestinal polyps (P<0.05; Fig. 1B). The serological levels of CST4, CEA, AFP, CA199, CA125, CA153 and CA724 exhibited a statistically significant increase in patients with digestive system malignancies compared to those with non-gastrointestinal polyps (P<0.05; Fig. 1A-G). The serological levels of CST4, CEA, CA199 and CA125 exhibited a statistically significant increase in patients with digestive system malignancies compared to those with gastrointestinal polyps (P<0.05; Fig. 1A and C-E).

Application of CST4 and related tumor markers in the clinical diagnosis of malignant digestive tumors. Through the analyses of relevant data, it was found that the positive rates of CST4, CEA, CA199, CA125 and CA724 in the observation group were significantly higher than those in the control group (P<0.01), while the positive rates of AFP and CA153 were not significantly different between the two groups (P>0.05) (Table II). The ROC curves were evaluated for the aforementioned tumor markers (including CST4, CEA, AFP, CA199, CA125, CA153 and CA724) to examine the diagnostic value of these markers in cancer (Fig. 2). The results demonstrated that the AUC of CST4 was 0.6725, which was followed by CEA (AUC, 0.6638). Since the difference among the AUCs of each index was small, paired AUC comparisons were made to examine whether there were any differences. The results indicated that there were no significant differences in the paired comparisons between the AUC values of CST4, AFP, CEA, CA199, CA125, CA153 and CA724 (P>0.05; Table III).

Application of CST4 combined with related tumor markers in the clinical diagnosis of malignant digestive system tumors. In clinical practice, to improve the identification of patients with gastrointestinal tumors at an early stage, multiple tumor indicators are often combined in parallel for diagnosis (28,29). Before the introduction of CST4 as a routine diagnostic indicator at The Affiliated Chaohu Hospital of Anhui Medical University, there were three diagnostic groups (traditional groups) available for the diagnosis of malignant tumors of the digestive system: i) Group A, CEA + AFP; ii) Group B, CA199 + CA125 + CA153 + CA724; and iii) Group C, AFP + CEA + CA199 + CA125 + CA153 + CA724. After incorporating CST4 as a routine diagnostic indicator, it was introduced into the traditional groups with the aim to improve the diagnostic effect. The new groups involving CST4 are as follows: i) group D, CST4 + CEA + AFP; ii) group E, CST4 + CA199 + CA125 + CA153 + CA724; and iii) group F, CST4 + AFP + CEA + CA199 + CA125 + CA153 + CA724. The results of the data analysis demonstrated that the positive rate of these six groups in the observation group was significantly higher than that in the control group (P<0.001; Table IV). The ROC curves were evaluated for the aforementioned six diagnostic groups to examine their diagnostic value for cancer (Fig. 3). The results demonstrated that group F had the highest AUC (0.7776), which was followed by group C (AUC, 0.7730). Since the difference among the AUCs of each index was small, paired AUC comparisons were made to examine whether there were any differences. There were no significant differences in the paired comparisons between the AUC values of groups A, B, C, D, E and F (P>0.05; Table V).

Diagnostic efficacy of related tumor markers in the diagnosis of digestive system malignancies. In a single-index diagnosis, CST4 (38.00%) had the highest sensitivity, which was followed by CEA (31.00%); however, CA153 (100.00%) had the highest specificity, which was followed by AFP (99.00%). In addition, CEA (64.50%) had the highest accuracy in a single-index diagnosis, which was followed by CA199 (62.00%). Compared with the traditional groups of tumor markers (including AFP,

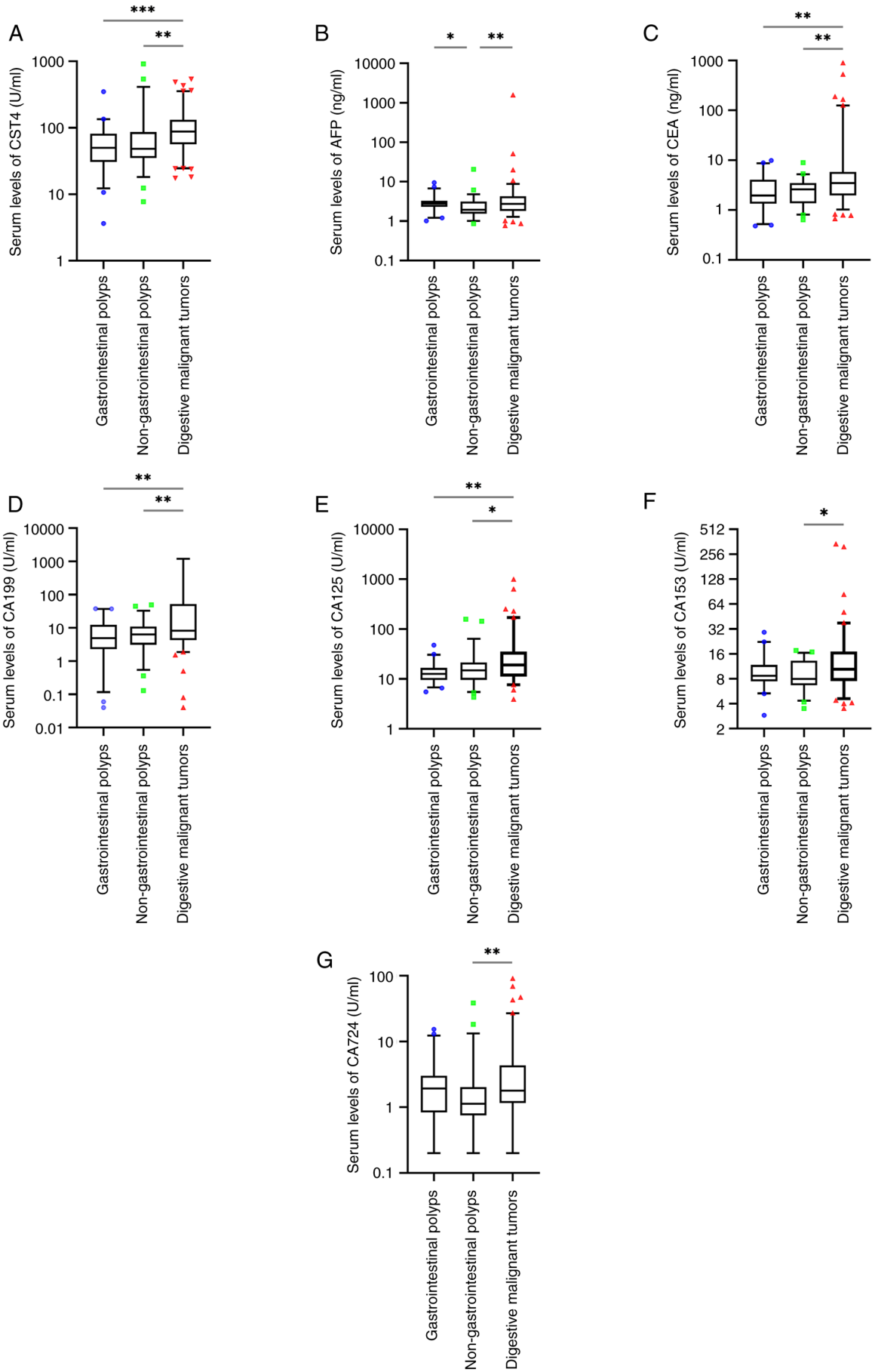

Figure 1. Serum levels of human (A) CST4, (B) AFP, (C) CEA, (D) CA199, (E) CA125, (F) CA153 and (G) CA724 in patients with digestive malignant tumors, gastrointestinal polyps and non-gastrointestinal polyps. *P<0.05; **P<0.01; ***P<0.001. CST4, cystatin-S; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

Table II. Comparison of related tumor markers in the diagnosis of digestive malignant tumors.

Biomarker	Group		χ^2	P-value
	Digestive malignant tumors, n (%)	Digestive benign diseases, n (%)		
CST4				
Positive	38 (38.00)	17 (17.00)	11.060	0.001
Negative	62 (62.00)	83 (83.00)		
AFP				
Positive	5 (5.00)	1 (1.00)	N/A	0.212
Negative	95 (95.00)	99 (99.00)		
CEA				
Positive	31 (31.00)	2 (2.00)	30.521	<0.001
Negative	69 (69.00)	98 (98.00)		
CA199				
Positive	26 (26.00)	2 (2.00)	23.920	<0.001
Negative	74 (74.00)	98 (98.00)		
CA125				
Positive	25 (25.00)	5 (5.00)	15.686	<0.001
Negative	75 (75.00)	95 (95.00)		
CA153				
Positive	5 (5.00)	0 (0.00)	N/A	0.059
Negative	95 (95.00)	100 (100.0)		
CA724				
Positive	18 (18.00)	6 (6.00)	6.818	0.009
Negative	82 (82.00)	94 (94.00)		

CST4, cystatin-S; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen. N/A, not applicable.

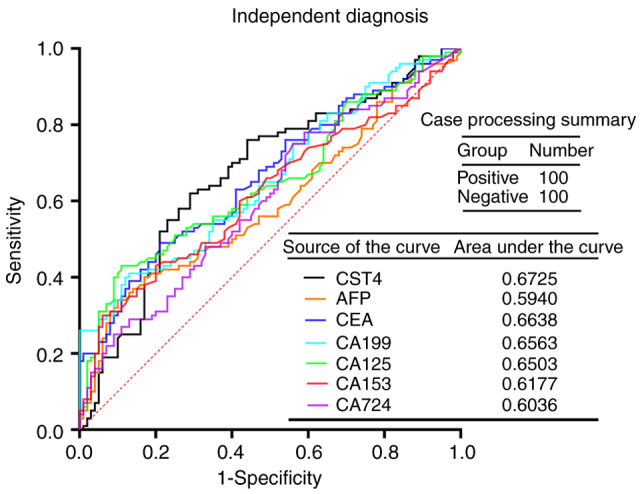

Figure 2. Receiver operating characteristic curves of CST4, AFP, CEA, CA199, CA125, CA153 and CA724 in distinguishing digestive malignant tumors from benign diseases. CST4, cystatin-S; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

CEA, CA199, CA125, CA153 and CA724), the introduction of the CST4 index improved the sensitivity (Table VI). The sensitivity of Group D increased by 24% compared to Group A, while Group E experienced a 13% increase in sensitivity compared to Group B Additionally, Group F observed a 9% increase in sensitivity when compared to Group C (Table VI).

Comparison of the sensitivity and specificity of CST4 and related tumor markers in digestive system cancer diagnosis. The sensitivity of AFP and CA153 in the observational group was significantly lower compared with that of CST4 (P<0.001; Fig. 4A), and the sensitivity of CA724 was also significantly lower compared with that of CST4 (P<0.05; Fig. 4A). The sensitivity of group C (including AFP, CEA, CA199, CA125, CA153 and CA724) was significantly higher than that of CST4 alone (P<0.01; Fig. 4A). In addition, the sensitivity of CST4 combined with the other related tumor markers was significantly higher than that of CST4 alone (P<0.001; Fig. 4A). The specificity of AFP, CA199 and CA153 was significantly higher compared with that of CST4 (P<0.01; Fig. 4B), and there was no significant difference between the specificity of CST4, CA125 and CA724 in the observation group (P>0.05; Fig. 4B). The specificity of CST4 combined with the other tumor markers was significantly lower than that of CST4 alone (P<0.05; Fig. 4B). In order to evaluate the sensitivity and specificity of diagnosis after the introduction of CST4 in the traditional groups, the sensitivity and specificity of group A vs. group D, group B vs. group E and group C vs. group F were compared, as shown in Fig. 4C and D. It was found that the sensitivity of the traditional marker groups was significantly

Table III. Comparison of AUCs of relevant indicators.

Groups compared	Z-value	P-value	AUC variance
CST4-AFP	1.390	1.000	0.079
CST4-CEA	0.161	1.000	0.009
CST4-CA199	0.332	1.000	0.016
CST4-CA125	0.451	1.000	0.022
CST4-CA153	1.032	1.000	0.055
CST4-CA724	1.229	1.000	0.069
AFP-CEA	-1.298	1.000	-0.070
AFP-CA199	-1.118	1.000	-0.062
AFP-CA125	-1.002	1.000	-0.056
AFP-CA153	-0.436	1.000	-0.024
AFP-CA724	-0.178	1.000	-0.010
CEA-CA199	0.160	1.000	0.008
CEA-CA125	0.260	1.000	0.014
CEA-CA153	0.913	1.000	0.046
CEA-CA724	1.161	1.000	0.060
CA199-CA125	0.119	1.000	0.006
CA199-CA153	0.789	1.000	0.039
CA199-CA724	0.956	1.000	0.053
CA125-CA153	0.635	1.000	0.033
CA125-CA724	0.816	1.000	0.047
CA153-CA724	0.249	1.000	0.014

CST4, cystatin-S; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen; AUC, area under the curve.

improved by the addition of CST4 (P<0.05; Fig. 4C); however, the specificity was significantly decreased (P<0.001; Fig. 4D). Detailed information on group distributions and comparisons is presented in Tables SII-SIX.

Association between CST4 and the clinical features of patients with malignant digestive tumors and benign diseases. The association between the serum CST4 level and the relevant clinical features of the observation and control groups was analyzed, to further explore the role of CST4 in malignant digestive tumors (Table VII). In patients with malignant digestive tumors and benign diseases, the positive rate of CST4 was independent of sex, age, diabetes and hypertension (P>0.05).

Association between clinical features and distant metastasis of malignant digestive tumors. In the observation group, 40 out of 100 patients developed distant metastases. As shown in Table VIII, distant tumor metastasis in the observation group was not associated with sex, age, hypertension or diabetes (P>0.05).

Association between the serum levels of tumor markers and distant metastasis of malignant digestive tumors. In the present study, the serum levels of AFP, CA153 and CA724 were not significantly different in the 40 patients with distant metastasis compared with the 60 patients without metastasis (P>0.05; Fig. 5B, F and G); however, the serum levels of CST4, CEA, CA199 and CA125 were significantly higher in patients with distant metastasis compared with those without metastasis (P<0.05; Fig. 5A and C-E). The positive rates of CST4, AFP, CA153 and CA724 in patients with distant metastasis were not significantly different from those in patients without distant metastasis (P>0.05; Table IX); however, the positive rates of CEA, CA199 and CA125 were significantly higher in patient with distant metastasis compared with those in patients without distant metastasis (P<0.01; Table IX).

Association between the serum levels of tumor markers and tumor types. The results demonstrated significant differences in the serologic levels of CST4, CA199 and CA153 between pancreatic cancer and gastrointestinal or non-gastrointestinal polyps (P<0.05; Fig. 6A, D and F). The serologic level of CA199 also exhibited a significant difference between pancreatic cancer and esophageal, stomach and rectal cancer (P<0.05; Fig. 6D). The serologic level of CA153 also exhibited a significant difference between pancreatic cancer and esophageal and stomach cancer (P<0.05; Fig. 6F). Significant differences were observed in the serologic levels of CA125 between patients with esophageal cancer and those with gastrointestinal polyps (P<0.05; Fig. 6E). There was a significant difference in serum CA724 levels between patients with esophageal cancer or stomach cancer, and those with non-gastrointestinal polyps (P<0.05; Fig. 6G).

Discussion

Due to the crucial role of cysteine protease in tumor regulation (10), cysteine protease inhibitors can serve as a reliable indicator of tumor progression. CST4, functioning as a potent cysteine protease inhibitor, holds great promise as a novel diagnostic marker for digestive system malignancies. By utilizing retrospective data analysis, the present study compared CST4 with other tumor markers to objectively evaluate its diagnostic efficacy and distribution among different patient populations. In the present study, the serum levels of CST4, CEA, AFP, CA199, CA125, CA153 and CA724 exhibited significant differences between patients without gastrointestinal polyps and those with gastrointestinal malignancies. Moreover, there were also significant differences in the levels of CST4, CEA, CA199 and CA125 between patients with gastrointestinal polyps and those diagnosed with gastrointestinal malignancies. The current findings are consistent with the results of previous studies (15,18), suggesting an important role of CST4 in the screening of gastrointestinal malignancies, as well as the ability to differentiate between gastrointestinal polyps and malignant tumors. In the present study, the serum levels of AFP and CA724 in patients without gastrointestinal polyps were significantly different from those in patients with digestive system malignancies, but there were no significant differences in these two markers between patients with digestive system malignancies and gastrointestinal polyps. AFP is widely used in the screening and diagnosis of hepatocellular malignancies (30), but its application in gastrointestinal polyps has been little studied; therefore, the reason for the aforementioned result is unclear. A previous study has shown that the expression level of CA199 in colon polyps is higher than that in normal colon mucosa (31), and another study has demonstrated that CA199 is closely related to the recurrence of colorectal polyps (32).

Table IV. Comparison of combined diagnostic groups in the diagnosis of malignant tumors of the digestive system.

Diagnostic group	Group		χ^2	P-value
	Digestive malignant tumors, n (%)	Digestive malignant tumors, n (%)		
A				
Positive	32 (32.00)	11 (11.00)	13.065	<0.001
Negative	68 (68.00)	89 (89.00)		
B				
Positive	50 (50.00)	13 (13.00)	31.723	<0.001
Negative	50 (95.00)	87 (99.00)		
C				
Positive	62 (62.00)	23 (23.00)	31.120	<0.001
Negative	38 (38.00)	77 (77.00)		
D				
Positive	56 (56.00)	27 (27.00)	17.321	<0.001
Negative	44 (44.00)	73 (73.00)		
E				
Positive	63 (63.00)	26 (26.00)	27.715	<0.001
Negative	37 (37.00)	74 (74.00)		
F				
Positive	71 (71.00)	35 (35.00)	26.014	<0.001
Negative	29 (29.00)	65 (65.00)		

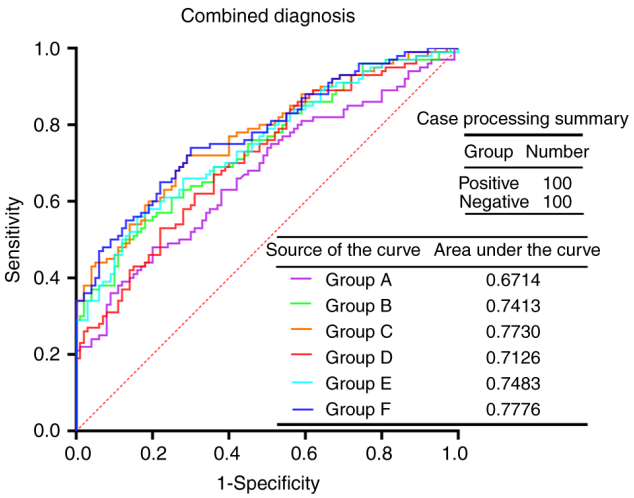

Figure 3. Receiver operating characteristic curves of six groups of combined indicators in distinguishing digestive malignant tumors from benign diseases.

Table V. Comparison of AUCs for relevant joint indicators.

Groups compared	Z-value	P-value	AUC variance
Group A-Group B	-1.634	1.000	-0.070
Group A-Group C	-2.716	0.099	-0.102
Group A-Group D	-1.013	1.000	-0.041
Group A-Group E	-1.723	1.000	-0.077
Group A-Group F	-2.778	0.082	-0.106
Group B-Group C	-1.402	1.000	-0.032
Group B-Group D	0.733	1.000	0.029
Group B-Group D	-0.312	1.000	-0.007
Group B-Group F	-1.401	1.000	-0.036
Group C-Group D	1.900	0.862	0.060
Group C-Group D	1.094	1.000	0.025
Group C-Group F	-0.228	1.000	-0.005
Group D-Group D	-0.904	1.000	-0.036
Group D-Group F	-2.250	0.366	-0.065
Group D-Group F	-1.160	1.000	-0.029

AUC, area under the curve.

The positive rates of CST4, CEA, CA199, CA125 and CA724 in patients with malignant digestive tumors were significantly higher than those in patients with benign diseases, while the positive rates of AFP and CA153 were not significantly different between the two groups. AFP is mainly used in the diagnosis of hepatocellular and pancreatic malignancies. However, the patients selected in the present study did not have hepatocellular malignancies, and the proportion of patients with pancreatic cancer was relatively low, which may be the reason for the low AFP positive rate. CA153 has been widely used in the diagnosis and evaluation of breast cancer and has also been applied in the diagnosis of ovarian, pancreatic, gastric and lung cancer (33). However, malignant tumors are not the only cause of increases in serum CA153; benign diseases, such as chronic active hepatitis, cirrhosis, sarcoidosis and megaloblastic anemia may also lead to changes in the CA153 level. Therefore, the false negative

Table VI. Evaluation of the diagnostic efficacy of related tumor markers in digestive malignant tumors.

Tumor marker	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	LR+	LR-
CST4	38.00	83.00	69.09	57.24	60.50	2.24	0.75
AFP	5.00	99.00	83.33	51.03	52.00	5.00	0.96
CEA	31.00	91.00	93.94	58.68	64.50	15.5	0.70
CA199	26.00	98.00	92.86	56.98	62.00	13.00	0.76
CA125	25.00	95.00	83.33	55.88	60.00	5.00	0.79
CA153	5.00	100.00	100.00	51.28	52.50	N/A	0.95
CA724	18.00	94.00	75.00	53.41	56.00	3.00	0.87
Group A	32.00	89.00	74.42	56.69	61.46	2.91	0.76
Group B	50.00	87.00	79.37	63.50	68.50	3.85	0.57
Group C	62.00	65.00	63.91	63.11	63.50	1.77	0.58
Group D	56.00	73.00	67.47	62.39	64.50	2.07	0.60
Group E	63.00	74.00	70.79	66.67	68.50	2.42	0.50
Group F	71.00	65.00	66.98	69.15	68.00	2.03	0.45

CST4, cystatin-S; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen; LR, likelihood ratio; +, positive; -, negative; PPV, positive predictive value; NPV, negative predictive value; N/A, not applicable.

and false positive rates of CA153 as a biomarker can both be high, which may be the reason for the low CA153 positive rate in the current study (33). Although in the present study the positive rate of CA724 was significantly different between the two patient groups, it has been reported that the CA724 level also increases in individuals with gout arthritis and benign diseases, and may also be affected by drugs (34). CEA is mainly used as a diagnostic indicator for colorectal cancer, but it is also elevated in other cancer types and diseases, such as pulmonary fibrosis and Alzheimer's disease (35). CA199 is widely distributed in normal human tissues and organs, and its distribution is closely related to genotype. CA199 biosynthesis depends on the enzymatic activity of fucosyltransferase-2 (FUT2) and fucosyltransferase-3 (FUT3) (36). The activity of both enzymes is determined by the FUT2 and FUT3 genotype. The presence of mutations in genotypes results in alterations in the activity of FUT2 and FUT3. The T59G mutation of the FUT3 gene has been shown to significantly affect patient serum CA199 levels (37). In addition to malignant tumors, other diseases, such as pancreatitis, hepatitis, cirrhosis and pulmonary fibrosis, also result in an increase or decrease in the CA199 serum level (38,39). In addition to being a traditional tumor marker, CA125 has also been used as an evaluation indicator of heart failure (40,41).

In the present study, the sensitivity of CST4 alone was the highest (38.00%), followed by CEA (31.00%), CA199 (26.00%) and CA125 (25.00%). The sensitivity of CST4 in patients with a digestive system malignancy was not significantly different from that of CEA, CA199 and CA125, but it was significantly higher than that of AFP, CA153 and CA724. The specificity of CA153 and AFP was the highest in patients with benign diseases of the digestive system (100.00 and 99.00%, respectively). However, when the ROC curve was constructed to comprehensively evaluate the diagnostic efficiency of the aforementioned indicators, it was found that there were no notable differences in the comprehensive performance of each diagnostic indicator. In addition, although the AUC values were different, statistically significant differences between were not observed. There are few published reference values for CST4, but the sensitivity of CST4 in detecting digestive system tumors in the present study was lower than that noted in the studies by Dou *et al* (15) and Cai *et al* (18) in patients with colorectal or gastric cancer, with no marked difference in specificity. In the study of Dou *et al* the ELISA detection system for CST4 showed significantly better sensitivities of 69.0 and 69.0%, and specificities of 85.6 and 83.6%, for gastric cancer and colorectal cancer, respectively. Additionally, the study conducted by Cai *et al* demonstrated that the AUC of serum CST4 in patients with early colorectal cancer was 0.927, exhibiting a sensitivity of 57.8% and a specificity of 95.3%. When compared with cystatin-SN (CST1), the specificity and sensitivity of CST4 was similar (33). In the study of Wang *et al* (42),the diagnostic sensitivity of CST1 for early esophageal squamous cell carcinoma was 31.25% (specificity 92.64%, AUC 0.654). When considering that the cases in the studies by Dou *et al* (15) and Cai *et al* (18) were mainly patients with colorectal and gastric malignancies, while other malignant tumors of the digestive system in addition to colorectal malignancies were included in the present study, the positive rate of detection may be decreased. In addition, we hypothesize that, as some patients included in the present study had undergone associated surgical and chemoradiotherapy treatment at the time of examination and the growth of tumor cells in the body was inhibited, this resulted in reduced cysteine protease secretion and thus indirectly reduced CST4 secretion.

Since multiple indicators are often combined in the diagnosis of digestive system tumors, six diagnostic groups were established in the present study (43,44). Among them, diagnostic group A is the traditional group used in previous large-scale screening, diagnostic group B is the glycogen marker group that has been commonly used in the past (45,46), diagnostic group C includes the six markers used in digestive

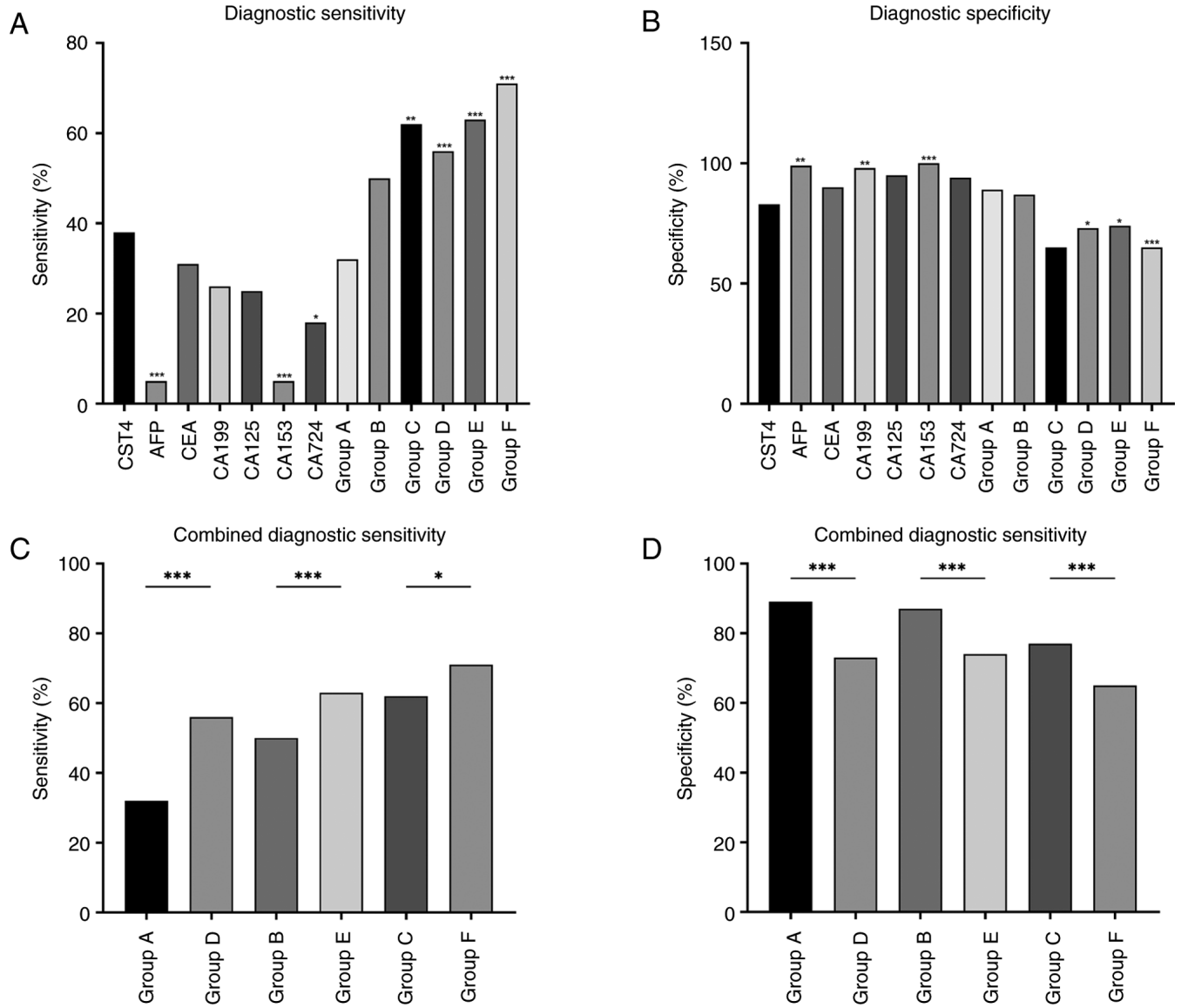

Figure 4. Comparison of the (A) sensitivity and (B) specificity of CST4 and related tumor markers in digestive system diagnosis. Comparison of the (C) sensitivity and (D) specificity between traditional groups and improved groups. *P<0.05, **P<0.01 and ***P<0.001 vs. CST4 or as indicated. CST4, cystatin-S; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

system tumor screening at The Affiliated Chaohu Hospital of Anhui Medical University, and the diagnostic groups D, E and F correspond to groups A, B and C combined with CST4, respectively. In the sensitivity and specificity analyses of the diagnostic groups, it was found that the sensitivity of the diagnostic groups was significantly increased following the inclusion of CST4, but the specificity was significantly decreased. Therefore, the introduction of CST4 may help to screen positive patients, but caution should be taken regarding the false positive results that may be produced. When evaluating the diagnostic efficacy of group E, it was observed that the inclusion of CST4 resulted in a sensitivity of 63.00%, specificity of 74%, accuracy of 68.50%, and LR+ of 2.42. In comparison to group B, there was a significant increase in sensitivity by 13%; however, specificity decreased by 13%. The accuracy remained unchanged while the LR+ decreased by 1.43. In addition, CST4 combined with group C (group F) had a sensitivity of 71.00%, an accuracy of 68.00% and an LR+ of 2.03, but the specificity was 18% lower compared with than of CST4 alone. ROC curves were constructed to analyze the diagnostic efficiency of the aforementioned groups, and the results demonstrated that there were no notable differences in the comprehensive performance between the diagnostic groups. Therefore, there was little difference in the diagnostic efficiency among the diagnostic groups, and the addition of CST4 exhibited no notable advantages.

The association between CST4 expression and the clinical characteristics of malignant digestive tumors and benign diseases were also evaluated in the present study. The results demonstrated that CST4 was not associated with age, sex, hypertension or diabetes. Since a considerable number of patients with malignant digestive tumors in the present study had distant metastases, the clinical features, serum levels and positive rates of tumor markers in these patients were further examined. The results demonstrated that distant metastasis was not associated with sex, age, hypertension or diabetes

Table VII. Association between the positive rate of cystatin-S and clinical data characteristics of patients with digestive malignant tumors and benign diseases.

	Digestive malignant tumors					Digestive benign diseases				
Characteristic	n	Positive	Negative	χ^2	P-value	n	Positive	Negative	χ^2	P-value
Sex										
Male	71	27	44	<0.001	0.993	58	12	46	1.332	0.248
Female	29	11	18			42	5	37		
Age, years										
<60	24	9	15	0.003	0.954	44	5	39	1.769	0.184
≥60	76	29	47			56	12	44		
Hypertension										
Yes	26	10	16	0.003	0.955	28	3	25	-	0.383
No	74	28	46			72	14	58		
Diabetes										
Yes	11	5	6	-	0.744	11	2	9	-	1.000
No	89	33	56			89	15	74		

Table VIII. Association between distant metastasis and clinical data characteristics of patients.

Characteristic	n	Patients with metastasis	Patients without metastasis	χ^2	P-value
Sex					
Male	71	26	45	1.166	0.280
Female	29	14	15		
Age, years					
<60	24	11	13	0.448	0.503
≥60	76	29	47		
Hypertension					
Yes	26	7	19	2.503	0.114
No	74	33	41		
Diabetes					
Yes	11	3	8	-	0.518
No	89	37	52		

in the observation group. The serum levels of CST4, CEA, CA199 and CA125 in the patients with distant metastasis were significantly higher than those in the patients without distant metastasis, while the serum levels of AFP, CA153 and CA724 were not significantly different. Subsequent investigations demonstrated that the positive rates of CEA, CA199 and CA125 in the patients with distant metastasis were significantly higher than that in the patients without distant metastasis, while there was no difference in the positive rates of CST4, AFP, CA153 and CA724 between the two groups. As most patients with distant metastasis are in the advanced stage of the disease and have low nutritional status and protein synthesis (47), this may lead to a decrease in CST4 secretion and thus a decrease of the positive detection rate.

The serum levels of various tumor markers in different tumor types were also analyzed in the present study, and it was found that there were no significant differences in the serum levels of CST4, AFP, CEA, CA125 and CA724 among the different types of digestive tract tumors assessed. The serum levels of CA199 were significantly higher in pancreatic cancer compared with esophageal, stomach and rectal cancer, and the serum levels of CA153 in pancreatic cancer were significantly higher than those in esophageal and stomach cancer.

The presence of advanced-stage disease, along with evident spread and metastasis at the time of diagnosis in some patients within the observation group, may contribute to a certain degree of elevation in the CST4 index among individuals with malignant tumors of the digestive system compared with the gastrointestinal and non-gastrointestinal polyps groups. However, it cannot be ruled out that malignant pancreatic tumor cells themselves may promote the upregulation of CST4. The high expression of CA199 and CA153 in pancreatic

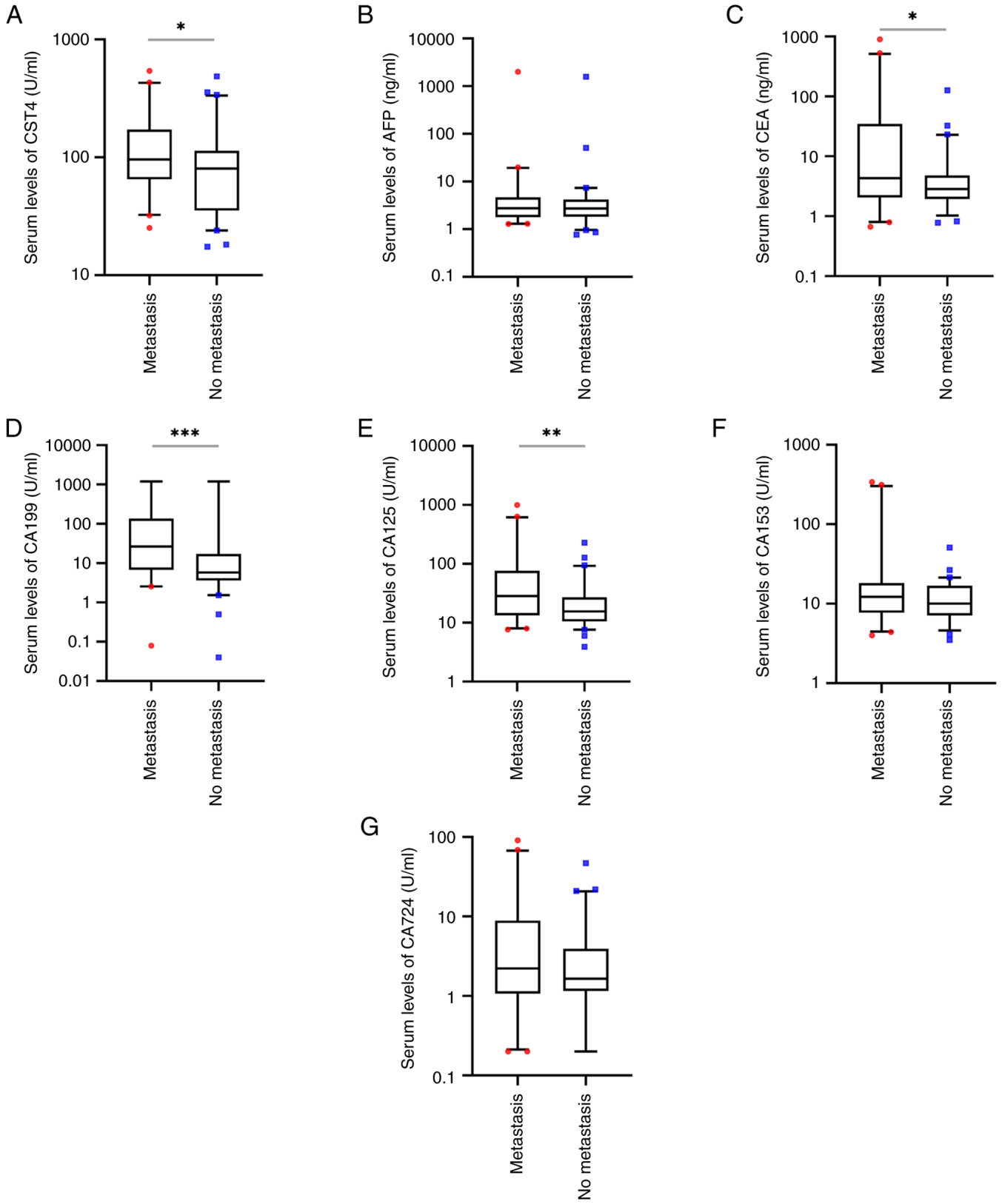

Figure 5. Serum levels of human (A) CST4, (B) AFP, (C) CEA, (D) CA199, (E) CA125, (F) CA153 and (G) CA724 in patients with and without distant metastasis. *P<0.05; **P<0.01; ***P<0.001. CST4, cystatin-S; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

cancer observed in the present study was also consistent with the results of previous studies (48,49). The results of the present study also demonstrated that the CST4 level in patients with malignant pancreatic tumor was significantly increased compared with that in the gastrointestinal and non-gastrointestinal polyps groups. In addition, the levels of CST4 were significantly increased in the colon cancer group compared with in the gastrointestinal polyp group. A previous

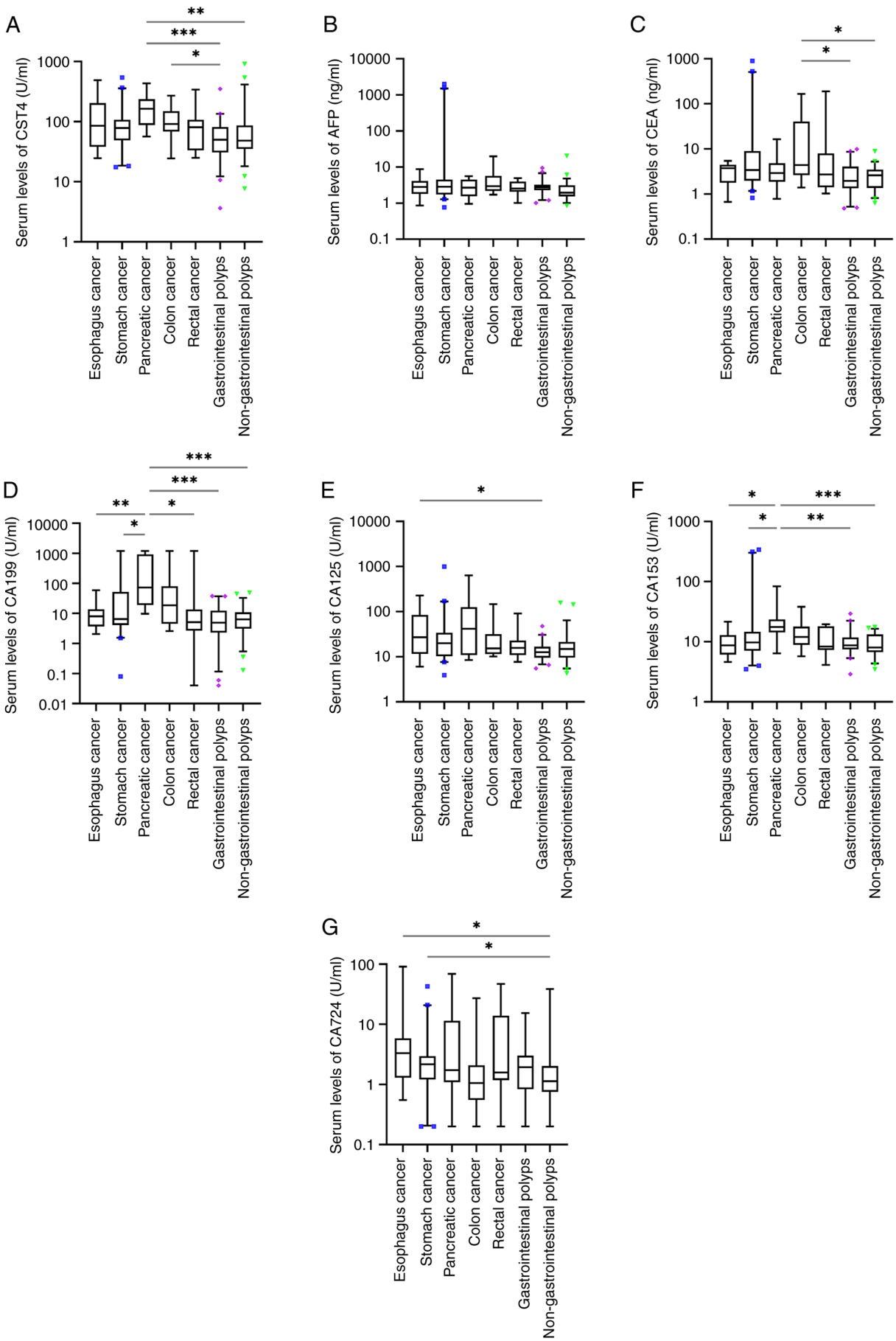

Figure 6. Serum levels of (A) CST4, (B) AFP, (C) CEA, (D) CA199, (E) CA125, (F) CA153 and (G) CA724 in patients with esophageal, stomach, pancreatic, colon and rectal cancer, gastrointestinal polyps and non-gastrointestinal polyps. *P<0.05; **P<0.01; ***P<0.001. CST4, cystatin-S; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

Table IX. Comparison of positive rates of related tumor markers in patients with and without metastasis.

	Metastasis, n (%)			
Biomarker	Yes	No	χ^2	P-value
CST4				
Positive	19 (47.50)	19 (31.67)	2.554	0.110
Negative	21 (52.50)	41 (68.34)		
AFP				
Positive	3 (7.50)	2 (3.33)	-	0.386
Negative	37 (92.50)	58 (96.67)		
CEA				
Positive	19 (47.50)	12 (20.00)	8.485	0.004
Negative	21 (52.50)	48 (80.00)		
CA199				
Positive	17 (42.50)	9 (15.00)	9.433	0.002
Negative	23 (57.50)	51 (85.00)		
CA125				
Positive	16 (40.00)	9 (15.00)	8.000	0.005
Negative	24 (60.00)	51 (85.00)		
CA153				
Positive	4 (10.00)	1 (1.67)	-	0.154
Negative	36 (90.00)	59 (98.33)		
CA724				
Positive	10 (25.00)	8 (13.33)	2.213	0.185
Negative	30 (75.00)	52 (86.67)		

CST4, cystatin-S; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; CA, carbohydrate antigen.

study on the early diagnosis of patients with colorectal malignant tumors has demonstrated that CST4 has good diagnostic efficacy (18). As few patients with rectal tumors were included in the present study, there was no significant difference in the CST4 levels between these patients and the gastrointestinal or non-gastrointestinal polyps patients groups. A previous study on CST1 in esophageal malignancies (42) demonstrated a significant elevation of CST1 levels in the group with esophageal malignancies compared to the group with esophageal benign lesions. However, there was no significant difference in the CST4 level between patients with esophageal cancer and the gastrointestinal or non-gastrointestinal polyps groups in the present study. Although CST1 and CST4 are tumor markers of the same type, their distinct characteristics may lead to different sensitivities in different tumor types. The inclusion of a larger cohort of patients with esophageal malignancies in future studies is warranted to further substantiate any potential disparities in the distribution patterns of CST1 and CST4 among these patients. The limitation of the present preliminary study is that the number of included patients with digestive system malignancies was small (100 patients), and that gallbladder and hepatocellular malignancies were not included, which may be the reason for the low positive rate of AFP. Since most patients had undergone a certain course of chemoradiotherapy or targeted therapy, it was not possible to further stratify the patients and analyze the expression of CST4 in patients at each stage. In addition, as certain patients were not at the affiliated Chaohu Hospital of Anhui Medical University for long-term treatment, and certain patients may refuse further treatment resulting in a loss of follow-up, association analyses of the treatment response, prognosis and survival rate could not be conducted for these patients.

The findings of the present study demonstrated a significant upregulation in the expression level of CST4 among patients diagnosed with malignant digestive tumors, as compared to those with gastrointestinal polyps and non-gastrointestinal polyps. The expression level of CST4 was not affected by age, sex, hypertension or diabetes, nor by gastrointestinal benign proliferative diseases during screening. Although CST4 was more sensitive than the other tumor markers when used alone, its specificity and accuracy exhibited no specific advantages. Therefore, it is suggested that CST4 could be combined with other tumor markers to establish more effective diagnostic tools, and to improve the accuracy of the diagnosis of malignant digestive system tumors. It is also necessary to establish an effective multi-index and multi-parameter combined detection model to improve the accuracy of cancer diagnosis.

In conclusion, the findings of the present study suggested that serum CST4 testing may be a promising and convenient diagnostic tool, but CST4 needs to be combined with other tumor markers to further improve its diagnostic efficacy. In addition, further large-scale, extensive, prospective, multi-center studies are required to confirm the clinical significance of serum CST4 testing in the diagnosis of digestive malignant tumors.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MZ and DZ conceived of the study; MZ, XF and DZ participated in the design of the study; DZ, XF, SX, ML, LG and RZ participated in data collection; XF and SX analyzed and interpreted the data; SX and ML drafted the manuscript; MZ, XF, DZ, SX, ML, LG and RZ revised and edited the manuscript. MZ and DZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethics approval was granted by The Ethics Committee of The Affiliated Chaohu Hospital of Anhui Medical University (approval no. KYXM-202201-058; Chaohu, China). The data utilized in the present study were obtained from the hospital's electronic medical record system; therefore, informed consent for participation was waived by the ethics committee.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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