

Original Article

Serum tumor markers used for predicting esophagogastric junction adenocarcinoma in esophageal malignancy

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Abstract: Background/aims: To evaluate a panel of complementary biomarkers for differential prediction of esophagogastric junction adenocarcinoma (EGAC) from other esophageal malignancy. Methods: Preoperative CA199, CEA, SCC and CA72-4 were evaluated among 410 patients (164 EGAC, 192 esophageal squamous cell carcinoma (ESCC) and 54 benign esophageal diseases). Kruskal-Wallis test, Wilcoxon rank-sum test, receiver operating characteristic curve and logistic regression analysis were applied. Results: The median CA19-9, CEA and CA72-4 levels were statistically higher in patients with EGAC than in those with ESCC (Wilcoxon rank sum test; $P < 0.001$, respectively). Being adjusted to optimal cut-off value, positive CA19-9, positive CEA, negative SCC and positive CA72-4 were significantly correlated with high risk of EGAC (Logistics regression; CA19-9 OR=9.600 $P < 0.001$; CEA OR=7.368 $P < 0.001$; SCC OR=2.754 $P < 0.001$; CA72-4 OR=2.487 $P = 0.001$). When applied as a markers panel, serum tumor markers show a medium sensitivity (65.2%) and a high specificity (79.7%) (AUC=0.791 $P < 0.001$) in EGAC prediction. Conclusions: Synchronous exertion of CA72-4, CEA, SCC, and CA19-9 could be a valuable method to distinguish esophagogastric junction adenocarcinoma from esophageal squamous cell carcinoma.

Keywords: Serum tumor markers, prediction, esophagogastric junction adenocarcinoma, esophageal squamous cell carcinoma

Introduction

Growing rapidly in Western countries [1], the incidence of the esophagogastric junction adenocarcinoma (EGAC) has been discussed in China. Study from China reported that the prevalence of EGAC, which had a yearly increase, was higher than esophageal squamous cell carcinoma (ESCC) and gastric carcinoma (GC) in some high risk areas [2, 3]. The healthy problems of EGAC draw the public attentions. Siewert, et al classified the EGAC into three types based purely on the anatomic location of the epicenter of the tumor or the location of the tumor mass [4]. Changing the classification slightly, NCCN 2015 defines these three subtypes of EGAC as: type I, the adenocarcinoma of the distal esophagus with the tumor center located within 1 to 5 cm above the anatomic esophagogastric junction (EGJ); type II, the true

carcinoma of the cardia with the tumor center within 1 cm above and 2 cm below the EGJ; type III, the subcardial carcinoma with the tumor center between 2 to 5 cm below the EGJ, infiltrating the EGJ and the distal esophagus from below [5]. Attributing to the complicated anatomical structure of EGJ, operative pathway for surgical operation requires differential diagnosis of EGAC from esophageal masses [6, 7]. The diagnosis of EGAC mainly depends on invasive examination, such as endoscopy and biopsy [7].

Serum biomarker is a useful indicator in diagnosis, surveillance and prognosis. However, there is few article focusing on the sensitive and specific tumor biomarkers for distinguishing EGAC from esophageal masses. In this study, we hypothesized that the combined use of several serum tumor markers (CA72-4, CEA,

Serum marker for esophagogastric junction adenocarcinoma

Table 1. Characteristics of the study population

	Benign disease (n=54)	Esophageal squamous cell carcinoma (n=192)	Esophagogastric junction adenocarcinoma (n=164)
Age (year)			
Median	56	60	62.5
Range	(20-76)	(40-82)	(37-84)
Sex			
Male	36	158	143
Female	18	34	21
Histological types of benign disease			
Dysplasia	10		
Chronic inflammation	24		
Hiatal hernia	5		
Benign tumor	15		
CA19-9 (U/mL)			
Mean	10.71	12.94	301.36
Range	0.6-27.59	0.6-160.5	0.6-38060
CEA (ng/mL)			
Mean	2.55	4.78	18.84
Range	0.718-8.59	0.406-293.4	0.2-538.2
SCC (U/mL)			
Mean	1.11	1.77	0.98
Range	0.3-4.9	0.3-31.3	0.3-4.5
CA72-4 (kU/mL)			
Mean	2.58	3.31	14.24
Range	0.736-73.240	0.579-32.7	0.327-629.7

SCC and CA19-9) may increase the sensitivity and accuracy for the diagnosis of EGAC. Thus, employing the panel of tumor markers, our research aims to distinguish EGAC from other malignant esophageal diseases, especially ESCC.

Methods

Study population

A total of 410 inpatients with preoperative serum available for protein analysis were enrolled from Cancer Hospital, Chinese Academy of Medical Sciences (CAMS) & Peking Union Medical College (PUMC), Beijing, China from July 2012 to May 2013. All patients were newly diagnosed and previously untreated. The pathological data for these patients was obtained by endoscopy and confirmed by biopsy. This study was approved by the Institute Review Board of the Cancer Hospital, CAMS & PUMC. Each patient was informed about the study and gave his/her consent.

Measurement of tumor markers

Four serum tumor markers, CEA, CA19-9, CA72-4 and SCC, were measured before treatment. CEA, CA72-4 and CA19-9 levels were investigated using a electrochemiluminescence immunoassay (ECLIA) (Roche, Germany), while SCC levels were investigated using a chemiluminescent enzyme immunoassay (CLEIA) (Abbott, USA). These assays were done at the Department of Clinical Laboratory, Cancer Hospital, Chinese Academy of Medical Science. The cut-off values for CEA, CA19-9, SCC, and CA72-4 were defined as 5.0 ng/mL, 37 U/mL, 1.5 U/mL and 9.8 kU/mL, respectively. All cut-off values were recommended by manufacturer, and confirmed by the Department of Clinical Laboratory. The sensitivity of the markers was calculated as the number of patients who showed elevated levels of each marker above the cut-off value divided by the total number of patients. The sensitivity of the combination of multiple markers was calculated as the percentage of patients who showed elevation in any of the combined markers.

Table 2. Higher CA19-9, CEA and CA72-4 levels are associated with EGAC rather than ESCC

	Benign disease (n=54)	Esophageal squamous cell carcinoma (n=192)	Esophagogastric junction adenocarcinoma (n=164)
^a CA19-9 (U/mL)	8.59	9.26 ^b	11.435 ^b
^a CEA (ng/mL)	2.565	2.435 ^b	3.19 ^b
^a SCC (U/mL)	0.9	1.1 ^b	0.8 ^b
^a CA72-4 (kU/mL)	1.675	1.875 ^b	2.795 ^b

^aKruskal-Wallis test was used to compare the difference of tumor markers between patients with different esophageal disease; ^bWilcoxon rank sum test was used to compare the difference of tumor markers between patients with esophagogastric junction adenocarcinoma, and esophageal squamous cell carcinoma, $P < 0.05$ was considered statistically significant.

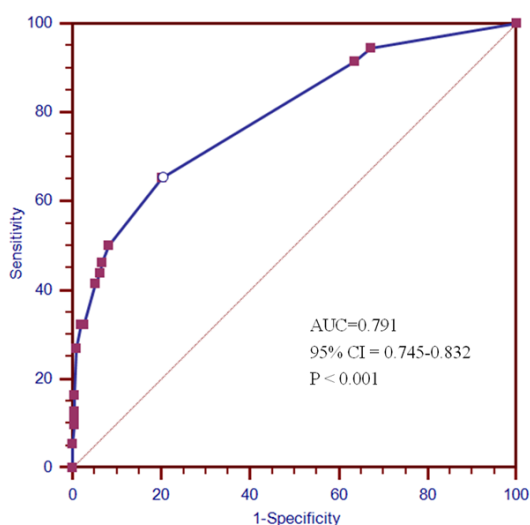


Figure 1. Area under the receiver operating characteristic curve values of markers panel used for predicting EGAC. Markers panel: AUC, 0.791 (95% CI, 0.745-0.832), $P < 0.001$.

Statistical analysis

All data ordered in an abnormal distribution are expressed as the Median (Range). The non-parametric Kruskal-Wallis test and Wilcoxon rank-sum test were used for the comparison of each original value of a tumor marker between groups. To investigate the diagnostic efficacy of tumor markers, a receiver operating characteristic (ROC) curve was constructed using the serum concentrations in both EGAC and ESCC to assess the predicting efficacy. Additionally, An optimal cut-off value, having the highest Youden Index, would be point nearest to the top of vertical axis in ROC. Logistic regression models were used to detect associations of tumor diagnosis with each of serum tumor marker and provided estimates of odds ratio (ORs) and

confidence intervals (CIs). All statistical analyses were conducted using SPSS 19.0 for Windows (SPSS Inc., USA). P values below 0.05 were considered statistically significant.

Results

Patient characteristics

Patient characteristics are shown in **Table 1**. A

total of 410 patients enrolled in the study. Of the patients enrolled, 54 patients (13.2%) had a diagnosis of benign esophageal disease. One hundred and ninety-two patients (46.8%) were diagnosed with esophageal squamous cell carcinoma, and 164 patients (40.0%) were diagnosed with esophagogastric junction adenocarcinoma.

Higher CA19-9, CEA and CA72-4 levels are associated with EGAC rather than ESCC

The median of tumor marker levels between patients with different types of underlying esophageal disease is shown in **Table 2**. There was a significant difference between tumor marker levels in those patients with benign lung disease, esophagogastric junction adenocarcinoma and esophageal squamous cell carcinoma (Kruskal-Wallis test, CA19-9: $P = 0.0019$; CEA: $P < 0.001$; SCC: $P < 0.001$; CA72-4: $P < 0.001$). There was a statistically significant difference between median tumor marker levels in patients with EGAC compared with those patients with ESCC (Wilcoxon rank sum test, CA19-9: $P < 0.001$; CEA: $P < 0.001$; SCC: $P < 0.001$, CA72-4: $P < 0.001$). The median values of CA19-9, CEA, CA72-4 are statistically higher in EGAC patients than in ESCC patients, while SCC was lower in EGAC.

Serum markers with adjusted cut-off value contribute to distinguishing EGAC from ESCC

A receiver operating characteristic (ROC) curve for tumor markers in distinguishing different malignance involvement is shown in **Figure 1**. Significance was observed regarding the area under curve (AUC) of serum markers (CA19-9 AUC=0.597 $P = 0.0017$; CEA AUC=0.643 $P < 0.001$; SCC AUC=0.634 $P < 0.001$; CA72-4 AUC

Serum marker for esophagogastric junction adenocarcinoma

Table 3. The optimal cut-off value of tumor markers to distinguish EGAC from ESCC

	AUC	95% confidence interval	P	Optimal cut-off value
CA19-9 (U/mL)	0.597	0.544-0.648	0.0017	34.61
CEA (ng/mL)	0.643	0.591-0.693	<0.001	4.97
SCC (U/mL)	0.634	0.582-0.685	<0.001	1.3
CA72-4 (kU/mL)	0.637	0.584-0.687	<0.001	4.1

AUC: Area under the receiver operating characteristic curve; Optimal cut-off value: the cut-off value representing a highest Youden Index, the point nearest to the top of vertical axis in receiver operating characteristic curve. $P < 0.05$ was considered statistically significant.

Table 4. Serum markers with adjusted cut-off value contribute to distinguishing EGAC from ESCC

Indicators	Odds ratio (OR)	95% confidence interval	P
CA19-9 >34.61	9.600	3.427-26.888	0.000
CEA >4.97	7.368	3.599-15.084	0.000
SCC <1.3	2.754	1.563-4.853	0.000
CA72-4 >4.1	2.487	1.437-4.304	0.001

Logistics regression was used to analysis the attribution of different serum tumor markers to predicting EGAC. Attribution expresses as odds ratio. $P < 0.05$ was considered statistically significant.

Table 5. Sensitivity and specificity of CA19-9, CEA, SCC, CA72-4 and the markers panel for predicting the diagnosis of EGAC

Tumor marker	Sensitivity (%)	Specificity (%)	Youden Index
CA19-9 >34.61	24.39	97.40	0.2179
CEA >4.97	38.41	94.74	0.3315
SCC <1.3	79.88	40.10	0.1998
CA72-4 >4.1	42.07	82.29	0.2436
Markers panel	65.24	79.69	0.4493

Youden Index: a single statistic that captures the performance of a diagnostic test. Youden Index = sensitivity + specificity - 1.

$P < 0.001$) (**Table 3**). To statistically develop the effective differential value of tumor markers, the study identified the point nearest to the top of vertical axis in ROC as the optimal cut-off value.

Logistic regression models identified the tumor markers as statistically significant between EGAC and ESCC (**Table 4**). In particularly, a positive CA19-9 beyond 34.61 U/ml had the highest risk of EGAC (OR=9.600, $P < 0.001$). Patients with CEA >4.97 or SCC <1.3 or CA72-4 may more likely EGAC (CEA OR=7.368 $P < 0.001$; SCC OR=2.754 $P < 0.001$; CA72-4 OR=2.487 $P = 0.001$). This result indicates CA199, CEA, SCC

and CA72-4 would be the effective risk indicators to EGAC.

Markers panel can be a helpful serum predictor for esophagogastric junction adenocarcinoma among esophageal malignancy

Median tumor markers values were markedly increased in patients with esophagogastric junction adenocarcinoma compared with patients with ESCC, as shown in **Table 2**. And positive CA19-9, CEA and CA72-4 showed the highest correlation with underlying esophagogastric junction adenocarcinoma (**Table 4**). Therefore, we hypothesized that a panel of serum markers may be used to predict the type of esophageal malignancy. Area under the receiver operating characteristic curve value was used for evaluate the efficiency of tumor markers after combined application. The area under the curve (AUC) was 0.791 (95% CI= 0.745-0.832 $P < 0.0001$) (**Figure 1**). Under the optimal cut-off value set, sensitivity and specificity from single predictive markers were compared with those of combination (**Table 5**). Markers panel conferred higher sensitivity of 65.24% and lower specificity of 79.69% after joint application. Considering the balance between sensitivity and specificity, Youden Index suggested that markers panel (0.4493) would be more effective

than single markers in serum predicting of esophagogastric junction adenocarcinoma.

Discussion

Serum biomarkers, with less trauma and inconvenience, have been a speedy approach for assistant diagnosis, surveillance and prognosis [8-10]. As a definition-renewed tumor in the diagnosis of gastrointestinal masses [5], EGAC has been discussed in the clinical practice only for a short period of time. Several indicators have been found as a prognostic value for EGAC patients [11, 12]. However, these studies were mainly focused on the relationship between the

serum biomarkers and the survival rate of patients with EGAC. Our study firstly discusses the indicating competence of serum biomarkers to distinguish EGAC from other esophageal masses. Our results exhibited that a difference of serum biomarkers between patients with EGAC and patients with ESCC is significant. Therefore, elevated serum biomarkers value is a helpful predictor of existence of EGAC.

Surgical strategy options for patients with esophageal malignancy were determined by extent of their lymphatic metastasis [13]. Considering the underlying lymph node involvement around gastric area in EGAC compared with ESCC, gastrectomy or semigastrectomy is recommended to assist tumor excision [14, 15]. Since the metastasis route is varied with the histopathological type, serological prediction before dissection facilitates the treatment options. As our results show, indicative risk on EGAC from elevated CA19-9 value was 9.6 times as high as value under cut-off (**Table 4**).

Gastrointestinal endoscope and endoscopic ultrasonography are the best preoperative choice in diagnosis of gastrointestinal masses [16]. Since the concept regarding esophagogastric junction is generalized recently, endoscopic definition of esophagogastric junction endoscopic detection still need some systematic training in the nationwide [17, 18]. Lack of standardization in EGAC differential diagnosis from esophageal carcinoma, especially ESCC, may cause misleading and decrease the specificity in primary hospital. Our data showed that optimal cutoff value of several common tumor markers resulted in high specificity of 0.80 and medium sensitivity of 0.65 (**Table 5**) for elevated tumor markers panel predicting EGAC. Although markers panel can not identify the precise location, the combination of endoscope and markers panel may increase the sensitivity and specificity of esophageal malignancy predictions.

Here, the limitations of this study have to be mentioned. Limitation of cases may obstruct the representativeness of the receiver groups because the cases merely come from single center. In addition, our research pertains to a retrospective study rather than a perspective one. It is promising that a prospective study could be designed to confirm the predictive value of biomarkers in clinics.

Conclusions

With the help of the ROC curve analysis and logistic regression analysis, our results suggest that the combined application of CA72-4, CEA, SCC, and CA19-9 could be a valuable method to distinguish patients with EGAC from those with ESCC. Further studies are recommended to confirm these results.

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Disclosure of conflict of interest

None.

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