Can preoperative CEA and CA19-9 serum concentrations suggest metastatic disease in colorectal cancer patients?

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Keywords: CA19-9 -CEA -Colorectal cancer -Liver metastases -Lymph nodes metastases

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Received:
17 October 2016
Accepted revised :
2 February 2017

Abstract

Objective: This study was designed to investigate the efficiency of preoperative serum carcinoembryonic antigen (CEA) and carbohydrate cancer antigen (CA19-9) levels for diagnosing synchronous liver metastases and lymph node in colorectal carcinoma (CRC) patients. **Subjects and Methods:** A total of 300 patients with histologically diagnosed CRC were included in this study between May 2014 and March 2015. The data were obtained from patient's medical records: medical history, demographics, tumor location, differentiation (grade), depth of the tumor (T), lymph node metastases (N), distant metastases (M), lymphatics, venous and perineural invasion, and disease stage. Tumor markers were measured with an electrochemiluminescent assay and the reference value was 5ng/ml for CEA and for Ca19-9, 37u/ml. **Results:** There was A high statistically significant difference in the levels of serum CEA and CA19-9 between different disease stages of CRC (P<0.001). Regarding different T stages of CRC, We noticed a significant statistical difference in CEA (stage I 3.76±8.73; II 5.68±17.27, III 7.56±14.81, and IV 70.90±253.23) and CA 19-9 levels (stage I 9.65 ±11.03, II 9.83±11.09; III 19.58±36.91, and IV 228.9±985.38, respectively). The mean CEA and CA19-9 serum levels were significantly higher in patients with regional lymph nodes involvement (CEA 37.21±177.85 vs 4.79±9.90, CA19-9 119.51±687.71 VS 12.24±17.69, respectively, P<0.05) and in liver metastases (CEA 86.56± 277.65 vs. 5.98± 12.98, and CA19-9 273.27±1073.46 vs. 4.98± 3142, respectively, with P<0.001) in comparison to patients without lymph node involvement and liver metastases. We noticed a cut-off value for lymph nodes involvement, for CEA and CA 19-9, 3.5 ng/mL and 7.5 U/mL, respectively. While, a cut-off value for the presence of synchronous liver metastases of these two markers was 3.5ng/mL AND 5.5 U/mL. **Conclusion:** Our study showed that tumor makers, CEA and CA19-9, can be used as diagnostic factors regarding the severity of CRC specifically to suggest metastatic disease in CRC.

Hell J Nucl Med 2017; 20(1): 41-45 *Epub ahead of print: 20 March 2017* *Published online: 20 April 2017*

Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer in both females and males in Western countries, with an incidence of about 9% estimated cancers, yearly [1]. According to the data of the Institute of Public Health of Serbia, CRC is the second leading malignancy after breast cancer in females, and after lung cancer in males, in Serbian population [2]. Extensive surgery is the leading strategy for the treatment of CRC. Lymph nodes involvement and of metastases, considerably affects the prognosis of these patients. Liver is the primary site for CRC metastases, detected in about 25% of the patients at initial diagnosis [3]. Although developments of new chemotherapeutics and enhanced surgical techniques have improved survival rates of CRC patients, there is still a need for reliable noninvasive markers for the prediction of liver lymph node and metastases [4].

Carcinoembryonic antigen (CEA) is a high molecular weight glycoprotein and the most widely used tumor marker in CRC patients [5]. It is produced by cells of the large intestine, it functions as intercellular adhesion molecule and promotes aggregation of human CRC cells [6]. Increased concentrations of CEA are rarely observed in early stages of the disease, but if detected may indicate a less favorable prognosis [7]. Recent studies showed that 15%-40% of CRC patients at initial diagnosis do not have elevated serum levels of CEA [7, 8]. Increased serum CEA may also be seen in many non-neoplastic conditions like: patients with inflammatory bowel disease, pancreatitis, chronic obstructive pulmonary disease, hepatitis, liver cirrhosis, hypothyroidism and in heavy smokers. The role of CEA in early diagnosis of CRC is controversial due to its insufficient sensitivity and organ specificity [9].

Carbohydrate antigen (CA19-9) is a high molecular weight glycoprotein, used in gastric, pancreatic malignancies and in CRC diagnostics. Although CA19-9 is non-specific for CRC, several studies showed that simultaneous assessment of CA19-9 and CEA may increase the diagnostic sensitivity in CRC. Since CRC is biochemically and molecularly a heterogeneous disease, CA19-9 as an intracellular adhesion molecule may influence cells synthetizing various tumor markers. [8, 9].

This study was designed to investigate the efficiency of preoperative serum CEA and CA19-9 levels as a tool for the assessment of lymph nodes and liver metastases newly diagnosed patients with CRC.

Patients and Methods

A total of 300 patients with histologically diagnosed CRC were included in this study between May, 2014 and March, 2015, 244 without liver metastases, and 56 with liver metastases.

All patients underwent curative surgery in the Clinic of Digestive System Surgery, Clinical Center of Serbia and also colonoscopy, abdominal ultrasonography, abdominal and pelvic CT, and chest radiography. Patients were staged in accordance with TMN and Dukes classification [10]. We investigated the association between serum concentrations of CEA and of CA19-9 and the CRC stage according to the American Joint Committee on Cancer, considering: cells differentiation, mucus production and TNM stage [10].

Peripheral blood 2mL samples were collected preoperatively the day before surgery from the cubital vein. For 300 of the patients the tumor markers were measured with an electrochemiluminescent assay using Roche Diagnostic reagent kits and Cobas 6000 automatic analyzer (Roche Diagnostics, Mannheim, Germany). In parallel we performed enzyme immunoassay (EIA) and electrochemiluminescent assay (Roche Diagnostics, Mannheim, Germany) in 20 patients. The reference values were SET TO 5ng/mL for CEA and for CA19-9, 37U/mL.

Patients meeting the following criteria were excluded from this study: a) Patients with recurrent CRC or with a 5-years history of another malignancy. b) Patients who had received chemotherapy, with hepatic, pancreatic, biliary, pulmonary or inflammatory bowel disease and c) patients with unresectable CRC. A written informed consent was obtained from each patient before the study. The study was approved by the Ethics Committee of the Clinical Center of Serbia.

Statistical analysis was carried out using the SPSS ver. 20.0 (SPSS Inc., Chicago, IL, USA). Patient's demographics, clinical and pathological characteristics were summarized. Continuous variables were expressed as mean ± standard deviation (SD. Normality of distribution was investigated by Kolmogorov-Smirnov test. The clinicopathological variables between the groups were compared using Mann-Whitney test and Krusal-Wallis test. The optimal cut-off values of tumor markers as prognostic variables were determined according to the receiver-operator characteristic (ROC) analysis. A value of $P<0.05$ was considered statistically significant.

Results

Demographic, clinical and pathological characteristics of CRC patients are presented in Table 1.

Table 1. *Characteristics of patients*

	n=300
Age, years (mean±SD)	62.39±11.495
Male/female (n, %)	182/118, 60/40
Disease stage (n, %)	
I	81, 27
II	72, 24
III	94, 32
IV	53, 17
Liver metastases (n,%)	
Yes	56, 19
No	244, 81
Tumor grade (n, %)	
low-grade	220, 73
medium-grade	66, 22
high-grade	14, 5

There was high statistically significant difference between serum CEA and CA19-9 and the different disease stages of CRC ($P<0.001$, Figures 1 and 2).

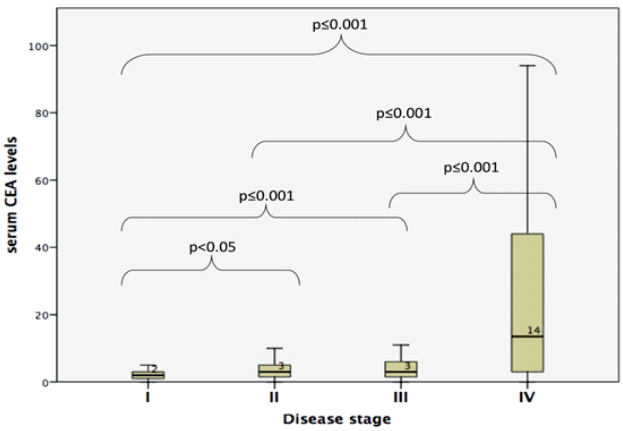

Figure 1. Levels of serum CEA between different disease stages of CRC.

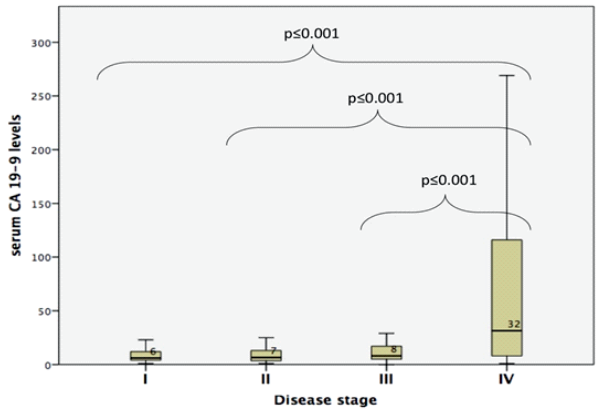

Figure 2. Levels of serum CA19-9 between different stages of CRC.

Examining serum CEA by T stage, we noted a significant sta-tistical difference in CEA levels among the different disease stages and especially comparing stages I, II and III to patients with IV stage (Figure 3). Average values of CEA in stages were (stage I 3.76±8.73; II 5.68±17.27, III 7.56±14.81, and IV 70.90 ±253.2)

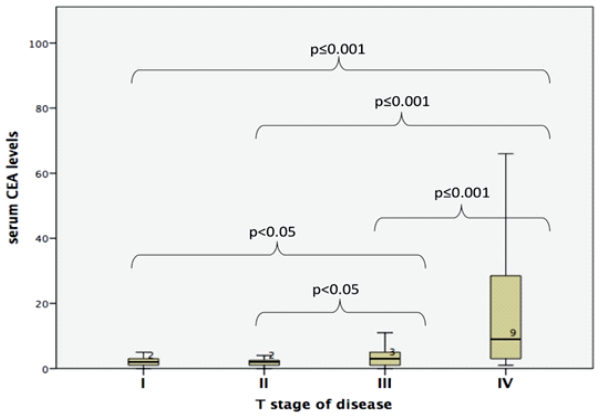

Figure 3. Levels of serum CEA by T stage

When we examined serum levels of CA19-9 by T stage, there was also a significant difference among different disease stages, especially between disease stage IV (Figure 4). Average values of CA 19-9 in stages were (stage I 9.65±11.03, II 9.83±11.09; III 19.58±36.91, and IV 228.9±985.38).

The mean CEA and CA19-9 serum levels were significantly higher in patients with regional lymph nodes involvement although SD was high (CEA 37.21±177.85 vs 4.79±9.90, CA 19-9 119.51±687.71 vs 12.24±17.69, respectively, with P< 0.05).

The mean CEA and CA19-9 serum levels were higher in patients with metastases (CEA 86.56±277.65 vs. 5.98± 12.98, and CA19-9 273.27±1073.46 vs. 4.98± 3142, respectively, with P<0.001). Levels of both markers were significantly elevated in the presence of metastases (P<0.001). No significant differences in CEA and CA19-9 levels were observed with respect to sex, age, smoking or tumor grade (P>0.05).

Spearman Rho Correlation revealed that CEA was positively correlated with CA19-9 (P < 0.001).

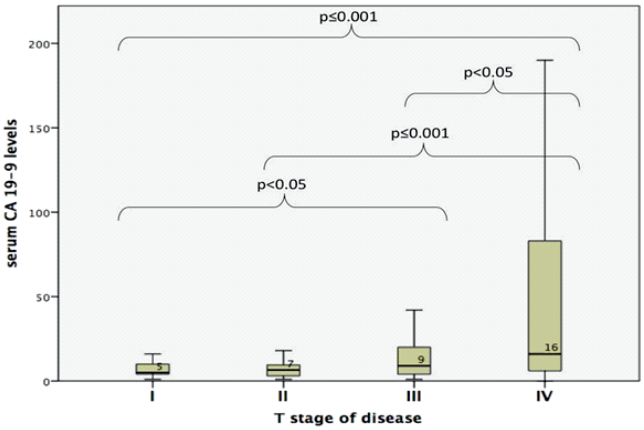

Figure 4. Levels of serum CA19-9 by stage in CRC patients

In 20 patients we investigated the correlation between CEA-EIA and CEA-RIA. Correlation levels were high with correlation coefficient was 0.882 (P<0.01). The sensitivity was similar in both assays (70% vs. 73%) at a cut-off level of 5ng/ml.

The ROC curve analysis for CEA in all disease stages had 53.15% sensitivity for a cut-off level of 2.5ng/mL, and overall positively for CA19-9 48.37% for a cut-off OF 8.5U/mL. The ROC curve analysis for CEA in stage IV for the cut-off level of 2.5ng/mL showed sensitivity of 76.2% and specificity of 59% with area under the curve (AUC) 0.738, and for CA19-9 for the cut-off level of 11.5U/mL showed sensitivity of 70% and specificity of 70% with AUC 0.740.

The ROC curve analysis for lymph nodes involvement for the CEA cut-off level of 3.5ng/mL showed sensitivity and specificity of 60% and 70% respectively and for the CA19-9, cut-off level of 7.5U/mL showed sensitivity of 70.3% and specificity of 53%.

The ROC curve analysis for the presence of synchronous liver metastases for the CEA cut-off level of 3.5ng/mL showed sensitivity of 76.8% and specificity of 71.8%, and for the CA19-9 cut-off level of 5.5 U/mL showed sensitivity of 80.4% and specificity of 40% respectively.

Discussion

At present it remains unclear whether CEA and CA19-9 can provide more information regarding the spread of the disease [11]. An ideal marker should be an inexpensive screening test which could identify population at risk of developing CRC. CEA and CA19-9 are not suggested as screening tests, and the data supporting their usage in determining whether to treat patients with an adjuvant therapy are also insufficient [11].

Our results showed that patients with disease stage I have significantly lower levels of serum CEA, when compared to patients with more advanced stages of CRC. These finding concur with the ones in the available literature [12]. In addi-

tion, studies have also showed that serum CEA levels have prognostic values, with higher CEA levels predicting a more advanced disease stage, which is in concordance with our results [13, 14].

Data in our study also show significantly higher levels of CA19-9, when comparing patients with disease stage I to ones with stage IV, in accordance with the previously published data [15].

Consistent with other studies, our findings confirmed that preoperative values of serum CEA and CA19-9 were significantly higher in stages III (P=0.02, P=0.05 respectively) and IV (P= 0.00 and P = 0.002 respectively) than in stage I [16].

Our study suggests that preoperative high levels of CEA and CA19-9 indicate lymphatic metastases, which is in concordance with a previous study of others [17].

There have been studies showing that degree of tumor differentiation is associated with higher serum CEA levels [18, 19]. Our results suggested no significant differences between histologically differentiated CRC, which is consistent with other reports [16, 20, 21]. In order to resolve this controversy there is a need for larger multicenter studies targeting the mechanisms of tumor marker production.

Distant organ metastases are the major cause of mortality among CRC patients. The most common sites of the CRC-associated metastases are liver, lung and the peritoneal cavity. More than 50% of CRC patients will eventually develop liver metastases, which are associated with an unfavorable prognosis [22, 23]. A newly published article by Pakdel et al. (2016) in 199 patients clearly emphasizes the association between preoperative serum CEA and synchronous liver metastases. In the above study, the authors concluded that patients with high preoperative serum CEA should have more intensive follow-up for the detection of synchronous liver metastases. Our results corresponded with the above and indicated that high pre operative serum CEA and CA19-9 concentrations showed synchronous liver metastasis [3]. We notice that in the above studies of both CEA and CA19-9 markers the number of patients studied was 142, 131 and 199 rather limited as related to the present study.

In a small group of patients (20 patients) for testing CEA we tried to compare with diagnostic value of using EIA and RIA. Both tests had similar sensitivity. Of course the RIA test is preferable as examining radiolabeled molecules but in cases where the RIA test is unavailable EIA test can be used in everyday practice [24].

Other studies showed different rates of positively for CEA and CA19-9 in patients with CRC and cut-off values of 3.56 ng/mL and 28 U/mL for CEA and CA19-9, respectively, with sensitivities of 56.2% and 36.4% or lower respectively [25].

The ROC curve analysis in our study for CEA in the diagnosis of CRC patients in different disease stages showed 53.15% sensitivity for a cut-off level of 2.5ng/mL, and an overall positively for CA19-9 of 48.37% for cut-off values of 8.5U/mL. Our lower cut-off values for CA19-9 as compared to the previously mentioned studies could be explained by the higher percentage of patients in our study with more advanced disease stages (III and IV). Sensitivity of CEA was 32.1% for stage I disease, 51.6% for stage II, 52.9% for stage III, and 76% for stage IV. Our results are in accordance with those of other researchers [26].

In conclusion, to the best of our knowledge the present study is the largest one conducted in Serbia and suggested that tumor makers, CEA and CA19-9, may indicate the risk of synchronous liver and lymph nodes metastases.

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Portait of Rudolph IV: The founder of the first University in Vienna-Austria, c 1360. Tempera on parchment on a wood panel. Vienna, Domund Diozesan museum.