

**PREDICTIVE VALUE OF CARCINOEMBRYONIC AND CARBOHYDRATE ANTIGEN 19-9 RELATED TO SOME CLINICAL, ENDOSCOPIC AND HISTOLOGICAL COLORECTAL CANCER CHARACTERISTICS****PREDIKTIVNE VREDNOSTI KARCINOEMBRIONSKOG I KARBOHIDRATNOG ANTIGENA 19-9 U ODNOSU NA NEKE KLINIČKE, ENDOSKOPSKE I HISTOLOŠKE KARAKTERISTIKE KOLOREKTALNOG KANCERA***Ratko Tomašević<sup>1</sup>, Tomica Milosavljević<sup>2</sup>, Dragoš Stojanović<sup>3</sup>, Zoran Gluvić<sup>1</sup>, Predrag Dugalić<sup>1</sup>, Ivan Ilić<sup>1</sup>, Radosav Vidaković<sup>1</sup>**<sup>1</sup>Clinical Hospital Center Zemun, Belgrade, Clinic of Internal Medicine, University of Belgrade, School of Medicine**<sup>2</sup>Clinical Center of Serbia, Gastroenterology and Hepatology Clinic, University of Belgrade, School of Medicine**<sup>3</sup>Clinical Hospital Center Zemun, Belgrade, Surgery Clinic, University of Belgrade, School of Medicine***Summary**

**Background:** Colorectal cancer (CRC) is an important oncological and public health problem worldwide, including Serbia. Unfortunately, half of the patients are recognized in an advanced stage of the disease, therefore, early detection through specific tumor biomarkers, such as carcinoembryonic (CEA) and carbohydrate antigen 19-9 (CA 19-9), is the only way to cope with CRC expansion.

**Methods:** Our cross-sectional study evaluated the influence of some clinical, endoscopic and histological characteristics of CRC on CEA and CA 19-9 serum levels, to determine whether these biomarkers could be related to CRC detection. The study included 372 participants: 181 suffered from CRC and 191 participants were controls. Endoscopic and histological examinations were used for CRC diagnosis, while additional ultrasound and abdominal computerised tomography imaging were used for staging the disease. Measurement of CEA and CA 19-9 was performed after CRC confirmation.

**Results:** Age, gender, tumor localization, macro-morphological and histological characteristics did not influence biomarkers serum levels. Both were significantly higher ( $p < 0.01$ ) in patients with Dukes D stage of CRC compared with controls. Sensitivity (76.8%) and specificity (76.6%) of CEA alone were higher than for CA 19-9, but with no statistical significance. Furthermore, sensitivity of CEA alone

**Kratak sadržaj**

**Uvod:** Kolorektalni karcinom (CRC) značajan je onkološki i zdravstveni problem kako u svetu tako i u Srbiji. Nažalost, polovina bolesnika se otkriva u uznapredovaloj fazi bolesti, stoga je rano otkrivanje preko specifičnih tumorskih markera, kao što su karcinoembrionski (CEA) i karbohidratni antigen 19-9 (CA 19-9), od izuzetnog značaja u borbi protiv ekspanzije CRC-a.

**Metode:** U ovoj unakrsnoj studiji smo ispitivali uticaj nekih kliničkih, endoskopskih i histoloških karakteristika CRC-a na serumske vrednosti CEA i CA 19-9, kako bi se utvrdilo da li ovi biomarkeri imaju sposobnost da detektuju CRC. U studiju je uključeno 372 ispitanika; 181 su bili pacijenti sa CRC-om a 191 subjekti u kontrolnoj grupi. Endoskopskim i histološkim pregledima je postavljena dijagnoza CRC-a a dopunskim ultrazvučnim pregledom i kompjuterizovanom tomografijom je utvrđivan stadijum bolesti. Serumske koncentracije CEA i CA 19-9 su određivane posle postavljanja dijagnoze CRC-a.

**Rezultati:** Starosna dob, razlike među polovima, lokalizacija tumora, njegove makromorfološke i histološke karakteristike ne utiču na vrednosti serumskih koncentracija biomarkera. Obe su bile značajno povišene ( $p < 0,01$ ) kod bolesnika u Dukes D stadijumu CRC-a u poređenju sa kontrolnom grupom. Senzitivnost (76,8%), kao i specifičnost (76,6%) markera CEA je bila viša nego kod CA 19-9, ali bez sta-

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in the Dukes A/B group was similar to the entire CRC patient group.

**Conclusions:** Although not recommended as a screening method for the general population, elevated values of each biomarker indicate further diagnostic procedures and their simultaneous testing can improve the diagnostic sensitivity in early detection of CRC, as shown by the united analysis (AUC 0.842).

**Keywords:** carcinoembryonic antigen, carbohydrate antigen 19-9, colorectal cancer, tumor markers

## Introduction

Colorectal cancer (CRC) has the third highest incidence rate and the fourth highest mortality rate, when compared with other cancers. It is estimated that there will be 1.2 million new cases reported annually and 0.6 million deaths worldwide (1). CRC has the first highest incidence rate and the second highest mortality rate in the European Union, with approximately 334 thousand new cases and 149 thousand deaths in 2008. Incidence of CRC increases with age; it is the highest between 70 and 74 years of age in both men and women (1). In Serbia, CRC is the second leading cancer (2, 3), with an incidence rate of 33.5/100,000. This places Serbia in the group of modestly high rate CRC countries, similarly to some countries of Central and Eastern Europe, Austria, USA and Poland (1). The Cancer Registry in Serbia shows an increase in the number of patients suffering from CRC, similar to some other countries of Central and Eastern Europe (4).

The majority of CRC cases arise sporadically, as a result of accumulation of genetic variations in cooperation with some other risk factors, such as biological hypersensitivity, unhealthy eating and alcohol consumption habits, smoking, presence of other diseases and stressful life moments (5). The most important prevention procedures for CRC are screening programs of the general population for early colonic adenoma and *in situ* CRC detection (6, 7). Still, half of the investigated patients are at stage I/II when diagnosed, and the other half are at stage III/IV, when the disease is already disseminated and consequently with low survival rate (8). According to World Health Organization recommendations, each country organizes screening programs adjusted to its own capabilities. Among the recommended procedures, faecal occult blood test and colonoscopy are the »gold standard« for early detection of CRC (8). Despite screening procedures for prevention, the results often showed a delay in CRC diagnosis (9).

Carcinoembryonic antigen (CEA) is found in embryonic and tumorous colonic tissue. Its blood concentration rises with tumor mass enlargement; hence, it is significantly positive only in 28% of CRC patients at the early stage of the disease. The predictive value is significantly reduced for early stage CRC

tističke značajnosti. Senzitivnost markera CEA kod grupe pacijenata sa stadijumom Dukes A/B je bila slična kao u ukupnoj grupi CRC pacijenata.

**Zaključak:** Povišene vrednosti svakog od dva biomarkera su indikacija za dalje dijagnostičke procedure a njihovo istovremeno testiranje može poboljšati dijagnostičku senzitivnost u ranom otkrivanju CRC-a, kao što to pokazuje kombinovana analiza (AUC 0,842).

**Ključne reči:** karcinoembrijski antigen, karbohidratni antigen 19-9, kolorektalni kancer, tumorski markeri

detection (10). An increase in tumor marker value depends on tumor growth and spreading, so its sensitivity rises to approximately 90% in advanced stages of CRC (11). Elevated carbohydrate antigen 19-9 (CA 19-9) level suggests its colonic tumor origin. It is less sensitive than CEA in the early stages of CRC, but conversely, in later stages of disease, its significance increases (12). CEA can indicate tumor progression or relapse, as well as assess the efficacy of treatment (13). Apart from tumor mass influence, some additional factors can increase the values of biomarkers, such as other diseases, habits, and tumor clinical characteristics (14).

The aim of this study was to examine the association of some demographic, clinical, endoscopic and histological parameters with CEA and CA 19-9 serum levels. In addition, we aimed to determine tumor marker levels at different stages of CRC, and also to investigate the significance of CEA and CA 19-9 as tumor markers in the detection of patients with curative stages of CRC.

## Material and Methods

### *Subjects and methods*

This cross-sectional study was performed in the Department of Gastroenterology and Surgery of Zemun Clinical Center (KBC Zemun), Serbia during 2014 and 2015. The study included 372 participants; 181 suffered from histologically confirmed CRC and 191 were controls. Out of 181 patients with the diagnosis of CRC, 72 were females and 109 males, with an average age of  $66.7 \pm 9.2$  years. Out of 191 controls admitted to the hospital due to non-neoplastic indications, 96 were women and 95 men, with an average age of  $62.8 \pm 10.9$  years. The control group consisted of orthopaedic non-trauma and trauma patients, with various fractures and injuries, as well as patients with some mechanical general surgical condition, such as hernias. Both groups were age- and gender-matched. The study was approved by the Ethics Committee of the School of Medicine, University of Belgrade, and informed consent was obtained from all subjects who participated in this study.

### Determination of CEA and CA 19-9 markers

Standard laboratory and imaging diagnostic procedures were carried out in both groups depending on clinical signs and indications. Blood samples were drawn to determine the levels of CEA and CA 19-9. Serum concentration of CEA and CA 19-9 were measured by chemiluminescent immunoassay (»DCI – 600« Beckman Coulter) with cut-off values 5 ng/mL (CEA) and 35.4 U/mL (CA 19-9), respectively.

### Medical procedures

Endoscopic procedures were carried out in the group of patients presenting with symptoms of colonic diseases, in accordance with good clinical practice. CRC diagnosis was confirmed by histological examination of tissue samples obtained during endoscopy of the colon and re-examination of the material obtained by surgical resection in surgical patients. In all patients with CRC, ultrasound and multi-sliced computed abdominal tomography were performed and correlated with the stage of the disease. Exclusion criteria for the study were the existence of medical history of cancer in other organs and diseases of the liver and pancreas. The following characteristics were observed: demographic (gender, age), clinical (staging, surgical feasibility), endoscopic (tumor localization – right colon, left colon, rectum, macroscopic aspect of the tumor: presence of luminal stenosis, proliferative or ulcerative type) and histological (well, moderately and poorly differentiated tumors). Dukes classification, modified by Astler-Coller, was used to determine the stage of the disease (15, 16). The Dukes A group included tumors infiltrating the mucosa and submucosa through the muscular layer, without metastases in regional lymph nodes. The Dukes B stage tumor invaded completely the bowel wall, but with no metastases in lymph nodes, Dukes C tumors with metastases in regional lymph nodes or distant organs (Dukes D).

### Statistical analysis

Data are shown as mean  $\pm$  standard deviation for normally-distributed variables and as absolute and relative frequencies for categorical variables. Tumor markers CA 19-9 and CEA deviated from normal distribution and are presented as geometrical mean and 95<sup>th</sup> confidence interval. For inter-group comparison we have used Student-t test and ANOVA, with distinct *post hoc* test where appropriate, and chi-square test for frequency data comparison.

We performed receiver operating characteristic (ROC) curves analysis and sensitivity and specificity calculation within a group consisting of the control group and all CRC patients (Group I), and within a group consisting of the control group and patients classified as Dukes A/B (Group II). Additionally, we used ROC analysis to test CEA and CA 19-9 capability to discriminate subjects with and without CRC. The SPSS 18.0 software package (SPSS, Chicago, IL, USA) and Medcalc software, version 13.2 were used for all calculations.

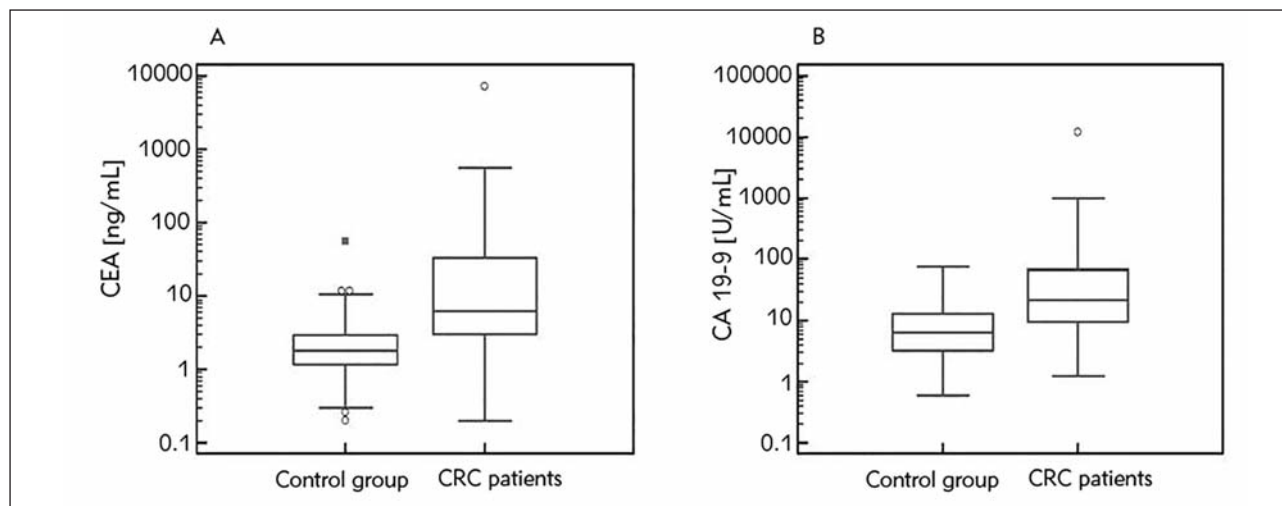
### Results

Results presented in *Table I* show values of CEA and CA 19-9 in the whole control group, as well as in female and male subjects separately. The values of

**Table I** CEA and CA 19-9 values in control group.

	CEA	CA 19-9
Control	1.82 (1.64–2.02)	7.03 (6.20–7.97)
Female	1.64 (1.41–1.91)	6.96 (5.75–8.42)
Male	2.01 (1.73–2.34)	7.10 (6.00–8.40)

Values presented as geometric mean and 95% confidence interval.

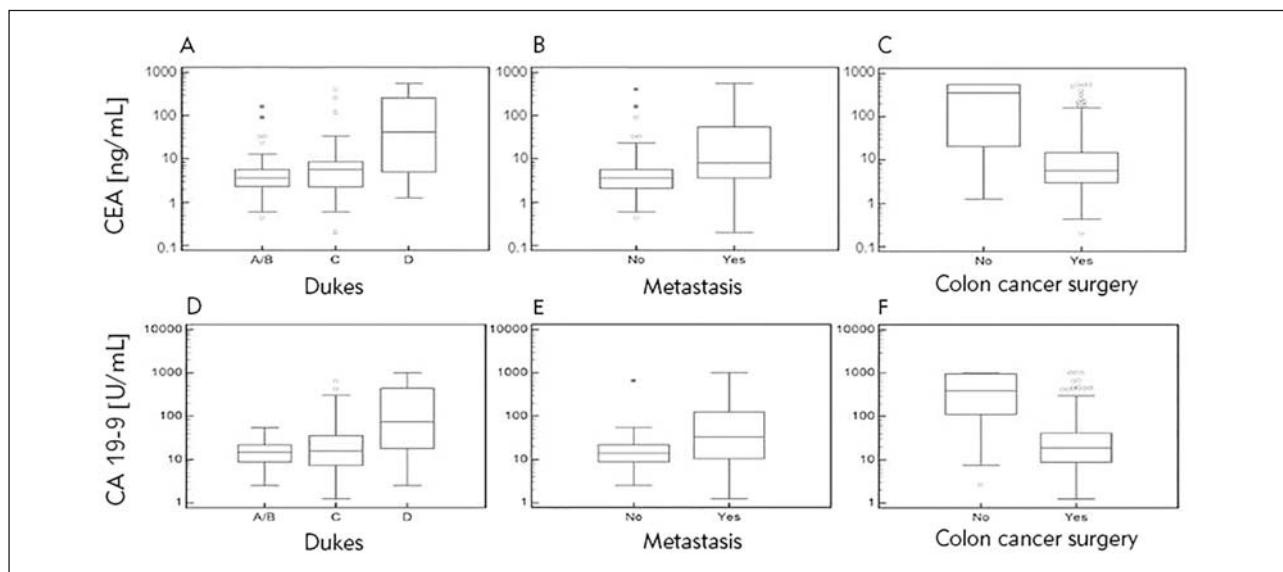


**Figure 1** CEA (A) and CA 19-9 (B) values in control group and CRC patients.

**Table II** CEA and CA 19-9 values in CRC group and number of values higher than cut-off.

	Number of patients	CEA (ng/mL)	Number of values >5 (ng/mL)	CA 19-9 (U/mL)	Number of values >35.4 (U/mL)
Patients	181	10.29 (7.82–13.56)	102 (56.3%)	28.79 (22.59–36.71)	67 (37.0%)
Female	72 (39.8%)	10.45 (7.13–15.31)	41 (37.6%)	28.44 (20.38–39.69)	27 (24.8%)
Male	109 (60.2%)	10.07 (6.81–14.88)	61 (84.7%)	29.34 (20.61–41.76)	40 (55.6%)
Age					
≤60 years	45 (24.9%)	8.62 (5.31–13.98)	22 (48.9%)	23.79 (15.34–36.88)	14 (31.1%)
61–70 years	74 (40.9%)	11.21 (7.05–17.84)	41 (55.4%)	23.84 (16.56–34.32)	30 (40.5%)
>70 years	62 (34.2%)	10.63 (7.05–17.84)	39 (62.9%)	41.08 (25.79–65.46)	23 (37.1%)
Tumor stage – Dukes					
A/B	43 (23.8%)	4.12 (2.84–5.97)	13 (30.2%)	13.66 (10.78–17.32)	4 (9.3%)
C	70 (38.7%)	5.19 (3.81–7.07)	38 (54.3%)	17.35 (12.71–23.70)	18 (25.7%)
D	68 (37.6%)	37.22 (22.77–60.84) **	51 (75.0%) **	77.72 (49.09–123.03) **	45 (66.2%) **
Histological examination (report)					
Poorly differentiated	16 (8.8%)	7.69 (3.00–19.72)	7 (43.7%)	27.03 (11.29–64.74)	6 (37.5%)
Moderately differentiated	108 (59.7%)	10.99 (7.63–15.82)	64 (59.2%)	28.45 (20.53–39.44)	42 (38.9%)
Well differentiated	57 (31.5%)	9.87 (6.02–16.20)	31 (54.4%)	29.98 (19.74–45.54)	19 (33.3%)
Macroscopic tumor appearance					
Luminal stenosis	90 (49.7%)	12.51 (8.48–18.43)	56 (62.2%)	25.99 (18.38–36.73)	30 (33.3%)
Polypoid type	76 (42.0%)	8.35 (5.32–13.11)	37 (48.7%)	29.93 (20.58–43.51)	31 (40.8%)
Ulcerative type	15 (8.3%)	9.24 (4.05–21.07)	9 (60.0%)	43.86 (16.25–118.40)	6 (40.0%)
Tumor localization					
Rectum	60 (33.2%)	11.21 (6.93–18.14)	35 (58.3%)	32.00 (20.81–49.20)	23 (38.3%)
Left colon	70 (38.7%)	12.26 (7.61–19.75)	40 (57.1%)	24.76 (16.54–37.06)	29 (41.4%)
Right colon	51 (28.1%)	7.33 (4.54–11.84)	27 (52.9%)	31.30 (19.92–49.21)	15 (29.4%)
Metastases					
Yes	130 (71.8%)	13.75 (9.86–19.17) **	86 (66.2%) **	36.42 (26.91–49.30) **	62 (47.7%)**
No	51 (28.2%)	4.18 (2.90–6.02)	16 (31.4%)	13.85 (10.97–17.50)	5 (9.8%)
Colon cancer surgery					
Yes	160 (88.4%)	7.73 (6.01–9.95)	86 (53.7%)	21.77 (17.39–27.26)	48 (30.0%) **
No	21 (11.6%)	91.34 (31.82–262.19) **	16 (76.2%) *	242.4 (113.5–517.3) **	19 (90.5%) **

Continuous variables are expressed as geometric mean and 95% confidence interval derived from log-normal distribution. Categorical variables are expressed as absolute and relative frequencies \* $p < 0.05$ , \*\* $p < 0.01$ .



**Figure 2** CEA (A-C) and CA 19-9 (D-F) values in patients according to Dukes classification, presence of metastases and following colon cancer surgery.

**Table III** The results of ROC analysis, sensitivity and specificity for individual and combined serum detection of CEA and CA 19-9.

	AUC (95% CI)	Sensitivity (95% CI)	Specificity (95%)
CEA			
Group I	0.815 (0.772–0.853)	76.8 (70.0–82.7)	76.6 (69.9–82.4)
Group II	0.728 (0.666–0.784) #	76.7 (61.4–88.2)	66.7 (59.5–73.3)
CA 19-9			
Group I	0.768 (0.722–0.810)	69.1 (61.8–75.7)	73.3 (66.4–79.4)
Group II	0.720 (0.658–0.776) #	76.7 (61.4–88.2)	60.7 (53.4–67.7)
CEA + CA 19-9			
Group I	0.842 (0.801–0.877) *	73.5 (66.4–79.8)	83.3 (77.2–88.2) *
Group II	0.773 (0.714–0.825) #	72.1 (56.3–84.7)	73.3 (66.4–79.4) *

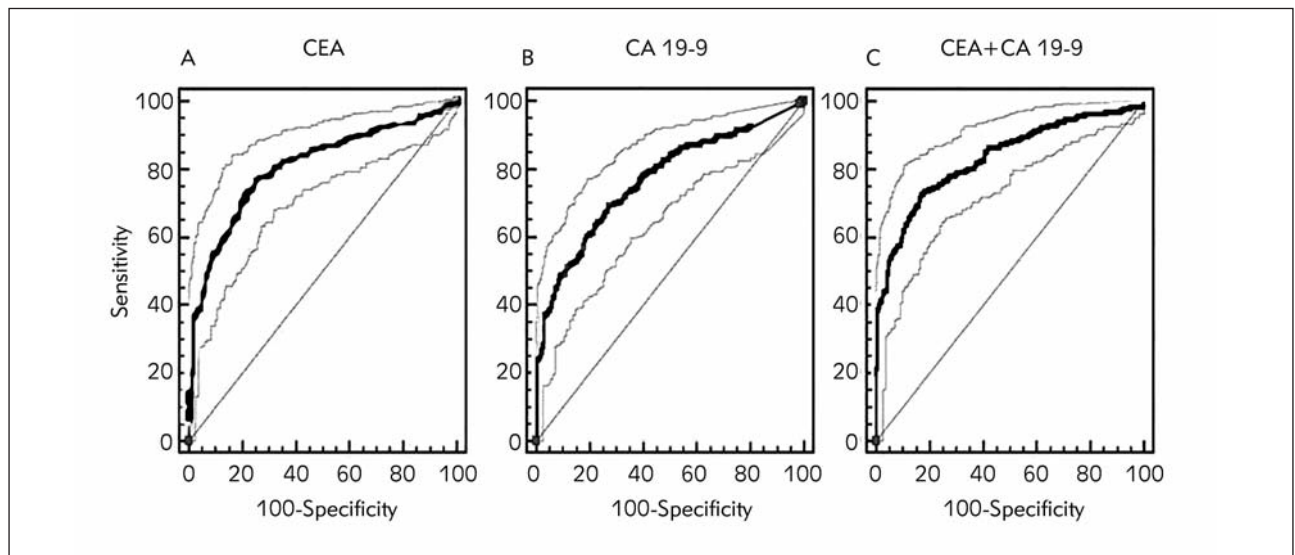
Group I: Control group plus all patients (n=372), Group II: Control group plus patients classified as Dukes A/B (n=234), \*p<0.05 combined vs individual detection of tumor markers, # p<0.05 Group I vs Group II.

both tumor markers were not significantly different between female and male subjects.

The levels of CEA and CA 19-9 in the CRC group were significantly higher compared with the control group (Figure 1). There was no significant difference between female and male subjects in the patient group (Table II). In patients divided according to age (<60, 60–70 and >70 years), histological examination (poorly, moderately and well differentiated), macroscopic tumor appearance (luminal stenosis, polypoid and ulcerative type) and tumor localization (rectum, left and right colon), there was no significant difference in CA 19-9 and CEA values, and

the numbers of the measured concentration for both markers were higher than the cut-off.

In patients with the D tumor stage (Dukes classification A/B, C and D), significantly higher CA 19-9 and CEA level was observed (Figure 2). Results for the level of both markers for patients with A/B and C tumor stage were similar. Furthermore, patients with tumor stage D also had a significantly higher level of both markers which was beyond cut-off for both tumor markers. Patients with metastases showed significantly higher values of CA 19-9 and CEA than patients without metastases (Figure 2), with almost 50% higher levels of CA 19-9 and more than 50% higher lev-



**Figure 3** ROC curves for single detection of CEA (A) and CA 19-9 (B) and combined detection of CEA plus CA 19-9 (C).

els for CEA than cut-off. Patients with inoperable CRC had extremely higher levels of both markers compared with the patients that had undergone surgery (Figure 2). Patients with inoperable cancer had 90% higher values of CA 19-9 and 76% higher values of CEA than cut-off, respectively.

In further analysis, we performed ROC analysis and sensitivity and specificity calculation in the group consisting of control group and all patients (Group I) and in the group consisting of control group and patients classified as Dukes A/B (Group II). In group I, the sensitivity (76.8%), as well as specificity (76.6%) for CEA alone was higher, but not significantly compared with CA 19-9 (Table III). In contrast, the sensitivity of combined serum concentration of CA 19-9 and CEA was 73.5%, lower than the sensitivity of CEA, but higher than the sensitivity of CA 19-9. Specificity of the combined detection of both tumor markers was 83.3%, and higher than for the detection of CEA and CA 19-9 alone. Results obtained for Group II patients (classified as Dukes A/B) show that the sensitivity of CEA alone was the same as in Group I, as well as the sensitivity of CA 19-9 in Group II. The specificity of CEA and CA 19-9 in Group II was lower than in Group I. The sensitivity and specificity of the combined detection of CEA and CA 19-9 in Group II were lower than in Group I.

Results presented in Figure 3 show that CA 19-9 alone had the lowest discriminatory power in Group I, while the combined detection of CEA and CA 19-9 had the significantly highest discriminatory potential in Group I. The values of AUCs in Group II were significantly lower than the values in Group I for both individual and combined detection of CEA and CA 19-9 (Figure 3).

## Discussion

Results presented in this study show that there is no correlation between increased levels of CEA, CA 19-9, and number of »above the cut-off« values with age, macroscopic and histological characteristics of the tumor, as well as with its localization. We found no differences in the levels of both tumor markers, i.e. the levels of both tumor markers in the Dukes A/B and C were similar. Subjects that suffered from Dukes D CRC had significantly higher levels for both tumor markers and a higher number of »above the cut-off« values than subjects that suffered from other Dukes stages. The highest distinction power between patients with CRC and healthy subjects is shown by the united analysis of both tumor markers (CEA and CA 19-9).

The gender differences analysis in our patients with CRC has shown that men are 1.5 times more often prone to disease than women. Our results are consistent with the results from other studies, showing that men are 1.5 times more often affected than women, in North America and Western Europe, and 1.6 times more often in Australia (1). Also, mortality rates in these patients are similar in relation to gender. In addition, men worldwide have an increased CRC mortality rate, from 1.2 to 1.6, compared with women (1). Furthermore, the results show that women have a lower risk of CRC until menopause when compared with men, but later in life the risks are equalized (17). In our study, we found that the levels of both tumor markers were not significantly different nor in the control group, as well as the values in CRC patients between female and male subjects.

Age is an important risk factor for CRC. There are very rare cases of developing CRC before the age of 40 years, while in the coming decades that num-

ber may increase significantly. Accordingly, 90% of CRC is detected in people older than 50 years (18). In Serbia, CRC was most commonly detected between 70 and 75 years of age (1), and in our study the average age of patients was 66.7 years. Our results show no statistically significant differences in the values of CA and CA 19-9 related to age. In this group of patients, the highest average value of the CA 19-9 marker was in subjects aged over 70 years (41.08 U/mL), and for CEA in patients between 60 and 70 years of age (11.21 ng/mL). These results suggest that age is not a significant factor influencing the serum concentrations of tested tumor markers in patients with CRC. These findings are consistent with the results reported by Duffy (19).

Our results, related to the analysis of the correlation between tumor markers and colonoscopy findings of tumor localization, show that the most frequent localization was on the left colon (38.7%), followed by the rectum (33.2%) and on the right colon (28.1%). Serum concentrations of CEA and CA 19-9 did not show statistically significant differences in relation to the localization of the tumor.

Literature data related to the level of CEA and tumor localization vary from study to study. For example, Vukobrat-Bijedic et al. (20) found that the highest value of CEA is on the right colon tumor localization, while Wilson et al. (21) found that high serum CEA concentrations are more common in tumors of the left colon. Colon tumor can lead to stenosis of the lumen, have a polypoid appearance or be of the ulcerative type. In our study, colon stenosis was found in 49.7% of patients, polypoid appearance had 42% occurrence, and the rarest, ulcerative type of tumor was detected in 8.3% of our patients. The highest value of CEA was found in patients with luminal stenosis and the highest level of CA 19-9 in patients with the ulcerative tumor types, but without any statistical significance. Similar results were reported by Li et al. (22). In addition, Sugarbaker found that obstruction of the CRC process provides the highest concentration of CEA. Decompression after operative treatment reduces the serum value of CEA (23).

In our study, we did not find any correlation between histological differentiation (well, moderately and poorly differentiated type of tumor) and an increase in the value of CEA and CA 19-9. Results reported by Bhatnagar et al. (24) show that well-differentiated tumors have a higher production of CEA. Furthermore, Pakdel et al. (25) suggested that the serum concentration of CEA is produced by tumor cells and that it depends on differentiation, vascularization and elimination, which is under the control of special molecular processes (GPI – PLD enzymes) but the mechanism is not fully understood.

According to the Dukes classification, 43 of the patients included in the presented study (23.8%) were at stage A/B, 70 (38.7%) were at stage C and 68

(37.6%) were at stage D. Mean values of CEA (37.2 ng/mL) and CA 19-9 (77.72 U/mL) were highest in patients in Dukes D stage. The values in this group were significantly higher than the values of these tumor markers in stage A/B and C. Groups with stage A/B and C did not have a statistically significant difference in the mean concentration of the marker. The number of patients with the values of the markers above the cut-off limits was also significantly higher in patients in Dukes D stage. Our findings show that the concentrations of tumor markers are increased with the degree of tumor progression and significantly increased with the emergence of metastases in distant organs (liver, lungs). Thus, in our study, there is a statistically significant difference between patients with metastases and patients without metastatic disease.

In our study, out of the total number of 181 patients with CRC, 160 had surgical treatment (88.4%) while 21 (11.6%) were classified as inoperable. The results show that the levels of both markers, CEA and CA 19-9, were statistically significantly higher in the group of inoperable patients (91.34 ng/mL and 242.4 U/mL, respectively). High concentrations of the tumor markers CEA and CA 19-9 in patients with CRC are signs of advanced disease and poor prognosis (19). Elevated values of the tumor markers in the lower stages of the disease (Dukes B) may indicate the need for adjuvant chemotherapy following surgery (13). It has been shown that CEA is an independent prognostic factor but also a predictive factor in patients with clinical D stadium, Dukes B (13).

Although the biomarkers CEA and CA 19-9 are today widely used in the diagnosis, staging and CRC screening (26), results obtained by using ROC curves analysis show that CEA can provide more power of discrimination between diseased and healthy patients than CA 19-9 (the areas under the ROC curve (AUC) were 0.815 and 0.768 for CEA and CA 19-9, respectively) (Table II, Figure 3).

Our findings are consistent with the results reported by others, also showing that CA 19-9 is less sensitive than CEA in the diagnosis of CRC (27). However, the combined detection of CEA and CA 19-9 has a significantly higher potential of separation (AUC 0.842) in subjects with and subjects without CRC (Figure 3). When the ROC analysis is applied only to patients with Dukes stage A/B (Group II), AUC values were significantly lower, for both individual (CEA 0.728, CA 19-9 0.720), and combined detection of CEA and CA 19-9 (AUC 0.773). In Group II, CEA sensitivity alone was the same, the specificity of both CEA and CA 19-9 was lower and the sensitivity and specificity of the combined detection of CEA and CA 19-9 were also lower in relation to the Group I, which was the group with patients suffering from CRC in all stages of the disease (Table II). In addition to the widely used tumor markers CEA and CA 19-9, there

is an indication of the importance of new biomarkers that might be able to improve the diagnosis of CRC (28, 29). Studies by other authors suggest that, although elevated to a significant proportion in pre-clinical CRC, CEA alone is not sufficient as a screening tool. Therefore, experts are working on a basis for a panel of biomarkers that, combined with CEA, would improve early detection of CRC (30).

Our results, together with literature data (19), suggest that when the lack of sensitivity and specificity of CEA is combined with the low prevalence of CRC in an asymptomatic population, CEA cannot be used as a screening method (19). Considering that in our study the sensitivity of CEA is similar in patients classified as Dukes A/B as in the whole CRC patient group, we suggest that CEA might be useful for detection of both CRC

and patients in early stages. Although testing for CEA in the general population is not recommended as a screening method, if the levels of CEA markers are elevated, even just above the cut-off limit, it is an indication to consider the need for further diagnostic procedures, however, taking into account the general condition and comorbidities. In conclusion, simultaneously testing CEA and CA 19-9 can improve safety and increase diagnostic sensitivity in identifying people with CRC.

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### Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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