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Changes in lecithin: cholesterol acyltransferase, cholesteryl ester transfer protein and paraoxonase-1 activities in patients with colorectal cancer

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ABSTRACT

Background: Previous studies revealed decreased level of high-density lipoprotein cholesterol (HDL−C) as important factor for development of colorectal cancer (CRC). Quantity and structure of HDL particles depend on activities of lipid transfer proteins lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP), but this topic is largely unexplored in CRC. The main objective of this study was to investigate activities of LCAT and CETP in patients with CRC. Additionally, we analyzed activity of paraoxonase-1 (PON-1), as a main carrier of HDL-antioxidant function.

Materials and methods: Ninety-nine CRC patients and 101 healthy individuals were included. LCAT and CETP activities were assessed by measuring rates of formation and transfer of cholesteryl esters. PON-1 paraoxonase and arylesterase activities were measured.

Results: Lower levels of HDL-C ($p < .001$) were observed in cohort of patients, alongside with decreased LCAT ($p < .050$) and increased CETP activity ($p < .050$). Both PON-1 activities were diminished in CRC ($p < .050$ and $p < .001$ respectively). Univariate logistic regression singled out HDL-C level (OR = 0.218, $p < .001$), CETP activity (OR = 1.010, $p < .01$) and mass (OR = 0.994, $p < .001$) as possible markers of elevated CRC risk. CETP mass maintained its predictive significance when adjusted for traditional risk factors and level of oxidative stress (OR = 0.993, $p < .001$; OR = 0.982, $p < .050$, respectively).

Conclusion: Our results demonstrated increased CETP and decreased LCAT and PON-1 activities in CRC patients. In preliminary analysis CETP mass was identified as potential significant predictor of CRC development, suggesting that alterations in HDL-C levels, alongside with changes in HDL structure might have a role in carcinogenesis.

1. Introduction

Colorectal cancer (CRC), being the third leading cause of malignancies in males and second in females [1], is one of the most important health problems of the modern world. It is a multifactorial disease which is connected with several genetic mutations [2,3]. However, comprehensive researches have singled out many cases without clearly identified genetic causes, suggesting that environmental factors could be equally important as the inheritance [4,5]. Risk factors such as age, low fiber diet, obesity, low physical activity and alcohol are associated with increased risk for development of CRC [5].

Review studies emphasized the significance of hyperlipidemia in pathogenesis of CRC, mostly in development of adenomatous polyps that precedes the onset of malignancy [6]. Therefore, changes in plasma lipid levels could be of analytical interest for prediction of the risk for the development of CRC. Indeed, numerous studies have analyzed

Abbreviations: CRC, colorectal cancer; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; ROS, reactive oxygen species; PAB, prooxidant/antioxidant balance; PON-1, paraoxonase-1; LDL-C, low-density lipoprotein cholesterol; FC, free cholesterol; CE, cholesteryl esters; ANCOVA, Analysis of covariance; BMI, body mass index; ρ, Spearman's rank correlation coefficient; OR, Odds ratios; 95% CI, 95% confidence intervals; VLDL, very low density lipoprotein

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associations of dyslipidemia and CRC risk, but the obtained results were conflicting. Still, more recent meta-analysis has revealed high levels of total cholesterol (TC) and triglycerides (TG), alongside with low concentration of high-density lipoprotein cholesterol (HDL-C), as determinants of increased risk for development of CRC [7]. In addition, Park et al. recently proposed a scoring system for prediction of advanced CRC neoplasm which included concentrations of TG and HDL-C [8]. However, the exact mechanism by which different components of dyslipidemia contribute to the development of CRC is still largely unknown. Moreover, previous studies were usually based on estimation of routine lipid status, while advanced lipid testing is rarely done in this population.

In spite of inconsistent results for other lipid markers, decreased HDL-C is repeatedly recognized as an important factor for development of colon cancer [9]. Having this in mind, it could be of special interest to additionally explore the activities of lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP), as two key modulators of HDL particle morphology and HDL-C levels. Investigating CETP activity in CRC could be particularly important, knowing that this lipid transfer protein connects different classes of lipoproteins and responds to changes in plasma lipid levels. Since hypertriglyceridemia provokes changes in CETP activity, which in turn affects HDL-C level, such analysis could also provide a better insight into complex disturbances of lipid profile in CRC. Although CETP activity is widely explored in cardiovascular diseases, according to our current knowledge, no data is available regarding this topic in CRC patients.

Significantly disrupted lipid balance is often accompanied with unstable pro-oxidant/antioxidant status. It is known that reactive oxygen species (ROS) primarily cause damage of cell membranes, DNA impairment and lipid peroxidation [10,11], altogether resulting in defective antioxidant protection system and enhanced onset of malignancies [12,13]. Up till now, numerous molecules were proposed as markers of elevated oxidative stress and weakened antioxidative defence, but it has been suggested that concomitant measurement of prooxidant/antioxidant balance (PAB) provides the most complete information on redox state of the organism [14]. On the other hand, antioxidative defence system in plasma contains an ample of various compounds, including HDL particles. HDL is unique among lipoproteins since it contains a battery of antioxidant molecules, while the main carrier of HDL antioxidative potential is the enzyme paraoxonase-1 (PON-1). It has been shown that altered HDL particle composition and HDL-C level affect PON-1 activity in atherosclerosis-related diseases [15], but studies of PON-1 in CRC patients were not conducted so far.

Since, dyslipidemia and elevated oxidative stress are considered as two etiological factors that contribute to the development of CRC, our goal was to simultaneously investigate lipid transfer proteins LCAT and CETP, as well as PON-1 activity, in an attempt to shed light on the role of structural and functional alterations of HDL in the onset of CRC. In addition, we sought to explore whether any of these parameters has a potential to be recognized as a candidate prognostic marker for CRC development and to preliminary evaluate their diagnostic accuracy for potential use in clinical practice.

2. Subjects and methods

2.1. Patients

For this study, we enrolled 126 patients who underwent elective resection for CRC in period from July 2014 to January 2016 at the Clinic for General Surgery, Military Medical Academy in Belgrade. Inclusion criteria were as follows: adult age, the first onset of the disease, no other malignant diseases in personal anamnesis, no previous treatment with neoadjuvant therapy, no severe physical disability and no use of anti-hyperlipidemic medication. Postoperative histological diagnosis was obtained for each patient. In 5 cases routine histological analysis of resected tissue ruled out CRC, while in rest of 121 cases adenocarcinoma was histopathologically confirmed. Routine questionnaire, containing basic information on age, anthropometric characteristics and lifestyle habits, was fulfilled for each patient by trained medical stuff. Due to uncomplete data, 16 patients were excluded from the study and uncompleted laboratory analyses cause exclusion of 6 more samples, so the final group consisted of 99 patients with CRC.

The control group consisted of 101 adult healthy volunteers who attended regular medical check-up at Medigroup General Hospital in Belgrade. All participants met the eligible criteria: absence of any present or previous malignant disease, absence of chronic heart, kidney or liver disease and no use of any anti-hyperlipidemic drugs. The same questionnaire as for the CRC patients was fulfilled for all healthy subjects. All participants signed an informed consent prior to the enrollment. The entire study protocol was designed and conducted according to the ethical guidelines defined by the Helsinki Declaration and approved by the local ethical committee.

2.2. Laboratory analyses

Blood samples were collected from CRC patients immediately before surgical procedure and after a 12-h fasting period, while in control subjects blood samples were drawn at the commencement of medical examination, after an overnight fasting. The samples were taken into evacuated tubes containing EDTA and serum sample tubes. Plasma and serum were separated by immediate centrifugation at 1500 $\times g$ for 10 min at 4 °C. Aliquots of each sample were stored at −80 °C. The samples were thawed immediately before analyses.

Concentrations of glucose, total proteins, albumin, TC, TG, low-density lipoprotein cholesterol (LDL-C) and HDL-C were assessed by routine laboratory methods on ILab 300+ (Instrumentation Laboratory, Diamond Diagnostics, USA). A procedure previously described by Asztalos and Fielding was used for LCAT and CETP activities determination [16]. Since LCAT is responsible for esterification of cholesterol in plasma during maturation of HDL particles, measuring of LCAT activity was based on determining the difference in the amount of free cholesterol (FC) before and after incubation of samples on 37 °C [16]. LCAT activity was expressed as a total amount of formed cholesteryl esters (CE) upon thermal activation of the enzyme. CETP mass was determined by commercial ELISA method (Cell Biolabs,Inc.), while CETP activity was assessed as CETP mediated transfer of CE between HDL and TG-rich lipoproteins, prior and after the incubation of samples on 37 °C for 2 h. Total CETP activity was analyzed regardless of the direction of CE transfer. The calculation was made according to the following equation:

$$\begin{aligned}\text{CETP activity} = &[(\text{initial plasma FC} - \text{final plasma FC})] \\ &- [(\text{final HDL} - \text{TC} - \text{final HDL} - \text{FC}) \\ &- (\text{Initial HDL} - \text{TC} - \text{Initial HDL} - \text{FC})]\end{aligned}$$

PON-1 paraoxonase activity was determined towards substrate paraoxon and its conversion to p-nitrophenol anion, which absorption maximum was kinetically followed on 405 nm [17]. Arylesterase activity of PON-1 was assessed by measuring of the rate of phenylacetate hydrolysis through spectrophotometric determination of an increase in phenol concentration at 270 nm [18]. In order to determine PON-1 phenotype, we plotted arylesterase against paraoxonase activity [19]. Two distinct PON-1 phenotypes were separated: phenotype A with low paraoxonase activity and phenotype B with high paraoxonase activity. A modified method of Hamidi-Alamdari et al. [14] was used to obtain PAB values. This method is based on the use of tetramethylbenzidine cation for reaction with prooxidans, and antioxidants at the same time. Prooxidant capacity was calibrated according to hydrogen peroxide, and anti-oxidant capacity calibration was performed according to uric acid.

2.3. Statistical analysis

Data that followed normal distribution are shown as mean ± standard deviation, while for log-normally distributed data, geometrical mean with 95% confidence interval was used. Median with interquartile range was used for presenting asymmetrically distributed data. Since age differences between our groups were significant, parametric and non-parametric ANCOVA were applied for further analyzing of data, with age as a covariate. Spearman correlation analysis was performed for investigating possible associations between biochemical parameters with LCAT, CETP mass and its activity, PON-1 activity and values of PAB. Univariate and multivariate binary logistic regression analysis was applied for estimation of parameters' ability to predict CRC development. Discriminative abilities of examined parameters were assessed by Receiver Operating Characteristics (ROC) curves, as global measures of diagnostic accuracy. According to established criteria, Area Under the Curve (AUC) was used for evaluation of diagnostic accuracy [20]. Statistical analyses were done by using IBM® SPSS® model 22.0. Differences at $P < .05$ were considered as significant.

3. Results

Basic anthropometric parameters and results of biochemical analyses are presented in Table 1. The examined groups didn't differ by gender, but there was a significant age difference. In order to minimize the influence of age to other results, all further analyses were performed with correction for age. BMI values were similar among the analyzed groups. Concentrations of total proteins and albumin were significantly lower in patients with CRC. Glucose levels did not show significant differences among patients and control group. Concentration of TG was similar between the groups, while concentrations of TC, LDL-C and HDL-C were statistically lower in patients.

Next, we examined activities of lipid transfer proteins and PON-1, as well CETP mass and PAB levels in our two groups (Table 2). CETP mass was lower, while CETP activity was significantly higher; LCAT was less active in patients with CRC. We have also examined parameters of prooxidant/antioxidant status. Statistically significant differences among our cohorts were demonstrated regarding PAB and both paraoxonase and arylesterase PON-1 activities. PAB levels were higher, while PON-1 activities were lower in CRC patients when compared to healthy subjects. Finally, even if we did observe higher prevalence of PON-1 phenotype A, characterized by lower paraoxonase activity in CRC patients, the difference in phenotypic distribution among the groups did not reach statistical significance (Table 2).

Table 1
Basic anthropometric parameters and biochemical results in analyzed groups.

	CRC patients ($N = 99$)	Control group ($N = 101$)	P
Age (years)[#]	64.9 ± 10.7	54.6 ± 7.72	< 0.001
Gender (m/f)[&]	66/33	56/45	0.113
BMI (kg/m^2)	25.2 ± 3.28	25.8 ± 3.32	0.052
Albumin (g/L)	39.1 ± 4.97	47.3 ± 4.03	< 0.001
Total proteins (g/L)	65.8 ± 7.26	72.7 ± 7.07	< 0.001
Glucose (mmol/L)	5.81 ± 1.42	5.49 ± 0.900	0.567
TC (mmol/L)	4.52 ± 1.17	5.59 ± 1.00	< 0.001
TG (mmol/L)[*]	1.29 (1.20–1.38)	1.24 (1.15–1.34)	0.357
HDL-C (mmol/L)	1.04 ± 0.392	1.33 ± 0.501	< 0.001
LDL-C (mmol/L)	2.86 ± 1.03	3.66 ± 0.929	< 0.001

Data are presented as mean ± standard deviation and compared by ANCOVA with age as covariate.

[*] Data are presented as geometrical mean (95% confidence interval for mean).

[#] Comparison was performed by Student *t*-test.

[&] Data are presented as absolute frequencies and comparison was performed by Chi-square test.

In a cohort of CRC patients, we observed strong positive correlation between LCAT and CETP ($\rho = 0.588$, $P < .001$). Correlations of PON-1 paraoxonase activity with TC ($\rho = 0.350$, $P < .01$) and HDL-C ($\rho = 0.277$, $P < .05$) were significant. PON-1 arylesterase activity significantly correlated with HDL-C ($\rho = 0.285$, $P < .05$). LCAT positively correlated with TC concentrations ($\rho = 0.232$, $P < .05$). In addition, we sought for correlations of PAB with LCAT, CETP and PON-1 activities. In patients group, there were no significant correlations of PAB and other specified parameters (data not shown). However, CETP mass significantly positively correlated with PAB ($\rho = 0.321$, $P < .05$).

When we analyzed correlations in healthy subjects, we found positive correlations of BMI with glucose ($\rho = 0{,}215$, $P < .050$) and TG ($\rho = 0{,}294$, $P < .01$), while negative between BMI and HDL-C ($\rho = -0.274$, $P < .01$). TG levels were in negative correlation with HDL-C ($\rho = -0.544$, $P < .001$), but in positive with LDL-C ($\rho = 0.354$, $P < .001$) and LCAT ($\rho = 0.329$, $P < .01$). Concentration of HDL-C negatively correlated with TG ($\rho = -0.544$, $P < .001$), LDL-C ($\rho = -0.210$, $P < .05$) but also with LCAT activity ($\rho = -0.220$, $P < .050$). Positive correlation was noticed between HDL-C and PAB ($\rho = 0.352$, $P < .050$). We have shown that there is a significant positive correlation between LCAT and CETP activities ($\rho = 0.412$, $P < .001$) as well as between LCAT activity and concentrations of LDL-C ($\rho = 0.358$, $P < .01$). No significant associations between PAB and LCAT and CETP activities were found in the control group (data not shown). In addition, except for positive correlation between PON-1 arylesterase activity and TC ($\rho = 0.216$, $P < .05$), we did not find any significant correlations between the examined markers of lipid status and PAB with PON-1 paraoxonase or arylesterase activity in healthy individuals.

Next we explore differences in LCAT and CETP activities and CETP mass according to PON-1 phenotypes. In CRC patients, we did not find any significant differences in examined parameters among individuals with different PON-1 phenotypes (data not shown). In healthy individuals, CETP activity was significantly higher ($P < .05$) in subjects with PON-1 phenotype A (median: 62.58, interquartile range: 42.25–85.08 μmol/L/h) when compared to PON-1 phenotype B carriers (median: 33.25, interquartile range: 21.13–57.33 μmol/L/h). Other variables did not significantly differ among subjects with PON-1 phenotypes A or B (data not shown).

We used univariate logistic regression to identify possible prognostic markers of CRC. Odds ratios (OR) and 95% confidence intervals (95% CI) for activities of CETP, LCAT and PON-1, as well as CETP mass are presented in Table 3. Performed univariate analysis has singled out CETP activity and mass, HDL-C concentration, PAB levels and PON-1 arylesterase activity as significant variables, while OR values for LCAT and PON-1 paraoxonase activity did not reach statistical significance (Table 3).

To further explore possible independent potential of CETP activity and mass in prediction of CRC development, we made separate models of multivariate logistic regression analysis: the first with the adjustment for age, gender, TG, HDL-C and LDL-C levels, and the second with the adjustment for PON-1 arylesterase activity and PAB, as markers of oxidative stress. The above-mentioned parameters were selected for creating models due to their possible relevance in the development of CRC. CETP activity remained significant in the model 1. However, in the model 2, when adjusted for parameters of oxidative stress, CETP activity lost its significance (Table 4).

Similar analysis, performed for exploration of possible role of CETP mass in prediction of risk for CRC development, was presented in Table 5. Equally to CETP activity, CETP mass was significant predictor in the model 1. However, even after inclusion of PAB and PON-1 arylesterase activity (Model 2), CETP mass retained its significance in prediction of CRC risk. In addition, after adjustment for serum albumin levels, as indicators of synthetic liver function, CETP mass remained significant marker in logistic regression analysis (OR: 0.994; CI: 0.990–0.998; $P < .01$).

Table 2
Lipid transfer proteins, PAB and PON-1 in analyzed groups.

	CRC patients (N = 99)	Control group (N = 101)	P
LCAT activity (μmol/L/h)	77.4 (50.0–105.7)	96.2 (72.9–116.5)	< 0.050
CETP activity (μmol/L/h)	64.8 (40.4–100.2)	48.5 (22.3–77.4)	< 0.050
CETP mass (ng/mL)	315.8 (194.7–469.4)	597.5 (462.1–794.4)	< 0.001
PAB (HKU)	434.6 (183.4–739.4)	66.0 (50.9–92.6)	< 0.001
PON-1 paraoxonase activity (U/L)	193.5 (136.0–574.5)	296.5 (214.3–646.0)	< 0.050
PON-1 arylesterase activity (kU/L)	90.60 (62.50–114.80)	114.30 (101.25–134.60)	< 0.001
PON-1 phenotype A (%)*	70.18	64.10	0.360

Data are presented as median (interquartile range) and compared by non-parametric ANCOVA with age as a covariate.
* Data are presented as relative frequencies and compared by Chi-square test.

Table 3
Univariate binary logistic regression analysis for associations between the examined markers and risk for development of CRC.

Parameter	OR	95% CI	P
CETP activity (μmol/L/h)	1.010	(1.004–1.017)	< 0.01
CETP mass (ng/mL)	0.994	(0.992–0.996)	< 0.001
LCAT activity (μmol/L/h)	1.002	(0.997–1.006)	0.468
HDL-C (mmol/L)	0.218	(0.104–0.458)	< 0.001
PON-1 paraoxonase activity (U/L)	0.999	(0.998–1.000)	0.058
PON-1 arylesterase activity (U/L)	0.971	(0.959–0.983)	< 0.001
PON-1 phenotype A (%)	0.778	(0.374–1.621)	0.503
PAB (HKU)	1.068	(1.035–1.102)	< 0.001

Variables are entered as continuous, except for PON-1 phenotype (1-A phenotype, 0-B phenotype).

Table 4
Multivariate binary regression analysis for independent associations between CETP activity and risk for development of CRC.

Parameter	OR	95% CI	P
	Model 1		
CETP activity (μmol/L/h)	1.010	1.001–1.020	< 0.05
Age (years)	1.123	1.070–1.177	< 0.001
Gender (m/f)	0.678	0.268–1.713	0.411
LDL-C (mmol/L)	0.426	0.274–0.660	< 0.001
HDL-C (mmol/L)	0.145	0.046–0.453	< 0.001
TG (mmol/L)	0.759	0.349–1.651	0.487
	Model 2		
CETP activity (μmol/L/h)	0.984	0.961–1.007	0.160
PON-1 arylesterase activity (U/L)	0.997	0.964–1.031	0.868
PAB (HKU)	1.074	1.029–1.120	< 0.01

Enter model.
All variables are entered as continuous except for: gender (0-female, 1-male).

Table 5
Multivariate binary regression analysis for independent associations between CETP mass and risk for development of CRC.

Parameter	OR	95% CI	P
	Model 1		
CETP mass (ng/mL)	0.993	0.990–0.996	< 0.001
Age (years)	1.139	1.078–1.204	< 0.001
Gender (m/f)	0.643	0.224–1.845	0.411
LDL-C (mmol/L)	0.481	0.305–0.759	< 0.01
HDL-C (mmol/L)	0.160	0.047–0.548	< 0.01
TG (mmol/L)	1.354	0.564–3.249	0.498
	Model 2		
CETP mass (ng/mL)	0.982	0.966–0.997	< 0.05
PON-1 arylesterase activity (U/L)	0.968	0.923–1.015	0.181
PAB (HKU)	1.093	1.018–1.175	< 0.05

Enter model.
All variables are entered as continuous except for: gender (0-female, 1-male).

Finally, we analyzed discriminative abilities of LCAT, CETP and PON-1 activities, as well as CETP protein and PAB levels. The results are present in Fig. 1. According to established criteria, AUC between 0.7 and 0.8 defines good diagnostic accuracy, AUC between 0.8 and 0.9 designates very good diagnostic accuracy, while AUC between 0.9 and 1.0 is a marker of excellent diagnostic accuracy. Our results demonstrated that PAB has excellent accuracy as potential diagnostic marker for CRC, while diagnostic accuracy for CETP protein level might be characterized as very good.

4. Discussion

In this study we analyzed activities of proteins and enzymes involved in remodeling and anti-oxidative function of HDL particles in patients with CRC. Although alterations of lipid status are commonly seen and widely explored in CRC, there is a lack of studies analyzing more profound aspects of lipid profile. Having in mind significance and prevalence of this malignancy in modern world, an insight into fundamental changes of lipoprotein metabolism in CRC patients could be important for better understanding of the etiology and prognosis of CRC.

Analyses of total proteins, albumin and glucose levels in our study yielded the expected results (Table 1). Contrary to that, routine lipid status determination provided an outcome that is a subject of many debates. As opposed to numerous scientific records, which showed high levels of TC and LDL-C (21), these parameters, including HDL-C, were markedly reduced in our CRC patients (Table 1). This might be a consequence of poor nutritional status that is common in CRC patients, suggesting that disturbances in lipid profile are the consequence of cancer, rather than the cause of the disease [22].

The results of previous studies concerning lipid status are divergent; from the conclusions that elevated levels of lipid parameters are associated with tumor metastasis [21], to the findings that down-regulated TC synthesis has an impact on colorectal tumor cell's survival [23]. However, recently published results of Abaza et al. are similar to ours. Namely, in the mentioned study a decrease in TC, HDL-C and LDL-C in CRC was evident, alongside with lower levels of TG and apolipoproteins AI and B-100 as well [24]. Apart from probable influence of reduced nutritional status, it is also possible that reduction of TC, LDL-C and HDL-C levels arises as a consequence of the fact that carcinogenic cells need cholesterol for its own growth and proliferation [25]. Thus, higher consumption of cholesterol by malignant cells could lead to the depletion of plasma cholesterol resources.

Changes of HDL-C levels in malignant diseases are particularly interesting. In numerous studies reduced HDL-C levels are consistently linked to colorectal carcinoma and adenomatous polyps development [26,27]. In addition, a study by Yun Wang et al. demonstrated an increase of HDL-C levels after adjuvant chemotherapy in patients with CRC [28]. The interest in HDL-C as a prognostic marker increased after findings that its protective role in cancer arises from its antiangiogenic influence through ApoAI mediated inhibition of matrix-metalloproteinase 9 and myeloid derived suppressor cells [29]. However, in spite of

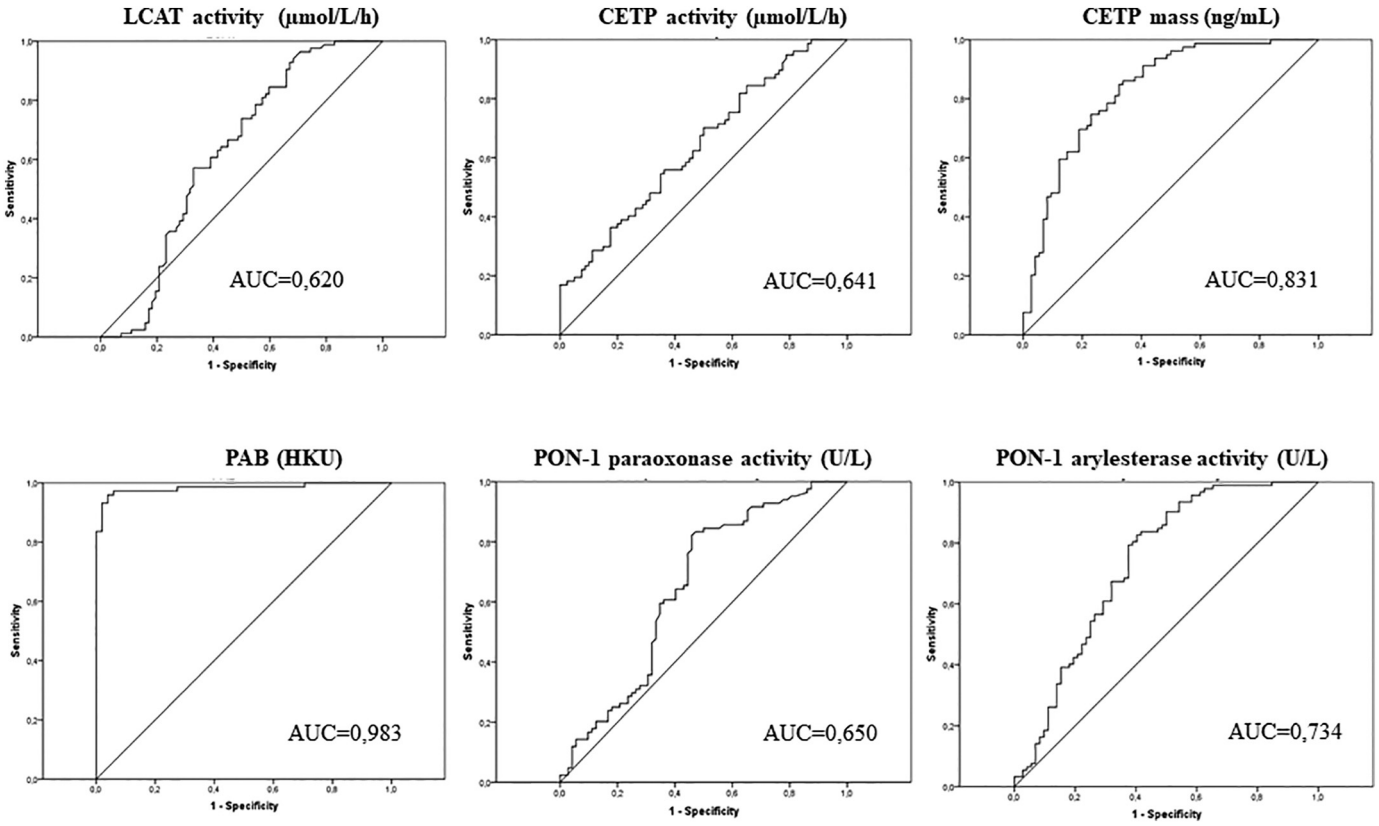

Fig. 1. Diagnostic accuracy for the examined potential markers of CRC.

numerous evidences associating levels of HDL-C with protective actions in cancer development, there is a lack of investigations regarding key HDL particle modulators.

LCAT is the main enzyme responsible for the cholesterol esterification in plasma and many studies have shown its significance in cardiovascular diseases [30]. Our results demonstrated decreased LCAT activity in patients with CRC (Table 2). It is well known that LCAT deficiency is associated with profound disturbance in HDL particle maturation and consequently with HDL-C reduction [31]. Our findings suggest that one of possible reasons for depleted HDL-C levels in CRC could be diminished LCAT activity. Interestingly, LCAT activity positively correlated with TC level in CRC patients. Therefore, we could also raise a possibility that reduction in plasma cholesterol level, as seen in our CRC patients, could be a driven force for lowering of LCAT activity, which will ultimately result in lower HDL-C concentration. Moreover, Xin Zhang et al. [32] found that the levels of serum free cholesterol were significantly higher in parallel with lower concentration of TC in CRC patients, thereby confirming our previous hypothesis. Still, the exact molecular mechanisms leading to decreased LCAT activity in this group of patients remains to be elucidated. Unexpectedly, LCAT activity correlated negatively with HDL-C, but positively with TG levels in healthy subjects. However, such associations were described previously [33] and they could be explained as a compensatory elevation of LCAT activity in an attempt to increase HDL-C concentration.

CETP is involved in transfer of cholesteryl esters from HDL particles to very low density lipoprotein (VLDL) and LDL, in exchange for TG. Activity of CETP is largely explored in cardiovascular diseases [34,35], but so far there is no data concerning this lipid transfer protein in CRC. Yet, acknowledging the bulk of evidences for decreased HDL-C levels in CRC, it would be important to explore CETP activity in these patients. According to our results, despite the reduced CETP protein level, the CETP activity remained significantly elevated in CRC, which may indicate the importance of this enzyme (Table 2). Since the liver is the main site of CETP synthesis, it is expectedly to find lower CETP mass in conditions characterized by a decreased synthetic function of liver, such is CRC. However, the observed discrepancy between CETP mass and activity in our study suggest that, in spite of reduced protein concentration, CETP activity might be upregulated in CRC, which should be further explored. Although comparable studies are not conducted in CRC, it is important to mention that CETP was recently identified as a contributor in developing and sustaining of breast cancer [36], mainly through its role in cholesterol uptake by the cancer cells. Knowing that all carcinogenic cells have increased need for cholesterol [37], the described mechanism might also be responsible for the contribution of CETP in CRC development. However, it should be mentioned that the observed discrepancy between CETP mass and activity in our patients could arise a consequence of stress conditions, which are typical for CRC. It is well known that CETP activity might fluctuate independently of protein level, as a response to alterations in the microenvironment and presence of activators or inhibitors. Thus, it is possible that the changes observed in our study simply represent the outcome of disturbed plasmatic circumstances. Therefore, it would be essential to explore whether the association of increased CETP activity with the risk for CRC development is simply a reflection of a more fundamental influence of cancer-associated stress on CETP function.

In addition, we should not neglect the role of CETP in HDL remodeling. Namely, increased CETP activity leads to the enrichment of HDL with TG, thereby compromising both structure and function of this lipoprotein [38]. Dysfunctional HDL is unable to perform antioxidative and anti-inflammatory actions, which triggers a cascade of events that are undoubtedly involved in atherogenesis [38], but it is also demonstrated that similar pro-oxidative and pro-inflammatory milieu contributes to the development of CRC [39,40]. In our study, increased PAB levels provide evidences for elevated oxidative stress in CRC, while diminished both PON-1 paraoxonase and arylesterase activities illustrate the impairment of HDL anti-oxidative function (Table 2). PON-1 is

of particular interest, since this enzyme represents a direct connection between lipid status and oxidative stress. Elevated PON-1 activity, together with decreased LCAT and increased CETP activity, is indicative for detrimental changes in both HDL structure and functionality. It has been known that altered HDL structure may compromise its functional properties [38]. In this context, it is important to mention that increased CETP activity was observed in healthy subjects with low-activity PON-1 phenotype A, while in patients, increased PAB values were associated with higher CETP mass. Although we were unable to directly measure the capacity of PON-1 binding with HDL, our findings might point out towards the association of altered lipid transfer proteins activities, changes in HDL structure and consequent detrimental effects on HDL anti-oxidative properties, reflected through lower PON-1 activity.

Taken all together, the observed results suggest that HDL particles could be placed in the center of complex interaction of dyslipidemia and oxidative stress during the development of CRC. Consequently, analysis of the structure and function of this lipoprotein might provide important data regarding individual propensity towards CRC development, as well as regarding the disease prognosis and treatment.

In order to further explore previous observations, we analyzed possible contribution of HDL-C, LCAT, CETP and PON-1 activities, as well as CETP protein levels in prediction of risk for development of CRC. Multivariate analysis have shown that CETP activity might have a potential for prediction of CRC development, even when adjusted for traditional risk factors, such are age, gender and lipid status. However, inclusion of markers of oxidative stress completely abolished the tentative independent contribution of increased CETP activity to the risk for CRC, suggesting that the observed changes in this lipid transfer protein are probably the result of stress conditions which are attributable to the disease itself. But, intriguing findings are recorded when we performed the same analysis for CETP serum levels. Namely, the adjustment for the extent of oxidative stress did not jeopardize possible independent contribution of decreased CETP levels to the risk of CRC development. Furthermore, after adjustment for serum albumin concentration, as an indicator of synthetic liver function, CETP mass retained significance, suggesting that the observed decrease might not be merely the outcome of declined liver function. Instead, decreased CETP levels might have independent potential in prediction of CRC, which should be evaluated in future prospective studies. In line with previous, CETP protein level was recognized as a marker with potential promising diagnostic role (Fig. 1). Apart from oxidative stress, which was continuously highlighted by previous and our results as an important hallmark of CRC, our finding implies a possibility that CETP as a modulator of lipid homeostasis might also be relevant in early prediction of risk and recognition of the disease onset.

Several limitations should be mentioned. First, patients were older than controls, which could influence the results. In order to minimize these effects, we performed analysis of covariance with age as a covariate. Then, the cross-sectional design of the study does not allow exploration of the effects of examined parameters on the disease progression. Finally, relatively small sample size might prevent detection of possible associations, which could otherwise be detected with a larger sample.

In conclusion, our results demonstrated increased CETP and decreased LCAT activity in patients with CRC, in parallel with elevated oxidative stress and diminished activity of anti-oxidant enzyme PON-1. CETP activity and protein levels were revealed as possible candidate markers for prognosis of CRC development, which should be confirmed by large prospective studies. In addition, our findings emphasized CETP protein levels, alongside with PAB levels as potentially useful diagnostic tools. Taken all together, our results suggest that alterations in HDL-C concentration, alongside with changes in HDL structure and consequently function, might have an important role in carcinogenesis. These findings emphasize the need for advanced lipid testing in this group of patients. Future studies conducted in larger cohorts of patients are needed to further extend and verify our preliminary findings.

Competing interest

Authors are familiar with policy on disclosure of potential conflicts of interest and all of them declare no conflicts of interest.

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Ethical approval

The entire study protocol was designed according to the Helsinki Declaration and approved by the local ethical committee (The Ethics committee of the Military Medical Academy; Ethics Committee reference number. 3000-1).

Authorship

MM performed laboratory measurements, statistical analysis and wrote the first draft of the manuscript. TG, SV, MM, AS contributed to experimental design and participated in laboratory analyses.DZ and BT were involved in protocol development, patient recruitment and data acquisition. JV, VSK and JKS provided intellectual guidance and critically reviewed the manuscript. AZ conceived and designated the study and critically reviewed the manuscript. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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