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Hyperhomocysteinemia and inflammatory biomarkers are associated with higher clinical SYNTAX score in patients with stable coronary artery disease

Hiperhomocisteinemija i biomarkeri inflamacije povezani su sa višim kliničkim SINTAKS skorom kod bolesnika sa stabilnom koronarnom arterijskom bolešću

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Abstract

Background/Aim. Previous studies have confirmed a positive correlation between homocysteine levels and a greater risk for acute coronary syndrome and stroke, but there are no available data to support an association between homocysteine and inflammatory markers and the severity of coronary artery disease according to the clinical SYNTAX score in patients with stable angina. The aim was to determine the association between homocysteine and inflammatory biomarker levels: interleukin (IL)-6, high sensitive C-reactive protein (hs-CRP), fibrinogen, erythrocyte sedimentation rate (ESR) and the severity of coronary artery disease according to clinical SYNTAX score. **Methods**. Eighty-two patients with stable angina pectoris (average age 65 ± 8 years, 28.9% females) underwent coronary angiography and were divided into three groups according to the clinical SYNTAX score: the group I < 22 (39 patients), the group II 23–32 (16 patients), the group III > 33 (27 patients). The severity and complexity of coronary artery disease were calculated by clinical SYNTAX score, multiplying the SYNTAX score with the modified ACEF score, based on the patients' left ventricular ejection fraction, age and creatinine clearance (derived with Cockcroft–Gault equation). **Results**. Homocysteine levels were significantly higher in patients with high clinical SYNTAX score [the group I: median (interquartile range – IQR): 10.20 (3.97), the group II: 10.45 (5.77), the group III: 14.70 (7.50), $p = 0.005$]. Patients in the group III had significantly higher homocysteine levels compared to the group I ($p = 0.001$). We also found a positive association between inflammatory biomarkers (IL-6, hsCRP, fibrinogen, ESR) and the severity of coronary artery disease according to the clinical SYNTAX score ($p = 0.017$, 0.001, 0.032, 0.049 respectively). We detected significantly lower plasma levels of vitamin B12 in the group III and group II in comparison with the group I (the group I: median (IQR): 238 (160), the group II: 171 (160), the group III: 172 (102), $p = 0.022$), which indicates its important role in homocysteine metabolism. **Conclusion.** The elevated plasma levels of homocysteine, IL-6, hsCRP, fibrinogen, ESR were detected in patients with high clinical SYNTAX score (> 33). Our results showed that hyperhomocysteinemia and some inflammatory biomarkers can predict more severe and extensive coronary artery disease in stable angina patients.

Key words:
coronary disease; inflammation mediators; homocysteine; angina, stable.

Apstrakt

Uvod/Cilj. Prethodne studije potvrdile su pozitivnu korelaciju između nivoa homocisteina i većeg rizika od nastanka akutnog koronarnog sindroma i moždanog udara, ali nije bilo istraživanja koja su ispitivala povezanost između vrednosti homocisteina i inflamacijskih markera i težine koronarne arterijske bolesti prema kliničkom SINTAKS skoru kod bolesnika sa stabilnom anginom pektoris. Cilj ovog istraživanja bio je da se utvrdi povezanost između koncentracije homocisteina i inflamacijskih biomarkera: interleukina (IL)-6, visoko senzitivnog C-reaktivnog proteina (hs-RCP), fibrinogena i sedimentacije eritrocita (SE) i stepena težine koronarne arterijske bolesti prema kliničkom SINTAKS skoru. **Metod.**

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Kod 82 bolesnika sa stabilnom anginom pektoris (prosečne starosti 65 ± 8 godina, 28,9% žena) urađena je koronarografija, nakon čega su podeljeni u tri grupe prema kliničkom SINTAKS skoru: I grupa < 22 (39 bolesnika), II grupa 23–32 (16 bolesnika), III grupa > 33 (27 bolesnika). Stepen težine koronarne arterijske bolesti određen je prema kliničkom SINTAKS skoru, množenjem SINTAKS I skora i modifikovanog ACEF skora, koji uzima u obzir ejekcionu frakciju leve komore, starost bolesnika i klirens kreatinina (izvedenog pomoću Cockcroft-Gault-ove jednačine). **Rezultati**. Vrednosti homocisteina bile su značajno više kod bolesnika sa visokim kliničkim SINTAKS skorom [I grupa: medijana (interkvartilini raspon – IQR): 10,20 (3,97), II grupa: 10,45 (5,77), III grupa: 14,70 (7,50), $p = 0{,}005$]. Bolesnici III grupe imali su značajno više vrednosti homocisteina u poređenju sa I grupom ($p = 0{,}001$). Takođe smo detektovali pozitivnu korelaciju između inflamacijskih markera (IL-6, hs-CRP, fibrinogena i SE) i težine koronarne arterijske bolesti prema kliničkom SINTAKS skoru (p = 0.017, 0.001, 0.032, 0.049 redom). Detektovali smo značajno niže vrednosti vitamina B12 u grupama III i II u odnosu na grupu I (I grupa: medijana (IQR): 238 (160), II grupa: 171 (160), III grupa: 172 (102), $p = 0{,}022$) što ukazuje na njegovu važnu ulogu u metabolizmu homocisteina. **Zaključak**. Povišene koncentracije homocisteina, IL-6, hsCRP, fibrinogena i SE u plazmi detektovane su kod pacijenata sa visokim kliničkim SINTAKS skorom (> 33). Naši rezultati pokazali su da hiperhomocisteinemija i pojedini inflamacijski biomarkeri mogu ukazati na prisustvo ozbiljnije i ekstenzivnije koronarne arterijske bolesti kod bolesnika sa stabilnom anginom pektoris.

Ključne reči:
koronarna bolest; zapaljenje, medijatori; homocistein; angina, stabilna.

Introduction

Amino acid homocysteine (HCy), participates in the initiation of endothelial dysfunction, and increases oxidative stress [1, 2], leading to accelerated atherosclerosis. HCy has been associated with hypercoagulability state and increased thrombus burden [3, 4] and it has been recognized as a risk factor for acute coronary syndrome and ischemic stroke [5, 6]. Recent studies [7, 8] concluded that hyperhomocysteinemia may develop as a consequence of chronic immune activation, which implies the importance of simultaneous measurement of both inflammatory markers and homocysteine levels, as well as vitamin B12 and folic acid.

Coronary artery disease (CAD) is mostly caused by atherosclerosis, which is considered to be an inflammatory disease [9, 10]. Inflammatory factors have a substantial role in the initiation and progression of CAD [11], and circulating markers reflect the inflammatory process within the coronary artery wall. During clinical practice, we have found that a certain number of patients without traditional risk factors for CAD had significant changes in coronary arteries. Thus, it is necessary to determine if some other risk factors may contribute to the formation and progression of CAD. Several studies have shown that fibrinogen is related to the increased cardiovascular (CV) risk [12] and plaque progression in patients with acute coronary syndrome and stable angina [13–15]. The AtheroGene study [16], which investigated 1,806 patients with documented CAD and stable angina pectoris, concluded that high fibrinogen and C-reactive protein (CRP) values were predictive for future CV risk, but did not provide additional information on top of traditional risk factors. CRP has been studied in patients with unstable and stable angina pectoris [17]. Very recent study [18] found a positive association between CRP levels and intrahospital mortality in patients with ST-elevation myocardial infarction. Another important inflammatory marker, interleukin (IL)-6, is engaged in pathogenesis of CAD, participating in plaque formation and its destabilization [19]. High levels of IL-6 have been detected in patients with unstable CAD, in comparison with stable angina patients [20].

The assessment of CAD severity can be done by using different scores, and according to the number of the diseased coronary arteries. The clinical SYNTAX (Synergy Between Percutaneous Coronary Intervention With Taxus and Cardiac Surgery) score (CSS), which combines SYNTAX I and modified the ACEF (age, creatinine, ejection fraction) score provides additional clinical characteristics based on the patients' left ventricular ejection fraction (LVEF), age and creatinine clearance (CrCl) derived using the Cockcroft-Gault [21] equation. It has been shown that CSS has predictive ability for adverse clinical outcome after percutaneous coronary intervention (PCI) [22–24] by incorporating clinical variables, but it also can be used in the assessment of the severity of CAD.

The aim of this clinical study was to investigate and determine the correlation between inflammation markers and metabolism of homocysteine and CAD and its severity in patients with stable angina pectoris.

Methods

The study included 82 patients with stable angina pectoris, and all had positive myocardial ischemia noninvasive tests, either on a treadmill exercise test or pharmacological echocardiography dobutamine stress test. Patients with acute coronary syndrome, active inflammatory diseases, infections and malignant diseases, as well as with previous myocardial infarction, history of coronary revascularization and severe valvular disorders were excluded. Several standard laboratory parameters were measured: fasting glucose, total and low density lipoprotein (LDL) cholesterol, triglycerides, creatinine, erythrocyte sedimentation rate (ESR), leukocyte count (Le), high sensitive C-reactive protein (hs-CRP) and fibrinogen.

The homocysteine level, expressed in μmol/L, was determined using a commercially available test on System Siemens nephelometric analyzer by immunonephelometry method in EDTA plasma samples. Sample coefficient of variation (CV) was 4.2%, and reference range 4.995–15.000 μmol/L. For serum IL-6 measurement we used DPC Immu-

lite 2000, Siemens analyzer by chemiluminescence immunometric assay. Sample coefficient of variation (CV) was 4.0%, and reference range 0.0–5.9 pg/mL.

Subjects assessed as positive for ischemic heart disease underwent coronary angiography in order to determine the severity of CAD according to the CSS [22, 23]. According to the severity of CAD, we divided all the patients into the three groups regarding the CSS: the group I (< 22 points), the group II (23–32 points), and the group III (> 33). We, also, estimated the severity of CAD according to the number of the affected coronary vessels (1-vessel, 2-vessel and 3-vessel disease). For the assessment of CAD severity, we used CSS, calculated multiplying the value of SYNTAX I score and modified ACEF score [24,25] based on the patients' left ventricular ejection fraction (LVEF), age and CrCl derived using the Cockcroft-Gault equation.

Statistical analysis was done using SPSS statistical software 25.0. Average values and standard deviation were used for data with a normal distribution. The median and interquartile range (IQR) were used for the data without normal distribution. A significant difference between the groups was measured using the Mann Whitney test for two independent groups and K independent samples (Kruskal Wallis) and categorical variables were compared by the chi-square test (χ^2).

The association between HCy serum levels and the severity of angina, clinical and anatomic SYNTAX score was estimated with logistic regression analysis. The results between groups were described as odds ratios (OR) (Mantel-Haenszel current OR) with a 95% confidence interval (CI). Cluster analysis with Ward's method was used for finding the cut-off points. For statistically significant differences we used $p < 0.05$.

Results

A total of 82 patients with the symptoms of stable angina (average age 65 ± 8 years, 28.9% females) underwent coronary angiography and were divided into three groups according to CSS: the group I (< 22; n = 39), the group II (23–32; n = 16), the group III (> 33; n = 27).

Patients' clinical characteristics and laboratory parameters from all the three groups are summarized in Table 1. There were significant differences between all three groups regarding age, physical activity, triglycerides, creatinine clearance and diastolic blood pressure on the admission to the hospital ($p < 0.05$). On the other hand, there was no statistically significant correlation between gender, active smoking, hypertension, family history, diabetes mellitus, fasting glucose, total and LDL cholesterol, atherosclerosis index, body mass index (BMI), acidum uricum, LVEF, end-diastolic, end-systolic diameter of left ventricular and the severity of CAD according to CSS. Homocysteine, inflammatory biomarkers (IL-6, hs-CRP, fibrinogen, ESR, leukocytes), folic acid, vitamin B12, prothrombin time (PT), activated partial thromboplastin time (APTT), as well as the number of affected and treated coronary arteries in all 3 groups are presented in Table 2.

There was a statistically significant positive correlation between homocysteine levels and the severity of CAD according to clinical SYNTAX score. Homocysteine levels were significantly higher in patients with high Clinical SYNTAX (> 33). Patients in group III had significantly higher HCy levels compared to group I (the group I: median (IQR): 10.20 (3.97), the group II: 10.45 (5.77), the group III: 14.70 (7.50), Kruskal Wallis test, $p = 0.005$); (Figure 1).

Then, we evaluated the odds ratio (OR) for CCS according to HCy values (I group HCy < 15 μmol/L, II group > 15 μmol/L) using multivariable logistic regression analysis (Mantel-Haenszel common OR with 95% confidence intervals). The patients with HCy > 15 μmol/L had more severe CAD according to CSS. We found that the OR between group III and group I was 8.125 with 95% CI (2.258–29.241, $p = 0.001$), and the relative risk was 4.695 (1.715–12.821). The high-risk patients for CAD were in the group with HCy values > 15 μmol/L (Figure 2). In multiple logistic regression analysis, where the Clinical SYNTAX score was a dependent variable, and homocysteine levels were independent variables we found statistically significant differences in HCy levels between group III (> 33) and group I (< 22); (Odds ratio = 1.230, 95% CI = 1.079–1.403, $p = 0.002$ and Odds ratio = 1.153, 95% CI = 1.015–1.309, $p = 0.028$, respectively). In multiple logistic regression analysis, where the multivessel disease was a dependent variable, and homocysteine levels were independent variables we found significant differences in HCy levels between 3-vessel and 2-vessel disease (Odds ratio = 1.217, 95% CI = 1.041–1.422, $p = 0.014$).

We detected significantly lower plasma levels of Vitamin B12 in group III compared to group I, which indicates its important role in HCy metabolism (the group I: median (IQR): 238 (160), the group II: 171 (160), the group III: 172 (102), Kruskal Wallis test, $p = 0.022$). Our results showed that HCy values were significantly higher in groups II and III, where vitamin B12 values were significantly lower. On the other hand, we did not find differences in folic acid values between all three groups (Table 2). We found that the inflammatory biomarkers (IL-6, hs-CRP, fibrinogen, ESR) were all in positive correlation with the severity of coronary artery disease according to CSS (Table 2). The presence of CAD was associated with higher values of IL-6 [the group I: median (IQR): 2.49 (2.67), the group II: 3.10 (3.91), the group III: 4.80 (4.52), Kruskal Wallis test, $p = 0.017$]; (Figure 3).

We detected significant differences in hs-CRP values between 3 groups, and additional statistical analysis showed differences between group III and group I, and group III and group II [the group I: median (IQR): 2.75 (5.77), the group II: 1.01 (2.78), the group III: 5.17 (8.84), Kruskal Wallis test, $p = 0.001$]. Comparison of the groups demonstrated significant differences in fibrinogen (Figure 4) and ESR values between group III and group I.

Fibrinogen in the group III was higher than in the groups I and II, which was statistically significant [the group I: median (IQR): 3.30 (0.90), the group II: 3.55 (0.85), the group III: 3.70 (0.60), Kruskal Wallis test; $p = 0.032$]. Patients with higher CSS had higher values of ESR [the group I: median (IQR): 18 (25), the group II: 22.5 (30), the group III: 26 (29), Kruskal Wallis test; $p = 0.049$].

Table 1

Patients' clinical characteristics and laboratory parameters from all the three groups according to the clinical SYNTAX score

Parameters	Clinical SYNTAX score			*p* (test)
	I (n = 39) < 22	II (n = 16) 23–32	III (n = 27) > 33	
Gender, n (%)				
female	8 (20.5)	1 (6.3)	10 (37.0)	> 0.05 (x^2)
male	31 (79.5)	15 (93.7)	17 (63.0)	
Age (years), median (IQR)	62.0 (13.0)	68.5 (11.5)	71.0 (9.0)	0.001 (KW)
Active smoking, n (%)	31 (79.49)	11 (68.75)	14 (51.85)	> 0.05 (x^2)
Hypertension, n (%)	38 (97.4)	13 (81.25)	26 (96.30)	> 0.05 (x^2)
Family history, n (%)	27 (69.23)	12 (75.00)	21 (77.78)	0.731 (x^2)
Diabetes mellitus, n (%)	13 (33.33)	3 (18.75)	11 (40.74)	0.332 (x^2)
Physical activity, n (%)	17 (43.59)	7 (43.75)	2 (7.41)	0.008 (x^2)
Glucose (fasting) (mmol/L), median (IQR)	6.00 (2.40)	5.60 (1.05)	6.40 (2.50)	> 0.05 (KW)
Triglycerides (mmol/L), median (IQR)	1.84 (1.21)	1.44 (0.86)	1.46 (0.75)	0.036 (KW)
Cholesterol (mmol/L), median (IQR)	5.24 (1.79)	5.29 (1.99)	4.74 (1.58)	> 0.05 (KW)
HDL cholesterol (mmol/L), median (IQR)	1.14 (0.31)	1.18 (0.23)	1.10 (0.36)	> 0.05 (KW)
LDL cholesterol (mmol/L), median (IQR)	3.00 (1.70)	3.00 (1.36)	2.92 (1.26)	> 0.05 (KW)
Atherosclerosis index, median (IQR)	2.98 (1.34)	2.59 (1.19)	2.32 (1.19)	> 0.05 (KW)
Atherogenic index of plasma, median (IQR)	0.22 (0.34)	0.17 (0.24)	0.11 (0.35)	> 0.05 (KW)
Body mass index (kg/m^2) median (IQR)	29.41 (5.07)	26.49 (4.74)	27.76 (4.80)	> 0.05 (KW)
Creatinine clearance (mL/min), median (IQR)	89.2 (15.4)	85.8 (21.3)	75.3 (29.8)	0.029 (KW)
Creatinine (μmol/L), median (IQR)	89.2 (15.4)	80.5 (18.0)	84.0 (29.0)	> 0.05 (KW)
Acidum uricum (μmol/L), median (IQR)	342 (136)	333 (120)	331 (147)	> 0.05 (KW)
Systolic blood pressure (mmHg), median (IQR)	140 (30)	130 (28)	140 (30)	0.004 (KW)
Diastolic blood pressure (mmHg), median (IQR)	80 (10)	80 (10)	80 (10)	> 0.05 (KW)
LVEF (%), median (IQR)	60.00 (2.00)	57.00 (5.00)	58.00 (5.00)	> 0.05 (KW)
End diastolic diameter (mm), median (IQR)	53.00 (5.00)	56.00 (4.25)	54.00 (9.00)	> 0.05 (KW)
End systolic diameter (mm), median (IQR)	34.00 (7.00)	35.50 (4.50)	35.00 (7.00)	> 0.05 (KW)

x^2 – chi-square test; KW – Kruskal Wallis test; IQR – interquartile range; HDL – high density lipoprotein; LDL – low density lipoprotein; LVEF – left ventricular ejection fraction.
***p* – values < 0.05 indicate significant differences regarding parameters among all 3 groups.**

Table 2

Laboratory parameters across the three groups of the clinical SYNTAX score

Parameter	Clinical SYNTAX score group			*p* (Kruskal Wallis test)
	I (< 22)	II (23–32)	III (> 33)	
Leukocytes (× 10^9), median (IQR)	7.03 (1.41)	6.71 (2.53	7.32 (2.74)	>0.05
ESR (mm/h), median (IQR)	18.0 (25.00)	22.5 (33.00)	26.0 (29.00)	0.049
C-reactive protein (mg/L), median (IQR)	2.75 (5.77)	1.01 (2.78)	5.17 (8.84)	0.001
Fibrinogen (g/L), median (IQR)	3.30 (0.90)	3.55 (0.85)	3.70 (0.60)	0.032
Interleukin-6 (pg/mL), median (IQR)	2.49 (2.67)	3.10 (3.91)	4.80 (4.52)	0.017
Homocysteine (μmol/L), median (IQR)	10.20 (3.97)	10.45 (5.77)	14.70 (7.50)	0.005
Folic acid (nmol/L), median (IQR)	14.6 (14.23)	13.1 (13.57)	14.02 (14.72)	>0.05
Vitamin B12 (pmol/L), median (IQR)	238 (160)	171 (160)	172 (102)	0.022
Prothrombin time (second), median (IQR)	1.05 (0.08)	1.01 (0.07)	1.02 (0.12)	> 0.05
Activated prothrombin time (second), median (IQR)	31.62 (5.77)	30.77 (5.28)	33.82 (9.70)	>0.05

IQR – interquartile range; ESR – erythrocytes sedimentation rate;
***p* – values < 0.05 indicate significant differences regarding parameters among all 3 groups.**

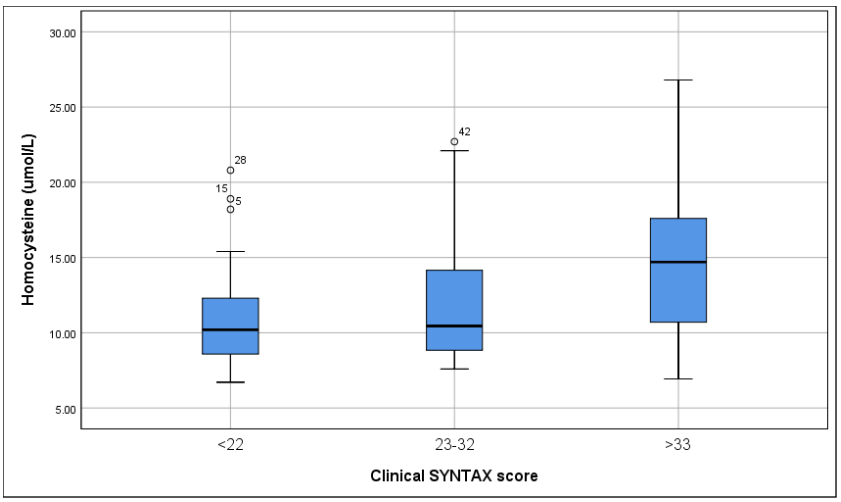

Fig. 1 – The correlation between plasma levels of homocysteine and the severity of coronary artery disease according to the clinical SYNTAX score.

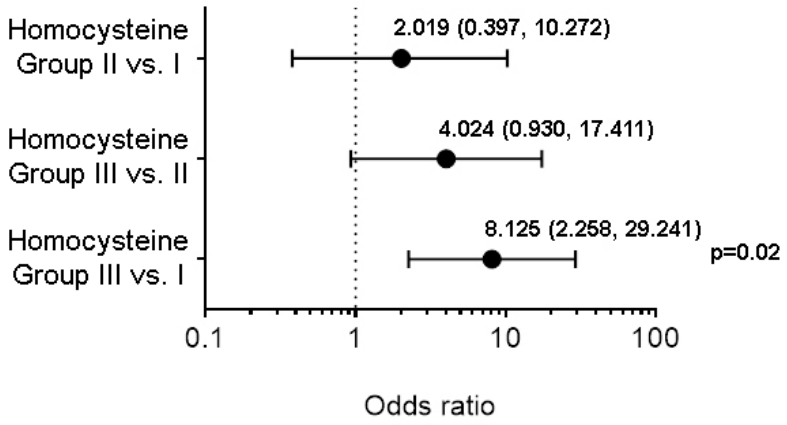

Fig. 2 – Odds ratio with 95% confidence intervals for the clinical SYNTAX score (CSS) according to the homocysteine (HCy) levels (HCy < 15 μmol/L, HCy > 15 μmol/L) in the study groups. Group I CSS < 22; Group II CSS 23–32; Group III CSS > 33.

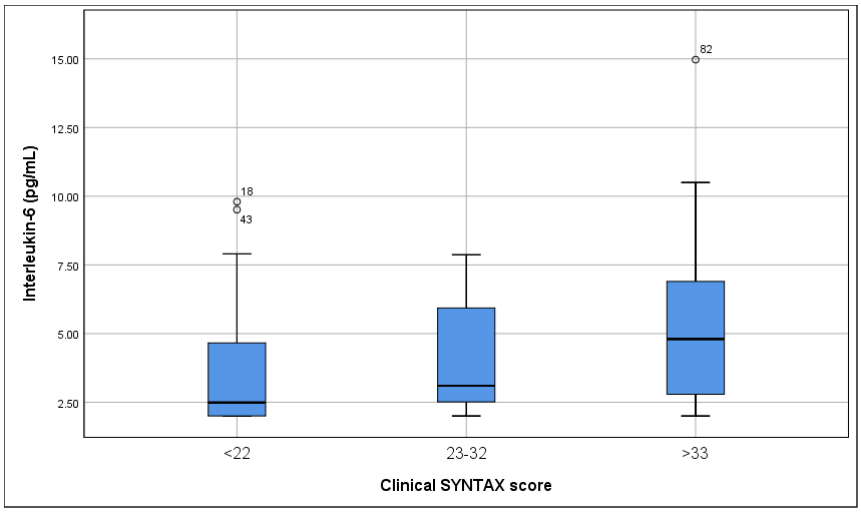

Fig. 3 – The correlation between plasma levels of interleukin-6 and the severity of coronary artery disease according to the clinical SYNTAX score.

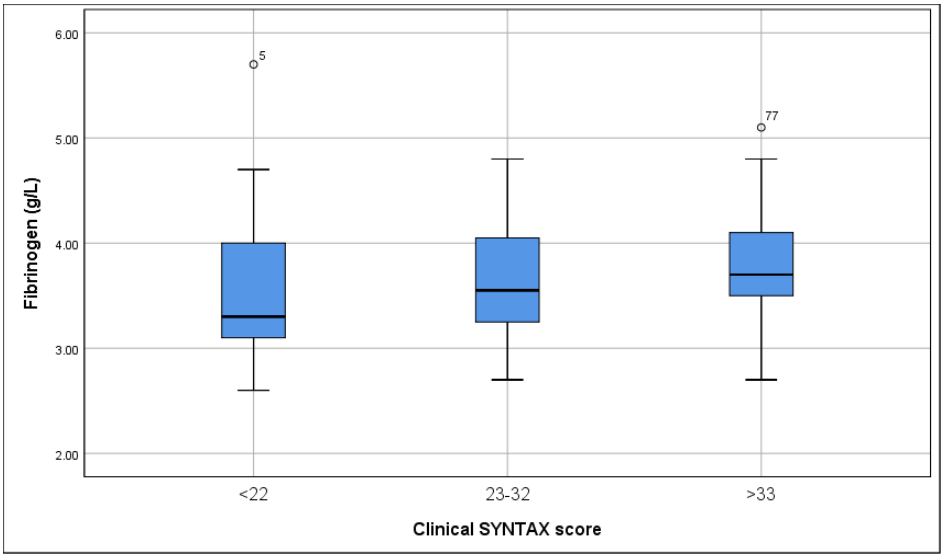

Fig. 4 – The correlation between plasma levels of fibrinogen and the severity of coronary artery disease according to the clinical SYNTAX score.

Discussion

The results of our study showed a significant correlation between the severity of CAD represented by the CSS and the levels of HCy and inflammatory markers (hs-CRP, ESR, IL-6, fibrinogen).

HCy reduces the production of nitric oxide (NO) and increases the proliferation of smooth muscle cells [26, 27]. HCy levels are influenced by vitamin B12 and folic acid, but also by a chronic immune response and renal function [28]. Unlike a recent study [29] in which a positive correlation between hyperhomocysteinemia and SYNTAX I score was found in patients with the acute coronary syndrome (ACS), we conducted the study where we found an association between the values of HCy and the CSS, but in stable angina patients. Two separate studies [30, 31] have shown that HCy levels were higher in patients with the three-vessel disease compared to those with single-vessel CAD. A positive correlation between hyperhomocysteinemia and acute coronary syndrome can be explained by its role in oxidative stress, endothelial dysfunction, and his prothrombotic activity, inducing the progression of stable to unstable atherosclerotic plaque [13, 14]. McCully [32] has shown that hyperhomocysteinemia can lead to accelerated atherosclerosis in the general population. The study we have done showed a positive correlation between HCy levels and the severity of CAD according to the CSS in patients with stable angina pectoris.

The results of our research are consistent with the results of the previous one, but a positive correlation was found, not only with HCy levels but also with the concentrations of the investigated inflammatory markers. Additional statistical analysis of the groups according to the CSS showed that the levels of the inflammatory markers (hs-CRP, ESR, IL-6, fibrinogen) were in correlation with the serum HCy levels and that a significant difference was detected between the group III and the group I. One explanation could be the synergistic action of HCy and inflammatory markers on the inflammation process in the blood vessel wall, which was the conclusion of the recent study [19] that detected the association of moderate hyperhomocysteinemia and cellular immune-mediated activity. Another assumption of the study was that the accumulation of HCy in the vessel wall might be due to a deficiency of vitamin B 12 which is related to chronic activation of the immune system. The results of a recent study [33] have shown that hyperhomocysteinemia in older patients with ACS is a significant predictor of total mortality and major adverse cardiovascular events (MACE). Our study included patients with stable angina pectoris, and it has shown the average age of 70 years, in the group III that had significantly higher levels of HCy than the group I, with an average age of 62 years, which is consistent with the fact that HCy levels raise with aging. We detected significantly lower plasma levels of vitamin B12 in the group III and the group II in comparison with the group I, which indicates its important role in HCy metabolism.

It is well known that inflammation is the initial step in atherosclerotic plaque formation, progression, and development of arterial thrombus burden [34]. Inflammatory mediators have an essential role in plaque destabilization and consequence rupture [35].

Some cohort studies [36] revealed that patients with multiple traditional risk factors did not have CAD, and that is one of the reasons why we conducted a study where we investigated traditional risk factors on one side, and HCy levels and the inflammatory markers on the other. Patients with more severe CAD (CSS > 33) were older, which can be explained by the cumulative effects of different CV risk factors in an extended period. Elderly patients have a high incidence of CAD and worse cardiovascular prognosis [37].

The results of our study showed significant correlation between inflammatory markers (hsCRP, ESR, IL-6, fibrinogen) and the severity of CAD according to the CSS ($p < 0.05$).

CRP is a biomarker of systemic inflammation, and elevated levels are detected in different conditions, such as in-

fection, injury and other inflammatory stimuli [38]. Recent study [18] involved patients with ST-elevation acute myocardial infarction (STEMI), and detected higher intrahospital mortality in those with higher CRP levels on admission to hospital. Other study [39] also showed a positive correlation between CRP and recurrent coronary events in ACS patients, but our study, to the best of our knowledge, was the first conducted to establish an association between CRP levels and the severity of CAD according to the CSS in patients with stable angina pectoris. In the early stage of inflammation, CRP provokes endothelial dysfunction, and therefore accelerates atherosclerosis by reducing NO release. Some studies [40, 41] have shown that high CRP levels are associated with future cardiovascular events in patients with unstable and stable coronary disease, but this is the first study in which the CSS was used for the severity assessment of CAD.

ESR has a positive association with traditional risk factors: gender, age, total cholesterol, body mass index (BMI), diabetes, and active smoking [42]. Reykjavik Study [43] has shown that ESR was an independent long-term predictor of CAD in both men and women. The results of our study are consistent with the study [44] in which ESR was related to the extent of atherosclerosis of coronary artery, but, unlike this study, we found an association with the extent of CAD according to the more accurate CSS.

IL-6 plays an important role in the pathogenesis of CAD [45], directly, leading to endothelial dysfunction, macrophage/ monocyte initiation, extracellular matrix degradation, and indirectly, stimulating the synthesis of coagulation factors. IL-6 also initiates the synthesis of other inflammatory markers in the liver [46]. The MESA study (Multi-Ethnic Study of Atherosclerosis) [47] with 6,617 participants without CV disease after 13.2 years of follow-up revealed a strong association and predictive value of IL-6 in the development of CV disease, heart failure, and total mortality. A large meta-analysis [48], which included 17 studies with 5,730 patients with CAD and 19,038 subjects in the control group, detected a strong association between IL-6 concentrations and CAD. Our results indicated that the elevated IL-6 values in the highest tercile were in positive correlation with CAD severity, and the CSS values were consistent with these findings. The elevated concentrations of IL-6 are detected at the very beginning of the inflammation in response to tissue damage and are a "warning signal" for the entire organism [49]. Concentrations of IL-6 correlate with obesity, which can explain the increased risk of CAD in obese patients. Our results are consistent with the previous study [50] because we found that 57.32% of patients were overweight (BMI 25–29.9 kg/m^2), and 26.83% were obese (BMI > 30 kg/m^2). Also, IL-6 stimulates the synthesis of the CRP [51], which can explain the results of our study where patients with a more severe CAD (CSS > 33), with high values of IL-6, also had elevated CRP values. The results of our study are entirely consistent with a recent study [52], which detected the elevated values in 100 patients with coronary angiography proven CAD, but we found a positive association with CAD severity according to CSS ($p < 0.05$). An explanation for the above may be the fact that the CSS takes into account the patients' age, renal function, and left ventricular ejection fraction.

A meta-analysis [53] comprising 31 studies with 154,211 subjects detected the correlation between fibrinogen concentration and the risk of CAD, stroke and other vascular mortality. A recent study [54] on 3,545 patients with stable angina pectoris during 7.3–10.2 years of follow-up showed that fibrinogen is a long-lasting independent marker of acute myocardial infarction and total mortality, and fibrinogen concentrations were the highest in patients with coronary angiographically most complex CAD, but not according to more sensitive CSS. Tabakci et al. [55] detected the severity and complexity of CAD in 134 patients, but patients were divided into three groups according to the values of SYNTAX score (SS control group = 0, SS intermediate group < 22, SS high-risk group > 22). In our study, we detected significantly higher fibrinogen values in the group of patients with the CSS > 33. De Luca et al. [56] detected a correlation between the severity of CAD by the number of affected blood vessels and the elevated fibrinogen levels. Very recent study [57] with 440 patients with acute myocardial infarction in whom 36 (8.2%) were identified as myocardial infarction with nonobstructive coronary arteries (MINOCA) and compared with myocardial infarction patients with obstructive CAD (MICAD), showed a significant increased fibrinogen concentration in both groups, which may be due to a myocardial infarction. Fibrinogen, as a precursor of fibrin, increases plasma viscosity, erythrocyte aggregation and has a thrombogenic potential because it connects thrombocytes in the formation of thrombus [58]. By comparing fibrinogen values among three groups, we found the highest values in patients with the CSS > 33, compared to small and intermediate-scale groups (< 32). A study of Cappelletti et al. [59] on 574 subjects, who performed coronary angiography, found that the elevated fibrinogen levels were associated with a critical narrowing of the main tree of the left coronary artery and the proximal segment of the left anterior descending artery (LAD). The results of our study was shown that there was a correlation between the concentration of fibrinogen and other investigated markers (ESR, CRP, IL-6) with significant stenosis (> 50%) of coronary arteries when we divided the subjects according to the CSS. The results of our study are in agreement with the results of other studies [60, 61], which have shown that baseline fibrinogen values may indicate the existence of a significant CAD and have the prognostic significance of future CV diseases.

To our knowledge, this is the first study where a significant difference in HCy and inflammatory marker levels were found between three groups according to the CSS. The results obtained in this study are consistent with previous studies, but we used the CSS for the assessment of the severity of CAD which included patients' clinical features like left ventricular ejection fraction, age, and creatinine clearance, besides anatomical variables.

Conclusion

The elevated plasma levels of homocysteine, IL-6, CRP, fibrinogen, ESR were detected in patients with high CSS (> 33), which confirmed previous findings that long-term, chronic inflammation of the coronary wall arteries preceded the formation of atherosclerotic plaques. We detected significantly lower plasma levels of vitamin B12 in the group III and the group II in comparison to the group I. Our results showed that both hyperhomocysteinemia and some inflammatory biomarkers could predict more severe and extensive CAD in stable angina patients. Higher values of tested parameters can be a useful prognostic indicator of the development of more severe clinical picture in patients with CAD.

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