Relationship between neutrophil-to-lymphocyte ratio and impaired myocardial perfusion in cardiac syndrome X

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Introduction

Cardiac syndrome X (CSX) has been defined as typical exertional angina, ST-segment depression on exercise stress tests, and normal coronary arteries after exclusion of spontaneous or inducible epicardial coronary spasm1. A wide range of pathophysiological mechanisms involving inflammation, changes in microvascular tonus, impaired coronary flow reserve, insulin resistance, altered sodium-hydrogen exchange activity, and increased sensitivity of cardiac pain have been described in several studies2-6. Among these mechanisms, microvascular ischemia mediated by inflammation and endothelial dysfunction proposed as the main factor underlying CSX2. Importantly, Maseri et al7 explained that in CSX, microvascular dysfunction is distributed patchily across the myocardium. Hence, in patients with CSX, diminished contractility in small myocardial areas might be dealt with normal, or even increased contractility of the adjacent interposed myocardial segments. For the same reason, metabolites released by minor ischemic areas might be diluted in the total coronary blood flow that also drains normal myocardial areas. Myocardial blush grade (MBG) is a recognized angiographic parameter of microvascular perfusion8. In a recent study9, we found that myocardial blush was decreased in patients with CSX and that a low MBG was a useful imaging tool for a diseased microvascular network in the catheterization laboratory. These results are in agreement with those of Atmaca et al10.

The neutrophil-to-lymphocyte ratio (NLR) is a surrogate marker of inflammation in a variety of cardiovascular disorders, including stable coronary artery disease and acute coronary syn-
dromes\textsuperscript{11-13}, carotid artery disease and stroke\textsuperscript{14-15}, and hypertension\textsuperscript{16-17}. The NLR is elevated and correlated with the carotid intima-media thickness in patients with CSX\textsuperscript{18}. However, to the best of our knowledge, no trials have evaluated the role of the NLR in determining the angiographic myocardial perfusion in patients with CSX. Accordingly, the aim of this study was to compare the NLRs in patients with CSX and control subjects and to seek its potential associations with microvascular perfusion as assessed by the MBG.

**Patients and Methods**

**Patient Characteristics**

The protocol of this bicentric, cross-sectional, observational, retrospective study was approved by the institutional review board of Baskent University, Ankara, Turkey (project number: KA14/414) and performed in accordance with the guidelines proposed in the Helsinki Declaration. Written informed consent was obtained from all the participants. The study population comprised 120 patients who underwent coronary angiography on an outpatient basis at the cardiology departments of Baskent and Gazi University Medical Schools. Sixty consecutive patients (54.1 ± 7.8 years of age, 49 females) diagnosed with CSX according to the presence of typical exercise-induced angina pectoris, transient ischemic ST-segment depression (> 1 mm) during the treadmill exercise test, and angiographically normal coronary arteries constituted the study group. The control group comprised 60 consecutive age- and sex-matched patients with anginal chest pain whose coronary arteries were found to be normal and who had no inducible ischemia on the exercise stress test.

Patients with the following were excluded from the study: previous myocardial infarction, unstable chest pain, any coronary artery atherosclerotic lesions (including plaques and ectasia), coronary vasospasm, moderate to severe valvular heart disease, left ventricular systolic dysfunction by echocardiography (ejection fraction < 0.40), renal dysfunction (serum creatinine > 1.5 mg/dl for men and > 1.4 mg/dl for women), hepatic insufficiency (liver function tests greater than twice the upper limit of the laboratory reference range), malignancies, acute or chronic infectious or inflammatory diseases, hematological disorders, or steroid therapy. Patients for whom the Thrombolysis In Myocardial Infarction (TIMI) frame count (TFC) and MBG were not computable due to technical problems were also excluded.

Hypertension was defined as the current use of antihypertensive drugs or systolic blood pressure of > 140 mm Hg or diastolic blood pressure of > 90 mm Hg measured at the outpatient clinic. Diabetes mellitus was defined as a fasting blood glucose of ≥ 126 mg/dl or the use of antidiabetic medication. Smoking was described as the regular use of tobacco.

**Treadmill Exercise Test**

Treadmill exercise tests were carried out in accordance with the modified Bruce protocol\textsuperscript{19}. All patients had refrained from ingesting food, alcohol, or caffeine or smoking within 3 hours of the test. Beta-blockers and non-dihydropyridine calcium channel blockers had been stopped for 72 hours before the test. The target heart rate was at least 85% of the age-predicted heart rate (220 – age in years). This protocol involved three stages, each lasting 3 minutes. The first stage was performed at 1.7 mph with a 0% grade, and the second at 1.7 mph with a 5% grade. In the third stage, the gradient is increased to 10% while the speed was maintained. All subjects continued walking for a 1-minute cool-down period after reaching the target heart rate. The total exercise time was 8 to 12 minutes. ST-segment depression was measured at 60 ms after the J point in all 12 electrocardiographic leads. A horizontal or downsloping ST-segment depression of ≥1 mm was accepted as significant.

**Coronary Angiography**

Coronary angiograms were obtained via a femoral approach using the standard Judkins technique\textsuperscript{20} without the use of nitroglycerin, adenosine, or a calcium channel blocker during the procedure. The angiographic records were reviewed by two experienced angiographers (A.Y. and A.A.) who were blinded to the laboratory and exercise test data. The coronary arteries were accepted as normal in the absence of any luminal narrowing or irregularities. A hyperventilation test was performed to exclude coronary artery spasm. For evaluation of the epicardial flow, TFC was calculated as described by Gibson et al\textsuperscript{21}. The mean TFC was calculated from the average of the TFC values of the right coronary artery (RCA), left circumflex artery (Cx), and left anterior descending artery (LAD) (a corrected TFC [c TFC] was used for the LAD by divid-
ing the TFC by 1.7). The MBG was quantified using the method described by van’t Hoff et al. (MBG = 0 when no contrast density is present, MBG = 1 when minimal contrast density is present, MBG = 2 when moderate to less-than-normal contrast density is present, and MBG = 3 when normal contrast density is present). In accordance with the literature, impaired myocardial perfusion was defined as an MBG score of less than 3, and normal myocardial perfusion was defined as an MBG score of 3 in all coronary territories9,10.

**Laboratory Measurements**

Blood samples for biochemical and hematological analyses were withdrawn from the antecubital vein without stasis after a 12-hour overnight fasting period and within 48 hours before the angiographic procedures. Ethylenediaminetetraacetic acid (EDTA)-anticoagulated tubes were used for complete blood count examinations, and blood samples were centrifuged at 3000 rpm for 5 minutes within 30 minutes after collection. The blood leukocyte and platelet counts, hemoglobin levels, and other whole blood count parameters (blood samples anticoagulated with K3EDTA) were measured in a blood cell counter using an Abbott Cell-Dyn® 3700 System (Abbott Diagnostics, Santa Clara, CA, USA).

**Statistical Analysis**

The SPSS statistical software package was used for statistical analyses (PASW Statistics for Windows, Version 18.0; SPSS Inc., Chicago, IL, USA). Normality of the distribution of the continuous variables was evaluated using the Kolmogorov-Smirnov test and histograms. Continuous variables are presented as mean ± standard deviation and were compared with Student’s t-test. Categorical data are presented as numbers and percentages and were compared using the chi-squared test (and Fisher’s exact test if needed). Correlation analyses were performed using Pearson’s test. A multivariate logistic regression model was created (including potential confounders of impaired MBG) to identify the independent predictors of impaired myocardial perfusion.

**Results**

The baseline characteristics of the patients with CSX and control subjects are presented in Table I. No differences in age, sex, diagnosis of hypertension and diabetes, smoking status, baseline biochemical parameters, ejection fraction, and medications were found between the two groups.

Comparison of the hematological indices revealed a higher NLR in the patients than in the control subjects (1.98 ± 0.77 vs 1.72 ± 0.55, respectively; \( p = 0.04 \)) (Figure 1, Table II). The other hematological parameters did not differ between the groups (Table II). Thirty-eight patients with CSX (63.3%) had deteriorated myocardial perfusion according to the MBG scores. When the patients with CSX were divided into two groups according to their myocardial perfusion.
dial perfusion, patients with impaired perfusion had a higher NLR than that of patients with normal perfusion (2.13 ± 0.82 vs 1.71 ± 0.59, respectively; \( p = 0.028 \)) (Figure 2, Table II).

A negative correlation was present between the NLR and total MBG score in patients with CSX (\( p = 0.027, r = -0.29 \)); however, the TFC values (separately for the LAD, Cx, and RCA and the mean TFC value) were not correlated with the NLR (\( p > 0.05 \) for all). The TFC value in each coronary artery and the mean TFC value of the patients with CSX are presented in Table II. Logistic regression analysis showed that the NLR was an independent and negative predictor of myocardial tissue perfusion (\( p = 0.027; \) beta,

| Table II. Hematological indices and angiographic parameters of the study patients. |
|---------------------------------|---------------------------------|---------------------------------|
|                                 | Cardiac syndrome X (n= 60) | Control group (n= 60) | \( \rho \) |
| White blood cell count, /mm\(^3\) | 6914 ± 1512 | 7210 ± 2176 | 0.39 |
| Neutrophil-to-lymphocyte ratio | 1.98 ± 0.77 | 1.72 ± 0.55 | 0.04 |
| Platelets, 103/mm\(^3\) | 270 ± 63 | 268 ± 58 | 0.87 |
| Platelet-to-lymphocyte ratio | 133 ± 53 | 120 ± 42 | 0.17 |
| Mean platelet volume, fl | 9.24 ± 1.41 | 9.28 ± 1.53 | 0.88 |
| Hemoglobin, g/dl | 13.57 ± 1.38 | 13.77 ± 1.48 | 0.45 |

|                                 | CSX with IMP (n= 38) | CSX with NMP (n= 22) | \( \rho \) |
| White blood cell count, /mm\(^3\) | 7110 ± 1369 | 6575 ± 1712 | 0.19 |
| Neutrophil-to-lymphocyte ratio | 2.13 ± 0.82 | 1.71 ± 0.59 | 0.028 |
| Platelets, 103/mm\(^3\) | 269 ± 67 | 273 ± 57 | 0.82 |
| Platelet-to-lymphocyte ratio | 133 ± 57 | 132 ± 45 | 0.95 |
| Mean platelet volume, fl | 9.46 ± 1.61 | 8.85 ± 0.84 | 0.11 |
| Hemoglobin, g/dl | 13.47 ± 1.58 | 13.75 ± 0.95 | 0.44 |
| TFC – LAD (c) | 21.18 ± 4.94 | 20.34 ± 2.40 | 0.46 |
| TFC – Cx | 25.78 ± 5.74 | 23.73 ± 4.68 | 0.16 |
| TFC – RCA | 21.87 ± 5.94 | 20.45 ± 4.18 | 0.33 |
| Mean TFC | 22.94 ± 4.12 | 21.51 ± 2.22 | 0.14 |

CSX: cardiac syndrome X, NMP: normal myocardial perfusion, IMP: impaired myocardial perfusion, TFC: Thrombolysis In Myocardial Infarction frame count (c: corrected), LAD: left anterior descending artery, Cx: circumflex artery, RCA: right coronary artery. Data are presented as mean ± standard deviation.
−1.057; odds ratio, 2.878; 95% confidence interval, 1.129-7.335) (Table III).

Discussion

In the present study, we evaluated the potential association between inflammation and myocardial microvascular circulation in patients with CSX. The NLR, an inflammatory marker, was higher in these patients than in control subjects, and there was a significant association between an increased NLR and angiographically proven impaired myocardial microcirculation. These results indicate that inflammation might be a part of the unclear pathogenesis of CSX as shown in obstructive atherosclerotic cardiovascular diseases. To the best of our knowledge, this is the first study to show this relationship.

The exact pathophysiological mechanisms of CSX have not yet been identified. Nevertheless, changes in microvascular tonus, impaired coronary flow reserve and insulin resistance, altered sodium-hydrogen exchange activity, and increased sensitivity of cardiac pain have been proposed as the plausible explanations of this clinical entity. According to the current data, in patients with CSX, microvascular dysfunction is distributed patchily in the myocardium and it is mediated by inflammation and oxidative stress. Recio-Mayoral et al. evaluated 21 patients with CSX and showed that the coronary flow reserve (CFR), as measured by positron emission computed tomography was lower in patients with CSX. Moreover, patients with CSX with a CRP level of >3 mg/dl had more prominent impairment in CFR, and there was a negative correlation between the CFR and CRP level. The authors considered that inflammation contributes to the modulation of the microvascular circulation in patients with CSX. Rinkevich et al. identified abnormal coronary autoregulation as assessed by myocardial contrast echocardiography in 18 women with CSX. They speculated that the coronary resistance vessels might be the site of the microvascular abnormality. Demir et al. reported increased concentrations of ischemia-modified albumin, high-sensitivity CRP, and oxidative stress parameters in 32 patients with CSX. Our results support these findings by providing a significant association between inflammation as evidenced by increased NLR and impaired myocardial tissue perfusion as evidenced by decreased MBG score.

The NLR is a readily available marker calculated from whole blood count parameters and has recently emerged as a surrogate marker of inflammation in a variety of cardiac and noncardiac disorders. Because inflammation plays key roles in the development, progression, and acute complications of cardiovascular diseases, many clinical trials have focused on the NLR. This marker has been shown to be associated with worse outcomes in patients with acute coronary syndromes, the severity of coronary artery disease in patients with stable and unstable chest pain, the prognosis of asymptomatic carotid artery disease and stroke, and blood pressure variability as well as diastolic dysfunction in hypertensive patients.

The NLR has also been examined in a few studies of patients with CSX. Demirkol et al. showed that patients with CSX had higher NLRs than did control subjects. They also found a positive correlation between the carotid intima-media thickness and the NLR. In a study investigating the relationship between the NLR and heart rate recovery after exercise, patients with CSX were found to have higher NLRs than the control group. Similarly, in a study by Tenekecioglu et al. the total white blood cell count, NLR, and CRP level were found to be higher in patients

<table>
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<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>p</th>
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<td>Age</td>
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<td>0.924-1.092</td>
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<td>Sex</td>
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<td>Diabetes mellitus</td>
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<td>0.479</td>
<td>0.114-2.008</td>
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<td>0.956</td>
<td>0.843-1.085</td>
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<td>Neutrophil-to-lymphocyte ratio</td>
<td>2.878</td>
<td>1.129-7.335</td>
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</table>

CI: confidence interval.
with CSX. These studies indicate the importance of inflammation in the development of CSX. The present findings support the previously published data but approach the issue from a new perspective. Namely, our main finding is the association between the NLR and impaired myocardial perfusion as assessed by the MBG. However, we did not show a relationship between the NLR and epicardial coronary blood flow as assessed by the TFC score. Hence, we can argue that subclinical inflammation might result in impairment of microvascular circulation as reported by Rencio-Mayoral et al\(^5\) and Rinkevich et al\(^6\), although coronary epicardial blood flow is normal. Undoubtedly, this issue should be clarified through large-scale clinical trials. We believe that our study may inspire further trials to investigate the mechanisms involved in the development of CSX and evaluate the possible role of inflammation in this entity. As a low-cost and readily available examination, the NLR might be used for this evaluation based on the results of our study.

Although MBG is a useful imaging tool for evaluation of diseased microvascular networks in the catheterization laboratory\(^9,10\), a combination of the MBG with echocardiographic and/or angiographic measurement of the CFR could have confirmed and strengthened our findings regarding the myocardial microcirculation. Most researches conducted in patients with coronary artery disease have revealed that the neutrophil counts and NLR are independent predictors of cardiovascular outcomes when analyzed together with other inflammatory markers such as the CRP level\(^27\). Even so, the use of additional inflammatory biomarkers could render our results more contributory.

**Conclusions**

In patients with CSX, we demonstrated a relationship between NLR and impaired myocardial microcirculation. As a low-cost and readily available examination, NLR might be used for this evaluation.

**Conflict of Interest**

The Authors declare that they have no conflict of interests.

**References**


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