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Predictors of coronary collaterals in patients with non ST-elevated acute coronary syndrome: the paradox of the leukocytes

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Abstract

Aim of the study: *Atherosclerosis represents active inflammation in which leukocytes play significant role. Coronary collateral development is a response to myocardial ischaemia. In this study we aimed to investigate the association of the leukocytes with coronary collateral development in patients with non ST-elevated acute coronary syndromes (NST-ACS).*

Material and methods: *A total of 251 consecutive patients were hospitalized in our hospital with a diagnosis of NST-ACS. The blood samples were collected 1-hour after admission to the hospital and peripheral leukocytes (neutrophils, monocytes and lymphocytes) were examined. All patients underwent coronary angiography. The coronary collateral vessels (CCV) are graded according to the Rentrop scoring system.*

Results: *Group 1 consisted of 146 patients with Rentrop 0 and Group 2 consisted of 105 patients with Rentrop 1, 2 and 3. The presence of CCV was significantly associated with neutrophil count, lymphocyte count, monocyte count and neutrophil-lymphocyte ratio (NLR). In subgroup analyses, higher NLR was significantly associated with good CCV development in patients with NST-ACS.*

Conclusions: *Higher neutrophil count, monocyte count and NLR and lower lymphocyte count on admission, were associated with the presence of CCV in patients with NST-ACS. High NLR may predict good collateral development in patients with NST-ACS.*

Key words: *leukocytes, angiogenesis, coronary collateral vessel.*

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Introduction

Atherosclerosis represents an active inflammation in which leukocytes play a significant role [1]. Neutrophil count, lymphocyte count and neutrophil-to-lymphocyte ratio (NLR) are readily available biomarkers that give important information about the inflammatory activity during the acute phase of the coronary syndrome [2]. Neutrophil to lymphocyte ratio was also reported as an independent predictor for mortality and myocardial infarction in coronary artery diseases [3].

Coronary collateral development is a response to myocardial ischaemia [4]. This adaptive circulation protects the myocardium from ischaemic episodes, improves the myocardial contractility and reduces anginal symptoms and cardiovascular events [5, 6]. Well-developed coronary collateral prolongs the substantial time from the onset of a myocardial infarct to successful coronary reperfusion [7].

The role of the inflammation in myocardial ischaemia which is the primary stimulant for coronary collateral development, has been reported previously [8]. Despite similar degrees of coronary stenosis, the discrepancy in collateral formation may be related to different effects of inflammatory cells [8].

In this study we aim to investigate the association of white blood cell subtypes and NLR with coronary collateral development in patients with non ST-elevated acute coronary syndromes (NST-ACS).

Material and methods

A total of 251 consecutive patients were hospitalised in our hospital with a diagnosis of NST-ACS. Acute coronary syndrome (ACS) was defined as presentation with symptoms of ischaemia in association with electrocardiograph-

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ic changes, positive cardiac enzymes, new documentation of coronary artery disease or the previous coronary artery disease diagnosis as defined by similar previous studies [9]. Symptomatic patients were assessed by a standard diagnostic flowchart that included clinical and electrocardiographic monitoring as well as biochemical measurements of myocardial necrosis markers. Patients diagnosed with acute myocardial infarction (AMI) with non-ST elevation (non-STEMI) (n = 154), and unstable angina (UA) (n = 92) were included in this study. The study protocol was approved by the institutional review board in our centre, and informed consent was obtained from all patients. Demographic characteristics, medical histories, laboratory studies including leukocyte counts and peripheral differential counts, and a variety of hospital outcome data were collected. Clinical information included data on systemic hypertension (HT), diabetes mellitus (DM), hyperlipidaemia, smoking, previous history of coronary artery disease (CAD), including coronary angioplasty or myocardial revascularisation, and early family history of CAD. Diabetes mellitus was determined by physician report and was based on a fasting blood sugar level ≥ 126 mg/dl or the use of an anti-diabetic medication. Hypertension was physician-reported for systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or use of anti-hypertensive agents. Hyperlipidaemia was physician reported for total cholesterol ≥ 200 mg/dl, low-density lipoprotein level ≥ 130 mg/dl, or use of cholesterol-lowering medication. Family history was self-reported when a first order relative had suffered cardiovascular death, myocardial infarction (MI), or coronary revascularisation before age 65. Smoking included active or previous (> 10 pack-years) tobacco use. The blood sample for neutrophil count, lymphocyte count and NLR assessment was collected 1 hour after admission to the hospital. In our hospital, blood samples are collected from the ante-cubital vein by an atraumatic puncture and are sent to the laboratory for analysis within 1 hour after collection. Venous blood is collected in a tube containing K3 EDTA for measurement of haematological indices in all patients undergoing the coronary angiography. Haematological indices are evaluated from CBC (complete blood count) analysis performed by a Coulter LH 780 Hematology Analyser (Beckman Coulter Ireland Inc. Mervue, Galway, Ireland). Coronary angiographies were performed in our clinic using the standard Judkins technique [10]. The coronary artery angiography films were reviewed by two experienced cardiologists blinded to the clinical and demographic data for all patients. The recorded data also included the number of diseased vessels, the vessel to which the collaterals were connected, and the grade of coronary collateral circulation. Vessels exhibiting a 70% or greater reduction in lumen diameter were classified as significant CAD. In subjects with more than one significant CAD vessel, the vessel with the highest collateral grade was chosen for analysis. Collateral flow was graded according to the Rentrop classification: 0 = no filling of any collateral circulation, 1 = filling of side branches of the artery to be perfused by collateral circulation, 2 = partial filling of the epicardial artery by collateral circulation, and 3 = complete filling of the epicardial artery by collateral circulation [11]. Patients were then classified according to presence of collateral circulation as either collaterals absent (grade 0 collateral circulation) (Group 1) or collaterals present (grade 1, grade 2 or grade 3 collateral circulation) (Group 2). In subgroup analyses, the patients were divided into two groups according to the status of the coronary collateral development; patients with poor coronary collaterals (patients with Rentrop 0 and Rentrop 1) and patients with good collaterals (patients with Rentrop 2 and Rentrop 3). Patients with a history of trauma, surgery, neoplasm, or infectious disease in the last 30 days prior to hospitalisation as well as current use of immunosuppressants (including systemic, inhaled and topical corticosteroids) and chronic systemic inflammatory diseases such as rheumatoid arthritis, chronic obstructive pulmonary disease, asthma etc. were excluded from the study. We also excluded any patients with history of post-admission complications such as cardiogenic shock, serious arrhythmias with haemodynamic instability, or heart failure.

Statistical analysis

The primary outcome of interest was the presence of coronary collateral vessels (CCVs), defined as a Rentrop grade ≥ 1. Normal distribution was assessed by the Kolmogorov-Smirnov test. Variables which did not follow a normal distribution were expressed as median and inter-quartile range, whereas other continuous variables were expressed as means ± standard deviation; categorical variables were expressed as proportions. Continuous variables were compared by Student's t-test or Mann-Whitney U test as appropriate. Chi-square test was used to compare categorical data. The Pearson's (for parametric variables) and Spearman's (for nonparametric variables) rank correlation coefficient was used for correlation analysis between Rentrop score (ordinal variable, grade 0-3) and other variables. The relation between the degree of CCVs and the cardiac troponin I (cTnI), total number of diseased vessels, white blood cell count, neutrophil count and monocyte count was quantified with linear logistic regression analysis with adjustment for gender, age, a history of MI. Odds ratios (ORs) and 95% confidence interval are presented. All p values were two-sided with a significance level of $p < 0.05$. The Statistical Package for the Social Sciences 20.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical analysis.

Results

Clinical characteristics

The baseline characteristics of the subjects are presented in Tables 1 and 2. There was no gender differ-

Table 1. Baseline clinical characteristics of patients

Variable	Group 1 (Rentrop 0)	Group 2 (Rentrop 1, 2, 3)	*p*
Gender (M/F), *n*	108/38	83/22	0.35
Age (years)	60.2 ±11.8	62.5 ±12.5	0.13
BMI (kg/m^2)	27.6 ±3.8	27.2 ±3.7	0.42
HT (*n*, %)	79 (54%)	49 (46%)	0.24
DM (%)	35 (24%)	33 (31%)	0.19
Total cholesterol (mg/dl)	197 ±48	195 ±53	0.78
LDL cholesterol (mg/dl)	124 ±44	124 ±42	0.98
HDL-C (mg/dl)	43 ±16	42 ±12	0.49
TG (mg/dl)	146 ±65	164 ±88	0.18
Smoking (*n*, %)	53 (36%)	47 (44%)	0.78
Previous PCI, *n* (%)	15 (10%)	13 (12%)	0.98
Previous CABG, *n* (%)	1 (0.6%)	1 (0.9%)	0.81
Total diseased coronary arteries (*n*)	1.82 ±0.76	2.20 ±0.82	< 0.001
Severely diseased coronary			
LAD	66	35	0.143
Cx	47	38	
RCA	33	32	
Presence of ischaemic ECG changes (%)	72%	73%	0.62
CK (U/l)	374 ±151	382 ±132	0.97
CK-MB (U/l)	39 ±25	40 ±28	0.72
Troponin I (ng/ml)	5.2 ±1.1	9.9 ±4.5	0.04

Table 2. Baseline blood parameters of patients

Variable	Group 1 (Rentrop 0)	Group 2 (Rentrop 1, 2, 3)	*p*
Hemoglobin (g/dl)	13.3 ±2.8	12.9 ±1.6	0.21
White blood cell count (10^3/µl)	9.31 ±2.62	10.24 ±2.68	0.006
Neutrophil count (10^3/µl)	5.74 ±2.12	7.54 ±2.87	0.002
Lymphocytes count (10^3/µl)	2.41 ±1.02	1.81 ±0.85	0.08
Monocyte count (10^3/µl)	0.66 ±0.21	0.78 ±0.32	0.005
Neutrophil/lymphocyte ratio	3.18 ±1.25	4.68 ±2.13	0.001
Platelet count (× 10^9/l)	238.3 ±66.8	235.8 ±69.9	0.77
MPV (fL)	8.58 ±0.92	8.68 ±1.10	0.45
MCV (µ^3)	88.1 ±8.3	89.06 ±6.1	0.29
RDW (%)	14.11 ±1.2	14.46 ±1.3	0.026

Table 3. Correlations of CCV presence with clinical and laboratory variables

Variable	r	p
Age	0.083	0.18
Troponin	0.146	0.02
Total diseased coronary	0.229	0.001
Neutrophil	0.218	0.001
Lymphocyte	–0.295	0.001
NLR	0.391	0.001
Monocyte	0.187	0.003
Coronary thrombus	0.253	0.001

ence between groups 1 and 2 ($p = 0.35$). The patients with CCVs were non-significantly older than the patients without CCVs ($p = 0.13$). Significant differences between the groups were not observed for body mass index (BMI), HT, DM, heart rate, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), smoking, creatine kinase (CK), creatine kinase isoenzyme MB (CK-MB), previous MI history, previous coronary artery by-pass greft (CABG) history, previous percutaneous coronary intervention (PCI) history, presence of ischaemic electrocardiography (ECG) changes, time interval between chest pain and admision to hospital and platelet count. Compared to patients without collaterals, cTnI was higher in patients with collaterals (5.2 ±1.1 vs. 9.9 ±4.5; $p = 0.04$). The patients with collaterals had a higher total number of diseased coronaries (2.20 ±0.82 vs. 1.82 ±0.76; $p < 0.001$), total leukocyte count (10.24 ±2.68 vs. 9.31 ±2.62; $p = 0.006$), red cell distribution width (RDW) (14.46 ±1.32 vs. 14.11 ±1.19; $p = 0.026$), neutrophil count (7.54 ±2.87 vs. 5.74 ±2.12; $p = 0.002$) neutrophil-lymphocyte ratio (4.68 ±2.13 vs. 3.18 ±1.25; $p = 0.001$), monocyte count (0.78 ±0.32 vs. 0.66 ±0.21; $p = 0.005$) than the patients without collaterals. Lymphocyte count was lower in patients with collaterals when compared with the patients without collaterals (1.81 ±0.85 vs. 2.61 ±1.02 respectively; $p = 0.08$).

In the correlation analysis, while the presence of CCVs was significantly positively correlated with the total number of diseased vessels, the presence of coronary thrombus, to-

Table 4. Clinical characteristics of patients with poor and good collaterals

Variable	Poor collateral (Rentrop 1)	Good collateral (Rentrop 2, 3)	*p*
Gender (M/F), *n*	45/15	38/7	0.24
Age (years)	64.6 ±12.6	59.7 ±11.8	0.042
HT (*n*, %)	32 (53%)	17 (38%)	0.11
Diabetes mellitus (%)	23 (38%)	10 (22%)	0.07
Total cholesterol (mg/dl)	194 ±57	197 ±46	0.75
LDL cholesterol (mg/dl)	122 ±44	127 ±39	0.52
Smoking (*n*, %)	22 (36%)	25 (55%)	0.054
Previous PCI, *n* (%)	8 (13%)	7 (15%)	0.74
Previous CABG, *n* (%)	1 (1.6%)	0 (0%)	0.38
Total diseased coronary arteries (*n*)	2.04 ±0.82	2.3 ±0.81	0.094
Coronary thrombus (%)	68%	74%	0.026
Presence of ischaemic ECG changes (%)	75%	71%	0.84
Troponin (ng/ml)	8.7 ±3.2	11.8 ±5.5	0.068

Table 5. Blood parameters of patients with poor and good collaterals

Variable	Poor collateral (Rentrop 1)	Good collateral (Rentrop 2, 3)	*p*
Hemoglobin (g/dl)	12.99 ±1.86	12.96 ±1.89	0.92
White blood cell count (10^3/µl)	9.85 ±2.66	10.01 ±2.51	0.51
Neutrophil count (10^3/µl)	6.72 ±2.17	7.1 ±1.56	0.048
Lymphocytes count (10^3/µl)	2.08 ±0.63	1.68 ±0.67	0.042
Monocyte count (10^3/µl)	0.66 ±0.21	0.78 ±0.32	0.047
Neutrophil/lymphocyte ratio	3.18 ±1.25	4.68 ±2.13	0.003
Platelet count (× 10^9/l)	229.1 ±65.2	244.9 ±75.7	0.26
MPV (fL)	8.71 ±1.12	8.63 ±1.07	0.71
MCV (μ^3)	88.9 ±7.1	89.3 ±4.7	0.70
RDW (%)	14.47 ±1.2	14.46 ±1.3	0.95

tal leukocyte count, neutrophil count, monocyte count and neutrophil-lymphocyte ration, it was significantly negatively correlated with lymphocyte count (Table 3). The total number of diseased vessels was significantly positively correlated with cardiac troponin I ($r = 0.146$, $p = 0.025$). Cardiac troponin I was also significantly correlated with total leukocyte count, neutrophil count, and NLR ($r = 0.326$, $p < 0.001$; $r = 0.343$, $p < 0.001$; $r = 0.239$, $p < 0.001$; respectively). The presence of coronary thrombus was significantly positively correlated with neutrophil count ($r = 0.310$, $p < 0.001$), NLR ($r = 0.252$, $p < 0.001$) and platelet count ($r = 0.295$, $p < 0.001$).

In the subgroup analysis of the patients with CCVs, the patients with good CCVs (Rentrop grade 2 and 3) were younger than the patients with poor CCVs (Rentrop grade 1) ($p < 0.05$). The total number of diseased coronaries between the patients with good CCVs and poor CCVs was not statistically different ($p = 0.094$). While the monocyte count and the neutrophil count was significantly increased in the group with good CCVs, the lymphocyte count was significantly lower in the group with good CCVs. Neutrophil to lymphocyte ratio was significantly higher in the group with good CCVs as compared to the group with poor CCVs (4.68 ±2.13 vs. 3.18 ±1.25 respectively; $p = 0.003$). Coronary thrombus was detected more frequently in the group with good CCVs when compared to the group with poor CCVs ($p = 0.026$) (Tables 4 and 5).

Logistic regression analysis

Age, total leukocyte count, cTnI, NLR, monocyte count, neutrophil count, lymphocyte count, red cell distribution width (RDW) and total number of diseased vessels were included in our univariate analysis. Neutrophil to lymphocyte ratio, monocyte count, neutrophil count, lymphocyte count and total number of diseased vessels were found significant in univariate analysis. In a multivariate logistic regression model with a forward stepwise method, neutrophil count (OR = 1.997, 95% CI: 0.001-0.171, $p = 0.047$), lymphocyte count (OR = 2.012, 95% CI: 0.006-0.583, $p = 0.045$), NLR (OR = 5.244, 95% CI: 0.207-0.457, $p < 0.0001$), and monocyte count (OR = 2.733, 95% CI: 0.128-0.790, $p = 0.007$) remained associated with the development of CCV after adjustment for variables found to be statistically significant in univariate analysis (Table 6). In the multivariate regression analysis of the subgroups with good CCVs and poor CCVs; we found that only NLR significantly predicts good CCVs within the NST-ACS patients (Table 7).

Discussion

The principal finding of our study is that increased total number of diseased coronaries, neutrophil count, monocyte count and NLR are predictors of coronary collateral development in patients with NST-ACS. In subgroup analyses, the increased NLR predicted the patients with good coronary collaterals in patients with NST-ACS.

Leukocytes are rapidly released into the circulation during periods of acute stress, such as trauma or acute

Table 6. Multivariate logistic regression analysis of collateral circulation (good collateral group as reference group)

	Univariate analysis		Multivariate analysis (forward)	
	OR (95% CI)	***p* value**	**OR (95% CI)**	***p* value**
Troponin	1.058 (0.004-0.012)	0.291	–	–
Total number of diseaesed coronary arteries	2.008 (0.002-0.234)	0.046	–	–
White blood cell count	–1.539 (0.078-0.010)	0.125	–	–
Neutrophil count	–1.997 (0.001-0.171)	0.047	–2.510 (0.023-0.190)	0.013
Lymphocyte count	2.012 (0.006-0.583)	0.045	1.979 (0.001-0.570)	0.049
Monocyte count	2.733 (0.128-0.790)	0.007	2.803 (0.132-0.754)	0.005
Neutrophil/lymphocyte ratio	5.244 (0.207-0.457)	< 0.0001	5.386 (0.214-0.461)	< 0.0001
RDW	1.778 (0.007-0.144)	0.077	–	–

Table 7. Logistic regression analysis of good collateral circulation

Model	**Unstandardized coefficients**		**Standardized coefficients**	***t***	**sig.**	**95% CI interval for B**	
	B	**std. error**	β			**lower bound**	**upper bound**
(Constant)	1.101	0.546		2.017	0.046	0.017	2.186
Age	–0.009	0.005	–0.163	–1.737	0.085	–0.019	0.012
Troponin	0.004	0.004	0.082	0.867	0.388	–0.005	–0.20
Neutrophil	–0.123	0.052	–0.357	–2.369	0.020	–0.226	–0.020
Lymphocyte	0.374	0.196	0.379	1.909	0.059	–0.015	0.764
NLR	0.220	0.072	0.705	3.065	0.003	0.077	0.362
Monocyte	0.304	0.185	0.151	1.645	0.103	–0.063	0.670
Total diseased coronary	–0.060	0.075	–0.074	–0.790	0.432	–0.209	0.090

Dependent variable: good collateral

and other markers (e.g., CD11b/CD18) of polymorphonuclear and monocyte activation has been reported in patients with acute ischaemic heart diseases [12, 13]. Coronary collateral vessels confine myocardial ischaemia and have an influence on the prevention of myocardial infarct extension, and preserve the myocardium in patients with ACS [14-17]. Despite the fact that the underlying mechanisms are not fully revealed, myocardial ischaemic episodes, growth factors [especially, granulocyte-colony stimulating factor (G-CSF), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF)], various cell types such as endothelial, monocytes, smooth muscle, shear stress and cytokines are likely factors that are responsible for collateral development during myocardial hypoxia [19-21]. Also, the duration of angina pectoris and the extent of CAD are evident determinants of CCVs. Previously, the researchers have found that in the group with adequate collateral development, the Gensini score and relatedly the atherosclerosis severity was significantly higher than the group with inadequate collateral development [22]. In our study, also, the total number of diseased coronaries was higher in patients with CCVs as compared to the patients without CCVs.

Various studies have been published about the role of monocytes in he development of collateral angiogenesis [23, 24]. Previously, the increased neutrophil count and NLR was found to be associated with poor collateral development in the patients with stable CAD [25]. Paradoxically, the neutrophil count and NLR was significantly increased in patients with CCVs in our study. We also found that increased NLR were significantly related with development of good coronary collaterals in patients with non ST-elevated acute coronary artery disease. Increased leukocytes and its subtypes have a major role in modulating the inflammatory response in the atherosclerotic acute ischaemic process [3]. During the ischaemic process, these inflammatory cells (neutrophils and monocytes) release metalloproteinases that dissolve the surrounding matrix

and the basal membrane of the preformed vessel. Ischaemia sensitises the local vascular endothelial cells to the chemotactic and proliferative effects of various growth factors by upregulating their receptors. Granule proteins and cytokines from neutrophils induce monocyte recruitment. Endothelial cells migrate, proliferate and subsequently form a new vessel with a lumen [26]. Increased reactive oxygen species (ROS) production, MCP-1, VEGF and GCSF are at the corner stones of this angiogenic response. Granulocyte-colony stimulating factor promotes neovascularisation by releasing VEGF from neutrophils and bone marrow-derived cells of haematopoietic lineage [27, 28]. The mechanisms that the neutrophil uses to kill microorganisms also has the potential to injure ischaemic tissue under special circumstances. Its paradoxical role in the pathophysiology of disease was particularly reported in some circumstances; diabetic retinopathy, sickle cell disease, renal microvasculopathy, stroke and acute coronary artery syndromes. In the study by Zhao *et al.*, in the myocardium of post-MI patients, angiogenesis was mostly active in the infarcted myocardium in the first week coincident with increased neutrophilic activation and increased production of ROS, and antioxidant treatment significantly supressed this microvascular angiogenesis [29]. Leukocyte infiltration in the early phase of ischaemia is harmful, but this might also be an essential process for myocardial repair, in which angiogenesis, fibrosis and consequently tissue healing takes place [30]. Inflammation after tissue ischaemia, was associated with subsequent angiogenesis [31]. Thus, angiogenesis after myocardial ischaemia seems to be an substantial part of cardiac remodelling that responds to the injured tissue. In the study by Egami *et al.* [32] the researchers have observed that, in P-selectin knockout (P-selectin$^{-/-}$) mice, there was a decreased tissue inflammation and in response to this, decreased angiogenic cytokine release and consequently, ischaemia-induced angiogenesis was significantly impaired. That study demonstrated the importance of inflammation in angiogenesis. On the other hand, recently, there have been many published studies which report the role of neutrophils in the pathophysiology of angiogenesis [33, 34]. Neutrophils were reported to have the capability to polarise to different phenotypes during angiogenesis [35]. While different neutrophil phenotypes were suggested for tumoural angiogenesis, this also might be considered for post-ischaemia angiogenesis; this requires further molecular biological studies. Besides the change of the phenotype of the neutrophils, the apoptosis of the neutrophils is delayed by the cytokines that results in functional longevity and modulation of the neutrophils' functions [36]. In patients with acute coronary syndromes, the lifespan of the neutrophils was significantly lengthened as a result of the delay of polymorphonuclear neutrophil apoptosis as compared to the patients with stable angina or healthy controls. The cytokines interferon γ (IFN-γ), GM-CSF and interleukin 1β (IL-1β) induced apoptosis delay in leukocytes [36]. This phenomenon might be responsible for the increase of neutrophil count and NLR in CCV development in patients with unstable coronary artery disease when compared to the patients with stable coronary artery disease. There might be a subclinical chronic inflammatory response in patients with unstable CADs; the leukocyte counts at admission may signify a 'subclinical chronic inflammatory response' rather than an acute inflammatory response to myocardial ischaemic attacks in patients with unstable coronary artery disease. Incidentally, compared to the blood neutrophils, those associated with coronary plaques, exhibited increased telomerase activity, which is also a significant marker reflecting prolonged neutrophil lifespan [37]. These data may provide an explanation for the response of such a chronic process (collateral development) to an 'acute' alteration in CBC (leukocyte increase). Apart from the cytokines mentioned above, some soluble mediators from activated platelets have an inhibitory impact on the apoptosis process, significantly prolonging leukocyte survival [36]. In relation to activated platelets, in our study, there was significant association between coronary thrombus formation and the neutrophil count, NLR and lymphocyte count.

Furthermore, there are published studies about neutrophil subpopulations [38, 39]. In the study by Christoffersson *et al.* [39], the researchers found evidence for multiple neutrophil subtypes and proposed a subset of neutrophils with angiogenic properties, releasing MMP-9 (matrix metalloproteinase 9). They proposed that the neutrophils may be plastic and could adopt to their environment by changing their phenotypes as mentioned above. Christoffersson *et al.* reported that neutrophil which releases MMP-9 aids in the development of new vasculature in transplanted pancreatic islets. Matrix metalloproteinase 9 is highly expressed in the vulnerable regions of the atherosclerotic plaque, and it has been suggested that it is causally involved in plaque rupture. These angiogenic neutrophils may play a role in CCV formation in the patients with acute coronary syndromes as well. During the atherosclerosis process, starting from the plaque activation, neutrophils may facilitate their phenotypes and may induce angiogenesis in severely diseased coronaries to relieve ischaemia in the related myocardial area. Our study is an observational study, and we hypothesise that during acute coronary syndromes, the leukocytes have a role not only in the vulnerability of the atherosclerotic plaques but also in the recruitment of the vessel formation.

There are some limitations to our study. Firstly, the number of patients is low, and an increased number of patients are needed. Secondly, we could not measure some inflammatory markers in our study, such as highly sensitive C-reactive protein (CRP), interleukins or chemokines (MCP-1) because of the technical insufficiency in our hospital. Thirdly, angiography may not detect most collaterals

situated intramurally. Therefore, the collaterals visualised by angiography may not accurately quantify collateral circulation. But the effect of this problem on the collateral score would be the same in the two groups and thus should not change the interpretation of our results. Finally, the present study is a retrospective, observational one. However, the angiographic and clinical data belong to the same period and come from the same laboratory.

Conclusions

In patients with NST-ACS, the increased neutrophil count, monocyte count and NLR may guide clinicians in their opinion about the development of CCVs in severely diseased coronaries. High NLR may predict good collateral development in patients with NST-ACS.

Authors declare no conflict of interest.

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