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Total leukocyte count, leukocyte subsets and their indexes in relation to cardiovascular risk in adolescent population

A. KLISIC[1], I. RADOMAN VUJAČIĆ[2], L.J. VUČKOVIĆ[3], A. NINIC[4]

[1]Center for Laboratory Diagnostics, Primary Health Care Center, University of Montenegro-Faculty of Medicine, Podgorica, Montenegro
[2]Clinical Center of Montenegro, Department of Internal medicine, University of Montenegro -Faculty of Medicine, Podgorica, Montenegro
[3]Clinical Center of Montenegro, Department of Pathology, University of Montenegro-Faculty of Medicine, Podgorica, Montenegro
[4]Department for Medical Biochemistry, University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia

Abstract. – OBJECTIVE: No studies investigated total leukocytes, their subpopulations and novel indexes based on different ratios of leukocyte subsets concerning cardiovascular risk (CV) risk in late adolescents. Therefore, the aim of the present study was to explore such potential relationships.

PATIENTS AND METHODS: A total of 156 adolescents were included. CV risk score was calculated by summarizing each risk factor (i.e., female sex, low high density lipoprotein cholesterol (HDL-c), high non-HDL-c, smoking, blood pressure, and fasting glycemia). Adolescents were divided into a low CV risk score (i.e., -2≤ CV risk score ≤1) and moderate/higher CV risk score (i.e., CV risk score ≥ 2). White blood cell count (WBC) and its subsets were analyzed on an automatic device. The indexes were calculated.

RESULTS: Total and differential WBC counts except basophil count were higher in moderate/higher CV risk participants. Multivariate binary regression analysis showed that total WBC count independently increased CV risk score by 1.623 times (p=0.001). Neutrophil and eosinophil counts (p=0.027 and p=0.010, respectively) were independently able to increase CV risk score by 1.486 and 1.556 times, respectively. On the contrary, indexes were not independently correlated with CV risk.

CONCLUSIONS: WBC, neutrophil, and eosinophil count are the independent predictors of increased CV risk in adolescents. The associations may indicate the different pathways that lead to CV disease in adulthood.

Key Words:
Adolescents, Cardiovascular risk, Inflammation.

Introduction

Identification of cardiovascular (CV) risk in adolescence is usually neglected. It often tracks into adulthood, leading to CV diseases and, in some cases, to increased mortality[1]. Hence, it is of great importance to treat this population promptly and adequately to avoid/reduce potential consequences late in life.

Obesity represents the major CV risk factor in youngsters[1]. We have recently reported a wide spectrum of biomarkers that are highly related to obesity-induced inflammation in young[2-4] and adult[5] population. It is known that increased inflammation, in addition to increased reactive oxygen species, could affect pathways of insulin signaling and lead to related cardiometabolic disturbances[6-9]. Accordingly, obesity needs to be targeted as the first line of treatment in adolescents with increased CV risk. In addition, preventive strategies to reduce obesity are needed[10,11], but special attention should also be paid to the recognition of the low cost and easily measured biomarkers that can indicate increased CV risk and that can be widely available in the primary care settings.

In line with this, in everyday practice, markers of inflammation that include total white blood cell count (WBC) and leukocyte subsets have been extensively explored in the adult population. However, as far as we know, such examinations are not investigated thoroughly in late adolescents. Moreover, recent studies[12-15] have suggested leukocyte indexes as more reliable parameters

Corresponding Author: Aleksandra Klisic, MD, Ph.D; e-mail: aleksandranklisic@gmail.com

that can enable better insight into various diseases than WBC alone. These indexes have been mostly explored in malignant and other chronic diseases in adults[12-15]. The proposed indexes include neutrophil to lymphocyte ratio (NLR), derived NLR (dNLR), neutrophil to monocyte ratio (NMR), monocyte/granulocyte to lymphocyte ratio (M/GLR), monocyte to lymphocyte ratio (MLR), basophil to lymphocyte ratio (BLR), basophil to neutrophil ratio (BNR), and basophil to monocyte ratio (BMR).

Concerning such information, we hypothesize that total leukocyte count, leukocyte subsets, and their indexes could be related to higher CV risk in the adolescent population. Therefore, the aim of the present study was to explore such potential relationships.

Patients and Methods

Study Population

The cohort comprised a total of 156 adolescents (i.e., from the 3rd and the 4th grades of the two secondary schools in Podgorica, Montenegro). Each teenager underwent physical examination, anthropometric measurements, and blood sampling procedure. The Ethical Committee of the Primary Health Care Center, Podgorica, Montenegro, gave approval for the study protocol. Each adolescent has signed an informed consent to participate, and for those <18 years, parental written consent was provided, also. Healthy adolescents between the ages 16-19 years who voluntarily accepted to participate in the study and that kept their body weight stable in the last 3 months were included. Those who had any acute/chronic inflammatory and cardiometabolic disease, who used any medications in the last 3 months, as well as those who reported cigarette smoking or alcohol consumption were excluded from further participation. Notably, we excluded all those examinees with hs-CRP higher than 10 mg/L to minimize the potential bias of other factors related to increased inflammation.

Anthropometric Measurements

The anthropometric measurements were obtained in the morning after an overnight fast. Participants were barefooted and with light clothing when measuring body weight and body height. Waist circumference was obtained with the tape over the abdomen after removal of clothing. The measurements were taken at the midpoint between the lowest rib and the iliac crest at the end of normal expiration, as previously described[3].

Body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared (kg/m^2).

Biochemical and Hematological Analyses

Blood sampling was provided in the morning after an overnight fast of at least 8 hours, immediately after the anthropometric measurements were taken. The two samples were obtained from each participant. One sample was provided in the tube with a serum separator and clot activator for the determination of hs-CRP, fasting glucose, and lipid parameters. The other sample was obtained in the tube with K_2EDTA for the determination of WBC and its subsets.

The samples with serum separator and clot activator were left to clot within 30 minutes and thereafter underwent centrifugation for 10 minutes at 3000xg (at room temperature). Afterward, serum levels of hs-CRP, fasting glucose, and lipid parameters were determined on Roche Cobas c501 chemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The whole blood samples were immediately analyzed on a Sysmex XT-4000i analyzer (Sysmex Corporation, Kobe, Japan) for WBC and their subsets as a part of a complete blood cell count.

The indexes were calculated[13] as follows: NLR=neutrophil to lymphocyte ratio, derived NLR (dNLR)=neutrophils/(WBC-neutrophils), NMR=neutrophil to monocyte ratio, M/GLR=-monocyte/granulocyte to lymphocyte ratio, MLR=monocyte to lymphocyte ratio, BLR=basophil to lymphocyte ratio, BNR=basophil to neutrophil ratio, BMR=basophil to monocyte ratio.

CV risk score was modified according to McMahan et al[16] and was calculated by summarizing of each risk factor: female sex (points= -1), low high density lipoprotein cholesterol (HDL-c) (<1.04 mmol/L points=1, between 1.04-1.55 mmol/L points=0, >1.55 mmol/L points= -1) high non-HDL-c (<3.4 mmol/L, points=0, between 3.4-4.0 mmol/L, points=2, between 4.1-4.8 mmol/L, points=4, between 4.9-5.6 mmol/L, points=6 and >5.7 mmol/L points=8), smoking (points=1), blood pressure (BP) (normal BP <130/85 mmHg, points=0, high systolic BP≥130 mmHg and/or diastolic BP≥85 mmHg, points=4) and fasting glucose (≥5.6 mmol/L, points=5), as reported previously[17]. Since we have excluded adolescents who were smokers to diminish other

bias factors that might influence inflammation levels, we have calculated 0 points for each participant's smoking status. Accordingly, adolescents were divided into a low CV risk score (i.e., -2≤ CV risk score ≤1) and moderate/higher CV risk score (i.e., CV risk score ≥2).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS, IBM, Chicago, IL, USA) version 22 was used for data analysis. The normality of the variables was assessed by the Kolmogorov-Smirnov test. Due to skewed data distribution, Mann Whitney *U* test was used to compare continuous variables between the two groups. Medians and 25^{th}-75^{th} percentiles were used for data presentation. The difference between categorical variables was assessed using the Chi-square test for contingency tables, and the data were presented as absolute frequencies. To evaluate the association between total leukocyte and subset counts and leukocytes' indexes (dependent, continuous variables) and CV risk score (categorical variable defined as 0 – low CV risk and 1 – moderate/higher CV risk), binary regression analysis was performed. Models in the binary regression analysis consisted of the main markers adjusted for gender and CV risk factors such as BMI and hs-CRP. Data from regression analyses were given as the estimated odds ratios (ORs) and 95% confidence intervals (CI). Explained variation in CV risk was presented as Nagelkerke R^2. *P* less than 0.05 was set as the significance level.

Results

Table I shows descriptive characteristics, leukocyte subsets, and indexes of low *vs.* moderate/higher CV risk in the study cohort. Participants with moderate/higher CV risk score had significantly higher BMI, waist circumference, and hs-CRP than participants in the low CV risk group. Total and differential WBC except basophil count were also higher in moderate/higher CV risk participants. However, of all calculated indexes, BNR and BMR were lower in participants with moderate/higher CV risk.

Our further intention was to determine the potential relation between all studied markers and CV risk score. To achieve this aim, we firstly performed the univariate binary regression analysis (Table II). Higher total and differential WBC counts except basophils increased the CV risk score. An increase in WBC, neutrophil, lymphocyte, monocyte, eosinophil counts, NLR, and M/GLR were associated with 1.809, 1.814, 1.794, 1.476, 1.665, 1.469, and 1.423 times, respectively increased likelihood to exhibit moderate/higher CV risk score (Table II). As demonstrated by Nagelkerke R^2 for each

Table I. General demographic and leukocyte subsets and their ratios in adolescents according to CV risk score.

	Low CV risk score (-2 ≤ CV risk score ≤ 1)	Moderate/Higher CV risk score (CV risk score ≥ 2)	*p*
N (female/male)	109 (91/18)	47 (35/12)	0.190
Age, years	18 (17-19)	17 (16-19)	0.461
BMI, kg/m^2	22.2 (20.5-25.2)	26.4 (23.3-30.8)	< 0.001
Waist circumference, cm	79 (75-86)	93 (81-100)	<0.001
hsCRP, mg/L	0.41 (0.30-0.72)	1.17 (0.52-2.58)	< 0.001
WBC (×10^9/L)	6.45 (5.80-7.39)	8.25 (6.95-9.77)	< 0.001
Neutrophil count (×10^9/L)	3.29 (2.75-3.96)	4.38 (3.23-5.51)	< 0.001
Lymphocyte count (×10^9/L)	2.34 (1.98-2.63)	2.61 (2.28-3.02)	< 0.001
Monocyte count (×10^9/L)	0.57 (0.47-0.68)	0.68 (0.56-0.78)	0.001
Eosinophil count (×10^9/L)	0.14 (0.10-0.20)	0.19 (0.13-0.29)	0.001
Basophil count (×10^9/L)	0.044 (0.023-0.068)	0.040 (0.026-0.053)	0.413
NLR=Neu/Lymph	1.46 (1.15-1.81)	1.53 (1.14-2.29)	0.322
dNLR=Neu/(WBC-Neu)	1.07 (0.87-1.37)	1.12 (0.84-1.55)	0.375
M/GLR=(WBC-Lymph)/Lymph	1.83 (1.44-2.16)	1.78 (1.45-2.77)	0.322
NMR=Neu/Mono	5.82 (4.96-7.24)	6.57 (5.22-8.41)	0.196
MLR=Mono/Lymph	0.24 (0.21-0.28)	0.25 (0.20-0.34)	0.587
BNR=Baso/Neu	0.014 (0.008-0.019)	0.008 (0.005-0.013)	0.011
BLR=Baso/Lymph	0.019 (0.010-0.027)	0.015 (0.009-0.024)	0.158
BMR=Baso/Mono	0.081 (0.041-0.109)	0.056 (0.037-0.086)	0.050

Data are given as median (interquartile range) and compared by Mann-Whitney test. Categorical data are presented as absolute frequencies and compared with Chi-square test for contingency tables.

Table II. Univariate binary logistic regression analysis for the associations of white blood cells and subsets and CV risk score in adolescents.

Predictors	Adjusted OR (95% CI)	p	Nagelkerke R^2
WBC ($\times 10^9$/L)	1.809 (1.423- 2.300)	< 0.001	0.246
Neutrophil count ($\times 10^9$/L)	1.814 (1.362-2.417)	< 0.001	0.175
Lymphocyte count ($\times 10^9$/L)	1.796 (1.115-2.891)	0.016	0.054
Monocyte count ($\times 10^9$/L)	1.476 (1.184- 1.838)	0.001	0.125
Eosinophil count ($\times 10^9$/L)	1.665 (1.247-2.224)	0.001	0.123
Basophil count ($\times 10^9$/L)	0.975 (0.856-1.110)	0.700	0.001
NLR=Neu/Lymph	1.469 (1.008-2.412)	0.045	0.041
dNLR=Neu/(WBC-Neu)	1.880 (0.986-3.586)	0.055	0.035
M/GLR=(WBC-Lymph)/Lymph	1.423 (1.015-2.020)	0.041	0.043
NMR=Neu/Mono	1.120 (0.961-1.306)	0.145	0.019
MLR=Mono/Lymph	1.233 (0.912-1.668)	0.174	0.017
BNR=Baso/Neu	0.703 (0.462-1.072)	0.102	0.027
BLR=Baso/Lymph	0.802 (0.590-1.092)	0.161	0.019
BMR=Baso/Mono	0.937 (0.868-1.012)	0.099	0.027

marker, WBC, neutrophil, lymphocyte, monocyte, and eosinophil counts could explain 24.6%, 17.5%, 5.4%, 12.5%, 12.3%, 4.1%, and 4.3%, respectively variation in CV risk score.

Our next step was to examine potential independent associations and predictions of examined markers on the CV risk score. Accordingly, we performed multivariate binary regression analysis. Each model consisted of significant predictors in univariate analysis, and markers related to CV risk (such as BMI and hs-CRP) were included in multivariate binary regression analysis. Also, we included gender in each model due to the small number of males in the tested cohort. According to Model 1, a higher total WBC count independently increased CV risk score by 1.623 times. This model could explain 43% of variation in the CV risk score. Higher neutrophil and eosinophil counts within Model 2 were independently able to increase CV risk score by 1.486 and 1.556 times, respectively. Model 2 could also explain variation in CV risk score by 43%, the same as the Model 1. However, lymphocyte and monocyte counts, as well as NLR and M/GLR after adjustment for gender, BMI, and hs-CRP were no longer significant predictors of CV risk score (Model 2, 3, and 4, Table III).

Discussion

To our knowledge, this is the first study that investigated total leukocytes and their subpopulations in relation to CV risk in late adolescents.

Table III. Multivariate binary logistic regression analysis for the associations of white blood cells, its subsets and indexes and CV risk score in adolescents.

	Adjusted OR (95% CI)	p	Nagelkerke R^2
Model 1			
WBC ($\times 10^9$/L)	1.623 (1.234-2.134)	0.001	0.430
Model 2			
Neutrophil count ($\times 10^9$/L)	1.486 (1.045-2.113)	0.027	0.430
Lymphocyte count ($\times 10^9$/L)	1.324 (0.706-2.484)	0.382	
Monocyte count ($\times 10^9$/L)	1.038 (0.778-1.386)	0.798	
Eosinophil count ($\times 10^9$/L)	1.556 (1.109-2.184)	0.010	
Model 3			
NLR = Neu/Lymph	1.283 (0.849- 1.939)	0.236	0.349
Model 4			
M/GLR=(WBC-Lymph)/Lymph	1.263 (0.868-1.837)	0.222	0.350

Model 1 consisted of WBC, gender, BMI and hsCRP. Model 2 consisted of neutrophil, lymphocyte, monocyte, eosinophil counts, gender, BMI and hsCRP. Model 3 consisted of NLR, gender, BMI and hsCRP. Model 4 consisted of M/GLR, gender, BMI and hsCRP.

It is also the first one that explored novel indexes based on different ratios of leukocyte subsets in relation to CV risk in teenagers. Our results reveal that WBC, neutrophil, and eosinophil count are the independent predictors of increased CV risk in this population group.

Our findings of the association of WBC and neutrophil count with higher CV risk are in line with a recent population-based cohort study in adults. Namely, Groot et al[18] reported an increase in WBC across the CV disease continuum, which was mostly dependent on the neutrophil count. Similarly, an increase in the left ventricular mass index, as well as increased carotid intima-media thickness, along with increased WBC quartiles in children, was shown[19].

Obesity is the major CV risk factor in adolescence[20-22]. Enlarged adipocytes are characterized by enhanced immune response followed by increased neutrophil infiltration and polarization of macrophages[23], thus leading to increased inflammation. Indeed, some earlier studies[6,11] reported higher neutrophil count in the obese state, whereas some others did not[24]. The different duration of obesity might explain such discordant results since it is assumed that, in early phases, the inflammation reflected with the high neutrophil count in circulation could not occur yet.

Neutrophils represent the most abundant subpopulation of WBC in circulation and are involved in the acceleration of each stage of atherosclerosis[25,26]. This is mainly enabled due to its ability to foster recruitment of monocytes, as well as to activate macrophages in the subendothelium. Their cytotoxicity favors tissue damage leading to increase secretion of interleukine precursors derived from macrophages. This suggests that neutrophils may play a direct role in the destabilization of the atherosclerotic plaque[25].

However, concerning the eosinophil count, the results of previous studies in the adult population are discrepant. In a study that included more than 5,000 patients that underwent coronary angiography, eosinophil count was inversely related to coronary artery disease[27]. Similarly, Semerano et al[28] reported that high eosinophil counts are predictors of better stroke outcomes and lower mortality. On the contrary, Fajar et al[29] reported that eosinophils were positively related to coronary heart disease, whereas Kang et al[30] showed that both lower and higher eosinophils and their changes during the first 3 months after hemodialysis initiation were related to higher all-cause mortality in patients on hemodialysis.

Such controversies can be attributed to differences in study samples and many confounding factors, such as comorbidities, medications use, ethnic differences, etc. In the current investigation, we included normal weight and overweight/obese otherwise healthy teenagers and tried to minimize any bias factor that might affect leukocytes and their subsets. Peterson et al[31] have revealed that eosinophils have the ability to synthesize and secrete bioactive molecules that exhibit cytotoxic properties in CV disease. Moreover, it was shown that eosinophils have the ability to activate platelets, which may further initiate thrombin formation[32].

Previous studies[13,14] have suggested that indexes calculated as ratios of different WBC subsets show better diagnostic performance than total WBC and their subpopulation. However, despite the fact that novel indexes are shown to be reliable indicators of disease severity in some studies in adults[12-15,33], the current research has failed to demonstrate their superiority over WBC and its subsets (i.e., neutrophils and eosinophils) in youngsters. This is opposite to the findings of Hlapčić et al[13], who have recently reported that NLR, as well as dNLR and M/GLR (as modified NLR parameters), show a good capability in identifying chronic obstructive pulmonary disease patients. In line with this, Rajwa et al[12] reported that among examined indexes, only dNLR was shown to be an independent prognostic factor for cancer-specific survival and overall survival in renal cell carcinoma patients after nephrectomy.

Our study has a cross-sectional design, and therefore, the cause-effect of the relationship between WBC and some of their subpopulations and CV risk cannot be confirmed. At the period the current study was conducted, we were limited with other diagnostic methods, such as carotid intima-media thickness measurement[20]. Accordingly, this is a suggestion for future studies. As far as laboratory parameters are concerned, in this cohort, in addition to WBC and its subsets, we have measured hs-CRP. Hs-CRP represents well-established and the best-validated inflammation marker for CV risk estimation. However, the measurement of some other inflammation parameters might contribute to deeper insight into the young population's cardiometabolic risk. Also, we have included only a minor group of subjects with moderate/higher CV risk, especially male teenagers, as most of them were not willing to participate in the study, such as adolescent girls. Therefore, a relatively larger sample size of ado-

lescents is recommended when evaluating such a potential relationship. Importantly, follow-up studies in the young population are needed to explore the relationship between WBC, their subsets and indexes, and CV disease onset and progression late in life.

Conclusions

We observed that the associations of WBC, neutrophils, and eosinophils in the young population may indicate the different pathways that lead to CV disease in adulthood. These hematological parameters are low cost, easily measured, and widely available in primary care settings. Further studies on larger sample sizes are needed to confirm our results.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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