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Novel leukocyte and thrombocyte indexes in patients with prediabetes and type 2 diabetes mellitus

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Abstract. – OBJECTIVE: To our knowledge, there are no studies that investigated the relationship between diabetes mellitus type 2 (T2D) and some novel indexes, such as monocyte/granulocyte to lymphocyte ratio (M/GLR), derived neutrophil to lymphocyte ratio (dNLR), and platelets to neutrophil ratio (PNR). The aim of this study was to examine the association between these novel indexes and glycated hemoglobin (HbA1c) in patients with prediabetes and T2D.

PATIENTS AND METHODS: A total of 827 participants were consecutively recruited. According to the American Diabetes Association (ADA) criteria, participants were divided into control, prediabetes, and T2D group.

RESULTS: White blood cell count (WBC), neutrophil count, NLR, dNLR, and M/GLR were higher in T2D patients than in the other two groups, whereas PNR was the lowest in T2D group. Lymphocyte count was higher in prediabetes and T2D patients than in control group. Multivariable ordinal regression analysis showed that WBC, neutrophil count, lymphocyte count, NLR, dNLR, and M/GLR were positively associated [OR (95% CI) 1.287 (1.191-1.390), $p<0.001$; 1.427 (1.275-1.594), $p<0.001$; 1.347 (1.130- 1.606), $p=0.001$; 1.350 (1.090-1.670), $p=0.006$; 1.662 (1.189-2.326), $p=0.003$; 1.275 (1.057-1.540), $p=0.012$, respectively] with HbA1c. However, PNR was negatively associated with HbA1c [0.987 (0.981-0.993), $p<0.001$].

CONCLUSIONS: Novel, modified NLR indexes, such as dNLR and M/GLR were independently correlated with HbA1c. Also, PNR showed superiority over platelets (PLT) in relation to HbA1c. These novel indexes might give a significant contribution to the timely recognition of disturbances of glucose homeostasis in patients with prediabetes and overt diabetes.

Key Words:
Leukocytes, Platelets, Inflammation, Diabetes.

Introduction

Type 2 diabetes mellitus (T2D) is a worldspread metabolic disorder that is tightly related to chronic low-grade inflammation[1,2]. Insulin resistance is known to be the main feature of T2D[1]. Due to many complications of the mentioned disorder (among them cardiovascular disease as the most common), it is important to gain better recognition into insulin signaling pathways and to explore thoroughly biomarkers that can have reliable diagnostic potential for T2D onset and progression[1].

It is established that the adipose tissue of the visceral region secretes a large number of pro-inflammatory cytokines[3,4]. Among them, the most studied are interleukin (IL)-6 and tumor necrosis factor-α (TNF-α), which can potentiate immune response due to increased neutrophil infiltration and macrophages polarization[3,4]. Some of them also act as proatherogenic and prothrombotic as well, since they influence megakaryocyte proliferation[5]. Namely, they act with thrombopoietin and therefore, stimulate megakaryocytopoiesis, with a concomitantly increasing in a number of PLT[6]. Therefore, PLT are shown to play a critical role in inflammation leading to atherothrombosis onset[7].

In the last two decades several hematological markers, such as total white blood cell count (WBC) and PLT and their subsets have been widely investigated in relation to various chronic disorders, in young[8,9] and adult populations[10-12]. Interestingly, recent studies[13-16] suggested several indexes derived from the WBC, PLT, and their subsets, are shown to be better determinants of many chronic diseases than each parameter alone.

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To our knowledge, there are no studies that investigated the relationship between T2D and some novel indexes, such as monocyte/granulocyte to lymphocyte ratio (M/GLR), derived neutrophil to lymphocyte ratio (dNLR), and platelets to neutrophil ratio (PNR).

Therefore, we aimed at exploring their potential relationship with glucoregulation in patients with prediabetes and T2D. We hypothesize that these low-cost and easy-to-measure hematological parameters and their indexes could be reliable markers in T2D, especially due to their availability and easy calculation of their indexes in a primary care setting.

Patients and Methods

Study Population

A total of 827 participants were consecutively recruited in the study when visiting the primary care setting. The study was approved by the Ethical Committee of the Primary Health Care Center, Podgorica, Montenegro. Each participant provided written informed consent.

Participants filled in questionnaires about lifestyle, illnesses, medications use, smoking habits, and alcohol consumption. Anthropometric measurements (body height and body weight) were obtained from each examinee and body mass index (BMI) was calculated. Measurements were performed in the morning after an overnight fasting in light clothing, immediately after the blood samples were taken.

According to the American Diabetes Association Standards of Diabetes Care criteria[17], all participants were divided into diabetes-free individuals (as the control group), and those with prediabetes and T2D, respectively.

Exclusion criteria were as follows: type 1 diabetes mellitus, pregnancy, acute/chronic inflammatory disease, thyroid disorders, hepatic and kidney disease, malignant disease.

Biochemical and Hematological Analyses

Venipuncture was performed after an overnight fasting of at least 8 hours, between 07:00-10:00 a.m. The sample in the tube with clot activator and serum separator was taken for fasting glucose, whereas the other one was taken in the tube with dipotassium ethylenediaminetetraacetic acid (K_2EDTA) for measurement of CBC (complete blood cell count), its subsets, and glycated hemoglobin (HbA1c).

After being left to clot within half an hour, the samples for fasting glucose determination were centrifugated for 10 minutes at 3000xg. The samples for CBC were determined immediately on a Sysmex XT-4000i analyzer (Sysmex Corporation, Kobe, Japan).

Serum levels of fasting glucose and HbA1c were measured on Roche Cobas c501 chemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

The indexes were calculated[8,9,16] as follows: M/GLR (monocyte/granulocyte to lymphocyte ratio)= (WBC-lymphocytes)/lymphocytes, NLR = neutrophil to lymphocyte ratio, derived NLR (dNLR) = neutrophils/(WBC-neutrophils), PNR = PLT/neutrophils ratio, PLR = PLT/lymphocytes ratio, and MPV/PLT = mean platelet volume to platelet ratio.

Statistical Analysis

The data distribution was checked using the Kolmogorov-Smirnov test. Differences in continuous variables between examined groups were tested by the Kruskal-Wallis test for 3 groups and the Mann-Whitney U-test for two groups. The results were reported as median (interquartile range). Categorical data were reported as absolute frequencies and compared by the Chi-square test for contingency tables. The correlation of HbA1c with independent variables was assessed by Spearman's correlation analysis, and data from this analysis were reported as correlation coefficient (ρ). Univariable and multivariable ordinal logistic regression analysis were conducted to assess the associations of HbA1c (dependent variable coded as follows: 1 – control group, 2-prediabetes, and 3 - diabetes) with hematological parameters (independent variables). Associations were reported as Odds Ratio (OR), with 95% Confidence Intervals (CI). The explained variation in HbA1c levels was given by Nagelkerke R^2 value. Statistical analysis was performed using SPSS software version 21.0 (SPSS Inc., IBM Corp., Armonk, NY, USA). The statistical difference between examined data was set at a $p < 0.05$.

Results

Laboratory and clinical data of the population are given in Table I. Females and males were equally distributed in the examined groups. Glucose and HbA1c levels were significantly different between all three groups, being the highest in the T2D group and the lowest in the control

Table I. Demographic and laboratory characteristics of the examined groups.

	Control group N = 359	Prediabetes N = 167	T2D N = 301	p
Male/Female, N	162/197	75/92	145/156	0.540
Age, years	62 (54-69)	65 (58-71)[a*]	66 (59-72)[a*]	< 0.001
BMI, kg/m^2	28.55 (26.29-31.44)	28.60 (26.11-31.51)	30.94 (27.65-33.75)[a*,b*]	< 0.001
Glucose, mmol/L	5.6 (5.3-5.9)	6.5 (6.1-6.8)[a#]	8 (6.9-9.2)[a*,b*]	< 0.001
HA1c, %	5.2 (4.9-5.4)	5.8 (5.7-6.0)[a#]	6.9 (6.3-8.2)[a*,b*]	< 0.001
Smokers, N (no/yes)	280/79	122/45	253/48[a†,b*]	0.015
Antihyperglycemics, N (no/yes)	359/0	167/0	21/280[a#,b#]	< 0.001
Insulin, N (no/yes)	359/0	167/0	230/71[a#,b#]	< 0.001
Antihyperlipidemics, N (no/yes)	241/118	121/46	157/144[a#,b#]	< 0.001
Antihypertensives, N (no/yes)	60/299	52/115[a#]	51/250[b#]	< 0.001

Continuous data are presented as median (interquartile range) and compared by Kruskal-Wallis and *post hoc* Mann-Whitney U test. Categorical data are presented as absolute frequencies and compared by Chi-square test for contingency tables. [a]Significantly different from the control group. [b]Significantly different from prediabetes. *$p < 0.01$; #$p < 0.001$; †$p < 0.05$.

group. Patients with prediabetes and T2D were significantly older than participants of the control group. BMI levels were higher in T2D patients than in the other two groups. Significantly more smokers, antihyperlipidemic, insulin, and antihyperglycemic users were among T2D patients than in other groups. More antihypertensive users were in the prediabetic patients compared to the control group and in T2D compared to prediabetic patients.

Leukocyte and thrombocyte count and indexes of the examined population were reported in Table II. T2D patients had higher WBC count than prediabetic patients and the control group. Also, WBC count was higher in prediabetic patients than in the control group. The examinees of the control group, prediabetic and T2D patients did not differ in PLT, PDW, MPV, PLR and MPV/PLT. Neutrophil count, NLR, dNLR, and M/GLR were the highest in T2D patients than in two other groups, whereas PNR was the lowest in T2D group. Lymphocyte count was higher in prediabetes and T2D patients than in control group.

The statistically significant positive correlations were observed between HbA1c and the following markers: age, BMI, glucose, WBC count, neutrophil count, lymphocyte count, NLR, dNLR, M/GLR. Significant negative associations were evident between HbA1c and PLR, PNR (Table III).

Further, we wanted to test in-depth associations of HbA1c and hematological markers (as the predictor variables), which showed a significant correlation with HbA1c. Therefore, we performed univariable ordinal regression analysis (Table IV). In the univariable ordinal regression analysis

Table II. Leukocytes and thrombocytes count and indexes in the examined groups.

	Control group	Prediabetes	T2D	p
WBC (×10^9/L)	6.32 (5.34-7.34)	6.72 (5.55-8.12)[a*]	7.29 (6.11-8.99)[a*,b#]	< 0.001
Neutrophil count (×10^9/L)	3.18 (2.58-3.95)	3.26 (2.72-4.36)	3.74 (3.05-5.00)[a*,b†]	< 0.001
Lymphocyte count (×10^9/L)	2.25 (1.92-2.73)	2.45 (1.92-2.73)[a*]	2.51 (2.03-3.12)[a*]	< 0.001
NLR = Neu/Lymph	1.44 (1.11-1.81)	1.40 (1.09-1.85)	1.55 (1.18-2.01)[a#,b*]	0.003
dNLR = Neu/(WBC-Neu)	1.05 (0.84-1.29)	1.07 (0.85-1.36)	1.14 (0.91-1.44)[a#, b*]	0.002
M/GLR = (WBC-Lymph)/Lymph	1.78 (1.40-2.18)	1.72 (0.85-1.36)	1.91 (1.49-2.38)[a#, b*]	0.005
PLT	235 (200-271)	229 (191-280)	237 (203-278)	0.314
PDW (fL)	13.5 (13.0-14.0)	13.7 (13.1-14.2)	13.6 (13.0-14.2)	0.467
MPV (fL)	10.0 (9.5-10.5)	9.9 (9.4-10.6)	10.1 (9.5-10.5)	0.533
PLR = PLT/Lymph	101.06 (80.28-126.17)	96.94 (77.40-117.25)	95.38 (75.58-117.56)	0.057
PNR = PLT/Neu	73.77 (59.03-90.90)	68.20 (54.98-87.68)	62.10 (49.67-78.45)[a*,b#]	< 0.001
MPV/PLT	0.043 (0.036-0.051)	0.044(0.034-0.054)	0.042 (0.034-0.052)	0.512

Data are presented as median (interquartile range) and compared by Kruskal-Wallis and Mann-Whitney *post hoc test*. [a]Significantly different from the control group. [b]Significantly different from prediabetes. *$p < 0.05$; #$p < 0.01$; †$p < 0.001$.

Table III. Spearman's correlation analysis of HbA1c and tested markers.

	ρ	p
Age, years	0.122	< 0.001
BMI, kg/m^2	0.187	< 0.001
Glucose, mmol/L	0.816	< 0.001
WBC (×10^9/L)	0.261	< 0.001
Neutrophil count (×10^9/L)	0.247	< 0.001
Lymphocyte count (×10^9/L)	0.159	< 0.001
NLR = Neu/Lymph	0.097	0.006
dNLR = Neu/(WBC-Neu)	0.101	0.004
M/GLR=(WBC-Lymph)/Lymph	0.092	0.009
PLT	0.055	0.117
PDW (fL)	0.032	0.360
MPV (fL)	0.017	0.634
PLR = PLT/Lymph	-0.096	0.006
PNR = PLT/Neu	-0.202	< 0.001
MPV/PLT	-0.041	0.249

WBC, neutrophil count, lymphocyte count, NLR, dNLR, M/GLR were positively associated with HbA1c. However, PNR was negatively associated with HbA1c.

Possible independent associations between HbA1c and hematological markers, which were significantly related to HbA1c in univariable analysis, were further tested in multivariable ordinal regression analysis. Variables significantly different between tested groups (BMI, smoking, antihypertensive therapy, antihyperlipidemic therapy) were included in the multivariable ordinal regression models as covariates. All hematological markers (predictors) significantly associated with HbA1c in univariable analysis kept their independent association with HbA1c when tested in models (Table V).

Adjusted ORs for WBC, neutrophil count, lymphocyte count, NLR, dNLR, M/GLR were 1.287, 1.427, 1.347, 1.350,1.662, respectively demonstrated that a rise in each hematological marker by 1 unit increased the probability for higher HbA1c concentration by 28.7%, 42.7%, 24.7%, 35%, 66.2%, respectively. However, rise in PNR by 1 unit, increase the probability for lower HbA1c by 1.3%. Nagelkerke R^2 for WBC, neutrophil count, lymphocyte count, NLR, dNLR, M/GLR, PNR were 0.167, 0.165, 0.127, 0.123, 0.124, 0.121 and 0.140, respectively that indicated that 16.7%, 16.5%, 12.7%, 12.3%, 12.4%, 12.1% and 14% of variation in HbA1c levels could be explained by each hematological marker, respectively.

Discussion

As far as we know, there are no studies investigating the relationship between HbA1c and some novel hematological indexes, such as derived neutrophil to lymphocyte ratio (dNLR), monocyte/granulocyte to lymphocyte ratio (M/GLR), and platelets to neutrophil ratio (PNR). Moreover, the current study is among the rare ones exploring a variety of other hematological parameters in relation to the different levels of glucoregulation (i.e., including patients with prediabetes and overt T2D as compared with diabetes-free individuals).

The main results of this study show that WBC, neutrophils, lymphocytes, and their indexes significantly rose across the examined groups, being the highest in patients with T2D, as compared to control and prediabetes groups. Regarding PLT and its indexes, only PNR showed significantly lower values in T2D, as compared to corresponding subgroups. On the other hand, the PLR showed the tendency of decreasing across the groups, but this difference did not reach statistical significance.

Furthermore, multivariable ordinal regression analysis confirmed that each mentioned hematological parameter that differed significantly across the examined groups kept the independent relationship with HbA1c.

Table IV. Odds ratios after univariate ordinal logistic regression analysis for CBC parameters associations with HbA1c.

Predictors	Unadjusted OR (95%CI)	p	Nagelkerke R^2
WBC	1.289 (1.201-1.385)	< 0.001	0.072
Neutrophil count	1.438 (1.296-1.594)	< 0.001	0.071
Lymphocyte count	1.363 (1.155- 1.610)	< 0.001	0.019
NLR = Neu/Lymph	1.432 (1.172-1.749)	< 0.001	0.018
dNLR = Neu/(WBC-Neu)	1.799 (1.310-2.470)	< 0.001	0.019
M/GLR = (WBC-Lymph)/Lymph	1.350 (1.131-1.611)	0.001	0.019
PLR = PLT/Lymph	0.997 (0.994-1.000)	0.084	0.004
PNR = PLT/Neu	0.985 (0.980-0.991)	< 0.001	0.041

OR: Odds Ratio; CI: Confidence interval.

Table V. Odds ratios after multivariate ordinal regression analysis for associations of CBC parameters with HbA1c.

Predictors	Adjusted OR (95%CI)	p	Nagelkerke R^2
WBC	1.287 (1.191-1.390)	< 0.001	0.167
Neutrophil count	1.427 (1.275-1.594)	< 0.001	0.165
Lymphocyte count	1.347 (1.130- 1.606)	0.001	0.127
NLR = Neu/Lymph	1.350 (1.090-1.670)	0.006	0.123
dNLR = Neu/(WBC-Neu)	1.662 (1.189-2.326)	0.003	0.124
M/GLR = (WBC-Lymph)/Lymph	1.275 (1.057-1.540)	0.012	0.121
PLR = PLT/Lymph	0.997 (0.993-1.001)	0.098	0.116
PNR = PLT/Neu	0.987 (0.981-0.993)	< 0.001	0.140

Each predictor was tested in a Model which included ages and BMI (continuous variables), and smoking, antihypertensive therapy, antihyperlipidemic therapy (categorical variables).

Some other studies reported higher NLR and lower PLR in patients in prediabetes and early stages of diabetes. However, PLR significantly increased in the later stages of the disease[10]. In line with this, higher levels of PLT and PLR were observed in complications of diabetes, such as diabetic retinopathy[18]. Recently, Chen et al[19] have shown that higher NLR and PLR predict mortality in subjects with diabetic foot ulcers.

Some other investigations[20] also reported higher WBC and neutrophils in patients with hyperglycemia. However, they failed to show the difference in NLR between normoglycemic and hyperglycemic subjects but reported a lower PLR ratio in subjects with hyperglycemia. Moreover, they showed no difference in NLR and PLR in examined groups (HbA1c ≥7% *vs.* HbA1c <7%). The mentioned study[20] included a smaller number of participants (n=278) than the current study did, which might explain discordance with the results of our study.

A few studies[15,16] proposed indexes calculated as ratios of leukocytes and their subpopulations as better diagnostic markers than WBC and each subset. Accordingly, Rajwa et al[15] demonstrated the superiority of dNLR over other examined indexes for prediction of overall survival and cancer-specific survival in patients with renal cell carcinoma. Furthermore, a Croatian study[16] has recently reported that NLR and modified NLR parameters (i.e., dNLR and M/GLR) displayed good ability for discriminating patients with chronic obstructive pulmonary disease. On the contrary, our recent study[8] that investigated the relationship between novel leukocyte indexes and cardiovascular risk (CV) in the adolescent population has failed to confirm such a relationship. In the latter study, only WBC, neutrophil, and eosinophil count were shown to be the independent predictors of increased CV risk. The relatively small sample size of the adolescents (n=156), especially those with higher CV risk (n=47), may be the reason for such discrepancies since our current study has included much more participants than the previous one (n=827).

Visceral adipose tissue secrets a large number of pro-inflammatory cytokines[21]. The latter contributes to the enhancement of immune responses attributed to increased neutrophil infiltration and macrophages polarization[22]. The ability of neutrophils to secrete free radicals (i.e., superoxide anions), pro-inflammatory cytokines, and a large number of proteolytic enzymes, along with its capability to infiltrate the vascular wall triggers endothelial damage which precedes atherosclerosis onset[23]. This might partially explain higher WBC, neutrophils, and their indexes in patients with prediabetes and T2D, since patients with T2D had higher BMI compared to corresponding groups. Additionally, it cannot be excluded that some of them have diabetic complications, which can further aggravate the inflammation cascade.

In parallel with the increase in pro-inflammatory cytokines (e.g., IL-6, TNF-α, etc.), enhanced process of thrombosis occurs[23] since many of those cytokines contribute to prothrombotic milieu characterized by platelet hyperactivity. Hence, the lower PNR ratio in T2D in our study might be partially explained by platelet aggregation caused by insulin resistance, as well as with deficient insulin secretion (the two major characteristics of T2D). Namely, insulin resistance diminishes beneficial properties of insulin, such as inhibition of platelet aggregation and hyperglycemia-induced oxidative stress, due to increased production of free radicals[7,24]. Some studies reported higher PLT and PLR in patients with longer duration of T2D and with diabetic complications[10,18,19]. It can be partly explained by the ability of omental adipose tissue to secrete

thrombopoietin[25], with a consequent increase in insulin resistance and PLT. Additionally, insulin resistance favors the shortening of PLT life, which is followed by the increased number of PLT[26]. Besides that, it is assumed that PLT exert insulin receptors and in the insulin-resistant state, as previously stated, the beneficial properties of insulin are diminished. This state is accompanied by increased nitric oxide (NO), due to the activation of endothelial NO synthase, with concomitant vasoconstriction and sensitization of PLT to the aggregation, thus increasing CV risk[7].

As stated before, a relatively large sample size represents one of the strengths of the current study. Moreover, we included a variety of hematological parameters and their indexes (among them, even those not investigated before in relation to glucoregulation, such as dNLR, M/GLR, and PNR). On the other hand, a cross-sectional design is a limiting factor that cannot confirm the causal link between hematological parameters, their indexes, and glucoregulation in patients with prediabetes and T2D. Also, we were limited to the data about diabetic complications in T2D patients which might further explain the obtained results of this study.

Conclusions

This is the first study demonstrating an independent relationship between novel, modified NLR indexes, such as dNLR and M/GLR, in patients with prediabetes and T2D. Additionally, no previous study has investigated over PNR in relation to glucoregulation.

PNR showed superiority over PLT and PLR in relation to HbA1c. These novel indexes might add a significant contribution to the early recognition of glucose disturbances in patients with prediabetes and overt diabetes. Longitudinal multicenter studies are needed to confirm our findings.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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