Arh. farm. 2022; 72: 193 – 199 *Review article/Pregledni rad*

White blood cell count, its subsets, and their indexes in type 2 diabetes mellitus

Aleksandra Klisić*[1], Jelena Kotur-Stevuljević[2], Ana Ninić[2]

[1]University of Montenegro – Faculty of Medicine, Primary Health Care Center, Trg Nikole Kovacevica 6, 81000 Podgorica, Montenegro

[2]University of Belgrade – Faculty of Pharmacy, Department for Medical Biochemistry, Vojvode Stepe 450, 11221 Belgrade, Serbia

*Corresponding author: Aleksandra Klisic, e-mail: aleksandranklisic@gmail.com

Abstract

Due to the increased prevalence of obesity-related complications, especially type 2 diabetes mellitus (DM2), great attention has been paid to the variety of adipokines and cytokines, i.e. biomarkers, which are secreted by the enlarged visceral adipose tissue. Those pro-inflammatory mediators diminish insulin sensitivity via impairing insulin signalling pathways. If these processes persist, insulin resistance and DM2 occur, with a typical finding of low-grade chronic inflammation. The current review focuses on some hematological indices that reflect the inflammation level, such as white blood cell count, its subsets, and derived indexes. Those parameters are easy to measure, cost-effective, and widely available in a primary care setting. A deeper knowledge of the changes in the mentioned hematological parameters in DM2, in addition to glucoregulation biomarkers, may enable physicians to act promptly when patients with this metabolic disorder are concerned.

Keywords: diabetes, leukocytes, obesity, neutrophils, inflammation

https://doi.org/10.5937/arhfarm72-36386

Introduction

The prevalence of type 2 diabetes mellitus (DM2) is increasing rapidly all over the world (1). Moreover, the increase in its complications, especially cardiovascular ones, is also related to an increase in mortality. Therefore, there is an urgent need to early recognize and promptly treat patients with DM2, in order to prevent and/or postpone its complications.

It is already known that the majority of patients with DM2 are obese. Central obesity, i.e. enlarged visceral adipose tissue, and concomitant insulin resistance are tightly interconnected. The proposed mechanisms are processes of inflammation and oxidative stress, which may contribute to many impaired metabolic signalling pathways (1,2).

In a normal-weight state, i.e. lean state, the metabolic homeostasis between macrophages and small adipocytes is evident. Actually, innate immune cell subsets secrete some of the cytokines that act in an anti-inflammatory manner [such as interleukin (IL)-4, IL-5, IL-10 and IL-13], thus playing a preventive role in immune cell activation related to inflammation and maintaining insulin sensitivity. However, with the enlargement of adipocytes, as well as with their increase in number during obesity, enhanced expression of immune receptors, followed by the recruitment of immune cells, as well as secretion of pro-inflammatory cytokines and adipokines, with concomitant hypoxia, are reported (3).

White blood cell count and its subsets in the obese state and DM2

It is important to note that quantitative and qualitative transformations which immune cells undergo, along with an increased infiltration of macrophages and neutrophils into adipose tissue, are the key components of pathological activation of the immune system during obesity onset and its progression (3).

Neutrophils are among the first ones that infiltrate adipose tissue. The recruitment of macrophages occurs in the later phase of this process.

Morevore, increased lipolysis in enlarged adipocytes during the insulin-resistant state leads to higher free fatty acids in circulation, which lead to the activation of M1-like macrophages. Those macrophages further secrete tumor necrosis factor-alpha (TNF-α), thus causing the progression of chronic low-grade inflammation (3), while at the same time being involved in the progression of insulin resistance and atherosclerosis onset (4).

Enlarged adipocytes and macrophages also secrete IL-8, which represents a potent chemoattractant of neutrophils. The latter ones can be further self-recruited via increased production of some other neutrophil chemoattractants, but they may also lead to the increased secretion of IL-1b, another pro-inflammatory cytokine, thus activating other immune cells and adipocytes (4).

In a nutshell, not only does adipose tissue secrete a large number of pro-inflammatory mediators, but the latter further promote the inflammatory cascade, thus

creating a vicious circle between low-grade systemic inflammation, pathological activation of the immune system, and metabolic disorders (1,5,6).

Although great attention has been paid to many pro-inflammatory biomarkers (especially adipokines and cytokines) that might impair insulin signalling pathways over the last several decades, there are still some hematological biomarkers that are easy to obtain, cost-effective, and widely available, even in a primary care setting, which can contribute significantly to the early recognition of patients with a high risk of DM2. Those biomarkers are often neglected considering the metabolic risk, even though it is established that visceral adipose tissue has proinflammatory, proatherogenic and prothrombotic properties (1,5). In line with this, this review covers some of the main aspects of complete blood count, differential blood count, and its indexes in relation to DM2.

Ohshita et al (7) examined WBC in patients with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). Accordingly, they reported higher WBC in patients with IGT than in those with IFG. They also reported a correlation between WBC and metabolic syndrome components, such as body mass index, fasting insulin, HDL-cholesterol, LDL-c, triglycerides and blood pressure.

Naredi et al (8) showed an association between higher WBC, even within the normal range, and chronic complications in DM2, and suggested that WBC can be a reliable parameter for the prediction of micro and macrovascular complications in such patients. Indeed, a meta-analysis that included a total of 85,040 non-DM2 patients and 8,647 DM2 patients confirmed the association between WBC and DM2 (9).

Novel indexes derived from white blood cell count subsets in type 2 diabetes

Previous studies reported higher WBC with concomitant higher neutrophils in obesity and DM2 (10-14), but also in other diseases characterized by low-grade chronic inflammation (15-17). Increased infiltration of neutrophils and polarization of macrophages occurs in enlarged adipose tissue (3,18). Those processes enhance the immune response and are highly correlated with increased TNF-α and IL-6, which visceral adipose tissue secrete. Neutrophils have the ability to secrete pro-inflammatory cytokines, free radicals, as well as a number of proteolytic enzymes. They also have properties that allow them to infiltrate the vascular wall, which represents a trigger for endothelial damage, thus preceding the onset of atherosclerosis and cardiovascular diseases (3,18).

Several investigations were focused on some novel indexes derived from WBC subsets in different chronic diseases. Interestingly, those studies reported their superiority over WBC and their indexes in the mentioned disorders (19-21).

These novel indexes include and are calculated as follows: NLR=neutrophil to lymphocyte ratio, derived NLR (dNLR)=neutrophil count/(WBC-neutrophil count), NMR=neutrophil to monocyte ratio, MLR=monocyte to lymphocyte ratio, BLR=basophil

to lymphocyte ratio, BMR=basophil to monocyte ratio, M/GLR=monocyte/granulocyte to lymphocyte ratio, BNR=basophil to neutrophil ratio (19-22).

However, to the best of our knowledge, there have been no studies that investigate this issue in relation to glucoregulation. This was a hint for the recent study by Klisic et al. (13) in which several novel indexes, such as NLR and modified NLR parameters (i.e., dNLR and M/GLR), were investigated in a relatively large sample (i.e., nearly 830 participants) of patients with prediabetes and DM2.

Accordingly, higher WBC, lymphocytes, neutrophils, NLR, dNLR and M/GLR in the DM2 group, compared with patients with prediabetes and control groups, was shown. Moreover, these parameters were independently associated with glycated hemoglobin (13).

Higher NLR was shown to be an independent predictor of mortality in patients with diabetic complications, such as diabetic foot ulcers (23).

Mendes et al. (12) also showed higher neutrophils and WBC in patients with hyperglycemia, compared to their normoglycemic counterparts. On the other hand, they did not find a difference in NLR between the examined groups.

A recent study conducted in the adolescent population reported higher WBC and its subsets (except for basophils) in youngsters with moderate/higher cardiovascular risk scores, compared to their counterparts with a low cardiovascular risk score (16). Even more, eosinophils, neutrophils, and WBC were shown to be independent predictors of increased cardiovascular risk. The obtained results suggest that even the young population exhibits higher WBC and its subsets in relation to an increased risk of cardiovascular disease. However, the latter study failed to confirm any association between NLR, dNLR, and M/GLR and cardiovascular risk (16).

These discrepancies in results related to neutrophil indexes might be attributed to the small sample size of the examined population, as well as the different ages of the examinees.

Given the fact that neutrophils play an important role in obesity, it would be expected that the mentioned novel neutrophil indexes deserve attention, and larger multicentric longitudinal studies are needed to confirm their role in the diagnosis and monitoring of patients with DM2.

Better recognition of changes of these hematological biomarkers in metabolic disturbances, in addition to glucoregulation parameters, may enable clinicians to act in a timely manner when patients with DM2 are concerned.

References

1. Papaetis GS, Papakyriakou P, Panagiotou TN. Central obesity, type 2 diabetes and insulin: exploring a pathway full of thorns. Arch Med Sci. 2015;11:463-482.
2. Klisic A, Kavaric N, Stanisic V, Vujcic S, Spasojevic-Kalimanovska V, Ninic A, et al. Endocan and a novel score for dyslipidemia, oxidative stress and inflammation (DOI score) are independently correlated with glycated hemoglobin (HbA1c) in patients with prediabetes and type 2 diabetes. Arch Med Sci. 2020;16:42-50.
3. Daryabor G, Kabelitz D, Kalantar K. An update on immune dysregulation in obesity-related insulin resistance. Scand J Immunol. 2019;89(4):e12747.
4. Blaszczak AM, Jalilvand A, Hsueh WA. Adipocytes, Innate Immunity and Obesity: A Mini-Review. Front Immunol. 2021;12:650768.
5. Galicia-Garcia U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, et al. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020;21:6275.
6. Čolak E, Pap D. The role of oxidative stress in the development of obesity and obesity-related metabolic disorders. J Med Biochem. 2021;40:1–9.
7. Ohshita K, Yamane K, Hanafusa M, Mori H, Mito K, Okubo M, et al. Elevated white blood cell count in subjects with impaired glucose tolerance. Diabetes Care. 2004;27(2):491-496.
8. Naredi M, Jhavar D, Krishnan D. Study of relationship between WBC count and diabetic complications. Int J Adv Med. 2017;4(4):1128-1132.
9. Gkrania-Klotsas E, Ye Z, Cooper AJ, Sharp SJ, Luben R, Biggs ML, et al. Differential white blood cell count and type 2 diabetes: systematic review and meta-analysis of cross-sectional and prospective studies. PLoS One. 2010;5(10):e13405.
10. Rodríguez-Rodríguez E, López-Sobaler AM, Ortega RM, Delgado-Losada ML, López-Parra AM, Aparicio A. Association between Neutrophil-to-Lymphocyte Ratio with Abdominal Obesity and Healthy Eating Index in a Representative Older Spanish Population. Nutrients. 2020;12(3):855.
11. Klisic A, Kavaric N, Bjelakovic B, Soldatovic I, Martinovic M, Kotur-Stevuljevic J. The association between retinol-binding protein 4 and cardiovascular risk score is mediated by waist circumference in overweight/obese adolescent girls. Acta Clin Croat. 2017;56:92-98.
12. Mendes BB, Oliveira ACR, Alcântara KC. Comparison of the neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in normoglycemic and hyperglycemic subjects. Einstein (Sao Paulo). 2019;17:eAO4403.
13. Klisic A, Scepanovic A, Kotur-Stevuljevic J, Ninic A. Novel leukocyte and thrombocyte indexes in patients with prediabetes and type 2 diabetes mellitus. Eur Rev Med Pharmacol Sci. 2022;26.
14. Anık A, Çelik E, Anık A.The Relation of Complete Blood Count Parameters with Metabolic and Clinical Parameters in Overweight and Obese Children. J Pediatr Res. 2021;8:161-170.
15. Dağdeviren M, Akkan T, Yapar D, Karakaya S, Dağdeviren T, Ertuğrul D, et al. Can neutrophil/lymphocyte ratio be used as an indicator of inflammation in patients with hyperthyroidism? J Med Biochem. 2020;39:7–12.
16. Klisic A, Radoman Vujačić I, Vučković Lj, Ninic A. Total leukocyte count, leukocyte subsets and their indexes in relation to cardiovascular risk in adolescent population. Eur Rev Med Pharmacol Sci. 2021;25:3038-3044.

17. Liu J, Liu X, Li Y, Quan J, Wei S, An S, et al. The association of neutrophil to lymphocyte ratio, mean platelet volume, and platelet distribution width with diabetic retinopathy and nephropathy: a meta-analysis. Biosci Rep. 2018;38:72.
18. Lolmède K, Duffaut C, Zakaroff-Girard A, Bouloumié A. Immune cells in adipose tissue: key players in metabolic disorders. Diabetes Metab. 2011;37:283-290.
19. Hlapčić I, Vukić Dugac A, Popović-Grle S, Markelić I, Rako I, Rogić D, et al. Influence of disease severity, smoking status and therapy regimes on leukocyte subsets and their ratios in stable chronic obstructive pulmonary disease. Arch Med Sci. doi:10.5114/aoms.2020.100720.
20. Sevencan NO, Ozkan AE. Associations between neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, albuminuria and uric acid and the estimated glomerular filtration rate in hypertensive patients with chronic kidney disease stages 1-3. Arch Med Sci. 2019;15:1232-1239.
21. Turcato G, Sanchis-Gomar F, Cervellin G, Zorzi E, Sivero V, Salvagno GL, et al. Evaluation of Neutrophil-lymphocyte and Platelet-lymphocyte Ratios as Predictors of 30-day Mortality in Patients Hospitalized for an Episode of Acute Decompensated Heart Failure. J Med Biochem. 2019;38:452-460.
22. Rajwa P, Życzkowski M, Paradysz A,Slabon-Turska M, Suliga K, Bujak K, et al. Novel hematological biomarkers predict survival in renal cell carcinoma patients treated with nephrectomy. Arch Med Sci. 2020;16:1062-1071.
23. Chen W, Chen K, Xu Z, Hu Y, Liu Y, Liu W, et al. Neutrophil-to-Lymphocyte Ratio and Platelet-to-Lymphocyte Ratio Predict Mortality in Patients with Diabetic Foot Ulcers Undergoing Amputations. Diabetes Metab Syndr Obes. 2021;14:821-829.

Ukupan broj leukocita, diferencijalna krvna slika i njihovi indeksi u diabetes mellitus-u tip 2

Aleksandra Klisić*[1], Jelena Kotur-Stevuljević[2], Ana Ninić[2]

[1]Dom zdravlja Podgorica, Univerzitet Crne Gore - Medicinski fakultet,
Trg Nikole Kovačevića 6, 81000 Podgorica, Crna Gora

[2]Univerzitet u Beogradu - Faramaceutski fakultet, Katedra za Medicinsku biohemiju,
Vojvode Stepe 450, 11221 Beograd, Srbija

*Autor za korespondenciju: Aleksandra Klisić, e-mail: aleksandranklisic@gmail.com

Kratak sadržaj

Zbog povećane prevalence komplikacija povezanih sa gojaznošću, naročito diabetes mellitus-a tip (DM2), posebna pažnja se posvećuje različitim adipokinima i citokinima, tj. biomarkerima koje luči uvećano visceralno masno tkivo. Pomenuti medijatori inflamacije smanjuju insulinsku senzitivnost vršeći uticaj na signalne puteve insulina. Ukoliko ovi procesi potraju duže dolazi do pojave insulinske rezistencije i DM2, sa tipičnim nalazom hronične inflamacije niskog stepena. Ovaj revijalni članak akcenat stavlja na određene hematološke parametre koji odražavaju stepen inflamacije, kao što je broj leukocita, njihove podvrste, kao i indeksi izvedeni iz istih. Ovi parametri su lako merljivi, ekonomski isplativi i široko dostupni u ambulantama primarne zdravstvene zaštite.

Bolje poznavanje promena navedenih hematoloških parametara u DM2, pored biomarkera glikoregulacije, može omogućiti lekarima pravovremenu reakciju kada se radi o pacijentima sa ovim metaboličkim poremećajem.

Ključne reči: dijabetes, leukociti, gojaznost, neutrofili, inflamacija