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Assessment of white blood cell distribution as a prognostic factorin type 2 diabetes mellitus and its complications - literature overview

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Abstract

Introduction:
Type 2 diabetes (T2DM) mellitus is the chronic, low-grade inflammatory disorder, which is considered to be the leading cause of morbidity and mortality worldwide. Wherefore, it is indispensable to look for new, non-invasive and widely available markers that will allow for

early detection of predisposition to the development of diabetes as well as its macro- and microvascular complications.

Aim of the study:
The aim of our study was to present the role of white blood cells (WBC) count as a prognostic factor in type 2 diabetes mellitus. Moreover, we discussed the perspectives for the usefulness of WBC subtypes and neutrophil to lymphocyte ratio (NLR) as a marker of glycemic control as well as an indicator of the risk of developing diabetes complications.

Description of knowledge:
The abundant number of previous studies revealed that the elevated level of total WBC count strongly correlates with the predisposition to prediabetes and T2DM development. There is also association between the insulin resistance as well as the function and mass of β-cells. The numerous research confirm that the increase in NLR may be the useful laboratory tool to evaluate the glycemic control and the effectiveness of antidiabetic treatment. NLR ratio as a marker, which directly reflects the level of inflammation is considered to indicate the risk of development of cardiovascular complications, diabetic peripheral neuropathy or nephropathy, even in the early stage of T2DM duration.

Conclusions:
Measurements of WBC count and changes in the number of subpopulations of them seem to be a useful, widely accessible marker of development of T2DM as well as its complications and may be helpful in management of T2DM patients.

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia, impaired glucose tolerance and reduced insulin levels as a consequence [1]. The latest epidemiological data shows that a global prevalence of diabetic patients estimate around 382 million people (8.3%) in 2013, and is expected to rise to 592 million by 2035 [2]. The major risk factors for the development of T2DM are environmental background, especially abdominal obesity, low physical activity level as well as genetic factors. However, recent scientific reports put particular emphasis on the low-grade inflammation as a principal component of the pathophysiology of diabetes type 2 as well its complications [3].

The leading hypothesized mechanism, which may explain insulin resistance and β-cell dysfunction in the course of T2DM is the local production of pro-inflammatory cytokines, chemokines, activation of inflammatory signalling networks as a response for stressful factors (excessive levels of nutrients, including glucose and free fatty acids) for insulin-sensitive tissue such as adipose tissue, liver, muscles in obese humans. It provides to recruited immune cells and contributes to secretion of cytokines and chemokines from the adipose tissues into the circulation and promotes whole-body inflammation [4]. That is why, the reduction in pancreatic β-cell mass and impaired insulin secretion is observed.

So far, various inflammatory markers were tested for their usefulness in clinical practice to predict the risk of developing T2DM. The prospective, nested case-control study revealed that elevated level of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) and C-reactive protein (CRP) may predict the risk of T2DM, however the limitation of that research was performing only in the group of healthy middle-aged women [5]. The results of recent years' research have suggested the association between total peripheral white blood cells (WBC) count as a marker of subclinical inflammation and diabetes risk. The recent meta-analysis from 20 cross-sectional and prospective cohort observational studies revealed that elevated level of almost all of subpopulation of white blood cells (granulocytes, lymphocytes)

strongly correlates with the risk of T2DM development. The researchers underscore that there is no relationship between the level of monocytes and the incidence of T2DM, probably due to the fact that these cells migrate to the inflamed tissue, primarily fat, differentiate into macrophages, and therefore the level of blood monocyte is not a representative [6].

Considering the results of experimental and epidemiological studies, measurements of white blood cells count as well as distribution of the WBC subpopulations as a commonly used and widely accessible test, will be an assistance in prediction of the risk of T2DM. Furthermore, the role of the neutrophil lymphocyte ratio (NLR), which is directly proportional to the degree of inflammation, is particularly emphasized as a potential indicator not only of the development of cardiovascular complications in the course of diabetes [7], but also can be used to evaluate the monitoring of the effectiveness of antidiabetic therapy, the alignment of carbohydrate metabolism and the other micro- and macrovascular complications of diabetes such as peripheral neuropathy, nephropathy, retinopathy [8-9].

Aim of the study

The aim of our study was to present the role of WBC count as a prognostic factor in T2DM. Furthermore, we discussed the future perspectives for the usefulness of WBC subtypes and NLR as a marker of glycemic control as well as an indicator of the risk of developing diabetes complications.

Materials and methods

The available literature was subjectively selected due to its usefulness in showing clinical approach to the role of WBC count and their subtypes as a marker of T2DM and its complications. Additionally, literature which reveals inconsistency in results was shown as well. Articles in the EBSCO and the PubMed database have been analyzed using keywords: diabetes mellitus type 2, diabetic complications, white blood cells and prognostic marker.

Description of knowledge

White blood cells as a predictor of T2DM development

T2DM, as well as the other accompanying disorders that are components of the metabolic syndrome (abdominal obesity, hypertension, lipid disorders) are considered for abnormalities in the immune system as the leading pathogenetic causes [10]. Several studies revealed that obesity can promote the development of T2DM, however, only recent reports show the role of inflammatory processes within excessive adipose tissue in the development of insulin resistance and pancreatic B-cell dysfunction. Gu Y. et al. in the prospective study proved that obese patient with the elevated level of WBC count have a greater risk of T2DM development (27/1000 person/year) in contrast to participants who are obese, but it was not accompanied by elevated levels of WBC at the same time (22/1000 person/year) [11].

A prospective population-based cohort study (n = 30.447 participants) reported that the total leukocytes, neutrophils, lymphocytes count is association with the increased risk of T2DM incidence in the follow-up of 14 years [12]. Moreover, the correlation between missense polymorphism R262W in the SH2B adaptor protein 3 gene responsible for coding a protein, which main function is to depress the proliferation of hematopoetic cell with the fasting blood glucose level and the prediction of T2DM presence was analyzed, but no relationship was found. However, the authors underline that it may be a result of a limitations in statistical power as well as the pleiotropic effects [13].

There is also a consideration in the scientific literature whether WBC count may be an independent, especially from obesity and age risk factor of diabetes mellitus [13]. The results show that the level of WBC above 6.91 x 10^9/l seems to be an accurate prognostic factor and besides the increased level of WBC above 1.0 x 10^9/l increase the diabetes risk in

approximately 7.6%. It is worth emphasizing, however, that such correlations were obtained only in the case of young healthy male participants [14].

The significant role in the prediction of T2DM incidence may also play the distribution of white blood cell subtypes, especially the proportion between neutrophils and lymphocyte levels [14]. Based on studies, it is known that in healthy subjects the NLR strongly negatively correlates with the fasting blood glucose level, however, this relationship is not observed in the group of prediabetic and diabetic patients [15]. Moreover, the total WBC count seems to be a predictor of β-cell dysfunction as well as NLR may indicate the level insulin sensitivity [16].

Summarizing, the WBC count and the distribution of their subtypes could be an accurate prognostic marker of the risk of T2DM development.

White blood cells count as a useful tool for glycemic control

Epidemiologic data indicate that the intensive glycemic control is crucial to significantly reduced the mortality as well as complications of long-standing diabetes [17]. Currently, the recommended rate for routine assessment of glycemic control within 2 to 3 months is the percentage of glycated hemoglobin (HbA1c), however, this parameter may be influenced by a variety of factors, including age, ethnicity, smoking, conditions that alter red cell turn-over, such as hemolytic anemia, structural hemoglobinopathies, major blood loss and blood transfusions [18]. Research shows that any reduction in HbA1c levels by up to 1% causes a significant reduction in the risk of developing diabetic cardiovascular complications as well as microvascular disorders. Intensive glycemic control in diabetic patient may benefit in the form of reduction of microvascular complications by up to 25% and the risk of myocardial infarction by 16% [17].

Considering the above data, there is a strong extremity to search for new, widely available markers that will allow even more accurate glycemic control. A comparative cross-sectional study performed on the group of 296 participants obviously displayed the statistically significant difference in hematological parameters of diabetic patients compared to controls, especially in the group of patients showing high blood glucose levels [19]. The retrospective, cross-sectional study performed among Arabs revealed that patients with poorly controlled T2DM (HbA1c ≥ 7%) displayed small, but statistically significant increase level of absolute neutrophil ($4.34 \times 10^9/l \pm 1.46 \times 10^9/l$) and lymphocyte ($2.50 \times 10^9/l \pm 0.75 \times 10^9/l$) count compared to diabetic patients with the normalized blood glucose level ($4.03 \times 10^9/l \pm 1.39 \times 10^9/l$; $2.32 \times 10^9/l \pm 0.68 \times 10^9/l$ respectively) [20]. Another study demonstrates that patients who developed symptoms of ketoacidosis or ketosis, due to poorly glycemic control, had more median leukocytes ($13.32 \times 10^9/l$ and $6.60 \times 10^9/l$, respectively) and median neutrophils ($11.12 \times 10^9/l$ and $4.12\ 10^9/l$, respectively), but fewer median eosinophils ($0.028 \times 10^9/l$ and $0.072 \times 10^9/l$, respectively) compared to non-diabetic ketosis and control groups. The authors suggest that an elevated level of total WBC, with an increased neutrophil count and reduced eosinophils count, may be a clear signal in clinical practice to assess the risk of acute diabetes complications due to high glucose levels in the blood [21].

Recent studies show that perhaps a more reliable marker than the total WBC count would be the NLR, because it has a superior predictive, diagnostic and discriminative ability. It is also worth noting that NLR is a much more accessible, cheap and easy to detect marker, which has comparable clinical significance with other inflammatory agents such as CRP, IL-6, TNF-α. The first retrospective study, which aimed to clarify whether there is a correlation between the level of glycated hemoglobin and thus the glycemic control and the NLR, was performed on a group of 71 patients with T2DM. Researchers divided patients into two groups depending on the HbA1c level (≤ 7% - regulated, > 7% - unregulated). The mean glucose levels were 187.1 ± 54.9 mg/dl and 216.0 ± 76.4 mg/dl in the first and second group

respectively. In addition, the study showed that poor blood glucose regulation correlates with the decreased lymphocyte counts and increased neutrophil counts as well as the increased of NLR consequently [8]. The observational study on the group of 330 patients with excellent, poor and worst control T2DM revealed that increased NLR level is directly associated with elevated HbA1c and worst glycemic control [22]. Moreover, the investigators suggest that NLR together with fasting glucose level or CRP may be an independent predictor of glycemic control.

These changes in WBC distribution in favor of neutrophils are mainly caused by decreased production of interleukin 2 (IL-2) or decreased expression of interleukin-2 receptor (IL-2R), which leads to disorders of lymphocyte proliferation and reduction of their number in relation to neutrophils [23].

White blood cells count and distribution – association with diabetic complications

Chronic complications of diabetes are the main challenge of contemporary diabetology. Difficulties in achieving criteria for alleviating diabetes cause that the micro- (retinopathy, nephropathy, peripheral neuropathy) and magroangiopathic (ischemic heart disease, ischemic stroke, impaired flow in the vessels of the lower limbs, hypertension) complications of diabetes cannot be entirely prevented. The recent clinical observations show that the low-grade chronic inflammation is positively associated with the risk of both of above mentioned group of diabetic complications development [9]. There is increasing indications that the inflammation markers, such as C-reactive proteins, fibrinogen level are associate with the development of metabolic syndrome, coronary heart disease as well as all-cause mortality. In our review article, the role of elevated total white blood cells as well as the disorders of WBC subpopulations distribution and the role of them in the prediction of the development of T2DM complications has been discussed. The involvement of WBC, their level in the blood and subpopulation distribution in the pathogenesis of T2DM as well as its complications was summarized in the *Figure 1*.

Diabetes-induced cardiovascular complications

The magroangiopathic, particularly cardiovascular complications, observed even in the early stage of T2DM development are considered to be the crucial factors responsible for high mortality rate in diabetic patients. The contribution of the inflammatory processes and immune cells in the pathogenesis of cardiovascular events as well as the adverse effect after successfully treatment and higher mortality rate was mainly discussed as a result of elevated level of white blood cells or NLR value [24,25].

The available scientific data suggest that the higher level of WBC count in obese patient can determine the abnormal glucose metabolism, remodeling of heart and the vascular risk profile, especially the risk of atherosclerosis [27]. The other investigators displayed that the white blood cells count may reflect the relationship between inflammation in the course of T2DM and the risk of endothelial activation, vascular dysfunction and hypertension. It was also discovered that the elevated levels of WBC as well as diabetes can occur without insulin resistance [28].

Diabetic nephropathy

The development of diabetic nephropathy largely depends on the duration of the disease, as well as the degree of glycemic incompatibility and associated hypertension. Research shows that the leading role in the pathogenesis of renal dysfunction in the course of diabetes, apart from metabolic and hemodynamic factors, plays the persistent inflammation and glomerular infiltration by leukocytes, which are observed, interestingly, at early stage of the development of chronic kidney disease (CKD) [29]. Currently, it is considered that the

most accurate screening test is the assessment of urinary albumin excretion in patients, who have not yet been diagnosed with clinically evident proteinuria. However, in nearly 50% of patients with diabetic-induced CKD the significant deterioration of kidney function exists without significant proteinuria [30]. That is why, it is crucial to investigate the novel indicators of renal insufficiency.

Recent studies suggested the role of white blood cells in the pathogenesis of diabetic nephropathy. Moreover, it was observed that elevated level of WBC may predict mortality in patient with CKD. The cross-sectional study on 376 participants, including 272 diabetic patients with or without CKD and 104 healthy controls revealed that patients with T2DM and CKD had increased neutrophil, eosinophil and monocyte level and decreased lymphocyte count [30]. The authors emphasize that the NLR would be the most appropriate marker of glomerular filtration rate (GFR), even better than total white blood cells count. Another, cross-sectional study in a Chinese community-based population also confirms the results of previous research and indicate that measurements of WBC count in diabetic patients at an early stage of development may allow more accurately assess the risk of developing CKD and implement the appropriate preventive measures [31]. The researches also revealed that patients with the highest level of WBC ($\geq 7 \times 10^9$/l) had 1.49–fold greater odds of rapid eGFR decline, 2.01–fold greater odds of renal function decline (RFD) and 4.79-fold greater odds of new incident CKD.

Fukui M. et al. discovered the positive association between elevated level of eosinophils and degree of albumin excretion rate in male patients with T2DM. Furthermore, these widely accessible parameter proved to be more adequate and independent indicator than HbA1c as well as duration of T2DM [32].

The recent year studies strongly confirm the significance of distribution of WBC subpopulation among patient with diabetes mellitus. NLR was statistically higher in diabetic patient with albuminuria (2.83 ± 0.85) compared to the one without any laboratory signs of kidney injury (1.94 ± 0.65) [33]. The elevated levels of ANC as well as ALC in CKD patient was observed. However, the study did not display any strong association between normal and diabetic nephropathy groups in relation to age, sex, body mass index (BMI), waist-hip ratio (WHR), HbA1c as well as all of components of lipid profile. Sato H. et al. also emphasize the role of pre-dialysis NLR level as a useful and specific marker in T2DM patient, but they strongly suggest a NLR (≥ 3.5) as an accurate short-term predictor of all-cause mortality in patients with diabetic nephropathy. The researches had the other conclusions that the NLR is probably better marker of renal complications than total WBC count and moreover, the higher level of NLR in the group of patient demanding the dialysis in urgent mode was observed, probably due to the increased activity of inflammation processes in the response to severe uremic condition [34].

The results of latest research present not only the correlation between WBC subpopulation distribution or level of NLR with the biochemical parameters evaluating the renal function, but also compare them with morphometric parameters. It occurs that the lower lymphocyte, higher eosinophil, neutrophil count as well as increased NLR level correlate with higher glomerular basement membrane width (the parameter which also can indicate the risk of renal function loss) and percentage of normally fenestrated endothelial cells [35].

Diabetic peripheral neuropathy

Diabetic peripheral neuropathy (DPN) is the most common chronic complication of T2DM. The incidence of DPN among patients with T2DM is almost 45% [36]. Chronic hyperglycemia, oxidative stress, vascular factors, especially atherosclerosis and changes in the nerve nutrient vessels are considered to play a major role in the pathogenesis of this complication. It is a great difficulty in clinical practice that almost half of patients, who develop DNP do not initially experience any clinical symptoms. To confirm the diagnosis, the

nerve conduction and electromyographic examination are performed. However, the pathological changes may occur not only as sensory-motor neuropathy, but also it can affect the autonym nerves in the circulatory system, gastrointestinal tract, genitourinary system and ocular. According to previous studies the low-grade inflammatory background is taking into consideration as a cause of DPN appearance. Given the above data, as well as the spread of diabetes in the world, new, low-cost and widely available markers should be sought that will help predict and monitor the risk of neuropathy development in T2DM patients [36, 37].

Liu S. et al. performed the study on the group of 511 T2DM patients which had total WBC count within a normal range. It occurred that the nerve conduction velocity (NCV) as well as vibration perception threshold (VPT) firmly correlate with the level of NLR [37]. The patient who represented the group with the highest total WBC count ($7.09 \pm 1.96 \times 10^9$/l), the most elevated value of NLR (3.26 ± 0.98) has shown the incidence of neuropathy in approximately 65.50%, and additionally the statistically significance increase in NCV and VPT value (16.78 ± 10.92). Another authors also confirmed that hypothesis. Furthermore, the researches focus on the role of NLR, as well as the positive correlation with the highest level of neutrophils and negative correlation with the lowest lymphocyte counts in the indication of the risk of DPN in patients with newly diagnosed T2DM. To conclude, NLR might be use in the clinical practice as an independent predictor of DPN development and in relatively easy way contribute to monitor the course of T2DM [38].

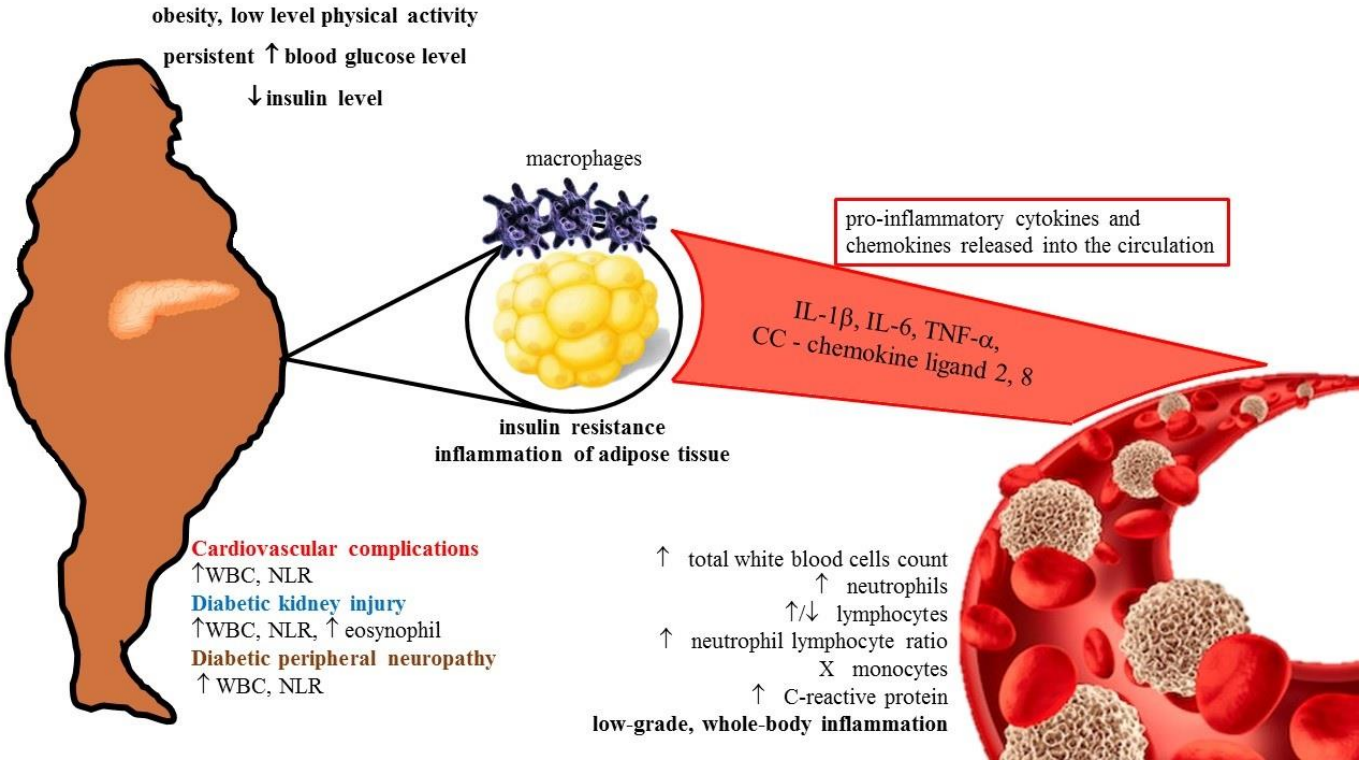

Figure 1. *The summary of involvement of white blood cells, their level in the blood and subpopulation distribution in the pathogenesis of T2DM as well as its complications.*

WBC - white blood cells, **NLR –** neutrophil lymphocyte ratio, **X** – lack of changes

Conlusions

T2DM as well as obesity are the chronic, low-grade inflammatory disorders, which are considered to be the leading causes of morbidity and mortality worldwide. Wherefore, it is

indispensable to look for new, non-invasive and widely available prognostic factors that will allow for early detection of predisposition to the development of diabetes as well as its macro- and microvascular complications. Measurements of WBC count and changes in the number of subpopulations of them seems to be a useful marker of β-cell dysfunction, and insulin resistance. Moreover, WBC count is considered as independent factor leading to development of T2DM. It is worth emphasizing that NLR, which is directly proportional to the degree of inflammation, closely correlates with the glycemic control, diabetic nephropathy, peripheral neuropathy, cardiovascular complications and may be helpful in management of T2DM patients.

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