Mini review

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Medical significance of simultaneous application of red blood cell distribution width (RDW) and neopterin as diagnostic/prognostic biomarkers in clinical practice

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Abstract: In our individual and collaborative studies, we have played a part in pioneering investigations on the usefulness of biomarkers – red blood cell distribution width (RDW) and neopterin. This mini review includes historical data on the topic and is related to the first contributions in this field, as well as to the possibilities for further improvement and simultaneous application of RDW and neopterin measurements in the prevention, prognosis and treatment of a great number of socially important disease conditions (arterial, cardiovascular, brain vascular, peripheral artery diseases, inflammations, autoimmune states, cancers and leukemias, addictions, etc.). When comparing the results obtained with the immunobiochemical biomarker neopterin with RDW, they are reported to be very similar as independent predictors of the same pathological states in the human body although their biomedical origins are very different. Both the parameters were until now successfully, but only separately used in medical practice. The combined use of these two biomarkers can shed some more light on their interrelationships and provide some clues as to how the interaction between immune system activation and red blood cells biology are intertwined.

Keywords: early diagnosis; laboratory diagnostic applications; neopterin; predictor of adverse outcomes; prognostic significance; red blood cell distribution width (RDW); treatment.

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Introduction

Red blood cell distribution width (RDW) is a hematologic index, automatically calculated by the common blood cell counters. RDW reflects the heterogeneity of erythrocyte volume and mean corpuscular volume (MCV) and is characterized as an electronic equivalent of erythrocyte anisocytosis as is registered light-microscopically in blood smears. As a coefficient of variation of erythrocyte volume (in percentage, %), RDW expresses the dispersion of the RBC volume curve obtained by using the formula: $RDW\% = SD \text{ of } MCV/MCV \times 100$, where $MCV =$ mean RBC volume; $SD =$ standard deviation of MCV. In different laboratories, RDW values are registered in the reference ranges between 11.5% and 14.5% [1].

In earlier hematological studies, RDW values were examined for the purposes of diagnosis, differential diagnosis and classification of anemia [2, 3]. Grant et al. [4] evaluated an inverse relationship between lung function and RDW in a population-based study.

In early 2006, we first proposed in the scientific literature RDW and MCV as hematometric erythrocyte indices to monitor the hemorheological properties of red blood cells (RBC) in chronic heroin addicts [5]. We observed increased RDW levels in chronic heroin users simultaneously with changes in RDW and MCV as bioindicators of negative effects of drugs on erythropoiesis. In conclusion, we recommended the results of our study be applied in medical practice in order to determine their clinical usefulness to optimize the treatment of some vascular/cardiovascular complications such as hypoxemia, major ischemic syndrome, circulatory disturbances, hypertension, cardiac arrythmia, acute myocardial infarction (AMI), etc. The prognostic value of RWD, as stated in our above-mentioned previous study has been recognized and this parameter was used for further studies by mainly cardiologists [6–11] (Table 1).

Recently, a large number of retrospective (and a few prospective) studies on the usefulness of RDW as an independent biomarker in the risk stratification and prevention of different socially important diseases in humans have been involved in the medical literature and clinical

Table 1: Pioneering studies of clinical significance and prognostic values of increased red blood cell distribution width (RDW) as biomarker in cardiovascular, brain vascular and peripheral vascular diseases.

Authors/Reference
[5] Elevated RDW levels in chronic heroin addicts: Savov Y, et al. Clin Hemorheol Microcirc 2006;35:129–33.
[6] RDW elevation in patients with NSTEMI: Azab B, et al. Cardiology 2011;119:72–80.
[7] High RDW levels in AMI and UAP: Gul M, et al. Coron Artery Dis 2012;23:330–6.
[8] RDW – prognostic significance in CAD: Isik T, et al. Coron Artery Dis 2012;23:51–6.
[9] RDW – in long-term prognosis of CVD: Isik T, et al. Cardiol J 2016;23:281–288.
[10] Predictive RDW values following TAVI: Magri CJ, et al. Int J Cardiol 2014;172:456–7.
[11] RDW – a biomarker of MSB in CAD: Magri CJ, et al. Postgrad Med J 2017;93:607–612.

AMI, acute myocardial infarction; CAD, coronary artery disease; CVD, cardiovascular disease; MSB, myocardial scar burden; NSTMI, non-ST elevated myocardial infarction; STEMI, ST elevated myocardial infarction; TAVI, trans-catheter aortic valve implantation; UAP, unstable angina pectoris.

practice [3, 12–23]. Many studies have reported that the increased levels of RDW are closely associated with inflammation [24–27] and oxidative stress [28]. All recent data have shown a strong and independent association between RDW and cardiovascular, brain vascular and peripheral artery diseases.

The clinical usefulness of increased RDW is also of great importance in the short- and long-term prognosis of vascular, cardiovascular, brain vascular and peripheral artery-diseases, in autoimmune and oxidative stress states, inflammatory diseases, malignancies (cancer and leukemia), etc. [3, 29]. In the pathological conditions described above the increased RDW has a high negative predictive value as an independent biomarker and represents a strong risk factor for negative outcomes not only in cardiac, arterial, brain, renal, hepatic, infectious diseases, etc., but also in the general middle-aged and older adult population [13, 14]. In this sense, the elevated RDW values are considered as features of morphological and/or functional (metabolic) imbalance in the human body. The exact biological, biochemical, cellular and molecular mechanisms remain unclear and should be elucidated in further studies.

From a hematological and hemorheological/biorheological point of view, in our opinion the fine biological mechanisms of increased RDW could be explained by pathophysiological changes in marrow/spleen erythropoiesis, in erythropoietin (EPO) synthesis and/or in RBC morphology, biochemistry, physiology and hemorheological properties. In this sense, RDW is not only a hematological/hematometrical, but also a hemorheological biomarker [5, 10].

In our pioneering studies on elevated RDW values (data obtained in chronic heroin addicts, see Savov et al. [5]), we supposed that increased RDW could be a biomarker of suboptimal nutritional status in heroin abusers related to the so-called metabolic syndrome (MS), as was described in the recent medical literature [30].

MS is a metabolic disorder with multi-factor pathology, in which biochemical, morphological and functional (including rheological) changes occur in erythrocytes. RBC disorders are connected to abnormal metabolism of lipids, e.g. in cases of diabetes type 2 and hypertension. The results pointed to some essential changes in the erythrocyte plasma membrane. The increased levels of cholesterol concentrations and lipid peroxidation lead to decreased fluidity of RBC membranes and decreased RBC membrane viscoelasticity and flexibility with a simultaneous increase in plasma membrane rigidity [17, 18]. These pathophysiological cellular events further lead to a decrease in the ability of erythrocytes to change their shape (decreased RBC deformability) and, finally, to disorders of blood flow in the macro- and preliminary in the microcirculation. In this sense, the functions of RBCs not only as transporters of gas and nutrients, but also as bioactive cells, playing a central role in cardiovascular homeostasis, could be considered. There is clinical evidence of correlations between RBC dysfunction and cardiovascular/vascular disease outcomes [31]. From the same relationships we have concluded that our results on elevated hematometric indices RDW and MCV in chronic heroin addicts could be associated with heroin-induced negative effects on early hematopoiesis (erythropoiesis) and especially on the structure and functions of RBC membranes – such data are of importance in medical practice [5].

From the biorheological/hemorheological point of view, the so-called pro-inflammatory profile of MS-patients could be closely related to the association of elevated RDW with decreased erythrocyte (RBC-) deformability. Higher anisocytosis (=increased RDW) seems to be simultaneously obtained with other severe morphological alterations in the erythrocyte shape and volume which also reduce RBC deformability. The loss of RBC plasma membrane flexibility is the main reason why in the peripheral blood of patients eythrocytes appeared with altered forms (spherocytes, elliptocytes, echinocytes, RBCs forming “rouleaux”, RBCs which are soon retained and destroyed in the spleen, etc.).

Recently, some studies reported that elevated (above 14%) RDW values are significantly related to decreased erythrocyte deformability [13, 14, 17–20]. In the biorheological and hemorheological laboratories, RBC deformability is measured by means of a microfluidic slit-flow ektacytometer and expressed as erythrocyte elongation index (EI): patients with elevated RDW showed lower EI, than controls.

EI correlates directly with MCV (mean RBC volume $p<0.05$) and with HDL-cholesterol ($p<0.05$) in patients with AMI [17]. The same authors showed in their multivariate regression model that only hemoglobin (Hb) and MCV could be independent predictors of elevated RDW values. The decreased EI is related to the high rigidity of erythrocyte membranes and could impair blood flow (preliminary in microcirculation), leading to an increased risk of thromboses and development of vascular/cardiovascular, brain-vascular, peripheral artery, etc. diseases. Horne et al. [21] reported that MCV is also a hematometric index and biomarker predicting adverse outcomes in chronic vascular diseases. In the recent study by Magri et al. [11], investigating RDW as a novel independent marker of cardiovascular disease (CVD) and especially of coronary artery disease (CAD), more hematometric indices such as MCV, Hb, platelet count (PLT), mean platelet volume (MPV), lipid profiles and other hematological and biochemical measurements were evaluated and statistically analyzed as additional strong and independent biomarkers associated with inflammation and oxidative stress. The normal reference range for RDW in Magri’s laboratory is between 11.5% and 14.5%. The authors [10, 11] also supported our previous data and conclusions [5] that rheological alterations could explain an association between high RDW levels and increased total blood viscosity and whole blood viscosity (WBV) in cases of altered RBC rheological properties that result in significant changes in the microcirculation with consequent sluggish blood flow and vessel occlusions.

The more interesting result obtained as well as a general conclusion made by Magri et al. [11] postulated that RDW was found to be an independent predictor of disease outcomes in cases of CAD together with MPV and LDL-cholesterol. In our studies [5, 32], we also demonstrated interesting relationships between simultaneously increased values of RDW, MCV, WBV and simultaneously increased values of RDW, MCV, WBV and MPV in cases of chronic heroin addiction. Additionally, our cytological studies of blood smears [33, 34] revealed simultaneous anisocytosis of erythrocytes and platelets in the peripheral blood smears of chronic heroin abusers, probably secondary to ineffective and/or disturbed bone marrow hematopoiesis/erythropoiesis and megacaryocytopoiesis under the influence of drugs. Our results related to the injuries of platelets in long-term heroin addicts were well supported by the recent data of Lu et al. [35]. Confirming our previous results that opioids induced distinct biological, morphological and functional platelet alterations, the authors obtained lower platelet counts and plasma brain-derived neurotrophic factor (BDNF) and transforming growth factor (TGF)-β1 levels in long-term, more than 6 years, heroin addicts. In both cases [33, 35], the conclusions and hypotheses were similar: platelet-protective agents might provide improvement in heroin addiction therapy. It is probable that the same hypothesis is valid in all cases of RDW elevation, because the injuries in bone marrow hematopoiesis act simultaneously on the stem cells and early progenitor from two different cell lines involved in erythropoiesis and in megacaryocytopoiesis and are evaluated *in vivo* and *in vitro*.

Other recent investigations [36, 37] also examined correlations between RBCs and PLTs and suggested that both cells are sensitive to the presence of pro-inflammatory cytokines like interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-1β (IL-1β). These and other cytokines could simultaneously affect both circulating RBCs and PLTs during inflammation and are closely related to the cardiovascular and vascular risks. A number of studies have shown that MPV is associated with cardiovascular risk factors and is a predictor of cerebrovascular disease [11, 16, 38]. The authors reported that higher PLT counts reflect underlying inflammation, as several inflammatory mediators and pro-inflammatory cytokines could influence bone marrow and spleen megacaryocytopoiesis, producing relative thrombocytosis in the blood stream and leading to the formation of platelet-rich thrombi on atherosclerotic plaques on the blood vessel walls. In this relationship, Cheng et al. [16] examined RDW to PLT ratio (RDW/PLT) as a novel noninvasive prognostic index.

Associations of RDW with other biomarkers of oxidative stress were also evaluated in cases of different vascular diseases. Oxidative stress may play a potential role in ischemic diseases with elevated RDW, as ischemic stroke, myocardial infarction, etc. [19, 39]. Oxidative stress is a pathological factor which directly injures RBC plasma membranes, which affects their survival leading to RBC senescence and shortened erythrocyte life [28, 40, 41].

Inflammation plus oxidative stress has been implicated in the hemorheology and pathophysiology of many cardiovascular, brain-vascular, peripheral artery and other non-hematological and hematological chronic diseases [10, 42–45].

In a great number of chronic inflammation states, significant correlations have been established between elevated RDW values and changed levels of other inflammatory biomarkers, namely, white blood cells (WBC), erythrocyte sedimentation [RBC sedimentation; erythrocyte sedimentation rate (ESR)], high sensitivity C-reactive protein (hsCRP) and pro-inflammatory cytokines (IL-2, IL-6, soluble TNF-receptor-1, etc.). The latter two can inhibit EPO-induced erythrocyte maturation leading to elevated RBC anisocytosis, B-type natriuretic peptides, triglycerides, lipoproteins and cholesterol [total, low density lipoprotein (LDL) and high-density lipoprotein (HDL) fractions] levels [6, 11, 18, 24, 27–29, 38, 40, 46–50].

Conclusions

Clinical studies have indicated that RDW is an independent biomarker of altered RBC hemorheological properties associated with changes in the WBV [5, 10]. From a biomedical point of view, RDW could be compared to the biochemical biomarker neopterin giving very similar results to those obtained by RDW and described as an independent predictor of the same pathological states in the human body. In addition to the existing data in the medical literature, only a limited number of simultaneous clinical studies could be obtained on the topics, namely, increased neopterin levels are associated with elevated RDW and comparative investigations of RDW and neopterin levels and concentrations of other pro-inflammatory biomarkers.

Neopterin (Figure 1) belongs to the group of chemical substances denominated as pteridines and is biosynthesized from guanosine triphosphate [51, 52]. Neopterin is primarily produced by human and primate monocytes/macrophages upon activation by pro-inflammatory stimuli like Th-1 type cytokine interferon-gamma (IFN-γ) [53, 54]. The concentrations of neopterin in the human

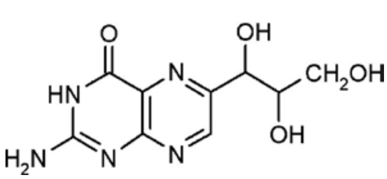

Figure 1: Neopterin (D-erythro-1′,2′,3′-trihydroxypropylpterin).

body increases simultaneously with IFN-γ levels, thereby neopterin assays help to monitor the activity of diseases associated with or inducible by IFN-γ as inflammation, malignancies, etc.

It should be kept in mind that neopterin is always formed together with its sister compound 7,8-diyhdroneopterin, which shares many chemical properties with neopterin. One exception is the fluorescence of neopterin but not in the dihydroform. Enzyme-linked immunosorbent assay (ELISA) methods measure exclusively neopterin levels in body fluids. Other assays measuring total neopterin present a sum of neopterin and 7,8-dihydroneopterin after oxidation [51–57].

Measurement of neopterin concentrations in human body fluids like blood plasma, serum, urine, cerebrospinal fluid, saliva, etc. provides information about the cellular immune system activation [53–56, 58] for the purpose of diagnosis, successful treatment and prognosis of inflammatory states, including atherosclerosis [56, 59, 60], malignancies (cancer and leukemia) [61], vascular (cardiovascular-, brain vascular, peripheral artery vascular) [59, 62, 63], neurological, autoimmune, and many other socially important diseases [e.g. acquired immune deficiency syndrome (AIDS); other viral and bacterial infections], aging-related disorders, including MS [56, 57, 60, 64–70].

In all pathological states examined in the medical scientific literature, the increased production of neopterin in the human body is associated with adverse outcomes and pure survival expectations as well as overall mortality in patients. Until now, it is comparable to high C-reactive protein (CRP) concentrations, but data on the comparison of neopterin with RDW values are still very scarce. We hypothesize that the conclusion from large future studies in this field might be that neopterin, RDW and CRP or hsCRP should be measured simultaneously in clinical laboratories. It will be more useful to monitor therapy and precise individual prognosis in individuals with elevated values obtained by simultaneous assays for these biomarkers.

Neopterin, applied *in vivo*, could suppress bone marrow erythropoiesis and simultaneously activate erythropoiesis in the spleen [71], probably leading to changes in the morphological properties of RBC and to RDW elevation. Inflammatory cytokine inhibition of erythropoiesis in patients has also been described [72].

As increased neopterin synthesis and high RDW values could be associated with elevated production of reactive oxygen and nitrogen species [reactive oxygen species (ROS), nitric oxide synthase (NOS), nitric oxide (NO), etc.] by activated immune cells, preferentially monocytes/macrophages, the lab results obtained could be interpreted not only as bioindicators of inflammation and cellular immune activation, etc., but also as biomarkers of oxidative stress, e.g. oxidative stress due to immune activation and inflammation [57, 58, 62, 73].

Additionally, data from the large population studies showed that the increased cellular activity of neopterin correlated with hyperlipidemia in patients with MS: elevated neopterin concentrations are associated with reduced HDL cholesterol [57, 64]. From a medical practical perspective, for the future, it is a very interesting fact that the functions of HDL cholesterol include anti-inflammatory and anti-oxidative activities in the human body. Thus, it will be important for laboratory medical practice to measure neopterin, in addition to cholesterols (especially to HDL cholesterol), preliminary in the elderly human populations and in the middle aged-individuals with suspected MS, and/or in the presence of other factors influencing cellular metabolism [57, 64, 74]. Earlier and recent studies including patients with HIV infection [57, 62, 64] have provided additional information on the interplay of immune response tested by neopterin assays and cholesterol metabolism, the neopterin concentrations were observed to be lower in individuals who used lipid-lowering drugs.

Neopterin testing is comparable to that of other inflammatory biomarkers

Elevated serum/plasma neopterin levels are associated with inflammation as well as with inflammatory responses in atherosclerosis: CAD, acute coronary syndrome (ACS), stable and/or unstable angina pectoris, cardiomyopathy, AMI, post-infarction states and other coronary events and cardiovascular risk assessment in older adults and the middle-aged human population [53, 55, 56, 58–60, 62, 63, 75–80]. The diagnostic value of neopterin was found to be similar to well-established markers in the medical laboratories such as CRP, hs-CRP, cholesterol plasma levels, etc.

In all cases of cardiovascular, brain-vascular and peripheral artery diseases, the elevated neopterin concentration serves as a biomarker of disease progression and unfavorable prognosis. In this respect, elevated neopterin concentrations correlated positively with increased plasma levels of hsCRP [69]. However, the direct contribution of CRP to atherogenesis is still unclear and debated [59]. The cellular and molecular mechanisms of a direct involvement of neopterin in atherogenesis through nuclear factor κB (NF-κB) could be better understood and clarified in the future, in view of its great potential as a bone marrow hematopoietic (granulocyte-macrophageal) growth factor, GM-CGF, as well as a bone marrow stromal cell growth factor [33, 81, 82]. The prospective and retrospective epidemiological studies on biomarkers neopterin and HDL cholesterol in patients with vascular/cardiovascular disease confirmed the direct association of neopterin with atherogenesis [33, 53, 56, 57, 59, 62, 64, 74–77, 82, 83].

Another interesting epiphenomenon is that low serum tryptophan and the kynurenine to tryptophan ratio (Kyn/Trp) could be predictors of acute coronary and cardiovascular events and are associated with higher mortality in patients with CVD [63, 69, 80, 84]. The explanation of this phenomenon is related to the inverse relationship found between serum neopterin and tryptophan: low serum tryptophan concentrations are associated with high levels of the inflammatory biomarker neopterin and with elevated hsCRP [69] which results from a parallel induction of neopterin formation and tryptophan breakdown by the interferon-inducible enzyme indoleamine 2,3-dioxygenase 1 (IDO-1).

The determination of neopterin in blood serum and in urine [51–57, 85], or in combination with other methods for the detection of pro-inflammatory and inflammatory biomarkers, could be of great importance for medical practice. In this respect, we detected elevated urinary neopterin levels in HIV-seronegative drug users (chronic heroin addicts) [55, 86]. In the last case, we observed an increased number of granulocytes (WBC), simultaneous anisocytosis of RBCs and PLTs [32, 86] and thus, increased RDW values [5].

General conclusions

Although it has been well established that elevated RDW and neopterin levels in humans are risk biomarkers in different pathological conditions, the fine biological – biochemical, cellular and molecular – mechanisms of this epiphenomenon are not fully established. The connection between RWD and neopterin is not obvious, and the biochemical link between the two biomarkers is unlikely to be direct. The critical role of the underlying inflammation, oxidative stress, alterations in hematopoiesis and metabolic imbalance (MS) in human body have been considered over the past decades for predicting the treatment responses, risk stratification and adverse outcomes in patients.

When used simultaneously and/or in combination with other biomarkers of inflammation (hsCRP, Kyn/Trp, some interleukins like IL-6, -8, -1β, and related hematometrical/hemorheological indices (MCV, PLT, MPV, PDW, NLR, PLR, etc.), RDW and neopterin could have higher predictive values for the correct diagnosis of different pathological states, monitoring of disease activity and response to therapy applied, as well as for complications and/or adverse outcomes and thus, prognosis of many diseases.

A strong-graded association of RDW with other hematometrical/hemorheological indices and its combination with biochemical biomarkers like neopterin and tryptophan breakdown (Kyn/Trp), might provide a new rational idea, namely to introduce simultaneous RDW- and neopterin measurements in an algorithm with eventual participation of additional biochemical biomarkers and hematometrical bioindices for the purpose of more precise diagnosis and prognosis of diseases including stratification of risk and prediction of adverse outcomes in different pathological states.

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