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Diagnostic and Prognostic Relevance of Red Blood Cell Distribution Width for Vascular Aging and Cardiovascular Diseases

Donatella Poz,[1] Elena De Falco,[2] Calogera Pisano,[3] Rosalinda Madonna,[4–6] Peter Ferdinandy,[7,8] and Carmela Rita Balistreri[9]

Abstract

Evidence suggests association of red blood cell distribution width (RDW) with cardiovascular diseases (CVDs). On the contrary, we underline that the sole RDW values cannot represent a valid CVD biomarker. High RDW values are expression of biological effects of a lot of both endogenous and exogenous factors (*i.e.*, age, sex, genetic background, inflammation, hormones, drugs, diet, exercise, hematological analyzers, and ranges of values), modulating the biology and physiology of erythrocytes. Thus, the singular monitoring of RDW cannot be used to predict cardiovascular disorders. Accordingly, we have reviewed the evidence for potential relationship of RDW values with alterations in the cardiovascular system (*i.e.*, regenerative capacity, endothelial turnover, and senescence of cardiovascular cells), associated with vascular aging and disease. In addition, we highlight the inevitable impact of biases in clinical application of RDW related to CVDs. Based on our thorough review of literature, we suggest a combined evaluation of RDW with other emerging biomarkers related to vascular aging and the diagnosis and prognosis of CVDs, including telomere length of leukocytes, circulating nucleated red blood cells (nRBCs) and endothelial progenitor cells (EPCs) in future large scale studies.

Keywords: RDW, CVDs, vascular aging, leukocyte telomere lengths, circulating endothelial progenitor cells and nucleated red blood cells

Definition, Features, and Routine (and Current) Clinical Applications of Red Blood Cell Distribution Width

The Red blood cell distribution width (RDW) is one of the parameters conventionally included in the complete blood count (CBC) reports. It is an index of variation in the size and shape of erythrocytes, corresponding to the degree of anisocytosis (increase in the variation of red blood cell size) (Fig. 1A). Thus, its values increase according to the heterogeneity in red cell size. RDW values are usually reported on CBC reports, to indicate an eventual anisocytosis, recognized as a useful diagnostic tool for hematological diseases, such as anemia. However, the initial microscopic evaluation, purposed by Price-Jones,[1] has reduced its diagnostic power due to the waste of time and the high intersubjectivity of visual inspection. To date, this laboratory approach has become obsolete and replaced by the use of modern hematology analyzers. They report the distribution of erythrocytes size in histograms, detecting

[1]Department of Laboratory Medicine, Institute of Clinical Pathology, Azienda Sanitaria Universitaria Integrata (ASUI) di Udine, Udine, Italy.
[2]Department of Medical-Surgical Sciences and Biotechnologies, ''Sapienza'' University of Rome, Latina, Italy.
[3]Cardiac Surgery, Tor Vergata University, Cardiochirurgia Policlinico Tor Vergata, Rome, Italy.
[4]Heart Failure Research, Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, Texas.
[5]Department of Internal Medicine, Cardiology, The University of Texas Health Science Center at Houston, Houston, Texas.
[6]Department of Neurosciences, Center of Aging Sciences and Translational Medicine, CESI-Met and Institute of Cardiology, Imaging and Clinical Sciences ''G. D'Annunzio'' University, Chieti, Italy.
[7]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary.
[8]Pharmahungary Group, Szeged, Hungary.
[9]Department of Pathobiology and Medical and Forensic Biotechnologies, University of Palermo, Palermo, Italy.

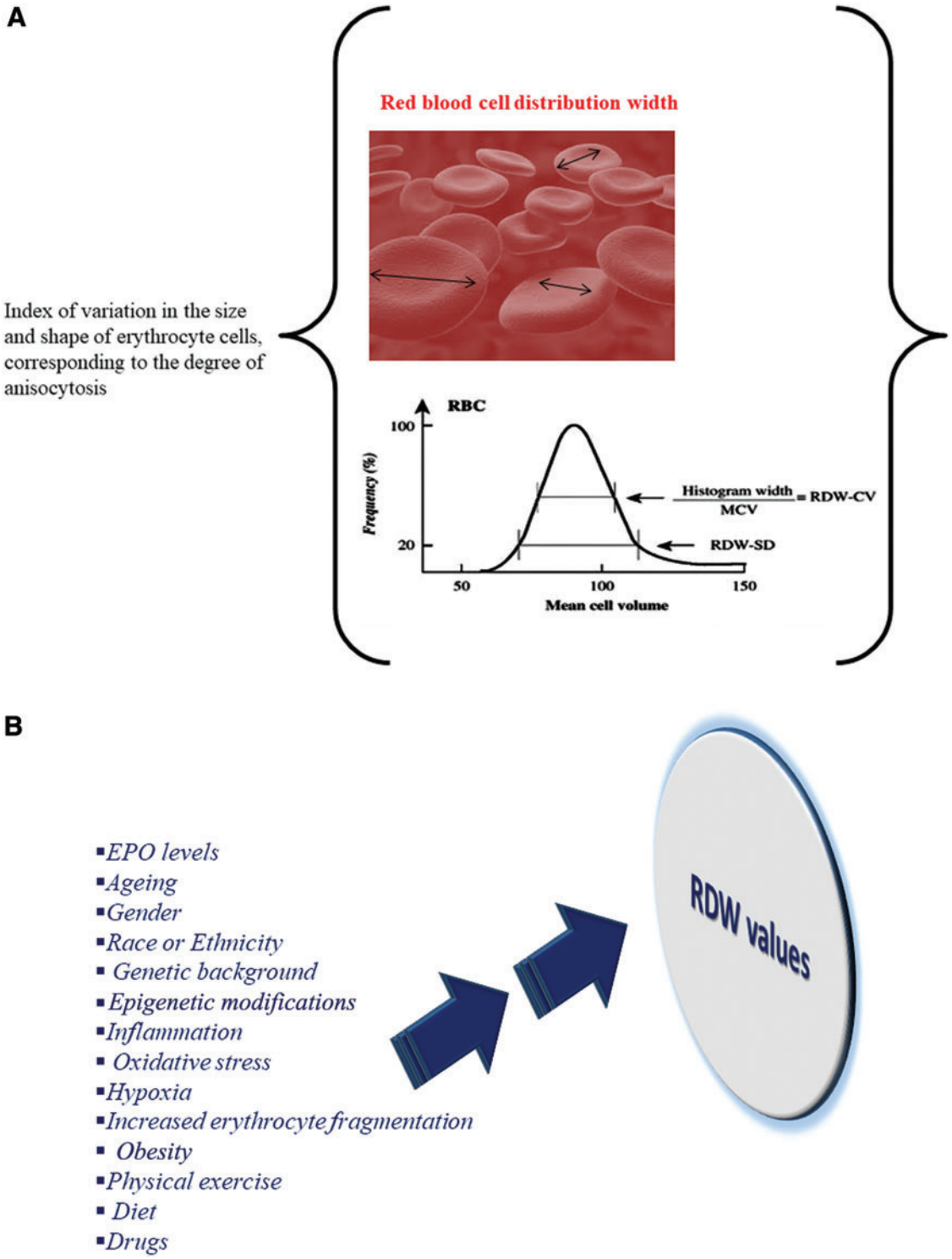

FIG. 1. **(A, B)**. Definition, calculation, and factors able to modulate RDW values. **(A)** the panel shows the significance and the calculation of the RDW values. RDW is a red blood cell parameter that measures variability of red cell volume/size (anisocytosis). Depending on the types of hematology analyzer instruments, RDW can be reported statistically as coefficient of variation (CV) and/or standard deviation (SD), RDW-CV and/or RDW-SD, respectively. RDW-SD takes measurements in ''fL'' and basically measures the width of red cells size distribution histogram. RDW-CV is expressed in percentage, and is calculated from MCV and standard deviation as follows: RDW-CV (in percentage) = 1 SD of RBC volume/ MCV × 100%. **(B)** The picture displays all endogenous and exogenous factors modulating the RDW values, as described in the text. RDW, red blood cell distribution width; MCV, mean corpuscular volume. Color images available online at www.liebertpub.com/rej

signals obtained from specific channels on a cell-by-cell analysis. Furthermore, they display RDW values within a reference range of 39–46 fL ± standard deviation (SD),[2] or as percentage (*SD of erythrocyte volume/mean corpuscular volume (MCV)* × *100*) (Fig. 1A), ranging between 12% and 15%.[3] However, the International Council for Standardization in Hematology (ICSH) has suggested a statistical method to standardize the RDW calculation,[4,5] therefore eliminating any potential discrepancies among clinical laboratories.[6,7] Despite this, its application still remains to be defined.

Regarding its routine clinical application, RDW was initially used as a suitable parameter for hematological diseases, particularly for anemia, since its values reflect anisocytosis. Accordingly, in 1983 Bessman et al. proposed a classification of anemia based on both the MCV and RDW parameters.[8] Specifically, they considered heterogeneous and homogeneous forms of anemia associated with increased (*e.g.*, in iron or cobalamin and folic acid deficiency) and normal RDW values (*e.g.*, heterozygote thalassemia, hypoproliferative conditions, or aplasia), respectively. However, it showed a high sensitivity but not a high specificity, given the wide distribution of the RDW values according to the disease. In addition, they reported a potential overlap between the different clinical conditions. In fact, RDW can increase in anemia due to chronic diseases and in about half of the heterozygote thalassemic subjects. Differently, normal RDW values are expressed in ~20% of patients with iron deficiency anemia. Moreover, in some cases, a double population of circulating erythrocyte can be detected in the periphery. In this circumstance, RDW cannot be considered an adequate biomarker of anisocytosis.[9,10] For these reasons, some groups have suggested to omit RDW values in hematological reports and to perform microscope evaluation only when indicated.[11] In addition, the RDW shows a little relevance in the differential diagnosis of microcytic anemia,[12] even if its usefulness as a general biomarker of red blood cell abnormality, when increased, has been maintained.

Since the mid-1920s, RDW has gained interest due to the emerging evidence about its association with different nonhematological clinical conditions, characterized by an inflammatory pathophysiology.[13] However, after the first initial evidence of a significant association between RDW and cardiovascular diseases (CVDs),[14] the role of RDW still remains under investigation in other human disorders, including for example diabetes and its complications (kidney disease, liver disease, cancer, and heart failure [HF])[15] or atrial fibrillation (AF) and stroke, chronic obstructive pulmonary disease and community-acquired pneumonia; or in critically ill patients: sepsis or septic shock, as well as in patients with HIV infection. Furthermore, an association of RDW with the prognosis or outcome of CVDs is emerging (see RDW: A Valid Biomarker for CVDs? section). For example, Abrahan et al. have recently performed a meta-analysis of 13 trials involving 10,410 patients with acute coronary syndrome (ACS), and demonstrated that low RDW values are associated with a significant reduced risk for major adverse cardiovascular events (RR 0.56, [95% CI: 0.51–0.61], $p < 0.00001$, $I^2 = 91\%$).[16] Moreover, many population-based longitudinal studies revealed an association between high RDW values and mortality risk.[15,17–20] However, its current interest as a prognostic indicator of mortality and morbidity for CVDs can be mitigated by fixing some critical aspects on RDW. Here, we highlight our concerns by first describing and discussing various endogenous and exogenous factors (*i.e.*, age, sex, genetic background, inflammation, hormones, drugs, diet, exercise, hematological analyzers, and ranges of values) and related mechanisms, which have been demonstrated to modulate the RDW values and to emphasize its relevance. Accordingly, we raise doubts about its association with CVDs. Specifically, we underline whether higher RDW values may really reflect alterations in the cardiovascular system (*i.e.*, regenerative capacity, endothelial turnover, and senescence of cardiovascular cells), as expression of vascular aging and disease, or show potential biases in their clinical application. Finally, we propose to combine the detection of standardized RDW values with other emerging biomarkers, including the length of leukocyte telomere, the circulating levels of nucleated red blood cells (nRBCs), and circulating endothelial progenitor cells (EPCs). Their combined measurement associated with an appropriate statistical analysis (*e.g.*, meta-analysis, linear regression, or other statistical tools) could result in a multibiomarker profile, and facilitate the development of a diagnostic/prognostic algorithm for vascular aging and CVDs.

Endogenous Factors Affecting RDW Values

A large array of endogenous factors affect the values of RDW in several pathologies (Fig. 1B). High RDW values can derive from an imbalanced erythropoiesis, where many physiological determinants are involved. In bone marrow microenvironment, erythropoiesis is finely regulated by humoral factors, first including erythropoietin (EPO). EPO plays a crucial role in production, maturation, and survival of red blood cells.[20,21] Accordingly, Kario et al. observed increased RDW values in EPO deficiency or hyporesponsiveness, characterized by a positive correlation between RDW values and EPO titers.[22] Afsar et al. recently confirmed these data by investigating patients affected by EPO hyporesponsiveness.[23]

Aging is another established determinant in RDW elevation. The first evidence, although not clearly justified, has been provided by Cheng et al. in the Third National Health and Nutrition Examination Survey 1998–1994 (NHANES III). In this broad survey, data from ~25,000 nationally US adults, stratified by age, sex, and race, were collected to obtain CBC reference interval diagrams.[24] Afterward, Patel's group collected data from the NHANES III and investigated RDW trend among 8175 adults over 45 years. The sample was divided according to RDW values in five quintiles (<12.60%, 12.60%–12.95%, 13.00%–13.40%, 13.45%–14.05%, and >14.05%, respectively). They found a progressive significant increment of age in RDW quintiles 1–5, respectively (p value <0.001).[16] Other groups reached the same conclusion. By dividing RDW in quartiles, they found that subjects in the highest RDW quartiles were significantly older than those in the lowest quartiles (with $p < 0.001$ for trend).[25–28] More recently, Lippi et al. carried out a retrospective analysis to establish the role of aging as a significant determinant of RDW. They collected results of hematological tests performed on a cohort of apparently

healthy blood donors aged >20 years over a 1-year period (*i.e.*, 1907 subjects: 562 females and 1345 males). It has been observed that the values of RDW steadily increase among different age groups ($p < 0.001$ for trend). In addition, subjects, aged ≥60 years or <60 years, exhibited higher median RDW values (14.6% and 13.2%, respectively). Interestingly, the percentage of subjects with RDW values above the conventional upper limit of the reference range (established at 14.6%) progressively increased ≥59% in people aged 80 years or older. In subjects aged less than 41 years, this percentage was ~6%; therefore, confirming the strict dependence of RDW on age. Moreover, older subjects exhibited a wider range of RDW values. Univariate and multiple regression analyses have confirmed similar results.[29] Based on Lippi's group observations, Alis et al. retrospectively investigated 809 healthy subjects (who were not blood donors) to further clarify the effect of aging on RDW values. Generally, blood donors are routinely subjected to marrow stimulation, resulting in an overestimation of the RDW value.[30] The results from Alis' group confirmed the previous findings from Lippi's group, showing a significant positive correlation between age and RDW values, especially in people older than 75 years.[30] Hoffmann et al. investigated 8089 subjects in a cohort study, achieving the same results from Lippi's and Alis' groups.[31] Thus, it is possible to affirm that there is a strong relationship between increased age and RDW elevation regardless of the analyzer used to measure RDW.[32]

In addition, there is a strong evidence of shortening telomeres with aging.[33] This leads to cellular senescence of hematopoietic progenitors, especially erythroid, and consequently impaired cell maturation, including red blood cells.[34]

The gender represents an important factor modulating RDW values, although data are still controversial. Females show a higher median RDW value compared with males (13.8% vs. 13.3%; p value = 0.001).[29] These results have been confirmed in other current studies (amply quoted in 30). However, previous investigations have demonstrated a nonrelationship between RDW and gender,[16,24,26–28] as also reported by Hoffmann et al. They found no significant gender-related differences in RDW-SD, with the exception for the 56–70 years age class (p value = 0.014).[32] Nonetheless, the difference observed was limited, and the very large number of subjects investigated ($N = 8089$ individuals) could explain the discrepancy observed among different studies described above.

Another factor impacting RDW values is represented by ethnicity, although less description is provided in the literature. One of the first investigations was reported by Saxena and Wong in the early nineties. They investigated 2142 subjects of four different ethnic cohorts (Whites, Afro Americans, Latin Americans, and Asians), observing significantly higher RDW values in Afro American subjects.[35] The same evidence was obtained from the group of Zalawadiya from a subanalysis of the NHANES III study. They retrieved higher mean RDW values with a higher baseline RDW value even in Afro Americans, while no significant discrepancies were found among other ethnic groups.[36] Thus, they hypothesized an important role of environmental and genetic factors, since African American people show a higher incidence and prevalence of nutritional deficits (*e.g.*, iron and folic acid[37]) as well as higher levels of chronic inflammation (as measured by C-reactive protein[38]). More recently, Loprinzi et al. collected data from seven 2-year cycles of NHANES and published an interesting paper. In the 1999–2012 period, they collected data from 34,171 adults with age ≥20 years (mean age of 46.7 years in the whole sample), demonstrating that the mean values of RDW and the prevalence of high RDW values had progressively increased from 1999 to 2012 in all sample, but especially in Afro Americans and women. Moreover, Afro Americans and women showed that RDW values rapidly change.[39] Thus, they assumed that inflammation, disorders in iron homeostasis, and/or resistance to EPO-induced anisocytosis may be involved.

Of note also is the increase of RDW values with obesity. In particular, Fujita et al.[40] have reported that RDW significantly rises in overweight adolescents (13.39 ± 0.10, $p = 0.015$) compared with normal-weight adolescents, while erythrocyte counts and hematocrit do not differ. Furthermore, they found positive significant correlations of RDW with biomarkers of inflammation. The potential causal relationship between obesity and RDW value was confirmed by demonstrating that nutritional changes in murine models increased RDW, whereas overweight *per se* did not change RDW. In 2014, Vayá et al.[41] reported similar results, examining obese patients before bariatric surgery ($n = 142$) and normal-weight controls ($n = 144$). Obese patients showed higher RDW values than controls ($p < 0.001$) without any correlations with blood inflammatory biomarkers (*i.e.*, C-reactive protein, fibrinogen, leukocytes, and neutrophils). In fact, only low serum iron (<62 μg/dL) and MCH (<28.14 pg) levels were associated with RDW >14% (OR 7.61, 95% CI: 1.93–30.04, $p = 0.004$; OR 5.67, 95% CI: 1.98–16.24, $p = 0.001$, respectively). However, a more recent study by Laufer Perl et al.[42] on a cohort of 3529 consecutive patients with metabolic syndrome (MetS) undergoing coronary angiography has shown that RDW ≥14% is independently associated with higher rates of MetS and long-term all-cause mortality. Likewise, another recent study with 223 participants demonstrated that neutrophil count, lymphocyte count, platelet count, platelet parameters, and RDW are significantly influenced by the body mass index (BMI) status.[43]

The underlying mechanisms related to the elevation of RDW under physiological conditions (*i.e.*, age, gender, and race), as well as in obesity, are not fully clarified. Certainly, the genetic background plays a key role in setting both physiological and pathological conditions. This has recently led Pilling et al.[44] to investigate the role of genetic factors in RDW increase. Specifically, they performed genetic analysis in a very large population cohort derived from the wide UK Biobank volunteer sample subjected to standardized RDW measurements. The results obtained demonstrated that 29.3% of the variation of RDW is associated with common genetic variants, and that this variation explained by the genetic variants increases with age, in contrast with the current knowledge that genetic effects decrease with aging. These data could be explained on the potential accumulation of detrimental aging-based effects over time.[45] Furthermore, the major number of genetic variants associated with RDW (in 71/194) has been previously demonstrated to be associated with different traits of the disease, such as metabolic syndrome, certain cancers and autoimmune diseases. Regarding CVDs, the analysis of genetic risk scores (GRSs),

highlighted in subjects with genetically increased risk of coronary heart disease (CHD) or cancer, demonstrated no association with significantly higher RDW values. In fact, they observed that only 6.6% of variation between RDW and CHD is explained by genetic background minimizing the role of genetics in red cell parameters and their influence on CHD.[40] Accordingly, the GRS analysis evidenced that either subjects with genetically lower and higher low-density lipoprotein (LDL) and/or triglyceride levels or systolic blood pressure showed higher RDW values. Several observational studies partially confirmed these data.[46] Specifically, they demonstrated that both LDL and high-density lipoprotein (HDL) cholesterol levels were negatively correlated with RDW values, whereas triglyceride levels were positively associated.[46] It is plausible that the relationship between lipids and RDW values is complex, and the positive epidemiological relationships observed between CVDs and RDW values could be strongly ascribable to other pathways and mechanisms, such as inflammation or environmental factors, as clearly evidenced in our recent papers.[47,48] Pilling et al. also reported that genetic variants associated with RDW were linked to specific molecular pathways, including iron homeostasis, several nucleosomes and histones, ribosomal RNA production, and telomere maintenance, whose length is a typical feature of cellular aging and senescence.[45] However, the causes still are uncertain,[49] and the longest telomeres have been linked to cancer risk.[50] In 2012, the group of Kozlitina reported that increased RDW values were associated with shorter telomeres in leukocytes, [see paragraph *Other Emerging Parameters: Leukocyte Telomere Length and nRBCs for Vascular Aging and CVDs*],[51] whereas Pilling's group did not confirm this association.[44]

The discrepancy of the data in several studies could be explained by genetic and epigenetic variants of factors affecting RDW. In fact, expression of genes is recognized to be the result of this complex mechanism of regulation, where the principal actors are the environmental and epigenetic factors.[52–54] Given their relevance, we stress their relationship with RDW in the specific paragraphs below.

Exogenous Factors Affecting RDW Values

Several evidence has shown the great impact of diet, exercise, and stress as major environmental modifiers of chronic inflammatory diseases, including CVDs.[55] Recently, Nahrendorf and Swirski elegantly reported[56] that lifestyle influences the molecular and cellular machinery of hematopoiesis, eventually leading to an altered number and phenotype of macrophages, and to a strong activation of neuroimmune and immune-metabolic axes. Based on this evidence, it is likely that life habits could also modulate indirectly RDW values. In 2013, Emans et al., by examining 17,533 European adults, demonstrated that RDW values were associated with physical inactivity, without affecting the relationship between RDW and HF.[57] Afterward, other independent groups showed that resistance training was favorably associated with RDW,[58] and the acute time of exercise reduced RDW values.[59] In 2015, the Loprinzi group also evaluated the association between objectively measured physical activity (accelerometry) and daily dietary patterns with RDW among a national sample of US adults.[60] They observed that physical activity, but not diet, seems to be inversely associated with low levels of RDW. As a result, they concluded that regular exercise can help preventing CVDs and mortality by changing the RDW.[60] In 2018, another group investigated[61] the effects of cigarette and hookah smoking on biochemical characteristics, such as RDW, in a representative population sample derived from the Mashhad stroke and heart atherosclerotic disorder (MASHAD) cohort study from northeastern Iran. Interestingly, they evidenced that RDW remained significant ($p < 0.001$) after multivariate analysis in cigarette smokers than in nonsmokers and between hookah smokers and nonsmokers ($p < 0.05$).

Among several exogenous factors influencing the elevation of RDW, drugs are of interest. Cytotoxic chemotherapies usually induce a decreased cell survival and an increased cellular fragmentation, including RBCs.[8] For example, capecitabine, a fluoropyrimidine carbamate, selectively activated after oral administration of 5-fluorouracil (5-FU) exerts its cytotoxic activity determining a defective DNA synthesis through the inhibition of thymidylate synthase (TS). An increase in MCV (without concomitant anemia or vitamin B_{12}/folate deficiency) is observed during capecitabine-based therapy. This could result in the inhibition of TS in erythroid progenitors, resulting in an impaired erythropoiesis in a time- and dose-dependent manner.[62,63]

Since hyperlactacidemia is involved in changing the RBC volume, drugs, able to increase the lactic acid concentration through metabolic interferences, could also cause a raise in anisocytosis, and consequently increase RDW values. As widely reviewed by Pham et al.,[64] the chronic use of metformin, nucleoside, and nucleotide reverse transcriptase inhibitor (NRTI)-based therapy (especially didanosine, stavudine, lamivudine, zidovudine, and abacavir, linezolid, isoniazid, valproate, propofol, and salicylates) could potentially result in RDW variation. Further investigations are needed to clarify all these issues.

The Potential Mechanisms Related to the Relationship Between Increased RDW Values and Endogenous and Exogenous Factors

Elevated levels of RDW have been associated with a wide range of endogenous and exogenous factors (as mentioned above), but the mechanisms and the related pathways involved remain to be fully elucidated. Systemic inflammation has been suggested as a principal mechanism related to RDW increase.[12,65–67] Accordingly, in 2008 Tonelli's group[65] examined the association between RDW and the risk of all-cause mortality and adverse cardiovascular outcomes in 4111 participants with coronary disease free of HF at baseline. They used Cox proportional hazards models to examine the association between RDW and adverse clinical outcomes. They observed a significant association between RDW and the adjusted risk of all-cause mortality (hazard ratio per percentage increase in RDW, 1.14; 95% CI: 1.05–1.24), concluding that this relationship may be the result of systemic inflammation. However, this hypothesis was not confirmed by these researchers. Accordingly, no inflammatory plasma biomarkers were detected in this study.[65] Later, Lippi's group obtained interesting results on RDW from 3845 adult outpatients during a 3-year period. Precisely, they confirmed the working hypothesis from Tonelli's group,

first demonstrating a graded association between RDW and high-sensitivity C-reactive protein (hsCRP), and erythrocyte sedimentation rate (ESR), two robust inflammatory biomarkers.[12] Moreover, it has been shown that inflammation could also affect iron metabolism, further impairing the maturation of erythrocyte[66] or lowering RBC survival, and leading to a more mixed population of RBC volumes in peripheral blood.[67]

It has also been highlighted that oxidative stress influences RDW.[68] Oxidative stress is known to increase the levels of reactive oxygen species (ROS), commonly causing cellular damage. Furthermore, it mediates negative effects on erythrocyte survival, thus increasing RBC turnover.[68] The evidence, on low serum antioxidant concentration and the inverse correlation with RDW, corroborates these claims.[69] Interestingly, it has also been demonstrated that in Fanconi anemia (FA), a condition known to exhibit a dysfunctional response to oxidative stress, RDW values are increased with a strict relationship with the progression of hematological diseases, including anemia.[70]

The increase in erythrocyte fragmentation, excluding hemolytic anemia, is another important condition responsible for the anisocytosis of both RBC and RDW values in several chronic diseases. In cancer patients, near to inflammation and nutritional deficiencies, an increased fragmentation of RBC is often due to cytotoxic chemotherapies.[8] Differently, in advanced chronic liver disease, increased RBC fragments are mainly due to expanded plasma volume and/or hypersplenism, which lowers the half-life of RBC by increased spleen sequestration and destruction.[71]

Chronic hypoxia represents a significant factor associated with the increase of the RDW values. It is conceivable that the release of both proinflammatory cytokines and lactic acidosis, caused by tissue hypoperfusion, plays a central role.[68] There is evidence that the uptake of RBC lactate exerts an increase in the volume of erythrocyte.[69]

Thus, these observations lead to the conclusion that a variety of determinants are able to induce high RDW values. As a consequence, both sensitivity and specificity of RDW as mirror of inflammatory states still are to be fully clarified.

The Key Relevant Action of Epigenetic Factors in the Increase of RDW Values

Epigenetic factors, including DNA methylation, histone modifications, chromatin remodeling, and microRNA (miRNA), play a key role in the activation of biological mechanisms related to the increase of RDW values.[52] Accordingly, recent investigations[72–76] have underlined the relevance of epigenetic factors in regulating erythropoiesis and the production of mature red blood cells and their parameters, such as RDW. Papageorgiou's group[74] has demonstrated the key role of DNMT1, a DNA methyltransferase, in embryonic development and cellular growth and differentiation in many somatic tissues in mammals, as well as in the developmental silencing of fetal β-type globin genes in the adult stage of human erythropoiesis. In addition, the group of Liu[72] has investigated the role of DNA methylation in erythrocyte production by human embryonic stem cells (hESCs). They observed a negative correlation between DNA methylation and gene expression during the later differentiation stage. Furthermore, erythropoietic genes with differentially methylated CpG sites that were primarily enriched in nonisland regions were upregulated, and demethylation of their gene bodies was associated with the presence of enhancers and DNase I hypersensitive sites. Moreover, they demonstrated that the components of JAK-STAT-NF-κB signaling (key genes for erythropoiesis) were DNA hypomethylated and upregulated. This also supports the role of inflammation in affecting the homeostasis of production of red blood cells and their parameters.

So far, there have not been specific investigations showing a direct link between endogenous and exogenous factors, epigenetic modifications, and the related increase of RDW values. Thus, additional future studies in this field are encouraged. However, Rosa-Garrido et al.[55] and Wallace et al.[77] have strongly suggested to consider the epigenetic signature as an additional innovative and predictive biomarker for the management/outcome and diagnosis of CVDs.

RDW: A Valid Biomarker for CVDs?

In recent years, many acute and chronic CVDs, including ACS, ischemic cerebrovascular disease, peripheral artery disease (PAD), HF, and AF, have been associated with high RDW values.[78,79] Table 1 shows the related current evidence.[80–108] In addition, some meta-analysis studies confirm this relationship. For example, Su et al.[109] investigated the possible association between high levels of RDW and the mortality, and the cardiovascular risk of developing other CVDs in patients with ACS, conducting a meta-analysis of 22 studies with 80,216 participants. The data obtained demonstrated a significant relationship between high levels of RDW and high risk for mortality and ACS complications.[109] Similar results were found in evaluation of the role of RDW on the prognosis of HF, by performing a meta-analysis of a total of 17 studies and 18,288 patients.[110] Specifically, this study reports that individuals with high levels of RDW may have a nonpositive prognosis compared with those with low RDW values.[111] In 2016, Luo et al. evidenced in another meta-analysis, including 32 studies, the potential association between high RDW values and high mortality in noncardiovascular patients in critical or acute conditions.[112]

These encouraging results suggest the prognostic significance of the RDW values for CVDs. However, further studies are needed to exclude all factors (described above) that could affect RDW values. In addition, further investigations are needed to find out the potential mechanisms involved, to use RDW values in the management of patients with CVDs. Accordingly, the biological causes were only postulated at moment, including the high release of EPO under hypoxia, the small reduction in RBC turnover, the increase in ROS concentration, age, aging, genetic background, ethnicity, inflammation, and other factors as reported above. Nevertheless, RDW seems to show some advantages when compared with other biomarkers conventionally used in the diagnosis and prognosis of CVDs. First, RDW values provide useful information on poor CVD prognosis, even if the presence of anisocytosis may be considered a nonspecific biomarker. Second, high RDW values in patients with a first overt of cardiovascular event can indicate timely treatments. In fact, Lippi has underlined

Table 1. Red Blood Cell Distribution Width in Cardiovascular Diseases (See References[80–108])

Cardiovascular diseases	*Findings*
Acute coronary syndrome	Significant association between the presence of ACS and high level of RDW Association between RDW and the risk of first-ever event of MI in the general population. The combined measurements of both troponin T and RDW have allowed us to diagnose MI with greater sensitivity than the analysis of troponin T alone in patients admitted to intensive care unit for chest pain. High level of RDW predicts both in hospital and long-term cardiovascular mortality in patients with ACS. The levels of RDW predict the development of stent thrombosis in patients with STEMI undergoing primary percutaneous coronary intervention
Heart failure	Significant association between the onset of HF and high level of RDW RDW and NT-proBNP improve the accurate rate of diagnosis and reduce the misdiagnosis in HF patients at admission Increased RDW is a strong independent predictor of morbidity and mortality in HF patients High RDW levels on admission predict prolonged LOS in HF patients
Atrial fibrillation	Significant association between high level of RDW and the onset of AF associated or not to valvular disease. High level of RDW predicts adverse outcomes in patients with AF High level of RDW predicts the onset of atrial fibrillation in patients undergoing CABG
Ischemic cerebrovascular disease	Significant association between high level of RDW and incidence of stroke in patient with HF Significant association between high level of RDW and incidence of stroke in patient with AF Significant association between high level of RDW and incidence of stroke in patient with ACS Significant association between high level of RDW and incidence of stroke in general population RDW is a promising, easy, rapid, and inexpensive index to distinguish stroke from stroke mimes (such as multiple sclerosis and epilepsy) in young patients.
Peripheral artery disease	Significant association between high level of RDW and the prevalence of PAD Role of RDW in the diagnosis of PAD

ACS, acute coronary syndrome; STEMI, ST elevation myocardial infarction; MI, myocardial infarction; HF, heart failure; LOS, length of hospital stay; BNP, brain natriuretic peptide; CABG, coronary artery bypass grafting; AF, atrial fibrillation; PAD, peripheral artery disease.

the use of RDW values as a surrogate biomarker for treatment targets in dyslipidemic patients.[113] However, it remains to demonstrate whether the close association between RDW and CVDs is causal, or it represents the consequence of specific conditions and/or the biological actions of several endogenous/exogenous factors, which occur and act during vascular aging and the onset of CVDs, such as the role of inflammatory cytokines, oxidative stress, poor metabolic status, and increased RBC turnover, as mentioned above.

Consistent with these considerations, in the next paragraph we discuss the potential biases in the clinical use of RDW.

Potential biases in the clinical use of RDW for CVDs

From a prognostic point of view, laboratory tests have always been considered as a powerful tool to prevent and/or to monitor relevant disorders, such as CVDs. Over the years, new cardiac biomarkers for CVDs have been derived from laboratory parameters routinely used in noncardiac disorders, particularly in the hematology field. As a consequence, the prognostic values of RDW in the CVDs have been well established. However, there is currently no real clinical application and implementation for several reasons. First, there is no unanimous consensus regarding the physiological reference interval of the RDW values, as well as in laboratory equipment and statistical interpretations, which provide variable results.[110] In contrast, some meta-analyses revealed that an unfavorable prognosis in subjects with HF, ACS, ischemic cerebrovascular disease, PAD, HF, AF, hypertrophy, or after cardiac valvular surgery is parallel to elevated values of RDW,[111,114–116] which in turn correlates with canonical cardiac biomarkers, including mid-regional proadrenomedullin (MR-proADM), soluble urokinase plasminogen activating receptor and copectin.[88] This has improperly strengthened the prognostic relevance of RDW in several types of CVDs. Thus, it is rightly acknowledged as a tool to increase the accuracy of the diagnosis in the presence or absence of a history of cardiac disorders.[26,81] However, the RDW values show dynamic significances and changes. During hospitalization, RDW values may change over time, especially in acute conditions,[117,118] indicating that its reliability as a clinical parameter is relevant for long monitoring periods. One of the main clinical biases is currently based on uncertain explanations regarding the association between RDW and CVDs. Furthermore, the RDW values are not always correlated with the type of CVD to a similar extent. For example, coronary atherosclerotic events are less associated with RDW values than stroke, myocardial infarction (MI), or thrombosis.[119] Similarly, it is unclear whether the RDW values can adequately reflect the progression of carotid plaque, a significant risk factor for ischemia and stroke. This suggests that the severity of the injury or the etiopathology of CVDs could represent a significant injury in association with other clinical determinants.

More importantly, the identification of the underlying biological mechanisms in the correlation between RDW and CVDs remains to be clarified. Given that the RDW values derive from the biology of erythrocytes, we should consider all related alterations that can influence the cardiovascular status of a subject. Furthermore, the major number of mechanisms ascribable to the increase in RDW values is associated with CVDs. Accordingly, chronic anemia causes a direct modification of the size of the erythrocytes, with consequent increase of the RDW values and compromised hemodynamic compensatory response. In fact, anemia is a biomarker of HF and left ventricular hypertrophy, where the cardiac tissue is stressed by the overload of blood to preserve the oxygen supply to the tissue.

Moreover, the immune system seems to play a key role in determining the variations of RDW values. In this regard, inflammation caused by increased levels of known cytokines, involved in the pathogenesis of CVDs, including IL-6, CRP, and TNF-α, has been reported to inhibit the maturation of erythrocytes. As a result, a decreasing fraction of the mature red blood cells would imply the recruitment of reticulocytes from the bone marrow into the systemic circulation and the consequent increase of the RDW values.[120–122] Although the inflammatory status cannot be assumed as homogeneous among patients, the quantification of the RDW values mirrors the metabolic life of circulating erythrocytes (130 days). This provides a significant prognostic advantage, since the monitoring of erythrocytes in the blood can be constantly performed over a longer time period than canonical cardiac biomarkers.[123] Furthermore, inflammation is able to significantly alter oxidative stress and platelet activity during the onset and progression of CVDs.[66,124] These processes stream into the periphery, and influence the physiology of erythrocytes and consequently the RDW values.[125] Additional mechanisms influencing RDW values, may be ascribable to additional variables, which may include potential pathological changes in the peripheral vascular system, such as edema, but also those related to biochemical changes in the hemoglobin molecule or serum iron saturation levels, reflect RDW values.[18]

Notably, although a close relationship between CVDs and inflammation is evident, not all cytokines positively correlate with RDW values. For example, the levels of IL6 and IL1 are directly and inversely proportional to the values of RDW, while levels of TNF-α have been reported as unaltered.[110]

A further important prejudice, concerning the role of RDW values as a prognostic factor in CVDs, certainly consists in the hemodynamic status of patients, which can influence their values.[126,127] For example, atherosclerosis, one of the main causes of blood flow modification in CVDs, can trap the erythrocytes inside the plaque, thus altering the microcirculation and the hemodynamic parameter. Elevated RDW values may be associated with thromboembolic events[128] or with the atherosclerotic profile of patient, turning into a prognostic indicator of potential adverse clinical outcomes in HF independently of the subject's anemia state[129]] and consequently hemoglobin concentration.[18] However, a recent study has reported that the RDW/hemoglobin ratio[127] and the blood concentration,[130,131] whose decrease is concomitant with the high RDW values during hospitalization,[117] would allow us to predict the outcome in a dose-independent dependence. This suggests that many other parameters should be employed to normalize the RDW values, to improve their prognostic impact. This consideration is reinforced by other studies. Thus, our message is that RDW cannot potentially be used as a prognostic indicator alone, but likely combined with other cardiac biomarkers, such as natriuretic peptides, ANP and BNP, it is known to significantly influence the prognostic features of HF.[114] Accordingly, here, we propose an alternative multibiomarker profile, as extensively described below.

Other important clinical biases are related either to the nutritional status of patients or to the level of free cholesterol. Vitamin D3 deficiency causes serious damage to hematopoiesis, inducing inflammation and alteration of angiogenesis, both representing a prerequisite to CVDs. Likewise, alterations in cholesterol levels deform the erythrocyte membrane, allowing accumulation in atherosclerotic plaque or compromising cardiac tissue perfusion.[132] The duration of the follow-up also influences the qualitative and quantitative assessment of RDW values in subjects with CVDs. Longer follow-up periods reduce interpatient heterogeneity,[133] which is always a critical issue to be addressed when a risk of optimized stratification in patients is desirable. As a result, meta-analysis studies confirm that this hypothesis, particularly when CVDs are further categorized by their etiology, is known to differently influence the evolution of these disorders. In fact, patients diagnosed with coronary artery disease (CHD) or dilated cardiomyopathy (DCM) exhibit a decreased survival rate with higher RDW values than patients with cardiac valvular disease (VHD).[133] This implies that multiple criteria included with least routine cardiac biomarkers, etiology of CVDs, and longer follow-ups must be combined with RDW.

Furthermore, we cannot exclude to consider that specific comorbidities, such as diabetes, exacerbate the inflammatory state in patients with CVDs and alter the RDW values among patients.'[121,122,134,135] In particular, the incidence of diabetes has been found to be linked to elevated values of RDW.[136,137] Given that diabetes represents a main cause of endothelial dysfunction, the evaluation of the end-stage renal disease (ESRD) should be combined with the RDW value. Accordingly, high RDW values are reported in patients with ESRD as demonstrated by Pandolfi's group.[138]

So far, a final conclusion on the role of RDW as additional parameter to include in the panel of routine cardiac biomarkers rather than as autonomous biomarker has not yet been reached. This uncertainty will not be clarified until clinicians adopt larger samples in their studies. Furthermore, the physiopathological scenario of CVDs is extremely complex, therefore the singular monitoring of red blood cells cannot represent the most accurate strategy for predicting cardiac disorders. More importantly, the biology and physiology of erythrocytes are influenced by various variables, including age, sex, hormonal factors, exercise, and lifestyle,[139,140] as already reported. For instance, the exogenous or endogenous resistance to EPO (caused by anemia and inflammation),[141] but its activity, is not related to the RDW values.[142] Finally, there is no general consensus on the intrinsic influence of pharmacological treatments on RDW values. Patients with CVDs are normally treated with a multiple drug regimen, ranging from angiotensin-converting enzyme (ACE) inhibitors to beta-blocker diuretics.[140] The modality by which drugs can alter bone-marrow-derived hematopoiesis, reflecting on metabolism and physiology, is yet to be fully explained. To the best of our knowledge, there are few current studies reporting a direct correlation

between drugs and RDW values. However, some meta-analysis studies have shown that some drugs are able to influence the RDW values as demonstrated for digoxin, warfarin, beta-blockers but diuretics.[25,34] Interestingly, statin[143] and antiplatelet therapy[144] are associated with increased levels of RDW, implementing the role of RDW in CVDs. The reason for underlying differences between drugs has never been investigated. Specific pharmacological treatments are likely to exert a more profound impact, as they influence the shape and size of erythrocytes, or alter their lipid content and proteins mediated by calcium signaling. In the next future, it will be essential to understand and establish a defined threshold value of RDW as an indication of mere erythrocyte adaptation in response to drug regimen during follow-up, rather than as a predictor of CVDs.

RDW as a Predictive Biomarker for Vascular Aging and Onset of CVDs?

Current evidence supports a significant association of elevated RDW values with CVDs and their prognosis. Several questions remain open. Thus, in the next future, it would be interesting to find if the RDW could be considered as a predictor of vascular aging (see its description in Box 1 and Box 2 for facilitating the understanding of the concepts stressed in this section) to early diagnose the onset of CVDs. To date, there are no studies on this specific issue. However, interesting observations have been reported in two recent studies (Fig. 2A). The first study was conducted in 2015 by Vrtovec's group.[145] They have investigated the possible

Box 1. Vascular Aging [See References[163–166,168–171]]

The term *vascular aging* indicates the process of gradual remodeling and progressing toward an unhealthy/diseased state, which characterizes the cardiovascular system with advancing age. It is recognized to play a central role in declining health and mortality in older people, and is accompanied by well-defined changes. Specifically, endothelial cells undergo senescence and manifest significant changes in their properties, resulting in impairment of the vascular functionality and neoangiogenic capability. This aging-dependent impairment of endothelial functions (*defined as endothelial dysfunction*) is considered a key factor contributing to vascular dysfunctions, which is responsible not only for the development of cardiovascular diseases (CVDs) but also for several age-related diseases, being the principal component of the stroma of all the tissues and organs, the vascular system included. Several mechanisms have been described to control aging-related endothelial cell senescence, including telomere shorting, microRNAs, mitochondrial dysfunction, DNA instability, DNA damage, and microenvironmental stressors, such as hypoxia. In addition, another mechanism contributing to endothelial dysfunction is the impairment of cardiovascular self-repair system. Accordingly, aging, but also other factors related to the process, affects cardiovascular repair by evocating the development of an imbalance of damage and self-repair, responsible for advancing age of onset and progression of CVDs. Precisely, cardiac or other stem (*i.e.*, CD34 stem cells) and progenitor cells (*i.e.*, endothelial progenitor cells [EPCs]) show impaired functions with age. This determines incapacity of counteracting damage evocated by age-related risk factors. Accumulation of damages consequently is inevitable. Thus, endothelial dysfunction occurs, and determines onset of vascular damage and its complications, including CVDs or other age-related diseases.

Box 2. Cardiovascular Repair: The Role of Endothelial Progenitor Cell and Its Features in Everyone[168–171]

It is important to precise that some individuals, even in the presence of potent risk factors, remain sheltered from consequences of cardiovascular alterations. The potential reason has been attributed to substantial ability of having an efficient cardiovascular self-repair, which appears to be prevalently modulated by genetic background, environmental and epigenetic factors. As a result, the interest in cardiovascular repair is increasing. It has led to evidence that three major processes drive it: (i) replacement (tissue transplant), (ii) rejuvenation or restoration (activation of resident or not stem and progenitor cells via autocrine, paracrine, or endocrine mechanisms; modulation of apoptosis, inflammation, angiogenesis, or metabolism), and (iii) regeneration (progenitor or stem cell engraftment forming differentiated cardiovascular cells). The three different entities may singularly function or be interlinked. However, their mechanisms remain to be determined. Furthermore, in the regeneration, hematopoietic stem and progenitor cells (HSCs and HPCs) seem to have a crucial role. HSCs and HPCs are, indeed, becoming the potential therapy's agents for improving reparatory mechanisms in the heart and vascular system. Many studies have investigated their role in different CVDs, such as acute coronary syndromes, stroke, limb ischemia, and cardiac nonischemic injury. Discordant results have been obtained. Thus, their real contribution is uncertain until now. However, it has been observed that cardiovascular risk factors induce impairment in their circulating levels and function. In contrast, physical exercise and statins mediate their improvement. Of note, it is also their contribution to physiological, endothelial, and cardiac renewal, as observed in healthy subjects. Among the HSCs and HPCs, EPCs are the most widely studied adult human progenitor cell subpopulation up to now. They are maintained within bone marrow (BM) stem cell niches and released upon induced mobilization.

Box 3. CD34 Stem Cells and Progenitors[168–171]

Hematopoietic stem cells (HSCs) expressing the classical CD34 marker (or more immature CD133 marker) are the principal source of endothelial progenitor cells (EPCs). They are maintained within bone marrow (BM) stem cell niches and released upon induced mobilization. This has led to define EPCs as $CD34^+$ or $CD133^+$ cells. HSC contribution to neovascularization has been initially evaluated in animal models, and after in human. The promising results obtained have led to several clinical studies on progenitor cell therapy (see references[168,170]). As mentioned above, the aging process affects their functions, reducing their capacity to produce new blood cells of all the lineages, and increasing the release of cells from myeloid lineage as response to increase of low grade of systemic inflammation with advancing age.

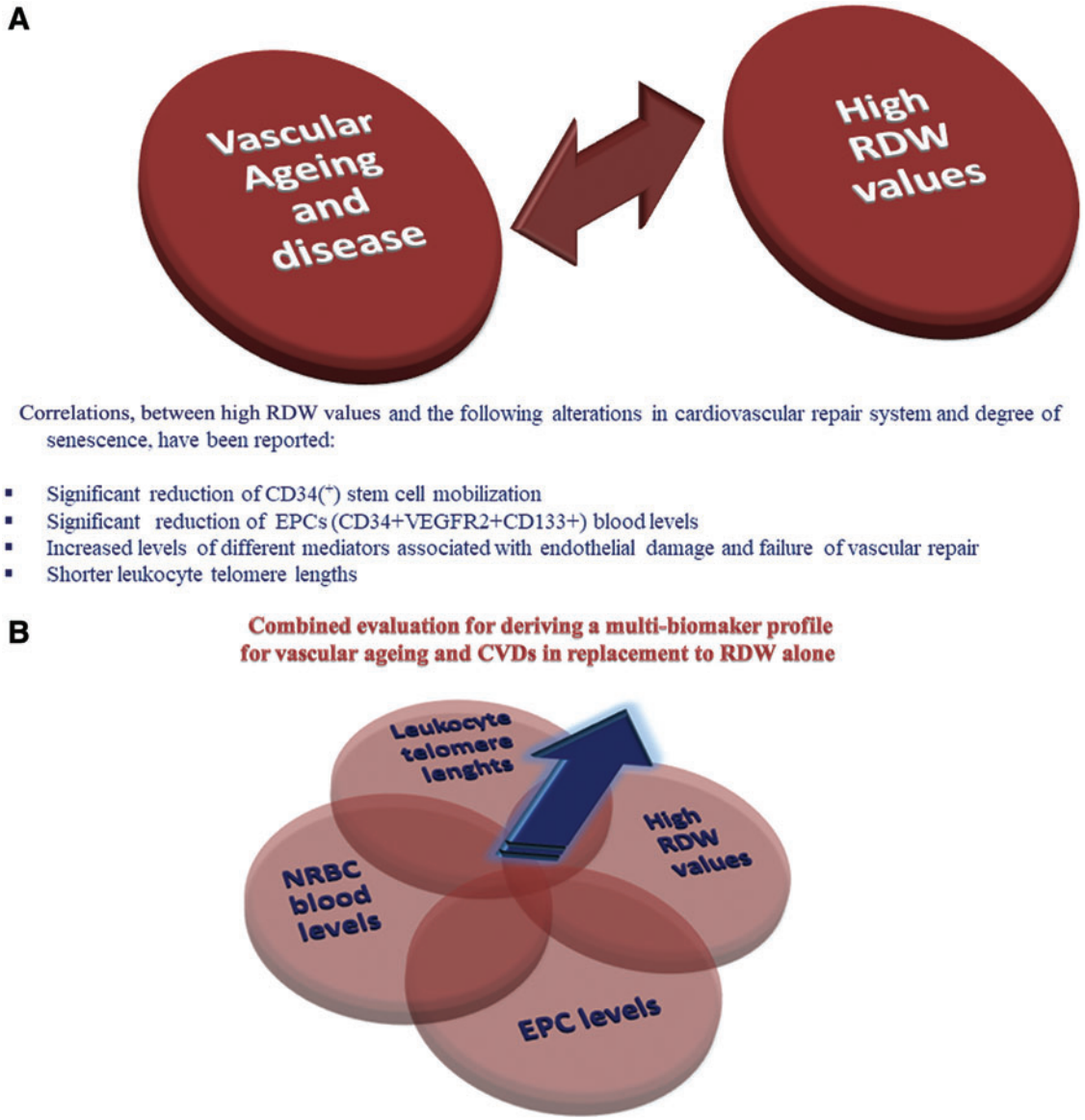

FIG. 2. **(A, B)** High RDW values as predictive biomarker for vascular aging and onset of CVDs? **(A)** To support the potential role of RDW as predictive biomarker of vascular aging and CVD onset, there is an established evidence. It demonstrates correlations between high RDW values and the specific alterations in cardiovascular repair system and degree of senescence. Specifically, they include reduction of $CD34^+$ cell mobilization, reduced EPCs ($CD34^+VEGFR2^+CD133^+$) blood levels, increased levels of different mediators associated with endothelial damage and vascular repair failure, and shorter leukocyte telomere lengths. **(B)** Our recommendation: combined evaluation of RDW values with leukocyte telomere length and circulating levels of nRBCs and EPCs in the hope to derive a potentially novel multibiomarker profile for the future development of an algorithm for vascular aging and CVDs. CVD, cardiovascular disease; EPC, endothelial progenitor cell; nRBC, nucleated red blood cell. Color images available online at www.liebertpub.com/rej

reduction of CD34 (+) stem cell mobilization in patients with advanced chronic HF, which was significantly decreased in 32% of the 44 patients enrolled. Furthermore, multivariate analysis has shown that RDW was an independent predictor of CD34 (+) stem cell mobilization (Box 3) decrease, suggesting its potential role as a predictive biomarker.[145] However, these results were obtained with a small sample size, therefore it is required to include additional sample size sets to confirm the results. The second study was conducted by Rodriguez-Carrio et al.,[146] considering the relevance of chronic inflammation and the reduction of EPC levels according to the onset of CVDs.[139,140,147–149] In fact, EPCs are significantly associated with endothelial damage and vascular repair failure, a hallmark of vascular aging and remodeling during the onset of several CVDs. In this study, Rodriguez-Carrio et al. optimized their study model by screening 194 patients with rheumatoid arthritis (RA), in which the endothelial damage and vascular repair failure parallelly occur, but also exacerbated by advanced state of RA severity and related to CVD onset. Their analysis showed that patients with RA showed elevated values of RDW, which were significantly correlated with both

reduced percentage of EPCs and increased levels of different mediators associated with endothelial damage and vascular repair failure (Fig. 2A). Specifically, the findings obtained evidenced that RDW was independently associated with an EPC depletion in the whole group (β [95% CI]: −3.537 [−6.162 to −0.911], $p = 0.009$) after adjusting for clinical parameters, disease duration, treatments, and traditional CVD risk factors.

This small evidence leads to a new vision of RDW as a predictor of vascular aging and onset of CVDs, although further studies are needed.

Other Emerging Parameters: Leukocyte Telomere Length and nRBCs for Vascular Aging and CVDs

It is well recognized that *telomere (the TTAGGG DNA repeats at the ends of chromosomes)* length shortening is a typical feature of cellular aging and its specific biomarker.[150] Telomere length is the result of the endogenous activity of the telomerase enzyme in stem or progenitor cells as well as of the biological effects of exogenous oxidative or inflammatory stressors, which can accelerate the shortening of telomeres.[150] Interestingly, centenarians and their offspring show longer telomere lengths than controls. This seems to be the result of higher education levels and a reduced cognitive decline with age.[151,152] In contrast, an increased evidence reports a significant association of short telomere lengths of leukocytes with several age-related diseases,[153] such as CVDs. Thus, the content of telomere in blood circulating leukocytes of an individual accurately reflects the biological age of the vascular wall, as suggested in our studies.[154,155]This could lead to the hypothesis to integrate telomere lengths of leukocytes and RDW, extending the panel of predictive biomarkers for vascular age-dependent CVDs. As a result, some studies have examined the relationship between telomere length and RBC parameters showing interesting data. Among these, results by Kozlitina and Garcia[51] performed in the large multiethnic population (=3.302 participants, 18–85 years of age) of the Dallas Heart Study 2 may be considered particularly relevant. They demonstrated a marginal association of shorter telomere lengths with lower red blood cell counts and a strict significant association of shorter telomere lengths with higher average red cell sizes (measured by MCV), increased RDW values, higher hemoglobin levels, and lower platelet count (Fig. 2A). Similar results have been obtained by Mazidi et al.,[156] in 2017, performing a study in a sample of 8892 healthy US adults. In particular, they determined the relationship between length of leukocyte telomere and CBC parameters, RDW included, in both sexes. The linear regression adjusted for age, race, gender, and BMI demonstrated that there was a significant negative relationship of length of leukocyte telomere with RDW ($\beta = -0.031$, 95% CI: −0.054 to −0.003), and monocyte count ($\beta = -0.051$, 95% CI: −0.422 to −0.142), mean cell hemoglobin ($\beta = -0.051$, 95% CI: −0.038 to −0.011) and while there was a significant positive relationship with basophil ratio ($\beta = 0.046$, 95% CI: 0.049–0.171). Thus, they concluded with the suggestion that telomere attrition may be a marker for reduced proliferative reserve in hematopoietic progenitor cells.[156] These promising results encourage further investigations to validate the usefulness of combining the RDW and the shortening of telomere lengths of leukocytes as predictive biomarkers of vascular age-dependent CVDs. However, some questions remain opened in using leukocyte telomere length as a valid biomarker, including differences observed among study populations, measurement methods, and statistical modeling, as well as changes with age and high interindividual variability, linked to basic biology and effective biological age of leukocytes examined in and among individual studies.[157–159] Consistent with these observations, it is necessary to point that the use of blood samples is only valid if telomere length estimated in peripheral leukocytes is the appropriate measure for the phenotype investigated. Whether telomere length measured in peripheral leukocytes is a surrogate marker for other tissues requires more investigation.[157–159]

Recently, the nRBCs are emerging as additional RBC parameters whose association has been found with various diseases, such as cancer, congestive HF, acute and chronic anemia, and other hematological disorders.[160,161] In healthy adults, nRBCs are absent in the peripheral blood, but they can be detectable at the onset of several diseases. The mechanisms involved in such process are not clear. However, clinical conditions such as hypoxemia or infection, which are known to decrease tissue oxidation and to increase concentrations of EPO, IL-3, and IL-6, respectively, appear to be the cause.[160] Furthermore, the presence of nRBC in a chronic pathology is usually associated with an unfavorable prognosis and precisely with an increased risk of mortality, probably due to exacerbated hypoxic and inflammatory lesions. It has also been suggested that the prognostic involvement of nRBCs is independent of other clinical and laboratory risk parameters. This leads to speculation about the introduction of nRBCs as a powerful biomarker of acute physiology and chronic health assessment systems (APACHE), such as APACHE II,[162] and the possibility of improving them. Furthermore, the assessment of nRBC in the blood represents a relatively early event before serious clinical outcome. Thus, the screening for nRBC could help identify high-risk patients in an early phase of the disease. Further studies are needed to clarify whether evaluation of nRBC levels could help improve the management of patients, and to introduce this parameter as decisive in clinical severity assessment guidelines and systems.

Recommendations: Combining RDW Values, Leukocyte Telomere Length, and Circulating Levels of nRBCs and EPCs

In this review, we have highlighted that RDW cannot be used as a definite prognostic indicator for vascular aging and CVDs, encouraging the association with other biomarkers and coherently with the complexity of the physiopathology of vascular aging and CVDs. Several risk factors, different molecular and cellular mechanisms, and a large number of pathways drive vascular aging and diseases.[47,163–166] Thus, singular monitoring of red blood cells cannot represent the most accurate strategy for predicting cardiac disorders. Consequently, the identification of appropriate biomarkers remains an important field of research, even if their detection is improved over the past 30 years,[167] facilitating more specific approaches of screening, management, and outcome. Despite the vertiginous increase of biomarkers of

different molecular nature (*i.e.*, genetic, epigenetic, protein, lipoprotein, receptor, etc.) and of different clinical significance (*i.e.*, predictive, diagnostic, prognostic, risk and association, therapeutic, etc.), there are several concerns about their real utility and impact on CVDs and their appropriateness in mirroring a specific clinical phase. For this reason, the development of multibiomarker profiles and algorithms is becoming the main objective of research for both vascular aging and CVDs. In contrast, there is a common consensus among the diverse international CVD guidelines to employ a precise panel of biomarkers and algorithms for specific CVDs to facilitate measures and patient's management, outcome, and therapy, therefore decreasing the economic burden for the National Health Systems.

Here, we propose to combine the evaluation of RDW values with leukocyte telomere length and circulating levels of nRBCs and EPCs, attempting to obtain a potentially novel multibiomarker profile for the future development of an algorithm for vascular aging and CVDs (Fig. 2B). Our approach certainly requires additional and future studies, which, in parallel, quantify the four parameters proposed and determine their associations with vascular aging and CVDs, using apposite statistical appraisals. Certainly, meta-analysis or other secondary (*i.e.*, data mining) analyses, and large cohorts of patients and controls, will be mandatory. Unfortunately, the number of current literature studies, which executed this kind of evaluation, is very limited for assessing these statistical evaluations. Specifically, there are only two studies (*i.e.*, Kozlitina and Garcia, and Mazidi et al.,[51,156] as mentioned in Other Emerging Parameters: Leukocyte Telomere Length and nRBC for Vascular Aging and CVDs section), which cotemporally analyzed leukocyte telomere length and RDW values, and precisely using as participants healthy subjects, and one from Rodriguez-Carrio et al.,[146] which investigated the association of RDW with EPC cells (see RDW as a Predictive Biomarker for Vascular Aging and Onset of CVDs? section). They reported promising results, as described above. In addition, there are not any investigations on the relationship of RDW with nRBCs, or with other parameters suggested. Nevertheless, to support our recommendations there are the results of a cross-sectional study that we have recently performed in 80 individuals affected by ascending aorta aneurysm (AAA) and 70 control patients, where RDW, leukocyte telomere length, and EPC levels have been measured in parallel to verify their association with the AAA risk. The data obtained, by using univariate and multivariate regression analyses, have shown that the three examined parameters are independently associated with the AAA risk (data not shown). However, the study is still ongoing, and the date obtained are partial. We think progressively to test all the four parameters, even if the major limitations are the costs of the investigations, time, and the amount of blood required for these assessments. It should be also interesting to evaluate the epigenetic factors stressed in the Wallace review.[77]

Thus, future studies are necessary, such as randomized studies and multicenter studies with homogeneous populations, to eliminate confounding factors, which could invalidate the data obtained. Furthermore, for the development of algorithms it will be required to stratify the risk among several classes (*i.e.*, low-, medium-, and high-risk classes), to optimize the therapeutic and lifestyle measures to correct the cardiovascular risk.

Concluding Remarks

In this review, we analyzed the role of RDW value in vascular aging and CVD context, highlighting more limitations than useful benefits. In line with this, we have suggested that RDW should be used in combination with more specific cardiovascular clinical parameters, to obtain a multibiomarker profile, which might be more appropriate. Certainly, additional and more extensive studies are mandatory to validate and confirm our suggestion.

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Authors' Contribution

Dr. Balistreri was involved in conception and study design. Drs Poz, Balistreri, and De Falco were involved in drafting the article. Dr. Pisano was only involved in summarizing the evidence about RDW in CVDs in Table 1. Prof. Ferdinandy and Drs. Balistreri, De Falco, and Madonna contributed to the critical revision of the text of article. Dr. Balistreri gave the final approval of the version to be published. All authors participated in the study, and they read and approved the final article.

Author Disclosure Statement

PF is the founder and CEO of Pharmahungary, a Group of R&D companies. The other authors have no conflicts of interest to disclose.

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Address correspondence to:
Carmela Rita Balistreri
Department of Pathobiology and Medical and Forensic Biotechnologies
University of Palermo
Corso Tukory, 211
Palermo 90134
Italy

E-mail: carmelarita.balistreri@unipa.it