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Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases

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Running Title: the limits of RDW as predictor of morbidity and mortality for CVD: our critical vision

The authors declare to have no conflict interests.

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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, haematological 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 ageing and disease. In addition, we also highlight the inevitable impact of biases in clinical application of RDW related to CVDs.

Based on our revision 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 (nRBC) and endothelial progenitor cells (EPCs). Promising data deriving from additional future studies could permit both to propose them as a multibiomarker profile and create an appropriate algorithm, which can facilitate the diagnosis and to predict the prognosis of different CVDs based on vascular aging.

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

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1.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) (see Fig. 1A). Thus, its values increase according to the heterogeneity in red cell size. RDW values are usually reported on CBC reports, in order 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 inter-subjectivity 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 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) x 100*), (see 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 suitable parameter for hematological diseases, and particularly for anemia, since its values reflect anisocytosis. Accordingly, in 1983 Bessman and collaborators proposed a classification of anemia based on both the MCV and RDW parameters [8]. Specifically, they considered heterogeneous and homogenous forms of anemia associated with increased (e.g. in iron or cobalamin and folic acid deficiency) and normal RDW values (e.g. heterozygote thalassemia, hypo-proliferative 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 also 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

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are expressed in about 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 non-hematological 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) [15] or atrial fibrillation and stroke, chronic obstructive pulmonary disease (COPD) and community-acquired pneumonia (CAP); 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 also emerging (see paragraph 5). For example, Abrahan and coworkers, 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 to 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 firstly describing and discussing various endogenous and exogenous factors (i.e. age, sex, genetic background, inflammation, hormones, drugs, diet, exercise, haematological analyzers, and ranges of values) and related mechanisms, which have been demonstrated to modulate the RDW values and to impact its relevance. Accordingly, we raise doubts about its association with CVDs. Specifically, we underline, whether higher RDW values may really reflect both alterations in the cardiovascular

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system (i.e. regenerative capacity, endothelial turnover, and senescence of cardiovascular cells), as expression of vascular ageing 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 cell (nRBC) 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 into a multi-biomarker profile, and facilitate the development of a diagnostic/prognostic algorithm for vascular ageing and CVDs.

2. Endogenous factors affecting RDW values

A large array of endogenous factors affects the values of RDW in several pathologies (see 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, firstly including erythropoietin (EPO). EPO plays a crucial role in production, maturation, and survival of red blood cells [20, 21]. Accordingly, Kario and coworkers observed increased RDW values in EPO deficiency or hypo-responsiveness, characterized by a positive correlation between RDW values and EPO titers [22]. Afsar and colleagues recently confirmed this data, by investigating patients affected by EPO-hypo-responsiveness [23].

Aging is another established determinant in RDW elevation. The first evidence, although not clearly justified, has been provided by Chang and coworkers in the Third National Health and Nutrition Examination Survey 1998-1994 (NHANES III). In this broad survey, data from about 25000 nationally US adults, stratified by age, sex, and race were collected to obtain complete blood count reference interval diagrams [24]. Afterward, Patel's group carried on 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 (less than 12.60%, 12.60% to 12.95%, 13.00% to 13.40%, 13.45% to 14.05%, and greater than 14.05% respectively). They found a progressive significant increment of age in RDW quintiles 1 through 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

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RDW quartiles were significantly older than those in the lowest quartiles (with p<0.001 for trend) [25-28]. More recently, Lippi and coworkers carried out a retrospective analysis in order 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 younger than 60 years old, 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 up to 59% or more in people aged 80 years or older. In subjects aged less than 41 years, this percentage was about 6%, therefore, confirming the strict dependence of RDW from age. Moreover, older subjects exhibited a wider range of RDW values. Univariate and multiple regression analysis have confirmed similar results [29]. Based on Lippi's group observations, Alis and coworkers 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 and coworkers investigated 8089 subjects in a cohort study, achieving the same results from Lippi's and Alis' group**s** [32**]**. Thus, it is possible to affirm that there is a strong relationship between increased age and RDW elevation regardless the analyzer used to measure RDW[31].

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 an higher median RDW value compared to 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 non-relationship between RDW and gender [16, 24, 26-28] as also reported by Hoffmann and

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coworkers. They found no significant gender-related differences in RDW-SD, with exception for the 56-70 years age class (p value 0= 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 above described.

Another factor impacting RDW values is represented by ethnicity, although very low is known in 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 sub-analysis 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 shows 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 and colleagues collected data from seven 2-years cycles of NHANES and published an interesting paper. In the 1999-2012 period, they collected data from 34,171 adults with age ≥20years (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 erythropoietin-induced anisocytosis may be involved.

Of note also is the increase of RDW values with obesity. In particular, Fujita and co-workers [40] have reported that RDW significantly raises in overweight adolescents (13.39 ± 0.10, P = 0.015) compared to normal-weight adolescents (13.07 ± 0.09) and), whereas erythrocyte counts and haematocrit 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, Vayà and co-workers [41] reported similar results, examining obese

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patients before bariatric surgery (n=142) and normo-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 and colleagues [42] on a cohort of 3,529 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 and co-workers [44] to investigate on 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 the 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 (GRS), highlighted in subjects with genetically increased risk for 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

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either subjects with genetically lower and higher 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 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 and co-workers also reported that genetic variants associated with RDW were linked to specific molecular pathways, including iron homeostasis, several nucleosome 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 [51, see below], whereas Pilling's group did not confirm this association [44].

The discrepancy of the data, in the several studies performed, could be justified by the fact that the biological effects mediated by genetic variants, and in the specific case, by the genetic variants associated with RDW, can occur/ or cannot, because controlled by the fine interplay between environmental and epigenetic factors. In fact, gene 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 of follow reported.

3. 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 haematopoiesis, eventually leading to an altered number and phenotype of macrophages, and to a strong activation of neuro-immune and immune-

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metabolic axes. Based on this evidence, it likely is that life habits could also modulate indirectly RDW values. In 2013 Emans and co-workers, by examining 17,533 European adults, demonstrated that RDW values were associated with physical inactivity, without affecting the relationship between RDW and heart failure [57]. Afterward, other independent groups showed that resistance training was favourably 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 U.S. 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 North-eastern Iran. Interestingly, they evidenced that RDW remained significant ($p<0.001$) after multivariate analysis in cigarette smokers than non-smokers and between hookah smokers and non-smokers ($p<0.05$).

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, included RBCs [8]. For example, capecitabine, a fluoropyrimidine carbamate selectively activated after oral administration to 5-fluorouracil (5-FU), exerts its cytotoxic activity determining a defective DNA synthesis through the inhibition of thymidilate synthase (TS). An increase in MCV (without concomitant anemia or vitamin B12/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 via metabolic interferences, could also cause a raise anysocytosis, and consequently increase RDW values. As widely reviewed by Pham and coworkers [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.

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4. The potential mechanisms related to the relationship between RDW increased values and endogenous and exogenous factors

Elevated levels of RDW have been associated with a wide range of endogenous and exogenous factors (as abovementioned), but the mechanisms and the related pathways involved remain to be fully elucidated. Systemic inflammation has been suggested as the 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, who were free of heart failure 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 percent increase in RDW, 1.14; 95% confidence interval, 1.05 to 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, firstly 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 been also 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, about low serum antioxidant concentration and the inverse correlation with RDW, corroborates these claims [69]. Interestingly, it has been also demonstrated that in Fanconi Anemia (FA), a condition known to exhibit a

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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.

4.1 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 during 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 non island regions, were up-regulated, and demethylation of their gene bodies was

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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 additionally supports the role of inflammation in affecting the homeostasis of production of red blood cells and their parameters.

So far, there are not 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 and co-workers [55] and Wallace and co-workers [77] have strongly suggested to consider the epigenetic signature as an additional innovative and predictive biomarker for the management/outcome and diagnosis of CVDs.

5. RDW: a valid biomarker for CVDs?

In recent years, many acute and chronic CVDs, including acute coronary syndrome (ACS), ischemic cerebrovascular disease, peripheral artery disease (PAD), heart failure (HF), atrial fibrillation (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 and co-workers [109] investigated the possible association between high levels of RDW and the mortality, and the cardiovascular risk for 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 non-positive prognosis compared to those with low RDW values [111]. In 2016, Luo and colleagues evidenced in another meta-analysis including 32 studies, the potential association between high RDW values and high mortality in non-cardiovascular 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 (above described) that could affect RDW values. In addition, further investigations are needed to find out the

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potential mechanisms involved, in order 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 to other biomarkers conventionally used in the diagnosis and prognosis of CVDs. Firstly, RDW values provide useful information on poor CVD prognosis, even if the presence of anisocytosis may be considered a non-specific biomarker. Secondly, high RDW values in patients with a first overt of cardiovascular event can indicate timely treatments. In fact, Lippi has underlined 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 abovementioned.

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

5.1 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 non-cardiac disorders, particularly in the haematology field. As a consequence, the prognostic values of RDW in the CVDs have been well established. However, there is currently no a real clinical application and implementation, for several reasons. Firstly, 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]. On the other hand, some meta-analyses revealed that a unfavourable prognosis in subjects with HF, ACS, ischemic cerebrovascular disease, PAD, HF, AF, hypertrophy or after cardiac valvular surgery is

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parallel to elevated values of RDW [111, 114-116], which in turn correlates to canonical cardiac biomarkers, including mid-regional pro-adrenomedullin (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 to the type of CVDs 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 etio-pathology 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, which can influence the cardiovascular status of a subject. Furthermore, the major number of the mechanisms ascribable to the increase in RDW values is also associated with CVDs. Accordingly, chronic anaemia causes a direct modification of the size of the erythrocytes, with consequent increase of the RDW values and compromised hemodynamic compensatory response. In fact, anaemia is a biomarker of HF and left ventricular hypertrophy, where the cardiac tissue is stressed by the overload of blood in order 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

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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 haemoglobin 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 haemodynamic 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 anaemia state [129] and consequently haemoglobin concentration [18]. However, a recent study has reported that the RDW/haemoglobin ratio [127] and the blood concentration [130, 131], whose decrease is concomitant with the high RDW values during hospitalization [117], would allow to predict the outcome in a dose-independent dependence. This suggests that many other parameters should be employed to normalize the RDW values, in order to improve their prognostic impact. This

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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, known to significantly influence the prognostic features of HF [114].Accordingly, here, we propose an alternative multi-biomarker profile, as extensively described below.

Other important clinical biases are related either to the nutritional status of patients and level of free cholesterol. Vitamin D3 deficiency causes serious damage to haematopoiesis, inducing inflammation and alteration of angiogenesis, both representing a prerequisite to CVDs. Likewise, alterations in cholesterol level 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 inter-patient 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 this hypothesis, particularly when CVDs are further categorized by their aetiology, 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, aetiology of CVDs and longer follow-ups, must be combined with RDW.

Furthermore, we cannot exclude to consider that specific co-morbidities, such as diabetes, exacerbate the inflammatory state in patients with CVDs and alter the RDW values among patients [134,135, 121, 122]. 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 with endothelial dysfunction , the evaluation of the end-stage renal disease (ESRD) should be combined with the RDW value. Accordingly, high RDW values 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 will adopt larger samples in their studies. Furthermore, the physio-pathological scenario of CVDs is

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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 life style [139, 140] as already reported. For instance, the exogenous or endogenous resistance to erythropoietin (caused by anaemia 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 haematopoiesis, 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 values, implementing the role of RDW in CVDs. The reason of underlying differences between drugs has never been investigated. Specific pharmacological treatment 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 signalling. 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.

6. 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 understand if the RDW could be considered as a predictor of vascular aging (see its description in the Box-1 and Box-3 for facilitating the understanding of the concepts stressed in this section) in order 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 the

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Vrtovec's group [145]. They have investigated the possible 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 (see Box-2) decrease, suggesting its potential role as a predictor biomarker [145]. However, these results were performed in a small sample size, therefore requiring to include additional sample size sets in order to confirm the results. The second study was conducted by Rodriguez-Carrio and collaborators [146], considering the relevance of chronic inflammation and the reduction of endothelial progenitor cells (EPCs) levels according to the onset of CVDs [139,140 147-149]. In fact, EPCs are significantly associated with endothelial damage and vascular repair failure, an hallmark of vascular aging and remodelling during the onset of several CVDs. In this study, Rodriguez-Carrio and colleagues optimized their study model, by screening 194 patients with rheumatoid arthritis (RA), in which the endothelial damage and vascular repair failure parallel 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 to 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, -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.

7. Other emerging parameters: leukocyte telomere length and nucleated red blood cells (NRBC) 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

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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 multi-ethnic 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 telomeres lengths with higher average red cell sizes (measured by MCV), increased RDW values, higher haemoglobin levels, and lower platelet count (Fig 2A). Similar results have been obtained by Mazidi and co-workers [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 complete blood count parameters, RDW included, in both sexes. The linear regression adjusted for age, race, gender and body mass index, demonstrated that there was a significant negative relationship of length of leukocyte telomere with RDW (β = -0.031, 95% CI: -0.054; -0.003), and monocyte count (β = -0.051, 95% CI: -0.422; -0.142), mean cell hemoglobin (β = -0.051, 95% CI: -0.038; -0.011) and while there was a significant positive relationship with basophil ratio (β = 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 modelling, as well as changes with age and high

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inter-individual variability, linked to basic biology and effective biological age of leukocytes examined in and among individuals studies [157-159]. Consistent with these observations, it 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 nucleated red blood cells (nRBC) is emerging as additional RBC parameter, whose association has been found with various diseases, such as cancer, congestive HF, acute and chronic anaemia and other haematological disorders [160, 161]. In healthy adults, nRBC are absent in the peripheral blood, but they can be detectable at 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 erythropoietin, 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 unfavourable prognosis and precisely to an increased risk for 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 to 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.

8. Our proposal: 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 the definite prognostic indicator for vascular aging and CVDs, encouraging the association with other

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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 biomarkers) and of different clinical significance (i.e. predictive, diagnostic, prognostic, risk and association, therapeutic, etc, biomarkers), there are several concerns about their real utility and impact in CVDs and their appropriateness in mirroring a specific clinical phase. For this reason, the development of multi-biomarker profiles and algorithms is becoming the main objective of research for both vascular ageing and CVDs. On the other hand, there is a common consensus among the diverse international CVD guidelines to employ a precise panel of biomarkers and algorithms for specific CVDs in order to facilitate measures and patient's management, outcome and therapy and 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 multi-biomarker profile for the future development of an algorithm for vascular ageing and CVDs (see Fig. 2B). Our approach certainly requires additional and future studies, which, in parallel, quantify the four parameters proposed and determine their associations with vascular ageing 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 and co-workers studies [51, 156], as mentioned in paragraph 7), which co-temporally analysed leukocyte telomere length and RDW values, and precisely using as participants healthy subjects, and one from Rodríguez-Carrio and co-workers[146], which investigated the

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association of RDW with EPC cells (see paragraph 6). They reported promising results, as above described. In addition, no studies there are about the relationship of RDW and nRBCs, as well as investigations on all the four parameters, which can consent to examine the weight of RDW than other biomarkers for vascular ageing and CVDs, and if it is much higher or not. Nevertheless, to support of our proposal 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 in order to verify their association with the AAA risk. The data obtained, by using an univariate and multivariate regression analysis, have shown that the three examined parameters are independently associated with the AAA risk (data not shown) [168]. However, the study is still ongoing and the date 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, multicenter studies with a very homogenous populations in order to eliminate confounding factors, which could invalidate the data obtained. Furthermore, for the development of algorithms will be required to stratify the risk among several classes (i.e. low, medium and high risk classes), in order to optimize the therapeutic and lifestyle measures to correct the cardiovascular risk.

9. Concluding remarks

In this review we analysed 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, in order to obtain a multi-biomarker profile, which might be more appropriate. Certainly, additional and more extensive studies are mandatory to validate and confirm our suggestion

Conflict of interest

The authors have no conflicts of interest to disclose.

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Author's contribution

Dr Balistreri was involved in conception and study design. Drs Poz, Balistreri and De Falco were involved in drafting the manuscript. 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 in the critical revision of the text of manuscript. 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 manuscript

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This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

References

[1] Price-Jones C. Red blood cell diameters. London : Humphrey Milford, Oxford University Press; 1933. p. 102.

[2] C. Briggs BB. Basic Hematological Techniques. In: Elsevier, editor. Dacie and LEwis Practical Hematology. 11th ed: Churchill Livingstone Edinburgh; 2012.

[3] R. McPherson MP. Henry's clinical diagnosis and mangment by laboratory methods. In: Saunder, editor. 22nd ed. Philadelphia: Elsevier; 2011. p. 1568.

[4] C.E. McLAREN BH, J.A. KOEPKE,R.M. ROWAN, P.J. McKAY, B.R. ORTNER, and M.L. BISHOP. Analysis of red blood cell volume distributions using the ICSH reference method: detection of sequential changes in distributions determined by hydrodynamic focusing. Clinical & Laboratory Haematology. 1993;15:12.

[5] ICSH recommendations for the analysis of red cell, white cell and platelet size distribution curves. Methods for fitting a single reference distribution and assessing its goodness of fit. International Committee for Standardization in Haematology. ICSH Expert Panel on Cytometry. Clinical and laboratory haematology. 1990;12:417-31.

[6] Buttarello M, Plebani M. Automated blood cell counts: state of the art. American journal of clinical pathology. 2008;130:104-16.

[7] Lippi G, Pavesi F, Bardi M, Pipitone S. Lack of harmonization of red blood cell distribution width (RDW). Evaluation of four hematological analyzers. Clinical biochemistry. 2014;47:1100-3.

[8] Bessman JD, Gilmer PR, Jr., Gardner FH. Improved classification of anemias by MCV and RDW. American journal of clinical pathology. 1983;80:322-6.

[9] Flynn MM, Reppun TS, Bhagavan NV. Limitations of red blood cell distribution width (RDW) in evaluation of microcytosis. American journal of clinical pathology. 1986;85:445-9.

[10] Bessman JD, Gilmer PR, Jr., Gardner FH. Too early to put down RDW for discriminating iron deficiency and thalassemia. American journal of clinical pathology. 1986;86:693-5.

[11] Cappelletti P. Linee guida per il referto ematologico-GdSE-SIMEL. Riv Med Lab-JLM. 2002;3:7.

[12] Sahli CA, Bibi A, Ouali F, et al. Red cell indices: differentiation between beta-thalassemia trait and iron deficiency anemia and application to sickle cell disease and sickle cell thalassemia. Clinical chemistry and laboratory medicine. 2013;51:1595-603

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[13] Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relation between red blood cell distribution width and inflammatory biomarkers in a large cohort of unselected outpatients. Archives of pathology & laboratory medicine. 2009;133:628-32.

[14] Fukuta H, Ohte N, Mukai S, et al. Elevated plasma levels of B-type natriuretic Peptide but not C-reactive protein are associated with higher red cell distribution width in patients with coronary artery disease. International heart journal. 2009;50:301-12.

[15] Felker GM, Allen LA, Pocock SJ, et al. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. Journal of the American College of Cardiology. 2007;50:40-7.

[16]Abrahan LL 4th, Ramos JDA, Cunanan EL, Tiongson MDA, Punzalan FER. Red Cell Distribution Width and Mortality in Patients With Acute Coronary Syndrome: A Meta-Analysis on Prognosis. Cardiol Res. 2018;9:144-152

[17] Perlstein TS, Weuve J, Pfeffer MA, Beckman JA. Red blood cell distribution width and mortality risk in a community-based prospective cohort. Archives of internal medicine. 2009;169:588-94.

[17] Patel KV, Ferrucci L, Ershler WB, Longo DL, Guralnik JM. Red blood cell distribution width and the risk of death in middle-aged and older adults. Archives of internal medicine. 2009;169:515-23.

[18] Allen LA, Felker GM, Mehra MR, et al. Validation and potential mechanisms of red cell distribution width as a prognostic marker in heart failure. Journal of cardiac failure. 2010;16:230-8.

[19] Makhoul BF, Khourieh A, Kaplan M, Bahouth F, Aronson D, Azzam ZS. Relation between changes in red cell distribution width and clinical outcomes in acute decompensated heart failure. International journal of cardiology. 2013;167:1412-6.

[20] Fried W. Erythropoietin and erythropoiesis. Experimental hematology. 2009;37:1007-15.

[21] Lippi G, Franchini M, Salvagno GL, Guidi GC. Biochemistry, physiology, and complications of blood doping: facts and speculation. Critical reviews in clinical laboratory sciences. 2006;43:349-91.

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[22] Kario K, Matsuo T, Nakao K, Yamaguchi N. The correlation between red cell distribution width and serum erythropoietin titres. Clinical and laboratory haematology. 1991;13:222-3.

[23] Afsar B, Saglam M, Yuceturk C, Agca E. The relationship between red cell distribution width with erythropoietin resistance in iron replete hemodialysis patients. European journal of internal medicine. 2013;24:e25-9.

[24] Cheng CK, Chan J, Cembrowski GS, van Assendelft OW. Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. Laboratory hematology : official publication of the International Society for Laboratory Hematology. 2004;10:42-53.

[25] Alattar FT, Imran NB, Patel P, Usmani S, Shamoon FE. Red cell distribution width (RDW) correlates with markers of diastolic dysfunction in patients with impaired left ventricular systolic function. International journal of cardiology Heart & vasculature. 2016;10:13-6.

[26] Chen PC, Sung FC, Chien KL, Hsu HC, Su TC, Lee YT. Red blood cell distribution width and risk of cardiovascular events and mortality in a community cohort in Taiwan. American journal of epidemiology. 2010;171:214-20.

[27] Qiao R, Yang S, Yao B, Wang H, Zhang J, Shang H. Complete blood count reference intervals and age- and sex-related trends of North China Han population. Clinical chemistry and laboratory medicine. 2014;52:1025-32.

[28] Borne Y, Smith JG, Melander O, Engstrom G. Red cell distribution width in relation to incidence of coronary events and case fatality rates: a population-based cohort study. Heart. 2014;100:1119-24.

[29] Lippi G, Salvagno GL, Guidi GC. Red blood cell distribution width is significantly associated with aging and gender. Clinical chemistry and laboratory medicine. 2014;52:e197-9.

[30] Alis R, Fuster O, Rivera L, Romagnoli M, Vaya A. Influence of age and gender on red blood cell distribution width. Clinical chemistry and laboratory medicine. 2015;53:e25-8.

[31] Hoffmann JJ, van den Broek NM, Curvers J. Reference intervals of extended erythrocyte and reticulocyte parameters. Clinical chemistry and laboratory medicine. 2012;50:941-8.

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[32] Hoffmann JJ, Nabbe KC, van den Broek NM. Effect of age and gender on reference intervals of red blood cell distribution width (RDW) and mean red cell volume (MCV). Clinical chemistry and laboratory medicine. 2015;53:2015-9.

[33] Codd V, Nelson CP, Albrecht E, et al. Identification of seven loci affecting mean telomere length and their association with disease. Nature genetics. 2013;45:422-7, 7e1-2.

[34] Xi Q, Liu Z, Zhao Z, Luo Q. Red blood cell distribution width predicts responsiveness of acute pulmonary vasodilator testing in patients with idiopathic pulmonary arterial hypertension. Clinica chimica acta; international journal of clinical chemistry. 2015;446:272-6.

[35] Saxena S, Wong ET. Heterogeneity of common hematologic parameters among racial, ethnic, and gender subgroups. Archives of pathology & laboratory medicine. 1990;114:715-9.

[36] Zalawadiya SK, Veeranna V, Panaich SS, Afonso L, Ghali JK. Gender and ethnic differences in red cell distribution width and its association with mortality among low risk healthy United state adults. The American journal of cardiology. 2012;109:1664-70.

[37] Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. Jama. 1997;277:973-6.

[38] Khera A, McGuire DK, Murphy SA, et al. Race and gender differences in C-reactive protein levels. Journal of the American College of Cardiology. 2005;46:464-9.

39] Loprinzi PD, Loenneke JP, Ahmed HM, Blaha MJ. Sex and Race-Ethnicity Secular Trends in Mean and Elevated Red Blood Cell Distribution Width Among Adults in the United States, 1999-2012. Ethnicity & disease. 2016;26:45-50.

[40] Fujita B, Strodthoff D, Fritzenwanger M, et al., Altered red blood cell distribution width in overweight
adolescents and its association with markers of inflammation. Pediatr Obes. 2013;8:385-91

[41] Vayá A, Alis R, Hernandez-Mijares A, et al. Red blood cell distribution width is not related with inflammatory parameters in morbidly obese patients. Clin Biochem. 2014 Apr;47(6):464-6.

[42]Laufer Perl M, Havakuk O, Finkelstein A, et al. High red blood cell distribution width is associated with the metabolic syndrome. Clin Hemorheol Microcirc. 2015;63(1):35-43

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[43]Furuncuoğlu Y, Tulgar S, Dogan AN, Cakar S, Tulgar YK, Cakiroglu B. How obesity affects the neutrophil/lymphocyte and platelet/lymphocyte ratio, systemic immune-inflammatory index and platelet indices: a retrospective study. Eur Rev Med Pharmacol Sci. 2016;20(7):1300-6.

[44] Pilling LC, Atkins JL, Duff MO, et al. Red blood cell distribution width: Genetic evidence for aging pathways in 116,666 volunteers. PloS one. 2017;12:e0185083.

[45] Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153:1194-217.

[46] Lippi G, Sanchis-Gomar F, Danese E, Montagnana M. Association of red blood cell distribution width with plasma lipids in a general population of unselected outpatients. Kardiologia polska. 2013;71:931-6.

[47] Balistreri CR, Ruvolo G, Lio D, Madonna R. Toll-like receptor-4 signaling pathway in aorta aging and diseases: "its double nature". Journal of molecular and cellular cardiology. 2017;110:38-53.

[48] Pisano C, Balistreri CR, Ricasoli A, Ruvolo G. Cardiovascular Disease in Ageing: An Overview on Thoracic Aortic Aneurysm as an Emerging Inflammatory Disease. Mediators of inflammation. 2017;2017:1274034.

[49] F.Y. Fyhrquist OJS. Modifiable factors influencing telomere lenght and aging. In: Springer, editor. Inflammation, Aging, and Oxidative Stress. 1st ed: Humana Press; 2016. p. 67-80.

[50] Telomeres Mendelian Randomization C, Haycock PC, Burgess S, et al. Association Between Telomere Length and Risk of Cancer and Non-Neoplastic Diseases: A Mendelian Randomization Study. JAMA oncology. 2017;3:636-51.

[51] Kozlitina J, Garcia CK. Red blood cell size is inversely associated with leukocyte telomere length in a large multi-ethnic population. PloS one. 2012;7:e51046.

[52] Tammen SA, Friso S, Choi SW. Epigenetics: the link between nature and nurture. Mol Aspects Med. 2013;34(4):753-64.

[53]Mirando MA, Swanson KS. Companion Animals Symposium: nutrition special needs-the relationship between novel ingredients, environment, and gene expression. J Anim Sci. 2013;91(6):2947-8.

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[54]Fairfax BP, Knight JC. Genetics of gene expression in immunity to infection. Curr Opin Immunol. 2014;30:63-71

[55] Rosa-Garrido M, Chapski DJ, Vondriska TM. Epigenomes in Cardiovascular Disease. Circ Res. 2018;122:1586-1607

[56] Nahrendorf M, Swirski FK. Lifestyle effects on hematopoiesis and atherosclerosis. Circulation research. 2015;116:884-94.

[57] Emans ME, Gaillard CA, Pfister R, et al. Red cell distribution width is associated with physical inactivity and heart failure, independent of established risk factors, inflammation or iron metabolism; the EPIC-Norfolk study. International journal of cardiology. 2013;168:3550-5.

[58] Loprinzi PD, Loenneke JP, Abe T. The association between muscle strengthening activities and red blood cell distribution width among a national sample of U.S. adults. Preventive medicine. 2015;73:130-2.

[59] Lippi G, Salvagno GL, Danese E, Tarperi C, Guidi GC, Schena F. Variation of red blood cell distribution width and mean platelet volume after moderate endurance exercise. Advances in hematology. 2014;2014:192173.

[60] Loprinzi PD, Hall ME. Physical activity and dietary behavior with red blood cell distribution width. Physiology & behavior. 2015;149:35-8.

[61]Saffar Soflaei S, Darroudi S, Tayefi M, et al. Hookah smoking is strongly associated with diabetes mellitus, metabolic syndrome and obesity: a population-based study. Diabetol Metab Syndr. 2018;10:33.

[62] Karvellas CJ, Sawyer M, Hamilton M, Mackey JR. Effect of capecitabine on mean corpuscular volume in patients with metastatic breast cancer. American journal of clinical oncology. 2004;27:364-8.

[63] Wenzel C, Mader RM, Steger GG, et al. Capecitabine treatment results in increased mean corpuscular volume of red blood cells in patients with advanced solid malignancies. Anti-cancer drugs. 2003;14:119-23.

[64] Pham AQ, Xu LH, Moe OW. Drug-Induced Metabolic Acidosis. F1000Research. 2015;4.

[65] Tonelli M, Sacks F, Arnold M, et al. Relation Between Red Blood Cell Distribution Width and Cardiovascular Event Rate in People With Coronary Disease. Circulation. 2008;117:163-8.

[66] Weiss G, Goodnough LT. Anemia of chronic disease. The New England journal of medicine. 2005;352:1011-23.

[67] Kiefer CR, Snyder LM. Oxidation and erythrocyte senescence. Current opinion in hematology. 2000;7:113-6.

[68] Friedman JS, Lopez MF, Fleming MD, et al. SOD2-deficiency anemia: protein oxidation and altered protein expression reveal targets of damage, stress response, and antioxidant responsiveness. Blood. 2004;104:2565-73.

[69] Semba RD, Patel KV, Ferrucci L, et al. Serum antioxidants and inflammation predict red cell distribution width in older women: the Women's Health and Aging Study I. Clinical nutrition. 2010;29:600-4.

[70] Sousa R, Goncalves C, Guerra IC, et al. Increased red cell distribution width in Fanconi anemia: a novel marker of stress erythropoiesis. Orphanet journal of rare diseases. 2016;11:102.

[71] Kimber C, Deller DJ, Ibbotson RN, Lander H. The Mechanism of Anaemia in Chronic Liver Disease. The Quarterly journal of medicine. 1965;34:33-64.

[72]Liu Z, Feng Q, Sun P, et al. Genome-wide DNA methylation drives human embryonic stem cell erythropoiesis by remodeling gene expression dynamics. Epigenomics. 2017;9(12):1543-1558.

[73]van Rooij FJA, Qayyum R, Smith AV, et al. Genome-wide Trans-ethnic Meta-analysis Identifies Seven Genetic Loci Influencing Erythrocyte Traits and a Role for RBPMS in Erythropoiesis. Am J Hum Genet. 2017;100(1):51-63.

[74] Papageorgiou DN, Karkoulia E, Amaral-Psarris A, et al. Distinct and overlapping DNMT1 interactions with multiple transcription factors in erythroid cells: Evidence for co-repressor functions. Biochim Biophys Acta. 2016;1859:1515-1526.

[75] Yao H, Goldman DC, Fan G, Mandel G, Fleming WH. The Corepressor Rcor1 Is Essential for Normal Myeloerythroid Lineage Differentiation. Stem Cells. 2015;33:3304-14.

[76] Ginder GD. Epigenetic regulation of fetal globin gene expression in adult erythroid cells. Transl Res 2015;165:115-25.

[77]Wallace RG, Twomey LC, Custaud MA, Turner JD, Moyna N, Cummins PM, Murphy RP. The role of epigenetics in cardiovascular health and ageing: A focus on physical activity and nutrition. Mech Ageing Dev. 2017. pii: S0047-6374(17)30233-6.

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
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Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)

[78] Saliba W, Barnett-Griness O, Elias M, Rennert G. The association between red cell distribution width and stroke in patients with atrial fibrillation. The American journal of medicine. 2015;128:192 e11-8.

[79] Danese E, Lippi G, Montagnana M. Red blood cell distribution width and cardiovascular diseases. Journal of thoracic disease. 2015;7:E402-11.

[80] Lippi G, Filippozzi L, Montagnana M, et al. Clinical usefulness of measuring red blood cell distribution width on admission in patients with acute coronary syndromes. Clinical chemistry and laboratory medicine. 2009;47:353-7.

[81] Skjelbakken T, Lappegard J, Ellingsen TS, et al. Red cell distribution width is associated with incident myocardial infarction in a general population: the Tromso Study. Journal of the American Heart Association. 2014;3.

[82] Hu GX, Zhang J, Tian YG, Li YH, Mou L, Qiao LJ. Diagnostic value of joint detection of homocysteine and RDW CV on acute myocardial infarction. European review for medical and pharmacological sciences. 2016;20:4124-8.

[83] Bujak K, Wasilewski J, Osadnik T, et al. The Prognostic Role of Red Blood Cell Distribution Width in Coronary Artery Disease: A Review of the Pathophysiology. Disease markers. 2015;2015:824624.

[84] Uyarel H, Ergelen M, Cicek G, et al. Red cell distribution width as a novel prognostic marker in patients undergoing primary angioplasty for acute myocardial infarction. Coronary artery disease. 2011;22:138-44.

[85] Gul M, Uyarel H, Ergelen M, et al. The relationship between red blood cell distribution width and the clinical outcomes in non-ST elevation myocardial infarction and unstable angina pectoris: a 3-year follow-up. Coronary artery disease. 2012;23:330-6.

[86] Sun XP, Chen WM, Sun ZJ, et al. Impact of red blood cell distribution width on long-term mortality in patients with ST-elevation myocardial infarction. Cardiology. 2014;128:343-8.

[87] Tuncez A, Cetin MS, Cetin EH, Yilmaz S, Korkmaz A, Ucar FM. Association between RDW and stent thrombosis in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention. Medicine. 2017;96:e5986.

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)

[88] Lippi G, Cervellin G. Risk assessment of post-infarction heart failure. Systematic review on the role of emerging biomarkers. Critical reviews in clinical laboratory sciences. 2014;51:13-29.

[89] Inuzuka R, Abe J. Red blood cell distribution width as a link between ineffective erythropoiesis and chronic inflammation in heart failure. Circulation journal : official journal of the Japanese Circulation Society. 2015;79:974-5.

[90] Tseliou E, Terrovitis JV, Kaldara EE, et al. Red blood cell distribution width is a significant prognostic marker in advanced heart failure, independent of hemoglobin levels. Hellenic journal of cardiology : HJC = Hellenike kardiologike epitheorese. 2014;55:457-61.

[91] Dai Y, Konishi H, Takagi A, Miyauchi K, Daida H. Red cell distribution width predicts short- and long-term outcomes of acute congestive heart failure more effectively than hemoglobin. Experimental and therapeutic medicine. 2014;8:600-6.

[92] Liu S, Wang P, Shen PP, Zhou JH. Predictive Values of Red Blood Cell Distribution Width in Assessing Severity of Chronic Heart Failure. Medical science monitor : international medical journal of experimental and clinical research. 2016;22:2119-25.

[93] Adamsson Eryd S, Borne Y, Melander O, et al. Red blood cell distribution width is associated with incidence of atrial fibrillation. Journal of internal medicine. 2014;275:84-92.

[94] Gungor B, Ozcan KS, Erdinler I, et al. Elevated levels of RDW is associated with non-valvular atrial fibrillation. Journal of thrombosis and thrombolysis. 2014;37:404-10.

[95] Liu T, Shao Q, Miao S, et al. Red cell distribution width as a novel, inexpensive marker for paroxysmal atrial fibrillation. International journal of cardiology. 2014;171:e52-3.

[96] Chaikriangkrai K, Valderrabano M, Bala SK, et al. Prevalence and Implications of Subclinical Coronary Artery Disease in Patients With Atrial Fibrillation. The American journal of cardiology. 2015;116:1219-23.

[97] Ertas G, Aydin C, Sonmez O, et al. Red cell distribution width predicts new-onset atrial fibrillation after coronary artery bypass grafting. Scandinavian cardiovascular journal : SCJ. 2013;47:132-5.

[98] Korantzopoulos P, Liu T. RDW as a marker of postoperative atrial fibrillation. International journal of cardiology. 2015;191:109.

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[99] Balta S, Demir M, Demirkol S, Arslan Z, Unlu M, Celik T. Red cell distribution width is related to stroke in patients with heart failure. Clinical and applied thrombosis/hemostasis : official journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis. 2015;21:190.

[100] Kaya A, Isik T, Kaya Y, et al. Relationship between red cell distribution width and stroke in patients with stable chronic heart failure: a propensity score matching analysis. Clinical and applied thrombosis/hemostasis : official journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis. 2015;21:160-5.

[101] Soderholm M, Borne Y, Hedblad B, Persson M, Engstrom G. Red cell distribution width in relation to incidence of stroke and carotid atherosclerosis: a population-based cohort study. PloS one. 2015;10:e0124957.

[102] Demir R, Saritemur M, Atis O, et al. Can we distinguish stroke and stroke mimics via red cell distribution width in young patients? Archives of medical science : AMS. 2015;11:958-63.

[103] Jia H, Li H, Zhang Y, Li C, Hu Y, Xia C. Association between red blood cell distribution width (RDW) and carotid artery atherosclerosis (CAS) in patients with primary ischemic stroke. Archives of gerontology and geriatrics. 2015;61:72-5.

[104] Turcato G, Cappellari M, Follador L, et al. Red Blood Cell Distribution Width Is an Independent Predictor of Outcome in Patients Undergoing Thrombolysis for Ischemic Stroke. Seminars in thrombosis and hemostasis. 2017;43:30-5.

[105] Haltmayer M, Mueller T, Luft C, Poelz W, Haidinger D. Erythrocyte mean corpuscular volume associated with severity of peripheral arterial disease: an angiographic evaluation. Annals of vascular surgery. 2002;16:474-9.

[106] Mueller T, Luft C, Haidinger D, Poelz W, Haltmayer M. Erythrocyte mean corpuscular volume associated with the anatomical distribution in peripheral arterial disease. VASA Zeitschrift fur Gefasskrankheiten. 2002;31:81-5.

[107] Zalawadiya SK, Veeranna V, Panaich SS, Afonso L. Red cell distribution width and risk of peripheral artery disease: analysis of National Health and Nutrition Examination Survey 1999-2004. Vascular medicine. 2012;17:155-63.

[108] Ark HYV. Red Cell Distribution Width Predicts Length of Stay in Patients with Acutely Decompensated Heart Failure. European Journal of Health Sciences. 2015;1:8.

[109] Su C, Liao LZ, Song Y, Xu ZW, Mei WY. The role of red blood cell distribution width in mortality and cardiovascular risk among patients with coronary artery diseases: a systematic review and meta-analysis. Journal of thoracic disease. 2014;6:1429-40.

[110] Li N, Zhou H, Tang Q. Red Blood Cell Distribution Width: A Novel Predictive Indicator for Cardiovascular and Cerebrovascular Diseases. Disease markers. 2017;2017:7089493.

[111] Huang YL, Hu ZD, Liu SJ, et al. Prognostic value of red blood cell distribution width for patients with heart failure: a systematic review and meta-analysis of cohort studies. PloS one. 2014;9:e104861.

[112] Luo R, Hu J, Jiang L, Zhang M. Prognostic Value of Red Blood Cell Distribution Width in Non-Cardiovascular Critically or Acutely Patients: A Systematic Review. PloS one. 2016;11:e0167000.

[113] Lippi G. Red blood cell distribution width and mean platelet volume: Surrogate markers for, or treatment targets in, dyslipidemia? Clinical biochemistry. 2015;48:555-6.

[114] Sarikaya S, Sahin S, Akyol L, et al. Is there any relationship between RDW levels and atrial fibrillation in hypertensive patient? African health sciences. 2014;14:267-72.

[115] Duchnowski P, Hryniewiecki T, Kusmierczyk M, Szymanski P. Red cell distribution width is a prognostic marker of perioperative stroke in patients undergoing cardiac valve surgery. Interactive cardiovascular and thoracic surgery. 2017;25:925-9.

[116] Shao Q, Korantzopoulos P, Letsas KP, et al. Red blood cell distribution width as a predictor of atrial fibrillation. Journal of clinical laboratory analysis. 2018.

[117] Ferreira JP, Girerd N, Arrigo M, et al. Enlarging Red Blood Cell Distribution Width During Hospitalization Identifies a Very High-Risk Subset of Acutely Decompensated Heart Failure Patients and Adds Valuable Prognostic Information on Top of Hemoconcentration. Medicine. 2016;95:e3307.

[118] Kim CH, Park JT, Kim EJ, et al. An increase in red blood cell distribution width from baseline predicts mortality in patients with severe sepsis or septic shock. Critical care. 2013;17:R282.

[119] Zoller B, Melander O, Svensson P, Engstrom G. Red cell distribution width and risk for venous thromboembolism: a population-based cohort study. Thrombosis research. 2014;133:334-9.

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[120] Spiropoulos A, Goussetis E, Margeli A, et al. Effect of inflammation induced by prolonged exercise on circulating erythroid progenitors and markers of erythropoiesis. Clinical chemistry and laboratory medicine. 2010;48:199-203.

[121] Bozkurt B, Mann DL, Deswal A. Biomarkers of inflammation in heart failure. Heart failure reviews. 2010;15:331-41.

[122] Okonko DO, Marley SB, Anker SD, Poole-Wilson PA, Gordon MY. Suppression of erythropoiesis in patients with chronic heart failure and anaemia of unknown origin: evidence of an immune basis. International journal of cardiology. 2013;166:664-71.

[123] Shemin D, Rittenberg D. The life span of the human red blood cell. The Journal of biological chemistry. 1946;166:627-36.

[124] Azab B, Torbey E, Hatoum H, et al. Usefulness of red cell distribution width in predicting all-cause long-term mortality after non-ST-elevation myocardial infarction. Cardiology. 2011;119:72-80.

[125] Mozos I. Mechanisms linking red blood cell disorders and cardiovascular diseases. BioMed research international. 2015;2015:682054.

[126] Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple parameter with multiple clinical applications. Critical reviews in clinical laboratory sciences. 2015;52:86-105.

[127] Hou H, Sun T, Li C, et al. An overall and dose-response meta-analysis of red blood cell distribution width and CVD outcomes. Scientific reports. 2017;7:43420.

[128] Cha MJ, Lee HS, Kim HM, Jung JH, Choi EK, Oh S. Association between red cell distribution width and thromboembolic events in patients with atrial fibrillation. European journal of internal medicine. 2017;46:41-6.

[129] Arbel Y, Weitzman D, Raz R, et al. Red blood cell distribution width and the risk of cardiovascular morbidity and all-cause mortality. A population-based study. Thrombosis and haemostasis. 2014;111:300-7.

[130] van der Meer P, Postmus D, Ponikowski P, et al. The predictive value of short-term changes in hemoglobin concentration in patients presenting with acute decompensated heart failure. Journal of the American College of Cardiology. 2013;61:1973-81.

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[131] Davila C, Reyentovich A, Katz SD. Clinical correlates of hemoconcentration during hospitalization for acute decompensated heart failure. Journal of cardiac failure. 2011;17:1018-22.

[132] Tziakas DN, Chalikias GK, Stakos D, et al. Independent and additive predictive value of total cholesterol content of erythrocyte membranes with regard to coronary artery disease clinical presentation. International journal of cardiology. 2011;150:22-7.

[133] Zhang Y, Wang Y, Kang JS, et al. Differences in the predictive value of red cell distribution width for the mortality of patients with heart failure due to various heart diseases. Journal of geriatric cardiology : JGC. 2015;12:647-54.

[134] Xanthopoulos A, Giamouzis G, Melidonis A, et al. Red blood cell distribution width as a prognostic marker in patients with heart failure and diabetes mellitus. Cardiovascular diabetology. 2017;16:81.

[135] Sanchez-Chaparro MA, Calvo-Bonacho E, Gonzalez-Quintela A, et al. Higher red blood cell distribution width is associated with the metabolic syndrome: results of the Ibermutuamur CArdiovascular RIsk assessment study. Diabetes care. 2010;33:e40.

[136] Solak Y, Yilmaz MI, Saglam M, et al. Red cell distribution width is independently related to endothelial dysfunction in patients with chronic kidney disease. The American journal of the medical sciences. 2014;347:118-24.

[137] Engstrom G, Smith JG, Persson M, Nilsson PM, Melander O, Hedblad B. Red cell distribution width, haemoglobin A1c and incidence of diabetes mellitus. Journal of internal medicine. 2014;276:174-83.

[138] Di Pietro N, Giardinelli A, Sirolli V, et al. Nitric oxide synthetic pathway and cGMP levels are altered in red blood cells from end-stage renal disease patients. Molecular and cellular biochemistry. 2016;417:155-67.

[139] Angelini F, Pagano F, Bordin A, Picchio V, De Falco E, Chimenti I. Getting Old through the Blood: Circulating Molecules in Aging and Senescence of Cardiovascular Regenerative Cells. Frontiers in cardiovascular medicine. 2017;4:62.

[140] Angelini F, Pagano F, Bordin A, et al. The Impact of Environmental Factors in Influencing Epigenetics Related to Oxidative States in the Cardiovascular System. Oxidative medicine and cellular longevity. 2017;2017:2712751.

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[141] van der Putten K, Braam B, Jie KE, Gaillard CA. Mechanisms of Disease: erythropoietin resistance in patients with both heart and kidney failure. Nature clinical practice Nephrology. 2008;4:47-57.

[142] Emans ME, van der Putten K, van Rooijen KL, et al. Determinants of red cell distribution width (RDW) in cardiorenal patients: RDW is not related to erythropoietin resistance. Journal of cardiac failure. 2011;17:626-33.

[143] Kucera M, Balaz D, Kruzliak P, et al. The effects of atorvastatin treatment on the mean platelet volume and red cell distribution width in patients with dyslipoproteinemia and comparison with plasma atherogenicity indicators--A pilot study. Clinical biochemistry. 2015;48:557-61.

[144] Xiong XF, Yang Y, Chen X, et al. Red cell distribution width as a significant indicator of medication and prognosis in type 2 diabetic patients. Scientific reports. 2017;7:2709.

[145] Poglajen G, Sever M, Cernelc P, Haddad F, Vrtovec B. Increased red cell distribution width is associated with poor stem cell mobilization in patients with advanced chronic heart failure. Biomarkers : biochemical indicators of exposure, response, and susceptibility to chemicals. 2015;20:365-70.

[146] Rodriguez-Carrio J, Alperi-Lopez M, Lopez P, et al. Red cell distribution width is associated with endothelial progenitor cell depletion and vascular-related mediators in rheumatoid arthritis. Atherosclerosis. 2015;240:131-6.

[147] De Falco E, Avitabile D, Totta P, et al. Altered SDF-1-mediated differentiation of bone marrow-derived endothelial progenitor cells in diabetes mellitus. Journal of cellular and molecular medicine. 2009;13:3405-14.

[148] De Falco E, Carnevale R, Pagano F, et al. Role of NOX2 in mediating doxorubicin-induced senescence in human endothelial progenitor cells. Mechanisms of ageing and development. 2016;159:37-43.

[149] De Falco E, Porcelli D, Torella AR, et al. SDF-1 involvement in endothelial phenotype and ischemia-induced recruitment of bone marrow progenitor cells. Blood. 2004;104:3472-82.

[150] Shay JW. Telomeres and aging. Current opinion in cell biology. 2017;52:1-7.

[151] Diez Roux AV, Ranjit N, Jenny NS, et al. Race/ethnicity and telomere length in the Multi-Ethnic Study of Atherosclerosis. Aging cell. 2009;8:251-7.

[152] Martorana A, Balistreri CR, Bulati M, et al. Double negative (CD19+IgG+IgD-CD27-) B lymphocytes: a new insight from telomerase in healthy elderly, in centenarian offspring and in Alzheimer's disease patients. Immunology letters. 2014;162:303-9.

[153] Rizvi S, Raza ST, Mahdi F. Telomere length variations in aging and age-related diseases. Current aging science. 2014;7:161-7.

[154] Balistreri CR, Pisano C, Martorana A, et al. Are the leukocyte telomere length attrition and telomerase activity alteration potential predictor biomarkers for sporadic TAA in aged individuals? Age. 2014;36:9700.

[155] Balistreri CR, Pisano C, Merlo D, et al. Is the mean blood leukocyte telomere length a predictor for sporadic thoracic aortic aneurysm? Data from a preliminary study. Rejuvenation research. 2012;15:170-3.

[156] Mazidi M, Penson P, Banach M. Association between telomere length and complete blood count in US adults. Arch Med Sci. 2017;13:601-605.

[157]Mather KA, Jorm AF, Parslow RA, Christensen H. Is telomere length a biomarker of aging? A review. J Gerontol A Biol Sci Med Sci. 2011;66:202-13.

[158]Sanders JL, Newman AB. Telomere length in epidemiology: a biomarker of aging, age-related disease, both, or neither? Epidemiol Rev. 2013;35:112-31.

[159]Chilton W, O'Brien B, Charchar F. Telomeres, Aging and Exercise: Guilty by Association? Int J Mol Sci. 2017;18, pii: E2573.

[160] Danise P, Maconi M, Barrella F, et al. Evaluation of nucleated red blood cells in the peripheral blood of hematological diseases. Clinical chemistry and laboratory medicine. 2011;50:357-60.

[161] Monteiro Junior JG, Torres Dde O, da Silva MC, et al. Nucleated Red Blood Cells as Predictors of All-Cause Mortality in Cardiac Intensive Care Unit Patients: A Prospective Cohort Study. PloS one. 2015;10:e0144259.

[162] Hwang SY, Lee JH, Lee YH, Hong CK, Sung AJ, Choi YC. Comparison of the Sequential Organ Failure Assessment, Acute Physiology and Chronic Health Evaluation II scoring system, and Trauma and Injury Severity Score method for predicting the outcomes of intensive care unit trauma patients. The American journal of emergency medicine. 2012;30:749-53.

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
This paper has been peer-reviewed and accepted for publication, but has yet to undergo copyediting and proof correction. The final published version may differ from this proof.

[163] Olivieri F, Pompilio G, Balistreri CR. Endothelial progenitor cells in ageing. Mechanisms of ageing and development. 2016;159:1-3.

[164] Regina C, Panatta E, Candi E, et al. Vascular ageing and endothelial cell senescence: Molecular mechanisms of physiology and diseases. Mechanisms of ageing and development. 2016;159:14-21.

[165] Madonna R, Novo G, Balistreri CR. Cellular and molecular basis of the imbalance between vascular damage and repair in ageing and age-related diseases: As biomarkers and targets for new treatments. Mechanisms of ageing and development. 2016;159:22-30.

[166] Balistreri CR, Madonna R, Melino G, Caruso C. The emerging role of Notch pathway in ageing: Focus on the related mechanisms in age-related diseases. Ageing research reviews. 2016;29:50-65.

[167] Dhingra R, Vasan RS. Biomarkers in cardiovascular disease: Statistical assessment and section on key novel heart failure biomarkers. Trends in cardiovascular medicine. 2017;27:123-33.

[168] Balistreri CR, Pisano C, Massoud R, Dolci S, Ruvolo G. A revolutionary predictor multi-biomarker profile for sporadic ascending aorta aneurysm: red blood cell distribution along with leukocyte telomere length, telomerase activity and hs-CRP blood levels. Inter. J. Cardiology 2018 submitted

[169]Balistreri CR, Buffa S, Pisano C, Lio D, Ruvolo G, Mazzesi G. Are Endothelial Progenitor Cells the Real Solution for Cardiovascular Diseases? Focus on Controversies and Perspectives. Biomed Res Int. 2015;2015:835934.

[170]Balistreri CR (Ed.).Endothelial progenitor cells (EPCs) in ageing and age-related diseases: from their physiological and pathological implications to translation in personalized medicine [Special issue]. Mech Ageing Devolop (2016)159(1-80).

[171]Balistrieri CR Endothelial progenitor cells: a new real hope or only an unrealizable dream? Springer International Publishing, Dordrecht (2017)**:** pgg.1-80

[172] Balistrieri CR An overview on adult stem/progenitor cells as potential drivers of tissue ageing/disease: Endothelial progenitor cells as typical examples J Hematol Clin Ther 2018, 1: 8-11

Rejuvenation Research
Diagnostic and prognostic relevance of Red blood cell distribution width for vascular aging and cardiovascular diseases (DOI: 10.1089/rej.2018.2094)
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Box-1: Vascular aging [see references 163-166, 169-172]

The term *vascular ageing* indicates the process of gradual remodelling and progressing towards an unhealthy/diseased state, which characterizes the cardiovascular system with advancing age. It is recognised 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 neo-angiogenic capability. This ageing-dependent impairment of endothelial functions (*defined as endothelial dysfunction*) is considered a key factor contributing to vascular dysfunctions, which is responsible not only of the development of CVDs, but also of 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 ageing-related endothelial cell senescence, including telomere shorting, microRNAs, mitochondrial dysfunction, DNA instability, DNA damage and micro-environmental stressors, such as hypoxia. In addition, another mechanism contributing to endothelial dysfunction is the impairment of cardiovascular self repair system. Accordingly, ageing, but also other factors related to process, affect cardiovascular repair by evocating the development of an imbalance of damage and self repair, responsible with advancing age of onset and progression of CVDs. Precisely, cardiac or other stem (i.e. CD34 stem cells) and progenitors cells (i.e. endothelial progenitor cells-EPCs) show impaired functions with age. This determines incapacity of counteracting damages 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: CD34 Stem cells and progenitors [169-172]

Hematopoietic stem (HSCs) express 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 169-171). As abovementioned, the ageing 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

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Box-3: Cardiovascular Repair: the role of EPC Cells and its features in everyone [169-172]

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 to have an efficient cardiovascular self-repair, which appears to be prevalently modulated by genetic background, environmental and epigenetic factors. As result, the interest on 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 non ischemic injury. Discordant results have been obtained. Thus, their real contribution is until now uncertain. 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 also is their contribution in 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.

Legend: 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, peripheralartery disease.

Table 1. Red blood cells distribution width in cardiovascular diseases (see references 80-108)

CARDIOVASCULAR DISEASES	FINDINGS
	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.
Acute coronary syndrome	The combined measurements of both troponin T and RDW have allowed todiagnose MI with greater sensitivity than the analysis of troponin T alone in patients admitted tointensive care unit for chest pain.
	High level of RDW predict 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
	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
Heart failure	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
	Significant association between high level of RDW and the onset of AF associated or not to valvular disease.

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	High level of RDW predict adverse outcomes in patients with AF
Atrial fibrillation	High level of RDW predict the onset of atrial fibrillation in patients undergoingCABG
	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
Ischemic cerebrovascular disease	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.
	RDW is a predictor of mortality and morbidity in patients with stroke undergoing or not to thrombolysis.
Peripheralartery disease	Significant association between high level of RDW and the prevalence of PAD
	Role of RDW in the diagnosis of PAD

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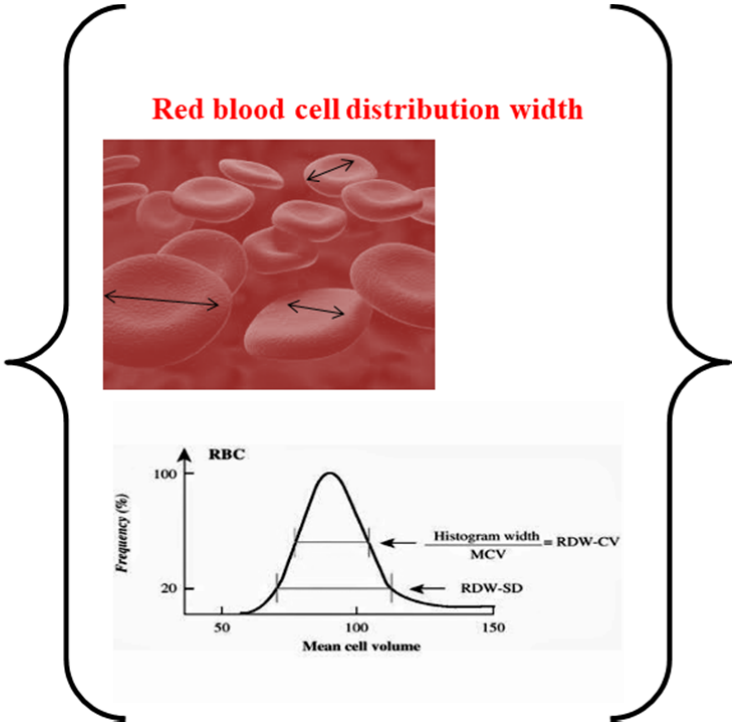
Figure legends



Index of variation in the size and shape of erythrocyte cells, corresponding to the degree of anisocytosis

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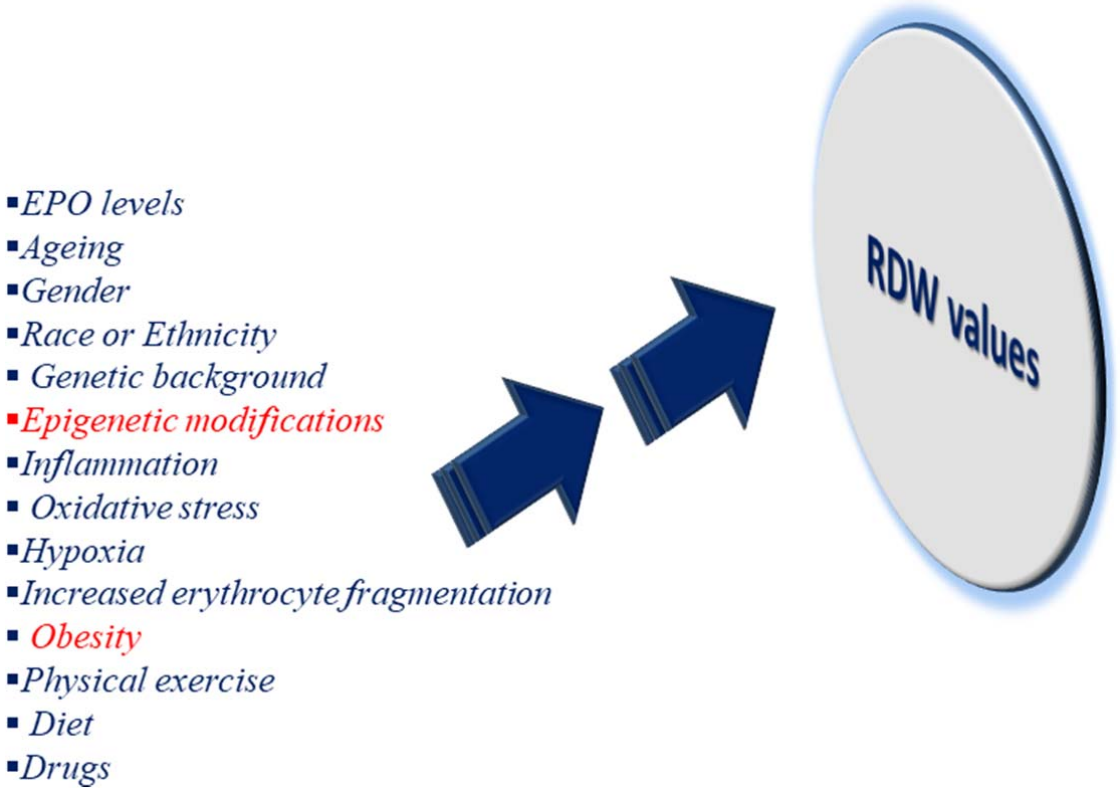
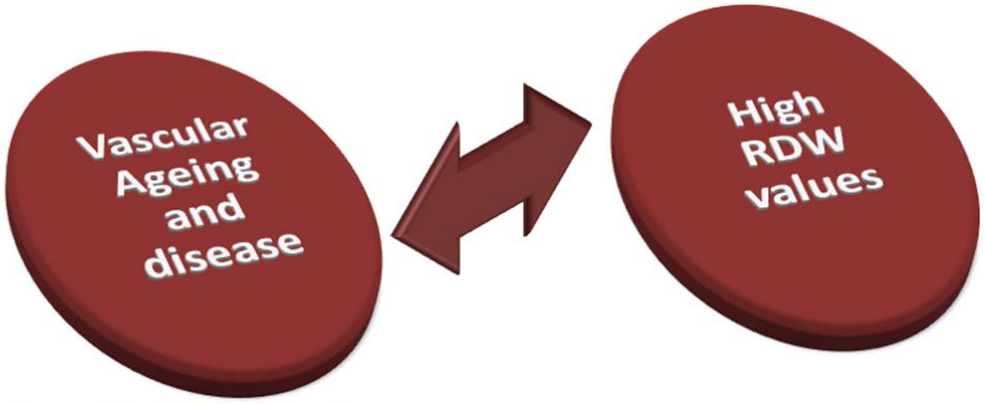


Figures 1A-B. Definition, calculation and factors able to modulate RDW values. **(A)** the panel shows the significance and the calculation of the RDW values. RDW is a red blood cell parameter that measures variability of red cell volume/size (anisocytosis). Depending on the types of hematology analyzer 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 x 100%. (B) The picture displays all endogenous and exogenous factors modulating the RDW values, as described in the text.



Correlations, between high RDW values and the following alterations in cardiovascular repair system and degree of senescence, have been reported:

- Significant reduction of CD34(+) stem cell mobilization
- Significant reduction of EPCs (CD34+VEGFR2+CD133+) blood levels
- Increased levels of different mediators associated with endothelial damage and failure of vascular repair
- Shorter leukocyte telomere lengths

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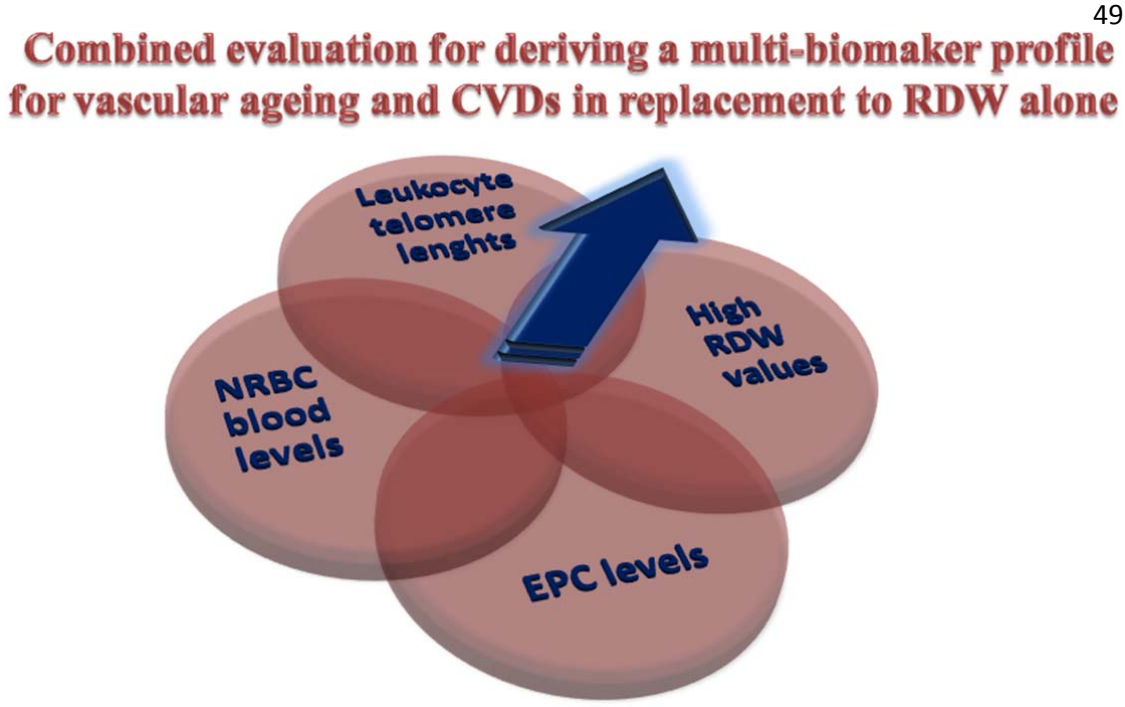

Figures 2A-B. High RDW values as predictive biomarker for vascular aging and onset of CVDs? (A) To support of the potential role of RDW as predictive biomarker of vascular ageing and CVD onset, there is an established evidence. It demonstrates correlations between high RDW values and the specific alterations 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 proposal: combined evaluation of RDW values with leukocyte telomere length and circulating levels of nRBCs and EPCs in the hope to derive a potentially novel multi-biomarker profile for the future development of a algorithm for vascular ageing and CVDs