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Red cell distribution width is associated with cardiovascular risk in adults

A medida da amplitude da distribuição do tamanho dos eritrócitos está associada ao risco cardiovascular em adultos

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Abstract *Red cell distribution width (RDW) is a measure of erythrocyte size variability. Recent studies have shown that RDW is a predictive, and prognostic marker of mortality and cardiovascular (CVD) events in the general population and in CVD patients. This study aimed to investigate the association between RDW and CVD risk in a large sample of adults. A subsample of CVD free participants of the ELSA-Brasil cohort were included (n=4,481). In the cross-sectional approach, multiple regression analysis was used to investigate the association between RDW and the Framingham Risk Score (FRS). Linear mixed effect model evaluated whether baseline RDW predicted changes in CVD risk after about four-year follow up. Cross-sectional analysis showed that RDW was independently associated with FRS, participants in the fourth-quartile of RDW distribution had a 29% higher FRS than those in the first-quartile RDW (p<0.001). A longitudinal analysis revealed that RDW remained associated with increased FRS. In this large cohort of adult Brazilians, RDW was independently associated with increased CVD risk, as measured by the FRS, both at baseline and after four-year follow-up. However, RDW did not predict change in CVD risk in this short-term follow up.*

Key words *Erythrocyte indices, Cardiovascular diseases, Risk*

Resumo *Estudos recentes têm mostrado que o RDW (do inglês Red Cell Distribution Width) é um marcador preditivo e prognóstico de mortalidade e eventos cardiovasculares (DCV) na população geral e em pacientes com DCV. Este estudo teve como objetivo investigar a associação entre RDW e risco de DCV em uma grande amostra de adultos. Foram incluídas uma subamostra de participantes sem DCV da coorte ELSA-Brasil (n=4.481). Na abordagem transversal, a análise de regressão múltipla foi usada para investigar a associação entre o RDW e o Escore de Risco de Framingham (ERF). O modelo linear de efeito misto foi usado para avaliar se o RDW basal previa mudanças no risco de DCV após cerca de quatro anos de acompanhamento. A análise transversal mostrou que o RDW foi independentemente associado ao ERF, os participantes no quarto quartil da distribuição do RDW tiveram um ERF 29% maior do que aqueles no primeiro quartil RDW (p<0,001). Na análise longitudinal, o RDW permaneceu associado ao aumento do ERF. Nesta grande coorte de adultos brasileiros, o RDW foi independentemente associado ao aumento do risco de DCV, medido pelo ERF, tanto no início quanto após quatro anos de acompanhamento. No entanto, RDW não previu mudança no risco de DCV neste seguimento de curto prazo.*

Palavras-chave *Índices eritrocitários, Doenças cardiovasculares, Risco*

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Introduction

Red cell distribution width (RDW) is a quantitative measure of erythrocyte size variability and reflects differences in erythrocyte size within a sample, or the degree of anisocytosis. RDW is a cost-efficient measure that is easily obtained, as most hematology analyzers provide RDW values as part of a complete blood count test. RDW is routinely used in differential diagnosis of anemic conditions such as thalassemia, megaloblastic anemia, chronic disease-related anemia, and iron deficiency anemia[1].

Recent studies indicate that RDW is a predictor of morbidity and mortality in several conditions, including cancer, autoimmune, respiratory and infectious diseases, end-stage renal disease and diabetes[2-5], among others. Moreover, the degree of anisocytosis is thought to be a risk factor for increased mortality and morbidity in the general population, especially in patients suffering from cardiovascular diseases (CVD), including acute coronary syndrome, peripheral arterial disease, atrial fibrillation, heart failure, hypertension[6]. Also, independent correlations between RDW and clinical conditions, lifestyle behaviors, and biomarkers have been demonstrated in different studies, suggesting that RDW elevation (meaning high variability in erythrocyte size) may be related to inflammatory status, oxidative stress, and endothelial dysfunction, which in turn raises the risk of developing CVD[7-9]. Studies have demonstrated that anisocytosis may be directly involved in the pathogenesis of cardiovascular disorders, since deformed erythrocytes (a common finding in anisocytosis) may: (1) lead to increased blood viscosity and compromised microcirculatory blood flow; (2) present more aggregation and endothelial adhesion, and (3) contribute to the atherosclerotic process via neutralization of vasodilator mediators and lipid accumulation in atherosclerotic lesions[6,7]

According to the World Health Organization, 17.5 million people die each year from CVD, an estimated 31% of all deaths globally. Over 75% of CVD deaths occur in low- and middle-income countries[10]. Thus, CVD prevention is paramount and its effectiveness depends upon the identification of asymptomatic individuals with increased risk of cardiovascular events. The Framingham Risk Score (FRS) is a widely used algorithm that estimates the risk of atherosclerotic disease. The FRS final score estimates the individual 10-year probability of CVD development in patients without a previous diagnosis of CVD[11].

Thus, this study aims to evaluate the association between RDW and FRS in participants of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) using cross-sectional and longitudinal approaches.

Methods

Study population

ELSA-Brasil is a prospective multicenter cohort study developed in partnership with the Ministry of Health and the Ministry of Science and Technology, and designed to investigate the incidence and risk factors of chronic diseases, particularly CVD and diabetes mellitus (DM), in Brazilian adults[12,13].

The ELSA-Brasil sample consists of 15,105 male and female civil servants aged 35-74 years. At baseline assessment (2008-2010) from research centers and higher education institutions in six states from three regions of Brazil, all participants underwent standardized interviews, physical examination, and laboratory testing. In the first follow-up assessment a second round of interviews, physical examination, and laboratory testing was conducted (2012-2014). The second follow-up assessment happened between 2016 and 2018; however, the data is not yet available. Detailed information about the ELSA-Brasil design and cohort profile can be found elsewhere[12,13].

ELSA-Brasil was approved by the Human Research Ethics Committees at the participating institutions and by the National Research Ethics Commission (CONEP). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. All participants provided written consent including permission for the storage of biological samples for future studies.

Participants

The studied sample is a subpopulation of the ELSA-Brasil cohort comprising 5,176 volunteers from the Federal University of Minas Gerais (UFMG) and Federal University of Rio Grande do Sul (UFRGS). These two centers were selected due to the availability of automated counters of the same brand and model, i.e., the use of the same RDW measurement methods because different methodologies may interfere with RDW values[1,14]. Of the 5176 eligible participants, 40 were excluded due to lack of RDW information

or RDW values <10% or >21%. A total of 609 participants were excluded due to the presence of CVD or lack of information on CVD and 46 participants who underwent bariatric surgery were also excluded (Figure 1).

The remaining 4,481 participants enrolled were stratified for cardiovascular risk by FRS. The following variables were considered for risk assessment: age, gender, total cholesterol, high-density lipoprotein (HDL) cholesterol, smoking, diabetes, systolic blood pressure and use of antihypertensive drugs[11]. Of the 4,481 eligible participants, 50 died and 280 were lost or refused to participate in the second examination (2012-2014). Thus, 300 people were lost at the first follow-up assessment.

Sociodemographic, clinical, and lifestyle variables

Sociodemographic variables including age, sex, skin color/race (White, Black, Brown, Indigenous and Asian descent), level of education, and cigarette and alcohol consumption were self-re-

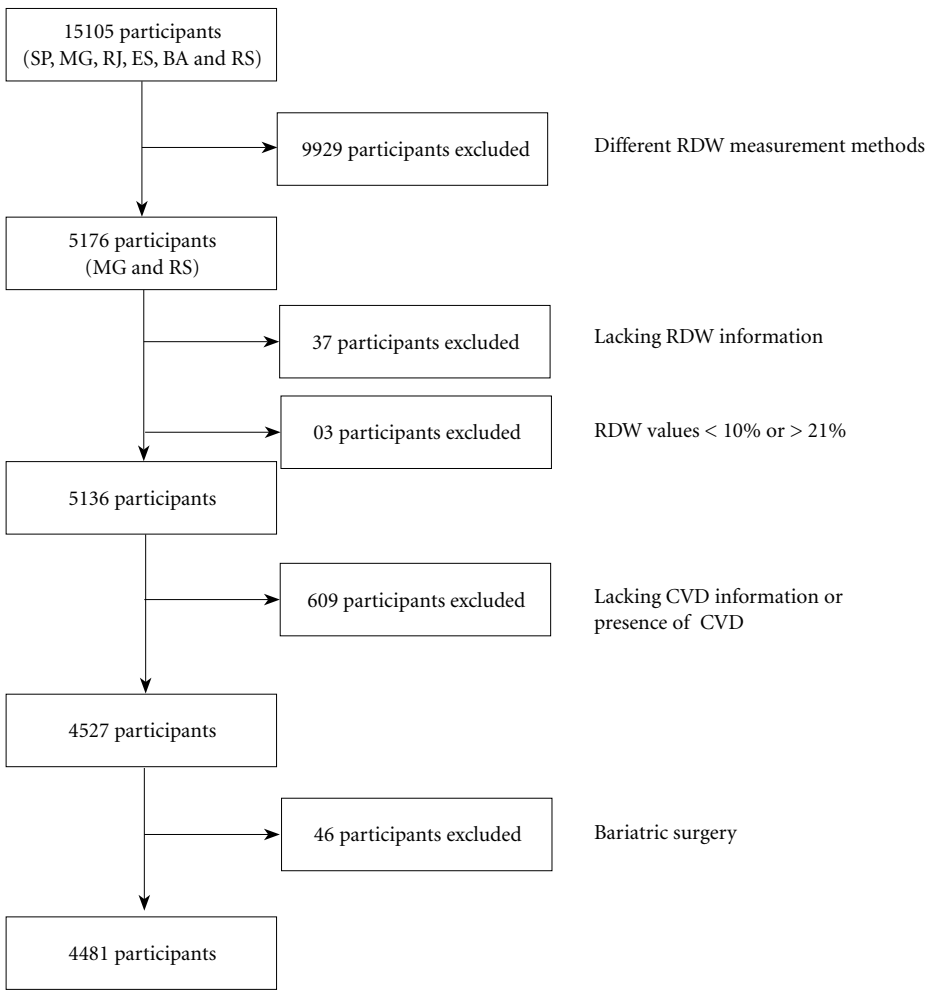

Figure 1. Enrollment flowchart for the study population.

SP: São Paulo, MG: Minas Gerais, RJ: Rio de Janeiro, ES: Espírito Santo, BA: Bahia and RS: Rio Grande do Sul. RDW: Red Cell Distribution Width, CVD: Cardiovascular Disease.

Source: Authors.

ported. If men reportedly consumed ≥210 g alcohol/week and if women reportedly consumed ≥140 g alcohol/week, they were considered excessive drinkers. Additional variables of interest included level of physical activity, based on the International Physical Activity Questionnaire (IPAQ)[15]; body mass index (BMI, kg/m²)[16]; and estimated glomerular filtration rate (eGFR) defined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[17]. DM was defined according to the American Diabetes Association (ADA) criteria or by self-report of previous diagnosis of DM and/or use of insulin or oral hypoglycemic agents. Impaired glucose tolerance (IGT) was defined as glucose levels >140 mg/dL and <200 mg/dL 2 h after overload with 75 g of anhydrous glucose according to ADA criteria. Hypertension was defined by self-report of medical diagnosis of hypertension, or use of antihypertensive agents, or blood pressure equal to or above 140/90 mmHg from three different measurements[13]. CVD was defined as reported surgical heart revascularization and/or medical diagnosis of acute myocardial infarction, and/or peripheral arterial disease, and/or stroke, and/or heart failure, and/or electrocardiography abnormalities consistent with myocardial infarction according to the Minnesota code[18].

Blood samples

Venous blood samples were collected in the morning after a 12- to 14-h fasting period in compliance with the Clinical and Laboratory Standards Institute (CLSI) – Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture: Approved Standard[19]. Participants were asked to stop taking multivitamins and vitamin C 24 h prior to blood collection and to restrain from physical activity during the fasting period. Venipuncture was performed using a butterfly needle for multiple vacuum collections and tourniquet application for a maximum of 1 min. Sample tubes containing ethylenediaminetetraacetic acid tripotassium salt (EDTA) were identified with bar codes and kept at room temperature until complete blood count (CBC) tests were performed. CBCs were conducted at local UFMG and UFRGS teaching hospital laboratories. Plasma/serum samples for biochemical analysis were stored at -80°C and sent to the ELSA-Brasil central laboratory at the University of São Paulo Teaching Hospital[20].

Red cell distribution width (RDW)

RDW was measured on SYSMEX XE 2100 D automated blood analyzers (Sysmex, Kobe, Japan). This system uses electrical impedance for erythrocyte count, size and volume determination and generates a histogram of the coefficient of variation for red cell distribution width (RDW-CV) that is mathematically derived from mean corpuscular volume (MCV) measured in fL. The RDW-CV is expressed in percentage and calculated with the following formula: RDW-CV (%) = one standard deviation divided by/MCV × 100. Values are expressed as percentages and reference intervals may vary from 10.7-12.9% to 13.8-15.3% (lower and upper ranges, respectively) depending on the analyzer used[14,21].

Biochemical analyses

An Advia 1200 automated biochemistry analyzer (Siemens, Deerfield, IL, USA) was used to determine fasting and overload glucose levels, HDL cholesterol, total cholesterol, C-reactive protein (CRP) was measured using nephelometry on a BN II nephelometer (Siemens, Vienna, Austria). Glycated hemoglobin (HbA1c) was measured using a high-performance liquid chromatography (HPLC) assay on a Variant™ II system (Bio-Rad, Hercules, CA, USA).

Statistical analysis

Variable distributions are reported as medians and interquartile ranges (continuous variables) or absolute values and frequencies (categorical variables). Baseline characteristics were compared across these quartiles using the chi-square test for categorical variables and the analysis of variance (ANOVA) test for continuous variables.

In both cross-sectional and longitudinal analyses, the relationship of RDW and FRS was analyzed using FRS as continuous variable, and RDW as a categorical variable grouped into quartiles: Q1=11.10-12.69%; Q2=12.70-12.99%; Q3=13.00-13.49%; and Q4=13.50-20.50%. FRS (response variable) was log-transformed to normalize its distribution.

Simple linear regression was used to assess the association between the FRS and the clinical, socio-demographic, lifestyle, and laboratory variables. From this analysis, we identified confounding factors and the adjustment models for the multivariate regression. The variables with

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potential confounding effect were those that correlated with the RDW with p<0.1 and those that were not part of the FRS to avoid over adjustment (hemoglobin, self-reported race/skin color, level of education, alcohol consumption, BMI, physical activity, MCV, eGFR, platelets and CRP). The final model retained all variables which remained associated with the FRS at the level of p<0.05.

Additionally, multiple linear regression analysis was used to estimate the independent association of RDW (explanatory variable) with the FRS at baseline, after adjusting for potential confounders, i.e., variables that are not part of the FRS, but can increase CVD risk and may be related to RDW. Having the FRS as the response variable, the following regression models were run: Model 1: RDW; Model 2: Model 1 + skin color/race, level of education, BMI, CRP, hemoglobin and alcohol consumption.

Independent association between RDW and changes in the trajectory of FRS after four-year follow up was investigated using linear mixed models. In these models, the response variable (FRS) was assessed on the baseline visit and at the second visit, whereas explanatory variables were only measured at the baseline visit. Change in FRS with time was assessed by entering an interaction term between RDW and time between visits in the final model, considering the significance level of p<0.05. The following variables were entered in each model: Model 1: RDW adjusted by the follow-up time; Model 2: Model 1 + skin color/race, level of education, BMI, CRP, hemoglobin, alcohol consumption and interactions (hemoglobin and time and BMI and time). Linear mixed models easily accommodate unbalanced, unequally spaced observations, and consequently are ideal tools for analyzing longitudinal data[22]. In mixed models, the interaction term between a fixed effect covariate and time evaluates whether this covariate is a predictor of the longitudinal changes in the response variable (FRS). Thus, the interaction terms between time and all of the aforementioned fixed effect variables were evaluated, but only the statistically significant (p<0.05) ones were retained in the final model. In all models, we included the intercept as a random term, which allows each participant's baseline value to vary from the population average, but kept the slope fixed and equal to β.

Statistical assumptions to perform regressions were checked by residual analysis. Statistical analyses were performed using R statistical software package version 3.4.2[23].

Results

Descriptive analysis

The sociodemographic, lifestyle, clinical, and laboratory characteristics of participants stratified by baseline RDW quartiles are shown in Table 1. The median age ranged from 51 to 53 years, most participants were women (54.47%), self-reported white (58.74%), had completed higher education (57.38%), and had never smoked (57.84%).

Significant differences were observed among the RDW quartiles: as RDW values increased, there was a gradual increase in the proportion of patients with comorbidities, such as DM and hypertension. In addition, among individuals in the 4th quartile of RDW there was significantly higher frequency of participants who engaged in low-intensity physical activity, were smokers, and had higher total cholesterol (Table 1). Median RDW was 13.0% (interquartile range: 12.7-13.5%; Table 1). RDW medians and interquartile ranges did not differ significantly (p=0.180) between UFMG [13.1% (12.7-13.6%)] and UFRGS [13.0% (12.7-13.5%)] populations.

Cross-sectional analysis

Multiple regression models revealed an independent association between RDW and FRS with an upward gradient, even after adjusting for confounders: FRS increases with increasing RDW quartile, although only the 3rd and 4th quartiles remained statistically significant in the fully adjusted model (Model 2). In total, Model 2 explained 29% of the variability in the FRS in the study sample (Table 2).

Longitudinal analysis

Linear mixed regression models also confirmed the findings of the cross-sectional analysis, showing significant associations between RDW and FRS after adjusting for confounding variables, both at baseline assessment and at the first follow-up assessment. The interaction term between the RDW and the covariable time was not statistically significant, indicating that the slope of the association did not change over time. Thus, although Model 2 accounts for about 29% of the FRS variability, RDW was not able to predict a worsening in the cardiovascular risk in the longitudinal trajectory of these individuals over four years (Table 3).

Table 1. Summary of participant characteristics in the baseline assessment.

Characteristic	RDW quartiles (%)				
	Q1 11.1-12.69 N=1,362	**Q2 12.7-12.99 N=887**	**Q3 13.0-13.49 N=1,198**	**Q4 13.5-20.5 N=1,034**	**p-value**
Sex, women	50.44%	53.10%	53.51%	62.09%	<0.001
Age	51.00 [44.00-58.00]	51.00 [45.00-59.00]	52.00 [46.00-58.00]	53.00 [46.00-59.50]	<0.001
Self-rated race/skin color					<0.001
White	63.73%	62.34%	60.10%	47.49%	
Brown	23.35%	24.01%	23.54%	28.53%	
Black	8.88%	9.24%	13.27%	21.47%	
Other[1]	4.04%	4.40%	3.09%	2.51%	
Level of education					0.002
University degree	59.62%	58.62%	58.85%	51.64%	
Incomplete elementary school	3.60%	5.07%	5.26%	6.19%	
Complete elementary school	5.73%	6.76%	6.18%	6.38%	
Complete high school	31.06%	29.54%	29.72%	35.78%	
Smoking status					<0.001
Never smoked	60.35%	56.82%	58.51%	54.64%	
Former smoker	28.71%	32.81%	28.96%	28.53%	
Current smoker	10.94%	10.37%	12.52%	16.83%	
Physical activity[2]					0.457
Low	74.06%	73.73%	74.37%	75.80%	
Moderate	18.15%	18.94%	17.45%	18.37%	
High	7.79%	7.33%	8.18%	5.83%	
Alcohol consumption[3], yes	9.18%	8.34%	9.02%	7.93%	0.688
BMI (kg/m²)	25.41 [23.05-28.05]	25.86 [23.44-29.97]	26.23 [23.57-29.41]	27.01 [24.18-30.54]	<0.001
Diabetes mellitus[4]	13.88%	15.14%	14.94%	19.34%	0.002
Hypertension[5]	10.35%	10.48%	10.77%	13.64%	0.050
Hemoglobin (g/dL)	14.30 [13.50-15.40]	14.20 [13.30-15.20]	14.20 [13.30-15.20]	13.70 [12.70-14.70]	<0.001
MCV (fL)	89.40 [87.00-91.80]	88.60 [86.30-90.80]	88.20 [85.50-90.80]	86.70 [82.90-89.70]	<0.001
Total cholesterol (mg/dL)	206.00 [185.00-232.00]	210.00 [183.00-240.00]	212.00 [188.00-240.00]	213.00 [186.00-241.00]	0.005
HDL cholesterol (mg/dL)	53.00 [45.00-63.00]	54.00 [45.20-64.00]	55.00 [46.00-65.00]	56.00 [47.00-67.00]	<0.001
CRP (mg/dL)	1.17 [0.61-2.51]	1.32 [0.70-2.67]	1.46 [0.74-3.20]	2.05 [0.91-4.31]	<0.001
eGFR[6]>60 mL/min	96.55%	95.71%	95.16%	95.16%	0.267

Continuous variables presented as median and interquartile ranges [IQ]. Categorical variables: frequencies (%). [1]Indigenous, Asian, and individuals who did not declare skin color/race; [2]Physical activity based on the International Physical Activity Questionnaire (IPAQ); [3]Excessive drinker (men≥210 g alcohol/week; women≥140 g alcohol/week); [4]Diabetes mellitus: defined according to American Diabetes Association (ADA) criteria or by self-report of previous diagnosis of DM and/or use of insulin or oral hypoglycemic agents; [5]Hypertension: defined as any of the following: self-report of previous diagnosis of hypertension, or use of antihypertensive agents, or blood pressure≥140\90 mmHg from three measurements; [6]Estimated glomerular filtration rate defined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. BMI - body mass index; MCV - mean corpuscular volume; HDL - high-density lipoprotein; CRP - c-reactive protein; eGFR - estimated glomerular filtration rate.

Source: Authors.

Table 2. Multiple regression models for the association between RDW and cardiovascular risk by FRS quartile adjusted for different variables.

Model	FRS				R^2Aj
	Q1 11.1-12.69 N=1,362	Q2 12.7-12.99 N=887	Q3 13.0-13.49 N=1,198	Q4 13.5-20.5 N=1,034	
	e^{β}; p-value [95%CI]	e^{β}; p-value [95%CI]	e^{β}; p-value [95%CI]	e^{β}; p-value [95%CI]	
1[1]	Reference	1.062; 0.079 [0.993-1.136]	1.083; 0.011 [1.018-1.153]	1.290; ≤0,001 [1.206-1.380]	0.299
2[2]	Reference	1.060; 0.088 [0.991-1.134]	1.087; 0.008 [1.021-1.153]	1.299; ≤0.001 [1.215-1.390]	0.298

Regression coefficients (β) were log-transformed. [1] Model 1: RDW; [2] Model 2: model 1 + hemoglobin, race, level of education, BMI, CRP, physical activity, and alcohol consumption. RDW - Red Cell Distribution Width; FRS - Framingham Risk Score; e^{β} - Exponential of Beta Coefficient; BMI - Body Mass Index; CRP - C-Reactive Protein. Multiple linear regression significant at p≤0.05.

Source: Authors.

Table 3. Mixed regression models for the association between RDW and cardiovascular risk by FRS quartile adjusted for different variables.

Model	FRS			
	Q1 11.1-12.69 N=1,274	Q2 12.7-12.99 N=818	Q3 13.0-13.49 N=1,112	Q4 13.5-20.5 N=947
	e^{β}; p-value [95%CI]	e^{β}; p-value [95%CI]	e^{β}; p-value [95%CI]	e^{β}; p-value [95%CI]
1[1]	Reference	1.051; 0.122 [0.986-1.121]	1.083; 0.007 [1.021-1.149]	1.280; ≤0.001 [1.201-1.364]
2[2]	Reference	1.062; 0.070 [0.994-1.134]	1.085; 0.008 [1.021-1.153]	1.290; ≤0.001 [1.208-1.377]
2[2]	Interaction terms time	(p-value) 0.236	(p-value) 0.455	(p-value) 0.057

Regression coefficients (β) were log-transformed. [1]Model 1: crude model adjusted for follow-up time and RDW; [2]Model 2: model one + hemoglobin, race, level of education, BMI, CRP, physical activity, alcohol consumption, and hemoglobin × time and BMI × time interactions. RDW - Red Cell Distribution Width; FRS - Framingham Risk Score; e^{β} - Exponential of Beta Coefficient; MCV - Mean Corpuscular Volume; TSH - Thyroid-Stimulating Hormone; ALT - Alanine Aminotransferase; BMI - Body Mass Index; CRP - C-Reactive Protein. Mixed linear regression significant at p≤0.05.

Source: Authors.

Discussion

In this study we found a positive association in which higher RDW is associated with higher CVD risk, as measured by FRS, even after adjusting for confounding variables, both in the cross-sectional and longitudinal analyses. However, RDW values could not predict a worsening of CVD risk in the longitudinal trajectory of these individuals over four years.

The cardiovascular risk of participants in the fourth quartile RDW was on average 29% higher compared to those in the first quartile RDW, both at baseline and after four-year follow-up, replicating the result observed in cross-sectional analysis (Table 2). For instance, a RDW≥13.5% would increase the 10-year CVD risk from 10% to 12.9%, which may considerably influence clinical decision making. Shah *et al.*[24] found that RDW values greater than 14.5% were associated with coronary disease death, whereas Sharma and Agrawal[25] reported that an RDW>14% was associated with elevated CRP, non-ST elevation myocardial infarction, and unstable angina. In

our study, we observed an RDW≥13.5% (Q4) to be associated with a higher CVD risk as measured by the 10-year FRS.

The independent association between RDW and CVD has been demonstrated in different studies and population groups. A systematic review and meta-analysis of 80,216 participants and 22 studies from different countries showed that high RDW values are associated with increased risks of mortality and cardiovascular events in patients with coronary heart disease (CHD)[26]. Moreover, diverse studies with large cohorts and 5- to 15-year follow-up showed that RDW is associated with increased risk of myocardial infarction and death from CHD in adults independent of other risk factors[27,28]. A 14-year follow up study of 7,005 healthy subjects classified at baseline, according to the FRS for CHD, found that the addition of RDW to the CHD-FRS helped to reclassify intermediate-risk category to high-risk category of death from CHD. The authors concluded that RDW improved the accuracy of FRS in predicting cardiovascular mortality[24]. Although we have the positive association between higher RDW and higher FRS in the transverse and longitudinal analysis, the interaction term between RDW and CVD risk showed that RDW did not predict a significant worsened CVD risk over the four-year follow-up period. It is possible that the four-year follow-up was insufficient to capture the RDW's influence on FRS over time. The prediction onset for CVD risk can range between 2 and 45 years, with most studies predicting CVD outcomes after an analysis of, on average, 5 to 10 years. Studies investigating an association between RDW and CVD have frequently a follow-up between 1 and 15 years. In addition, the FRS was designed after the analysis of a 10-year time period[29,30].

In the present study, individuals with the highest values of RDW (Q4) also had lower MCV values, higher CRP values and higher prevalence of DM and hypertension, when compared to the other RDW quartiles (Table 2). Evidence suggest that the erythropoiesis may be affected by inflammatory status via the following mechanisms: direct myelosuppression of erythroid precursor cells, decreased renal erythropoietin production, decreased iron bioavailability, increased erythropoietin resistance in erythroid precursor cells leading to impaired erythrocyte maturation, ineffective erythropoiesis and anisocytosis[31]. Oxidative stress may lead to cytoskeletal rearrangement, lipid loss and erythrocyte membrane asymmetry; erythrocytes then become more rigid and start to differ in size, with resulting anisocytosis. Oxidated erythrocytes present increased aggregation and endothelial adhesion, triggering a vicious cycle of oxidative damage and endothelial dysfunction[7].

To reduce the risk of confounding, we adjusted the models for several variables that may potentially affect the risk of cardiovascular events. We also adjusted the analysis for CRP, a biomarker of systemic low-grade inflammation, a potential mediator in the association between RDW and CVD risk. In the model for the longitudinal analysis without CRP, RDW remained independently correlated with FRS (Q4, e^{β}=1,299; p≤0.001). This result shows that even though inflammation has been implicated in anisocytosis, and may directly impact cardiovascular risk, in our study it did not affect the association between RDW and FRS. Hence, anisocytosis *per se* may be directly involved in the pathogenesis of cardiovascular disorders. As state before, deformed erythrocytes, a common finding in anisocytosis, may (1) lead to increased blood viscosity and compromised microcirculatory blood flow; (2) present more aggregation and endothelial adhesion, and (3) contribute to the atherosclerotic process via neutralization of vasodilator mediators and lipid accumulation in atherosclerotic lesions[32].

Being a parameter that is part of the automated blood count, RDW has several advantages, including low intra-individual biological variation, low cost, ease of interpretation, wide availability, and the fact that it does not require specific skills or instrumentation; all of which argue in favor of its use in clinical practice. However, some precautions are necessary to use RDW as a predictor marker. For example, different approaches are used for measuring erythrocyte size (i.e., electrical impedance or optical techniques). Also, there is no universal consensus whether RDW shall be expressed in standard deviation (RDW-SD) or as coefficient of variation (RWD-CV) of erythrocyte volumes[33]. Therefore, the standardization of the analytical method is crucial for use in clinical practice.

Potential confounding factors such as ferritin, vitamin B12, and folate serum levels were not measured and thus not included in the analysis, which might limit our findings. However, vitamin B12 or folate deficiency is unlikely in this study population, since subjects with macrocytosis and anemia were controlled at the models of adjustments. The multicenter nature of the ELSA-Brasil study and the limited stability of whole blood samples required that blood tests be performed in different laboratories in each

ELSA-Brasil investigation centers with different hematologic analyzers. However, all ELSA-Brasil research centers followed the same protocols, complying with the recommendations of the Clinical and Laboratory Standards Institute and the Brazilian Society of Clinical Pathology/Laboratory Medicine for carrying out laboratory tests, in order to minimize analytical and pre-analytical tests. Differences in cell counting instruments and RDW determination between laboratories precluded the inclusion of the entire ELSA-Brasil cohort in this study. Moreover, the follow-up time of the cohort of the present study may have been short since the interval between exposure to risk factors and the development of cardiovascular disease is generally longer.

In this large cohort of adult Brazilians, RDW was independently associated with increased CVD risk as measured by the FRS both at baseline and after four-year follow-up, but did not predict change in the FRS with time. A new assessment, after ten years of follow-up of the ELSA-Brasil cohort, may clarify the relationship between the increase in RDW and the worsening of CVD risk as measured by FRS, and whether time is an important variable in this relationship. Studying this relationship may improve the predictive accuracy of current CVD risk stratification.

Collaborations

NM Carvalho, CB Maluf, SM Barreto and PG Vidigal contributed to the conception or design of the work; acquisition, analysis and interpretation of the data; drafted manuscript and critically revised the manuscript. DRM Azevedo contributed to the design of the work; contributed to analysis and interpretation of the data. RCP Reis and CD Castilhos contributed to analysis and interpretation of the data and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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