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ARTYKUŁ ORYGINALNY / *ORIGINAL ARTICLE*

Association of red blood cell distribution width with plasma lipids in a general population of unselected outpatients

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Abstract

Background and aim: Increased values of red blood cell distribution width (RDW) are frequent in patients suffering from cardiovascular disorders, and are associated with traditional or less conventional risk factors. Nevertheless, limited and controversial information exists on the association between anisocytosis and plasma lipids.

Methods: We performed a retrospective search to retrieve test results of RDW and plasma lipids of unselected outpatients aged 18 years or older referred for routine testing over a six-month period. No restrictive inclusion or exclusion criteria were applied for extracting data of total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, total to HDL-cholesterol ratio (COL/HDL) and atherogenic index of plasma (AIP). Cumulative results were retrieved for 4,874 outpatients (2,150 men, 2,724 women).

Results: A significantly higher median RDW was found in females than in males (13.2 vs. 13.1; $p < 0.01$). After stratification of the study population into RDW quartiles, significant differences were observed in all parameters, except triglycerides and COL/HDL in men. RDW was negatively correlated with haemoglobin and mean corpuscular volume (MCV), and positively associated with age. After comparison of lipid values across RDW quartiles by multivariate logistic regression adjusted for age, haemoglobin and MCV, the RDW was negatively associated with HDL-cholesterol (OR 3.20 in females and OR 1.67 in males), and positively associated with AIP (1.53 in females and 1.43 in males), hypertriglyceridaemia (OR 1.66) and COL/HDL (OR 1.28) in women.

Conclusions: Higher RDW is associated with a globally unfavourable lipid profile, especially in women.

Key words: red blood cell distribution width, cardiovascular risk, lipids, cholesterol

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INTRODUCTION

The red blood cells (RBCs), also known as erythrocytes, have a typical diameter between 6 and 8 μm and a thickness of 2 μm, with a usual mean corpuscular volume (MCV) of nearly 90 femtolitres [fL]. RBC width (RDW) is conventionally defined as the index of the erythrocyte heterogeneity (i.e. anisocytosis), and is calculated by division of standard deviation (SD) of RBC volume by MCV (i.e. [SD of MCV/MCV] × 100) [1].

Although the normal range of RDW is between 11% and 14%, several pathophysiological conditions may substantially influence RBC size and erythrocyte heterogeneity, thus contributing to increases in the RDW [1]. This erythrocyte parameter is widely used in combination with other RBC indices to identify the potential cause of anaemia in clinical and laboratory practice. Increased values have however been recently reported in several cardiovascular disorders such as ischaemic heart disease, acute and chronic heart failure, peripheral occlusive disease, stroke, pulmonary embolism, and pulmonary arterial hypertension, wherein it is also associated with all-cause, cardiac and non-cardiac mortality [2].

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RDW has also been found to be an independent predictor of worse reperfusion in patients with acute myocardial infarction treated with a primary coronary intervention [3], mortality in patients undergoing percutaneous coronary intervention [4], mortality in patients with pulmonary embolism [5], and finally with in-hospital mortality in hospitalised patients [6]. Nevertheless, it still remains unclear whether anisocytosis may be considered the cause, or rather a simple epiphenomenon, of underlying pathological conditions such as inflammation, impaired renal function, oxidative damage and others. Higher RDW has in fact been associated with a variety of traditional cardiovascular risk factors, including high-sensitive C reactive protein (hs-CRP) [7], impaired renal function [8], diabetes [9], hypertension [10], obesity and smoking [11], as well as with obstructive sleep apnoea syndrome [12] and vitamin D deficiency [13], two conditions increasingly associated with cardiovascular disease [14, 15]. Limited and controversial information is available, however, on the association between anisocytosis and plasma lipids in the general population, since the vast majority of the available studies have assessed the relationship between RDW and hypercholesterolaemia, and less frequently with other lipid variables.

Therefore, the aim of this investigation was to assess the existence of potential epidemiological associations between RDW and conventional (i.e. total cholesterol, low density lipoprotein [LDL]-cholesterol, high density lipoprotein [HDL]-cholesterol and triglycerides) or innovative lipid parameters (i.e. total to HDL-cholesterol ratio [COL/HDL] and atherogenic index of plasma [AIP]) in a large Italian population of unselected outpatients.

METHODS

Data extraction

We performed a search on the database of the Laboratory Information System of the Clinical Laboratory of the Academic Hospital of Verona (Italy). We retrieved haematological data as well as test results of plasma lipids of a whole cohort of unselected outpatients aged 18 years or older. All these patients had been referred by general practitioners for routine blood testing over the previous six months (i.e. from January to June 2012). No restrictive inclusion or exclusion criteria were applied for data extraction, since the aim of this study was to assess the relationship between RDW and lipid variables in an unselected population. Venous blood samples were routinely drawn in the morning after an overnight fast. Plasma lipids were assayed by enzymatic methods on a Roche Cobas System (Roche Diagnostics GmbH, Mannheim, Germany), whereas haematological testing was performed on Advia 2120 (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). LDL-cholesterol and AIP were calculated with the Friedewald's formula [16], and the equation log (triglycerides/HDL-cholesterol) [17], respectively. Quality of results throughout the study period was validated by regular internal quality control procedures and participation in an External Quality Assessment Scheme. The study was carried out in accordance with the Declaration of Helsinki and under the terms of all relevant local legislation.

Statistical analysis

The significance of differences was assessed by either a Wilcoxon-Mann-Whitney test or one-way analysis of variance (ANOVA). Multivariable logistic regression analysis was also performed to assess the potential association between RDW, as dependent variable, and the value of each plasma lipid variable, by also entering age, haemoglobin and MCV as covariates in the model. The guidelines from the American Heart Association (AHA) and the American College of Cardiology (ACC) [18], and the US-National Cholesterol Education Programme Adult Treatment Panel III (NCEP-ATP III) [19], were used to define undesirable values of plasma lipid variables. Since the goodness-of-fit tests for normal distribution showed that all parameters were not normally distributed ($p < 0.05$ for all), the median and interquartile range (IQR) were used for descriptive analyses. The statistics was carried out with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK) and MedCalc Version 12.3.0 (MedCalc Software, Mariakerke, Belgium).

RESULTS

Overall, cumulative results for complete haematological testing and plasma lipids were retrieved for 4,874 unselected outpatients aged 18 years or older (2,150 men and 2,724 women). A slightly but significantly higher median RDW value was found in females (13.2; IQR 12.7–13.8%) than in males (13.1; IQR 12.7–13.7%; $p < 0.01$), so that we decided to separate the population into genders for further statistical analysis. Significant differences between genders were also observed for all parameters tested, except for age (Table 1). Moreover, after stratification of the study population into quartiles of RDW presented in Table 1, significant differences in the value distribution of all parameters were observed, except for triglycerides and COL/HDL in men (Table 2). Subjects with RDW in the top quartiles were older than those with a RDW in the lower quartiles. From the bottom to the top quartile of RDW, we observed a significant decrease of haemoglobin, MCV, total cholesterol, LDL-cholesterol, HDL-cholesterol, and AIP in both males and females. The median value of triglycerides and COL/HDL significantly increased among the RDW categories in women, but not in men. A strong, independent relationship was then observed in both genders between RDW and haemoglobin (beta coefficient –0.30; $p < 0.01$ in males; and beta coefficient –0.35; $p < 0.001$ in females), MCV (beta coefficient –0.26; $p < 0.01$ in males, and beta coefficient –0.25; $p < 0.001$ in females) and age (beta coefficient 0.09; $p < 0.01$ in males, and beta coefficient 0.06; $p < 0.001$ in females).

In multivariable logistic regression after adjustment for age, values of haemoglobin and MCV, the RDW was still

Table 1. Between-gender comparison of haematological and lipid parameters in a large population of unselected outpatients

	Males (median and IQR)	Females (median and IQR)	P
N	2,150	2,724	
Age [years]	63 (49–72)	63 (48–72)	0.112
Haemoglobin [g/L]	148 (138–156)	135 (127–142)	< 0.01
MCV [fL]	92 (89–95)	91 (88–94)	< 0.01
RDW [%]	13.1 (12.7–13.7)	13.2 (12.7–13.8)	< 0.01
Total cholesterol [mmol/L]	4.90 (4.14–5.62)	5.26 (4.56–6.01)	< 0.01
LDL-cholesterol [mmol/L]	2.88 (2.24–3.54)	3.00 (2.42–3.69)	< 0.01
HDL-cholesterol [mmol/L]	1.37 (1.14–1.63)	1.66 (1.35–1.97)	< 0.01
Triglycerides [mmol/L]	1.10 (0.81–1.50)	1.02 (0.75–1.38)	< 0.01
COL/HDL (ratio)	3.52 (2.88–4.30)	3.14 (2.59–3.83)	< 0.01
AIP (ratio)	–0.09 (–0.28 to 0.11)	–0.21 (–0.40 to –0.02)	< 0.01

IQR — interquartile range; MCV — mean corpuscular volume; RDW — red blood cell distribution width; LDL — low density lipoprotein; HDL — high density lipoprotein; COL/HDL — total to HDL-cholesterol ratio; AIP — atherogenic index of plasma

significantly associated with HDL-cholesterol, triglycerides, COL/HDL and AIP in women, as well as with HDL-cholesterol and AIP in men (Table 3). We thereby calculated the RDW-attributable risk for undesirable levels of these variables according to well established guidelines of the AHA/ACC [18] and NCEP [19] (i.e. HDL-cholesterol ≤ 1.04 mmol/L, hypertriglyceridaemia ≥ 1.7 mmol/L, COL/HDL ≥ 3.5 and AIP > 0). The resulting odds ratio (OR) and 95% confidence interval (95% CI) of fourth vs. first RDW quartile were 3.20 (95% CI 2.04–5.01; $p < 0.01$) in women and 1.67 (95% CI 1.20–2.33; $p < 0.01$) in men for low HDL-cholesterol, 1.53 (95% CI 1.19–1.97; $p < 0.01$) in women and 1.43 (95% CI 1.11–1.84; $p < 0.01$) in men for undesirable AIP value, 1.66 (95% CI 1.24–2.23; $p < 0.01$) for hypertriglyceridaemia and 1.28 (95% CI 1.03–1.61; $p = 0.03$) for increased COL/HDL in women.

DISCUSSION

Irrespective of a causal or casual (i.e. epidemiological) relationship [20], increased RDW values are widely acknowledged to be a hallmark of several cardiovascular disorders, wherein it also represents a significant predictor of cardiovascular and all-cause mortality [21, 22]. High RDW has been associated with conventional cardiovascular risk factors. Nonetheless, scarce and even controversial information has been provided on the relationship between this important RBC index and traditional or innovative lipid parameters in the general population [2].

In the present study, which we believe to be one of the largest ever published on RDW in non-hospitalised populations, we have found convincing interactions between increased RDW and low HDL-cholesterol values in both genders. Additional positive relationships were found with AIP in both genders, as well as with hypertriglyceridaemia and increased COL/HDL in women. In an earlier trial, involving 4,111 subjects with coronary disease free of heart failure at baseline, Tonelli et al. [22] reported lower values of total and LDL-cholesterol in the highest compared to the lowest RDW quartile, whereas HDL-cholesterol values did not differ among quartiles of RDW. In multivariable linear regression analysis, however, none of these plasma lipids remained significantly associated with RDW. In agreement with our findings, Förhécz et al. [23] found a strong, inverse association between tertiles of RDW and total cholesterol in patients with systolic heart failure, which disappeared in the fully adjusted multiple linear regression model (beta coefficient 0.135; $p = 0.137$). Similarly, a lower prevalence of hypercholesterolaemia has also been reported by Perlstein et al. [11] in 15,852 participants of the National Health and Nutrition Examination Survey (NHANES) III, by Chen et al. [24] in a 3,226 member community cohort in Taiwan, and by Bonaque et al. [25] in 698 consecutive outpatients with chronic heart failure, although none of these studies adjusted the RDW values for other potentially confounding variables. Interestingly, and in agreement with our findings, Çetin et al. [26] observed that HDL-cholesterol was lower in patients with coronary atherosclerotic disease than those with normal coronary arteries, while RDW values were higher in patients with cardiovascular disease than those without, independently from nonspecific inflammation and circulating inflammatory cells. It is then noteworthy that, at variance with these findings, Zalawadiya et al. [27] reported increased values of total and HDL-cholesterol across RDW quartiles in a more recent analysis of the NHANES III, including 15,460 subjects.

Our results have some meaningful implications. First, we have confirmed that age, haemoglobin and MCV may be strong determinants of RDW in the general population. Therefore, the results of epidemiological studies assessing this parameter in health and disease should be always adjusted for these variables in order to produce meaningful information. We have also found that the RDW values are significantly different between genders, and are significantly and inde-

Table 2. Laboratory tests results stratified according to quartiles of RDW in a large population of unselected outpatients

Quartiles of RDW	1st	2nd	3rd	4th	P
N					
Males	575	544	545	486	
Females	702	750	641	631	
Age [years]					
Males	56 (46–68)	61 (48–70)	65 (54–73)	69 (57–74)	< 0.01
Females	60 (45–69)	63 (49–72)	65 (53-73)	64 (47-75)	< 0.01
Haemoglobin [g/L]					
Males	151 (143–157)	151 (143–157)	148 (139–156)	138 (125–149)	< 0.01
Females	137 (131–143)	137 (131–144)	135 (129–142)	125 (116–135)	< 0.01
MCV [fL]					
Males	91.9 (89.4–94.9)	92.0 (89.0–95.0)	92.0 (89.4–94.8)	91.0 (85.7–94.8)	< 0.01
Females	91.8 (89.6–94.5)	91.5 (88.9–94.2)	90.9 (88.1–93.7)	88.5 (83.5–92.8)	< 0.01
Total cholesterol [mmol/L]					
Males	5.05 (4.30–5.72)	4.92 (4.20–5.65)	4.90 (4.20–5.62)	4.60 (3.83–5.49)	< 0.01
Females	5.41 (7.74–6.04)	5.31 (4.66–6.06)	5.33 (4.61–6.11)	4.97 (4.25–5.70)	< 0.01
LDL-cholesterol [mmol/L]					
Males	3.02 (2.38–3.66)	2.93 (2.28–3.53)	2.89 (2.27–3.56)	2.63 (1.94–3.35)	< 0.01
Females	3.10 (2.51–3.73)	3.06 (2.46–3.78)	3.08 (2.48–3.75)	2.80 (2.26–3.39)	< 0.01
HDL-cholesterol [mmol/L]					
Males	1.40 (1.19–1.66)	1.37 (1.14–1.63)	1.35 (1.13–1.64)	1.34 (1.09–1.58)	0.04
Females	1.71 (1.40–2.02)	1.71 (1.42–2.00)	1.63 (1.32–2.00)	1.53 (1.22–1.86)	< 0.01
Triglycerides [mmol/L]					
Males	1.08 (0.80–1.43)	1.09 (0.82–1.52)	1.11 (0.82–1.52)	1.14 (0.82–1.57)	0.11
Females	0.97 (0.70–1.32)	0.98 (0.72–1.35)	1.04 (0.78–1.49)	1.05 (0.81–1.45)	< 0.01
COL/HDL (ratio)					
Males	3.51 (2.94–4.37)	3.59 (2.89–4.30)	3.51 (2.87–4.31)	3.42 (2.78–4.27)	0.82
Females	3.08 (2.56–3.78)	3.09 (2.55–3.77)	3.19 (2.65–3.92)	3.22 (2.59–3.90)	0.01
AIP (ratio)					
Males	–0.12 (–0.30 to –0.07)	–0.10 (–0.28 to –0.11)	–0.10 (–0.28 to –0.12)	–0.06 (-0.25 to –0.14)	0.04
Females	–0.25 (–0.43 to–0.05)	–0.24 (–0.42 to –0.05)	–0.18 (–0.39 to 0.02)	–0.16 (–0.34 to 0.03)	< 0.01

RDW — red blood cell distribution width; MCV — mean corpuscular volume; LDL — low density lipoprotein; HDL — high density lipoprotein; COL/HDL — total to HDL-cholesterol ratio; AIP — atherogenic index of plasma

pendently associated with four lipid parameters in women, and two lipid variables in men, thus contributing to generate a globally unfavourable lipid profile, especially in women. This is important information, inasmuch as the vast majority of the studies that have assessed the biological role and the prognostic significance of RDW in cardiovascular disorders have not partitioned the population into genders, although the relative weight of the different cardiovascular risk factors as well as the potential causes of anisocytosis are rather different between males and females. It is hence noteworthy that therapeutic interventions aimed at correcting RDW-associated cardiovascular risk may require different strategies, wherein the association of increased RDW with hypertriglyceridaemia and increased COL/HDL values seem a prerogative of the female gender.

We are aware, however, that there may be some limitations to this study. In fact, these findings are limited to the cohort studied here, which included non-hospitalised European adults, and may hence not be entirely applicable to other ethnic origins. Moreover, the results could only be standardised for age and gender, but not for other demographic or clinical variables, so it remains to be established whether the relationship between anisocytosis and blood lipids is causal or rather an epiphenomenon.

Table 3. Multivariate regression analysis (standardised beta coefficient and statistical significance) between red blood cell distribution width and values of plasma lipids in a large population of unselected outpatients after adjustment for age, haemoglobin and mean corpuscular volume

	Males	Females
Total cholesterol	–0.01; p = 0.60	–0.03; p = 0.07
LDL-cholesterol	–0.01; p = 0.43	–0.02; p = 0.38
HDL-cholesterol	–0.13; p = 0.01	–0.28; p < 0.01
Triglycerides	0.11; p = 0.07	0.26; p < 0.01
COL/HDL	0.05; p = 0.08	0.11; p < 0.01
AIP	0.24; p < 0.01	0.62; p < 0.01

LDL — low density lipoprotein; HDL — high density lipoprotein; COL/HDL — total to HDL-cholesterol ratio; AIP — atherogenic index of plasma

CONCLUSIONS

Higher RDW is associated with a globally unfavourable lipid profile, especially in women.

Conflict of interest: *none declared*

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Zależność między szerokością rozkładu krwinek czerwonych a stężeniem lipidów w osoczu w niewyselekcjonowanej grupie pacjentów leczonych ambulatoryjnie

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Streszczenie

Wstęp i cel: Zwiększona szerokość rozkładu krwinek czerwonych (RDW) często występuje u osób z chorobami sercowo-naczyniowymi i wiąże się z tradycyjnymi lub mniej konwencjonalnymi czynnikami ryzyka. Jednak dane na temat zależności między anizocytozą i stężeniem lipidów w surowicy są nieliczne i często sprzeczne.

Metody: Autorzy wyszukali w sposób retrospektywny wyniki pomiarów RWD i oznaczeń lipidów w osoczu w niewyselekcjonowanej grupie chorych leczonych ambulatoryjnie, których kierowano na rutynowe badania w okresie 6 miesięcy. Nie przyjęto ścisłych kryteriów włączenia ani wykluczenia podczas wyszukiwania danych dotyczących stężeń cholesterolu całkowitego, cholesterolu frakcji LDL, cholesterolu frakcji HDL, współczynnika cholesterolu całkowitego do frakcji HDL (COL/HDL) i wskaźnika aterogenności osocza (AIP). Łącznie zgromadzono dane 4874 osób (2150 mężczyzn).

Wyniki: U kobiet mediana RDW była istotnie większa niż u mężczyzn (13,2 *vs*. 13,1; $p < 0,01$). Po stratyfikacji badanej populacji na kwartyle RDW stwierdzono istotne różnice w zakresie wszystkich parametrów, z wyjątkiem triglicerydów i współczynnika COL/HDL u mężczyzn. Wykazano istnienie odwrotnej zależności między RDW a stężeniem hemoglobiny i średnią objętością krwinek czerwonych (MCV) oraz prostej zależności między RDW a wiekiem. Po porównaniu wartości lipidów między poszczególnymi kwartylami RDW metodą wieloczynnikowej regresji logistycznej i skorygowaniu danych względem wieku, stężenia hemoglobiny i MCV stwierdzono odwrotną zależność między RDW a stężeniem cholesterolem frakcji HDL (OR 3,20 u kobiet i OR 1,67 u mężczyzn) oraz prostą zależność między RDW a AIP (1,53 u kobiet i 1,43 u mężczyzn), hipertriglicerydemią (OR 1,66) i współczynnikiem COL/HDL (OR 1,28) u kobiet.

Wnioski: Autorzy podsumowali, że większa wartość RDW wiąże się ogólnie z niekorzystnym profilem lipidowym, zwłaszcza u kobiet.

Słowa kluczowe: szerokość rozkładu krwinek czerwonych, ryzyko sercowo-naczyniowe, lipidy, cholesterol

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