Red cell distribution width and its polygenic score in relation to mortality and cardiometabolic outcomes

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Introduction: Elevated red cell distribution width (RDW) has been associated with a range of health outcomes. This study aims to examine prognostic and etiological roles of RDW levels, both phenotypic and genetic predisposition, in predicting cardiovascular outcomes, diabetes, chronic kidney disease (CKD) and mortality.

Methods: We studied 27,141 middle-aged adults from the Malmö Diet and Cancer study (MDCS) with a mean follow up of 21 years. RDW was measured with a hematology analyzer on whole blood samples. Polygenic scores for RDW (PGS-RDW) were constructed for each participant using genetic data in MDCS and published summary statistics from genome-wide association study of RDW ($n = 408,112$). Cox proportional hazards regression was used to assess associations between RDW, PGS-RDW and cardiovascular outcomes, diabetes, CKD and mortality, respectively.

Results: PGS-RDW was significantly associated with RDW (Pearson's correlation coefficient = 0.133, $p < 0.001$). RDW was significantly associated with incidence of stroke (hazard ratio (HR) per 1 standard deviation = 1.06, 95% confidence interval (CI): 1.02–1.10, $p = 0.003$), atrial fibrillation (HR = 1.09, 95% CI: 1.06–1.12, $p < 0.001$), heart failure (HR = 1.13, 95% CI: 1.08–1.19, $p < 0.001$), venous thromboembolism (HR = 1.21, 95% CI: 1.15–1.28, $p < 0.001$), diabetes (HR = 0.87, 95% CI: 0.84–0.90, $p < 0.001$), CKD (HR = 1.08, 95% CI: 1.03–1.13, $p = 0.004$) and all-cause mortality (HR = 1.18, 95% CI: 1.16–1.20, $p < 0.001$). However, PGS-RDW was significantly associated with incidence of diabetes (HR = 0.96, 95% CI: 0.94–0.99, $p = 0.01$), but not with any other tested outcomes.

Discussion: RDW is associated with mortality and incidence of cardiovascular diseases, but a significant association between genetically determined RDW and incident cardiovascular diseases were not observed. However, both RDW and PGS-RDW were inversely associated with incidence of diabetes, suggesting a putative causal relationship. The relationship with incidence of diabetes needs to be further studied.

Abbreviations
AF, Atrial fibrillation; CE, Coronary events; CI, Confidence interval; CKD, Chronic kidney disease; GWAS, Genome-wide association studies; HF, Heart failure; HR, Hazard ratio; MDCS, The Malmö Diet and Cancer study; MI, Myocardial infarction; PGS, Polygenic score; RDW, Red cell distribution width; VTE, Venous thromboembolism.

Introduction

Red cell distribution width (RDW) is a measure of the heterogeneity of the red cell volumes, which is commonly used for subclassifying different types of anaemia clinically. High RDW has been associated with several adverse health events, such as incidence of stroke (1), coronary events (CE) (2, 3), diabetes (4) and all-cause mortality (5) in general populations. Several possible explanations for the relationship between RDW and health outcomes have been proposed, including inflammation (6), oxidative stress and altered erythrocytes survival (7, 8). Nevertheless, owing to the inherent limitations of observational studies, the causal relationships between RDW and health outcomes remain uncertain.

In the past decades, large-scale genome-wide association studies (GWAS) have identified hundreds of genetic variants that are associated with cardiometabolic diseases (9) and with RDW (10, 11). Based on that, Mendelian randomization (MR) studies are able to conduct examinations of the causal relationship between RDW, venous thromboembolism (VTE) (12) and haemorrhagic strokes (13). However, the application of MR analysis has often been limited to genome-wide significant variants, potentially leading to decreased statistical power due to the relatively small contribution of individual genetic variants in explaining the variance of complex traits (14). On the other hand, polygenic score (PGS), which aggregate the cumulative effects of many genetic variants, is able to capture an increased probability of disease with reasonable statistical power (14–16). PGS is becoming popular in predicting complex diseases such as type-2 diabetes, coronary artery disease and inflammatory bowel disease (17). Association between PGS for RDW and cardiovascular diseases has also been examined in an interesting cross-sectional study suggesting the possibility of using genetic susceptibility to infer causal relationships (18).

Our objective was to investigate the relationship between phenotypically measured and genetically determined RDW and various health outcomes, including diabetes, incidence of chronic kidney disease (CKD), a range of cardiovascular diseases such as atrial fibrillation (AF), stroke, myocardial infarction (MI), heart failure (HF), VTE, and all-cause mortality. We conducted our research using data from a general population study, the Malmö Diet and Cancer study (MDCS, $n = 27,141$) with a follow-up period of up to 27 years.

Methods

Study population

The Malmö Diet and Cancer study (MDCS) is a large prospective cohort study from the city of Malmö, Sweden. During 1991–1996, all men born 1923–1945 and women born 1923–1950 living in the city of Malmö were invited in the screening center to participate in the study. The overall participation rate was 40.8% (19). A total of 30,446 participants underwent the baseline examinations. Here, we excluded subjects with missing genotype data, resulting in 28,977 subjects included. Additionally, subjects with missing information on RDW ($n = 1,820$) or outliers of RDW were excluded ($n = 16$). In total, 27,141 subjects were included in the final study sample for analysing associations with incidence of CE, stroke, AF, HF, VTE, CKD, diabetes and mortality. Since diabetes or a CVD event potentially could affect RDW and life span of the red cells (8), we excluded individuals with endpoints before the baseline examination, resulting in slightly different numbers for the various outcomes (range 25,929–27,141) (**Supplementary Figure 1**: study flow chart for study selection).

A study of representativity and mortality in participants and non-participants has been published. Prevalence of smoking and obesity was comparable in MDCS and another study from the same city—a postal survey with participation rate of 75% (20). However, the mortality rates were lower in MDCS participants than in non-participants, suggesting that MDCS is a comparably healthy cohort (19).

All participants provided and signed written consent and the study conforms to the principles of the Declaration of Helsinki. This study was approved by the Ethical Committee at Lund University (LU 51–90, LU 2009/633, LU 2011/356).

Baseline examinations and red cell distribution width measurements

The baseline information was collected using extensive questionnaires, blood sample collection and physical examination. Standing height and weight were recorded with light clothing and no shoes. BMI was calculated as weight/height^2 (kg/m^2). Non-fasting blood samples were taken during the baseline examinations. Serum and plasma were separated within one hour and stored at −80°C until the analyses. RDW-SD was used in this study, measured in fresh and heparinized blood by a Sysmex K1000 counter (www.sysmex.com). RDW-SD was defined as the width (fL) of the red cell distribution curve at the height of 20% above the baseline (21). Smoking status was ascertained from self-administered questionnaires, which were divided into three groups (regular or occasional smokers, non-smokers and missing). Apolipoprotein A1 (ApoA1) and B (ApoB) were analysed using Quest Diagnostics (San Juan Capistrano, CA). Fasting plasma glucose (FPG) was measured using HemoCue (HemoCue AB, Ängelholm, Sweden). HbA1c was measured by ion exchange chromatography, using the Swedish Mono-S standardization system. Baseline prevalent diabetes was collected by self-reported history of use of anti-diabetic medication or physician's diagnosis of diabetes from questionnaire.

Genotyping and quality control of the Malmö diet and cancer study

The subjects included in the MDCS were genetically tested using Illumina GSA v1 genotyping array. Prior to imputation, quality controls on the genotyped single nucleotide polymorphism (SNP) were conducted by removing low quality variants [mismatched

probe, minor allele frequency (MAF) ≤ 0.01, incorrect assignment of allelic variant, failed genotype calling or p-value from Hardy-Weinberg Equilibrium test less than 1×10^{-15}]. Samples exhibiting evidence of gender mismatch or possessing an overall sample call rate below 90% were excluded from the analysis. Population structure was characterized using principal component analysis (PCA) by implementing the FlashPCA (22) on the genotyped data. Data were imputed with reference panel of Haplotype Reference Consortium (HRC r1.1) using the Michigan Imputation Server (23).

Polygenic scores (PGS) for RDW

A Bayesian regression framework PRS-continuous shrinkage (PRS-CS) (24) was conducted to build PGS for RDW. Restricted to common variants (MAF > 0.05) that are biallelic, PRS-CS was used to infer posterior effect sizes of SNP which were based on linkage disequilibrium reference panel of HapMap3 in a European sample and GWAS summary statistics on RDW (n = 408,112) (25), where age, sex and principal components (PCs) were adjusted in the GWAS analyses. Using the PLINK (version 1.90) (26), PGS for RDW were constructed for 28,977 MDCS samples by aggregating the obtained posterior effect sizes for 1,009,029 SNPs. PGS for RDW is a relative measure in arbitrary units. High PGS for RDW represent high RDW levels.

Endpoint ascertainment

The outcomes of interest in this study were, incidence of CKD, diabetes, cardiovascular outcomes (including HF, CE, stroke, AF, VTE) and all-cause mortality. All participants were followed from the baseline examinations until the event of interest, emigration, or last follow-up date (31st December 2018), whichever came first. For VTE, the participants were followed up till 31st December 2013.

In this study, information regarding all-cause mortality was retrieved from the Swedish Cause of Death Register (CDR) (27), which covers all deaths among Swedish citizens. Death certificates with information about underlying causes of death were written by a registered physician and coded according to the *International Classification of Diseases*, 9th and 10th revision (ICD-9 and ICD-10) codes.

Information regarding incident CE, stroke, AF, HF, CKD, or VTE were obtained from national patient registers. The Swedish inpatient register has been in operation during the follow-up period and data from this registry has been reported to have acceptable validity for epidemiological research (28). For incident CE, data linkage with the Swedish patient register and the CDR were used to retrieve cases (27, 28). Incidence of CE were defined as fatal or non-fatal MI (ICD-9 code 410 or ICD-10 code I21) or death due to ischemic heart disease (ICD-9 codes 410-414; ICD-10 codes I21-I25).

Stroke was defined as codes 430, 431,434 or 436 (ICD-9), or I60-61 or I63–64 (ICD-10). Ischemic stroke was defined as 434 (ICD-9) or I63 (ICD-10) (28, 29).

The diagnostic code for AF was 427D for ICD-9 and I48 for ICD-10 (30, 31). HF was defined as a primary diagnosis of ICD-9 code 428 or ICD-10 code I50 (32). VTE included deep vein thrombosis (DVT) and pulmonary embolism (PE), which was defined as ICD-9 code: 415B (PE), and 451 (DVT); and ICD-10 code: I26 (PE), and I80 (DVT) as the primary diagnosis (33).

Incident CKD was defined as code: 585-586 (ICD-9) and N18 and N19 (ICD-10). Incidence of CKD was retrieved from Swedish patient register (34) and the Swedish renal registry was searched for any additional CKD cases (35).

Incident diabetes was defined using several registers, including the Malmö HbA1c register, the Swedish national Diabetes register, the Swedish patient register, the CDR and the Swedish drug prescription register which have been described previously (36).

Statistical analysis

Means ± standard deviations (SDs) were reported for continuous variables and numbers with percentages were reported for categorical variables. The Pearson and Spearman correlation were performed for the correlations of two continuous variables. Cox regression models were implemented to obtain hazard ratios (HR) with 95% confidence interval (CI) per 1-SD increase of RDW and PGS for RDW, respectively. Adjustments were kept to a minimum and limited to a few key confounders known from the literature (Model 1 was crude model; model 2 was additionally adjusted for age, sex, smoking status, BMI, and diabetes). For PGS for RDW, the models were additionally adjusted for the first 5 principal components (PCs) of the population structure, since genetic ancestry may explain associations between variants and a specific phenotype. Interactions between age and sex and RDW or PGS-RDW, respectively, were tested using multiplicative interaction terms in Cox regressions with adjustments in model 2.

A p value <0.05 was considered significant. All statistical analyses were carried out using SPSS V.28 (IBM, Armonk, New York, USA).

Results

Baseline characteristics of study population

Baseline characteristics across the quartiles of PGS-RDW are shown in **Table 1**. Across the quartiles of PGS-RDW, we found that there were significant relationships between PGS-RDW and RDW, haemoglobin, Apo A1, and HbA1c. The distribution of RDW and PGS-RDW in this study is illustrated in **Supplementary Figures 2**, **3**. From the figure, we could see that PGS-RDW was normally distributed and associated with RDW in the current study.

The correlation coefficients between RDW and PGS-RDW, respectively, and other risk factors are presented in **Table 2**. The Pearson's correlation of PGS-RDW and RDW were 0.133, $p < 0.001$. RDW was significantly correlated to age, BMI, HbA1c and glucose. There was a significant correlation between PGS-RDW and HbA1c ($r = 0.101$, $p < 0.001$, $n = 5,186$) and BMI ($r =$

TABLE 1 The characteristics of study population across the quartile of PGS-RDW.

	Total	Q1	Q2	Q3	Q4	p-for trend
Participants (numbers)	27,141	6,785	6,785	6,786	6,785	
RDW (fL)	40.7 ± 3.40	40.2 ± 3.41	40.6 ± 3.32	40.9 ± 3.40	41.3 ± 3.36	<0.001
PGS-RDW	−0.06 ± 0.46	−0.64 ± 0.24	−0.20 ± 0.09	0.09 ± 0.09	0.52 ± 0.22	<0.001
Age (years)	58.2 ± 7.61	58.3 ± 7.59	58.3 ± 7.63	58.0 ± 7.62	58.2 ± 7.61	0.302
Male sex (n, %)	10,692, 39.4	2,707, 39.9	2,629, 38.7	2,620, 38.6	2,736, 40.3	0.106*
BMI (kg/m^2)	25.7 ± 3.97	25.7 ± 3.97	25.7 ± 3.92	25.7 ± 3.97	25.8 ± 4.03	0.135
Diabetes (n, %)	827, 3.0	211, 3.1	214, 3.2	199, 2.9	203, 3.0	0.867*
Smoking (n, %)	7,562 (27.9)	1,879 (27.7)	1,844 (27.2)	1,963 (28.9)	1,876 (27.6)	0.263
Hemoglobin (mg/dl)	141.8 ± 12.1	142.7 ± 12.2	141.9 ± 12.1	141.4 ± 12.0	141.1 ± 11.9	<0.001
Anemia (n, %)	807 (3.0)	181 (2.7)	178 (2.6)	213 (3.1)	235 (3.5)	0.010
Apo A1 (mg/dl)	156.8 ± 28.2	155.8 ± 27.6	156.9 ± 28.3	157.4 ± 28.6	157.2 ± 28.3	0.008
Apo B (mg/dl)	107.2 ± 26.1	107.3 ± 26.0	107.2 ± 26.0	107.7 ± 25.9	106.5 ± 26.4	0.066
HbA1c (%, n = 5,189)	4.8 (4.5, 5.1)	4.7 (4.4, 5.0)	4.8 (4.5, 5.1)	4.8 (4.5, 5.1)	4.9 (4.6, 5.2)	<0.001
Glucose (mmol/L, n = 5,193)	4.9 (4.6, 5.3)	4.9 (4.6, 5.3)	4.9 (4.6, 5.3)	4.9 (4.6, 5.3)	4.9 (4.6, 5.3)	0.379

The results were presented as means ± standard deviations for continuous variable and numbers, percentages for categorical variables.
Q, quartile; RDW, red cell distribution width; PGS, polygenic score; BMI, body mass index; Apo A1, Apolipoprotein A1; Apo B, Apolipoprotein B; HbA1c, hemoglobin A1c.
*The results were obtained by Chi-Square.

TABLE 2 The correlations between PGS-RDW, RDW and several factors.

Variables		RDW	Age	BMI	Hemoglobin	Apo A1	Apo B	HbA1c	Glucose
Numbers		n = 27,141	n = 27,141	n = 27,099	n = 27,007	n = 26,659	n = 26,656	n = 5,186	n = 5,193
PGS-RDW	R1	0.133	−0.004	0.014	−0.059	0.013	−0.012	0.101	0.003
	P	<0.001	0.461	0.024	<0.001	0.028	0.044	<0.001	0.808
	R2	0.135	−0.005	0.013	−0.054	0.016	−0.012	0.149	−0.013
	P	<0.001	0.368	0.035	<0.001	0.011	0.058	<0.001	0.353
RDW	R1	–	0.118	−0.118	−0.053	0.121	−0.066	0.051	−0.088
	P	–	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
	R2	–	0.112	−0.124	−0.054	0.123	−0.066	0.190	−0.071
	P	–	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

R$_1$, Pearson's correlation coefficient; R$_2$, Spearman's correlation coefficient; RDW, red cell distribution width; PGS, polygenic score; BMI, body mass index; Apo A1, Apolipoprotein A1; Apo B, Apolipoprotein B; HbA1c, hemoglobin A1c.

0.014, $p = 0.024$), but no significant correlation with fasting glucose ($r = 0.003$, $p = 0.808$).

Cardiometabolic outcomes predicted by PGS-RDW and RDW

Elevated RDW was significantly associated with incidence of stroke, AF, HF, VTE, CKD and all-cause mortality. These relationships remained significant after additional adjustments for risk factors (age, sex, smoking status, BMI, and diabetes) (Table 3 and Figure 1). RDW was inversely associated with incidence of diabetes (adjusted HR: 0.87, 95% CI: 0.84–0.90, $p < 0.001$).

For PGS-RDW, we found an inverse association with incidence of diabetes [adjusted HR: 0.96 (95% CI: 0.94–0.99), $p = 0.011$ in model 2] (adjusted for age, sex, PCs 1–5, smoking status, BMI) (Table 3 and Figure 1). PGS-RDW was not significantly associated with stroke, AF, VTE, HF, CKD or mortality after full adjustments (Table 3 and Figure 1).

The sex-specific relationships between RDW, PGS-RDW and outcomes are presented in Supplementary Table 1. There was a significant interaction between RDW and sex with respect to mortality, indicating a stronger relationship in men. For incidence of diabetes, the relationship with RDW was stronger in women (Supplementary Table 1). We also tested the age-specific relationships between RDW, PGS-RDW and outcomes. There were significant interactions between RDW and age with respect to mortality and incidence of diabetes. The associations with mortality were stronger in individuals aged <60 years, while the reduced risk of diabetes was stronger in individuals aged ≥60 years (Supplementary Table 2).

Sensitivity analyses

As PGS-RDW was significantly associated with HbA1c, we further examined the relationships between PGS-RDW and incidence of diabetes after excluding diabetes cases whose diabetes diagnosis was based on high HbA1c values in the HbA1c register. The results showed that RDW and PGS-RDW were still significantly associated with incident diabetes: adjusted HR per 1 SD increase in PGS-RDW: 0.96, 95% CI: 0.92–0.99, $p = 0.013$; HR per 1 SD increase in RDW: 0.89, 95% CI: 0.85–

TABLE 3 The associations between PGS-RDW, RDW and mortality and cardiometabolic outcomes in the MDCS.

Outcomes (events/individuals, n/n)	Per 1 SD increase of PGS-RDW[a]	Per 1 SD increase of RDW[b]
All-cause mortality (11,179/27,141)		
HR (95% CI) Model 1	1.01 (0.99–1.03)	1.31 (1.29–1.33)
HR (95% CI) Model 2	1.01 (0.99–1.03)	1.18 (1.16–1.20)
Incidence of CE (3,384/26,621)		
HR (95% CI) Model 1	1.02 (0.99–1.06)	1.10 (1.07–1.14)
HR (95% CI) Model 2	1.02 (0.99–1.06)	1.03 (0.99–1.06)
Incidence of stroke (3,124/26,846)		
HR (95% CI) Model 1	0.99 (0.96–1.03)	1.15 (1.11–1.19)
HR (95% CI) Model 2	0.99 (0.96–1.03)	1.06 (1.02–1.10)
Incidence of AF (5,195/26,867)		
HR (95% CI) Model 1	1.00 (0.97–1.03)	1.12 (1.09–1.15)
HR (95% CI) Model 2	1.00 (0.97–1.02)	1.09 (1.06–1.12)
Incidence of CKD (1,761/27,134)		
HR (95% CI) Model 1	1.00 (0.96–1.05)	1.10 (1.04–1.15)
HR (95% CI) Model 2	1.00 (0.96–1.05)	1.08 (1.03–1.13)
Incidence of HF (1,992/27,062)		
HR (95% CI) Model 1	0.99 (0.95–1.03)	1.21 (1.16–1.26)
HR (95% CI) Model 2	0.99 (0.95–1.03)	1.13 (1.08–1.19)
Incidence of diabetes (4,427/25,929)		
HR (95% CI) Model 1	0.97 (0.94–1.00)	0.85 (0.82–0.88)
HR (95% CI) Model 2	0.96 (0.94–0.99)	0.87 (0.84–0.90)
Incidence of VTE (1,332/26,943)		
HR (95% CI) Model 1	1.00 (0.95–1.06)	1.23 (1.17–1.30)
HR (95% CI) Model 2	1.00 (0.95–1.06)	1.21 (1.15–1.28)

RDW, red cell distribution width; PGS, polygenic score; HR, hazard ratio; CI, confidence interval; CE, coronary events; AF, atrial fibrillation; CKD, chronic kidney disease; HF, heart failure; VTE, venous thromboembolism.
[a]Model 1 was adjusted for PC1-5; Model 2 was additionally adjusted for age, sex, BMI, smoking and diabetes.
[b]Model 1 was crude model; Model 2 was additionally adjusted for age, sex, BMI, smoking and diabetes.

0.92, $p < 0.001$. As anaemia is a risk factor for RDW and cardiometabolic disease, we also additionally adjusted for anaemia in the last models of associations between RDW, PGS-RDW and cardiometabolic diseases, but the results were almost unchanged (data not shown).
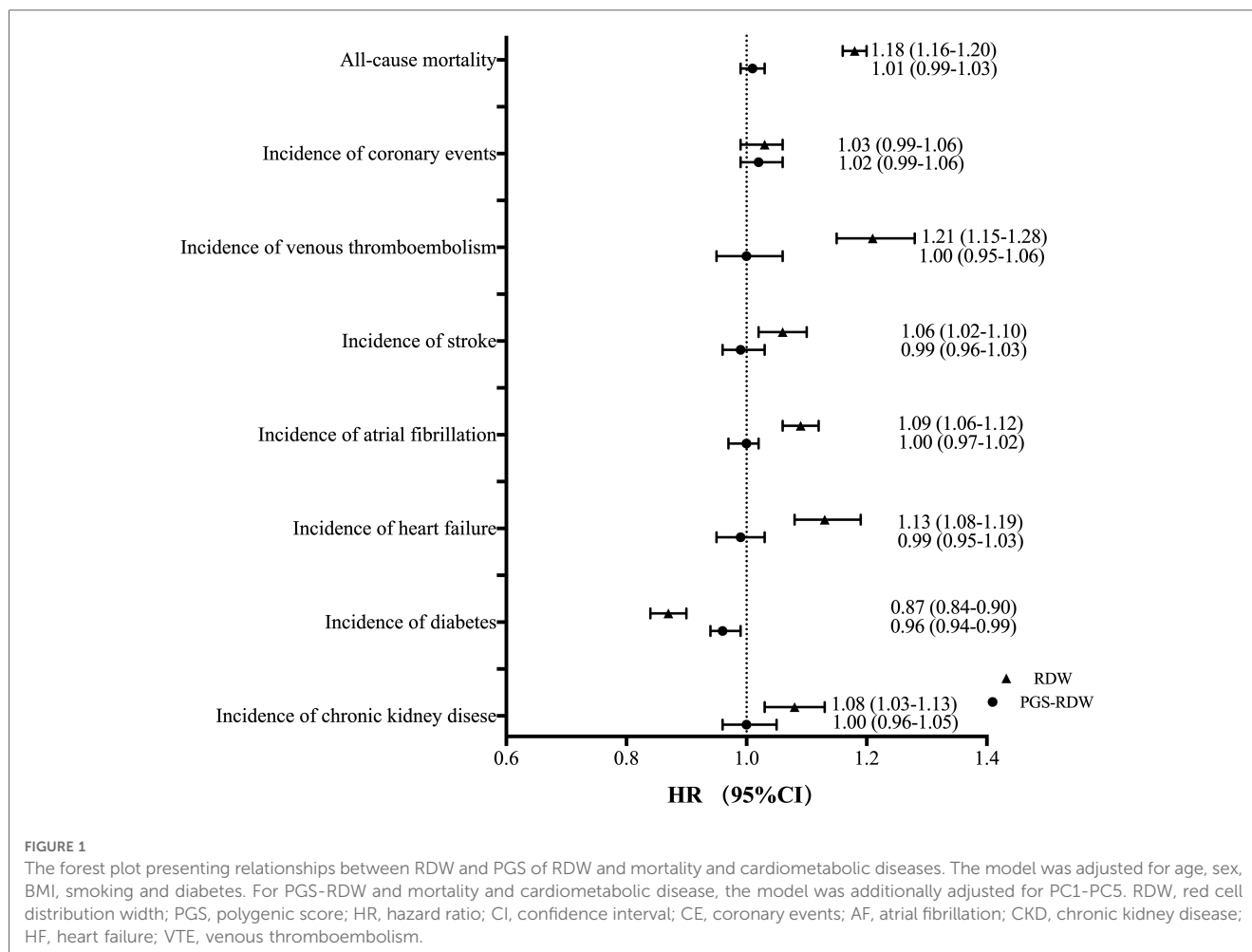
Discussion

RDW has been related to risk of cardiometabolic diseases and mortality in several previous studies (1, 4, 5), but possible etiological relations between RDW and cardiometabolic diseases remain unclear. We found significant relationships between RDW and incidence of several adverse events, such as incidence of CKD, CVDs and all-cause mortality in the MDCS. Interestingly, with exception of diabetes, PGS-RDW was not significantly associated with incidence of these outcomes. For incidence of diabetes, there was a significant inverse association with both RDW and PGS-RDW after adjustments for potential risk factors. The results show that RDW is an unspecific risk marker for various adverse health outcomes but is probably not causally related to mortality and CVD. However, the nature of

the inverse relationship between RDW and incidence of diabetes is of interest and warrants further studies.

Several observational studies have found that RDW is independently associated with CVDs, including MI (2), stroke (1, 37), AF (38), HF (39), VTE (40) and mortality (41, 42). The present results of RDW confirm and expand on previous results from MDCS, as well as other cohort studies. Several potential mechanisms were proposed to contribute to these associations, such as inflammation (6) and oxidative stress (7), which could affect red blood cell (RBC) production and erythropoiesis, and thereby RDW. There are also some previous studies of genetically predicted-RDW in relation to CVDs (12, 18, 43, 44). A study from the UK Biobank reported that genetically increased RDW was not predictive for incident cardiovascular disease. Furthermore, genetic risk score of LDL and genetic risk score of systolic blood pressure were inversely associated with RDW. The authors suggested that relationship between RDW and cardiovascular outcomes may partly be related to aging (43). Another previous study (18) showed non-significant relationships between genetically-increased-RDW and CVDs. In accordance with the previous studies, we did not find any significant relationship between PGS-RDW and CVDs in this study with individual follow-up data. This suggests that although RDW could be a valuable marker of predicting incident of CVDs, it is probably not causally related to increased CVD or mortality risks.

Inflammation or oxidative stress have been reported to increase RDW, as inflammation might be related to reduced RBC formation (6). A previous study (45) reported that the low response to erythropoietin treatment in patients with low-grade inflammation could lead to low red cell turn-over. As inflammation is commonly seen in many diseases, including CVDs, it is presumable that this might lead to high RDW in individuals. Besides, a previous study of the associations between common demographic and clinical characteristics (including laboratory tests) with variability in RDW, indicated that only small percentage of the observed variation in RDW was explained by routinely assessed clinical or laboratory variables [including inflammation markers, such as white blood cells (WBC), etc.] (46). Also, our previous studies reported RDW was associated with mortality and abdominal aortic aneurysm after multiple adjustments of routine laboratory parameters (i.e., WBC, etc.) (5, 47). Hence, other routine laboratory parameters do not seem to fully explain the relationships between RDW and mortality or cardiovascular outcomes. The relationships between RDW and outcomes might be driven by many factors including inflammatory factors. In addition, it is also possible that unmeasured confounders could explain the associations between RDW and all-cause mortality and major cardiovascular events in observational studies. More research is needed in this regard.

RDW has previously been linked to adverse outcomes in patients with CKD or with impaired kidney function (48–50). We observed significant relationships between RDW and incidence of CKD in the MDCS, however, we did not find any significant relationship between PGS-RDW and incident CKD and the results suggest that RDW could be a biomarker for CKD risk in the general population, but probably not a causal factor for incident CKD.

FIGURE 1
The forest plot presenting relationships between RDW and PGS of RDW and mortality and cardiometabolic diseases. The model was adjusted for age, sex, BMI, smoking and diabetes. For PGS-RDW and mortality and cardiometabolic disease, the model was additionally adjusted for PC1-PC5. RDW, red cell distribution width; PGS, polygenic score; HR, hazard ratio; CI, confidence interval; CE, coronary events; AF, atrial fibrillation; CKD, chronic kidney disease; HF, heart failure; VTE, venous thromboembolism.

The inverse relationship between RDW and diabetes is in accordance with a previous study from the present cohort, but in contrast to results from a Chinese study (51). In our study we also found a significant inverse relationship between PGS-RDW and incidence of diabetes. The relationship between RDW and diabetes is complex. First, many studies have shown positive relationship between RDW and HbA1c (52), similarly to our findings. In our study we also found a significant relationship between HbA1c and PGS-RDW. This is probably explained by a relationship between RDW and increased life span of the circulating red cells (8). Longer red cell lifespan will result in a higher proportion of red cells being exposed to glucose for long time (4). Individual differences in erythrocyte lifespan can be large enough to explain variations in HbA1c (53). However, in our study, the relationship between PGS-RDW and incidence of diabetes persisted in a sensitivity analysis when we excluded diabetes cases who were identified using the register of HbA1c measurements. Another important factor is that hyperglycaemia might affect erythrocytes in several aspects (54), such as increased volumes, reduced deformability and osmotic stability of the red cells (55), which might lead to shorter red cell life span and higher concentrations of reticulocytes. Hence, presence of hyperglycaemia could potentially modify the relationships between RDW and glucose or HbA1c. The present study of an initially non-diabetic population

found significant inverse relationships with incidence of diabetes, both for RDW and PGS-RDW. The results are supported by results from UK Biobank reporting an inverse relationship between the genetic risk scores for type 2 diabetes and RDW, although this became non-significant after adjustment for multiple comparisons (43). Nevertheless, the relationship between RDW and diabetes need to be further studied also with other types of study design.

Strengths and limitations

The large population-based cohort study with individual follow-up data is a major strength of this study. The PGS-RDW was constructed using about one million genetic polymorphisms, which should increase the statistical power. However, even though we used a large cohort with individual follow-up data, low statistical power remains a potential problem in Mendelian randomization studies.

The GWAS study we used to calculate the PGS-RDW, and the subjects of the present study were mostly from European ancestry (44). This limits the generalizability to subjects from non-European ancestry. Furthermore, the GWAS used to calculate PGS-RDW was based on RDW-CV, and not RDW-SD, which was used as measure of RDW in this cohort. However, RDW and PGS-RDW were also

significantly correlated in this study. Another limitation is potential horizontal pleiotropic effects. Especially for diabetes, more studies are needed to examine whether there are any causal relationships.

Conclusions

RDW is associated with mortality and increased incidence of CKD and different CVDs, but the non-significant relationship with PGS-RDW indicates that the relationships are not causal. However, both RDW and PGS-RDW were inversely associated with incidence of diabetes, indicating a potentially causal association. RDW is not causally involved in most CVDs but could still be a useful marker. The relationship with incidence of diabetes needs to be further studied.

Data availability statement

The data sets presented in this article are not publicly available because the data is owned by Lund University, and the research is coordinated by the steering committee of MDCS. Applications for research data can be addressed to Anders Dahlin, data manager, email: anders.dahlin@med.lu.se. Legal restrictions to data access may apply.

Ethics statement

The studies involving humans were approved by Ethical Committee at Lund University (LU 51-90, LU 2009/633, LU 2011/356). All participants provided and signed written consent and the study conforms to the principles of the Declaration of Helsinki. This study was approved by the Ethical Committee at Lund University (LU 51-90, LU 2009/633, LU 2011/356).

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2023.1294218/full#supplementary-material

References

1. Söderholm M, Borné Y, Hedblad B, Persson M, Engström G. Red cell distribution width in relation to incidence of stroke and carotid atherosclerosis: a population-based cohort study. *PLoS One*. (2015) 10(5):e0124957. doi: 10.1371/journal.pone.0124957

2. Skjelbakken T, Lappegård J, Ellingsen TS, Barrett-Connor E, Brox J, Løchen ML, et al. Red cell distribution width is associated with incident myocardial infarction in a general population: the Tromsø study. *J Am Heart Assoc*. (2014) 3(4):e001109. doi: 10.1161/JAHA.114.001109

3. Borné Y, Smith JG, Melander O, Engström G. Red cell distribution width in relation to incidence of coronary events and case fatality rates: a population-based cohort study. *Heart*. (2014) 100(14):1119–24. doi: 10.1136/heartjnl-2013-305028

4. Engström G, Smith JG, Persson M, Nilsson PM, Melander O, Hedblad B. Red cell distribution width, haemoglobin A1c and incidence of diabetes mellitus. *J Intern Med*. (2014) 276(2):174–83. doi: 10.1111/joim.12188

5. Pan J, Borné Y, Engström G. The relationship between red cell distribution width and all-cause and cause-specific mortality in a general population. *Sci Rep*. (2019) 9 (1):16208. doi: 10.1038/s41598-019-52708-2

6. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med*. (2005) 352 (10):1011–23. doi: 10.1056/NEJMra041809

7. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: a simple parameter with multiple clinical applications. *Crit Rev Clin Lab Sci*. (2015) 52(2):86–105. doi: 10.3109/10408363.2014.992064

8. Patel HH, Patel HR, Higgins JM. Modulation of red blood cell population dynamics is a fundamental homeostatic response to disease. *Am J Hematol*. (2015) 90(5):422–8. doi: 10.1002/ajh.23982

9. Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA, et al. 10 years of GWAS discovery: biology, function, and translation. *Am J Hum Genet*. (2017) 101(1):5–22. doi: 10.1016/j.ajhg.2017.06.005

10. Hodonsky CJ, Jain D, Schick UM, Morrison JV, Brown L, McHugh CP, et al. Genome-wide association study of red blood cell traits in hispanics/latinos: the hispanic community health study/study of latinos. *PLoS Genet*. (2017) 13(4): e1006760. doi: 10.1371/journal.pgen.1006760

11. Chen Z, Tang H, Qayyum R, Schick UM, Nalls MA, Handsaker R, et al. Genome-wide association analysis of red blood cell traits in African Americans: the COGENT network. *Hum Mol Genet*. (2013) 22(12):2529–38. doi: 10.1093/hmg/ddt087

12. He J, Jiang Q, Yao Y, Shen Y, Li J, Yang J, et al. Blood cells and venous thromboembolism risk: a two-sample Mendelian randomization study. *Front Cardiovasc Med*. (2022) 9:919640. doi: 10.3389/fcvm.2022.919640

13. Liu J, Chou EL, Lau KK, Woo PYM, Wan TK, Huang R, et al. A Mendelian randomization-based exploration of red blood cell distribution width and mean corpuscular volume with risk of hemorrhagic strokes. *HGG Adv*. (2022) 3 (4):100135. doi: 10.1016/j.xhgg.2022.100135

14. Richardson TG, Harrison S, Hemani G, Davey Smith G. An atlas of polygenic risk score associations to highlight putative causal relationships across the human phenome. *eLife*. (2019) 8:e43657. doi: 10.7554/eLife.43657

15. Mega JL, Stitziel NO, Smith JG, Chasman DI, Caulfield M, Devlin JJ, et al. Genetic risk, coronary heart disease events, and the clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. *Lancet*. (2015) 385 (9984):2264–71. doi: 10.1016/S0140-6736(14)61730-X

16. Seibert TM, Fan CC, Wang Y, Zuber V, Karunamuni R, Parsons JK, et al. Polygenic hazard score to guide screening for aggressive prostate cancer: development and validation in large scale cohorts. *Br Med J*. (2018) 360:j5757. doi: 10.1136/bmj.j5757

17. Sun J, Wang Y, Folkersen L, Borné Y, Amlien I, Buil A, et al. Translating polygenic risk scores for clinical use by estimating the confidence bounds of risk prediction. *Nat Commun*. (2021) 12(1):5276. doi: 10.1038/s41467-021-25014-7

18. Thayer TE, Huang S, Farber-Eger E, Beckman JA, Brittain EL, Mosley JD, et al. Using datasets to detangle the relationships between red cell distribution width and cardiovascular diseases: a unique role for body mass index. *Open Heart*. (2021) 8 (2):e001713. doi: 10.1136/openhrt-2021-001713

19. Manjer J, Carlsson S, Elmståhl S, Gullberg B, Janzon L, Lindström M, et al. The malmö diet and cancer study: representativity, cancer incidence and mortality in participants and non-participants. *Eur J Cancer Prev*. (2001) 10(6):489–99. doi: 10. 1097/00008469-200112000-00003

20. Manjer J, Elmståhl S, Janzon L, Berglund G. Invitation to a population-based cohort study: differences between subjects recruited using various strategies. *Scand J Public Health*. (2002) 30(2):103–12. doi: 10.1177/14034948020300020401

21. (March 2011) RDW-SD and RDW-CV: using this information practically. Sysmex Xtra Online March 2011. (2014).

22. Abraham G, Qiu Y, Inouye M. FlashPCA2: principal component analysis of biobank-scale genotype datasets. *Bioinformatics*. (2017) 33(17):2776–8. doi: 10.1093/bioinformatics/btx299

23. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, et al. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet*. (2016) 48 (10):1279–83. doi: 10.1038/ng.3643

24. Ge T, Chen CY, Ni Y, Feng YA, Smoller JW. Polygenic prediction via Bayesian regression and continuous shrinkage priors. *Nat Commun*. (2019) 10(1):1776. doi: 10. 1038/s41467-019-09718-5

25. Vuckovic D, Bao EL, Akbari P, Lareau CA, Mousas A, Jiang T, et al. The polygenic and monogenic basis of blood traits and diseases. *Cell*. (2020) 182 (5):1214–31.e11. doi: 10.1016/j.cell.2020.08.008

26. Shaun Purcell CC. PLINK v1.90b6.9. (2019). Available at: www.cog-genomics. org/plink/1.9/

27. Brooke HL, Talbäck M, Hörnblad J, Johansson LA, Ludvigsson JF, Druid H, et al. The Swedish cause of death register. *Eur J Epidemiol*. (2017) 32(9):765–73. doi: 10.1007/s10654-017-0316-1

28. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health*. (2011) 11:450. doi: 10.1186/1471-2458-11-450

29. Muhammad IF, Borné Y, Zaigham S, Söderholm M, Johnson L, Persson M, et al. Comparison of risk factors for ischemic stroke and coronary events in a population-based cohort. *BMC Cardiovasc Disord*. (2021) 21(1):536. doi: 10.1186/s12872-021-02344-4

30. Smith JG, Platonov PG, Hedblad B, Engström G, Melander O. Atrial fibrillation in the Malmö diet and cancer study: a study of occurrence, risk factors and diagnostic validity. *Eur J Epidemiol*. (2010) 25(2):95–102. doi: 10.1007/s10654-009-9404-1

31. Waldo AL, Feld GK. Inter-relationships of atrial fibrillation and atrial flutter mechanisms and clinical implications. *J Am Coll Cardiol*. (2008) 51(8):779–86. doi: 10.1016/j.jacc.2007.08.066

32. Ingelsson E, Arnlöv J, Sundström J, Lind L. The validity of a diagnosis of heart failure in a hospital discharge register. *Eur J Heart Fail*. (2005) 7(5):787–91. doi: 10. 1016/j.ejheart.2004.12.007

33. Manderstedt E, Halldén C, Lind-Halldén C, Elf J, Svensson PJ, Engström G, et al. Thrombomodulin (THBD) gene variants and thrombotic risk in a population-based cohort study. *J Thromb Haemost*. (2022) 20(4):929–35. doi: 10.1111/jth.15630

34. Bao X, Borné Y, Muhammad IF, Schulz CA, Persson M, Orho-Melander M, et al. Complement C3 and incident hospitalization due to chronic kidney disease: a population-based cohort study. *BMC Nephrol*. (2019) 20(1):61. doi: 10.1186/s12882-019-1248-7

35. Qureshi AR, Evans M, Stendahl M, Prütz KG, Elinder CG. The increase in renal replacement therapy (RRT) incidence has come to an end in Sweden-analysis of variations by region over the period 1991–2010. *Clin Kidney J*. (2013) 6(3):352–7. doi: 10.1093/ckj/sft032

36. Enhörning S, Sjögren M, Hedblad B, Nilsson PM, Struck J, Melander O. Genetic vasopressin 1b receptor variance in overweight and diabetes mellitus. *Eur J Endocrinol*. (2016) 174(1):69–75. doi: 10.1530/EJE-15-0781

37. Lappegård J, Ellingsen TS, Skjelbakken T, Mathiesen EB, Njølstad I, Wilsgaard T, et al. Red cell distribution width is associated with future risk of incident stroke. The Tromsø study. *Thromb Haemost*. (2016) 115(1):126–34. doi: 10.1160/TH15-03-0234

38. Adamsson Eryd S, Borné Y, Melander O, Persson M, Smith JG, Hedblad B, et al. Red blood cell distribution width is associated with incidence of atrial fibrillation. *J Intern Med*. (2014) 275(1):84–92. doi: 10.1111/joim.12143

39. Borné Y, Smith JG, Melander O, Hedblad B, Engström G. Red cell distribution width and risk for first hospitalization due to heart failure: a population-based cohort study. *Eur J Heart Fail*. (2011) 13(12):1355–61. doi: 10. 1093/eurjhf/hfr127

40. Zöller B, Melander O, Svensson P, Engström G. Red cell distribution width and risk for venous thromboembolism: a population-based cohort study. *Thromb Res*. (2014) 133(3):334–9. doi: 10.1016/j.thromres.2013.12.013

41. 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. *Arch Intern Med*. (2009) 169(5):515–23. doi: 10.1001/archinternmed.2009.11

42. Horne BD, Muhlestein JB, Bennett ST, Muhlestein JB, Jensen KR, Marshall D, et al. Extreme erythrocyte macrocytic and microcytic percentages are highly predictive of morbidity and mortality. *JCI Insight*. (2018) 3(14):e120183. doi: 10. 1172/jci.insight.120183

43. Pilling LC, Atkins JL, Duff MO, Beaumont RN, Jones SE, Tyrrell J, et al. Red blood cell distribution width: genetic evidence for aging pathways in 116,666 volunteers. *PLoS One*. (2017) 12(9):e0185083. doi: 10.1371/journal.pone.0185083

44. Astle WJ, Elding H, Jiang T, Allen D, Ruklisa D, Mann AL, et al. The allelic landscape of human blood cell trait variation and links to common complex disease. *Cell*. (2016) 167(5):1415–29.e19. doi: 10.1016/j.cell.2016.10.042

45. Kimachi M, Fukuma S, Yamazaki S, Yamamoto Y, Akizawa T, Akiba T, et al. Minor elevation in C-reactive protein levels predicts incidence of erythropoiesis-stimulating agent hyporesponsiveness among hemodialysis patients. *Nephron*. (2015) 131(2):123–30. doi: 10.1159/000438870

46. Guimarães PO, Sun JL, Kragholm K, Shah SH, Pieper KS, Kraus WE, et al. Association of standard clinical and laboratory variables with red blood cell distribution width. *Am Heart J*. (2016) 174:22–8. doi: 10.1016/j.ahj.2016.01.001

47. Xiao J, Borné Y, Gottsäter A, Pan J, Acosta S, Engström G. Red cell distribution width is associated with future incidence of abdominal aortic aneurysm in a population-based cohort study. *Sci Rep*. (2020) 10(1):7230. doi: 10.1038/s41598-020-64331-7

48. Yilmaz F, Sozel H. Red blood cell distribution width is a predictor of chronic kidney disease progression and all-cause mortality. *Bratisl Lek Listy*. (2021) 122 (1):49–55. doi: 10.4149/bll_2021_006

49. Deng X, Gao B, Wang F, Zhao M-h, Wang J, Zhang L. Red blood cell distribution width is associated with adverse kidney outcomes in patients with chronic kidney disease. *Front Med (Lausanne)*. (2022) 9:877220. doi: 10.3389/fmed.2022.877220

50. Lippi G, Targher G, Montagnana M, Salvagno GL, Zoppini G, Guidi GC. Relationship between red blood cell distribution width and kidney function tests in a large cohort of unselected outpatients. *Scand J Clin Lab Invest*. (2008) 68 (8):745–8. doi: 10.1080/00365510802213550

51. Gang L, Lifang W. Association of the elevated red blood cell distribution width with the risk of developing diabetes mellitus. *Intern Med*. (2016) 55(15):1959–65. doi: 10.2169/internalmedicine.55.5956

52. Veeranna V, Zalawadiya SK, Panaich SS, Ramesh K, Afonso L. The association of red cell distribution width with glycated hemoglobin among healthy adults without diabetes mellitus. *Cardiology*. (2012) 122(2):129–32. doi: 10.1159/000339225

53. Cohen RM, Franco RS, Khera PK, Smith EP, Lindsell CJ, Ciraolo PJ, et al. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA1c. *Blood*. (2008) 112(10):4284–91. doi: 10.1182/blood-2008-04-154112

54. Singh DK, Winocour P, Farrington K. Erythropoietic stress and anemia in diabetes mellitus. *Nat Rev Endocrinol*. (2009) 5(4):204–10. doi: 10.1038/nrendo. 2009.17

55. Knychala MA, Garrote-Filho MDS, Batista da Silva B, Neves de Oliveira S, Yasminy Luz S, Marques Rodrigues MO, et al. Red cell distribution width and erythrocyte osmotic stability in type 2 diabetes mellitus. *J Cell Mol Med*. (2021) 25 (5):2505–16. doi: 10.1111/jcmm.16184