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CLINICAL RESEARCH

Red blood cell distribution width in addition to N-terminal prohormone of B-type natriuretic peptide concentration improves assessment of risk of cardiovascular events in adult patients with congenital heart disease

La variation de la grosseur des hématies associée à la concentration du peptide natriurétique de type B améliore l'évaluation du risque d'événements cardiovasculaires chez les patients adultes avec cardiopathie congénitale

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Abbreviations: ACE, angiotensin-Converting Enzyme; ARB, Angiotensin II Receptor Blocker; CHD, congenital Heart Disease; GFR, glomerular Filtration Rate; NT-pro-BNP, N-terminal prohormone of B-type natriuretic peptide; RDW, Red Blood Cell Distribution Width; ROC, Receiver Operating Characteristic.

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KEYWORDS
Red blood cell distribution width;
Congenital heart disease;
Haemoglobin;
NT-pro-BNP;
Survival

Summary
Background. — Red blood cell distribution width (RDW) is a predictor of adverse outcomes in patients with heart disease.
Aim. — To establish predictors of high RDW values in patients with congenital heart disease (CHD), and their relationship with cardiovascular events.
Methods. — Overall, 561 patients with stable CHD who attended a single outpatient clinic and a matched control population of 2128 patients were studied. Exclusion criteria were renal failure, anaemia, receiving iron therapy and cyanosis. Blood tests included glucose, creatinine, iron, apoferritin, liver enzymes and a complete blood count. C-reactive protein and N-terminal prohormone of B-type natriuretic peptide (NT-pro-BNP) concentrations were also measured in patients with CHD. Major adverse cardiac events (MACE) were defined as cardiovascular/total mortality, arterial thrombotic events, arrhythmias, major bleedings, pulmonary embolism or heart failure needing hospital admission.
Results. — The median age in patients with CHD was 23 (17—36) years and the median follow-up time was 5.8 (3.2—8.7) years; 103 (4.8%) controls and 40 (7.1%) patients with CHD had an RDW > 15% ($P = 0.032$). During follow-up, MACE were reported in 48 patients. CHD of great complexity, cardiovascular risk factors, low haemoglobin concentration and high NT-pro-BNP concentration were risk factors for an RDW > 15%. Kaplan-Meier analysis showed a significantly worse cardiovascular outcome in patients with CHD with an RDW > 15% ($P < 0.001$). The multivariable survival analysis determined that age, CHD of great complexity, high NT-pro-BNP concentration and an RDW > 15% were independent predictive factors for MACE.
Conclusion. — RDW and NT-pro-BNP concentration are independent analytical predictors of MACE in patients with CHD.

MOTS CLÉS
Variation de la grosseur des hématies ;
Cardiopathie congénitale ;
Hémoglobine ;
NT-proBNP ;
Survie

Résumé
Contexte. — La variation de la grosseur des hématies (VGH) est un prédicteur d'événements secondaires dans les pathologies cardiaques.
But. — Etablir les prédicteurs de valeurs hautes de VGH chez les patients avec cardiopathies congénitales (CC) et leur relation avec les événements cardiovasculaires.
Méthodes. — Au total, 561 patients avec CC stable pris en charge en clinique ambulatoire et une population contrôle appariée de 2128 patients étaient étudiés. Les critères d'exclusion étaient une insuffisance rénale, une anémie, une thérapie par fer et une cyanose. Les tests sanguins incluaient le glucose, la créatinine, le fer, l'apoferritine, les enzymes hépatiques et une numération formule complète. La protéine-C-réactive et le NT-proBNP étaient aussi mesurés chez les patients avec CC. Les événements secondaires cardiaques majeurs (MACE) étaient définis comme étant la mortalité totale/cardiovasculaire, les événements thrombotiques artériels, les arythmies, les saignements majeurs, l'embolie pulmonaire ou l'insuffisance cardiaque nécessitant une hospitalisation.
Résultats. — L'âge médian des patients avec CC était de 23 (17—36) ans et le suivi médian de 5,8 (3,2—8,7) années; 103 (4,8 %) patients contrôles et 40 (7,1 %) patients avec CC avaient une VGH > 15 % ($p = 0,032$). Durant le suivi, des MACE étaient rapportés pour 48 patients. Une CC de grande complexité, des facteurs de risque cardiovasculaire, une hémoglobine basse et un NT-proBNP élevé étaient des facteurs de risque pour une VGH > 15 %. L'analyse Kaplan—Meier montrait une altération significative du pronostic cardiovasculaire chez les patients avec CC avec une VGH > 15 % ($p < 0,001$). L'analyse multivariée de survie déterminait que l'âge, une CC de grande complexité, un NT-proBNP élevé et une VGH > 15 % étaient des facteurs prédictifs indépendants pour les MACE.
Conclusion. — La VGH et le taux de NT-proBNP sont des prédicteurs analytiques indépendants de MACE chez les patients avec CC.

Background

Red blood cell distribution width (RDW), which is routinely determined and reported as a component of the standard complete blood count, measures the variation in the size of erythrocytes. Although a number of studies in the general population [1,2] and in patients with coronary artery disease [3], heart failure [4,5], pulmonary arterial hypertension [6] and congenital heart disease (CHD) [7,8] have linked high RDW values with a worse prognosis, there are confounding factors, such as renal failure, anaemia and cyanosis, which form part of the causal pathway of anisocytosis that may obscure the real effect of RDW on outcome. In fact, RDW plays an essential role in the differential diagnosis of anaemia [9,10], being an economic tool for detecting low serum apoferritin concentrations in patients with CHD who are cyanotic [11] — a population with a high percentage of anaemia despite optimizing their haemoglobin concentrations [12]. Therefore, RDW may be an epiphenomenon rather than an effective player in the pathogenesis of cardiovascular diseases [13].

The aims of this study were:

- to compare RDW values in young adult patients with CHD and a matched control population from the same geographical area;
- to determine predictors of high RDW values in patients with CHD and;
- to evaluate the role of RDW as an independent predictor of cardiovascular outcomes in the CHD population.

Methods

This study comprised an observational component that compared cases and controls, and a prospective component in which cases were followed up. Cases included clinically stable patients aged > 14 years, with structural CHD verified with imaging tests, and recruited from a single adolescent and adult CHD outpatient unit between January 2007 and December 2018. Controls were drawn from patients aged > 14 years attending primary health care centres in the same geographical area, between July 2017 and December 2018, for preventive activities or minor illnesses, such as anxiety, palpitations or muscle aches, and matched for age, sex, systemic arterial hypertension and diabetes mellitus to the patients with CHD. Exclusion criteria were severely decreased glomerular filtration rate (GFR), anaemia or cyanosis, which can all participate directly in the causal aeitiopathogenesis of anisocytosis [11,14,15]. Patients with an emergency admission, surgery or catheter intervention in the 3 months before the blood test or receiving iron therapy were also excluded from the study. Severe renal failure was defined as GFR < 30 mL/min/1.73 m^2 [16], anaemia as haemoglobin < 13 g/dL in men and < 12 g/dL in women [17] and cyanosis as basal haemoglobin oxygen saturation < 90% [18].

Clinical data

Cardiovascular imaging established the diagnosis of CHD, and patients were classified into diagnostic groups according to the underlying cardiac anatomy. Consistent with published classification schema, cardiac defects were categorized as being of simple, moderate or great complexity [19].

Arterial hypertension and diabetes mellitus were diagnosed according to the European Society of Cardiology guidelines [20—22] in patients with CHD and controls. Patients were considered as dyslipidaemic if they were receiving statin therapy, and as smokers if they were current smokers. Body weight and height were measured with the patients wearing light clothes and barefoot, and body mass index was calculated using the following formula: (weight in kg)/(height in m $\times$ height in m). GFR was calculated from serum creatinine using the Modification of Diet in Renal Disease Study equation [23]. The existence of atrial fibrillation or flutter was determined by electrocardiogram. Medical treatment in patients with CHD included aspirin or other antiplatelet agents, warfarin or other anticoagulants, beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), calcium channel blockers, loop diuretics and statins. Systemic ventricular dysfunction was documented as a left ejection fraction < 40% or a description consistent with moderate or severe systolic dysfunction and a tricuspid annular plane systolic excursion (TAPSE) of the right ventricle < 17 mm [24]. A cut-off value of 125 pg/mL was used for NT-pro-BNP concentration, as a lower concentration effectively rules out heart failure [25]. Systolic pulmonary artery pressure was calculated invasively by cardiac catheterization or non-invasively by Doppler echocardiography, and pulmonary hypertension was defined as an increase in mean pulmonary arterial pressure to $\geq$ 25 mmHg at rest [26].

Blood test

After an overnight fast of at least 10 h, blood samples were drawn for the measurement of glucose, creatinine, low-density lipoprotein cholesterol, iron, apoferritin, liver enzymes and a complete blood count in patients with CHD and controls. Total bilirubin, C-reactive protein and N-terminal prohormone of B-type natriuretic peptide (NT-pro-BNP) concentrations were also measured in patients with CHD. The tested analytes were obtained by spectrophotometry using Olympus AU 2700 equipment (Olympus Diagnostic, Hamburg, Germany) and NT-pro-BNP concentrations were measured by immunoassay with the Siemens Stratus CS Acute Care Diagnostic System (Siemens Healthcare Diagnostics, Inc., Newark, DE, USA). An automated complete blood count was processed within 30 min of venipuncture in ethylenediaminetetraacetic acid (EDTA) blood samples (K_3EDATA 7.2 mg vacuum blood collection tubes; Vacutest Kima, Arzergrande, PD, Italy) using a Coulter LH 750 Analyzer (Beckman Coulter, Fullerton, CA, USA). The same blood test equipment and references values were used for all patients with CHD and controls.

The reference range for RDW in our lab was 11.8—14.8%. We used, as an RDW cut-off value for patients with CHD, the 95th percentile of the RDW value in the control group (15.1%) rounded to the nearest unit to correspond to the cut-off point used by other authors [27].

Follow-up

After enrolment, patients with CHD were followed prospectively. Major adverse cardiac events (MACE) were defined as cardiovascular and non-cardiovascular death, arterial thrombotic events (myocardial infarction, stroke or peripheral vascular disease), pulmonary embolism, heart failure, arrhythmias or major haemorrhagic events requiring hospital admission. Cardiovascular events and stroke were described according to the Standardized Data Collection for Cardiovascular Trials Initiative (SCTI) and the US Food and Drug Administration (FDA) [28]. For patients with multiple events, event-free survival was defined as the time from enrolment to the occurrence of the first event. Patients without any cardiovascular events were censored at the end of the follow-up duration. Patients with CHD were followed up by reviewing the International Classification of Diseases, version 9 and version 10 (ICD-9-ES and ICD-10-ES) diagnostic coding system in the medicoadministrative data from our institution, clinical history or telephone calls. All participants provided informed consent, and the study was approved by the hospital's research ethics committee.

Statistical analysis

Continuous variables are presented as mean ± standard deviation or median (interquartile range), depending on the normality of distribution. Comparisons between two groups were performed using Student's *t* test for continuous variables or the Mann—Whitney U test for continuous non-parametric variables. Categorical values were compared using the χ^2 test. Logistic regression analysis was used to predict the dichotomous variable (RDW above and below 15%). The χ^2 test and linear regression analysis attempted to model the relationship between those variables that were significant in the univariate analysis. Crude odds ratios were obtained after considering the effect of variables that were significant in the univariate analysis, and adjusted odds ratios were obtained after including all variables that showed significance in the crude odds ratio analysis. A risk prediction model was obtained using the receiver operating characteristic (ROC) curve. To check the goodness-of-fit in the logistic regression model, the area under the ROC curve was calculated. Survival analysis was estimated by the Kaplan-Meier method and the log rank test. Univariable and multivariable Cox regression analyses were used to determine cardiovascular events during follow-up, and the results a*re* presented as hazard ratios. The 95% confidence interval was used to estimate the precision of the odds and hazard ratios. A *P*-value < 0.05 was considered statistically significant. Statistical Package for the Social Sciences (SPSS 24, Chicago, IL, USA) was used for data analysis.

Results

Study population

Overall, 670 out of 712 patients with CHD followed up in our outpatient CHD unit signed the informed consent and had blood samples drawn. A total of 561 of 670 patients with CHD were included in the study; 42 patients with cyanosis, 56 with anaemia, 11 receiving iron treatment and one with a GFR < 30 mL/min/1.73 m^2, who also had anaemia, were withdrawn in accordance with the exclusion criteria. The control population consisted of 2128 patients, after excluding patients with anaemia, renal failure or CHD and those who were receiving iron therapy. Table 1 shows CHD classification according to complexity: 318 (57%) patients had simple defects; 172 (31%) patients had moderate defects; and 71 (patients 13%) had CHD of great complexity.

Table 1 Congenital heart disease classification according to complexity.

	Number of patients
Simple complexity	318
Aortic valve disease	42
Pulmonary valve disease	61
Atrial septal defect	61
Ventricular septal defect	100
Ductus	14
Other simple defects	40
Moderate complexity	172
Subvalvular or supravalvular aortic stenosis	19
Coarctation of the aorta	52
Subvalvular or supravalvular pulmonary stenosis	8
Tetralogy of Fallot	51
Ebstein's anomaly	4
Atrioventricular septal defects	36
Sinus venosus septal defect	2
Great complexity	71
Dextro-transposition of the great arteries	24
Levo-transposition of the great arteries	8
Pulmonary atresia	3
Single ventricle	6
Double outlet right ventricle	8
Tricuspid atresia	2
Truncus arteriosus	2
CHD with pulmonary arterial hypertension	18
Total number of patients with CHD	561

CHD: congenital heart disease.

Blood test in patients with CHD and controls

Median RDW values were 13.4% (12.9—13.8%) in the control group and 13.3% (12.9—13.9%) in patients with CHD (*P* = 0.099). In the control group, 103 (4.8%) patients had an RDW > 15%, whereas in the CHD group, 40 (7.1%) patients had an RDW > 15% (*P* = 0.032). No significant differences were

Table 2 Demographic, clinical and blood test data in patients with congenital heart disease and the control population.

	Control population	Patients with CHD	P^a
	($n = 2128$)	($n = 561$)	
Age (years)	23 (17—35)	23 (17—36)	0.495
Male sex	1268 (60)	319 (60)	0.243
Arterial hypertension	263 (12)	70 (12)	0.939
Diabetes mellitus	99 (5)	22 (4)	0.458
Smoker	246 (12)	25 (5)	< 0.001
Laboratory results			
Glucose (mg/dL)	93 (88—100)	94 (88—99)	0.374
Creatinine (mg/dL)	0.8 ± 0.2	0.9 ± 0.2	< 0.001
GFR (mL/min/1.73 m^2)	118 (104—136)	94 (81—117)	< 0.001
Haemoglobin (g/dL)	14.7 ± 1.3	14.6 ± 1.4	0.424
LDL cholesterol (mg/dL)	96 (78—122)	92 (73—114)	0.004
MCV (fl)	88 (85—91)	89 (86—92)	0.001
MCH (pg)	30 (29—31)	30 (29—32)	< 0.001
RDW (%)	13.4 (12.9—13.8)	13.3 (12.9—13.9)	0.099
Leucocytes (%)	7.2 (6.0—8.5)	7.0 (5.9—8.6)	0.071
Platelets ($10\hat{3}/\mu L$)	240 ± 55	231 ± 61	0.004
Iron (μg/dL)	87 (64—114)	84 (61—107)	0.009
Apoferritin (ng/mL)	57 (30—106)	43 (24—77)	< 0.001
ALT (IU/L)	18 (13—28)	17 (14—26)	0.995
AST (IU/L)	21 (18—26)	22 (19—27)	0.030
Medical treatment			
Antiplatelet	22 (1)	46 (8)	< 0.001
Oral anticoagulation	8 (0.5)	64 (11)	< 0.001
Beta-blocker	51 (2)	75 (13)	< 0.001
ACE inhibitor/ARB	119 (5)	74 (13)	< 0.001
Calcium channel blocker	38 (2)	16 (3)	0.109
Statin	153 (7)	38 (7)	0.733

Data are expressed as median (interquartile range), number (%) or mean ± standard deviation. ACE: angiotensin-converting enzyme; ALT: alanine aminotransferase; ARB: angiotensin II receptor blocker; AST: aspartate aminotransferase; CHD: congenital heart disease; GFR: glomerular filtration rate; LDL: low-density lipoprotein; MCH: mean corpuscular haemoglobin; MCV: mean corpuscular volume; RDW: red blood cell distribution width.

[a] Categorical variables were evaluated by Pearson's χ^2 test; continuous data with normal distribution were compared by Student's *t*-test and continuous data without normal distribution by the Mann—Whitney test.

seen between patients with CHD and controls in terms of haemoglobin concentrations (Table 2).

Predictors of high RDW values in patients with CHD

Patients with CHD with an RDW > 15% had hypertension and diabetes significantly more frequently, CHD of greater complexity, a worse New York Heart Association functional class, lower haemoglobin and iron concentrations and higher C-reactive protein and NT-pro-BNP concentrations than patients with CHD with RDW ≤ 15%. In addition, patients with CHD with an RDW > 15% used significantly more medical treatment (oral anticoagulation, beta-blockers, ACE inhibitors/ARBs and loop diuretics) and had mechanical valve prostheses, atrial fibrillation/flutter and pulmonary arterial hypertension significantly more frequently than patients with CHD with an RDW ≤ 15%. Also, patients with CHD with an RDW > 15% had a significantly higher rate of cardiovascular events than patients with CHD with an RDW ≤ 15% (Table 3).

Patients with CHD of great complexity had a significantly ($P < 0.001$) worse New York Heart Association functional class (> II), used more medical therapy (oral anticoagulation, beta-blockers, ACE inhibitors/ARBs and diuretics) and had pulmonary arterial hypertension more frequently than patients with CHD of mild or moderate complexity. On the other hand, the linear regression analysis explained the relationship between haemoglobin concentration and mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, iron ($P < 0.001$) and C-reactive protein ($P = 0.010$) concentrations. After modelling the relationship between variables in patients with CHD, the binary logistic regression analysis showed that having CHD of great anatomical complexity, systemic arterial hypertension or diabetes mellitus, a low haemoglobin concentration or a high NT-pro-BNP concentration (> 125 pg/mL) were predictive factors for a high RDW value (> 15%) (Table 4). The area under the ROC

Table 3 Demographic, clinical and blood test data in patients with congenital heart disease, according to red blood cell distribution width values.

	Patients with CHD		P^a
	RDW ≤ 15%	RDW > 15%	
	(*n* = 521)	(*n* = 40)	
Age (years)	22 (17—35)	26 (17—45)	0.127
Male sex	300 (58)	19 (48)	0.215
BMI (kg/m^2)	23 (20—26)	23 (21—26)	0.881
CHD of great complexity	61 (12)	10 (25)	0.015
NYHA functional class ≥ II	136 (26)	17 (42)	0.025
Arterial hypertension	61 (12)	9 (22)	0.047
Diabetes mellitus	18 (4)	4 (10)	0.040
Smoker	23 (4)	2 (5)	0.050
Laboratory results			
Glucose (mg/dL)	94 (88—99)	95 (87—104)	0.327
Creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.2	0.707
GFR (mL/min/1.73 m^2)	94 (81—118)	92 (76—110)	0.481
LDL cholesterol (mg/dL)	92 (73—115)	95 (72—117)	0.839
Haemoglobin (g/dL)	14.7 ± 1.3	13.5 ± 1.4	< 0.001
Haematocrit (%)	43 (40—45)	41 (38—43)	0.002
MCV (fl)	89 (86—92)	85 (82—91)	< 0.001
MCH (pg)	31 (29—32)	29 (27—31)	< 0.001
MCHC (g/dL)	34 (34—35)	33 (32—34)	< 0.001
RDW (%)	13.2 (12.8—13.8)	16.4 (15.5—17.6)	< 0.001
Leucocytes (%)	7 (6—8)	6 (5—9)	0.565
Platelets (10$\hat{3}$/μL)	231 ± 58	229 ± 93	0.839
Total bilirubin (mg/dL)	0.7 (0.5—0.9)	0.8 (0.5—1.5)	0.202
ALT (IU/L)	17 (14—25)	20 (14—38)	0.138
AST (IU/L)	22 (19—26)	24 (19—31)	0.138
Iron (μg/dL)	85 (62—107)	64 (33—90)	0.007
Apoferritin (ng/mL)	43 (24—76)	37 (16—80)	0.614
C-reactive protein (mg/L)	0.1 (0.06—0.4)	0.5 (0.1—0.9)	0.001
NT-pro-BNP (pg/mL)	62 (19—143)	295 (52—1023)	< 0.001
Treatment			
Antiplatelet	42 (8)	4 (10)	0.667
Oral anticoagulation	48 (9)	16 (40)	< 0.001
Beta-blocker	63 (12)	12 (30)	0.001
ACE inhibitor/ARB	62 (12)	12 (30)	0.001
Calcium channel blocker	13 (2)	3 (7)	0.067
Loop diuretic	42 (8)	15 (37)	< 0.001
Statin	34 (6)	4 (10)	0.399
Mechanical valve prosthesis	21 (4)	5 (12)	0.014
Systemic ventricular dysfunction	87 (18)	7 (18)	0.989
Atrial fibrillation/flutter	16 (3)	6 (15)	< 0.001
Pulmonary arterial hypertension	13 (2)	5 (14)	0.001
Clinical outcome (MACE)	38 (7)	10 (25)	< 0.001

Data are expressed as median (interquartile range), number (%) or mean ± standard deviation. ACE: angiotensin-converting enzyme; ALT: alanine aminotransferase; ARB: angiotensin II receptor blocker; AST: aspartate aminotransferase; BMI: body mass index; CHD: congenital heart disease; GFR: glomerular filtration rate; LDL: low-density lipoprotein; MACE: major adverse cardiovascular events; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; NT-pro-BNP: N-terminal prohormone of B-type natriuretic peptide; NYHA: New York Heart Association; RDW: red blood cell distribution width.

[a] Categorical variables were evaluated by Pearson's χ^2 test; continuous data with normal distribution were compared by Student's *t*-test and continuous data without normal distribution by the Mann—Whitney test.

Table 4 Binary logistic regression analyses in patients with congenital heart disease to predict a red blood cell distribution width value > 15%.

	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
CHD of great complexity	2.514 (1.171—5.396)	0.018	3.024 (1.048—8.441)	0.036
Cardiovascular risk factors[a]	2.855 (1.386—5.881)	0.004	4.703 (1.695—12.837)	0.002
Mechanical prosthesis	3.401 (1.210—9.563)	0.020	3.284 (0.592—13.912)	0.130
Atrial/flutter fibrillation	5.570 (2.048—15.147)	0.001	2.008 (0.431—7.964)	0.341
Haemoglobin (g/dL)	0.548 (0.428—0.703)	< 0.001	0.595 (0.410—0.830)	0.004
NT-pro-BNP > 125 pg/mL	3.924 (1.775—8.677)	< 0.001	2.845 (1.069—7.927)	0.038

CHD: congenital heart disease; CI: confidence interval; NT-pro-BNP: N-terminal prohormone of B-type natriuretic peptide; OR: odds ratio.
[a] Cardiovascular risk factors include arterial hypertension and diabetes mellitus.

curve was 0.834 (95% confidence interval 0.751—0.917), with a sensitivity of 0.769, a specificity of 0.772 and a negative predictive value of 0.978.

Cardiovascular outcome

Follow-up time to composite cardiovascular events was 5.8 (3.2—8.7) years, and no patient was lost to follow-up. MACE were reported in 48 patients during follow-up; two of these patients had a history of MACE at baseline because of cardiovascular events: stroke ($n = 1$); and peripheral artery disease ($n = 1$). The distribution of MACE was as follows: 18 patients had arterial thrombotic events (14 strokes, two myocardial infarctions and two cases of peripheral artery disease); 16 patients died, including 10 cardiac deaths and six deaths of extracardiac origin; seven patients had heart failure; four patients had arrhythmias requiring hospital admission; two patients had a pulmonary embolism; and one patient had a major haemorrhagic event. No significant differences were seen in RDW values between patients with CHD with MACE before ($n = 17$) or after ($n = 31$) 1 year of follow-up: 13.8% (12.9—15.5%) vs 13.5% (13.2—14.3%), respectively ($P = 0.069$). On the other hand, in the Kaplan—Meier analysis, patients with CHD with an RDW > 15% had a significantly worse cardiovascular outcome than those with an RDW < 15%, as seen in Fig. 1 ($P < 0.001$). Similarly, when RDW was analysed in a multivariable Cox model including clinical and blood test data, the association between RDW and worse prognosis remained (Table 5).

Discussion

This study has demonstrated that the combination of RDW and NT-pro-BNP has additional value in predicting prognosis in patients with CHD. In this context, many recent studies have reported an association between RDW and adverse cardiovascular outcomes, both in control populations and in patients with cardiovascular disease [1—8,29,30]. However, there is little information regarding the association between RDW and NT-pro-BNP concentrations and the outcome of patients with CHD.

Different factors may explain why the CHD group, unlike the control population, presented a significantly greater number of individuals with RDW values > 15%, despite the

Table 5 Univariate and multivariable Cox regression analysis of variables associated with major adverse cardiovascular events in patients with congenital heart disease.

	Univariate analysis		Multivariable analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age (years), per 1-year increase	1.055 (1.039—1.072)	< 0.001	1.040 (1.024—1.068)	< 0.001
Male sex	0.956 (0.516—1.771)	0.886		
CHD of great complexity	4.435 (2.348—8.376)	< 0.001	3.589 (1.874—6.866)	< 0.001
Cardiovascular risk factors[a]	1.559 (0.746—3.259)	0.238		
Mechanical prosthesis	2.609 (0.921—7.390)	0.071		
Atrial fibrillation/flutter	5.300 (2.200—12.655)	< 0.001	0.812 (0.295—2.234)	0.686
Haemoglobin, per 1-unit increase (g/dL)	1.056 (0.847—1.316)	0.628		
C-reactive protein, per 1-unit increase (mg/dL)	1.186 (0.929—1.515)	0.171		
NT-pro-BNP > 125 pg/mL	5.749 (2.370—13.945)	< 0.001	4.555 (1.857—11.158)	0.001
RDW > 15%	4.769 (2.273—10.006)	< 0.001	2.638 (1.179—5.905)	0.018

CHD: congenital heart disease; CI: confidence interval; HR: hazard ratio; NT-pro-BNP: N-terminal prohormone of B-type natriuretic peptide; RDW: red blood cell distribution width.
[a] Cardiovascular risk factors include arterial hypertension and diabetes mellitus.

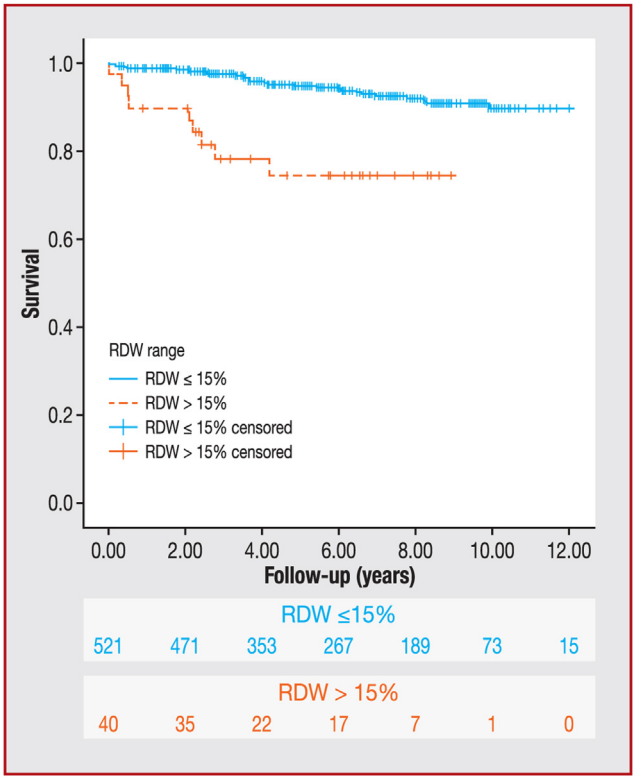

Figure 1. Kaplan–Meier curve shows major adverse cardiovascular events in patients with congenital heart disease with red blood cell distribution width (RDW) > 15% (dashed green line) and RDW ≤ 15% (continuous blue line) during follow-up (log-rank test $P < 0.001$).

fact that RDW median values did not show statistical differences between these groups:

- a lower haemoglobin concentration, as iron deficiency is frequent in patients with CHD [11], even after ruling out patients with anaemia or cyanosis or those receiving iron therapy, as in our series; in fact, iron deficiency is one of the most common causes of elevated RDW, as a result of the increase in erythrocyte size variability caused by microcytic red blood cells as iron deficiency ensues [7];
- heart failure that, through several potential causes that hamper erythrocyte maturation (e.g. nutritional deficiencies, renal dysfunction, hepatic congestion, inflammatory stress), may favour a change in RDW variables [4,30–35]; in fact, although many patients with CHD do well, heart failure remains a common, difficult and often final complication of CHD [36] and;
- systemic arterial hypertension and diabetes mellitus, as both these components of the metabolic syndrome have been associated with high RDW values [37].

On the other hand, although mechanical heart valve prostheses (particularly those with a smaller diameter [38]) may induce haemolysis, and having an atrial arrhythmia may favour an increase in erythrocyte volume in patients with chronic atrial fibrillation [39], no significant differences were seen in our multivariable analysis. Similarly, although it has been reported that RDW correlates with liver disease in hospitalized patients, no significant differences were seen in liver enzyme or bilirubin concentrations between patients with CHD with low or high RDW values.

In relation to survival, RDW values have been associated with an increased mortality risk in the general population [1,2], and with an improvement in the ability of the Framingham risk score model, after its inclusion, to predict cardiovascular mortality in a healthy cohort in the USA [40]. Also, in patients with chronic [4] or acute [5] heart failure, a previous myocardial infarction [41] or idiopathic pulmonary arterial hypertension [6], RDW has been shown to be an useful circulating biomarker in predicting survival. Likewise, Baggen et al. [29], in a CHD cohort, came to the conclusion that RDW was significantly associated with cardiovascular events, independent of NT-pro-BNP concentrations and other prognostic markers. Similarly, Alshawabkeh et al. [7], in a group of patients with CHD of moderate or great complexity, found that elevated RDW was an independent predictor of all-cause mortality or non-elective cardiovascular hospitalization. Yang et al. [8] reached similar conclusions in patients with CHD with Eisenmenger syndrome. For our part, we found that in patients with CHD without cyanosis or anaemia, RWD remained an independent predictor of MACE, together with other factors, such as age and NT-pro-BNP concentration. Therefore, and unlike other studies where patients with cyanosis or anaemia were included (both of which are associated with a high risk of cardiovascular events [8,42]), we found that RDW and NT-pro-BNP concentration kept their additional value for predicting CHD outcome regardless of other factors, such as age, classic cardiovascular risk factors and CHD complexity.

Study limitations

There were, however, limitations to our study that may have affected our findings. Firstly, only a small number of patients had an RDW > 15%, and a low number of MACE were observed during follow-up (typical of a young population). Nonetheless, we think that the sample size was large enough to draw a link between RDW and cardiovascular events in young adult patients with CHD without anaemia or cyanosis. Secondly, the lack of serial RDW determinations prevented us from determining the association between recent changes in RDW and the cardiovascular event. Finally, patients with CHD are a heterogeneous population, so it may be difficult to draw final conclusions about the overall prognosis.

Conclusions

RDW is a widely available test that may be used, together with NT-pro-BNP concentration, as a predictor of cardiovascular events in young adult patients with CHD without anaemia or cyanosis. RDW values may reflect in patients with CHD a wide spectrum of pathophysiological processes, such as cardiac complexity, iron deficiency, heart failure or metabolic syndrome, which may explain the association between RDW and worse outcome.

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Disclosure of interest

The authors declare that they have no competing interest.

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