Journal of Cardiac Failure Vol. 17 No. 8 2011

Determinants of Red Cell Distribution Width (RDW) in Cardiorenal Patients: RDW is Not Related to Erythropoietin Resistance

MIREILLE E. EMANS, MD,[1,2] KARIEN VAN DER PUTTEN, MD,[2,8] KARLIJN L. VAN ROOIJEN, MD,[2,5] ROB J. KRAAIJENHAGEN, PhD,[3] DORINE SWINKELS, MD, PhD,[4] WOUTER W. VAN SOLINGE, MD, PhD,[5] MAARTEN J. CRAMER, MD, PhD,[1] PIETER A.F.M. DOEVENDANS, MD, PhD,[1] BRANKO BRAAM, MD, PhD,[6] AND CARLO A.J.M. GAILLARD, MD, PhD[2,7]

Utrecht, Amersfoort, and Amsterdam, The Netherlands; and Edmonton, Canada

ABSTRACT

Background: Studies have shown that red cell distribution width (RDW) is related to outcome in chronic heart failure (CHF). The pathophysiological process is unknown. We studied the relationship between RDW and erythropoietin (EPO) resistance, and related factors such as erythropoietic activity, functional iron availability and hepcidin.

Methods and Results: In the Mechanisms of Erythropoietin Action in the Cardiorenal Syndrome (EPO-CARES) study, which investigates the role of EPO in 54 iron-supplemented anemic patients with CHF and chronic kidney disease (CKD) (n = 35 treated with 50 IU/kg/wk Epopoetin beta, n = 19 control), RDW was not associated with EPO resistance. We defined EPO resistance by EPO levels ($r = 0.12$, $P = .42$), the observed/predicted log EPO ratio ($r = 0.12$, $P = .42$), the increase in reticulocytes after 2 weeks of EPO treatment ($r = -0.18$, $P = .31$), and the increase of hemoglobin after 6 months of EPO treatment ($r = 0.26$, $P = .35$). However, RDW was negatively correlated with functional iron availability (reticulocyte hemoglobin content, $r = -0.48$, $P < .001$, and transferrin saturation, $r = -0.39$, $P = .005$) and positively with erythropoietic activity (soluble transferrin receptor, $r = 0.48$, $P < .001$, immature reticulocyte fraction, $r = 0.36$, $P = .01$) and positively with interleukin-6 ($r = 0.48$, $P < .001$). No correlation existed between hepcidin-25 and RDW.

Conclusions: EPO resistance was not associated with RDW. RDW was associated with functional iron availability, erythropoietic activity, and interleukin-6 in anemic patients with CHF and CKD. (*J Cardiac Fail 2011;17:626–633*)

Key Words: Anemia, hepcidin, iron metabolism.

Red blood cell distribution width (RDW) is routinely performed as part of a complete blood cell count and quantifies the variability in size of circulating red blood cells (ie, anisocytosis), defined as the standard deviation of erythrocyte size divided by the mean corpuscular volume (MCV). Recently, researchers have reported an independent

From the [1]Department of Cardiology, University Medical Centre, Utrecht, The Netherlands; [2]Department of Internal Medicine, Meander Medical Centre, Amersfoort, The Netherlands; [3]Department of Clinical Chemistry, Meander Medical Centre, Amersfoort, The Netherlands; [4]Department of Clinical Chemistry, Radboud University Nijmegen Medical Centre, The Netherlands; [5]Department of Clinical Chemistry and Haematology, University Medical Centre, Utrecht, The Netherlands; [6]Division of Nephrology and Immunology, University of Alberta, Edmonton, Canada; [7]Department of Nephrology, VU University Medical Centre, Amsterdam, The Netherlands and [8]Department of Nephrology, Leiden University Medical Center, The Netherlands.

Manuscript received January 1, 2011; revised manuscript received April 5, 2011; revised manuscript accepted April 11, 2011.

Reprint requests: Carlo A.J.M. Gaillard, MD, PhD, Department of Nephrology, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam. Tel: +31204444312; Fax: +314444960. E-mail: C.Gaillard@vumc.nl

Supported by the Dutch Heart Foundation, The Hague, the Netherlands (grant number 2005B192) and by an unrestricted grant from Roche, the Netherlands.

Conflict of Interest: None.

See page 632 for disclosure information.

doi:10.1016/j.cardfail.2011.04.009

association between RDW and the risk of adverse outcomes in patients with chronic and acute heart failure,[1–4] in patients with stable coronary artery disease,[5] and even in a community-based cohort.[6–8] The pathophysiological mechanism responsible for the association between RDW and adverse outcomes, is open to question. Anemia is highly prevalent in chronic heart failure (CHF) and is associated with morbidity and mortality.[9] Most factors that cause anemia through ineffective red cell production or increased red cell destruction could cause anisocytosis. Importantly, however, RDW remained an independent predictor of outcome after adjusting for hemoglobin level.[1]

Erythropoietin (EPO) resistance, that is the inadequate bone marrow response to *endogenous* and/or *exogenous* EPO leading to an impaired red blood cell line, plays an important role in anemia of CHF and chronic kidney disease (CKD).[10] Resistance to EPO is associated with morbidity and mortality.[11] Several authors hypothesize that EPO resistance could explain the association between RDW and outcome.[2,4,12] Inflammation and disordered iron metabolism are factors that can cause EPO resistance and indeed, recent studies have shown that inflammatory markers, EPO levels, and decreased functional iron availability correlate with RDW.[2,4] However, no direct data are available as to the association between RDW and EPO resistance. The Mechanisms of Erythropoietin Action in the Cardiorenal Syndrome (EPOCARES) study created an opportunity to investigate the association between RDW and EPO resistance in iron-supplemented, EPO-naive patients with CHF and CKD.[11] Because a universally accepted definition of EPO resistance does not exist, we estimated EPO resistance in three ways using the log observed/predicted ratio (O/P), which reflects the EPO level for the degree of anemia[13]; the extent in increase of reticulocyte count, soluble transferrin receptor or immature reticulocyte fraction after 2 weeks of exogenous EPO treatment; and the hemoglobin increase after 6 months of EPO treatment. In addition, we investigated the role of associated factors, such as inflammation, erythropoietic activity (rate of red cell production), functional iron availability, and hepcidin.

Methods

Study Design and Patients

The study design of the EPOCARES study (ClinicalTrials.gov number NCT 00356733) has been published elsewhere.[14] In short, the EPOCARES study is an open-label, prospective, randomized trial, in which patients with CHF, CKD (glomerular filtration rate by Cockroft-Gault equation of 20 to 70 mL/min) and mild anemia (hemoglobin 10.3 to 12.6 g/dL for men and 10.3 to 11.9 g/dL for women) are included to test the hematopoietic and nonhematopoietic responses to EPO treatment. Exclusion criteria, among others, were erythropoietic therapy within 6 months, bleeding, hemolysis, hemoglobinopathies, chronic inflammatory disease, or malignancy. Hemoglobin (Hb) level for inclusion was measured after at least 4 weeks of oral iron supplementation, if tolerated. The diagnostic criteria for CHF were those recommended by the European Society of Cardiology guidelines.[15] Patients with heart failure with reduced left ventricular ejection fraction (HFREF) as well as patients with preserved left ventricular ejection fraction (HFPEF) were included.[16] The Medical-Ethical Committee approved the protocol of the study and informed consent was obtained from all subjects. Procedures were in accordance with the Helsinki Declaration and all patients gave written consent.

Patients were randomized on a 1:1:1 basis, after they had been clinically stable on maximal tolerated doses of a ß-blocker, an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker for at least 4 weeks. One group received 50 IU·kg·wk Epopoetin beta (Neorecormon, Roche Pharmaceuticals) and their Hb was kept at baseline level by phlebotomies during the first 6 months. One group received 50 IU/kg/wk Epopoetin beta and their Hb could rise (to a certain safety level). The third group received standard treatment. Most biochemical measurements were performed at baseline, after 2 weeks and monthly thereafter. Blood samples were drawn between 8 and 9 AM in supine position and stored at −80°C until analysis.

Biomarker Analysis

Levels of Hb, hematocrit, MCV, RDW, white blood cells, platelets, reticulocyte count and reticulocyte hemoglobin content (RET-He) were measured with the use of a Sysmex XE-2100 hematology analyzer (Toa Medical, Kobe, Japan).

High sensitivity C-reactive protein (hs-CRP) was determined by particle-enhanced immunonephelometry using a standard Cardio-Phase hs-CRP for BNII (Dade Behring Holding GmbH, Liederbach, Germany). Plasma interleukin-6 (IL-6) levels (pg/mL) were measured in duplo using a commercially available high sensitive ELISA kit (R&D Systems, Minneapolis, MN).

As a marker of total iron stores,[17] ferritin was determined using a sandwich immunoassay on an Acces®2 immunoanalyzer within a Dx automated system from Beckman Coulter (Brea, CA). Functional iron availability was determined by measuring transferrin saturation (TSAT), soluble transferrin receptor (sTfR) and RET-He. TSAT was calculated from serum iron and transferrin estimates obtained with standard methods on a Beckman Coulter Dx. sTfR assay was performed with an immunoassay on a BNPro-Spec nephelometer from Siemens (Marburg, Germany). RET-He was performed using flow cytometric analysis with Ret-Search (II)® dye on a Sysmex XE-2100 hematology analyzer (Toa Medical, Kobe, Japan).

Erythropoietin Levels, Erythropoietic Activity, and EPO Resistance

Serum EPO levels were measured by a 2-site sandwich chemiluminescent immunoassay on an IMMULITE 2000 platform (Siemens Healthcare Diagnostics, Breda, the Netherlands). As markers of erythropoietic activity, we measured sTfR and assessed the ratio of young immature reticulocytes (IRF). Reticulocytes have variable amounts of RNA, which correlates with their maturation. The fluorescent intensity of a reticulocyte, measured by using a fluorescent polymethine dye, is proportional to the quantity of RNA. Reticulocytes are thus divided in the most immature, moderately immature (together comprising IRF), and mature reticulocytes. An increase in IRF precedes the increase in reticulocyte count and is therefore used as a marker of erythropoietic activity.[18,19]

EPO resistance was measured in multiple ways. *Endogenous* EPO resistance was determined by defining the EPO levels for the degree of anemia, by calculating the observed/predicted log (EPO) ratio (O/P ratio). EPO levels were defined as inappropriate

at an O/P ratio <0.80. The O/P ratio can be calculated as follows: O/P ratio = (log(observed Epo))/(log(predicted Epo)]. To predict EPO levels, we used the regression equation as defined by Opasich et al: log(Epo) = 4.746−(0.275 × Hb).[13] Patients were stratified by O/P ratio at inclusion. *Exogenous* EPO response was measured as the increase of reticulocyte count, sTfr, and IRF after 2 weeks of EPO treatment and by assessing the Hb response after 6 months of EPO treatment.

Hepcidin-25

Serum hepcidin-25 measurements were performed by a combination of weak cation exchange chromatography and time-of-flight mass spectrometry.[20] Serum hepcidin-25 concentrations were expressed as nmol/l. The lower limit of detection of this method was 0.5 nM; average coefficients of variation were 2.7% (intra-run) and 6.5% (inter-run). The median reference level of serum hepcidin-25 as measured at 8:30 AM is 2.9 nM, range 0.5 to 8.2 nM.[21,22]

Statistical Analysis

Data are presented as medians with inter-quartile ranges for non-normally distributed variables and means ± standard deviation (SD) for normally distributed continuous variables. Unpaired data were compared with the unpaired *t*-test or the Mann-Whitney U test. For paired data we used the paired *t*-test or Wilcoxon rank test. Pearson correlation coefficient was used to test univariate correlation with RDW in normally distributed variables. Skewed variables were log-transformed. Analysis of variance was applied on parametric variables. Differences were considered significant when $P < .05$. Following univariate correlations, variables with a P value $<.05$, were entered into a multivariable linear regression model with stepwise forward selection process, to identify independent predictors of RDW. For statistical analyses the Statistical Package for Social Sciences (SPSS, Chicago, IL) version 17 was used.

Results

Population Characteristics

The original study population of the EPOCARES study comprising 62 patients. Five patients withdrew their informed consent and 1 patient was excluded because of malignancy (diagnosed on routine X-ray). Baseline RDW data were missing for 2 patients. Baseline characteristics of the 54 patients for this study are presented in Table 1, divided according to tertiles of RDW. Univariate linear correlations are listed in Table 2. In Table 3, the multivariable regression analysis is shown.

All patients had CKD, CHF, and anemia, as shown by the decreased estimated glomeral filtration rate (eGFR), using the modified diet in renal disease formula, higher NT-proBNP the lower LVEF, and the lower Hb levels. Vitamin B12 and folate levels were normal and hemolysis was absent, as measured by lactodehydrogenase levels. CRP and hs-CRP levels were only slightly elevated, showing that the study involved chronic stable patients without evident inflammation. At baseline there was no significant difference in all previously mentioned variables among the 3 study groups.

RDW at Baseline

The median RDW value at baseline was 14.0% (inter-quartile range 13.3-15.1), and 26% of the patients had a level above the upper limit ($>15\%$), which corresponds with data from other studies.[1−5,23] In our normocytic anemic population, a higher RDW was not associated with baseline Hb levels, hematocrit or MCV. RDW showed no correlation with renal function, as measured by creatinine, Cockroft-Gault equation, modified diet in renal disease formula, or cystatin C, nor with cardiac function as measured by left ventricular ejection fraction or NTproBNP levels at baseline (Tables 1 and 2).

Iron Metabolism

In these patients on oral iron supplementation (if tolerated) without overt inflammation, ferritin at baseline was <100 ng/mL in 22 of the patients (41%), indicating that some patients may have been (relatively) iron deficient despite oral supplementation. Indeed, also baseline TSAT levels were low in some patients ($<20\%$ in 26 of the patients or $<15\%$ in 8 patients). However, in the 35 patients that received EPO, there was no significant decrease in RET-He after 2 weeks of EPO treatment ($P = .32$). This indicates that there was no iron-restricted erythropoiesis in these patients. Therefore no direct evidence of decreased functional iron availability in the patients that received EPO was observed.[24] At baseline there was no difference in the variables for iron metabolism between the EPO-treated groups versus the control group, therefore we conclude that there was no iron-restricted erythropoiesis in the whole study population. Total iron store variables at baseline did not correlate with RDW (Tables 1 and 2). However, functional iron availability was negatively associated with RDW; patients in the highest RDW-tertile had both significant lower TSAT level and RET-He levels at baseline, as demonstrated by univariate correlation in Table 2 and further depicted in Figure 1A. Baseline hepcidin-25 showed no significant correlation with RDW.

Inflammation

A positive correlation was observed between RDW and IL-6 at baseline, but this was not the case with CRP, nor with hs-CRP. There was a negative correlation between IL-6 with RET-He (respectively, $P = .03$, $r = -0.30$). As previously described, there was no correlation between both IL-6 and hs-CRP with hepcidine-25 (respectively, $P = .93$, $r = -0.12$ and $P = .27$, $r = 0.20$) in our population.[25]

EPO Levels, Erythropoietic Activity, and EPO Resistance

There was a positive correlation between RDW and erythropoietic activity as measured by sTfr and IRF at baseline (univariate correlation Table 2 and scatterplot in Fig. 1B and 1C), but this was apparently not related to

Table 1. Baseline Characteristics of Patients from the EPOCARES Study, Stratified by RDW Values

Characteristics*	All Patients n = 54	First Tertile RDW < 13.4%	Second Tertile RDW 13.4-14.7%	Third Tertile RDW ≥ 14.8 %	*P* Value
Age (y)	74 (69-80)	72 (68-75)	77 (71-82)	77 (66-81)	.27
Male sex, n (%)	35 (65%)	12 (67%)	12 (67%)	11 (61.1%)	.49
Hemoglobin (g/dL)	11.8 ± 0.9	11.6 ± 0.9	12.1 ± 0.9	11.8 ± 0.9	.36
Hematocrit (%)	0.35 ± 0.03	0.35 ± 0.03	0.32 ± 0.12	0.34 ± 0.09	.71
MCV (/μm^3)	90.0 ± 4	90 ± 3	91 ± 5	89 ± 4	.60
MDRD (mL·min·1.73 m^2)	34.7 ± 13.8	30.5 ± 13.8	39.0 ± 15.3	34.7 ± 11.5	.18
Cockroft Gault (mL/min)	36.7 ± 14.9	33.9 ± 11.6	38.4 ± 17.2	37.8 ± 15.7	.62
Cystatin C (mg/L)	1.72 (1.36-2.47)	2.03 (1.50-2.72)	1.49 (1.08-2.45)	1.76 (1.48-2.37)	.43
NTproBNP (pg/mL)	1453 (718-2655)	1128 (482-1887)	1306 (718-2162)	2352 (926-6688)	.08
LVEF (%)	43.8 ± 11.7	45.7 ± 11.8	47.1 ± 10.3	37.9 ± 10.5	.97
Iron (μmol/L)	10 (8.8-14.0)	12 (10-14)	10 (9-15)	9 (8-12)	.08
Ferritin (ng/mL)	126 (75-175)	140 (68-198)	126 (90-195)	106 (55-141)	.46
Transferrin (g/L)	2.2 (2.0-2.5)	2.1 ± 0.23	2.3 ± 0.32	2.4 ± 0.51	.19
Transferrin saturation (%)	20 (15.8-25.0)	24 (18.8-29.0)	20 (16.5-25.3)	17 (14.0-20.3)	**.020**
Ret-He (fmol)	1.9 ± 0.14	1.94 ± 0.09	1.93 ± 0.14	1.80 ± 0.14	**.003**
Hepcidin-25 (nM)	5.9 (3.6-7.9)	6.9 (3.5-10.0)	5.8 (4.3-7.4)	5.0 (2.9-8.5)	.64
Soluble transferrin receptor (mg/L)	1.40 ± 0.47	1.19 ± 0.31	1.35 ± 0.42	1.67 ± 0.53	**.003**
Erythropoietin (IU/L)	13.0 (7.0-16.0)	11.5 (6.8-15.3)	14.5 (10.0-18.0)	12.5 (7.0-16.8)	.59
O/P ratio	0.78 ± 0.19	0.74 ± 0.18	0.80 ± 0.19	0.80 ± 0.20	.55
Reticulocyte count (10^{12}/L)	0.046 ± 0.015	0.038 ± 0.01	0.050 ± 0.01	0.049 ± 0.02	**.026**
IRF (%)	8.8 (5.4-11.4)	5.6 (3.8-9.2)	9.2 (6.4-11.2)	11.0 (7.4-14.7)	**.003**
CRP (mg/L)	6 (2-12)	2.5 (1.0-5.3)	7.0 (2.0-12.0)	7.0 (3.8-20)	.03
hs-CRP (mg/L) (n = 37)	5.8 (2.04-10.4)	2.4 (0.9-6.4)	6.2 (2.2-10.2)	7.2 (3.8-20.2)	.09
IL-6 (pg/mL)	3.66 (1.90-5.57)	2.5 (1.6-3.3)	3.66 (1.8-4.8)	7.1 (3.6-10.7)	**<.001**
Vitamin B12 (pg/mL)	277 (214-408)	277 (232-468)	239 (171-362)	329 (259-515)	.16
Folate (ng/mL)	17.5 (12.5-40.7)	20.2 (11.6-45.0)	16.5 (11.2-29.7)	21.7 (15.4-45.0)	.48
Lactate dehydrogenase (U/L)	408 (353-487)	397 (326-428)	422 (370-574)	411 (373-534)	.19
Albumin (g/L)	36.7 ± 3.0	37.0 ± 2.3	37.8 ± 2.3	35.4 ± 3.6	.045
Total cholesterol (mmol/L)	4.1 ± 1.1	4.6 ± 1.26	4.1 ± 0.98	3.6 ± 0.94	.044

MCV = mean corpuscular volume; NTproBNP = N-terminal pro-brain natriuretic peptide; LVEF = left ventricular ejection fraction; Ret-He = reticulocyte hemoglobin content; O/P ratio = log observed/predicted erythropoietin ratio; IRF = immature reticulocyte fraction; CRP = C-reactive protein; hs-CRP = high sensitivity CRP; IL-6 = interleukin-6; MDRD = estimated glomerular filtration rate by modified diet in renal disease formula.

*Values in mean ± standard deviation or median (interquartile range).

higher endogenous EPO levels, because there was no correlation between baseline EPO levels and RDW (Table 2).

The EPO level was defined for the degree of anemia by the baseline O/P ratio. Given that the average O/P ratio was only slightly below 0.80, the endogenous EPO production was partly preserved in this population with CKD and CHF. We found no correlation with RDW and *endogenous* EPO resistance as measured by the O/P ratio (Table 2).

Of the 54 patients, 35 patients received EPO treatment. After 2 weeks of EPO treatment, the reticulocyte count significantly increased ($P < .0001$), as did sTfR ($P < .0001$) and IRF ($P = .027$). Also, after 2 weeks of EPO treatment, RDW was significantly increased ($P < .001$). In the control group, without EPO treatment, the reticulocyte count ($P = .21$) did not change, nor did IRF ($P = .62$) or RDW ($P = .80$).

The magnitude of increase in reticulocyte count in the EPO treated group, however, did not correlate with the baseline RDW values. Neither did the extent in increase of sTfR and IRF after 2 weeks EPO treatment correlate with baseline RDW. These correlations between the increase in reticulocyte count, sTfR, and IRF after 2 weeks with log-transformed RDW at baseline are depicted in Figure 2A, 2B, and 2C.

In 17 of the 35 patients who received EPO treatment, the Hb was left to increase. After 6 months of EPO treatment, the Hb in these patients was significantly increased compared to baseline Hb ($P = .001$). The magnitude in increase of Hb after 6 months, showed no correlation with RDW at baseline ($P = .35$, $r = 0.26$). Neither was there any correlation with baseline RDW and the magnitude of increase in reticulocyte count, sTfR, and IRF after 6 months of EPO treatment (resp. $P = .54$, $P = .39$, and $P = .36$). These results show that there is neither a correlation between baseline RDW and response to *exogenous* EPO.

Multivariable Regression Model

After entering all baseline biomarkers with a significant univariate correlation with baseline RDW in a multivariable regression model, on a stepwise forward selection, sTfR, IL-6, RET-He, and IRF proved to be independent predictors of RDW (Table 3).

Discussion

A strong independent association exists between RDW, a measure of anisocytosis, and adverse outcomes in cardiovascular disease (eg, CHF). The underlying process that links RDW to outcome is unknown. One of the main findings of this study is that EPO resistance as measured by several different methods was not associated with RDW.

Table 2. Univariate Correlation Coefficients of Clinical and Biochemistry Variables with RDW, EPOCARES Study, n = 54

	RDW	
Variable	*r*	*P*
Age	0.25	0.076
Hemoglobin	−0.05	.72
Hematocrit	0.09	.52
MCV	−0.08	.58
MDRD	0.13	.37
Cockroft Gault	0.11	.43
Cystatin C	−0.26	.85
NTproBNP	0.24	.10
LVEF	−0.17	.22
Serum iron	−0.33	.02
Ferritin	−0.21	.16
Transferrin	0.34	.012
Transferrin saturation	−0.39	.005
Ret-He	−0.48	<.001
Hepcidin	−0.25	.07
Soluble transferrin receptor	0.48	<.001
Erythropoietin	0.12	.42
O/P ratio	0.11	.42
Reticulocyte count	0.13	.36
IRF	0.36	.01
CRP	0.23	.11
hs-CRP (n = 37)	0.27	.13
IL-6	0.48	<.001
Vitamin B12	0.25	.073
Folate	0.07	.61
Lactate dehydrogenase	0.21	.13
Albumin	−0.17	.23
Total cholesterol	−0.27	.055

MCV = mean corpuscular volume; NTproBNP = N-terminal pro-brain natriuretic peptide; LVEF = left ventricular ejection fraction; Ret-He = reticulocyte hemoglobin content; O/P ratio = log observed/predicted erythropoietin ratio; IRF = immature reticulocyte fraction; CRP = C-reactive protein; hs-CRP = high sensitivity CRP; IL-6 = interleukin-6; MDRD = estimated glomerular filtration rate by modified diet in renal disease formula.

All nonparametric variables were considered for analysis after logarithmic transformation.

In this stable patient group with both heart and renal failure, RDW was associated with functional iron availability and erythropoietic activity. After multivariate analysis, markers of functional iron availability, erythropoietic activity, and IL-6 were independent predictors of RDW. However, hepcidin levels were not significantly associated with RDW. This underscores earlier findings that, in low-inflammatory patient groups, hepcidin levels are not associated with markers of inflammation.[25,26]

Table 3. Stepwise Multivariate Linear Regression for RDW With Baseline Variables from the EPOCARES Study*, n = 54

Variable	Step No.	Multiple r^2	β Coefficient	*P*
Soluble transferrin receptor	1	0.218	0.483	<.001
Interleukin-6	2	0.356	0.394	<.001
Reticulocyte hemoglobin content	3	0.434	−0.309	<.001
Immature reticulocyte fraction	4	0.529	0.278	<.001

*All non-parametric variables were considered for analysis after logarithmic transformation.

EPO resistance, defined as an inadequate bone marrow response to *exogenous* or *endogenous* EPO, contributes to anemia[11] and is associated with increased mortality in patients with heart and/or kidney failure.[27,28] Indeed, EPO levels in patients with CKD and/or CHF are higher as compared to healthy controls, but inappropriately low for the degree of anemia.[10,13] This observation indicates a relative EPO deficiency as well as a reduced bone marrow response to *endogenous* EPO.[11] Approximately, 10% of CKD patients treated with *exogenous* EPO have an inadequate response, which leads to therapy with higher doses of exogenous EPO. In several studies, such as the Correction of Hemoglobin and Outcomes in Renal Insufficiency (CHOIR) study, the use of high-dose EPO in EPO-resistant patients was associated with increased morbidity and mortality.[27] Subsequently it was hypothesized by several authors that a disturbed bone marrow response to erythropoietin could explain the association between morphologic changes in the red blood cell (RDW) and cardiovascular risk.[2,4,12] However, in the current study, none of the markers of EPO resistance, estimating both the response to *endogenous* EPO as well as the response to *exogenous* EPO, was associated with RDW. Furthermore, in our EPO-naive patient group, treatment with exogenous EPO induced an increase in RDW. This contradicts an association between resistance to EPO and RDW.

We did find a significant correlation between RDW and markers of erythropoietic activity at baseline (IRF and sTfR). It is important to note that increased erythropoietic activity does not necessarily result in increased hemoglobin levels because the level of hemoglobin is determined by the red cell production and maturation rate and by the rate of red cell destruction. IRF is defined as the ratio of young, immature reticulocytes to the total number of reticulocytes. IRF is used to assess the degree of erythropoietic activity (eg, after chemotherapy).[18] Circulating reticulocytes shed the soluble transferrin receptor during their maturation sequence. This sTfR level correlates more strongly with corpuscular indices than with iron parameters and is used as a biomarker of increased erythropoietic activity.[29] Förhécz et al also reported a positive correlation between sTfR and RDW in a nonanemic cohort of patients with systolic heart failure.[2] This concept is further strengthened by our results, in which RDW is positively related to erythropoietic activity, as measured by both IRF and sTfR, in anemic patients with heart and kidney failure.

RDW is most commonly used in the differential diagnosis of iron deficiency anemia, in which MCV is decreased and RDW is increased. Decreased functional iron availability plays a role in anemia of CHF and CKD. We demonstrate a negative correlation between baseline TSAT, RET-He, sTfR, and RDW in our iron-supplemented patients. RET-He is considered to be a very sensitive indicator reflecting iron availability for erythropoiesis[30] and is an indicator for iron-restricted erythropoiesis in patients receiving EPO.[24] Compared with the use of ferritin or TSAT, it compared better in sensitivity and specificity.[17] Therefore, although 41% of our orally iron-supplemented patients had a ferritin

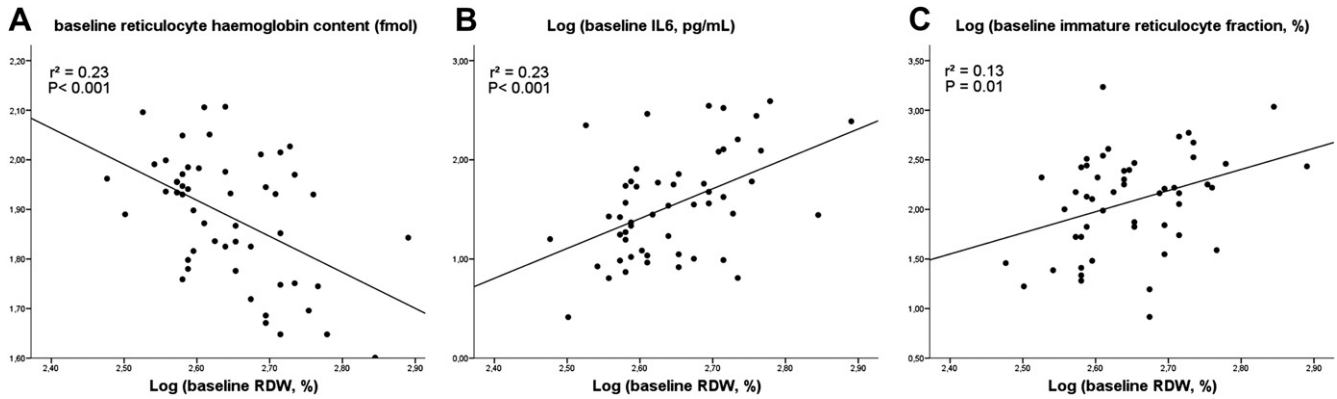

Fig. 1. The correlation between log-transformed baseline red cell distribution width (RDW) values and baseline levels of (A) reticulocyte hemoglobin content, (B) log-transformed interleukin-6, and (C) log-transformed immature reticulocyte fraction in 54 cardiorenal patients.

level < 100 ng/mL, indicating possible lower iron stores, the lack of decrease in RET-He after EPO therapy suggests that there was no overtly iron-restricted erythropoiesis. Because we found no association between ferritin levels and RDW, our data suggest that decreased functional iron availability but not iron stores play a role in higher RDW values. Our results correspond with recently published results,[2,4] describing a positive correlation between RDW and TSAT and/or sTfR, in symptomatic heart failure patients. In addition to these studies, we determined RET-He, a more sensitive marker of functional iron availability, which further substantiated the contention that increased RDW values are associated with decreased functional iron availability.

As mentioned earlier, in our study RDW was associated with markers of both erythropoietic activity and functional iron availability. Furthermore, a strong correlation was observed between RDW and IL-6. It has been hypothesized that the correlation between RDW and functional iron availability is mediated by hepcidin.[4] Hepcidin is upregulated by a number of stimuli (eg, anemia and inflammation [IL-6]). Hepcidin thus integrates input from erythropoietic and inflammatory pathways.[31] In patients with CKD,[32] it has been shown that hepcidin levels are higher compared to healthy controls, but in patients with CHF and anemia this was not confirmed.[26,33] In our patients with the combination of heart and kidney failure and anemia hepcidin-25 levels, were higher. Thus, hepcidin seems an obvious ''candidate-linking factor'' between inflammation and decreased functional iron availability, leading to higher RDW levels. However, our data show no clear correlation between hepcidin-25 and RDW. It should be noted that, because this is a small study and the *P* value ($P = .07$) approached significance, a weak association between RDW and hepcidin cannot be ruled out. Also, there was no correlation between hepcidin-25 and inflammation in our patient group. This finding was confirmed in another study and is in keeping with our earlier finding that in our group of stabile cardiorenal patients, with relatively low levels of inflammatory biomarkers, increased hepcidin levels were associated with markers of iron load (ferritin) rather than with markers of inflammation.[25,26] Thus, in our study, RDW was associated with IL-6 but not with other markers of inflammation. Although data exist that IL-6 can influence iron absorption during the hypoxic exposure, via a mechanism independent of hepcidin,[34] at this point it is unclear how IL-6 is related to an increase in RDW.

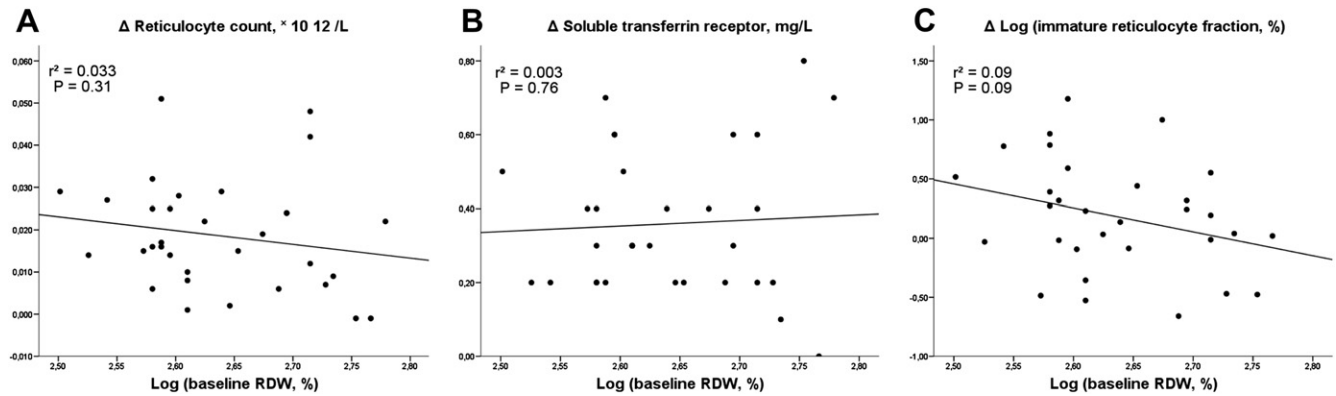

Fig. 2. The correlation between log-transformed baseline red cell distribution width (RDW) values and (A) the reticulocyte increase, (B) the increase in soluble transferrin receptor, and (C) the increase in immature reticulocyte fraction, after 2 weeks of erythropoietin treatment in 35 cardiorenal patients.

RDW is elevated in conditions of increased erythropoiesis/ineffective erythropoiesis and in conditions of increased red cell destruction. RDW increases when the relative number of larger and/or smaller red blood cells increases. Because RDW is not correlated to MCV in our patient group, it can be hypothesized that the changes in RDW are caused by both an increase in the relative number of large red cells as well as an increase of the relative number of small red cells in the peripheral blood. Indeed, MCV positively correlated with both RET-He ($r = 0.387$, $P = .004$) as well as with IRF ($r = 0.245$, $P = .08$). The absence of a correlation between RDW and MCV in our anemic patients thus may be due to a balanced net effect of increased erythropoietic activity leading to a higher MCV and decreased iron availability, leading to a lower MCV.

Finally, limitations of the study as result of sample size and selection bias need to be acknowledged. The cohort size of the 2-center EPOCARES study is rather small. Studying simple associations and constructing a multivariate model using a relatively small sample size is of limited value, although most of the associations we report are robust despite the small sample size. However, we cannot fully exclude the possibility that the lack of association between some parameters (eg, hepcidin, RDW) is due to lack of power. This patient group comprised anemic patients with both CHF and CKD; therefore, these results should be carefully interpreted and cannot be generalized to all patients with CHF, especially those patients without renal dysfunction. Also, the EPOCARES patients were receiving multiple drugs, including oral iron supplementation, throughout the study, and were in a relatively low inflammatory state. This might not fully represent daily clinical practice on which data of RDW as biomarker for outcome are based.

In conclusion, EPO resistance was not associated with RDW in these iron-supplemented anemic patients with CKD and CHF. However, RDW was associated with erythropoietic activity, decreased functional iron availability and IL-6. We found no significant correlation between hepcidin and RDW. In our view, as also pointed out by Allen et al, the association of RDW with outcome may imply that the erythrocyte may be viewed as a ''barometer'' of overall cardiovascular health.[4] Therefore mechanisms that cause changes in relative distribution of red cell size such as increased erythropoietic activity, increased red cell destruction, and reduced red cell half life should be investigated.

Acknowledgments

The authors thank Mrs. A. Diepenbroek for the excellent patient care assistance and data collection.

Disclosure

None.

References

1. Felker GM, Allen LA, Pocock SJ, Shaw LK, McMurray JJ, Pfeffer MA, et al. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. J Am Coll Cardiol 2007;50:40−7.
2. Forhecz Z, Gombos T, Borgulya G, Pozsonyi Z, Prohaszka Z, Janoskuti L. Red cell distribution width in heart failure: prediction of clinical events and relationship with markers of ineffective erythropoiesis, inflammation, renal function, and nutritional state. Am Heart J 2009;158:659−66.
3. Pascual-Figal DA, Bonaque JC, Redondo B, Caro C, Manzano-Fernandez S, Sanchez-Mas J, et al. Red blood cell distribution width predicts long-term outcome regardless of anaemia status in acute heart failure patients. Eur J Heart Fail 2009;11:840−6.
4. Allen LA, Felker GM, Mehra MR, Chiong JR, Dunlap SH, Ghali JK, et al. Validation and potential mechanisms of red cell distribution width as a prognostic marker in heart failure. J Card Fail 2010;16:230−8.
5. Tonelli M, Sacks F, Arnold M, Moye L, Davis B, Pfeffer M. Relation between red blood cell distribution width and cardiovascular event rate in people with coronary disease. Circulation 2008;117:163−8.
6. 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;169:515−23.
7. Patel KV, Semba RD, Ferrucci L, Newman AB, Fried LP, Wallace RB, et al. Red cell distribution width and mortality in older adults: a meta-analysis. J Gerontol A Biol Sci Med Sci 2010;65:258−65.
8. Perlstein TS, Weuve J, Pfeffer MA, Beckman JA. Red blood cell distribution width and mortality risk in a community-based prospective cohort. Arch Intern Med 2009;169:588−94.
9. Groenveld HF, Januzzi JL, Damman K, van Wijngaarden J, Hillege HL, van Veldhuisen DJ, et al. Anemia and mortality in heart failure patients a systematic review and meta-analysis. J Am Coll Cardiol 2008;52:818−27.
10. Belonje AM, Voors AA, van der Meer P, van Gilst WH, Jaarsma T, van Veldhuisen DJ. Endogenous erythropoietin and outcome in heart failure. Circulation 2010;121:245−51.
11. van der Putten K, Braam B, Jie KE, Gaillard CA. Mechanisms of disease: erythropoietin resistance in patients with both heart and kidney failure. Nat Clin Pract Nephrol 2008;4:47−57.
12. van Kimmenade RR, Mohammed AA, Uthamalingam S, van der Meer P, Felker GM, Januzzi JL Jr. Red blood cell distribution width and 1-year mortality in acute heart failure. Eur J Heart Fail 2010;12:129−36.
13. Opasich C, Cazzola M, Scelsi L, De Feo S, Bosimini E, Lagioia R, et al. Blunted erythropoietin production and defective iron supply for erythropoiesis as major causes of anaemia in patients with chronic heart failure. Eur Heart J 2005;26:2232−7.
14. van der Putten K, Jie KE, Emans ME, Verhaar MC, Joles JA, Cramer MJ, et al. Erythropoietin treatment in patients with combined heart and renal failure: objectives and design of the EPOCARES study. J Nephrol 2010;23:363−8.
15. Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the diagnosis and treatment of acute and chronic heart failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). Eur J Heart Fail 2008;10:933−89.
16. Paulus WJ, Tschope C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J 2007;28:2539−50.

17. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. Clin J Am Soc Nephrol 2006;1(Suppl 1):S4−8.
18. Briggs C. Quality counts: new parameters in blood cell counting. Int J Lab Hematol 2009;31:277−97.
19. Davis BH, Ornvold K, Bigelow NC. Flow cytometric reticulocyte maturity index: a useful laboratory parameter of erythropoietic activity in anemia. Cytometry 1995;22:35−9.
20. Kroot JJ, Laarakkers CM, Geurts-Moespot AJ, Grebenchtchikov N, Pickkers P, van Ede AE, et al. Immunochemical and mass-spectrometry-based serum hepcidin assays for iron metabolism disorders. Clin Chem 2010;56:1570−9.
21. Kroot JJ, Hendriks JC, Laarakkers CM, Klaver SM, Kemna EH, Tjalsma H, et al. (Pre)analytical imprecision, between-subject variability, and daily variations in serum and urine hepcidin: implications for clinical studies. Anal Biochem 2009;389:124−9.
22. Peters HP, Laarakkers CM, Swinkels DW, Wetzels JF. Serum hepcidin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. Nephrol Dial Transplant 2010;25: 848−53.
23. Al-Najjar Y, Goode KM, Zhang J, Cleland JG, Clark AL. Red cell distribution width: an inexpensive and powerful prognostic marker in heart failure. Eur J Heart Fail 2009;11:1155−62.
24. Schoorl M, Schoorl M, Nube MJ, Bartels PC. Erythropoiesis activity, iron availability and reticulocyte hemoglobinization during treatment with hemodialysis and in subjects with uremia. Clin Lab 2006;52: 621−9.
25. van der Putten K, Jie KE, van den Broek D, Kraaijenhagen RJ, Laarakkers C, Swinkels DW, et al. Hepcidin-25 is a marker of the response rather than resistance to exogenous erythropoietin in chronic kidney disease/chronic heart failure patients. Eur J Heart Fail 2010;12: 943−50.
26. Matsumoto M, Tsujino T, Lee-Kawabata M, Naito Y, Akahori H, Sakoda T, et al. Iron regulatory hormone hepcidin decreases in chronic heart failure patients with anemia. Circ J 2010;74:301−6.
27. Szczech LA, Barnhart HX, Inrig JK, Reddan DN, Sapp S, Califf RM, et al. Secondary analysis of the CHOIR trial epoetin-alpha dose and achieved hemoglobin outcomes. Kidney Int 2008;74:791−8.
28. Zhang Y, Thamer M, Stefanik K, Kaufman J, Cotter DJ. Epoetin requirements predict mortality in hemodialysis patients. Am J Kidney Dis 2004;44:866−76.
29. Choi JW. Serum transferrin receptor concentrations correlate more strongly with red cell indices than with iron parameters in iron-deficient adolescents. Acta Haematol 2003;110:213−6.
30. Bartels PC, Schoorl M, Schoorl M. Hemoglobinization and functional availability of iron for erythropoiesis in case of thalassemia and iron deficiency anemia. Clin Lab 2006;52:107−14.
31. Swinkels DW, Wetzels JF. Hepcidin: a new tool in the management of anaemia in patients with chronic kidney disease? Nephrol Dial Transplant 2008;23:2450−3.
32. Zaritsky J, Young B, Wang HJ, Westerman M, Olbina G, Nemeth E, et al. Hepcidin—a potential novel biomarker for iron status in chronic kidney disease. Clin J Am Soc Nephrol 2009;4:1051−6.
33. Divakaran V, Mehta S, Yao D, Hassan S, Simpson S, Wiegerinck E, et al. Hepcidin in anemia of chronic heart failure. Am J Hematol 2010;86:107−9.
34. Raja KB, Latunde-Dada GO, Peters TJ, McKie AT, Simpson RJ. Role of interleukin-6 in hypoxic regulation of intestinal iron absorption. Br J Haematol 2005;131:656-62.