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Red cell distribution width is associated with endothelial progenitor cell depletion and vascular-related mediators in rheumatoid arthritis

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ABSTRACT

Objectives: The role of Red Cell Distribution Width (RDW) as a predictor of cardiovascular (CV) events has been proposed in a variety of conditions, including Rheumatoid Arthritis (RA). However, the mechanisms underlying this effect are still unknown. Since inflammation and Endothelial Progenitor Cells (EPCs) imbalance have been reported in RA patients to be related to CV disease, we wondered whether RDW could be linked to endothelial repair failure in RA.

Methods: EPCs ($CD34^+VEGFR2^+CD133^+$) were quantified by flow cytometry in peripheral blood samples from 194 RA patients. IFNα, TNFα, IFNγ, IL-8, VEGF, GM-CSF, MCP-1, ICAM-1, EGF, Leptin and Resistin serum levels were quantified by immunoassays. Clinical and immunological parameters as well as history of traditional CV risk factors and CV events were registered from medical records. RDW was measured in complete blood cell count analyses.

Results: RDW was negatively related to EPC counts in patients with established disease (>1 year, $n = 125$) ($r = -0.306$, $p < 0.001$). Moreover, RDW was independently associated to an EPC depletion in the whole group (β [95% CI]: −3.537 [−6.162, −0.911], $p = 0.009$) after adjusting for clinical parameters, disease duration, treatments and traditional CV risk factors. Additionally, RDW was positively correlated with IFNα serum levels, a cytokine related to endothelial damage, and with IL-8, VEGF and neutrophil to lymphocyte ratio, thus supporting the association with inflammation and vascular remodelling.

Conclusions: RDW was associated to EPC depletion and increased levels of different mediators linked to endothelial damage and vascular repair failure, thereby shedding new light on the nature of RDW as CV-predictor.

1. Introduction

Red Cell Distribution Width (RDW) is a traditional marker of anisocytosis, widely used for anaemia diagnosis. However, recent evidence points to a role for RDW as a cardiovascular risk biomarker [1]. Additionally, other authors have linked high RDW measurements with increased markers of inflammation [2]. These findings make RDW an attractive candidate to be regarded as CV risk biomarker in autoimmune conditions and, especially, in Rheumatoid Arthritis (RA) [3]. We have recently looked into this possibility, concluding that RDW could be a CV risk biomarker in RA patients [4].

However, the actual mechanisms that underlie the predictive role of RDW as a CV risk biomarker in RA remain unclear. Disease activity [3] and inflammation [5] have been linked to CV disease in RA probably because of the effects they could cause on vascular damage and repair. In this scenario, circulating Endothelial Progenitor Cells (EPC) could play a pivotal role in endothelial damage repair. Decreased or functionally impaired EPCs have been reported in RA, associated in most cases with disease activity and inflammatory mediators [6–8], and linked to CV disease development [9–11]. In this context, we wondered whether RDW would be associated to an endothelial repair failure. Due to the growing relevance of EPCs in the field of rheumatic diseases, this would be of special interest in the clinical setting, since it would provide information regarding EPC imbalance through an easy, inexpensive

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and validated parameter.

On the other hand, although RDW has been associated with inflammatory burden, the exact mechanisms that underlie this effect remain unknown. Only associations with general markers of inflammation (that is, CRP and ESR) have been reported [2,12]. Due to the relevance of the cytokine network in RA pathogenesis [13] and CV disease susceptibility [14], dissecting the actual circuits within the inflammatory burden that account for the RDW values in RA, would provide valuable insights about the nature of RDW as a biomarker and, additionally, new strategies for CV risk management in RA.

Consequently, the main aim of this study was to explore the mechanisms that underlie the role of RDW as a CV risk biomarker in RA patients and evaluate its potential associations with proven biomarkers of endothelial damage and impaired vascular repair.

2. Material and methods

2.1. Patients

Our study involved 194 RA patients consecutively recruited from the Rheumatology Department at Hospital Universitario Central de Asturias (HUCA). All patients fulfilled American College of Rheumatology 2010 criteria. Routine clinical examination included DAS28 score calculation and assessment of ongoing therapies and concomitant traditional CV risk factors. Furthermore, clinical medical records were revised to register past CV events. Traditional CV risk factors and CV events were classified as previously described [10,15]. A fasting blood sample was obtained by venipuncture. A complete blood count (including RDW) was performed in an ADVIA 2120 automated haematology analyser (Siemens) in the haematology laboratory at the HUCA.

Approval for the study was obtained from the Regional Ethics Committee for Clinical Investigation, in compliance with the Declaration of Helsinki, and all the participants gave written informed consent.

2.2. Flow cytometry analysis

Circulating EPCs were quantified in blood samples by flow cytometry as previously described [10]. Briefly, 100 μl of whole blood were preincubated with FcR Blocking Reagent (Miltenyi Biotec) for 20 min at 4 °C. Then, anti-CD34 FITC (BD Biosciences), anti-VEGFR2 PE (R&D) and anti-CD133 APC (Miltenyi) or isotype-matched controls were added and incubated for 30 min at 4 °C. Finally, red cells were lysed and cells were washed twice with PBS. Stained cells were acquired in a FACS Canto II (BD Biosciences) at a low rate until more than 100,000 events in the lymphocyte gate and 100 events in the $CD34^+$ gate were registered. EPCs were identified as $CD34^+CD133^+VEGFR2^+$ cells within the lymphocyte gate.

2.3. Quantification of serum cytokine levels

Serum aliquots were obtained from RA patients and were stored at −80 °C until cytokine measurements were conducted. IFNα serum levels were measured in 194 patients (69 early and 125 long-standing RA, whereas other cytokines were analysed in 129 individuals (29 and 110, respectively). IFNα, IL-8, VEGF and GM-CSF serum levels were quantified using a Cytometric Bead Array Flex Set (BD) in a BD FACS Canto II flow cytometer using FCAP Array v.1.0.1, following the manufacturer's instructions. The theoretical detection limits were 1.25 pg/ml, 1.2 pg/ml, 4.5 pg/ml and 0.2 pg/ml, respectively.

IFNγ serum levels were assessed using an OptEIA kit (BD) following the manufacturer's instructions (detection limit: 0.58 pg/ml). Levels of ICAM-1, TNFα, MCP-1, EGF, leptin and resistin were quantified using Mini ELISA Development Kits (PeproTech), according to the manufacturer's instructions (detection limits were: 23 pg/ml, 3.9 pg/ml, 8 pg/ml, 8 pg/ml, 63 pg/ml and 24 pg/ml, respectively).

2.4. Statistical analysis

Data are expressed as median (interquartile range) or n (%), as appropriate. Since data were not normally distributed, spearman rank's test was used to analyse correlations and Mann–Withney U test was used to assess differences between groups. Variables were log-transformed to achieve normal distribution prior to multiple regression analyses. A p-value <0.050 was considered as statistically significant. Statistical analyses were performed under SPSS v. 19.0 and R software v. 2.15.1.

3. Results

3.1. EPC depletion correlates with RDW levels

Given the association between RDW and CV disease in RA patients, we aimed to evaluate whether this parameter could be related to EPC counts, a surrogate marker of endothelial repair. To this end we quantified circulating EPCs by flow cytometry in 194 RA patients (Table 1) with different disease duration (from 0 to 258 months). Our data revealed that EPC counts correlated negatively with disease duration (Fig. 1A). In fact, long-standing (>1 year) RA patients (n = 125) exhibited an EPC depletion compared to their early counterparts (0.025(0.027) vs 0.060(0.060)·10^3/μl, p = 0.012), whereas RDW did not differ between groups (p = 0.103).

Although the analysis of the whole RA group showed a negative association between RDW and EPC counts (r = −0.186, p = 0.014), this correlation was stronger in long-standing patients but was not detected in the early group (Fig. 1B). Multivariate regression analyses confirm that this effect was detected in long-standing patients even after adjusting for traditional CV risk factors (hypertension, dyslipidaemia, diabetes, BMI and smoking habit) and treatments (glucocorticoids, methotrexate, TNFα-blockers, tocilizumab and statins) as possible confounding variables (β[95% CI], p-value: −4.182 [−6.394, −1.970], p = 0.0003), but not in their early counterparts (−1.779 [−9.583, 6.025], p = 0.634). Interestingly, RDW did not correlate with total CD34+ or CD133+ progenitors (r = 0.044, p = 0.559; and r = 0.049, p = 0.441; respectively), thus confirming that this effect was specific for the EPC population and not due to a generalized mobilization of bone-marrow progenitors.

On the other hand, the analysis of clinical parameters revealed that RDW was associated with inflammation, disease activity and severity in the long-standing group, but not in the early stage disease group (Table 2). Interestingly, RDW was also associated with traditional CV risk factors (age and BMI) in these patients. Moreover, these results were consistent after controlling for traditional CV risk factors (β, p-value: age at sampling: 0.257, p = 0.037; disease duration: 0.367, p < 0.001; BMI: 0.435, p = 0.012; CRP: 0.249, p = 0.032; ESR: 0.259, p = 0.024; PGA: 0.415, p < 0.0001; DAS28: 0.272, p = 0.018 and HAQ: 0.267, p = 0.019), hence excluding any potential effect of comorbidities-derived inflammation. Therefore, a multivariate regression analysis using EPC counts as dependent variable was performed in the whole group, including the demographic and clinical parameters associated with RDW (as shown in Table 2) as well as traditional CV risk factors and immunomodulatory treatments as potential confounding variables. Our results confirm that RDW was independently associated with an EPC depletion (β [95% CI]: −3.537