European Journal of Internal Medicine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Original Article

Red cell distribution width and the risk of cerebral vein thrombosis: A case–control study

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ARTICLE INFO

Article history:
Received 17 March 2016
Received in revised form 2 October 2016
Accepted 20 October 2016
Available online xxxx

Keywords:
Intracranial sinus thrombosis
Venous thrombosis
Cerebrovascular disorders
Blood coagulation
Risk factors
Erythrocyte indices

ABSTRACT

Background: Red cell distribution width (RDW) is a marker of cardiovascular diseases and venous thromboembolism, but its role in cerebral vein thrombosis (CVT) is unknown.
Aims: To investigate whether high values of RDW are associated with an increased risk of CVT.
Methods: A case–control study of CVT patients (≥18 years-old) referred to our center contrasted with healthy individuals. Odds ratios (ORs) were calculated for RDW values >90th percentile by multivariable logistic regression and adjusted for demographic characteristics, hemorheological parameters, renal function, fibrinogen and CRP. Quartiles based on the distribution of RDW values were used in an additional model to assess a dose–response relationship. The risk of CVT associated with the combined presence of high RDW and either thrombophilia abnormalities or oral contraceptive use was also estimated.
Results: 143 cases (median age 36 years, 18–79) and 352 controls (42 years, 18–80) were investigated. RDW values >90th percentile (>14.6%) were associated with an increased risk of CVT (OR 2.44, 95% CI 1.39–4.28). The association remained after further adjustment for hemorheological parameters (OR 3.73, 95% CI 1.72–8.09), inflammatory markers (OR 3.77, 95% CI 1.72–8.25) and renal function (OR 3.62, 95% CI 1.53–8.55). The risk appeared restricted to these extreme levels (>14.6%), as there was no graded association between values of RDW and CVT risk over quartiles. There was a synergistic effect on the risk of CVT for the combination of high RDW and thrombophilia abnormalities (OR 33.20, 95% CI 6.95–158.55) or oral contraceptive use (OR 37.99, 95% CI 8.78–164.45).
Conclusions: Values of RDW >90th percentile are associated with CVT.

1. Introduction

Cerebral vein thrombosis (CVT) is a form of stroke whereby thrombosis occurs in the cerebral dural sinuses or veins. The incidence of CVT has been estimated at 3 to 12 cases per million per year, representing about 0.5–1% of all strokes, with the highest incidence in women [1]. CVT typically affects the young individuals and, despite its rare occurrence, is associated with mortality (up to 30%) and high rate of severe disability (up to 44%), partly related to the underlined disorder [2]. In fact, CVT may occur in association with cancers of multiple etiologies (brain tumors, but also hematologic malignancies and solid tumors at other sites), cerebral infections, traumas, vasculitis and inflammatory systemic disorders, risk factors that are shared with venous thrombosis at other sites (i.e., deep vein thrombosis of the lower limbs and pulmonary embolism) [2,3]. However, especially in western countries, oral contraceptive use, pregnancy and puerperium are the strongest non-specific risk factors for CVT, accounting for the high prevalence of young women within CVT patients [3,4]. Also thrombophilia abnormalities (such as factor V Leiden, prothrombin G20210A mutation, antithrombin, protein C or protein S deficiencies and the presence of lupus anticoagulant antibodies) have been confirmed to be a risk factor for CVT, but with a different degree of association as compared with venous thromboembolism [5,6]. Finally, in up to 30% of patients with CVT, there are no identifiable risk factors [7].

Red blood cell distribution width (RDW) is a measurement of the size variation of circulating red blood cells and an index of their heterogeneity [8]. It is a standard part of the routine complete blood count and together with the mean corpuscular volume (MCV), RDW is used as a predictor of an underlying anemia, being increased in iron-deficiency and megaloblastic anemias and normal in thalassemia and macrocytic anemias from other causes. A variety of conditions may determine a significant variation of the red blood cell size, generating a high RDW (anisocytosis), whereas a low RDW indicates a uniform size of red

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http://dx.doi.org/10.1016/j.ejim.2016.10.017
0953-6205/

blood cells. For instance, high RDW was independently associated with declining glomerular filtration rate, suggesting an inverse association between RDW and renal function [9]. Inflammation is also strongly related to ineffective erythropoiesis, and some studies showed that inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, inhibit red blood cell maturation, thus promoting anisocytosis [10,11]. Recently, high values of RDW have been associated with the risk of venous thromboembolism, i.e., deep vein thrombosis of the lower limbs and/or pulmonary embolism [12–14], and with the risk of incident cerebrovascular events, particularly ischemic stroke [15]. To date few data are available on RDW values in patients with CVT [16], and no studies have investigated the association between RDW and the risk of CVT.

To investigate whether high values of RDW are associated with an increased risk of CVT, a case–control study, based on a historical population with data previously collected, was carried out taking into account several possible confounders. The combined effect of high RDW values and other known risk factors for CVT (thrombophilia abnormalities and oral contraceptive use) was also evaluated.

2. Patients and methods

Patients aged more than 18 years with a first objectively diagnosed episode of CVT (through cerebral digital angiography, computed tomography angiography, magnetic resonance, or magnetic resonance angiography) consecutively referred to the outpatient clinic of our Center between January 2000 and March 2015 were included in the study [17]. Demographic data, thrombosis location and risk factors (cancer, infections, trauma, oral contraceptive use, pregnancy or puerperium) were recorded by interview and medical charts on the day of blood sampling. Only patients who underwent a complete blood count at our center, with RDW measurement, were included. Patients with previous episodes of arterial thrombosis (stroke, myocardial infarction or peripheral arterial thrombosis) or venous thromboembolism were excluded. Acquaintances or partners of cases in whom a history of arterial or venous thrombosis was excluded by a validated questionnaire were included as controls [18].

Individuals with overt cancer (including myeloproliferative neoplasm), hepatic or autoimmune diseases were excluded from the study due to the influence of these conditions on RDW values [19,20]. The study was approved by the Hospital Institutional Review Board and Ethics Committee, and all patients and controls signed the informed consent before inclusion in the study.

2.1. Laboratory tests

Blood samples were collected at morning after 8 h fasting period in vacuum tubes with EDTA as anticoagulant for automated complete blood count and without anticoagulant for biochemical analyses. For the thrombophilia screening, additional samples were collected into vacuum tubes containing 3.2% sodium citrate (Vacuette Premium tubes, Greiner Bio-One, Kremsmünster, Austria) and centrifuged within 15 min at 20 °C for 20 min at 2880 × g. Complete blood count, homocysteine and creatinine levels were tested at the same day of plasma collection. The remaining plasma obtained was aliquoted and snap-frozen in liquid nitrogen, and then stored at −80 °C until analysis. Creatinine clearance was calculated as a measure of renal function, following the Cockroft–Gault formula [21].

RDW was calculated by the automated hematological analyzer by dividing the standard deviation of the red blood cell volume (MCV) by the mean MCV, then multiplying the result by 100 in order to express it as a percentage (RDW% = [standard deviation MCV / mean MCV] × 100) [8]. RDW varies widely depending on the method used by different analyzers to calculate it [22]. With our automated hematological analyzer (Sysmex XE2100, Dasit Diagnostica), the lower and upper limits of the normal laboratory reference range are 11.5% and 14.5%, respectively. The between-run coefficient of variability (CV%) calculated by our laboratory for RDW is 3.0%.

DNA was isolated from 10 mL of EDTA-augmented blood and amplified by polymerase chain reaction as previously described [23].

Thrombophilia screening included: (I) DNA analysis for the 1691 guanine to adenine substitution in coagulation factor V gene (factor V Leiden) and for the 20210 guanine to adenine substitution in the 3′-untranslated region of the prothrombin gene [24,25]; (II) functional and antigenic (when required) assays for plasma fibrinogen, antithrombin, protein C and protein S [26]; (III) antiphospholipid antibodies (lupus anticoagulant, anticardiolipin and anti-β2 glycoprotein I IgG and IgM antibodies) [27]; (IV) plasma factor VIII levels [28] with high levels defined when exceeding the 95th percentile of the distribution among controls (166 IU/dL); (V) fasting and post-methionine load (3.8 g per square-meter of body surface area) with hyperhomocysteine defined when levels exceeded the 95th percentile of the homocysteine distribution among controls (24 and 29 μmol/L fasting levels and 40 and 37 μmol/L the difference between post-methionine load and fasting levels, for women and men, respectively) [29].

All samples were collected at least 3 months after CVT (median time 20.0 months, IQR 64.5) and after the termination of any anticoagulation therapy, in order to avoid changes in biological parameters related to the event [30].

2.2. Statistical analysis

Kolmogorov–Smirnov test was used to assess the normality of continuous variables distribution. We used median and interquartile range to describe continuous variables non-normally distributed, and count and percentage for categorical variables. Differences between groups were analyzed using the Student's *t*-test for continuous variables and chi-square test for categorical variables; a difference between groups with a p-value <0.05 was considered statistically significant. Multivariable logistic regression models were used to obtain odds ratios (ORs) and corresponding 95% confidence intervals (CIs) as measures of the relative risk for the association between RDW and CVT. All analyses were adjusted for sex, age and body mass index. Additional models included adjustments for hemoglobin levels, mean corpuscular volume, fibrinogen levels, homocysteine and red blood cell, white blood cell and platelet count, all as continuous variables. The main analyses were based on a dichotomous exposure, with a cut-off value set at the 90th

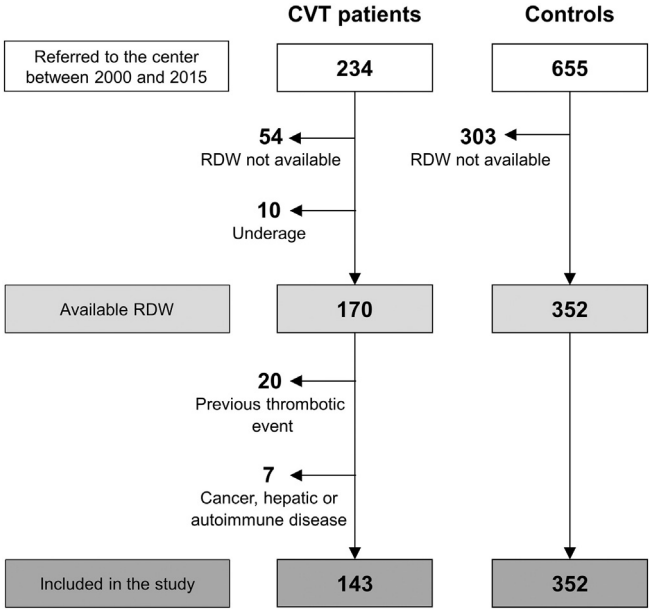

Fig. 1. Flow chart of the selection of the study population.

Please cite this article as: Maino A, et al, Red cell distribution width and the risk of cerebral vein thrombosis: A case–control study, Eur J Intern Med (2016), http://dx.doi.org/10.1016/j.ejim.2016.10.017