Hematologic variables and venous thrombosis: red cell distribution width and blood monocyte count are associated with an increased risk

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ONLINE SUPPLEMENT

METHODS

The Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA study) is a large population-based case-control study of which details were published elsewhere.[16,17] Briefly, between March 1999 and September 2004, consecutive patients with a first venous thrombotic event were recruited from six anticoagulant clinics in the Netherlands. Eligible participants were between 18 and 70 years of age at the time of their inclusion. Control subjects were either patients' partners or controls from random digit dialing (RDD). All participants provided informed consent and the study was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands.

Study participants

For logistic reasons, blood sampling was performed for patients and controls included until June 2002. For the current analysis, we included patients and controls who donated a blood sample, which was the case in 2473 (48%) out of the 5184 patients and 2935 (49%) out of the 5984 controls.

Questionnaire

All participants were asked to fill in a questionnaire that contained questions on recent (within three months prior to the event date) leg injury, surgery, pregnancy, or immobilization (plaster cast, bedridden at home, hospitalization), estrogen use (oral

contraceptives or hormonal replacement therapy) at time of the event date, obesity (body mass index [BMI] $\geq$ 30 kg/m^2), smoking and alcohol use, diagnosis of malignancy within five years before or within six months after the event date and presence of co-morbidities. The event date was defined as the date of diagnosis of the thrombotic event for patients and their partners, or date of completion of the questionnaire for the RDD controls.

Classical thrombotic risk factors referred to presence of history of cancer within previous 5 years of index data; surgical procedure, immobilization, hospitalization, fracture, pregnancy or use of plaster cast within previous 3 months of index data; use of hormone replacement therapy and pill at index data and history of travel within 2 months of index data.

Laboratory assays

Patients and their partners were invited for blood sample collection from 3 months after discontinuation of oral anticoagulant therapy. Exception to this applied to patients and controls who were on prolonged anticoagulation for clinical reasons. These individuals donated blood while on anticoagulation. . All assays were performed in an automated machine by laboratory technicians who were unaware of the case-control status of the samples.

Venous blood from fasting patients was collected in 1:10 volume of 0.106 mol/L trisodium citrate and immediately centrifuged for 10 minutes at 2500 X *g*. Blood was processed within 4 hours. Complete blood count analysis and red cell indices were

determined using a Beckman/Coulter MD-II Hematology Analyzer (Beckman Coulter, Woerden, Netherlands). Except for the red cell distribution width (RDW), mean corpuscular volume (MCV), mean hemoglobin volume (MCH) and mean corpuscular hemoglobin volume (MCHC) the final counts were multiplied by a factor of 1.1 in order to adjust the values due to the collection in sodium citrate.

C-reactive protein (CRP) was measured on stored (at −80°C) and previously unthawed samples by automated particle-enhanced immunoturbidimetric assay (Tina-quant® CRP detection method; Roche Diagnostics, West Sussex, UK).

Statistical analysis

Odds ratios and their 95% confidence intervals (95% CI) were calculated for erythrocytes and leukocytes counts, hematocrit, hemoglobin and red cell indices and adjusted for age, sex, malignancy, co-morbidities and CRP using logistic regression. Co-morbidities were defined as the presence of liver disease, renal disease, rheumatoid arthritis, multiple sclerosis, or a history of myocardial infarction or stroke. Additionally, since smoking is a risk factor for venous thrombosis and may affect the values of hematocrit, hemoglobin and erythrocyte counts, these variables were further adjusted for smoking. Smoking was added in the logistic regression as a categorical variable, defined as current, former or never smoker. Although anemia is not known to be a risk factor for venous thrombosis, it can act as a proxy for diseases that can alter the values of MCH, MCV and RDW and also affect venous thrombosis risk (e.g. malignancy). Thus, these variables were further adjusted for anemia. Anemia in men was defined as hematocrit or erythrocytes or

hemoglobin lower than 0.40 or 4.5 x 10^{12}/L or < 8.5 mmol/L, respectively. Anemia in women was defined as hematocrit or erythrocytes or hemoglobin lower than 0.35 or 4.0 x 10^{12}/L or < 7.5 mmol/L, respectively.

Since some hematological variables can vary with inflammation, infection and malignancy, the analysis was also adjusted for CRP. Because CRP levels were skewed to the left, we used the log transformed CRP in the model that provided a normal distribution.

Both RDD and patients' partners were considered as control subjects. Cut-off points of variables were established at the 1st, 5th, 95th, 97.5^{th} and 99th percentiles in the control subjects. There were no major differences between hematological variables by sex, except for hematocrit, hemoglobin and erythrocytes.

A subgroup analysis was also performed taking into account the occurrence of PE only, DVT with or without associated PE and unprovoked thrombosis. This analysis was not performed for hemoglobin, hematocrit and erythrocytes due to small numbers as this analysis had to be carried out sex-specifically.

Statistical analyses were performed with SPSS for Windows, release 20.0 (SPSS Inc, Chicago, Ill).