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Full Length Article

von Willebrand activation factor as a marker of mortality, cardiovascular events, and bleeding complications in patients treated with oral anticoagulants

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ABSTRACT

Background: Serious bleeding is a frequent and feared treatment complication in patients treated with oral anticoagulants (OACs). Levels of von Willebrand factor (VWF) antigen have been linked to the risk of bleeding complications, mortality, and cardiovascular events.
Objectives: In this longitudinal cohort study of evaluating patients treated with OACs, we aimed to evaluate the relationship between VWF displaying a glycoprotein Ib binding conformation (VWF activation factor) and the risk of cardiovascular events, bleeding complications, or all-cause mortality.
Materials and methods: Blood samples were collected at baseline in 356 patients on OACs. Patients were followed for an average of 48 months and bleeding complications leading to admission to hospital or death, cardiovascular events (myocardial infarction, ischemic stroke, and peripheral arterial emboli), and all-cause mortality were recorded and classified.
Results: During the study period, 47 bleeding complications, 84 cardiovascular events, and 97 deaths occurred. In multivariate Cox regression analyses, VWF activation factor was significantly associated with all-cause mortality (HR 1.62; 95% CI: 1.25–2.08) and cardiovascular events (HR 1.28; 95% CI: 1.01–1.63). There was no association observed between VWF activation factor and bleeding complications.
Conclusions: Patients with high levels of VWF activation factor had an increased risk of cardiovascular events and all-cause mortality during OAC treatment. The selectivity for thrombotic complications adds to the potential value of VWF activation factor as a biomarker or pharmacological target.

1. Introduction

von Willebrand factor (VWF) is an essential player in primary hemostasis. Deficiency or structural defects of VWF lead to an increased bleeding tendency, which is seen in patients with von Willebrand disease [1]. In humans, most of the circulating VWF originates from Weibel–Palade bodies in endothelial cells [2]. Upon secretion, VWF is cleaved by ADAMTS13 into multimers [3]. In epidemiological studies, levels of VWF are most often measured as its antigen and are associated with myocardial infarction [4], stroke [5,6], intracranial bleedings [7], and mortality [8]. Levels of VWF antigen were proposed to be a risk marker, when used in clinical risk scores, for thromboembolic events in patients with atrial fibrillation [5].

High levels of VWF antigen are associated with both thrombotic events and bleeding complications in patients treated with oral anticoagulants (OACs) [9,10]. The mechanism for this dual association is not clear and different pathways could contribute to high levels of VWF antigen. Increased levels of VWF due to endothelial dysfunction [11] could reflect an underlying atherosclerotic process with a greater risk of thrombotic events. An increase in arteriosclerosis was observed in ADAMTS13 −/− mice, which still produce VWF [12]. It is also possible that VWF could be an effector in thrombotic events, possibly by increased binding to platelets. Measurement of VWF antigen or multimers might not reflect the platelet binding capacity of the circulating VWF since smaller multimers could still retain some platelet binding capacity [13]. The proportion of high-affinity VWF, which is able to bind spontaneously to platelets, could be measured using a specific antibody-based ELISA [14]. Our hypothesis is that the proportion of high-affinity VWF, also called active VWF, is increased in patients with thrombotic events. The primary aim of this study was to investigate possible associations between active VWF and cardiovascular

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events, bleeding complications, or all-cause mortality in patients on OAC treatment.

2. Methods

2.1. Patients

The study cohort was recruited from the OAC clinic at Skellefteå County Hospital, Skellefteå, Sweden, in June 1996 and has in part been described in an earlier paper [10] Patients with a planned treatment time greater than 3 months were considered to be on long term treatment and eligible for the study; thus, consent forms were sent to them. Blood samples were obtained at inclusion for the 356 patients included in the final study cohort. All patients had been treated with OACs for at least 2 months prior to blood sampling. The indications for treatment and international normalized ratio (INR) at the time of blood sampling were available from registers at the OAC clinic. Additional data regarding diabetes, prior peptic ulcers, prior bleeding peptic ulcers, hypertension, weight, and height were available through questionnaires. The study was approved by the Research Ethics Committee of Umeå University. All patients provided written informed consent.

2.2. Blood sampling and laboratory procedures

Venous blood samples were drawn after a minimum of stasis and collected in standard siliconized, plasma tubes containing 0.13 mol/L sodium citrate. After centrifugation the plasma samples were frozen and stored at −70 °C until analyzed. The study cohort was analyzed at the same time and location. The laboratory staff had no knowledge of event status. The levels of VWF with high affinity to platelets (VWF activation factor) were determined by measuring the levels of activated VWF, which represents the active, GPIba-binding conformation with the AU/VWFa-11 llama-derived nanobody, as described by Hulstein et al. [14] The VWF activation factor was calculated by dividing the absorbance slope of a patient's sample by the slope of the corresponding standard sample (normal pooled plasma [NPP] from a pool of more than 32 adult donors). The intra assay coefficient of variation was 7.1% and the inter assay coefficient of variation was 13.7%.

The VWF antigen (kU/L) was measured using an enzyme-linked immunosorbent assay (ELISA) from DAKO in Denmark. High sensitivity C-reactive protein (hsCRP) was determined with an automated method (IMMULITE Diagnostic Products Corporation, USA). Creatinine was analyzed on a Hitachi 911 multi analyzer (Roche, Mannheim, Germany) with kits from Roche/Boehringer (Crea plus, enzymatic method). The estimated glomerular filtration rate (eGFR) was estimated using the four variable Modification of Diet in Renal Disease (MDRD) Study equation ($GFR = 175 \times \text{standardized serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times 1.212$ [if black] $\times$ 0.742 [if female]) [15]. The INR was determined using an Owren method with the reagent SPA 50 (Diagnostica Stago, Inc.) The INR was calibrated with a national standard.

2.3. Follow-up

Study inclusion began on the date of blood sampling, with the earliest date being June 1st 1996. The patients were followed longitudinally from June 1st 1996 until death, bleeding, cessation of OAC treatment, or until January 1st 2002. All medical records were reviewed during the study period. Bleeding complications causing admission to hospital or death, myocardial infarction, ischemic stroke, and peripheral arterial emboli were recorded and classified by a panel of three researchers (ML, JHJ, LJ). Clinically relevant bleeding was defined as fatal bleeding and/or symptomatic bleeding in a critical organ, or overt bleeding based on objective investigations leading to hospital admission or prolonged hospital care. All other bleeding events were classified as minor and were excluded. Cardiovascular events and mortality included myocardial infarction, stroke, transient ischemic attack, ruptured aortic aneurysm, and peripheral arterial emboli. Myocardial infarction and stroke were classified according to WHO criteria [16,17]. Peripheral arterial embolism and ruptured aortic aneurysm required clinical symptoms plus objective verification either on angiography, at operation or autopsy.

The cause of death was registered and classified according to the death certificate. In all but one case, the cause of death could be classified. Investigators classifying events were blinded to the biochemical results.

2.4. Statistical analysis

The distributions of VWF antigen, VWF activation factor, and hsCRP were skewed and, therefore, transformed using the natural logarithm (ln). Spearman correlation coefficients were used to evaluate potential relations between variables. Univariate Cox regression analysis was performed on each of the variables to estimate hazard ratios (HRs) and the 95% confidence interval (CI) with the increments of HRs presented for the standard deviations. Statistical analyses of VWF were performed using continuous variables and by categorizing the data into tertiles, with the lowest tertile as the reference group. Multivariate Cox regression analysis was performed to estimate the effects of different determinants when controlling for other factors. Age, hsCRP, and creatinine were possibly related to both VWF activation factor levels and outcome; therefore, they were considered to be potential confounders and were included in the multivariate analysis if the P-value in the univariate analyses was <0.20. Separate multivariate Cox regression models were performed on each of the variables: VWF activation factor and VWF antigen. The assumption of proportional hazard was verified graphically using Kaplan–Meier survival curves.

The VWF antigen and activation factor were dichotomized at the median and patients were classified into four groups according to high or low levels of antigen and activation factor. Direct age-adjustment was calculated using ten year intervals to present the effect of different levels of activity and antigen (Fig. 1). Cox regression analysis was used to test for significant differences between groups. A P-value <0.05 (two-sided) was considered statistically significant. PAWS version 18 was used for all statistical analyses. Individuals with missing values (none for VWF antigen, 11 for VWF activation factor, 2 for CRP, and 29 for eGFR) were excluded from the statistical analyses.

3. Results

Three-hundred-and-fifty six patients treated with OACs were enrolled in the study. Baseline characteristics of the patients are presented in Table 1. At the time of sampling, 9% of patients had an INR value <2.0, 5% had an INR value >3.5, and 86% were within the therapeutic range used at the time, INR 2.0–3.5. During the study period, 74% of patients had a stable INR within the therapeutic range when treated with an OAC in the Skellefteå OAC clinic. Patients were followed on average for 1449 days (range 19–2028 days). The VWF activation factor significantly correlated with age ($r = 0.16$), eGFR ($r = -0.18$), VWF antigen ($r = 0.43$), and diabetes ($r = -0.14$). There was no correlation between VWF activation factor and INR at the time of sampling.

3.1. All-cause mortality

A total of 97 patients died during OAC treatment. VWF activation factor was available for 92 cases. Mean time to event was 818 days.

Univariate and multivariate analyses are presented in Table 2. Hypertension and sex were not associated with the outcome and were, therefore, omitted from further analysis. The VWF antigen and activation factor, age, CRP, and eGFR were significantly associated with all-cause mortality in the univariate analysis. In the multivariate analysis, the highest tertile of VWF activation factor was significantly