Atherosclerosis 269 (2018) 144–150

Contents lists available at ScienceDirect

Atherosclerosis

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

Antithrombin levels are associated with the risk of first and recurrent arterial thromboembolism at a young age

F. Nanne. Croles [a, *], Janine E. Van Loon [b], Diederik W.J. Dippel [c], Moniek P.M. De Maat [a], Frank W.G. Leebeek [a]

[a] Department of Hematology, Erasmus University Medical Center, Room Na823, PO Box 2040, 3000CA Rotterdam, The Netherlands
[b] Department of Cardiology, Erasmus University Medical Center, Rotterdam, The Netherlands
[c] Department of Neurology, Erasmus University Medical Center, Rotterdam, The Netherlands

ARTICLE INFO

Article history:
Received 21 July 2017
Received in revised form
29 November 2017
Accepted 12 January 2018
Available online 16 January 2018

ABSTRACT

Background and aims: It is as yet unknown whether antithrombin levels are associated with arterial thromboembolism (ATE) at a young age. To investigate the association between antithrombin levels and premature and recurrent ATE, we performed a case-control study and a subsequent nested cohort study of premature coronary heart disease (CHD) patients.

Methods: In the case-control study, we included 571 patients who had a recent premature ATE, including CHD and ischemic stroke (IS), and 461 healthy controls. The association between antithrombin levels (dichotomized: ≤median *vs.* >median) and ATE was investigated. Subsequently we studied the association between antithrombin levels and recurrent cardiac events, ATE or death in a nested cohort of 323 CHD patients.

Results: Low antithrombin levels (≤median, 1.04 IU/mL) are associated with an increased risk of ATE (OR 1.46; 95% CI:1.09–1.96), after adjustment for classical cardiovascular risk factors. This was observed in the subgroups of CHD patients (1.43; 1.01–2.02) and IS patients (1.48; 1.01–2.19). CHD patients with low antithrombin levels had a higher risk of recurrent cardiac events (HR 2.16, 95% CI:1.07–4.38). Especially in women with low antithrombin levels, the risk of recurrent cardiac events was high (HR 5.97, 95% CI 1.31–27.13) as was the risk of recurrent ATE or death (HR 4.22, 95% CI 1.19–15.00).

Conclusions: Individuals with relatively low antithrombin levels have an increased risk for ATE at a younger age. CHD patients with low antithrombin levels, especially women, have a higher risk of recurrent cardiac events.

1. Introduction

Antithrombin is a strong inhibitor of blood coagulation through inactivation of mainly thrombin and factor Xa. The anticoagulant function of antithrombin *in vivo* is thought to be activated by heparan sulphate on the vascular endothelium [1]. It has been suggested that strongly reduced antithrombin levels as in hereditary antithrombin deficiency (around 50% of normal) are associated with arterial thrombotic events (ATE) [2]. Furthermore, a pooled analysis of four cohort studies showed that inherited thrombophilia, including antithrombin deficiency, increased the risk of ATE more pronouncedly in women than in men [3]. However, it is as yet unknown whether slightly reduced levels are associated with ATE. The process of atherosclerosis is driven by traditional cardiovascular risk factors, and the occurrence of ATE is typically caused by rupture of atherosclerotic lesions and subsequent thrombus formation. A lack of inhibition of thrombus formation, for instance by low antithrombin levels, may cause acceleration of thrombus formation and increased risk of occlusion of blood vessels. This may cause an increased risk of ATE [3]. Hemostatic factors are thought to play a more important pathophysiological role in ATE in young patients than in older patients because in young individuals atherosclerosis is not as extensive as in elderly. In addition, in young patients with myocardial infarction, normal coronary arteries are more often found than in older patients [4–6]. Indeed, levels of hemostatic factors such as fibrinogen, TAFI and ADAMTS13 have been associated with cardiovascular disease at a young age [7–9].

The mortality after myocardial infarction in young patients has

* Corresponding author.
E-mail address: f.croles@erasmusmc.nl (F.Nanne. Croles).

https://doi.org/10.1016/j.atherosclerosis.2018.01.014

been reported as high as 8% at 8 years after percutaneous coronary interventions [10]. Risk factors for mortality after a myocardial infarction include heart failure, ventricular arrhythmias, angina pectoris and re-infarction [11]. Finding more risk factors may allow for a more precise identification of patients at risk for recurrence or death in those with documented coronary heart disease. We hypothesize that low-normal antithrombin levels are associated with both a first and recurrent ATE at a young age, and may differ between sexes, as this has been shown before in other thrombophilias [3]. Therefore, we investigated the association of antithrombin levels with the risk of first and recurrent ATE at a young age, including a sex-specific analysis.

2. Patients and methods

2.1. Patients

We performed two related studies: first we performed a case-control study, and subsequently, we performed a follow-up (cohort-) study of the patients with coronary heart disease from the first study.

2.2. Case-control study

The 'Genetic risk factors for Arterial Thrombosis at a young age: the role of TAFI and other Coagulation factors (ATTAC)' study is a single-center, case-control study investigating the role of coagulation factors on incidence of ATE at a young age. The design of the study and recruitment of patients and controls have been reported elsewhere in detail [7,8]. In short, cases were consecutive patients who presented with a first acute arterial ischemic event in our center. Our center is a university hospital with a community function, located in the city centre. Our center employs no selection criteria of admission of cardiovascular patients, but young patients are over-represented. Patients were men aged 45 years or younger or women aged 55 or younger. The case-control study originally consisted of three subgroups: Group CHD: coronary heart disease, including acute myocardial infarction and unstable angina pectoris (CHD); group IS: ischemic stroke (IS) or transient ischemic attack (TIA); and group PAD: peripheral arterial disease (PAD). Patients were included 1–3 months after the event, to avoid a possible acute phase response on plasma levels of the parameters investigated. Controls were neighbours or friends of the patients. They fulfilled the same age criteria but did not have a history of ATE.

2.3. Follow-up study

The 368 subjects included in the ATTAC study who presented with coronary heart disease were asked to participate in a follow-up study as reported elsewhere in more detail [9]. These subjects were followed at the outpatient clinic (and by telephone in case this was not possible) for recurrent cardiac events and for any recurrent cardiovascular event or all-cause mortality.

Participants were included in the study after written informed consent, between October 2001 and June 2010. Both the ATTAC and the follow-up studies were approved by the institutional Medical Ethics Committee of the Erasmus MC, and both conform to the ethical guidelines of the 1975 declaration of Helsinki.

2.4. Definitions

The definitions used in this study have been reported elsewhere [8]. In short, CHD was defined as acute myocardial infarction (AMI) or unstable angina pectoris (UAP). AMI was defined as typical chest pain, with elevated cardiac markers (CK MB/troponin T), and/or characteristic electrocardiographic findings. UAP was defined as typical chest pain at rest. Transient ischemic attack (TIA) was defined as the acute onset of focal cerebral dysfunction, which could not be explained otherwise than by focal cerebral ischemia, as diagnosed by a neurologist. Symptoms had to be temporary and last less than 24 h. Ischemic stroke (IS) was defined as the acute onset of focal cerebral dysfunction as a result of cerebral ischemia, with symptoms lasting longer than 24 h. Brain imaging by CT-scan and MRI-scan had to be compatible with the diagnosis and were used to rule out intracerebral hemorrhage. Smoking status was defined as self-reported never, previous or current smoker. Hypercholesterolemia was originally defined as a subject-reported presence of hypercholesterolemia or the use of lipid lowering treatment on the day of the event. As most cases used cholesterol lowering medications at inclusion, cholesterol measurements were not representative of cholesterol levels before the event. Therefore, in the case-control study, cholesterol was not adjusted for in the analyses. In the follow-up study, as nearly all patients used lipid-lowering treatment, cholesterol levels were adjusted for in the analyses. Patients with a medical history of diabetes or using either oral anti-diabetic medication or insulin therapy on the day of the event were considered to have diabetes. Hypertension was defined by a systolic blood pressure $\geq$140 mmHg, and/or diastolic blood pressure $\geq$90 mmHg or the use of anti-hypertensive drugs.

In the follow-up study we used the endpoints recurrent cardiac event (defined as myocardial infarction or revascularization procedure) and any recurrent arterial thrombotic event or death (defined recurrent cardiac event, cerebrovascular event (IS or TIA) or all-cause mortality). At the time of inclusion in the study, direct acting oral anticoagulants were not yet registered in the Netherlands.

2.5. Blood sampling

Blood was collected 1–3 months after the event in sodium citrate (final concentration 0.105M) using a Vacutainer System (Beckton, Dickinson and Company, Plymouth, UK) and centrifuged at 2000*g* for 10 min at 4 °C. Plasma was additionally centrifuged for 10 min at 20,000*g* for 10 min at 4 °C and stored in aliquots at −80 °C. Technicians were not aware of the case–control status of the samples. Antithrombin activity measurements were performed using the factor Xa-based INNOVANCE® Antithrombin assay kit (Siemens). Measurements of samples were performed once, but AT values outside the reference range were performed in duplicate. Cholesterol and HDL were determined on Modular Analytics® (Roche Diagnostics, Mannheim, Germany).

2.6. Statistical analysis

Descriptive statistics were used in both studies. The data are presented as means ± standard deviation.

2.6.1. Case-control study

To compare levels of the normally distributed risk factors between groups, ANOVA's were performed with adjustment for age and sex. We performed logistic regression for ATE with antithrombin activity as a continuous variable and as a binominal variable, using the predefined cut-off-levels of the lower limit of the reference range ($\leq$0.80 U/mL) and the median antithrombin level of controls. Logistic regression analyses were adjusted for age, sex, BMI, smoking, family history, hypertension, diabetes mellitus. In addition, separate sex-specific analyses were performed, adjusted for the same cardiovascular risk factors.