Venous and arterial thromboembolism

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Venous and arterial thromboembolism: a questionable dichotomy

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Cover: Joris Swaak, Inge van Schouwenburg
Lay out: Joris Swaak, Inge van Schouwenburg
Printed by: Facilitair Bedrijf RuG, Groningen

ISBN: 978 90 367 5787 4

Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowlegded.

Printing of this thesis was financially supported by:
Boehringer Ingelheim b.v., Covidien Nederland b.v., GlaxoSmithKline, Pfizer b.v., Federatie van Nederlandse Trombosediensten, GUIDE/UMCG (Research Institute for Drug Exploration), University of Groningen and the Stichting tot Bevordering van Onderzoek en Onderwijs op het gebied van Haemostase, Trombose en Rheologie Groningen.

RIJKSUNIVERSITEIT GRONINGEN

Venous and arterial thromboembolism: a questionable dichotomy

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. E. Sterken,
in het openbaar te verdedigen op
woensdag 14 november 2012
om 11.00 uur

door

Inge Maaike van Schouwenburg

geboren op 2 juli 1984
te Meyrin, Zwitserland

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1 Introduction

Introduction

Venous thromboembolism (VTE) is a major health problem in Western countries.[1] Reported incidence rates for first VTE vary between 1.4 and 1.9 per 1000 person-years.[1–3] Venous thrombosis usually starts in the calf veins, from where it may extend and cause deep vein thrombosis (DVT) and subsequent pulmonary embolism (PE).

More than a century ago, Virchow postulated 3 main causes of thrombosis: stasis of the blood, changes in the vessel wall and changes in the composition of the blood.[4] In VTE, alterations in blood flow and composition of the blood are the main determinants for clot formation. Important risk factors for VTE are surgery, major trauma, prolonged immobilization, pregnancy, puerperium, the use of oral contraceptives or hormone replacement therapy, malignancy and older age.[5] Furthermore, abnormal pro- and anticoagulant factors increase thrombotic risk, i.e. factor V Leiden, prothrombin G20210A mutation, deficiency of antithrombin, protein C and S, and elevated levels of factor VIII.[5]

An episode of VTE can have serious consequences. Fifty percent of the patients with DVT develop post-thrombotic syndrome in subsequent years.[6,7] The post-thrombotic syndrome is characterized by injury to the venous valvular system. This causes venous insufficiency accompanied with chronic symptoms including pain, venous dilatation, edema, pigmentation, skin changes, and venous ulcers. The post-thrombotic syndrome is the major factor impairing quality of life in subjects with previous DVT.[7] Subjects with VTE are at high risk to develop recurrence, with incidence rates reported as high as 26 to 95 per 1000 person-years.[8–10] In addition, subjects with VTE have a high mortality rate. About 6% of the patients die in the first month after their event and this percentage increases up to 20% within the first year.[1] Although mortality rate decreases with time, it remains elevated up to eight years after VTE.[11] This higher mortality rate is not only explained by the high prevalence of malignancy in subjects with VTE.[11]

Recent findings suggest another important consequence of VTE; subjects with first VTE seem to be at increased risk to develop arterial thromboembolism (ATE).[12] In the past decades, VTE and ATE were seen as two different entities due to their different presentations and assumed different pathogenesis. ATE, of which myocardial infarction and ischemic stroke are most prevalent, is usually a result of vascular endothelial injury. In VTE, changes in stasis and composition of the blood are regarded the main determinants for clot formation. Important risk factors for ATE are hypertension, hyperlipidemia, diabetes, high body mass index and smoking.[13–15] These cardiovascular risk factors lead to endothelial injury, which evokes the formation of atherosclerotic plaques. This can result in full occlusion of the arteries. Unlike VTE, arterial disease develops gradually and at places where shear stress is high. Arterial thrombi are often referred to as white clots

as they consist mainly of platelets. In contrast, venous thrombi are referred to as red clots as they consist mainly of fibrin and red blood cells. For this reason, antiplatelet therapy is preferred in preventing ATE, whereas in VTE anticoagulant therapy is recommended.

Presently, ATE is the leading cause of death in most western countries.[16] Moreover, global cardiovascular deaths are projected to increase from 17.1 million in 2004 to 23.4 million in 2030.[16] The recent finding suggesting an association between VTE and ATE, therefore, is important and needs further examination.

The present thesis focuses on the relationship between VTE and ATE. First, we examined whether we could confirm the alleged increased risk of ATE after VTE. Second, we aimed to get more insight in the etiology and pathogenesis of the diseases to help us understand their association. For this reason, we studied the relationship between several established arterial cardiovascular risk factors and VTE risk. In addition, we studied the effect of abnormal levels of pro- and anticoagulant factors on the risk of ATE.

Insulin resistance

Several risk factors for ATE have been investigated with respect to their relationship with VTE but, up till now, only obesity has consistently been shown to be an independent risk factor for VTE.[17–20] Obesity itself is associated with several risk factors that influence the risk of ATE (e.g. systolic blood pressure, total cholesterol, high-density lipoprotein, triglycerides and, systemic inflammation).[21,22] Insulin resistance has been suggested as central factor underlying this multifaceted syndrome.[23–25]

Subjects with insulin resistance have a subnormal biological response to insulin levels with respect to glucose secretion and uptake. This results in elevated serum concentrations of insulin and, in some cases, to hyperglycemia. Insulin resistance is associated with endothelial damage[26–28] and increased levels of several prothrombotic factors (e.g. plasminogen activator inhibitor-1 (PAI-1),[29,30] fibrinogen[31,32] and von Willebrand factor (vWf)[29] antigen). This suggests that insulin resistance possibly not only influences the risk of ATE but also the risk of VTE.

The potential association of insulin resistance with VTE has only been investigated in one small case-control study.[33] This study had the unexpected finding that overweight subjects with VTE had lower insulin levels than overweight controls.[33] Other studies investigated the association of diabetes mellitus, fasting glucose and HbA1c with the risk of VTE.[34–36] In these studies, the focus was on measures of glycemia rather than on measures of insulin resistance. Importantly, insulin resistance only leads to hyperglycemia once pancreatic beta-cells start to fail.[37] Therefore, the question whether insulin resistance is a risk factor for VTE in a prospective setting remains unanswered to date.

(Apo-) lipoproteins

Cholesterol and triglycerides, together called lipids, fulfill important roles in cell membrane and hormone synthesis, and energy supply. However, abnormal levels will enhance atherosclerosis of the arteries, which eventually might lead to ATE.

Lipids are not soluble in water and therefore need to bind to apolipoproteins to be transportable through blood. Apolipoproteins contain phospholipids that enhance solubility. When lipids and apolipoprotein bind, they form lipoprotein. The most important lipoproteins are High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL). HDL extracts cholesterol from the cells and transports it back to the liver, which takes care of its excretion. As HDL reduces the levels of cholesterol in the tissues, high levels are protective for ATE. All other lipoproteins, of which LDL is most important, are atherogenic, meaning that they increase the risk of atherosclerosis and thereby the risk of ATE. These atherogenic lipoproteins transport lipids from the liver to the organs.

Lipid-lowering drugs are prescribed to reduce the increased risk of ATE due to high levels of atherogenic lipoproteins. Recently, the Jupiter trial showed that the use of lipid-lowering medicine also decreases the risk of VTE.[38] Two prospective, observational studies also reported a decreased risk of VTE associated with the use of the lipid-lowering statins.[39,40] Studies on the association between lipid profile and VTE, however, are inconsistent. Whereas some studies observe an increased risk of VTE in subjects with low HDL or high LDL levels,[41,42] others do not observe such an association.[18,20,43] A possible explanation for this inconsistency is that studies on the relationship between lipid profile and VTE mainly focused on classical lipoproteins (i.e. total cholesterol (TC), LDL, HDL and triglycerides (TG)).[18,20,44] However, in ATE, apolipoproteins and their ratios are possibly stronger predictors for the risk of ATE than the classical lipoproteins.[45–47] This may also apply to VTE. Therefore, we examined whether (apo-) lipoproteins and their ratios are related to VTE.

Albuminuria

In the kidney, blood is filtered to form urine. The glomerular filtration barrier prevents large proteins from leaving the circulatory system. One of these large proteins is albumin. Healthy subjects only have small amounts of albumin in their urine. In some subjects, however, high levels of urinary albumin are detected. A high level of albuminuria can indicate specific renal disease,[48–50] but is also seen as sensitive marker for generalized endothelial damage[51,52] and as such, urinary albumin levels can effectively predict ATE risk.[49,53–55]

Recently, our group showed that elevated albuminuria can also be used as a risk indicator for first VTE.[56] Subjects with microalbuminuria (30-300 mg/24h) were at a 2.3-fold increased risk to develop first VTE compared to subjects with albuminuria of

less than 15 mg per 24-hour urine collection, whereas people with macroalbuminuria (> 300 mg/24h) had a 3.0-fold increased risk compared to their healthy counterparts.

It is well recognized that many factors associated with an increased risk for first VTE are not related to a higher risk of recurrence.[57] This seemingly paradoxical fact has recently been explained as index-event bias.[58] The index-event bias explains the paradox by multicausalitity of a disease. Subjects who develop VTE, for example, have a certain risk profile, in which the presence of multiple risk factors will eventually lead to passing the threshold of developing VTE.[5] When a certain variable, for example albuminuria, is a strong risk factor, other risk factors do not have to be present for VTE to develop. Thus, subjects will develop first VTE based on either high levels of albuminuria or because of the presence of other risk factors. In this latter group, urinary albumin level is not necessarily elevated. For first VTE, patients are compared with the general population. Although not elevated in all VTE patients, high urinary albumin levels will be more common in patients then in the general population. Hence, albuminuria can distinguish between people at high and low risk for first VTE. In recurrent VTE, subjects are compared with others at high risk for VTE. This risk is either based on high urinary albumin level or on an otherwise high risk profile combined with low albumin levels. In other words, both subjects with high and subjects with low urinary albumin levels are at increased risk for recurrence. The first because of high urinary albumin levels, the latter because of the presence of other risk factors. Therefore, high urinary albumin levels no longer distinguish between subjects at high and low risk for recurrence.

Whether this index-event bias also applies to urinary albumin levels and the risk of recurrent VTE is unknown. This information, however, is important for the clinical management of patients with VTE. As mentioned before, subjects with VTE are at high risk to develop recurrence.[8–10] This risk can be reduced by prolonging anticoagulant therapy, but prolonging anticoagulation is accompanied by an increased risk of bleeding.[59,60] It is important that duration of anticoagulant therapy is balanced against the risk of bleeding. Therefore, it should be examined whether it is possible to distinguish between subjects at low and high risk for recurrence. For this reason, we studied the influence of increased urinary albumin levels on VTE recurrence.

Factor VIII

Factor VIII is an essential prothrombotic protein. Elevated levels are related to an increased risk of VTE.[61,62] Factor VIII level is partly genetically determined[63] but is also associated with an inflammatory state.[64] An inflammatory state probably mediates the development of ATE in subjects with arterial thrombotic risk factors.[65–69] This suggests that elevated factor VIII levels might also be associated with an increased ATE risk. Indeed, literature implies such an association.[61,70] However, it is unknown whether this

association is acquired or genetic. This question was addressed in a large cohort of families with thrombophilic defects.

Protein S

Protein S is a vitamin K-dependent anticoagulant protein. In the circulatory system protein S exists both in a free form and bound to protein C4b-binding protein. Only the free form is active in the inhibition of thrombus formation. Low levels of free protein S are associated with an increased risk of VTE.[71] As with Factor VIII level, free protein S level is partly genetically influenced[72] but is also associated with inflammatory state.[73] Again, as an inflammatory state probably mediates the development of ATE in the presence of arterial thrombotic risk factors,[65–69] free protein S level might also be a risk indicator for ATE risk, next to Factor VIII level. Most studies on free protein S and ATE risk are limited to case reports or small case series.[74–76] We investigated this issue in a large family cohort.

Outline of the thesis

In the present thesis the association between VTE and ATE is studied. First we describe whether we could confirm the alleged association between the two diseases in the population-based cohort of the PREVEND (Prevention of REnal and Vascular ENd-stage Disease) Study **(chapter 2)**. The PREVEND Study is a prospective, observational cohort study, originally designed to investigate the natural course of albuminuria and its relation with renal and cardiovascular disease **(see figure 1)**.[77] The study started in 1997 by inviting all inhabitants of the city of Groningen, the Netherlands, aged 28 to 75 years, to participate. Out of 85 421 subjects invited, 40 856 (48%) responded by sending a morning urine sample and answering a short questionnaire. The database of these 40 856 subjects is linked yearly to the database of the national registry of hospital discharge diagnoses (Prismant, Utrecht, the Netherlands) and the national mortality registry (Central Bureau of Statistics, The Hague/Heerlen, the Netherlands). This provided us with data on ATE. In addition to these databases, the database of the regional anticoagulation clinic was used to identify subjects with VTE. With the use of this data, we assessed the absolute and relative risks of ATE after prior VTE in order to investigate the magnitude of the association between the two diseases **(chapter 2)**.

After confirmation of the association between VTE and ATE we tried to find an explanation for this association. For this purpose we studied the relationship between several established arterial cardiovascular risk factors and VTE risk. In addition, we studied the effect of abnormal levels of pro- and anticoagulant factors on the risk of ATE.

In **chapter 3** and **chapter 4** the association between insulin resistance and VTE

and the association between lipid profile and VTE are described. To study these issues, we used a subset of the 40 856 subjects previously described. This subset forms the actual PREVEND cohort and consists of 8592 subjects who are selected to be intensively studied and followed over time. The selection is made based on urinary albumin level. All subjects with a urinary albumin concentration of 10 mg/l or greater who agreed to participate (*n* = 6000) are selected together with a random sample of 2595 subjects with a urinary albumin concentration of less than 10 mg/l. In these 8592 subjects, the relative risk of VTE is assessed in relation to different levels of insulin resistance and in relation to different levels of several lipid biomarkers.

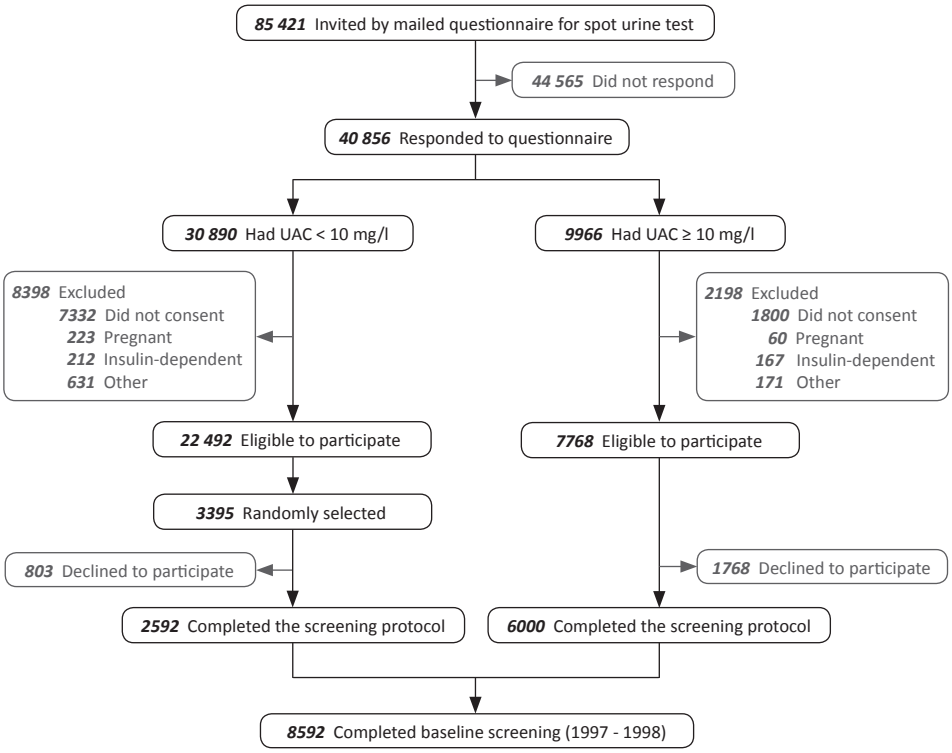

Figure 1 Flow diagram of the PREVEND Study Cohort

In **chapter 5** the association between urinary albumin level and the risk of recurrent VTE is assessed. Out of the baseline PREVEND cohort of 40 856 subjects, 597 developed VTE between 1997 and 2009. Those who had a history of VTE and those who were using ongoing anticoagulant therapy until the end of the study period are excluded. The remaining 351 subjects with first VTE are used to examine whether subjects with elevated albumin levels were at increased risk to develop recurrent VTE.

In **chapter 6** the relationship between factor FVIII and free protein S levels and ATE incidence is studied. This association is examined in pooled data of four large

retrospective family cohort studies.[61,78–81] These studies were performed in three University Medical Centers in the Netherlands. As no central lab was involved, only the data of the University Medical Center of Groningen is used to answer the present research question. This way the possibility of interlaboratory variability is excluded. The first family cohort study consists of first-degree relatives (i.e., offspring, siblings, and/or parents) of consecutive patients (probands) with documented VTE and established hereditary deficiencies of either antithrombin, protein C, or protein S. Due to the small number of antithrombin deficient probands, second-degree relatives (i.e., grandchildren and/or blood related uncles or aunts) with a deficient parent are also identified. Subjects were enrolled between April 1999 and July 2004.

The other three studies consist of first-degree relatives of consecutive patients with VTE or premature atherosclerosis (< 50 years of age) and the presence of either the prothrombin G20210A mutation, high levels of factor VIII at repeated measurements, or hyperhomocysteinemia. Enrollment in these studies started in May 1998 and was completed in July 2004.

Taken together 1468 relatives are included in the present study **(see figure 2)**. Probands are excluded to avoid referral bias. The relatives are tested for deficiencies of antithrombin, protein C and protein S, factor V Leiden, prothrombin G20210A, and high levels of factor VIII. Physicians at the thrombosis outpatient clinic collected detailed information on previous episodes of ATE, risk factors for atherosclerosis, and anticoagulant treatment by using a standardized questionnaire and examining medical records. This data is used to study the absolute and relative risks for ATE in subjects with high levels of factor VIII or low levels of free protein S.

In **chapter 7** results of previous chapters are summarized. All findings are discussed in **chapter 8**.

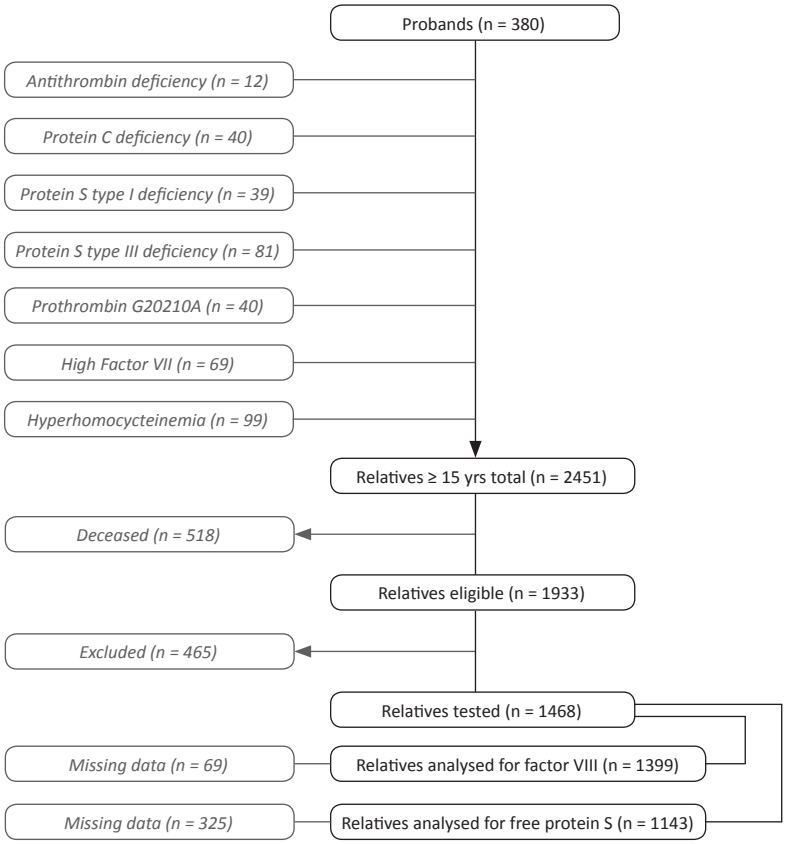

Figure 2 Flow diagram of the family cohort

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2

Increased risk of arterial thromboembolism after a prior episode of venous thromboembolism: Results from the PREVEND Study

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British Journal of Haematology 2012, in press

Abstract

Large population-based studies are needed to establish the magnitude and duration of the recently suggested association between arterial and venous thromboembolism. In 1997-1998, all inhabitants of Groningen, the Netherlands, aged 28-75 years (*n* = 85 421), were invited to participate in a study that followed and monitored responding subjects (*n* = 40 856) for venous and arterial thromboembolism until 2009. Thromboembolism was verified with national registries of hospital discharge diagnoses and death certificates, anticoagulation clinic and medical records. During a median follow-up of 10.7 years, 549 participants developed venous thromboembolism and 3283 developed arterial thromboembolism. Annual incidence of arterial thromboembolism after venous thromboembolism was 2.03% [95% confidence interval (CI), 1.48-2.71], compared to 0.87% (95% CI, 0.84-0.90) in subjects without venous thromboembolism. The hazard ratio (HR) of arterial thromboembolism after venous thromboembolism was 1.40 (95% CI, 1.04-1.88) after adjustment for age, sex and cardiovascular risk factors. This risk was highest during the first year after venous thromboembolism [annual incidence, 3.00% (95% CI, 1.64-5.04); adjusted HR, 2.01 (95% CI, 1.19-3.40)] and after an unprovoked event [annual incidence, 2.53% (95% CI, 1.68-3.66); adjusted HR, 1.62 (95% CI, 1.11-2.34)]. This study showed that subjects with venous thromboembolism are at increased risk for arterial thromboembolism, particularly in the first year after venous thromboembolism and after an unprovoked event.

Introduction

The concept that arterial and venous thromboembolism are separate pathophysiological entities has been challenged.[1] In 2003, Prandoni *et al*. were the first to report a twofold increased risk for the presence of atherosclerotic plaques in patients with unprovoked deep vein thrombosis.[2] Since then, several studies have examined the relationship between venous thromboembolism and the risk of subsequent arterial thromboembolism and confirmed a relationship between the two diseases. However, their generalizability is limited due to either a rather small sample-size,[3,4] a patient-based cohort[3,5] or a lack of controls.[6] Also, some studies were limited by possible misclassification of outcome events due to the retrospective way in which the cardiovascular events were obtained.[7,8] In a recent meta-analysis of Becattini *et al*.[9] no adjustments for age could be made. Age is a strong confounder to the risk of both venous and arterial thromboembolism, hence, based on this meta-analysis, we can not firmly conclude that the higher incidence of arterial thromboembolism after venous thromboembolism is truly related to previous venous thrombotic disease, as it can also merely be a result of ageing. The limitations of the abovementioned studies preclude an accurate estimation of the absolute incidences of cardiovascular arterial disease in patients with venous thromboembolism. This information, however, is important for the clinical management of these patients. A large population-based study was performed, in which the limitations discussed above were taken into account.[10] In this study of Sørensen *et al*., a two- to three-fold increased risk of arterial thromboembolism was found after first venous thromboembolism, predominantly in the first year following initial venous thromboembolism.[10] However, large population-based studies on this issue are still needed to further establish the magnitude and duration of the association between arterial and venous thrombosis.

The Prevention of REnal and Vascular ENd stage Disease (PREVEND) Study[11] offered us the opportunity to investigate the incidence of arterial and venous thrombotic disease in a large population-based cohort. We intended to use this study to advance our understanding of both arterial and venous thrombotic disease and provide further insight into the clinical course of patients with venous thromboembolism. Our aims were to establish whether venous thromboembolism is a risk factor for subsequent arterial thromboembolism, and to determine the absolute risk of arterial thromboembolism after venous thromboembolism, in a prospectively followed population-based cohort of more than 40 000 subjects.

Methods

Study population

This study was conducted on participants in the PREVEND Study, which was designed to prospectively investigate the natural course of albuminuria and its relationship with renal and cardiovascular disease in a large cohort drawn from the general population. Within the PREVEND Study design, arterial thromboembolic events were collected prospectively. Details of this study have been published previously[11] and can be found at http://www.prevend.org. In 1997-1998, all inhabitants of the city of Groningen, the Netherlands, aged 28 to 75 years (*n* = 85 421) were sent a postal questionnaire and a vial to collect an early morning urine sample. A total of 40 856 subjects (47.8%) responded. Their observation time started at study entry and ended at time of arterial thromboembolism, moving out of the city, death or end of study (January 2009).

All participants gave written informed consent. The PREVEND Study was approved by the local medical ethics committee and was conducted in accordance with the guidelines of the Declaration of Helsinki.

Definition of thrombotic events

To identify subjects with arterial and venous thromboembolism, the databases of the national registry of hospital discharge diagnoses (Prismant, Utrecht, the Netherlands) and death certificates (Central Bureau of Statistics, The Hague/Heerlen, the Netherlands) were linked yearly to the PREVEND database. In addition, the database of the regional anticoagulation clinic, which monitors the anticoagulant therapy of all inhabitants of the city of Groningen, was searched for venous events. When available, data on subjects with venous thromboembolism according to any of the abovementioned databases was confirmed by patients' medical records (*n* = 522). Arterial thromboembolism was predefined as acute myocardial infarction [International Classification of Diseases (ICD)-code 410], acute and subacute ischemic heart disease (411), occlusion or stenosis of the precerebral (433) or cerebral arteries (434), coronary artery bypass grafting (CABG) or percutaneous transluminal coronary angioplasty (PTCA), and other vascular interventions, such as percutaneous transluminal angioplasty or bypass grafting of aorta and peripheral vessels.[12]

Deep vein thrombosis had to be confirmed by compression ultrasound, and pulmonary embolism by ventilation/perfusion lung scanning, spiral computed tomography, or at autopsy. When deep vein thrombosis and pulmonary embolism were diagnosed simultaneously, this was classified as pulmonary embolism. Only deep vein thrombosis and pulmonary embolism were considered in the present study, other types

of venous thrombosis were not included. Venous thromboembolism was classified as being provoked when it had occurred at or within three months after exposure to an exogenous risk factor including surgery, trauma, immobilization for more than seven days, pregnancy, puerperium, the use of oral contraceptives or hormonal replacement therapy, or malignancy. Venous thromboembolism was classified as unprovoked when no such exogenous risk factor was present.

Measurements

The questionnaire provided information about the presence of established risk factors for cardiovascular disease. Subjects were classified as being diabetic when they gave a positive answer when questioned if they had been diagnosed with diabetes by a physician, regardless of the type of antidiabetic treatment. Subjects were considered hypertensive or dyslipidemic when they positively answered the question regarding whether high blood pressure or high cholesterol, respectively, had ever been measured. Those who reported smoking or having smoked cigarettes during the previous 5 years were regarded as smokers. A history of myocardial infarction or stroke was considered present if subjects positively answered the question regarding whether they ever suffered from myocardial infarction or ischemic stroke.

Morning urinary albumin concentration was established by a commercial immunoturbidimetry assay with sensitivity of 2.3 mg/l and inter- and intra-assay coefficients of variation of 2.2 and 2.6%, respectively (BN II, Dade Behring Diagnostica, Marburg, Germany).[11,13] First morning urine was used for analysis. Albuminuria was considered elevated at a concentration of 20 mg/l or more.[14]

Statistical analysis

We estimated the absolute risk of arterial thromboembolism in subjects with and without venous thromboembolism to assess whether venous thromboembolism is a risk factor for arterial thromboembolism. The absolute risk was expressed as an annual incidence and was calculated by dividing the number of arterial events by the time at risk. The 95% confidence intervals (CIs) around the annual incidences were assessed with the Poisson distribution assumption. A time-varying exposure Cox proportional hazard model was used to estimate whether venous thromboembolism was a risk factor for arterial thromboembolism. With this model, we accounted for differences in the onset of venous thromboembolism, i.e. subjects were allocated to the non venous thromboembolic group and added follow-up time to this group as long as they did not develop venous thromboembolism. At the time that subjects developed venous thromboembolism they switched to the venous thromboembolic group and started adding follow-up time to

this group. Adjustments were made for age, sex, hypertension, dyslipidemia, diabetes mellitus, smoking status, elevated albuminuria and history of arterial thromboembolism. Based on clinically relevant differences, preplanned sensitivity analyses were performed for the subgroups of venous thromboembolism (i.e., deep vein thrombosis *versus* pulmonary embolism and unprovoked *versus* provoked venous thromboembolism). Furthermore, *a priori* planned subanalyses were performed for the first year of follow-up after venous thromboembolism versus the rest of follow-up, to investigate the persistence of venous thromboembolism as a risk factor through time.

As hospitalization bias can cause misclassification of outcome events due to differences in monitoring subjects with or without venous thromboembolism, an additional sensitivity analysis was performed in which only the arterial thromboembolic outcome events myocardial infarction, ischemic stroke or death due to arterial thromboembolism were regarded. Categorical data are presented as counts and percentages, continuous variables as medians with interquartile ranges (IQR). Statistical significance was considered as 2-tailed $P < 0.05$. Statistical analyses were performed using PASW version 18.0 (IBM SPSS, Chicago, IL, USA) and SAS version 9.2 (SAS Institute, Inc, Cary, NC, USA).

Results

Study Population

Baseline characteristics are shown in **Table 1**. Out of 40 856 participants 46% was male. Median follow-up time was 10.7 (IQR, 8.8-11.0) years. Median age at enrollment was 60 (IQR, 48-68) and 48 (IQR, 39-60) years for subjects with and without venous thromboembolism, respectively. Venous thromboembolism occurred in 549 subjects at a median age of 64 years (IQR, 53-73). In 256 subjects the venous event was secondary to an external risk factor, 249 events were unprovoked. In 44 events the presence or absence of an external risk factor could not be assessed from medical records. Arterial thromboembolism occurred in 3283 subjects (ICD-code 410, 33%; 411, 29%; 433, 5%; 434, 14%; CABG, 8%; PTCA, 5%; other, 6%). Forty-five subjects with venous thromboembolism subsequently developed arterial thromboembolism at a median age of 72 years (IQR, 65-78). In the group without venous thromboembolism, 3238 subjects developed arterial thromboembolism at a median age of 69 years (IQR, 60-74). The type of thromboembolic event is shown in **Table 2**. Type of arterial thromboembolic event (ICD-9 coding) was equally distributed between the subjects with and without venous thromboembolism (data not shown).

In the patients with venous thromboembolism, the median treatment time with anticoagulation was 6 (IQR, 3-10) months. Of the 45 subjects who developed

arterial thromboembolism after venous thromboembolism, 17 subjects were still on anticoagulant therapy. One subject had stopped taking anticoagulant therapy < 1 month before developing arterial thromboembolism, in all others the interval between cessation of anticoagulation and arterial thromboembolism was longer than 1 month.

In 9547 out of 40 856 participants, follow-up ended prematurely at time of non-arterial and non-venous vascular event (*n* = 220, 0.5%), non-cardiovascular death (*n* = 2222, 5.4%) or moving out of the city (*n* = 7105, 17.4%).

Table 1 Baseline Characteristics

	Subjects with VTE	Subjects without VTE
TOTAL	**549 (100)**	**40 307 (100)**
Male	260 (47)	18 365 (46)
Age at enrollment, y	60 (48-68)	48 (39-60)
Cardiovascular risk factors		
Hypertension	197 (36)	11 642 (29)
Dyslipidemia	83 (15)	5566 (14)
Diabetes Mellitus	18 (3)	1033 (3)
Current Smokers	202 (37)	16 946 (42)
Microalbuminuria (≥ 20 mg/l)	60 (11)	3140 (8)
History of arterial thromboembolism	30 (6)	1749 (4)

VTE = venous thromboembolism. Continuous variables are presented as median (IQR), categorical variables as number (%)

Risk of arterial thromboembolism after venous thromboembolism

Figure 1 shows the risk of arterial thromboembolism after venous thromboembolism. The annual incidence of arterial thromboembolism after prior venous thromboembolism was 2.03% (95% CI, 1.48-2.71), compared to 0.87% (95% CI, 0.84-0.90) in subjects without venous thromboembolism. The crude hazard ratio (HR) of subsequent arterial thromboembolism was 2.24 (95% CI, 1.67-3.00; *P* < 0.001) in subjects with venous thromboembolism, compared to subjects without. After adjustment for age, sex, cardiovascular risk factors and previous arterial thromboembolism, the HR was 1.40 (95% CI, 1.04-1.88; *P* = 0.03). Within this model, age was a strong confounder as adjustment for age only resulted in a HR of 1.43 (95% CI, 1.06-1.92; *P* = 0.02).

Our preplanned subgroup analysis of venous thromboembolism indicated that differences between subjects with deep vein thrombosis and subjects with pulmonary embolism were minimal. With an adjusted HR of 1.62 (95% CI, 1.11-2.34; *P* = 0.01), subjects with unprovoked venous thromboembolism were seemingly at higher risk of arterial thromboembolism than subjects with provoked venous thromboembolism

[adjusted HR of 1.22 (95% CI, 0.71-2.11; *P* = 0.47)].

Risk of arterial thromboembolism was highest within the first year after venous thromboembolism with an annual incidence of 3.00% (95% CI, 1.64-5.04) and an adjusted HR of 2.01 (95% CI, 1.19-3.40; *P* = 0.01). This higher risk was predominantly found in subjects with deep vein or unprovoked thrombosis [adjusted HR of 2.68 (95% CI, 1.44-4.99; *P* = 0.002) and 2.91 (95% CI, 1.57-5.42; *P* < 0.001), respectively]. After 1 year of follow-up, the adjusted HR of arterial thromboembolism after venous thromboembolism decreased to 1.23 (95% CI, 0.86-1.75; *P* = 0.26).

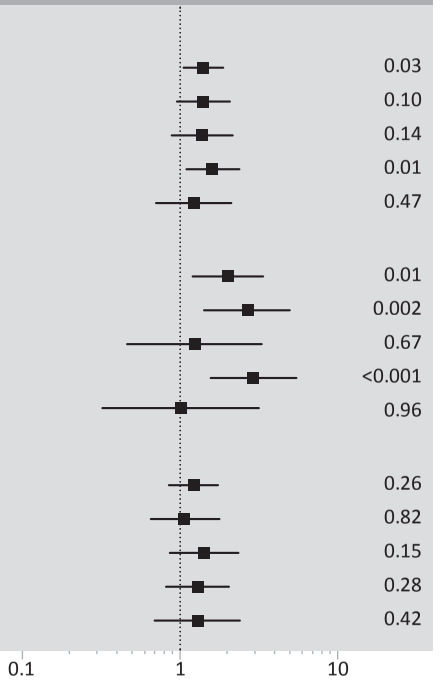
Table 2 Type of thrombotic event

	Number (%)
Venous thromboembolism	**549 (100)**
Deep vein thrombosis	313 (57)
Pulmonary embolism	175 (32)
Deep vein thrombosis and pulmonary embolism	61 (11)
Arterial thromboembolism	**3283 (100)**
Acute myocardial infarction	1079 (33)
Acute and subacute ischemic heart disease	966 (29)
Occlusion or stenosis of the precerebral arteries	173 (5)
Occlusion or stenosis of the cerebral arteries	448 (14)
Coronary artery bypass grafting	258 (8)
Percutaneous transluminal coronary angioplasty	149 (5)
Other vascular interventions	210 (6)

To explore the influence of misclassification due to hospitalization bias, a subanalysis was performed in which cardiovascular outcome was limited to myocardial infarction, ischemic stroke and cardiovascular death. Out of the 3283 subjects that developed an arterial event during follow-up, 1873 subjects developed myocardial infarction, ischemic stroke or cardiovascular death. Twenty-seven of these arterial thromboembolic events developed subsequent to venous thromboembolism, while 1846 subjects did not suffer from prior venous thromboembolism. The annual incidence of myocardial infarction, ischaemic stroke or cardiovascular death in subjects with previous venous thromboembolism was 1.22% (95% CI, 0.81-1.77), compared to 0.49% (95% CI, 0.47-0.52) in subjects without previous venous thromboembolism. The overall crude HR of myocardial infarction, ischemic stroke or cardiovascular death was 2.34 (95% CI, 1.60-3.42; *P* < 0.001) in subjects with venous thromboembolism, compared to subjects without. Multivariable analysis showed an overall adjusted HR of

1.42 (95% CI, 0.97-2.08; *P* = 0.07). Within the first year, this adjusted HR was 1.93 (95% CI, 0.96-3.87; *P* = 0.06). After 1 year of follow-up, the adjusted HR of myocardial infarction, ischemic stroke or cardiovascular death after venous thromboembolism decreased to 1.28 (95% CI, 0.81-2.01; *P* = 0.29).

	Observation years	No. ATE	Annual Incidence % (95% CI)	Crude Hazard Ratio* (95% CI)	Adjusted Hazard Ratio† (95% CI)	Decreased risk for ATE	Increased risk for ATE	*P*-value
Overall								
Venous thromboembolim (n = 549)	2222	45	2.03 (1.48-2.71)	2.24 (1.67-3.00)	1.40 (1.04-1.88)			0.03
Deep vein thrombosis (n = 313)	1378	25	1.81 (1.17-2.68)	1.99 (1.34-2.95)	1.40 (0.94-2.07)			0.10
Pulmonary embolism (n = 236)	844	20	2.37 (1.45-3.66)	2.59 (1.67-4.03)	1.39 (0.89-2.16)			0.14
Unprovoked VTE (n = 249)	1105	28	2.53 (1.68-3.66)	2.78 (1.92-4.04)	1.62 (1.11-2.34)			0.01
Provoked VTE (n = 256)	867	13	1.50 (0.80-2.56)	1.63 (0.95-2.82)	1.22 (0.71-2.11)			0.47
≤ 1 Year								
Venous thromboembolim	466	14	3.00 (1.64-5.04)	3.46 (2.04-5.84)	2.01 (1.19-3.40)			0.01
Deep vein thrombosis	271	10	3.69 (1.77-6.79)	4.24 (2.28-7.88)	2.68 (1.44-4.99)			0.002
Pulmonary embolism	195	4	2.05 (0.56-5.25)	2.34 (0.88-6.23)	1.24 (0.46-3.30)			0.67
Unprovoked VTE	221	10	4.52 (2.17-8.32)	5.17 (2.78-9.62)	2.91 (1.57-5.42)			<0.001
Provoked VTE	203	3	1.48 (0.30-4.32)	1.68 (0.54-5.21)	1.03 (0.33-3.20)			0.96
> 1 Year								
Venous thromboembolim	1756	31	1.77 (1.20-2.51)	1.92 (1.35-2.74)	1.23 (0.86-1.75)			0.26
Deep vein thrombosis	1107	15	1.36 (0.76-2.23)	1.47 (0.88-2.44)	1.06 (0.64-1.76)			0.82
Pulmonary embolism	649	16	2.47 (1.41-4.00)	2.67 (1.63-4.36)	1.43 (0.88-2.35)			0.15
Unprovoked VTE	884	18	2.04 (1.21-3.22)	2.22 (1.40-3.53)	1.29 (0.81-2.06)			0.28
Provoked VTE	664	10	1.51 (0.72-2.77)	1.62 (0.87-3.02)	1.29 (0.70-2.41)			0.42



VTE = venous thromboembolism, ATE = aterial thromboembolism, CI = confidence interval

*Reference group are those without venous thromboembolism; in the overall analysis (n = 40 307) 3238 subjects developed ATE in 370 529 years of observation time. †Reference group are those without venous thromboembolism, adjusted for age, sex, hypertension, dyslipidemia, diabetes mellitus, smoking status, elevated albuminuria and history of arterial thromboembolism

—■— Adjusted hazard ratio and 95% confidence interval

Figure 1 Risk of arterial thromboembolism after venous thromboembolism.

Discussion

This large population-based cohort study showed that subjects with previous venous thromboembolism are at increased risk to develop arterial thromboembolism. Although age was a strong confounder to this risk, the risk was still 1.4-fold increased after adjustment for age, sex, cardiovascular risk factors and history of arterial thromboembolism.

The overall absolute risk for arterial thromboembolism in subjects with venous thromboembolism was as high as 2.0% per year and even 3.0% during the first year after a diagnosis of venous thromboembolism. These values approach the absolute risk of recurrent venous thromboembolism.[15,16] These data indicate that clinicians should be aware of the possibility of arterial thromboembolism, as well as recurrent venous thromboembolism. It also implicates that the treatment of patients with venous thromboembolism may have to be reconsidered. A meta-analysis of Karthikeyan *et al*.[17] concluded that acetyl salicylic acid, known for its preventive effect for cardiovascular disease, is also effective in preventing venous thrombosis. As yet, however, there is insufficient evidence to advise a combination of vitamin K antagonists and antiplatelet therapy in order to prevent both recurrence and arterial thromboembolism after a first venous thromboembolism. Two ongoing studies (Warfasa, agnellig@unipg.it and Aspire, aspire@ctc.usyd) address this issue. Another option might be to prescribe statins to patients with venous thromboembolism. In addition to their lipid-lowering and cardioprotective capacity these drugs also appear to decrease the risk of venous thromboembolism.[18]

Our results showed that the risk of arterial thromboembolism is highest during the first year after venous thromboembolism, especially in patients with an unprovoked event. This finding is in accordance with other studies.[7,10] The early occurrence of cardiovascular events is difficult to understand, as patients with venous thromboembolism usually receive anticoagulant therapy in the first[3-6] months following their event and anticoagulant therapy is known to prevent cardiovascular events.[19] In the early 1960s it was observed that an increased risk of cardiovascular events occurred after cessation of oral anticoagulant therapy.[20] This high risk was assigned to a rebound effect on coagulant factors.[21–23] This notion, however, was not corroborated by others[24–26] and therefore remains controversial. In our cohort, only one subject developed arterial thromboembolism within a month after cessation of anticoagulant therapy, indicating that the presence of a rebound effect is unlikely.

Given these considerations, we hypothesize that the high risk of arterial thromboembolism within the first year after venous thromboembolism suggests that a joint mechanism relates the two diseases. The presence of underlying pathology

affecting the venous system might also affect the arterial system. The high risk of arterial thromboembolism in subjects with an unprovoked venous event compared to those with a provoked event supports this idea, as does our finding that the relationship between arterial and venous thromboembolism persists after adjustment for self-reported cardiovascular risk factors. Which underlying pathology could relate the two diseases cannot be concluded from our study. An explanation could be bodyweight. Obesity is related to a higher risk of arterial[27,28] and venous thromboembolism.[29,30] This might partly explain the relationship between arterial and venous thromboembolism through endothelial damage and/or the related changes in the levels of procoagulant proteins.[31,32] However, in a subset of patients for whom data on body mass index was available, adjustment for body mass index did not affect the risk of arterial thromboembolism after venous thromboembolism (data not shown).

Our study has both strengths and limitations. The strengths include the large population-based cohort, long follow-up time, prospectively collected data on arterial events, estimation of absolute risks and the adjustments made for age and sex in all analyses. A limitation of our study is that the data on cardiovascular risk factors were collected using self-reported histories at baseline. Data on the development of arterial cardiovascular risk factors during follow-up is not available. Furthermore, data regarding anticoagulant therapy were only available for the subjects who developed venous thromboembolism during follow-up. Hence, the use of anticoagulants was not included in our multivariable analyses and so we were not able to address the recent finding that anticoagulation therapy might accelerate arterial calcification.[33] The incidence of venous thromboembolism in our cohort may be underestimated as venous thromboembolism cases were retrospectively identified. Nonetheless, as compared with other prospective studies, our annual incidence of 0.15% is rather high as our cohort was confined to individuals younger then 75 years.[34] Lastly, the higher risk of arterial thromboembolism after venous thromboembolism may be spurious due to misclassification of arterial thromboembolism caused by hospitalization bias. Nonetheless, our subanalysis with outcome restricted to myocardial infarction, ischemic stroke and death due to arterial thrombosis, confirmed the primary analysis. Therefore, we conclude that misclassification was only marginal, if present. As shown in **Figure 1**, subgroup analyses were limited due to small numbers of arterial events, resulting in wider confidence intervals. For the same reason, we refrained from assessing differences in pulmonary embolism versus deep vein thrombosis and unprovoked versus provoked venous thromboembolism in this sensitivity analysis with restricted arterial outcome.

We conclude from this large cohort study that subjects with venous thromboembolism are at an increased risk to develop arterial thromboembolism. This risk is especially high in the first year after venous thromboembolism and after an unprovoked event. The risk persists after adjustment for age, sex, cardiovascular risk

factors and previous arterial thromboembolism. Our findings implicate that the care for patients with venous thromboembolism should not only focus on the prevention of recurrent venous thromboembolism but also on the prevention of arterial thromboembolism.

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3

Insulin resistance and risk of venous thromboembolism: results of a population-based cohort study

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Journal of Thrombosis and Haemostasis 2012; 10: 1012-1018

Abstract

Background Obesity is an established risk factor for venous thromboembolism (VTE), but it is uncertain how this is mediated. Insulin resistance has a central role in the pathophysiology of the metabolic effects of obesity.

Objective We aimed to investigate whether insulin resistance is a risk factor for VTE.

Methods For this analysis we used the PREVEND prospective community based observational cohort study. Insulin resistance was measured as HOMA-IR (homeostasis model assessment of insulin resistance) and fasting insulin. VTE was assessed using databases of the national registries of hospital discharge diagnoses, death certificates and the regional anticoagulation clinic.

Results Out of 7393 subjects, 114 developed VTE during a median follow-up of 10.5 years. High HOMA-IR was associated with increased risk of VTE after adjustment for traditional cardiovascular risk factors, CRP and markers of endothelial dysfunction (hazard ratio [HR], 1.38; 95% confidence interval [95% CI], 1.09-1.75; $P = 0.007$). When body mass index (BMI) was added to the model, BMI was a strong risk predictor for VTE (HR, 1.53; 95% CI, 1.24-1.88; $P < 0.001$) whereas HOMA-IR no longer showed such an association (HR, 1.11; 95% CI, 0.85-1.43; $P = 0.45$). Results were similar for fasting insulin.

Conclusion Our population-based cohort study shows an increased risk of VTE in subjects with increasing insulin resistance but not independently of BMI.

Introduction

Growing evidence shows a relationship between arterial and venous thromboembolism (VTE).[1] A possible explanation for this relationship is an overlap in risk factors. Several cardiovascular risk factors have been investigated regarding their association with VTE.[2] Overweight and obesity appeared to be strongly related to an increased risk of VTE.[2–5] Insulin resistance has a central role in the pathophysiology of the metabolic effects of overweight and obesity.[6–8] This raises the question whether insulin resistance is also a risk factor for VTE.

Insulin resistance is associated with endothelial damage[9–11] and increased levels of several prothrombotic factors (e.g. plasminogen activator inhibitor-1 [PAI-1],[12,13] fibrinogen[14,15] and von Willebrand factor [vWf][12] antigen. The potential association of insulin resistance with VTE has been investigated in only one relatively small case-control study.[16] This study had the unexpected finding that overweight subjects with VTE had lower insulin levels than overweight controls.[16] Therefore, the question of whether insulin resistance is a risk factor for venous thromboemoblism in a prospective setting remains unanswered to date.

The Prevention of REnal and Vascular ENd stage Disease (PREVEND) Study[17] is a large prospective population-based cohort study in which accurate measurements of fasting insulin and the homeostasis model assessment of insulin resistance (HOMA-IR) are available for over 7000 subjects. This gave us the opportunity to investigate the relationship between insulin resistance and risk of VTE at a population-based level.

Methods

Study population and design

This study was conducted with participants in the PREVEND Study. Details of this study have been published previously.[17] In 1997-1998, all inhabitants of the city of Groningen, the Netherlands, aged 28-75 years (n = 85 421), were invited to participate in this prospective cohort study, aimed at investigating the natural course of albuminuria and its relationship with renal and cardiovascular disease. A total of 40 856 subjects (47.8%) responded, filled out a questionnaire and had urinary albumin measured. Individuals with insulin-dependent diabetes mellitus were excluded from the PREVEND Study because the link between cardiovascular or renal disease and microalbuminuria in this subpopulation is well established. Pregnant women were excluded from the study too, as pregnancy may cause temporary microalbuminuria. All subjects with a urinary albumin concentration of 10 mg/l or greater (n = 7768) were invited and 6000

agreed to participate. A random control sample of 3394 subjects with a urinary albumin concentration of < 10 mg/l was invited and 2592 subjects agreed to participate. These 8592 subjects completed the screening protocol and form the baseline PREVEND cohort. For the present study, subjects with an invalid assessment of insulin resistance were excluded: subjects who had not explicitly stated that they had been fasting for at least 8 h prior to baseline blood sampling (*n* = 857), subjects who were using oral antidiabetic medication (*n* = 140) and subjects with missing values on glucose or insulin measurement at baseline (*n* = 202). The remaining 7393 subjects were included in the present analysis.

All participants gave written informed consent. The PREVEND Study was approved by the local medical ethics committee and was conducted in accordance with the guidelines of the Declaration of Helsinki.

Measurements

Participants visited the outpatient research unit twice for a baseline survey. All participants completed a questionnaire, height, weight and waist circumference were measured, an 8 h-fasting blood sample was drawn, and two 24-h urine samples were collected. Body mass index (BMI) was calculated as weight (kg) divided by square of height (m^2), and waist/height ratio was calculated as waist circumference (m) divided by height (m). During the first and second visit blood pressure was measured, in the supine position for 10 minutes with an automatic device (Dinamap XL Model 9300; Johnson-Johnson Medical, Tempa, USA). Blood pressure values are given as the mean of the last two recordings of both visits. High-sensitivity C-reactive protein (hsCRP) and urinary albumin concentration were determined by nephelometry (Dade Behring Diagnostic, Marburg, Germany). For urinary albumin concentration, the mean of the two 24-h samples was used for analysis. Total cholesterol and plasma glucose were measured by dry chemistry (Eastman Kodak, Rochester, USA), and triglycerides were measured enzymatically. High-density lipoprotein (HDL) was measured using a homogeneous method (direct HDL, Aeroset TM System; Abbott Laboratories, Abbott Park, Chicago, IL, USA). Plasma antigen level of PAI-1 was measured using an ELISA kit from Technoclone (Vienna, Austria).

Insulin Resistance

As measure for insulin resistance we used both fasting insulin level and HOMA-IR. HOMA-IR was calculated as: (glucose [mmol/l] × insulin [μU/ml])/22.5.[18] Insulin was measured with an AxSym® auto-analyzer (Abbott Diagnostics, Amstelveen, the Netherlands) with a threshold of 7 pmol/l and intra-assay and inter-assay coefficients of variation of 2.6% and 4.3%, respectively. This assay has virtually no cross-reactivity with pro-insulin

(0.016% at 106 pg/ml). The correction coefficient applied for calculation of HOMA-IR from insulin concentrations is 1 µU/ml = 6.00 pmol/l.[19]

Definition of thrombotic events

Subjects with VTE between study entry and 1 January 2009, were identified using the database of the regional anticoagulation clinic, which monitors the anticoagulant therapy of all inhabitants of the city of Groningen. In addition, the database of the national registry of hospital discharge diagnoses (Prismant, Utrecht, the Netherlands) was used. To identify fatal cases of VTE, the database of death certificates (Central Bureau of Statistics, The Hague/Heerlen, the Netherlands) was searched. When available, data on subjects with VTE according to any of the abovementioned databases was adjudicated by checking patients' medical records (*n* = 110) by an assessor blinded for insulin and glucose status of the subject under investigation. Deep vein thrombosis had to be confirmed by compression ultrasound, and pulmonary embolism by ventilation/perfusion lung scanning, spiral computed tomography, or at autopsy. When VTE had occurred at or within 3 months after exposure to an exogenous risk factor, including surgery, trauma, immobilization for more than 7 days, pregnancy, puerperium, the use of oral contraceptives or hormonal replacement therapy, or malignancy, this event was classified as being provoked. VTE was classified as unprovoked when no such risk factor was present.

Statistical analysis

To answer the question of whether insulin resistance increases the risk of VTE we used a Cox proportional hazard model. First, univariate analyses were performed for age, sex, BMI, insulin resistance, PAI-1 and all cardiovascular risk factors known to be associated with insulin resistance (including systolic blood pressure, total cholesterol, HDL, triglycerides and hsCRP), and their relationship with VTE. Second, insulin resistance was adjusted for age and sex **(Model 1)**. **Model 2** included a multivariable analysis, in which insulin resistance was additionally adjusted for systolic blood pressure, total cholesterol, HDL, triglycerides and hsCRP. In **Model 3**, additional adjustments were made for PAI-1. Finally, BMI was added to this model **(Model 4)**. These analyses were performed both with HOMA-IR and fasting insulin as measures for insulin resistance. In these analyses several variables were log transformed ($\log^e$) to approach a normal distribution (i.e. HOMA-IR, fasting insulin, systolic blood pressure, total cholesterol, HDL, triglycerides, hsCRP and PAI-1). After transformation these variables were included as continuous variables in the Cox proportional hazard model. First order interactions between HOMA-IR and the variables included in the models were calculated.

Because the variables under investigation have different units we choose to report hazard ratios per standard deviation (SD). Comparing the risk of VTE through increases by standard deviation enables good comparison of strength of associations for the various parameters under study. The HRs are reported with 95% confidence interval (95% CI). Observation time started with study entry and ended at time of VTE, end of study (January 2009), death or moving out of the city. Various sub-analyses were performed. Firstly, subjects with provoked and unprovoked VTE were analyzed separately. Secondly, because insulin resistance is known to be associated with higher urinary albumin excretion[20,21] and urinary albumin excretion is also related to an increased risk of VTE,[22] sensitivity analyses were performed with additional adjustments for urinary albumin excretion. Thirdly, analyses were repeated with waist/height ratio[23] and waist circumference[24] as measures for body density, because in arterial cardiovascular disease these measures are reported to be slightly better in estimating the cardiovascular risk. Lastly, designed-based sensitivity analyses were performed, using survey probability weights,[25] to correct for the enrichment of the cohort for subjects with high levels of urinary albumin excretion.

In the present study, we used a cohort that was originally composed with a different aim, namely, to investigate the natural course of albuminuria and its relationship with renal and cardiovascular disease. To answer the present research question, we included the maximum number of subjects participating in this cohort. Therefore, an *a priori* power and sample size calculation was not performed.

Correlations were determined between different variables, expressed as Spearman non-parametric correlation coefficients. Categorical data are presented as counts and percentages. Continuous variables are presented as medians with interquartile ranges (IQR) when non-parametric, and as mean with SD when parametric. Statistical difference was tested with the Mann-Whitney *U*-test, Student's *t*-test and chi-square test. A two-tailed $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed using PASW version 18.0 (IBM SPSS, Chicago, IL, USA).

Results

Study population

Baseline characteristics are shown in **Table 1**. Of the 7393 subjects, 49% were male. Mean age at enrollment was 50 (SD, 13) years. In 1841 subjects, follow-up ended prematurely at time of death (*n* = 509, 7%) or moving out of the city (*n* = 1332, 18%). During a median follow-up of 10.5 (IQR, 10.2-10.8) years, 114 subjects developed a VTE at a mean age of 62 (SD, 13) years. The annual incidence of VTE was 0.16% (95% CI, 0.13-0.19). Deep vein thrombosis occurred in 69 subjects, 33 subjects developed pulmonary embolism, and

Table 1 Baseline Characteristics

	VTE	No VTE	*P*-value
TOTAL	**114**	**7273**	
Female	53 (47)	3708 (51)	0.34
Age at enrollment, y	57 (12)	50 (13)	<0.001
Glucose level, mmol/l	5.0 (4.6-5.3)	4.7 (4.4-5.1)	<0.001
Insulin level, µU/ml	9.7 (7.2-15.6)	7.8 (5.5-11.6)	<0.001
HOMA-IR	2.2 (1.5-3.4)	1.6 (1.1-2.6)	<0.001
Systolic blood pressure, mmHg	135 (118-147)	125 (113-140)	0.001
Total cholesterol, mmol/l	5.8 (5.1-6.5)	5.6 (4.9-6.3)	0.01
HDL, mmol/l	1.2 (1.0-1.4)	1.3 (1.0-1.6)	0.08
Triglycerides, mmol/l	1.3 (1.0-1.9)	1.2 (0.8-1.7)	0.006
hsCRP, mg/l	1.8 (1.1-3.9)	1.3 (0.6-2.9)	<0.001
PAI-1 µg/l	103.8 (61.0-156.9)	73 (41.2-122.8)	<0.001
BMI, kg/m^2	29 (6)	26 (4)	<0.001
Waist circumference (cm)	97 (13)	88 (13)	<0.001
Waist/height ratio	56 (8)	51 (7)	<0.001
UAE, mg/24h	13.8 (8.1-43.5)	9.4 (6.3-17.3)	<0.001

VTE = venous thromboembolism, HOMA-IR = homeostasis model assessment of insulin resistance, HDL = high-density lipoprotein, hsCRP = high-sensitivity C-reactive protein, PAI-1 = plasminogen activator inhibitor-1 antigen, BMI = body mass index, UAE = urinary albumin excretion. Categorical data are presented as number (%), continuous variables are presented as medians (IQR) when non-parametric, and as mean (SD) when parametric

12 subjects developed both deep vein thrombosis and pulmonary embolism. Thrombosis at atypical sites did not occur. In 64 subjects VTE was secondary to an external risk factor, and 46 events were unprovoked. In four events the presence or absence of an external risk factor could not be assessed from medical records. Median glucose level was 4.7 (IQR, 4.4-5.1) mmol/l, median insulin level was 7.8 (IQR, 5.5-11.7) µU/ml and median HOMA-IR was 1.6 (IQR, 1.1-2.6). Compared to subjects without VTE, subjects who developed VTE had significantly higher age, HOMA-IR, systolic blood pressure, BMI, waist circumference, waist/height ratio, urinary albumin excretion and levels of glucose, insulin, total cholesterol, triglycerides, hsCRP and PAI-1.

Risk of VTE according to insulin resistance

A test for interaction of each risk factor with time showed that the proportional hazards assumption was not violated. Furthermore, there were no significant first order interactions between HOMA-IR and the variables included in the models. **Table 2** shows

the results of the univariate and multivariable analyses of HOMA-IR in relation to overall VTE. In the univariate model, increasing HOMA-IR, age, systolic blood pressure, total cholesterol, triglycerides, hsCRP, PAI-1 and BMI all significantly increased the risk of overall VTE. In **Model 2**, HOMA-IR was the only cardiovascular risk factor that significantly increased the risk of VTE, besides age and hsCRP (HR, 1.45; 95% CI, 1.16-1.81; *P* = 0.001). PAI-1 showed a significant correlation with HOMA-IR (ρ = 0.46, *P* < 0.001). When PAI-1 was added to the model **(Model 3)**, HOMA-IR still significantly increased the risk of VTE (HR 1.38; 95% CI, 1.09-1.75; *P* = 0.007). BMI was also strongly associated with HOMA-IR (ρ = 0.59, *P* < 0.001) and when added to the model, BMI appeared to be a strong risk predictor for VTE (HR, 1.53; 95% CI, 1.24-1.88; *P* < 0.001) whereas insulin resistance no longer showed such an association (HR, 1.11; 95% CI, 0.85-1.43; *P* = 0.45).

Similar results were found when the analyses were repeated with fasting insulin as measure for insulin resistance **(see Figure 1)**. In **Model 1**, fasting insulin increased the risk of VTE with an HR of 1.47 (95% CI, 1.22-1.76); *P*<0.001. In the multivariable **Model 2** this HR was 1.55 (95% CI, 1.24-1.93; *P* < 0.001). Further adjustment for PAI-1 showed an HR of 1.50 (95% CI, 1.19-1.90; *P* = 0.001). When BMI was added to this model, fasting insulin no longer increased the risk of VTE (HR 1.22; 95% CI, 0.94-1.57; *P* = 0.14).

When analyses were confined to unprovoked VTE, we found that HOMA-IR increased the risk after adjustment for age, sex, systolic blood pressure, HDL and total cholesterol, triglycerides, hsCRP and PAI-1 (HR, 1.64; 95% CI, 1.13-2.36; *P* = 0.009). After additional adjustment for BMI this HR decreased to a non-significant 1.25 (95% CI, 0.83-1.88; *P* = 0.28). When restricted to provoked VTE, the multivariate **Model 3** showed an HR of 1.20 (95% CI, 0.87-1.64; *P* = 0.26). This HR decreased after additional adjustment for BMI to 1.03 (95% CI, 0.73-1.45; *P* = 0.86).

When analyses were repeated with additional adjustments for urinary albumin level, similar results were found: the HR of HOMA-IR for overall VTE was 1.36 (95% CI, 1.07-1.72; *P* = 0.01) in **Model 3**. After additional adjustment for BMI, this HR was 1.09 (95% CI, 0.84-1.41; *P* = 0.51), in **Model 4**.

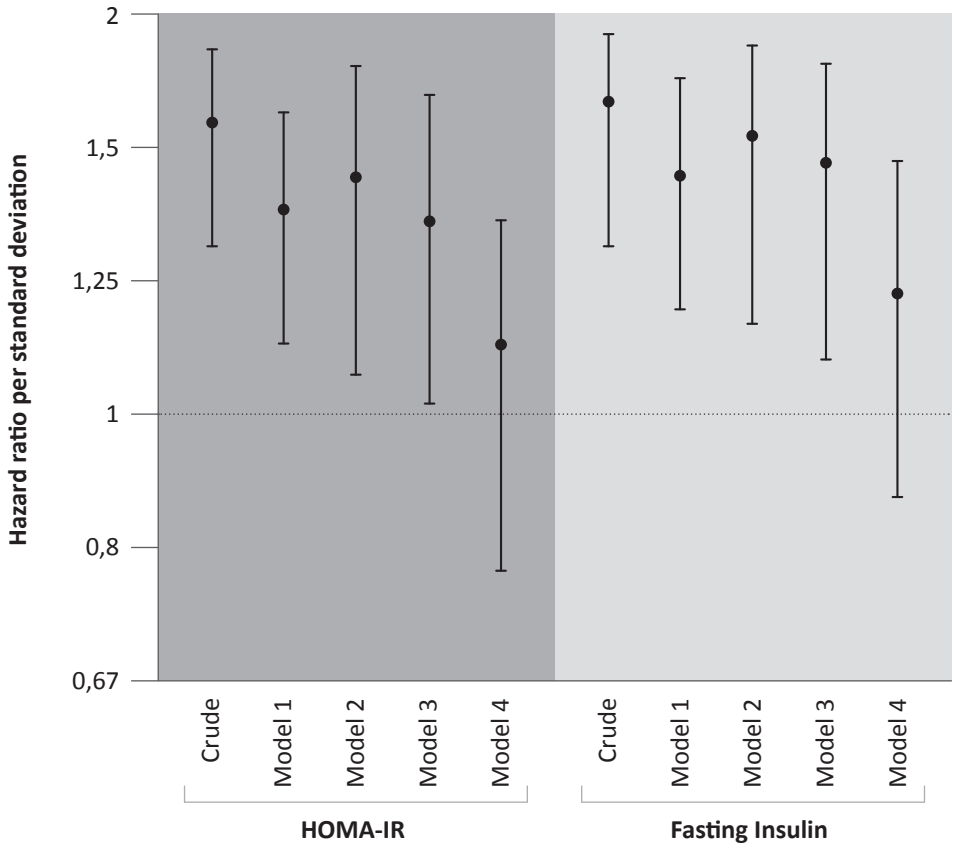
Sensitivity analyses were performed in which waist circumference and waist/height ratio were used instead of BMI. Both variables were strong univariate risk factors for overall VTE (HR, 1.85; 95% CI, 1.57-2.19; *P* < 0.001 and 1.84; 95% CI, 1.57-2.15; *P* < 0.001 for waist circumference and waist/height ratio, respectively). Waist circumference (ρ = 0.56, P < 0.001) and waist/height ratio (ρ = 0.59, *P* < 0.001) showed strong correlations with HOMA-IR and when added to the multivariable **Model 3**, waist circumference (HR, 1.55 [95% CI, 1.20-1.99], *P* = 0.001) and waist/height ratio (HR, 1.48; 95% CI, 1.16-1.89; *P* = 0.002) remained significantly associated with an increased risk of overall VTE, while HOMA-IR was no longer a risk factor (HR, 1.14; 95% CI, 0.89-1.48; *P* = 0.30 and 1.16; 95% CI, 0.90-1.49; *P* = 0.25 for waist circumference and waist/height ratio, respectively).

Table 2 Risk of overall venous thromboembolism according to HOMA-IR-level

	Univariate model		Model 1*		Model 2†		Model 3‡		Model 4§	
	Hazard Ratio (95%CI)	*P*-value	Hazard Ratio (95%CI)	*P*-value	Hazard Ratio (95%CI)	*P*-value	Hazard Ratio (95%CI)	*P*-value	Hazard Ratio (95%CI)	*P*-value
HOMA-IR (per SD)	1.58 (1.33-1.87)	<0.001	1.40 (1.17-1.68)	<0.001	1.45 (1.16-1.81)	0.001	1.38 (1.09-1.75)	0.007	1.11 (0.85-1.43)	0.45
Age (per SD)	1.86 (1.53-2.26)	<0.001	1.71 (1.41-2.09)	<0.001	1.78 (1.41-2.24)	<0.001	1.78 (1.41-2.26)	<0.001	1.77 (1.40-2.24)	<0.001
Sex (female)	0.82 (0.57-1.18)	0.28	0.95 (0.65-1.37)	0.76	0.91 (0.59-1.39)	0.65	0.92 (0.60-1.42)	0.70	0.83 (0.53-1.30)	0.42
SBP (per SD)	1.35 (1.14-1.61)	0.001	-	-	0.92 (0.74-1.15)	0.46	0.93 (0.75-1.16)	0.52	0.92 (0.73-1.15)	0.47
Total cholesterol (per SD)	1.27 (1.05-1.52)	0.01	-	-	1.02 (0.81-1.28)	0.90	0.99 (0.78-1.25)	0.93	0.98 (0.77-1.25)	0.87
HDL (per SD)	0.83 (0.69-1.00)	0.054	-	-	0.96 (0.74-1.24)	0.74	0.97 (0.74-1.26)	0.81	0.96 (0.73-1.26)	0.78
Triglycerides (per SD)	1.24 (1.04-1.47)	0.02	-	-	0.82 (0.62-1.09)	0.17	0.83 (0.63-1.11)	0.21	0.85 (0.63-1.13)	0.26
hsCRP (per SD)	1.48 (1.22-1.79)	<0.001	-	-	1.24 (1.00-1.54)	0.049	1.25 (1.00-1.56)	0.047	1.16 (0.92-1.46)	0.23
PAI-1 (per SD)	1.44 (1.21-1.72)	<0.001	-	-	-	-	1.11 (0.88-1.40)	0.36	1.02 (0.81-1.30)	0.86
BMI (per SD)	1.61 (1.42-1.83)	<0.001	-	-	-	-	-	-	1.53 (1.24-1.88)	<0.001

HOMA-IR = homeostasis model assessment of insulin resistance, SBP = systolic blood pressure, HDL = high-density lipoprotein, hsCRP = high-sensitivity C-reactive protein, PAI-1 = plasminogen activator inhibitor-1 antigen, BMI = body mass index, CI = confidence interval. In these analyses, HOMA-IR, SBP, total cholesterol, HDL, triglycerides, hsCRP and PAI have been logtransformed to approach a normal distribution. *Model 1: multivariable model including: HOMA-IR, age and sex. †Model 2: as model 1 with additional adjustment for SBP, total cholesterol, HDL, triglycerides and hsCRP. ‡Model 3: as model 2 with additional adjustment for PAI-1. §Model 4: as model 3 with additional adjustment for BMI

When the models were adjusted for the enrichment of the study cohort with participants with higher urinary albumin levels, using survey probability weights,[25] the corresponding HRs were 1.75 (95% CI, 1.26-2.42; *P* = 0.001) in **Model 3** and 1.29 (95% CI, 0.93-1.79; *P* = 0.13) in **Model 4**, in which BMI was added to the multivariable analysis.



HOMA-IR = homeostasis model assessment of insulin resistance
Model 1: multivariable model including: HOMA-IR, age and sex; Model 2: as model 1 with additional adjustment for systolic blood pressure, total cholesterol, high-density lipoprotein, triglycerides and high-sensitivity C-reactive protein; Model 3: as model 2 with additional adjustment for PAI-1; Model 4: as model 3 with additional adjustment for body mass index

Figure 1 Risk of overall venous thromboembolism according to level of insulin resistance

Discussion

The present study is the first population-based study investigating the relationship between insulin resistance and risk of VTE. Subjects with higher levels of insulin resistance had an increased risk of VTE, mainly driven by an increased risk of unprovoked events. The higher risk in subjects with higher levels of insulin resistance was independent of several risk factors related to insulin resistance, including age, sex, systolic blood pressure, HDL and total cholesterol, triglycerides, hsCRP and urinary albumin level. However, BMI and insulin resistance were strongly related and insulin resistance was not an independent risk factor for VTE when BMI was added to the model. BMI itself strongly increased the risk of VTE.

Only one relatively small case-control study reported on the relationship between insulin levels and VTE.[16] This study compared insulin levels between subjects with and without VTE. In normal weight subjects no difference in insulin level between patients and controls was found. In overweight subjects, the authors had the unexpected finding that patients had significantly lower levels of insulin when compared with controls.[16] Our study does not show such a relationship. A reason for this discrepancy could be the fact that multiple testing in small groups was performed in the case-control study, which may have caused chance findings. Aside from this case-control study, no other studies have previously investigated the relationship between insulin resistance and VTE. Two cohort studies investigated the relationship between glucose levels or HbA1c and VTE in subjects with the metabolic syndrome.[26,27] In accordance with our results, these studies show that BMI was the main determinant of the metabolic syndrome that was associated with the increased risk of VTE.[26,27] Unlike the present study, these studies did not examine insulin resistance, but beta-cell insufficiency and VTE. Importantly, insulin resistance only leads to hyperglycemia once pancreatic beta-cells start to fail.[28]

When studying insulin resistance, we also found that none of the cardiovascular or procoagulant risk factors independently increased the risk of VTE, except for BMI. A possible explanation for this finding could be the strong relationship between insulin resistance and BMI. Like insulin resistance, BMI itself is also related to endothelial damage and increased levels of several prothrombotic risk factors.[29–31] It is possible that the studies that showed a relationship between insulin resistance and prothrombotic state were actually looking at the relationship between BMI and prothrombotic state. However, the few studies that did adjust for BMI still found an independent relationship between insulin resistance and prothrombotic state.[12,29] It may well be that these prothrombotic changes are not severe enough to cause thrombosis or are counteracted by an increase in levels of anticoagulant factors. Our results thus suggest that insulin resistance is not the main pathway for the development of VTE in overweight or obese

subjects. Indeed, high levels of BMI can influence thrombotic risk through several other ways. For example, thrombotic risk increases with the level of physical inactivity[32] and physical inactivity is more common in subjects with high BMI.[33,34] Another explanation for the relationship between BMI and VTE could be an increase in FVIII level. This protrombotic factor is known to be elevated in subjects with high BMI[35,36] and could play a role in the development of VTE.

We acknowledge that our study has limitations. First, incidence of VTE may have been underestimated as cases were identified retrospectively. Nonetheless, the assessor who investigated patient charts was blinded to insulin and glucose status and there is no reason to assume an unequal distribution of missed events between different levels of insulin resistance. Second, the cohort under investigation is enriched for participant with higher levels of albuminuria. This enrichment, however, is unlikely to have influenced our risk estimates (i.e. HRs), as these estimates did not significantly change after accounting for study design. Third, insulin and glucose levels, as well as the relevant covariates used in our analyses, were measured only once at inclusion. Changes in the values of these variables were not considered. In our opinion, this does not weaken our finding that insulin resistance is not related to VTE when controlling for BMI. Despite the time-lag between data assessment and VTE occurrence, both univariate and multivariable analyses showed the hypothesized association between insulin resistance and VTE. After adjustment for BMI, insulin resistance was no longer associated with VTE. As insulin resistance and BMI were strongly correlated, we feel that repeated measurements will not influence this confounding effect. Fourth, we did not have information on long-term anticoagulant therapy for atrial fibrillation. Long-term anticoagulant therapy could influence VTE occurrence. However, as atrial fibrillation and insulin resistance are not related,[37] there is no reason to assume an unequal distribution of subjects on long-term anticoagulant therapy due to atrial fibrillation between subjects with different levels of insulin resistance. Therefore, this is not likely to have influenced our results.

Lastly, we investigated PAI-1 as pro-coagulant factor. Unfortunately, data on vWf and fibrinogen levels were not available, and we could therefore not adjust for these variables. Data on other acquired and inherited thrombophilia factors were not available either. However, these variables have no known relationship with insulin resistance, and are therefore not likely to be a source of confounding.

Strengths of this study are that to our knowledge it is the first study assessing insulin resistance as a risk factor for VTE. Moreover, the PREVEND cohort is a large population-based prospective cohort in which insulin resistance is assessed as both fasting insulin and HOMA-IR. For both variables similar results were obtained with respect to the association between insulin resistance and VTE, indicating that our findings are robust and likely to be true. Furthermore, detailed and accurate data on cardiovascular risk factors were available. This provided us with other measures of body density, namely

waist circumference and waist/height ratio, which strengthens our findings regarding the role of body density in the relationship between insulin resistance and VTE. Additionally, information on diabetes treatment was available which made it possible to exclude these subjects in our analysis.

In conclusion, we have shown an increased risk of VTE in subjects with increasing insulin resistance, but not independently of BMI. Our results suggest that insulin resistance is not essential for the development of VTE in overweight or obese subjects. Future studies should focus on other pathways that could explain the high thrombotic risk in these subjects.

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Lipid levels do not influence the risk of venous thromboembolism: results of a population-based cohort study

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Thrombosis and Haemostasis 2012, in press

Abstract

Studies on the association between lipid profile and venous thromboembolism (VTE) are inconsistent. This could be caused by classical lipoproteins being inferior to apolipoproteins as markers for VTE risk. Therefore, we examined whether apolipoproteins are more strongly related to VTE than lipoproteins. For this analysis we used the PREVEND prospective community based observational cohort study. Levels of apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), total cholesterol (TC), high-density lipoprotein (HDL), non-HDL, low-density lipoprotein (LDL), triglycerides (TG), lipoprotein(a), ApoB/ApoA1 and TC/HDL ratio were assessed. Subjects with VTE were identified using databases of the national registries of hospital discharge diagnoses, death certificates, and the regional anticoagulation clinic. Out of 7627 subjects, 110 developed VTE during a median follow-up of 10.5 years. In both univariate and multivariable analyses no significant associations between apolipoproteins and overall VTE were observed. Of the classical lipoproteins, TC, non-HDL, LDL, TG, and TC/HDL ratio were significantly associated with overall VTE in univariate analysis. Significant associations were no longer present in multivariable analysis. TGL and LDL were significantly associated with unprovoked VTE in univariate analysis. After adjustment for age and sex this significance was lost. No significant associations between (apo-) lipoproteins and provoked VTE were found. We conclude that apolipoproteins are not better in predicting VTE risk than the classical lipoproteins. Our population-based cohort study does not show an association between both apolipoproteins and the classical lipoproteins and VTE risk.

Introduction

Growing evidence shows a relationship between arterial and venous thromboembolism (VTE).[1] A possible explanation for this relationship is an overlap in risk factors, such as an abnormal lipid profile. Indeed, the Jupiter trial showed that the use of statins decreases the risk of VTE.[2] Two prospective, observational studies also reported a decreased risk of VTE associated with the use of statins.[3,4]

Studies on the association between lipid profile and VTE, however, are inconsistent.[5–9] These studies mainly focused on classical lipoproteins (i.e. total cholesterol [TC], low-density lipoprotein [LDL], high-density lipoprotein [HDL], triglycerides [TG] and lipoprotein (a) [lp(a)]).[5,6,10] However, in arterial thromboembolism, apolipoproteins and their ratios are possibly stronger predictors for the risk of arterial thromboembolism than the classical lipoproteins.[11–13] This may also apply to venous thromboembolism. Possibly, the inconsistent results on the association between lipid profile and VTE are caused by the use of inferior risk markers which could not optimally detect the effect of an abnormal lipid profile on VTE risk.

For this reason, we examined whether apolipoproteins are more strongly related to VTE than lipoproteins in the large population-based cohort of the Prevention of REnal and Vascular ENd stage Disease (PREVEND) Study.

Materials and Methods

Study population and design

This study was conducted on participants in the PREVEND Study. Details of this study have been published previously[14] and can be found at http://www.prevend.org. In 1997-1998, all inhabitants of the city of Groningen, the Netherlands, aged 28 to 75 years (*n* = 85 421), were invited to participate in this prospective cohort study, which was designed to investigate the natural course of albuminuria and its relation to renal and cardiovascular disease. A total of 40 856 subjects (47.8%) responded. Individuals with insulin-dependent diabetes mellitus were excluded from the PREVEND Study since the link between cardiovascular or renal disease and microalbuminuria in this population is well established. As pregnancy may cause temporary microalbuminuria, pregnant women were excluded from the study too. After these exclusions, all subjects with a urinary albumin concentration of 10 mg/l or greater (*n* = 7768) were invited and 6000 agreed to participate. A random control sample of 3394 subjects with a urinary albumin concentration of less than 10 mg/l was invited and 2592 subjects agreed to participate. Taken together, 8592 subjects took part in the baseline screening and constitute the

PREVEND cohort. For the present study, we excluded participants who were nonfasting at the time of first blood sampling (*n* = 428) and those who used lipid-lowering drugs according to either pharmacy or self-report (*n* = 537). This left us with 7627 patients for the present analysis.

All participants provided written informed consent. The PREVEND Study was approved by the local medical ethics committee and was conducted in accordance with the guidelines of the Declaration of Helsinki.

Measurements

Participants visited the outpatient research unit twice for baseline survey. All participants completed a questionnaire on demographics, cardiovascular disease history, smoking habits, alcohol consumption and medication use prior to their first visit. Height and weight were measured, an 8 hour-fasting blood sample was drawn and two 24-hour urine samples were collected. Body mass index (BMI) was calculated as weight (kg) divided by square of height (m^2). During the first and second visit blood pressure was measured, in supine position for 10 minutes with an automatic device (Dinamap XL Model 9300, Johnson-Johnson Medical, Florida, USA). Blood pressure values are given as the mean of the last two recordings of both visits. Hypertension was defined as systolic blood pressure of at least 140 mmHg, diastolic blood pressure of at least 90 mmHg, or the pharmacy-confirmed use of antihypertensive drugs.[15] Diabetes mellitus was diagnosed by fasting plasma glucose ≥ 7.0 mmol/l, according to 1997 American Diabetes Association criteria[16] or pharmacy-confirmed use of oral glucose-lowering drugs. Self-reported data on medication use were confirmed using pharmacy-dispensing information from all community pharmacies in the city of Groningen; drug use was available and complete for 85% of PREVEND participants. High-sensitivity C-reactive protein (hsCRP) and urinary albumin concentration were determined by nephelometry (Dade Behring Diagnostic, Marburg, Germany). For urinary albumin excretion, the mean of the two samples was used for analysis. Estimated glomerular filtration rate (eGFR) was estimated using the Modification of Diet in Renal Disease study equation, taking into account sex, age, race, and serum creatinine level.[17] Subjects were classified as smokers when they reported smoking cigarettes during the previous year.

(Apo-) lipoproteins

Serum samples for lipid and apolipoprotein measurements were stored at -80 ^{0}C until analysis. TC was measured by dry chemistry (Eastman Kodak, Rochester, NY, USA), TG were measured enzymatically. HDL was measured using a homogeneous method (direct HDL, AEROSET System; Abbott Laboratories, Abbott Park, IL, USA).[18] LDL was estimated

using the Friedewald formula.[19] NonHDL was calculated as the difference between TC and HDL. Serum apolipoprotein A1 (apoA1), apolipoprotein B (apoB), and lp(a) were determined by nephelometry applying commercially available reagents for Dade Behring nephelometer systems (BN II; Dade Behring, Marburg, Germany).[20]

Definition of thrombotic events

The database of the regional anticoagulation clinic, which monitors the anticoagulant therapy of all inhabitants of the city of Groningen, was used to identify participants of the PREVEND study who developed VTE between study entry and January 1, 2009. As an additional confirmation, the database of the national registry of hospital discharge diagnoses (Prismant, Utrecht, The Netherlands) was searched. Fatal cases were identified with the use of the database of the national registry of death certificates (Central Bureau of Statistics, The Hague/Heerlen, The Netherlands). When available, data on subjects with VTE according to any of the abovementioned databases was confirmed by patients' medical records (n = 89). This was carried out by an assessor blinded for the lipid profile of the subject under investigation. Only objectively verified symptomatic thromboembolic events were considered. Deep vein thrombosis had to be confirmed by compression ultrasound, and pulmonary embolism by ventilation/perfusion lung scanning, or by spiral computed tomography. When VTE had occurred at or within three months after exposure to an exogenous risk factor including surgery, trauma, immobilisation for more than seven days, pregnancy, puerperium, the use of oral contraceptives or hormonal replacement therapy, or malignancy, this event was classified as being provoked. VTE was classified as unprovoked when no such risk factor was present.

Statistical analysis

To examine the association between (apo-) lipoproteins and their ratios and VTE risk we used a Cox proportional hazard model. The following variables were examined: ApoA1, ApoB, TC, HDL, non-HDL, LDL, TG, lp(a), ApoB/ApoA1 and TC/HDL ratio. As the hazard ratio (HR) did not gradually increase with increasing levels of the (apo-) lipoproteins, these variables could not cot be included as continues variables. Therefore, the risk of VTE was analysed according to the tertiles of the (apo-) lipoproteins. Adjustments were made for age, sex, hypertension, diabetes, urinary albumin excretion, hsCRP and BMI. In these analyses urinary albumin excretion and hsCRP were log transformed ($\log^e$) to approach a normal distribution. First order interactions between the (apo-) lipoproteins and the variables included in the model were calculated. The HRs are reported with 95% confidence intervals (95% CI). Observation time started with study entry and ended at time of VTE, end of study (January 2009), death or moving out of the city.

Various subanalyses were performed. Firstly, subjects with provoked and unprovoked VTE were analysed separately. Secondly, a sensitivity analysis was performed, in which all analyses were repeated without excluding subjects that were originally excluded based on the use of lipid-lowering medication. Lastly, design-based sensitivity analyses were performed, using survey probability weights,[21] to correct for the enrichment of the cohort for subjects with high levels of urinary albumin excretion.

Categorical data are presented as counts and percentages. Continuous variables are presented as medians with interquartile ranges (IQR) when not normally distributed, and as mean with standard deviation (SD) when normally distributed. Statistical difference was tested with the Mann-Whitney *U* test, Student's t-test and Chi^2 test. A two-tailed $P < 0.05$ was considered statistically significant. Statistical analyses were performed using PASW version 18.0 (IBM SPSS, Chicago, IL, USA).

Results

Study population

Baseline characteristics are shown in **Table 1**. Of the 7627 subjects 49% were male. Mean age at enrollment was 49 (SD, 13) years. In 1949 subjects follow-up ended prematurely at time of death (*n* = 531, 7%) or moving out of the city (*n* = 1418, 19%). During a median follow-up of 10.5 (IQR, 10.2-10.8) years, 110 subjects developed VTE at a mean age of 61 (SD, 13) years. Median time from study inclusion to VTE was 4.9 (1.9-7.6) years. The annual incidence of VTE was 0.15 (95% CI; 0.12-0.18) per 100 person years. In 61 subjects VTE was secondary to an external risk factor, 44 events were unprovoked. In five events the presence or absence of an external risk factor could not be assessed from medical records. Compared to subjects without VTE, subjects who developed VTE had significantly higher TC, non-HDL, LDL, TG and TC/HDL ratio at baseline. They also had significantly higher BMI, hsCRP and urinary albumin excretion and lower HDL and eGFR. Furthermore, subjects with VTE were more often hypertensive and were older.

Risk of VTE according to (apo-) lipoproteins

Inclusion of time dependent covariates in the Cox proportional hazard model showed that the proportional hazards assumption was not violated. Furthermore, there were no relevant significant first order interactions between the (apo-) lipoproteins and the variables included in the models. **Table 2** shows the results of the univariate and multivariable analyses of the apolipoproteins in relation to overall VTE. In both univariate and multivariable analyses no significant associations between apolipoproteins and VTE were observed.

Table 1 Baseline Characteristics

	VTE (n = 110)	No VTE (n = 7517)	*P*-value
ApoA1(g/l)	1.33 (1.18-1.50)	1.36 (1.20-1.56)	0.30
ApoB (g/l)	1.07 (0.90-1.25)	1.00 (0.82-1.21)	0.05
ApoB/ApoA1	0.76 (0.64-0.98)	0.74 (0.58-0.93)	0.09
Total Cholesterol (mmol/l)	5.83 (5.08-6.80)	5.51 (4.87-6.30)	0.01
HDL (mmol/l)	1.22 (0.97-1.45)	1.28 (1.04-1.57)	0.03
Non-HDL (mmol/l)	4.64 (3.88-5.43)	4.23 (3.44-5.06)	0.001
LDL (mmol/l)	4.25 (3.60-5.10)	3.97 (3.23-4.74)	0.001
Triglycerides (mmol/l)	1.29 (0.95-1.93)	1.13 (0.82-1.65)	0.003
Lipoprotein (a) (g/l)	0.06 (0.03-0.14)	0.05 (0.02-0.14)	0.46
TC/HDL	4.65 (3.81-5.93)	4.32 (3.32-5.60)	0.003
Age at enrollment (years)	58 (13)	49 (13)	<0.001
Sex (male)	59 (54)	3681 (49)	0.34
Hypertension	46 (42)	2172 (29)	0.004
Diabetes Mellitus	3 (3)	222 (3)	1.00
UAE (mg/24h)	14.1 (8.1-51.9)	9.3 (6.3-16.8)	<0.001
hsCRP (mg/l)	1.8 (1.1-3.9)	1.3 (0.5-2.9)	<0.001
BMI	29 (5)	26 (4)	<0.001
eGFR	77.1 (15.6)	80.9 (14.4)	0.01
Smoking	33 (30)	2844 (38)	0.11

VTE = venous thromboembolism, HDL = high-density lipoprotein, LDL = low-density lipoprotein, 'Apo' = apolipoprotein, TC = total cholesterol, UAE = urinary albumin excretion, hsCRP = high-sensitivity C-reactive protein, BMI = body mass index, eGFR = estimated glomerular filtration rate. Categorical data are presented as number (%), continuous variables are presented as medians (IQR) when not normally distributed, and as mean (SD) when normally distributed

Table 2 Risk of venous thromboembolism related to apolipoproteins and their ratios

Variable	Crude Hazard Ratio (95% CI)	*P*-value	Adjusted Hazard Ratio† (95% CI)	*P*-value	Adjusted Hazard Ratio‡ (95% CI)	*P*-value
ApoA1 (n = 7243) §		0.49		0.56		0.85
2nd tertile	0.97 (0.61-1.52)		0.93 (0.59-1.48)		1.00 (0.62-1.60)	
3rd tertile	0.76 (0.46-1.24)		0.76 (0.45-1.27)		0.87 (0.51-1.49)	
ApoB (n = 7244)		0.10		0.91		0.77
2nd tertile	1.36 (0.81-2.29)		1.01 (0.59-1.71)		0.85 (0.50-1.44)	
3rd tertile	1.71 (1.04-2.80)		1.10 (0.66-1.83)		0.83 (0.49-1.41)	
ApoB/ApoA1 (n = 7243)		0.17		0.65		0.29
2nd tertile	1.59 (0.96-2.61)		1.22 (0.73-2.03)		1.02 (0.61-1.71)	
3rd tertile	1.48 (0.89-2.45)		1.01 (0.59-1.73)		0.72 (0.41-1.25)	

Apo = apolipoprotein, CI = confidence interval. §number of subjects included in univariate analysis. †Reference group are those within the first tertile. Adjustments are made for age and sex. ‡Reference group are those within the first tertile. Adjustments are made for age and sex, hypertension, diabetes, albuminuria, C-reactive protein, body mass index, estimated glomerular filtration rate and smoking

Table 3 shows the results of the univariate and multivariable analyses of the classical lipoproteins and their ratios in relation to overall VTE. In the univariate analyses, TC, non-HDL, LDL, TG and TC/HDL ratio were significantly associated with overall VTE. However, after adjustment for age and sex, a significant association was no longer present in all these variables. This was also true after additional adjustment for hypertension, diabetes, urinary albumin excretion, hsCRP and BMI.

When analyses were confined to subjects with unprovoked VTE we observed a significant association with TGL and LDL in the univariate analysis. After adjustment for age and sex no significant associations between unprovoked VTE and any of the (apo-) lipoproteins or their ratios remained. When confined to subjects with provoked VTE no significant associations between any of the variables and VTE was found in both univariate and multivariable analyses.

When a sensitivity analysis was performed in which subjects on lipid-lowering drugs were not excluded from analyses, similar results were found. In the univariate analyses non-HDL, LDL, TG and TC/HDL ratio were related to VTE risk. After adjustment for age and sex no significant relationship between any of the variables and VTE was found.

When the analyses were adjusted for the enrichment of the cohort with participants with higher urinary albumin levels, using survey probability weights,[21] similar results were obtained. In the multivariable analyses no significant relationship between any of the variables and VTE was found.

Table 3 Risk of venous thromboembolism related to classical lipoproteins and their ratios

Variable	Crude Hazard Ratio (95% CI)	*P*-value	Adjusted Hazard Ratio† (95% CI)	*P*-value	Adjusted Hazard Ratio‡ (95% CI)	*P*-value
Total Cholesterol (n = 7577) §		0.045*		0.61		0.78
2nd tertile	1.18 (0.71-1.95)		0.87 (0.52-1.45)		0.83 (0.49-1.41)	
3rd tertile	1.73 (1.09-2.74)		1.09 (0.68-1.77)		0.86 (0.52-1.43)	
HDL (n = 7445)		0.09		0.24		0.82
2nd tertile	0.91 (0.59-1.40)		0.96 (0.62-1.49)		1.17 (0.72-1.88)	
3rd tertile	0.59 (0.36-0.96)		0.66 (0.39-1.11)		1.08 (0.61-1.91)	
Non-HDL (n = 7412)		0.02*		0.61		0.80
2nd tertile	1.39 (0.83-2.32)		1.00 (0.59-1.69)		0.84 (0.49-1.45)	
3rd tertile	1.97 (1.22-3.18)		1.21 (0.73-2.01)		0.86 (0.50-1.46)	
LDL (n = 7412)		0.003*		0.27		0.93
2nd tertile	1.47 (0.86-2.49)		1.07 (0.62-1.84)		0.93 (0.53-1.63)	
3rd tertile	2.28 (1.40-3.72)		1.43 (0.85-2.40)		1.02 (0.59-1.75)	
Triglycerides (n = 7446)		0.01*		0.10		0.21
2nd tertile	2.11 (1.26-3.55)		1.76 (1.04-2.97)		1.36 (0.79-2.32)	
3rd tertile	2.16 (1.29-3.63)		1.62 (0.96-2.75)		0.92 (0.52-1.63)	
Lipoprotein (a) (n = 7243)		0.72		0.83		0.94
2nd tertile	1.13 (0.69-1.85)		1.10 (0.67-1.80)		1.00 (0.60-1.65)	
3rd tertile	1.21 (0.76-1.93)		1.15 (0.72-1.84)		1.08 (0.67-1.73)	
TC/HDL (n = 7412)		0.03*		0.26		0.31
2nd tertile	1.94 (1.18-3.21)		1.53 (0.92-2.57)		1.06 (0.62-1.81)	
3rd tertile	1.82 (1.09-3.03)		1.29 (0.75-2.21)		0.74 (0.42-1.32)	

HDL = high-density lipoprotein, LDL = low-density lipoprotein, TC = total cholesterol, CI = confidence interval. *Statistical significance at 2-tailed P ≤ 0.05. §number of subjects included in univariate analysis. †Reference group are those within the first tertile. Adjustments are made for age and sex. ‡Reference group are those within the first tertile. Adjustments are made for age and sex, hypertension, diabetes, albuminuria, C-reactive protein, Body Mass index, estimated glomerular filtration rate and smoking

Discussion

The present population-based cohort study did not show a stronger association with VTE for apolipoproteins than for classical lipoproteins. Both apo- and lipoproteins showed no association with VTE, neither did their ratios. When analysed separately for subjects with unprovoked or provoked VTE similar results were found.

An association between lipid levels and VTE risk was hypothesized based on the established relationship between cardiovascular disease and VTE.[1,22–24] Possibly, the relationship between the two diseases can be explained by an overlap in risk factors, such as an abnormal lipid profile. The lipid profile is largely influenced by food intake,[25] and dietary patterns are strongly related to the risk of cardiovascular disease.[26–28] Diet patterns might also be related to VTE risk.[29] However, the present study showed that, like many cardiovascular risk factors,[5,6] an altered lipid profile does not influence VTE risk. This might explain why the relationship between healthy diets and VTE risk was not always corroborated.[28,30] As abnormal lipid levels do not seem to influence VTE risk, we can conclude that the association between cardiovascular disease and VTE is not explained by an abnormal lipid profile.

Previously, a case-control study of 49 male VTE patients and matched controls, aged < 55 years, showed that VTE risk was elevated in subjects with an elevated ApoB/ApoA1 ratio. When analysed separately low ApoA1 was associated with increased VTE risk while high ApoB level was not.[8] On the contrary, Everett *et al.* reported that high levels of both ApoA1 and ApoB100 were associated with an increased risk of unprovoked VTE in women on hormone therapy.[7] In contrast to abovementioned studies, our study was performed in a population-based setting. This might explain the differences in outcome. Everett *et al.* also did not show a relationship between apolipoproteins and VTE in women who were not on hormone therapy.[7] Our findings are in accordance with the findings of a large population based cohort study in which no association between ApoA1 and VTE risk could be demonstrated.[31]

Although not confirmed in a meta-analysis,[13] two large case-control studies suggest that apolipoproteins are better than the classical lipid biomarkers in predicting the risk for arterial cardiovascular disease.[11,12] The pathophysiology behind this finding is not fully understood, but is partly explained by the fact that apolipoproteins better reflect an individual's atherogenic potential.[32] Atherogeneity will most likely not influence VTE risk, because atherosclerosis does not take place in the venous system. Still, statins reduce the risk of VTE.[2–4] Possibly, lipid biomarkers have other characteristics that could influence venous haemostasis. Indeed, it has been demonstrated that an elevated LDL level accelerates activation of prothrombin, factor X and factor VII, while HDL enhances the protein C anticoagulant pathway and reduces thrombin generation.[33] Furthermore,

a high level of TC enhances platelet thrombus formation.[34] However, the present study did not show an association between the lipid biomarkers and VTE risk. This suggests that the prothrombotic effects of an abnormal lipid profile might be too mild to actually influence VTE risk or that these effects are counteracted by other mechanisms. The reported decreased risk of VTE due to statins[2–4] is probably not caused by lowering lipid levels itself, but more likely through other properties of this medication. This theory is supported by the finding that of all lipid-lowering medication, only statins reduce the risk of VTE.[35] Statins have several other mechanisms that could reduce VTE risk. For example it induces Kruppel-Like Factor 2 expression, which in turn promotes thrombomodulin expression on endothelial cells, thereby enhancing the activity of the protein C anticoagulant pathway.[36] Furthermore, statins diminish levels of inflammatory markers[37,38] and they reduce tissue factor expression and thrombin generation.[39]

Our study has both strengths and limitations. Strength of the study is that all important lipid biomarkers and the ratios known to be related to arterial cardiovascular disease have been examined. Including all important lipid biomarkers and ratios strengthens the finding that an altered lipid profile does not influence VTE risk. Other strengths of our study are the large population-based cohort and the accurate information on the use of lipid-lowering medicine, which made it possible to exclude these subjects in our analysis.

A limitation of our study is that incidence of VTE may have been underestimated as cases were identified retrospectively. Nonetheless, the assessor who evaluated patient charts was blinded to lipid profile and there is no reason to assume an unequal distribution of missed events between different levels of (apo-) lipoproteins. Second, data regarding anticoagulant therapy were only available for the subjects who developed VTE during follow-up. Hence, the use of anticoagulants was not included in our multivariable analyses. Third, the cohort under investigation is enriched for participants with higher levels of albuminuria. This enrichment might have reduced generalizability. However, it is unlikely to have influenced our risk estimates (i.e., HRs) on the relationship between (apo-) lipoproteins and VTE, as these estimates did not significantly change after accounting for study design. Generalizability might have also been slightly reduced due to the inclusion criteria of the PREVEND Study, in which inclusion was limited to subjects aged 28-75 years at baseline. This might explain the somewhat low mean age at time of VTE found in our study, when compared to other studies.[40,41] Fourth, lipid profile was assessed at inclusion. It is possible that lipid profile has changed during follow-up due to the natural course of lipid biomarkers or lifestyle intervention.

The present study is important in understanding the pathophysiology of VTE. Our results show no association between either apolipoproteins or the classical lipoproteins and VTE risk. Apparently, the reported association between arterial cardiovascular disease and VTE is not explained by an altered lipid profile as shared risk factor. Future studies should focus on other mechanisms that can explain the association between

arterial cardiovascular disease and VTE to give us more insight into the pathophysiology of VTE.

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Elevated albuminuria associated with increased risk of recurrent venous thromboembolism: results of a population-based cohort study

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British Journal of Haematology 2012; 156: 667-671

Abstract

This study examined the risk of recurrent venous thromboembolism (VTE) in patients with elevated albuminuria. In 1997-1998, inhabitants of Groningen, the Netherlands, aged 28-75 years (*n* = 85 421), were invited to participate in the PREVEND (Prevention of REnal and Vascular ENd stage Disease) Study, an observational, population-based cohort study. Albuminuria was measured and VTE occurrence was monitored in responding subjects (*n* = 40 856). Patients with first VTE between study entry and January 2009, identified through databases of the national registry of hospital discharge diagnoses, death certificates, regional anticoagulation clinic and medical records, were used for analysis. Of 351 subjects with first VTE, 37 subjects developed a recurrence during a median follow-up period of 3.3 (interquartile range, 1.1-6.4) years. Annual incidence of recurrence in subjects with elevated albuminuria (≥ 20 mg/l) was 5.00% [95% confidence interval (CI); 2.16-9.85], compared to 2.38% (95% CI; 1.59-3.41) in subjects with normal albuminuria (< 20 mg/l). Hazard ratio for recurrence was 1.95 (95% CI; 0.89-4.30) after adjustment for age and sex. This hazard ratio was 3.35 (95% CI; 1.18-9.47) in patients with first unprovoked, and 1.12 (95% CI; 0.25-5.01) in those with a first provoked event. This study showed that subjects with elevated albuminuria who experience an unprovoked VTE are at an increased risk of recurrence, independent of age and sex.

Introduction

Venous thromboembolism (VTE) is a major health problem in Western countries.[1] Reported incidence rates for first VTE vary between 1.4 and 1.9 per 1000 person-years.[1–3] Subjects with VTE are at high risk to develop recurrent VTE, with incidence rates reported as high as 26-95 per 1000 person-years.[4–6] The risk of recurrence is especially high in subjects with first unprovoked VTE. These subjects have an almost twofold higher risk for recurrence than subjects with first provoked VTE.[5] Prolongation of anticoagulation could reduce this risk, but must be balanced with the risk of bleeding.[7–9] To optimize duration of anticoagulant therapy it is important to determine the risk of recurrence in subgroups of patients with VTE.

One of these subgroups could include subjects with an elevated urinary albumin concentration. Recently, we identified microalbuminuria as a risk indicator for first VTE.[10] Subjects with microalbuminuria were at a 2.3-fold increased risk to develop VTE compared to subjects with albuminuria < 15 mg/24 h urine collection, whereas people with macroalbuminuria had a 3.0-fold increased risk compared to their healthy counterparts. Higher levels of albuminuria were particularly associated with unprovoked events. The increased risk of thromboembolism in patients with elevated albuminuria has been postulated to be associated with endothelial injury and/or the related changes in the levels of procoagulant proteins.[10–13]

It is well recognized that many factors associated with an increased risk for first VTE are not related to a higher risk of recurrence.[14] This seemingly paradoxical fact has recently been explained as index-event bias.[15] However, there are no data on the influence of increased urinary albumin levels on recurrence of VTE. We therefore performed a population-based cohort study to determine whether subjects with elevated albuminuria have an increased risk of recurrent VTE.

Methods

Study population

This study was performed in participants of the Prevention of REnal and Vascular ENd stage Disease (PREVEND) Study. The PREVEND Study was designed to prospectively investigate the natural course of albuminuria and its relationship to renal and cardiovascular disease in a large cohort drawn from the general population. Details of this study have been published previously[16] and can be found at http://www.prevend.org. In 1997-1998, all inhabitants of the city of Groningen, the Netherlands, aged 28-75 years (*n* = 85 421) were sent a postal questionnaire and a vial for them to collect an early morning urine

sample. Out of 40 856 responding subjects (47.8%), 597 subjects developed VTE during follow-up. After exclusion of subjects with a history of VTE (*n* = 104) and subjects who were using ongoing anticoagulant therapy from first VTE until end of study (*n* = 132), 351 subjects with first VTE were eligible to participate, and used for analysis. Anticoagulant therapy was defined as therapeutic doses of vitamin K antagonists or low molecular weight heparin.

The PREVEND study was approved by the local medical ethics committee and was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants gave written informed consent.

Measurements

Urinary albumin concentration was established by a commercial immunoturbidimetry assay with sensitivity of 2.3 mg/l and inter- and intra-assay coefficients of variation of 2.2 and 2.6%, respectively (BN II, Dade Behring Diagnostica, Marburg, Germany). First morning urine was used for analysis. Elevated albumin concentration was defined as ≥ 20 mg/l, whereas a concentration of < 20 mg/l was defined as normal albuminuria.[17]

Definition of thrombotic events

Subjects with VTE between study entry and January 1, 2009 were identified using the databases of the national registry of hospital discharge diagnoses (Prismant, Utrecht, the Netherlands) and death certificates (Central Bureau of Statistics, The Hague/Heerlen, the Netherlands). Events and duration of anticoagulation were verified with the database of the regional anticoagulation clinic, which monitors the anticoagulant therapy of all inhabitants of the city of Groningen. When available, data on subjects with VTE according to any of the abovementioned databases was confirmed by accessing patients' medical records (*n* = 332). This was carried out by an assessor blinded for albuminuria status of the subject under investigation. Deep vein thrombosis had to be confirmed by compression ultrasound, and pulmonary embolism by ventilation/perfusion lung scanning, spiral computed tomography, or at autopsy. VTE was classified as 'provoked' when it had occurred at or within 3 months after exposure to an exogenous risk factor including surgery, trauma, immobilization for more than 7 days, pregnancy, puerperium, the use of oral contraceptives or hormonal replacement therapy, or malignancy. VTE was classified as 'unprovoked' when no such risk factor was present. Recurrence was established when demonstrated by objective techniques at another anatomical site than the first event, or at the same site, if previously repeated tests showed no residual venous thrombosis, or post thrombotic symptoms were not present before the second event.

Statistical analysis

To determine whether elevated urinary albumin concentration increases the risk of recurrent VTE we estimated the absolute risk of recurrent VTE in subjects with normal albuminuria and in subjects with elevated albuminuria. The absolute risk was expressed as an annual incidence and was calculated by dividing the number of subjects with recurrent venous thrombosis by the total number of observation years. Observation time started after discontinuation of anticoagulant therapy and ended at time of recurrence, restart of long-term (> 3 months) anticoagulant treatment for another indication, end of study (January 2009), death or moving out of the city. The 95% confidence intervals (CIs) around the annual incidences were assessed with the Poisson distribution assumption. A Cox proportional hazard model was used to estimate whether increased albuminuria was a risk indicator for recurrent VTE. Adjustments were made for sex and age at time of first VTE. A test for the interaction of each variable with time showed that the proportional hazards assumption was not violated. Furthermore, there were no significant interactions between the variables included in the models, including albuminuria with type of index VTE (provoked vs unprovoked). Despite the absence of statistical heterogeneity we had, however, *a priori* planned a subgroup analysis in subjects with provoked and unprovoked VTE separately. The rationale for this analysis was the clinically relevant difference in the risk of recurrence in subjects with either a first unprovoked or a first provoked VTE.[5]

Given that the time between albumin measurement and VTE differed between subjects and often covered several years, it was possible that the risk for recurrent VTE, associated with an increased urinary albumin concentration, was attenuated. To explore this possibility we performed a sensitivity analysis in which the risk of recurrence in patients with elevated albuminuria was assessed after adjustment for time from albumin measurement to first VTE and its interaction with albumin concentration.

To assess differences in duration of anticoagulant therapy between subjects with different levels of urinary albumin concentration a Mann-Whitney U test was performed.

Categorical data are presented as counts and percentages, continuous variables as medians with interquartile ranges (IQR). Statistical significance was considered as a two-tailed P <0.05. Statistical analyses were performed using PASW version 18.0 (IBM SPSS, Chicago, IL, USA) and SAS version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

Study population

Baseline characteristics are shown in **Table 1**. Of the 351 subjects, 49% were male. Median age at first VTE was 64 (IQR, 51-73) years. First VTE was secondary to an external risk factor in 170 subjects, and was unprovoked in 151 subjects. In 30 subjects the presence

or absence of an external risk factor could not be assessed from medical records. Median follow-up time was 3.3 (IQR, 1.1-6.4) years. Thirty-seven subjects developed a recurrent venous thrombosis at a median age of 69 (IQR, 59-74) years. Thirteen percent (*n* = 45) of the subjects had an urinary albumin concentration of ≥ 20 mg/l. Median time from albumin measurement to first venous thrombosis was 5.2 (IQR, 2.6-7.9) years, for recurrence this was 6.5 (IQR, 4.9-9.4) years. Median duration of treatment with vitamin K-antagonists did not differ between subjects with normal (< 20 mg/l) or elevated levels (≥ 20 mg/l) of albuminuria (6.0 (IQR, 3.9-6.2) and 5.9 (IQR, 3.1-6.2) months, respectively. *P* = 0.62). A total of 72 subjects were censored at time of death (*n* = 54, 15%) or moving out of the city (*n* = 18, 5%).

Table 1 Baseline Characteristics

	Total cohort	UAC < 20 mg/l	UAC ≥ 20 mg/l
TOTAL	**351**	**306**	**45**
Male (n, %)	171 (49)	145 (47)	26 (58)
Age at first VTE, y	64 (51-73)	64 (51-73)	68 (56-75)
Urinary albumin concentration, mg/l	5.93 (3.61-11.30)	5.28 (3.21-8.31)	41.20 (26.50-109.00)
Anticoagulant therapy first event			
< 3 months	36 (10)	32 (11)	4 (9)
3-6 months	149 (43)	128 (42)	21 (47)
6-12 months	140 (40)	123 (40)	17 (38)
> 12 months	26 (7)	23 (8)	3 (7)

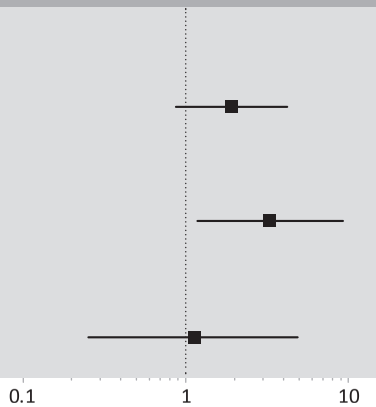
VTE = venous thromboembolism, UAC = urinary albumin concentration. Continuous variables are presented as median (IQR), categorical variables as number (%)

Risk of recurrent VTE in subjects with increased urinary albumin concentrations

Figure 1 shows the risk of recurrence in patients with elevated albuminuria. The annual incidence of recurrence in subjects with elevated albuminuria was twice that of subjects with normal albuminuria, 5.00 (95% CI; 2.16-9.85) versus 2.38 (95% CI; 1.59-3.41) per 100 person-years. After adjustment for age and sex the hazard ratio (HR) was 1.95 (95% CI; 0.89-4.30; *P* = 0.10).

Although heterogeneity of the effect of elevated albuminuria was not statistically significant, the increased risk was mainly powered by the subjects with a first unprovoked event. In this subgroup, the annual incidence was three times as high in subjects with elevated albuminuria as in subjects with normal albuminuria, 8.82 (95% CI; 3.24-19.21) versus 2.93 (95% CI; 1.67-4.75) per 100 person-years. In subjects with first unprovoked VTE, the adjusted (for age and sex) HR of recurrence in subjects with

Urinary Albumin Concentration	Observation years	No. events/ No. patients	Annual Incidence % (95% CI)	Crude Hazard Ratio (95% CI)	Crude Hazard Ratio* (95% CI)	Decreased risk for rVTE	Increased risk for rVTE	*P*-value
Overall								
< 20 mg/l	1221	29/306	2.38 (1.59-3.41)	*Reference*	*Reference*			
≥ 20 mg/l	160	8/45	5.00 (2.16-9.85)	2.10 (0.96-4.60)	1.95 (0.89-4.03)			0.10
Unprovoked								
< 20 mg/l	547	16/133	2.93 (1.67-4.75)	*Reference*	*Reference*			
≥ 20 mg/l	68	6/18	8.82 (3.24-19.21)	3.06 (1.19-7.83)	3.35 (1.18-9.47)			0.02
Provoked								
< 20 mg/l	516	12/145	2.33 (1.20-4.06)	*Reference*	*Reference*			
≥ 20 mg/l	81	2/25	2.47 (0.30-8.92)	1.09 (0.24-4.86)	1.12 (0.25-5.01)			0.89



rVTE = recurrent venous thromboembolism, CI = confidence interval
*Adjusted for age and sex

Figure 1 Risk of recurrent VTE in patients with elevated albuminuria

elevated albuminuria was 3.35 (95% CI; 1.18-9.47), as compared to subjects with normal urinary albumin levels (*P* = 0.02). In contrast, this adjusted HR was 1.12 (95% CI; 0.25-5.01) in subjects with first provoked VTE (*P* = 0.89).

A sensitivity analysis was performed to investigate whether the time lag between albumin measurement and VTE could have caused attenuation of the risk for recurrent VTE. This analysis showed no significant interaction between time from albumin measurement to first VTE and albumin level for risk of recurrence (*P* = 0.31).

Discussion

This population-based cohort study is the first to investigate the association between elevated levels of urinary albumin and risk of recurrent VTE. We observed an almost twofold increased risk of recurrent VTE in subjects with elevated albuminuria. Our planned subgroup analysis indicated a difference in this risk between subjects with first unprovoked and first provoked VTE. A 3.4-fold increased risk of recurrence was observed in subjects with elevated albuminuria who had an unprovoked first VTE. The annual incidence of recurrence in these subjects was as high as 8.8%. No relationship between albumin level and recurrence rate was found in subjects in whom first event was provoked.

The present study was based on our previous study in which we showed a significantly higher risk of first VTE in patients with micro- and macroalbuminuria.[10] Like in the present study, this relationship was mainly seen in patients with an unprovoked event. As the prevalence of recurrence in a population-based cohort is much lower than the prevalence of first VTE, we used a slightly different study design to assess the risk on recurrence to capture more events. In our previous study a subset of the 40 856 responders was used, consisting of 8574 subjects who were phenotyped in great detail.[10] In the present study all the 40 856 responders were observed for development of first VTE between study entry and January 2009. Those who developed first VTE were included in this study. Instead of 24-h urine measurement, spot urine measurements were available for these subjects, with corresponding cut-off values for clinical classification (elevated albuminuria defined as albuminuria > 30 mg/24h or > 20 mg/l, respectively).[16] Our previous study showed that this difference in albuminuria assessment did not materially affect outcome regarding the association between albuminuria and VTE.[10] Furthermore, 24-h urine measurement and spot urine measurement showed good correlation (r = 0.77).

Our study has both strengths and limitations. Its strengths are the large population-based cohort and the prospective way in which the subjects were followed. Furthermore, we have detailed information on the presence of risk factors for first VTE in the large

majority of subjects. This is important, as the unprovoked nature of the first event is the most important risk determinant for recurrence.[5]

A limitation of our study is that albumin measurements did not take place at the moment of VTE. It is possible that albumin status changed during this time. However, if this kind of misclassification had occurred, it has biased us towards the null. Also, our sensitivity analysis showed no interaction effect between albumin level and time from measurement to first VTE on the risk of recurrence. This suggests that time of albumin measurement has no major influence on the predictive value of albumin level for recurrent VTE. Similar long-term prognostic value of urinary albumin excretion has been observed for arterial cardiovascular outcome.[18] A second limitation is that the incidence of VTE in our cohort may be underestimated as VTE cases were identified retrospectively. However, the assessor who investigated patient charts was blinded to albuminuria status and there is no reason to assume an unequal distribution of missed events between the different subgroups of albumin level. Lastly, we only adjusted for age and sex as independent risk determinants for recurrence. We could not correct for other determinants (e.g. D-dimer level at the end of anticoagulant treatment, deficiencies of the natural anticoagulants antithrombin, protein C, protein S). However, these factors have no known relation with albuminuria, and are therefore not likely to be a source of confounding.

The present study identified elevated albuminuria as a risk indicator not only for first VTE but also for recurrence. Given that elevated albuminuria can distinguish between subjects with low or high risk for recurrence in patients with first unprovoked VTE, we can identify a subgroup of patients that might benefit from long-term treatment with anticoagulant therapy. It should be noted though that, due to small numbers, CIs were large. Large studies are needed to give a more precise estimation of the magnitude of the increased risk of recurrence after unprovoked VTE.

In conclusion, our population-based cohort study showed an increased risk of recurrent VTE in subjects with elevated albuminuria who developed a first unprovoked VTE. It remains to be examined whether this should influence treatment decisions.

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6

Associations between high factor VIII and low free protein S levels with traditional arterial thrombotic risk factors and their risk on arterial thrombosis: Results from a retrospective family cohort study

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Based on: Thrombosis Research 2010; 126(4): e249-54

Abstract

Introduction Whether high factor VIII and low free protein S levels are risk factors for arterial thrombosis is unclarified.

Material and Methods In a post-hoc analysis of a single-centre retrospective family cohort, we determined if these two proteins could increase the risk of arterial thrombosis. In total, 1399 relatives were analyzed.

Results Annual incidence in relatives with high factor VIII levels was 0.29% (95% CI, 0.22-0.38) compared to 0.13% (95% CI, 0.09-0.19) in relatives with normal factor VIII levels. In relatives with low free protein S levels, this risk was 0.26% (95% CI, 0.16-0.40), compared to 0.14% (95% CI, 0.10-0.20) in relatives with normal free protein S levels. Mean factor VIII levels adjusted for age and sex were 11 IU/dl, 18 IU/dl, and 21 IU/dl higher in relatives with hypertension, diabetes mellitus, and obesity as compared to relatives without these arterial thrombotic risk factors. Moreover, a dose response relation between increasing factor VIII and body mass index was found. None of these associations were shown for free
protein S.

Conclusions High factor VIII and low free protein S levels seemed to be mild risk factors for arterial thrombosis. High factor VIII levels were particularly observed in relatives with traditional arterial thrombotic risk factors. Free protein S levels were not influenced by these thrombotic risk factors. This assumes that low free protein S levels were genetically determined.

Introduction

Arterial thrombosis in subjects with arterial thrombotic risk factors is probably mediated by the presence of a prothrombotic and/or inflammatory state.[1-5] Factor VIII and free protein S levels are both part of the clotting cascade, but have been reported to be associated with an inflammatory state when levels are high (for factor VIII),[6] or low (for free protein S).[7] Both high factor VIII and low free protein S levels, however, are also partially genetically determined.[8,9] A high level of factor VIII is a well known risk factor for venous thrombosis,[10,11] and possibly for arterial thrombosis as well.[11,12] Whether low free protein S levels are a risk factor for arterial thrombosis is uncertain.[13] Most information on low free protein S levels to the risk of arterial thrombosis comes from case reports or small case series.[14-16]

We hypothesize that both high factor VIII levels and low free protein S levels increase the risk of arterial thrombosis either through a genetic or acquired link. Presence of a genetic association is assumed when protein levels are not influenced by (acquired) traditional arterial thrombotic risk factors. To test this hypothesis, we performed a retrospective study in a large series of families with thrombophilic defects to assess the risk of arterial thrombosis for different high factor VIII levels and low free protein S levels.

Materials and Methods

Data retrieval

This is a post-hoc analysis of pooled data from individual subjects of four large retrospective family cohort studies with various thrombophilic index defects from which outcomes have recently been published.[17,18] These studies were performed by three university medical centers (Groningen, Amsterdam and Maastricht). As no central lab was involved, we choose to include only the data obtained from subjects in our centre (Groningen) to exclude interlaboratory variability in the present study. All studies were performed at the same time and laboratory tests had not changed over time. The first study comprised first-degree relatives (i.e., offspring, siblings, and/or parents) of consecutive patients (probands) with documented venous thrombosis and established hereditary deficiencies of either antithrombin, protein C, or protein S. As the number of antithrombin deficient probands was small, second-degree relatives (i.e., grandparents and/or blood related uncles or aunts) with a deficient parent were also identified. They were enrolled between April 1999 and July 2004. The other three studies comprised first-degree relatives of consecutive patients with venous thrombosis or premature

atherosclerosis (< 50 years of age) and the presence of either the prothrombin G20210A mutation, high levels of factor VIII at repeated measurements, or hyperhomocysteinemia. Enrollment started in May 1998 and was completed in July 2004. Approval was obtained from the institutional review board of University Medical Centre Groningen.

Subjects

All relatives, identified by pedigree analysis, were 15 years of age or older and were contacted through the probands. Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Physicians at our thrombosis outpatient clinic collected detailed information on previous episodes of arterial thrombosis, risk factors for atherosclerosis, and anticoagulant treatment by using a standardized questionnaire (similar for all four studies) and reviewing medical records. Clinical data were collected before laboratory testing. Relatives were tested for deficiencies of antithrombin, protein C and protein S, factor V Leiden, prothrombin G20210A, and high levels of factor VIII. In addition, levels of free protein S were measured in most relatives. Due to shortage of stored plasma, free protein S levels could not be measured for all relatives. Factor VIII:C levels were measured by one-stage clotting assays (Behring, Marburg, Germany) and were considered 'high' at levels above 150 IU/dl to enable a comparison of results between this study and previous studies of ours.[11] Free protein S antigen levels were measured after precipitation of protein S complexed with C4b-binding protein with polyethylene glycol.[19] Normal ranges protein S were determined in 393 healthy blood donors, who had no (family) history of venous or arterial thrombosis and were neither pregnant, nor had used oral contraceptives for at least 3 months.[20] A free protein S level below 65 IU/dL was considered 'low', corresponding with the lower limit of the normal range in healthy volunteers. The CV of the free protein S assay was < 5%. Other laboratory tests, definitions of abnormal results, and criteria for inheritance of natural anticoagulant deficiencies have been described in detail elsewhere.[17,18] To avoid bias, all probands were excluded from the analyses. In addition, relatives with protein S deficiency type I were excluded from analysis when analyzing effects of free protein S. This was done as protein S deficiency is classified into protein S deficiency type I (recognized by decreased levels of both total and free protein S antigen levels) and type III (recognized by decreased free protein S and normal total protein S antigen levels).[21] Protein S deficiency type II, a functional protein S deficiency with reduced APC cofactor activity but normal total and free PS antigen levels, could not be determined due to the absence of a functional protein S assay in our hospital. Results of hereditary protein S type I deficiency on the risk of arterial thrombosis in this cohort have already been published and were therefore not primarily studied in the present study.[22] For similar reasons, we did not primarily study the effect of hereditary antithrombin or protein C deficiency,

and prothrombin G20210A on the risk of arterial thrombosis.[22,23] The number of factor V Leiden carriers in this cohort was too small to provide accurate relative risk estimates. Other studies and meta-analyses already showed that the increase in risk for arterial thrombosis in factor V Leiden carriers is negligible.[24] Although previous study questions of ours included whether low free protein S and high factor VIIII levels influence the risk of arterial thrombosis in thrombophilic families,[11,13] these studies did not provide answers to our current hypothesis, that is, whether high factor VIII or low free protein S levels are risk factors for arterial thrombosis through an acquired or genetic link.

Definitions

Coronary and peripheral arterial disease had to be symptomatic and angiographically proven, whereas myocardial infarction was diagnosed according to clinical, enzymatic and electrocardiographic criteria. Ischemic stroke was defined as the onset of rapidly developing symptoms and signs of loss of cerebral function, which lasted at least 24 hours and had an apparent vascular cause, as demonstrated by CT or magnetic resonance imaging. If a cerebral event completely resolved within 24 hours without cerebral lesions at scanning, it was classified as transient ischemic attack (TIA). Known risk factors for arterial thrombosis were recorded and included: hypertension, hyperlipidemia, the presence of diabetes mellitus, smoking habits or obesity defined as body mass index (BMI) > 30 kg/m^2.

Statistical analysis

We analyzed the absolute risk of first arterial thrombosis in relatives, comparing those who did or did not have high factor VIII levels or low free protein S levels, respectively. We performed a sensitivity analysis where myocardial infarction and ischemic stroke were analyzed separately.

As both factor VIII and free protein S levels are continuous variables, a dichotomous breakdown in the analysis may seem artificial. Therefore, we also analyzed factor VIII and free protein S as continuous variables. A further stratification into quartiles, to investigate whether there was a dose-response effect, was not feasible due to small numbers. Observation time was defined as the period from the age of 15 years until the first arterial thrombotic episode or until the end of study. The 95% confidence intervals (95% CIs) around the incidence rates were calculated under the Poisson distribution assumption. Relative risks were adjusted for age and sex with Cox regression. Our study cohort consisted of thrombophilic families and subjects were therefore prone to have multiple thrombophilic defects.[24,25] To provide as homogenous risk estimates as possible we therefore adjusted for antithrombin, protein C or protein S type I deficiency, factor V

Leiden and prothrombin G20210A with stepwise Cox regression. To account for the non-randomness of the relatives analyzed, relative risks were also adjusted for clustering of events within families by using random-effects Cox regression and the robust sandwich method in Stata.

Linear regression was used to determine the relationship between factor VIII levels and free protein S levels, respectively, combined with traditional arterial thrombotic risk factors. Adjustments were made for age and sex.

A cumulative distribution curve is a graphical representation in which two continuous variables can be compared directly. They were constructed to visualize a possible relationship between factor VIII and BMI, and free protein S and BMI and the occurrence of arterial thrombosis.

Continuous variables were expressed as mean values and standard deviations, categorical data as counts and percentages. Differences between groups were evaluated by the Student's *t*-test and by Fisher exact test for categorical variables. A 2-tailed *P*-value of less than 0.05 indicated statistical significance. The statistical software used was SPSS version 16.0 (SPSS, Chicago, Illinois, United States) and Stata version 10.1 (Stata Corp., College Station, Texas).

Results

Our study cohort comprised 2451 relatives aged 15 years or older, of 380 probands **(Figure 1)**. Of relatives, 518 (21%) had died before the start of the study. Another 465 relatives did not participate because of various reasons, including refusal, inability to give informed consent, or residence outside The Netherlands (exclusion rate 24%). Of 1468 relatives tested for thrombophilia, 1399 were analyzed on factor VIII (missing laboratory data, $n = 69$) and 1143 were analyzed on free protein S (94 relatives excluded with protein S type I deficiency and 231 relatives with missing laboratory data). Forty-six percent were male **(Table 1)**. Mean age at enrollment was 45 years. Mean observation period was 30 years. Arterial thrombotic events were documented in 86 relatives (6%) at a mean age of 57 years. In relatives with high factor VIII levels mean age of occurrence of arterial thrombosis was 60 years. Mean age of arterial thrombosis in relatives with low free protein S levels was 55 years. Mean factor VIII level was 146 IU/dl and mean free protein S level was 80 IU/dl.

Annual incidence of arterial thrombosis in relatives with high factor VIII levels was 0.29% (95% CI, 0.22-0.38) compared to 0.13% (95% CI, 0.09-0.19) in relatives with normal factor VIII levels [crude relative risk, 2.2 (95% CI, 1.4-3.4)] **(Table 2)**. When end-point myocardial infarction was chosen, this crude relative risk was 3.6 (95% CI, 1.6-8.0). For ischemic stroke, this crude relative risk was 2.0 (95% CI, 0.8-4.8).

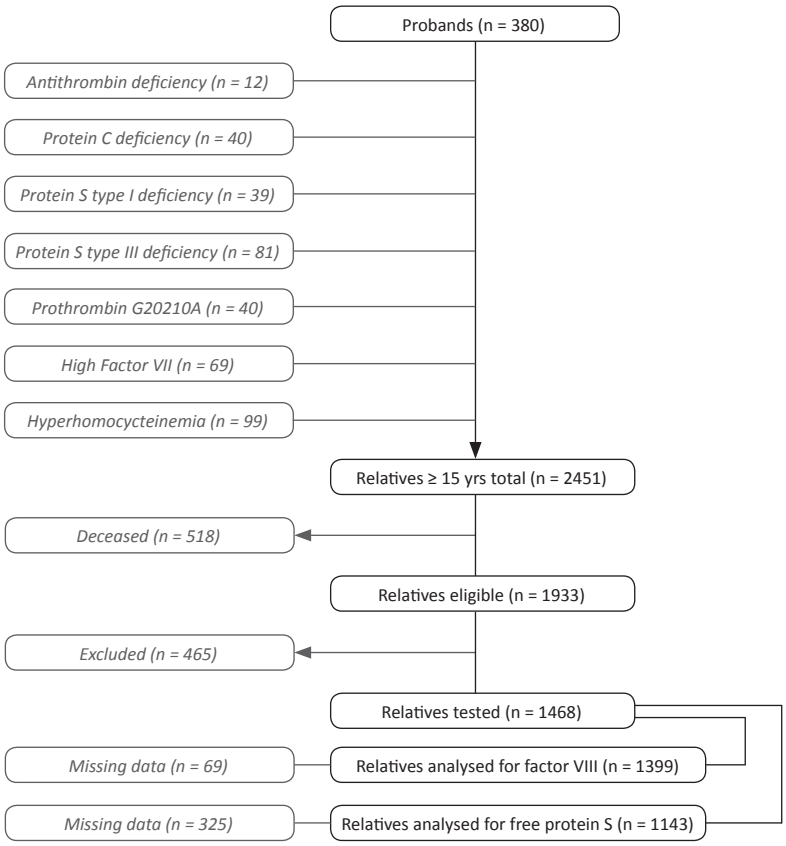

Figure 1 Flow diagram of the family cohort

Adjusted for age, sex and clustering of events within families, relative risk of arterial thrombosis in relatives with high factor VIII levels as compared to relatives with normal factor VIII levels was 1.5 (95% CI, 0.9-2.5). Age was the main determinant in the model that influenced this adjusted risk. After traditional arterial thrombotic risk factors were added in this model, relative risk was 1.4 (95% CI, 0.8-2.3). In relatives with low free protein S levels, annual incidence of arterial thrombosis was 0.26% (95% CI, 0.16-0.40), compared to 0.14% (95% CI, 0.10-0.20) in relatives with normal free protein S levels, crude relative risk 1.8 (95% CI, 1.1-3.1). When end-point myocardial infarction was chosen, this crude relative risk was 1.7 (95% CI, 0.7-4.2). For ischemic stroke, this crude relative risk was 2.1 (95% CI, 0.8-5.7). Adjusted for age, sex and clustering of events within families, relative risk of arterial thrombosis in relatives with low free protein S levels as compared to relatives with normal free protein S levels was 1.7 (95% CI, 1.1-3.1). After traditional arterial thrombotic risk factors were added in this model, relative risk was 1.7 (95% CI, 1.0-2.9). As oral contraceptives may increase factor VIII levels and decrease free protein S levels,[26] we performed a sensitivity analysis excluding

Table 1 Characteristics of 1399 relatives of probands with a thrombophilic defect

Male, n (%)	648	(46)
Age at enrollment, mean (SD)	45	(17)
Long-term vitamin K antagonists, n (%)	38	(3)
Oral contraceptive use, n (% women)	210	(28)
Arterial thrombosis, n (%)	86	(6)
Age at onset arterial thrombosis, mean (SD)	57	(13)
Classification		
Myocardial infarction, n (%)	32	(37)
Ischemic stroke, n (%)	21	(24)
Transient ischemic attack, n (%)	17	(20)
Peripheral arterial thrombotic event, n (%)	16	(19)
Thrombophilia		
Factor VIII, mean (SD)	146	(53)
Factor VIII > 150 IU/dl, n (%)	547	(39)
Free protein S*, mean (SD)	80	(20)
Free protein S < 65 IU/dl*, n (%)	259	(23)
Arterial thrombotic risk factors		
Hypertension, n (%)	236	(17)
Hyperlipidemia, n (%)	162	(12)
Diabetes mellitus, n (%)	58	(4)
Previous smokers, n (%)	295	(21)
Obesity (body mass index > 30 kg/m^2), n (%)	185	(13)

* 94 relatives with protein S type I deficiency excluded, total tested relatives 1143

all women who used oral contraceptives at time of enrollment. This did not change outcomes. Adjustments for various thrombophilic defects by stepwise Cox regression did also not change outcomes. When factor VIII and free protein S were analyzed as continuous variables in a Cox proportional-hazards model, the adjusted (for age, sex and clustering of events within families) relative risk of arterial thrombosis was 1.003 (95% CI, 0.999-1.007) for each increase of 1 IU/dl in the level of factor VIII and 0.989 (95% CI, 0.978-0.998) for each increase of 1 IU/dl in the level of free protein S.

Cumulative distribution functions of free protein S levels and increasing factor VIII levels were overlying **(Figure 2)**. Hence, no relationship between decreasing free protein S levels and increasing factor VIII levels was observed.

Table 3 shows mean factor VIII levels in relatives with hypertension, hyperlipidemia,

Table 2 Risk of arterial thrombosis in relatives with high factor VIII levels or low free protein S levels

	Observation years (relatives)	Relatives with event	Annual Incidence, % (95% CI)	Crude Relative Risk (95% CI)	Adjusted Relative Risk (95% CI)*	Adjusted Relative Risk (95% CI)†	Adjusted Relative Risk (95% CI)‡	*Adjusted Relative Risk (95% CI), adjusted for age*				
								Antithrombin deficiency	Protein C deficiency	Protein S type I deficiency	Factor V Leiden	Prothrombin G20210A
Factor VIII < 150 IU/dl	22465 (n = 852)	30	0.13 (0.09-0.19)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Factor VIII > 150 IU/dl	19388 (n = 547)	56	0.29 (0.22-0.38)	2.2 (1.4-3.4)	1.5 (0.9-2.8)	1.6 (0.9-2.8)	1.4 (0.8-2.3)	1.5 (0.9-2.4)	1.7 (1.0-3.0)	1.8 (1.0-3.0)	1.7 (1.0-3.0)	1.5 (0.9-2.4)
Free protein S > 65 IU/dl	26405 (n = 884)	38	0.14 (0.10-0.20)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Free protein S < 65 IU/dl	7968 (n = 259)	21	0.26 (0.16-0.40)	1.8 (1.1-3.1)	1.7 (1.1-3.1)	1.8 (1.0-3.2)	1.7 (1.0-2.9)	1.7 (1.0-3.0)	1.7 (1.0-3.0)	NA	1.7 (1.0-3.0)	1.7 (1.0-3.0)

CI = confidence interval. *Relative risk adjusted for age, sex and clustering of events within families. †Exluding estrogen users (n = 210), adjusted for age and sex and clustering of events within families. ‡Relative risk adjusted for age, sex and traditional arterial thrombotic risk factors. NA denotes not applicable; protein S type I deficient relatives were excluded when analyzing risk of arterial thrombosis for decreased free protein S levels

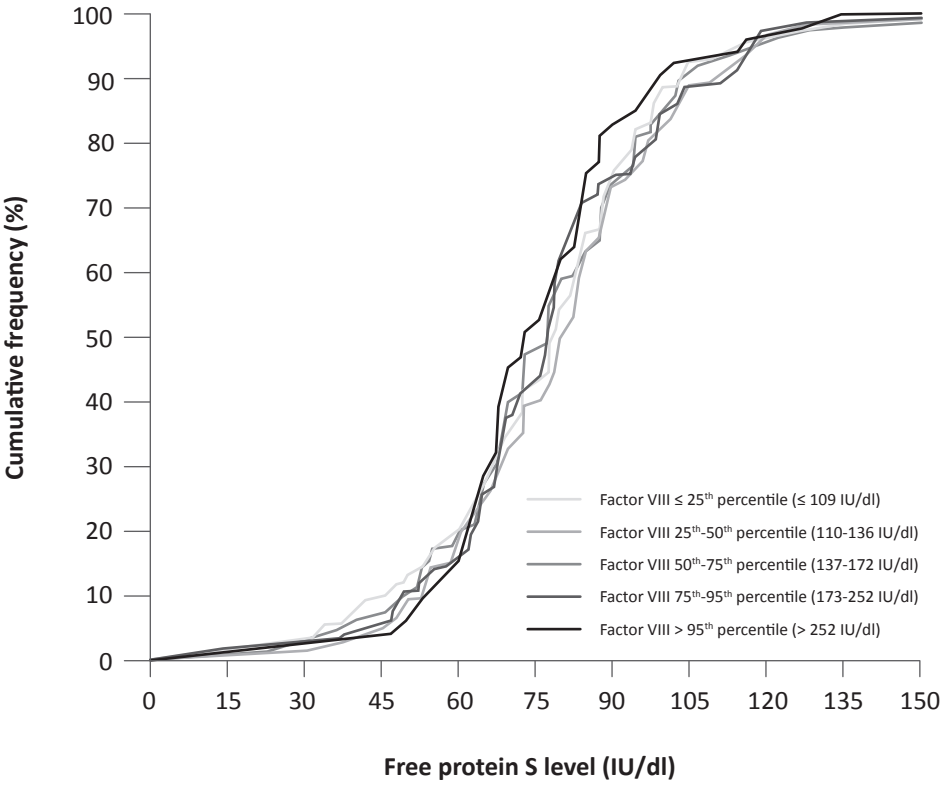

Figure 2 Cumulative distribution function of free protein S levels in relatives of probands with a thrombophilic defect

diabetes mellitus, obesity or previous smokers. Relatives with one of these traditional arterial thrombotic risk factors had mean factor VIII levels that were 24 IU/dl, 17 IU/dl, 31 IU/dl, 10 IU/dl and 26 IU/dl higher compared to relatives without exposure to these risk factors. After adjustment of age and sex, these levels were 11 IU/dl, 5 IU/dl, 18 IU/dl, 1 IU/dl and 21 IU/dl higher and still statistically significant for hypertension, diabetes mellitus, and obesity. Mean free protein S levels were similar in relatives who were exposed and not exposed to traditional arterial thrombotic risk factors, possibly except for relatives with obesity, as obese relatives appeared to have higher free protein S levels than non-obese relatives. Because we had continuous data on BMI, we could make cumulative distribution functions of factor VIII levels and free protein S levels on BMI. As shown in **Figure 3A**, an increase in factor VIII was associated with higher BMI. No such relationship was observed for free protein S levels **(Figure 3B)** suggesting that the earlier found increase of free protein S levels in obese versus non-obese relatives is a result of chance and not a real association.

Relative risk, adjusted for age, sex and clustering of events within families, for arterial thrombosis in relatives with hypertension was 1.8 (95% CI, 1.2-2.9) compared

Table 3. Association of factor VIII and free protein S levels on arterial thrombotic risk factors*

	Mean factor VIII IU/dl	Mean difference (95% CI)	Mean difference* (95% CI)	*P*-value*	Mean free protein S IU/dl	Mean difference (95% CI)	Mean difference* (95% CI)	*P*-value*
Hypertension yes	165	24	11		80	0	2	
Hypertension no	141	(16 to 31)	(3 to 19)	0.005	79	(-3 to 4)	(-1 to 6)	0.22
Hyperlipidemia yes	161	17	5		81	2	1	
Hyperlipidemia no	144	(8 to 26)	(-4 to 14)	0.25	79	(-6 to 2)	(-6 to 3)	0.50
Diabetes mellitus yes	175	31	18		81	2	3	
Diabetes mellitus no	144	(17 to 45)	(5 to 32)	0.009	79	(-5 to 9)	(-3 to 10)	0.32
Previous smokers	154	10	1		78	-1	-2	
Never smokers	144	(3 to 17)	(-6 to 10)	0.68	79	(-4 to 2)	(-5 to 2)	0.32
Obesity (BMI > 30 kg/m^2)	168	26	21		82	4	5	
No obesity (BMI < 30 kg/m^2)	142	(8 to 35)	(13 to 29)	< 0.001	78	(0 to 7)	(1 to 9)	0.01

CI = confidence interval, BMI = body mass index
* Adjusted for age and sex

to relatives with normotension. This risk remained unchanged after further adjustment for factor VIII. Relative risk for arterial thrombosis in relatives with hyperlipidemia, diabetes mellitus, previous smokers and obese relatives were 2.8 (95% CI, 1.8-4.4), 1.2 (95% CI, 0.6-2.5), 1.5 (95% CI, 1.0-2.2) and 1.5 (95% CI, 0.8-2.9) adjusted for age, sex and clustering of events within families and compared to relatives without the exposure. Additional adjustment for factor VIII did also not change these outcomes.

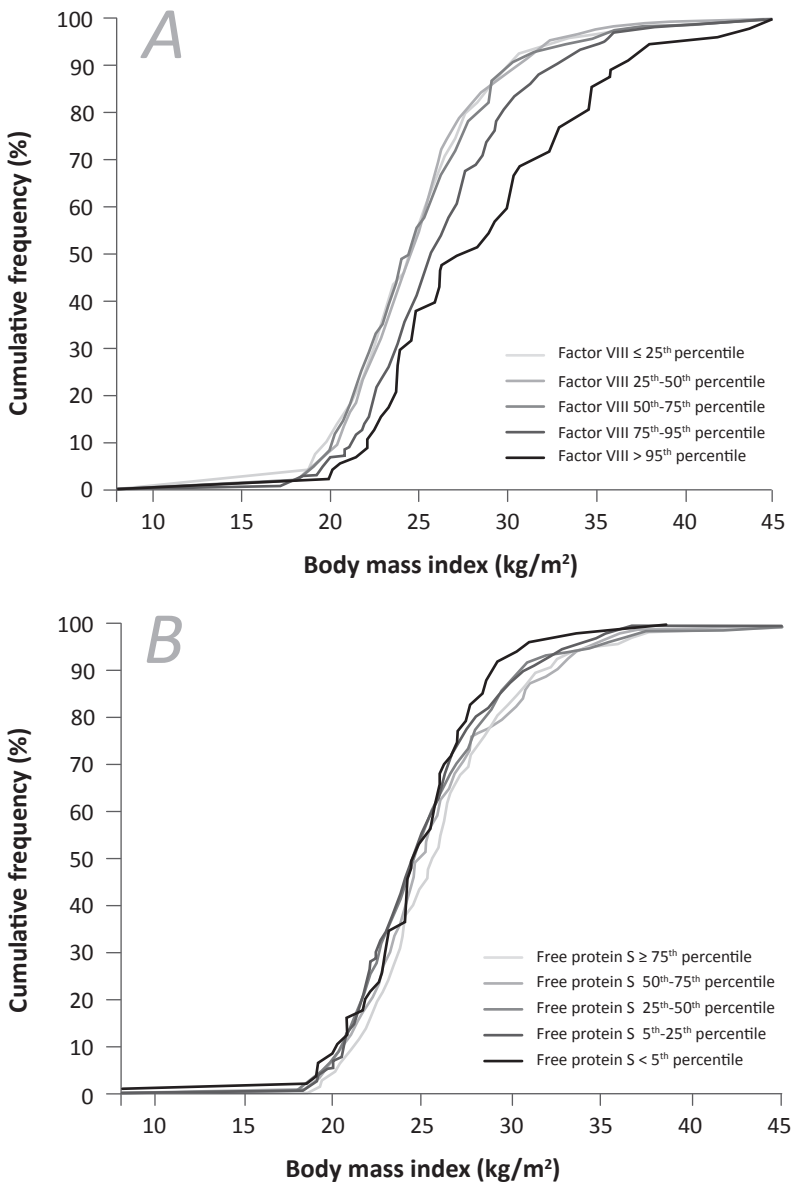

Figure 3 Cumulative distribution function of factor VIII levels (A) and free protein S levels (B) in relaives of probands with a thrombophilic defect according to body mass index

Discussion

This retrospective family study showed an approximately two-fold increased risk of arterial thrombosis in relatives with high factor VIII levels or low free protein S levels. Age had a strong effect on this risk in relatives with high factor VIII levels as, after adjustment, the risk dropped to 1.5. The risk of arterial thrombosis in relatives with low free protein S remained, however, unchanged after adjustment for age. Furthermore, factor VIII levels were higher in relatives with traditional arterial thrombotic risk factors, whereas no such association was observed for free protein S levels. Somewhat surprisingly, we could not demonstrate that higher factor VIII levels were associated with lower free protein S levels. This correlation could be expected as both thrombophilic abnormalities are associated with chronic inflammation[7,27] and chronic inflammation is considered to be a risk factor for arterial thrombosis.[3,4,27] On the other hand, that this association was not shown could be a consequence of the family design of our study. Although genotype-phenotype associations in relatives with low free protein S levels were not determined in this study, it is likely that a genetic factor is involved as free protein S levels and arterial thrombotic risk were not influenced by age, and no association between free protein S levels with traditional arterial risk factors was shown. In addition, mean free protein S levels in this study (80 IU/dl) showed a left shift compared with the normal population (mean 100 IU/dl) which is likely a result of including thrombophilic families.[17] Accordingly, one might expect the presence of low free protein S levels in relatives of patients with arterial thrombosis who have a family history of venous thrombosis or premature atherosclerosis. However, it cannot be concluded from this study whether testing for free protein S is useful for primary or secondary prevention of arterial thrombosis.

Several methodological aspects of our study warrant comment. First, because the study had a retrospective design, where traditional arterial thrombotic risk factors were self-reported and/or derived from medical records, it is possible that misclassification occurred. This might have led to slightly lower risks and differences conferred by traditional arterial thrombotic risk factors, but was probably reduced by using a standardized questionnaire. Second, height and weight were self-reported. As in general, subjects with underweight tend to overreport their body weight, while subjects with overweight tend to underreport their body weight,[28] actual risks and differences could be somewhat higher than reported if this phenomenon occurred. Third, referral bias may have been introduced by the university hospital setting. However, this was probably reduced by testing consecutive patients with thrombosis. Fourth, absolute risk estimates for arterial thrombosis were low in our study cohort. This clearly is a result of enrolling young relatives in the study as mean age at enrollment was 45 years in our cohort. Although generalizibility of our results for this reason is hampered (but also

because of the family cohort design) a family study of young participants is probably ideal to determine whether genetic variants are involved for arterial thrombotic disease occurrence as increasing age is strongly associated with an increased risk of arterial thrombosis.[25] Fifth, although we used a large study cohort and long follow-up period, we only observed a relative small number of arterial events (total *n* = 86) that resulted in relatively wide confidence intervals. Hence, our study results should be interpreted with caution.

Finally, in this retrospective study, factor VIII levels were influenced by age, diabetes mellitus, obesity and hypertension. Causal inference of high factor VIII levels on arterial thrombotic risk can therefore not be inferred. Nevertheless, it might be interesting for future studies to determine why factor VIII levels increase with age, hypertension, diabetes mellitus and obesity. Shear stress (for hypertension) or endothelial damage (for increasing age) might explain these findings, but these hypotheses have, as far as we know, not been studied yet in humans. Furthermore, ABO blood group plays a significant role on factor VIII levels. This topic could not be covered in our study, as blood group was not measured.

Although free protein S levels were stable over time, and were not influenced by traditional arterial thrombotic risk factors, which assumes that low free protein S levels are genetically determined, we cannot exclude the possibility that this is based on residual confounding. Whether low free protein S levels are genetically determined can only be inferred with certainty from genotype-phenotype studies. The present finding that low free protein S levels are associated with arterial thrombosis, and our similar finding in a previous study, but then on venous thrombotic risk, which was independent of traditional venous thrombotic risk factors,[17] could provide rationale for future studies to perform such a genotype-phenotype study.

We did not use a normal range of free protein S that was stratified on sex and age, although it is known that this is lower in premenopausal women than in men.[20] However, adjustment for sex and age did not change our outcomes. Furthermore, oral contraceptive use and hormonal replacement therapy decrease free protein S levels[26] and are known risk factors for venous thrombosis as well.[29] Therefore, we performed a sensitivity analysis excluding all women who used oral contraceptives at time of enrollment. This did not change outcomes.

In conclusion, both high factor VIII levels and low free protein S levels seemed to be mild risk factors for arterial thrombosis in thrombophilic families. High factor VIII levels were particularly observed in relatives with traditional arterial thrombotic risk factors. Hence, it is questionable whether a high factor VIII level itself increases risk of arterial thrombosis, or if this risk is explained by other, factor VIII associated arterial thrombotic risk factors, such as increasing age or hypertension. Free protein S levels were not influenced by traditional arterial thrombotic risk factors, which assumes that low free

protein S levels were genetically determined. Larger studies on this issue are required.

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7 Summary

Summary

Chapter 1

VTE and ATE are generally seen as two different diseases. In the past decade, however, this dichotomy has been questioned. **Chapter 1** described the latest findings on this topic and presented the outline of this thesis.

Chapter 2

In **chapter 2** the postulated relationship between ATE and VTE was examined. In a population-based cohort of 40 856 subjects, subjects with previous VTE were at increased risk to develop ATE. The annual incidence of ATE after VTE was 2.03%, compared to 0.87% in subjects without VTE. After adjustment for age, sex, cardiovascular risk factors and history of ATE, the relative risk was still 1.4-fold increased. The risk was highest during the first year after VTE, in which 3% of the subjects developed ATE. This incidence was two times higher than the incidence in subjects without prior VTE. Subjects who developed VTE in the absence of exogenous risk factors (unprovoked VTE) had the highest risk for ATE. The annual incidence of ATE was 2.53% in these subjects, which was 1.6 times higher than in subjects without prior VTE. Apparently, VTE is an important risk marker for subsequent ATE.

Chapter 3

Several risk factors for ATE have been investigated regarding their relationship with VTE, but only obesity has consistently been shown to be an independent risk factor for VTE. Little is known about what mediates the relationship between obesity and VTE. Obesity is associated with a combination of risk factors that influence the risk of ATE, such as hypertension, dyslipidemia, and systemic inflammation. Insulin resistance has been suggested as central factor underlying this multifaceted syndrome. Possibly, insulin resistance also influences the venous system. In **chapter 3** the relationship between insulin resistance and VTE risk was described. In a population-based cohort of 7393 subjects, higher levels of insulin resistance were indeed associated with a higher risk of VTE. This association remained significant after adjustment for age, sex, traditional cardiovascular risk factors, C-reactive protein, albuminuria, and plasminogen activator inhibitor-1. However, after additional adjustment for body mass index, insulin resistance was no longer associated with VTE risk, whereas body mass index itself appeared to be a strong risk predictor for VTE. Our results suggest that insulin resistance is not essential for the development of VTE in overweight or obese subjects.

Chapter 4

An altered lipid profile is a well-known risk factor for ATE. Studies on the association between lipid profile and venous thromboembolism (VTE) are inconsistent. This could be caused by classical lipoproteins being inferior to apolipoproteins as markers for VTE risk. In **chapter 4** we examined whether apolipoproteins are more strongly related to VTE than the classical lipoproteins. In a cohort of 7627 subjects, both univariate and multivariable analyses showed no significant associations between apolipoproteins and VTE. Several classical lipoproteins were significantly associated with VTE in univariate analyses. However, for all lipid biomarkers, significance was lost in multivariable analyses. When separate analyses were performed for subjects with provoked or unprovoked VTE, multivariable analyses similarly did not reveal any significant associations. We conclude that neither apolipoproteins nor classical lipoproteins predict VTE risk.

Chapter 5

In **chapter 5** the risk of recurrent venous thromboembolism (VTE) in patients with elevated albuminuria was investigated. Elevated albuminuria is a well-known risk factor for ATE. Recently our research group also observed an increased risk for VTE in subjects with elevated levels of albuminuria. Whether urinary albumin level also influences the risk of recurrent VTE has not been previously investigated. Out of the baseline population-based PREVEND cohort of 40 856 subjects, 351 developed first VTE in the study period and were included in the analysis. The annual incidence of recurrence in subjects with elevated albuminuria (≥ 20 mg/l) was 5.00%, compared to 2.38% in subjects with normal albuminuria (< 20 mg/l). After adjustment for age and sex, subjects with elevated albuminuria were at an almost two-fold increased risk to develop recurrence when compared to subjects with normal albuminuria. When subdivided into subjects with first provoked or unprovoked VTE, it appears that this increased risk was mainly explained by an increased risk in subjects with unprovoked VTE. Subjects with an unprovoked first VTE and elevated albuminuria were at a 3.4-fold increased risk to develop recurrence when compared to subjects with normal albuminuria. In subjects with first provoked VTE, urinary albumin level did not influence the risk of recurrence. In conclusion, urinary albumin level can distinguish between high or low risk for recurrence in subjects with first unprovoked VTE.

Chapter 6

A high factor VIII level and a low free protein S level are associated with an increased risk for VTE. It is not clear whether these variables also influence the risk of ATE. This issue was addressed in **chapter 6**. In a family cohort of 1468 subjects we observed that factor VIII

and free protein S levels were mildly associated with ATE risk. The annual incidence in relatives with elevated factor VIII levels was 0.29%, compared to 0.13% in relatives with normal factor VIII levels. In relatives with low free protein S levels, this risk was 0.26%, compared to 0.14% in relatives with normal free protein S levels. Hypertension, diabetes mellitus and obesity were associated with high levels of factor VIII, whereas free protein S levels did not show an association with any of the arterial cardiovascular risk factors. We postulated that low free protein S levels were genetically determined. We conclude that both elevated factor VIII levels and low free protein S levels are mild risk factors for ATE. However, it is questionable whether a high factor VIII level by itself increases ATE risk, or whether this increased risk is rather caused by the presence of other arterial thrombotic risk factors.

8

General discussion and future perspectives

Discussion and future perspectives

The association between arterial and venous thromboembolism

ATE and VTE were traditionally considered as two different diseases. In 2003, Prandoni *et al*. were the first to report data that questioned this idea.[1] Their data showed a two-fold increased risk for the presence of atherosclerotic plaques in subjects with unprovoked deep vein thrombosis.[1] As a result of this study, more studies followed that observed an increased risk of ATE after VTE.[2–8] In the present thesis, this finding was confirmed. Especially subjects with unprovoked VTE were at an increased risk to develop ATE and the risk of ATE was highest within the first year following VTE. This early occurrence of cardiovascular disease was also observed in a large 20-year cohort study.[9] This observation is even more remarkable as subjects with VTE receive anticoagulant therapy for three to six months following their event. Besides it protective effect on VTE, anticoagulant therapy is also known to reduce the risk of ATE. A large meta-analysis of 31 randomized trials showed that anticoagulant treatment of high or moderate intensity (international normalized ratio of 2.8-4.8 and 2-3, respectively) is effective in reducing the risk of myocardial infarction and ischemic stroke.[10]

A possible explanation for the early occurrence of ATE might be a rebound effect on coagulant factors.[11–13] In the early sixties an increased incidence of cardiovascular events was observed after cessation of oral anticoagulant therapy.[14] This notion, however, was not corroborated by others[15–17] and therefore remains controversial. In the PREVEND cohort, used for our study, the presence of a rebound effect is unlikely as, out of 45 subjects that developed ATE subsequent to VTE, only one subject developed ATE within a month after cessation of anticoagulant therapy.

An alternative explanation for the high incidence of ATE in subjects with previous VTE might be hospitalization bias. Patients with VTE may be diagnosed more often with subsequent ATE due to the fact that they are being extensively monitored after the development of VTE. Nonetheless, hard end points, like myocardial infarction, ischemic stroke and cardiovascular death should not be sensitive to such a bias. Both this thesis and previous research showed that the incidence of ATE was also higher in subjects with previous VTE, when restricted to these hard end points.[2,6,7] With this, the presence of a hospitalization bias, as explanation for the high incidence of ATE after VTE, becomes unlikely.

All studies regarding the relationship between ATE and VTE failed to take the use of anticoagulants into account.[2–8] Therefore, we cannot address the role of anticoagulant therapy itself on ATE risk. Recently, however, it has been suggested that long-term (> 10 years) use of vitamin-K antagonists accelerates arterial calcification.[18] Matrix gamma-carboxyglutamic acid (G1a) protein (MGP) is an important calcification inhibitor

and carboxylation of this protein is vitamin-K dependent. It is presumed that vitamin-K antagonists increase vascular calcification through inhibition of MGP.[19–21] Although we cannot exclude the possibility of arterial calcification in subjects with VTE, most likely this will not explain the high risk of subsequent ATE, given that standard therapy for VTE is only three to six months. Furthermore, abovementioned study investigated the effect of vitamin-K antagonists on arterial calcification in subjects aged younger than 55 years. Both VTE and ATE, however, occur considerably more often in subjects with older age. In older subjects, results on the relationship between long-term anticoagulant treatment and vascular calcification are contradicting.[19–22] Additionally, as mentioned before, vitamin-K antagonist appeared effective in ATE prevention, due to the anticoagulant effects of these drugs.[10]

Given these considerations the most likely hypothesis for the high risk of ATE within the first year after VTE is that a joint mechanism relates the two diseases. The presence of underlying pathology affecting the venous system might also affect the arterial system. The high risk of ATE in subjects with an unprovoked venous event compared to those with a provoked event, found in both the present thesis and other studies,[2,23] supports this idea.

Arterial thromboembolic risk factors and the risk of venous thromboembolism

Which underlying pathologic mechanism could relate the two diseases is not known. Possibly, VTE and ATE have common risk factors. Several previous studies investigated this possibility and examined the effect of numerous arterial cardiovascular risk factors on the risk of VTE. Only obesity has consistently been shown to be related to VTE risk.[24–27] Results on other classical ATE risk factors, like hypertension, dyslipidemia, smoking, and diabetes, are contradicting.[24–26,28]

In the present thesis we aimed to further explore the overlap between ATE and VTE risk factors. Because obesity is the only arterial cardiovascular risk factor that also clearly increased the risk of VTE,[25–27,29] we first investigated the relationship between insulin resistance and VTE. Insulin resistance plays a central role in the cardiovascular risk profile of obese subjects.[30–32] Furthermore, insulin resistance is related to endothelial damage and an increase in several prothrombotic factors.[33–39] Our study indeed showed that subjects with higher levels of insulin resistance were at increased risk for the development of VTE. This association remained significant after correction for several cardiovascular risk factors. However, significance was lost after correction for body mass index. It appeared that the level of insulin resistance was strongly associated with body mass index. These results suggest that insulin resistance does not explain the relationship between ATE and VTE. However, although high body mass index does not seem to increase VTE risk through insulin resistance, high body mass index itself was

indeed related to a higher VTE risk. There are several other ways in which high body mass index can influence the risk of VTE. For example, thrombotic risk rises with the level of physical inactivity[40] and physical inactivity is more common in subjects with high body mass index.[41,42] Another explanation for the association between body mass index and VTE could be an increase in factor VIII level. This prothrombotic factor is elevated in subjects with high body mass index[43,44] and possibly enhances the development of VTE in these subjects.

We further explored the relationship between ATE and VTE by investigating the association between lipid profile and VTE. We examined whether apolipoproteins are better at predicting VTE risk than the classical lipoproteins, and observed that this was not the case. In fact, apolipoproteins showed no relationship with VTE risk at all, nor did the classical lipoproteins in our cohort. This finding is remarkable as an altered lipid profile is known to affect haemostasis. Elevated LDL level accelerates activation of prothrombin, factor X and factor VII, whereas HDL is known to enhance the protein C anticoagulant pathway and to reduce thrombin generation.[45] Furthermore, a high level of total cholesterol enhances platelet thrombus formation.[46] The absence of an association between lipid profile and VTE risk suggests that these prothrombotic effects are probably too mild to actually influence VTE risk or that these effects are counteracted by other mechanisms. Our findings suggest that the reduced risk of VTE in subjects using lipid-lowering drugs[47–49] should be ascribed to other properties of this medication, and not to its influence on lipid levels. This theory is supported by the finding that statins are the only type of lipid-lowering drugs that reduce the risk of VTE.[50] Statins have several other mechanisms that could inhibit thrombus formation. Firstly, statins induce Kruppel-Like Factor 2 expression. Overexpression of Kruppel-Like Factor 2 promotes thrombomodulin expression on endothelial cells, thereby enhancing the activity of the protein C anticoagulant pathway.[51] Furthermore, it causes a decreased level of plasminogen activator inhibitor-1.[52] Secondly, statins are associated with diminished levels of inflammatory markers[53,54] and with reduced tissue factor expression and thrombin generation.[55]

Next, we investigated the relationship between albuminuria and the risk of recurrent VTE. High levels of urinary albumin are associated with an increased risk for ATE[56–59] and recently a relationship with VTE was also shown.[60] The increased risk of thromboembolism in patients with elevated albuminuria is hypothesized to be associated with endothelial injury and/or the related changes in the levels of procoagulant proteins.[60–63] As risk factors for first VTE do not always distinguish between people at lower or higher risk for recurrence, we investigated whether this was the case for albuminuria. It appeared that subjects with unprovoked first VTE and high urinary albumin levels were at increased risk to develop recurrent VTE when compared to subjects with normal albuminuria. These findings enable us to distinguish between people at high and low risk for recurrence.

This is important for the clinical management of VTE patients as it brings us closer to optimizing the balance between thrombotic and hemorrhagic risk. Furthermore, elevated albuminuria can be treated with other (non-anticoagulant) medicines, such as angiotensin converting enzyme inhibitors or angiotensin receptor blockers. Possibly, these medicines could also play a role in the treatment or prophylaxis strategies for VTE. This would be an interesting hypothesis to test.

Venous thromboembolic risk factors and the risk of arterial thromboembolism

In addition to examining whether arterial cardiovascular risk factors predispose to VTE, the relationship between VTE risk factors and ATE incidence might also be useful in explaining the association between the two diseases. Indeed, VTE risk factors like pregnancy and the use of oral contraceptives are related to an increased risk of ATE.[64–66] The same is true for thrombophilia. Although relative risks are small, elevated levels of factors VIII, IX, XI and fibrinogen are associated with an increased risk of ATE.[67,68] Furthermore, Factor V Leiden and the 20210A prothrombin mutation, both known to increase circulating thrombin generation, might slightly add to ATE risk.[69–71] The same applies to protein C and protein S type I deficiency.[72,73]

High factor VIII and free protein S levels are known risk factors for VTE.[74–76] We investigated whether these factors also influence ATE risk and whether this occurs through either a genetic or an acquired link. For this purpose, we used pooled data of four family cohorts. Analyses showed a mildly increased risk of ATE in subjects with high factor VIII or low free protein S levels. In the case of factor VIII, high levels were associated with the presence of several arterial thrombotic risk factors. Hence, it is questionable whether high factor VIII levels itself increases ATE risk, or whether this increased risk is explained by other, factor VIII related, arterial thrombotic risk factors. Free protein S levels were not associated with arterial thrombotic risk factors. As both free protein S levels and arterial thrombotic risk factors are linked to inflammation,[77–82] concomitant occurrence, however, is likely when low free protein S levels are acquired. The absence of such a balance, especially in a family cohort, assumes a genetic link. Furthermore, the association between free protein S level and ATE is not materially affected by age, which supports the assumption of genetic involvement.

Although free protein S level is related to both VTE and ATE, it is unlikely to entirely explain the association between the two diseases. First, its association with ATE is mild. Second, it is probably through a genetic link that free protein S levels influence ATE risk, and hereditary protein S deficiency is rare. Therefore, this will probably not explain the high ATE incidence in VTE subjects in the general population.

The study design used to examine the association between both factor VIII and free protein S levels and ATE was not optimal. Firstly, a retrospective study design was used and

factor VIII and free protein S measurements took place at end of study. As measurements took place after ATE occurrence we can not be sure that levels were the same at time of ATE. Of course this problem is relatively small when it concerns genetically influenced levels, but factor VIII and free protein S levels can also be influenced by environmental factors. Secondly, we used a family cohort of young participants. ATE incidence is low in young subjects as ATE incidence is strongly associated with increasing age. This design is likely to have reduced generalizability, however, a cohort as used for our analyses, is very helpful in identifying genetic defects. As well as ATE incidence, the prevalence of acquired risk factors for ATE also increases with age. Consequently, especially young subjects with ATE are useful to detect genetic influences. Of course, actual genotyping would solve this problem and would enable us to detect the effect of genetic defects in more generalizable cohorts, but this type of research is expensive and time-consuming. Hence, in the case of rare genetic defects, a study design like ours can be useful and can serve as first step in exploring a possible genetic association.

Conclusion and future perspective

From this thesis we can conclude that a relationship between VTE and ATE indeed exists. The early occurrence and high incidence of ATE after an unprovoked VTE both suggest that a joint mechanism underlies this relationship. Most likely, as both VTE and ATE are multicausal diseases, the relationship between the two is also explained by multiple factors. Data from this thesis suggests that insulin resistance and an altered lipid profile do not explain the association. However, the PREVEND Study has a limitation when trying to explain the association between ATE and VTE; some relevant variables, such as levels of insulin, glucose and apolipoproteins, were measured only once, at inclusion. Any changes in these variables could therefore not be accounted for when assessing the risk for either VTE or ATE. In the ideal situation assessments take place right before ATE or VTE occurrence in order to examine its influence on the event. To approach this ideal situation repeated measures are required. This would enable us to investigate not only the influence of abnormal levels, but also the influence of changes in levels.

A population-based cohort study, like the PREVEND Study, is perfectly valid to maintain the generalizability of study results. However, as VTE incidence is not extremely high, huge cohorts are needed in order to draw firm conclusions about the relationship between VTE and ATE. In illustration of this point; out of the 40 856 subjects that formed the baseline PREVEND Study cohort, only 45 subjects developed VTE and subsequent ATE in a follow-up period of approximately 11 years. Due to this small number of endpoints there are clear limits to the expansion of statistical models. To solve this problem even larger cohorts should be obtained, a task that requires enormous resources. A solution to this problem is to form a high risk population cohort, for example, a cohort with older

subjects, as age is known to strongly influence both VTE and ATE incidence. This way, a minimum number of participants and a maximum number of patients are included. However, although saving time and costs, a certain loss of generalizability will occur. Another method to increase power could be to pool data of several cohorts that used designs similar to that of the PREVEND Study.

Probably, a better solution is the use of existing ongoing prospective cohort studies. An example of such a study is the Lifelines study[83] which aspires to include 165 000 subjects to investigate the influence of hereditary and acquired factors on healthy aging. Every five years, this study intends to test a set of variables which could be of interest in exploring the association between VTE and ATE. If it is possible to link this database to the databases of the anticoagulant clinics and pharmacies, this study could be very valuable in exploring the association between VTE and ATE, while costs and time-consumption are minimal.

Data from literature and the present thesis suggest that obesity, vascular injury and inflammation might be of particular interest to explore in such a study, with respect to the association between VTE and ATE.[24,60,84–87] Especially the role of obesity might be intriguing as its prevalence increased exponentially in the past years. In 2009, almost fifty percent of the adult Dutch population was overweight or obese (body mass index ≥ 25 kg/m^2).[88] Another reason why overweight might be of special interest is that it can be measured and remedied without medical interfering.

Our study investigating the association between insulin resistance and VTE showed that body mass index measured at baseline is associated with an increased risk of VTE approximately five years later. This finding seems to indicate that overweight subjects generally remain overweight. Treatment and prophylactic strategies on lifestyle intervention, including diet and physical exercise, are daunting, but might gain huge benefit in the prevention of VTE as well as ATE.

Also without knowing the exact mechanism, the high incidence of ATE after VTE sheds a new light on the treatment of subjects with VTE. It implicates that the care for patients with VTE should not only focus on the prevention of recurrent VTE but also on the prevention of ATE. Possibly, the traditional treatment with vitamin-K antagonists can be extended with other medication. With this purpose, it might be valuable to screen subjects with VTE on arterial cardiovascular risk factors and treat them accordingly. This might even kill two birds with one stone as certain prophylactic strategies for ATE also reduce the risk for VTE. Statins, for example, are prescribed to normalize an altered lipid profile, but treatment also appears effective in reducing VTE risk.[47–49] Furthermore, it might be useful to extend the traditional anticoagulant treatment of VTE with acetyl salicylic acid, a drug that is widely used in the prevention of ATE. At this moment there is insufficient evidence to advise this combined antiplatelet and anticoagulant treatment strategy, however, two ongoing studies (Warfasa, agnellig@unipg.it and

Aspire, aspire@ctc.usyd) address this issue. Effects on arterial outcome are not available yet, but initial results show that aspirin reduces the risk of recurrence in subjects with unprovoked VTE without increasing the risk of bleeding, when given after a treatment period with anticoagulants.[89]

In conclusion, we can now firmly conclude that subjects with VTE are at increased risk for ATE. It is yet to be discovered what explains this association. Future studies should focus on reducing ATE risk after prior VTE by trying to explain the association and by investigating extended treatment strategies.

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9

Dutch summary

Samenvatting

Hart- en vaatziekten zijn doodsoorzaak nummer één in de westerse wereld. Onder deze noemer vallen onder andere een hartinfarct, herseninfarct, diep veneuze trombose en longembolie. Een hogere leeftijd is de belangrijkste risicofactor voor hart- en vaatziekten. Aangezien wij steeds ouder worden zullen deze ziekten een steeds belangrijker rol gaan spelen.

Bij veel vormen van hart- en vaatziekten is er sprake van overmatige stolselvorming waardoor een bloedvat (gedeeltelijk) afgesloten raakt. Wanneer dit het geval is dan spreken we van trombose. Trombose kan plaatsvinden in de slagaders (arteriën), die zuurstofrijk bloed naar de organen vervoeren, of in de aders (venen), die het bloed weer terug vervoeren naar het hart. In het eerste geval spreken we van arteriële trombose, in het tweede geval van veneuze trombose. Een hartinfarct en herseninfarct zijn voorbeelden van arteriële trombose. Veel voorkomende vormen van veneuze trombose zijn diep veneuze trombose in het been of longembolie.

Decennialang werd verondersteld dat arteriële en veneuze trombose twee verschillende ziektebeelden waren. De reden hiervoor was dat de beide typen trombose erg van elkaar verschillen met betrekking tot de risicofactoren, de ontstaanswijze en de samenstelling van het stolsel. Bekende risicofactoren voor arteriële trombose zijn roken, hoge bloeddruk, verhoogd cholesterol en diabetes. Deze factoren veroorzaken een beschadigde vaatwand. In reactie hierop blijven bloedplaatjes aan de wand kleven, wat langzaam maar zeker zorgt voor afsluiting van de slagader. De arteriële trombus bestaat dan ook met name uit bloedplaatjes. Bekende risicofactoren voor veneuze trombose zijn operaties, zwangerschap, gebruik van de anticonceptiepil, kanker, immobilisatie en afwijkende stollingsfactoren. Deze factoren hebben niet zo zeer invloed op de vaatwand, maar beïnvloeden de samenstelling van het bloed en de stroming van het bloed. Een veneuze trombus vormt zich in korte tijd en bestaat vooral uit rode bloedcellen en fibrinedraden.

De relatie tussen arteriële en veneuze trombose

In de afgelopen tien jaar is de oorspronkelijke tweedeling tussen arteriële en veneuze trombose in twijfel getrokken. Mogelijk zijn deze ziekten meer met elkaar verbonden dan wij altijd vermoedden. Dit vraagstuk werd in het huidige proefschrift nader onderzocht. Hierbij maakten wij gebruik van het grote populatie cohort van de PREVEND Studie. In 1997 is deze studie opgezet om de relatie tussen nierfunctie en arteriële trombose te onderzoeken. Alle inwoners van Groningen tussen de 28 en 75 jaar werden uitgenodigd om aan deze studie deel te nemen.

Ruim 40 000 mensen reageerden op deze uitnodiging en leverden een ingevulde vragenlijst en een urine monster in. Al deze mensen werden in de daaropvolgende jaren gevolgd en gecontroleerd op de ontwikkeling van arteriële trombose. In het kader van de huidige onderzoeksvragen zijn deze data gekoppeld aan de data van de trombosedienst, die de behandeling van veneuze trombose mede uitvoert. Met deze gegevens en de gegevens van de medische dossiers verzamelden wij van alle 40 000 deelnemers een compleet overzicht van alle nieuwe gevallen van arteriële en veneuze trombose vanaf 1997 tot 2009.

Deze gegevens hebben we gebruikt om te onderzoeken of de in eerdere studies gevonden relatie tussen arteriële en veneuze trombose ook aangetoond kon worden in ons grote populatiecohort. Inderdaad vonden we dat mensen met een veneuze trombose een verhoogd risico hadden op het ontwikkelen van een arteriële trombose in vergelijking tot de mensen die nooit veneuze trombose hadden gehad. Dit risico was voornamelijk hoog in het eerste jaar na veneuze trombose. Drie procent van de patiënten met veneuze trombose ontwikkelde een hartinfarct, herseninfarct of andere arteriële trombose in het daaropvolgende jaar. Dit percentage was twee keer zo hoog als dat van mensen zonder veneuze trombose. Patiënten die een spontane veneuze trombose hadden ontwikkeld, dus zonder een duidelijk aanwijsbare oorzaak, hadden het hoogste risico op arteriële trombose.

Deze bevindingen deden ons vermoeden dat er een onderliggende afwijking bestaat die zowel veneuze als arteriële trombose uitlokt. Mogelijk hebben beide ziekten toch gemeenschappelijke risicofactoren. Dit vraagstuk hebben we nader onderzocht.

Insuline resistentie

Glucose is een belangrijk bron van energie voor ons lichaam. Het is echter van belang dat de glucosespiegels in het bloed constant blijven. Om deze reden wordt er razendsnel insuline aangemaakt wanneer we glucose binnen krijgen via onze voeding. Insuline houdt de glucosespiegels op peil door de opslag van glucose in spieren en lever te stimuleren en de afbraak van glycogeen tot glucose te remmen. Wanneer iemand insulineresistent is, reageert het lichaam niet goed op insuline waardoor de cellen niet optimaal functioneren en de glucosespiegel in eerste instantie niet voldoende daalt. Dit compenseert het lichaam door nog meer insuline aan te maken. Wanneer het lichaam deze verhoogde insulineproductie niet meer aan kan zal dit resulteren in een verhoogde glucosespiegel. Wanneer de glucosespiegel een bepaalde grens overschrijdt spreken we van suikerziekte (Diabetes Mellitus). Hoge insuline- en glucosespiegels zorgen voor beschadigingen aan de vaatwand.

Mensen met overgewicht hebben een verhoogd risico op het ontwikkelen van arteriële trombose. Vaak gaat overgewicht gepaard met een scala aan arteriële

risicofactoren, zoals een verhoogde bloeddruk, verhoogd cholesterol en verhoogde ontstekingsactiviteit. Insulineresistentie speelt een sleutelrol in dit arteriële risicoprofiel.

Overgewicht verhoogt ook het risico op veneuze trombose. Mogelijk is insulineresistentie dus ook gerelateerd aan een verhoogd risico op veneuze trombose. Van de ruim 40 000 PREVEND deelnemers zijn 8500 deelnemers nader onderzocht op een aantal belangrijke arteriële risicofactoren, waaronder insulineresistentie. In deze groep onderzochten wij of insulineresistentie het risico op veneuze trombose beïnvloedde. Het bleek dat een sterkere mate van insulineresistentie inderdaad een verhoogd risico op veneuze trombose met zich meebracht. Deze relatie bleek echter afhankelijk te zijn van overgewicht. In andere woorden, mensen die insulineresistent zijn, hebben vaak ook overgewicht en waarschijnlijk is dit overgewicht de reden van het verhoogde risico op trombose. Onze studie bevestigt dat overgewicht zelf onafhankelijk gerelateerd is aan een verhoogd risico op veneuze trombose. Onze resultaten suggereren dat insulineresistentie niet essentieel is voor de ontwikkeling van veneuze trombose in mensen met overgewicht.

(Apo-) lipoproteïnen

Cholesterol en triglyceriden, samen bekend als lipiden, hebben een belangrijke functie in de energievoorziening en in de synthese van celmembraan en hormonen. Afwijkende lipidespiegels kunnen echter zorgen voor verkalking van de slagaders en geven daarmee een verhoogd risico op arteriële trombose. Lipiden zijn niet oplosbaar in water en binden daarom aan apolipoproteïnen om getransporteerd te kunnen worden door het bloed. Apolipoproteïnen bevatten fosfolipiden die de oplosbaarheid verbeteren. Wanneer lipiden en apolipoproteïnen binden, vormen ze samen lipoproteïne. De belangrijkste lipoproteïnen zijn High-Density Lipoproteïne (HDL) en Low-Density Lipoproteïne (LDL). HDL onttrekt cholesterol aan de cellen en transporteert het terug naar de lever die zorgt voor de uitscheiding ervan. HDL verlaagt de cholesterolspiegel en hoge HDL-spiegels hebben daarom een beschermend effect op het risico op arteriële trombose. Alle andere lipoproteïnen, waarvan LDL de belangrijkste is, zijn atherogeen. Dat wil zeggen dat ze het risico op aderverkalking verhogen en daarmee het risico op arteriële trombose. Deze atherogene lipoproteïnen transporteren lipiden van de lever naar de organen.

Recent is aangetoond dat lipidenverlagende middelen, de zogeheten statinen, het risico op veneuze trombose verlagen. De literatuur is echter inconsistent wat betreft het effect van een afwijkend lipidenprofiel op het risico van veneuze trombose. Waar sommige studies inderdaad een relatie aantonen, doen andere dat niet. Mogelijk komt dit omdat er vrijwel alleen onderzoek is gedaan naar de klassieke lipide biomarkers (o.a. HDL, LDL en totaal cholesterol). De klassieke lipide biomarkers zouden echter wel eens niet de beste maat kunnen zijn voor het risico op trombose. Sommige studies

laten zien dat apolipoproteïnen betere voorspellers zijn als het om het voorspellen van arteriële trombose gaat. In de 8500 nader onderzochte deelnemers van de PREVEND Studie onderzochten wij of apolipoproteïnen ook voor veneuze trombose betere risicovoorspellers zijn dan de klassieke lipide biomarkers. Dit bleek niet het geval. Zowel apolipoproteïnen als de klassieke lipide biomarkers waren niet gerelateerd aan veneuze trombose. Het positieve effect op het risico op veneuze trombose als gevolg van het gebruik van statinen ligt dus waarschijnlijk niet aan het lipidenverlagende effect, maar aan andere eigenschappen van deze medicijnen. We kunnen uit deze studie concluderen dat abnormale lipidenspiegels de relatie tussen arteriële en veneuze trombose niet kunnen verklaren.

Albuminurie

In de nieren wordt het bloed gefilterd en urine gevormd. Hierbij wordt de doorgang geblokkeerd voor grote eiwitten zodat deze de bloedstroom niet kunnen verlaten. Eén van deze grote eiwitten is albumine. Gezonde mensen hebben nauwelijks albumine in hun urine. In sommige gevallen is de albuminespiegel in de urine (albuminurie) echter verhoogd. Dit kan duiden op algehele vaatwandschade in het lichaam. Doordat de vaatwand in de nieren beschadigd is, kan het eiwit albumine in de urine lekken. Aangezien verhoogde albuminurie een marker is voor vaatwandschade is het een goede voorspeller voor het risico op arteriële trombose.

Onze studiegroep heeft recentelijk laten zien dat een hoge albuminespiegel in de urine ook een risicomarker is voor een eerste veneuze trombose. Het is echter bekend dat risicofactoren of -markers voor een eerste veneuze trombose vaak niet onderscheidend zijn voor het risico op een tweede veneuze trombose. Of dat het geval is voor verhoogde albuminurie is onbekend. Deze informatie is echter wel van groot belang voor de behandeling van patiënten met een eerste veneuze trombose. Na een eerste veneuze trombose krijgen patiënten antistollingsmiddelen toegediend. Deze behandeling duurt standaard drie tot zes maanden. Wanneer het risico op een tweede trombose heel hoog is, wordt een langduriger behandeling overwogen. Echter, antistollingsmiddelen gaan de vorming van nieuwe stolsels tegen, maar verhogen hiermee tegelijkertijd het risico op bloedingen. Om deze reden moet er een goede afweging gemaakt worden welke patiënten baat hebben bij een langdurige behandeling en welke, met het oog op de bloedingsneiging, beter niet langdurig behandeld kunnen worden. Daarom is het van belang patiënten te identificeren met een hoog risico op een tweede veneuze trombose.

Van het PREVEND populatiecohort van ruim 40 000 mensen ontwikkelden 351 mensen een eerste veneuze trombose tijdens de studieperiode. Deze mensen werden gevolgd op het ontwikkelen van een tweede veneuze trombose, wat bij 37 mensen werd vastgesteld. Onze studie heeft laten zien dat mensen met een spontane eerste veneuze

trombose, dus zonder bekende uitlokkende factor, een drie keer zo hoog risico hebben op het ontwikkelen van een tweede veneuze trombose wanneer hun albuminespiegel in de urine verhoogd is. Aangezien verhoogde albuminurie een marker is voor vaatwandschade zou dit kunnen betekenen dat vaatwandschade ook een rol speelt bij veneuze trombose, hetzij direct dan wel indirect door het lekken van eiwitten die de stolselvorming tegen gaan. Deze grote eiwitten verlaten het bloed normaal niet, maar als albumine in de urine terecht komt zou dit ook kunnen gelden voor andere eiwitten. Ook blijkt een verhoogde albuminurie gerelateerd aan hogere spiegels van eiwitten die de stolling juist bevorderen. Wat het precieze mechanisme is achter het verhoogde risico op een tweede veneuze trombose in mensen met een verhoogde albuminurie moet dus nog nader onderzocht worden, maar onze studie suggereert dat mensen met een spontane eerste veneuze trombose en verhoogde albuminurie mogelijk gebaat zijn bij een langdurige behandeling met antistollingsmiddelen.

Factor VIII en vrij proteïne S

De vorming van een (veneus) stolsel wordt gereguleerd door een groot aantal eiwitten die de stolling bevorderen of juist tegengaan. Factor VIII is een eiwit dat de stolling bevordert. Hoge spiegels van dit eiwit zijn dan ook gerelateerd aan een verhoogd risico op veneuze trombose. Proteïne S is een eiwit dat de vorming van een stolsel juist tegen gaat. Proteïne S bestaat in een vrije vorm en in een vorm waarin het gebonden is aan een ander eiwit. Alleen de vrije vorm speelt een rol in de remming van stolselvorming. Een lage vrij proteïne S-spiegel is geassocieerd met een verhoogd risico op veneuze trombose. De FVIII- en vrij proteïne S-spiegels zijn gedeeltelijk erfelijk bepaald en worden deels beïnvloed door ontsteking. Ontsteking speelt een belangrijke rol bij de ontwikkeling van arteriële trombose in mensen met arteriële risicofactoren. Dit zou kunnen betekenen dat hoge factor VIII-spiegels en lage vrij proteïne S-spiegels ook gerelateerd zijn aan een verhoogd risico op arteriële trombose. We hebben dit onderzocht in een groot familiecohort van vier gecombineerde studies. In deze studies zijn familieleden geïncludeerd van patiënten met veneuze trombose óf aderverkalking op jonge leeftijd, en daarnaast een afwijking in één van de stollingsfactoren. De inclusies vonden plaats tussen 1999 en 2004. Bij alle deelnemers werd een groot aantal stollingseiwitten gemeten. Van de ruim 1400 deelnemers werd onderzocht of ze in het verleden arteriële trombose hadden doorgemaakt en of er arteriële risicofactoren bij hen aanwezig waren. Met behulp van deze informatie onderzochten we of hoge factor VIII-spiegels en lage vrij proteïne S-spiegels gerelateerd waren aan een verhoogd risico op arteriële trombose. Ook onderzochten we of de afwijkende spiegels een associatie vertoonden met de aanwezigheid van arteriële risicofactoren. Was dit het geval dan beschouwden we de afwijking als verworven. Wanneer er geen relatie was tussen

de afwijkende stollingseiwitten en de arteriële risicofactoren, dan namen we aan dat de afwijkende spiegels niet door ontsteking werden veroorzaakt, maar door erfelijke factoren.

Ons onderzoek liet zien dat beide factoren inderdaad milde risicofactoren zijn voor arteriële trombose. Een hoge factor VIII-spiegel bleek echter ook gerelateerd te zijn aan de aanwezigheid van arteriële risicofactoren. Mogelijk verklaart dit de relatie tussen factor VIII en het verhoogde risico op arteriële trombose. Een lage vrij proteïne S-spiegel was niet gerelateerd aan de aanwezigheid van arteriële risicofactoren. Aangezien een lage vrij proteïne S-spiegel en arteriële risicofactoren beide gerelateerd zijn aan ontsteking is het waarschijnlijk dat deze gelijktijdig optreden wanneer de vrij proteïne S-spiegel laag is onder invloed van ontsteking. Aangezien dit niet het geval was in het onderzochte familiecohort veronderstellen wij dat de lage vrij proteïne S-spiegels in ons cohort erfelijk bepaald zijn.

Conclusie en toekomstperspectief

Uit dit proefschrift kunnen we concluderen dat de veronderstelde relatie tussen arteriële en veneuze trombose inderdaad bestaat. Het hoge risico op arteriële trombose na een spontane veneuze trombose en het feit dat dit risico met name hoog is vlak na de veneuze trombose lijken erop te wijzen dat de beide ziekten een gemeenschappelijk onderliggend mechanisme hebben. Onze studies suggereren dat insulineresistentie en een afwijkend lipiden profiel geen onderdeel uitmaken van dit gemeenschappelijke mechanisme. Een verlaagde vrij proteïne S-spiegel is wel gerelateerd aan zowel arteriële als veneuze trombose. Het is echter onwaarschijnlijk dat deze samenhang de relatie tussen de twee ziekten geheel verklaart. Ten eerste is er slechts een milde relatie tussen een verlaagde vrij proteïne S-spiegel en arteriële trombose. Ten tweede verloopt deze relatie waarschijnlijk via een genetische link en een erfelijk proteïne S tekort komt maar zelden voor. Dit zal de hoge incidentie van arteriële trombose in de algemene bevolking dus waarschijnlijk niet verklaren.

De onderzoeken in het huidige proefschrift en overige literatuur laten zien dat overgewicht, vaatwandschade en ontsteking mogelijk wel een rol spelen in de relatie tussen arteriële en veneuze trombose. Vooral overgewicht is een interessante factor voor nader onderzoek, gezien de stijgende prevalentie hiervan. Bovendien is overgewicht vast te stellen en te verhelpen zonder medisch ingrijpen.

Ook al kunnen we het mechanisme achter de relatie tussen arteriële en veneuze trombose nog niet doorgronden, de hoge incidentie van arteriële trombose na veneuze trombose werpt wel een nieuw licht op de behandelstrategieën van patiënten met veneuze trombose. Deze relatie suggereert dat we ons bij patiënten met een eerste veneuze trombose niet alleen moeten focussen op het voorkomen van een tweede

veneuze trombose, maar ook op het voorkomen van arteriële trombose. Mogelijk moeten we de behandeling van mensen met veneuze trombose uitbreiden. Het zou bijvoorbeeld nuttig kunnen zijn om mensen na hun eerste veneuze trombose te screenen op hun arteriële risicoprofiel en ze te behandelen daar waar nodig. Aangezien de medicijnen voor sommige arteriële risicofactoren ook het risico op veneuze trombose verlagen, zou dit zelfs twee vliegen in één klap slaan. Niet alleen het risico op arteriële trombose daalt, maar ook het risico op een tweede veneuze trombose.

Daarnaast zou het ook nuttig kunnen zijn om de huidige behandeling van veneuze trombose te verlengen met acetylsalicylzuur, een medicijn dat gebruikt wordt voor de preventie van arteriële trombose. Op het moment is er nog onvoldoende literatuur om een dusdanige combinatietherapie te adviseren, maar er zijn twee grote studies bezig dit te onderzoeken. Tot op heden zijn er nog geen resultaten bekend over het effect een combinatietherapie op arteriële trombose, maar de eerste resultaten laten zien dat het verlengen van de therapie van een eerste spontane veneuze trombose met acetylsalicylzuur, het risico op een tweede veneuze trombose verlaagt, zonder het risico op bloedingen te verhogen.

Concluderend kunnen we nu met absolute zekerheid zeggen dat arteriële en veneuze trombose gerelateerd zijn. Wat deze relatie verklaart moet nog nader onderzocht worden. Toekomstige studies zouden zich moeten richten op het reduceren van het risico van arteriële trombose bij mensen met een eerdere veneuze trombose. Dit kan bewerkstelligd worden door een verklaring te vinden voor de associatie tussen de beide ziekten en door aangepaste behandelstrategieën te onderzoeken.

List of Publications, Presentations and Awards

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Publications

van Schouwenburg I.M., Mahmoodi B.K., Gansevoort R.T., Muntinghe F.L.H., Dullaart R.P.F., Kluin-Nelemans H.C., Veeger N.J.G.M., Meijer K. (2012). Lipid levels do not influence the risk of venous thromboembolism. Results of a population-based cohort study. Thrombosis and Haemostasis 108(5). [Epub ahead of print]

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XXIII Congress of the International Society of Thrombosis and Haemostasis, Kyoto, Japan, 2011.

Population based cohort study on the risk of arterial thrombosis in subjects with previous venous thrombosis.
XXII Congress of the International Society of Thrombosis and Haemostasis, Boston, USA, 2009.

Awards

Young Investigator Award
XXIII Congress of the International Society of Thrombosis and Haemostasis, Kyoto, Japan, 2011.

Young Investigator Award
XXII Congress of the International Society of Thrombosis and Haemostasis, Boston, USA, 2009.

Acknowledgements

Dankwoord

Velen hebben mij geholpen bij de totstandkoming van dit proefschrift. Sommigen verrijkten mijn onderzoek met hun intellectuele kennis, anderen boden een luisterend oor en weer anderen zorgden voor de essentiële afleiding en gezelligheid. Graag wil ik jullie voor deze bijdragen bedanken.

Allereerst wil ik Jan bedanken voor het vertrouwen dat hij me heeft gegund toen hij mij, met als Bewegingswetenschappen niet de meest voor de hand liggende achtergrond, aannam als promovendus bij de stolling. Jan liet me vrij om de dingen te doen zoals ik ze voor me zag en zolang ik dat goed deed, was hij een tevreden man. Hij interesseerde zich voor wat je deed op en naast het werk en met een goed gevoel voor humor zorgde hij voor een ontspannen werksfeer. Een betere baas om mijn werkende leven mee te beginnen had ik me niet kunnen wensen.

Na het plotselinge overlijden van Jan heeft Hanneke zijn taak als promotor overgenomen. Geheel niet makkelijk, want Jan had heel wat promovendi onder zich en had vrijwel niks (zo niet, helemaal niks) gedocumenteerd. Een moeilijke taak dus, maar met verve uitgevoerd. Daarvan getuigt onder andere dit proefschrift. Hanneke, ondanks alle extra drukte nam de snelheid waarmee je op je mails reageerde geenszins af. Stuurde ik om 18:00 uur een artikel op, dan kon ik om 23:00 uur weer een gecorrigeerde versie in m'n inbox verwachten. Een zeer gewaardeerde eigenschap! Met een kritische blik heb je al mijn artikelen bekeken en in rap tempo van nuttig commentaar voorzien.

Karina, halsoverkop keerde jij naar Nederland terug om de Stolling met je expertise te verrijken. Toen bleek dat een schip met 5 kapiteins niet vooruit te branden was, heb ook ik daar aanspraak op gedaan. Met jou als begeleider voer het schip de laatste anderhalf jaar weer met volle vaart vooruit. Met behulp van jouw adviezen, feedback en nuchtere blik hebben we een aantal mooie artikelen weten te publiceren.

Ron, als tweede copromotor heb je een grote bijdrage geleverd aan dit proefschrift. Niet alleen leverde je me een grote hoeveelheid data, ook leerde je me alle ins en outs van deze enorm grote en ingewikkelde PREVEND database. Ik kon aankloppen wanneer ik wilde en het eerste wat je me op het hart drukte, was dat mijn bezoekjes laagdrempelig moesten zijn. Dit kon ik erg waarderen. Daarnaast vanzelfsprekend ook mijn grote dank voor een enorme hoeveelheid intellectuele input.

Graag wil ik de leden van de leescommissie, prof. dr. F.R. Rosendaal, prof. dr. H. ten Cate en prof. dr. B.H.R. Wolffenbuttel bedanken voor het lezen en goedkeuren van mijn proefschrift.

Ook mijn medeauteurs, dr. Stephan Bakker, dr. Friso Muntinghe, dr. Robin Dullaart en Margaretha Visser wil ik graag bedanken voor hun bijdrage aan mijn artikelen.

Ina, Tineke en Marian, secretaresses van de Stolling, naast het werk dat jullie me uit handen hebben genomen, wil ik jullie ook bedanken voor het enthousiasme elke keer wanneer ik mijn nieuwste aankopen bij jullie kwam showen. Gelukkig waren de kritieken altijd positief! Jullie zijn een leuk en gevarieerd groepje; Tineke recht door zee en slagvaardig, Marian een echte levensgenieter en Ina vrolijk en (soms iets te) goedlachs (een paar steekwoorden: wespensteek, opgezwollen gezicht, tranen over de wangen, rollend over de grond… Dat is nog een verbeterpunt). Geregeld ben ik bij jullie aan komen waaien wanneer ik in een schrijversblok zat, wanneer mijn hart gelucht moest worden of als ik toe was aan wat gezelligheid. Ik hoop dat m'n volgende werkplek net zo'n gezellig secretariaat heeft. Met een net zo goed gevulde snoeppot.
Ina, jij in het bijzonder hartelijk dank voor al het logistieke werk rondom mijn promotie.

Nic, bij jou heb ik heel wat uren aan je bureau doorgebracht. Allerlei computers en supersnelle systemen haalde je erbij om mijn ingewikkelde statistische vraagstukken te kunnen beantwoorden. En als ik vervolgens al weer vrolijk verder zat te typen op mijn kamertje, zat jij nog minstens 24 uur met de gebakken peren, omdat de volledige computercapaciteit ingenomen werd door mijn berekeningen. Bedankt voor deze opoffering. Ook wil ik je graag bedanken voor je hulp wanneer ik in totale statistische stress verkeerde en je mails begon te sturen met onderwerpen als 'PANIEK!' en 'HELP!' Problemen werden door jou rap de kop in gedrukt zodat de rust snel wederkeerde.

Khan, mijn steun en toeverlaat in onderzoeksland! Ik ken niemand die zo behulpzaam is als jij. Alles kan je te allen tijde bij je neerleggen en je bent bereid iedereen te helpen. Daarbij word je ook nog gesierd door een ernstige vorm van bescheidenheid, want wanneer ik je na een vragenvuur van een uur hartelijk bedank voor je hulp antwoord je standaard: 'Ja… ik heb eigenlijk niks gedaan…' Khan, je bent een bijzondere collega met een groot hart. Al moest ik je soms aan een interview onderwerpen om wat over je te weten te komen, ik vond het erg interessant en leuk om jou en jouw cultuur zo te leren kennen.

Marieke, met veel plezier heb ik m'n jaren bij de Stolling aan 't bureau tegenover jou doorgebracht. Samen deelden we kamers op congressen en hebben we heel wat lol

gehad! Naast veel gezelligheid bood je me ook steun. Wanneer ik zwaar in de stress was voor een praatje voor heel wat toehoorders, dan nodigde je me gewoon een nachtje uit in jouw super-de-luxe 4-persoons hotelkamer, zodat ik alle stressverhogende medepromovendi niet bij het ontbijt trof. Dat kon ik heel erg waarderen. Je bent een gedreven, betrouwbare en lieve collega met een goed luisterend oor!

Vladimir, je hebt me nogal wat restricties opgelegd op alles wat ik hier over je mag schrijven, dus helaas kan ik niet te veel uitweiden over waarom ik je zo waardeer. Laten we dus maar zeggen dat ik je waardeer omdat je bent zoals je bent. Je praat het liefst over alles waar vrijwel niemand iets over wil horen, maar gelukkig zijn jouw favoriete gespreksonderwerpen ook mijn favoriete gespreksonderwerpen, dus van onze maandagochtend-bijklets-sessies heb ik altijd erg genoten. Zelfs aan jouw wekelijkse doel om mij te laten gillen door het vertellen van onsmakelijke verhalen kijk ik met plezier terug. Daarnaast heb ik grote bewondering voor jouw omgang met kritiek. Een eigenschap waar ik zeker wat van kan leren.

Hilde en Sophie, jullie zijn met name belangrijk geweest bij de laatste loodjes. Alle MEGA belangrijke beslissingen die er gemaakt moesten worden -'Mat of glanzende voorkant?' '90 grams papier of 100 grams papier? Ja, ik weet wel dat je het verschil verder niet echt ziet, maar ja, het moet wel goed zijn.' 'Sophie, wat denk jij, moet dit misschien net ietsiepietsie roder zijn?' 'En moet dit stippellijntje misschien toch een doorgetrokken lijntje worden?'- werden met jullie hulp wat makkelijker.
Verder wil ik Hilde nog bedanken voor haar whizzkid-brein, waar ook ik van mee kon profiteren. Al vind ik mensen die zich verheugen op 'lekker een hele week statistieken' doorgaans een beetje eng, als ik een statistische mening nodig had, kon ik altijd even met je brainstormen.

Margriet, allereerst bedankt voor de samenwerking met de trombosedienst. Met jouw hulp is de basis voor mijn onderzoek gelegd. Daarnaast veel dank voor de gezelligheid die je meebracht op de kamer. Je gezonde zelfspot, je gevoel voor humor en je verhalen over je wilde jeugd maakten je een zeer welkome aanvulling op onze kamer.

Graag wil ik Nakisha, Anja en Lies bedanken voor de gezelligheid op congressen, cursussen en in het UMCG, de verpleegkundigen voor het eerste jaar dat ik bij jullie op de kamer heb doorgebracht, de labmedewerkers voor de borreltjes en taartjes die we samen hebben genuttigd, Marjan voor het congresplezier en je vrolijke lach die hier altijd door de kamer schalde, Joop voor de bijzondere, maar gezellige gesprekken, Lucia voor de meidenavonden en galajurk-keur-sessies, René voor je gezelschap bij de huisbezoeken en natuurlijk ook bij de congressen, Min Ki omdat je me introduceerde bij

de Stolling, Willem voor het feit dat je me bij m'n nekvel greep en met beide benen op de grond zette toen de stollingswereld onder m'n voeten weg dreigde te glijden en Heleen voor de keren dat je wat extra leven in de brouwerij op onze kamer bracht.

Bird, Es, Renée, Wouter, Rik en Jan, mijn vaste pauze-matties! De kopjes koffie en thee, de meetingen @ DE hoek, de PUUR, de fontein, de kinderspeelhoek en 'my place', de ontspannen wandelingetjes langs de meerkoetjes die we jaren lang voor waterhoentjes hebben uitgescholden, ons speelkwartiertje bij de YALP, springend en dansend rekensommetjes oplossen op t speelplein van de plaatselijke basisschool, met snoepjes strooien op de faculteit… Ze behoren zeker tot de leukste momenten van mijn onderzoeksbestaan! Bedankt!

Huize Vlassie, en dan met name Essie 1 en Essie 2, waar ik toch het grootste deel van mijn promotietraject bij in huis heb gewoond. Vele malen is mij gevraagd waarom ik nog steeds in mijn studentenhuis woon en of ik niet een keer iets anders wil. Met volle overtuiging zeg ik altijd 'NEE!' Al maken jullie me geregeld 's nachts wakker omdat jullie de sleutel voor de 300ste keer zijn kwijt geraakt, ik vind jullie cool, leuk en heel gezellig en heb het ontzettend naar m'n zin (gehad) bij jullie in huis! En bovendien, een zeer handige bijkomstigheid, die studenten van tegenwoordig hebben zeeën van tijd. Dus als ik weer eens wat laat van m'n werk kwam -'Nee, half 7 haal ik wel, ik doe dan ook wel even boodschappen.' 'Ok, half 7 lukt toch niet. 7 uur dan, inclusief boodschappen.' 'Half 8 ook goed? En kan jij misschien toch die boodschappen even doen?' 'Ok, 8 uur ben ik thuis, en dan heb ik ook hele erge honger, dus zullen we doen dat het eten dan al klaar is?' - dan kon ik altijd op ze rekenen. Thanks babes, voor al die lekkere maaltijden waarbij ik zomaar aan kon schuiven!

Dit laatste is overigens een punt waarop ik alle mensen met wie ik de afgelopen vier jaar heb gegeten moet bedanken. Wanneer ik net in een positieve en productieve vibe zat wou ik die altijd graag ten volle benutten, want je weet tenslotte nooit wanneer die weer komt, en helaas is mijn vibe altijd het best gebleken aan het eind van de middag/begin van de avond. Vele malen heb ik mensen dus op me moeten laten wachten… of voor me moeten laten koken. Hierbij ook met name een chapeau voor Maike, die geregeld uitgehongerd op me heeft staan wachten en er daarbij ook nog eens, in 99% van de gevallen, in slaagde haar irritatie hierover te verbergen.

DE groep; Marleen, Linda, Ankie, Maike, Marije, Rob, Jan-Maarten, Joeri, Thomas, Joris en Sander, bedankt voor jullie ontzettend hechte vriendschap. Ik ben blij dat we elkaar nog steeds zo veel zien en samen zo veel leuke dingen doen. De raarste dingen maak ik met jullie mee en het repertoire van hilariteiten blijft zich maar uitbreiden. Bedankt dat

ik met jullie heerlijk los kan gaan!
Thomas, jij in het bijzonder, bedankt voor het leveren van gtst-materiaal de afgelopen jaren. De hele afdeling heeft hier uitgebreid van genoten. ;)

BW-ers, vakantiechickies, jaarclub- en dispuutsgenootjes en alle andere niet tot een specifieke groep behorende vrienden en vriendinnen, bedankt voor de gezellige borreltjes, etentjes, vakanties en weekendjes weg. Ontspanning draagt net zoveel bij aan dit proefschrift als inspanning.

Teamies, bedankt dat ik de onderzoeksfrustraties er samen met jullie, op het veld, lekker uit kon slaan. Zo kon ik weer met hernieuwde energie verder.

Joris, many thanks voor alle hulp bij de lay-out van dit boekje. Het is heel mooi geworden en ik ben er heel blij mee. En blij met jou, want je hebt me bergen werk uit handen genomen. Om zo iets te maken voor de perfectionistische Inge is bepaald niet makkelijk, maar iets waar jij prima mee om kan gaan. Wanneer ik allerlei gestreste sms-jes stuurde of je hier en daar wel aan had gedacht, gevolgd door 10 mails met punten die je absoluut niet moest onderschatten wanneer je een proefschrift moest lay-outen, dan kreeg ik gewoon een sms-je terug met de tekst ‘Inge, ik druk elke maand een boekje. Het komt goed.’ En zo werd ik weer even op m’n plek gezet en gedwongen de controle wat te laten vieren. En goede les voor mij.

Slimme Jaap, jou wil ik ook bedanken, want aan de hand van het format van jouw eigen proefschrift, konden wij het mijne maken. Dat heeft heel veel tijd bespaard. En niet alleen bij de afronding van het proefschrift ben jij van nut geweest, want gedurende het hele traject kon ik jou met vragen bestoken wanneer ik dat wou. Naast Einstein ken ik niemand die zo intelligent is als jij, en ik vind het bewonderenswaardig hoe jij altijd voor je vrienden klaar staat om deze intelligentie met ze te delen.

Marije, bedankt voor het zijn van mijn paranimf. Bedankt voor het helpen organiseren, het nemen van alle beslissingen van levensbelang en het meedenken over hoe ik dingen het beste aan kon pakken. Heel fijn dat voor deze periode het ‘je moet leren je eigen keuzes te maken, dus ik zeg lekker niks’- embargo even opgeheven kon worden. ;) Mede dank zij jouw hulp gaan we er een mooie dag van maken en een heel mooi feestje (er vanuit gaande dat ik het vragenvuur overleef natuurlijk ;))

Lieve mama en papa, door jullie ben ik geworden wie ik ben. Van jullie heb ik geleerd nieuwsgierig te blijven naar het onbekende, door te zetten wanneer het even tegen zit en te streven naar perfectie. Alle drie even belangrijke eigenschappen bij het voltooien van dit proefschrift.

Martine, mijn allerliefste zus, de afgelopen vier jaar hebben we heel wat uren aan de telefoon doorgebracht. Kletsend over alles in ons leven, maar toch ook voor een groot deel over onze onderzoeken. Naast jou ken ik vrij weinig mensen die net zo enthousiast worden als ik van de mededeling 'Jaaaa, ik heb een significante relatie gevonden!!' Ook bespraken we urenlang onze frustraties wanneer we een oneindigheid naar het computerscherm hadden lopen staren zonder een intelligent idee te hebben gekregen, wanneer onze databases niet deden wat wij wilden of wanneer deadlines ons angstaanjagend in de ogen keken. Bedankt voor al je steun de afgelopen jaren en bij de laatste loodjes als mijn paranimf!

Lieke, jij bent gewoon heel leuk. En mijn allerliefste zusje. Qua onderzoek was je misschien niet degene waar ik het meest op terugviel, want al probeerden we het wel eens, meestal eindigde dit in een: 'Ja, Inge, daar moet je misschien Martine maar even voor bellen.' Maar indirect heb ook jij aan dit proefschrift bijgedragen. Onder andere door je relativerende blik, waardoor ik dingen weer in perspectief ging zien. Daarnaast zorgen je persoonlijkheid en humor altijd voor een lach op m'n gezicht. En zonder ontspanning ook geen inspanning, dus de uren dat jij onder dwang spelletjes met me hebt gespeeld mogen hier ook wel even geroemd worden.

Ik prijs mezelf heel gelukkig met twee van zulke lieve zusjes waarbij ik ALTIJD terecht kan!

Tot slot, iedereen bedankt die het heeft volgehouden tot hier door te lezen. :)