Thromboembolic disease of the venous and the arterial system:

two different entities or two different sides of the same coin?

Bakhtawar Khan Mahmoodi

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This thesis is dedicated to memories of my first supervisor, Prof. Dr. Jan van der Meer. I owe him for my research inspirations!

Professor J. van der Meer and B.K. Mahmoodi discussing an e-mail from the editor of Circulation journal.

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Chapter 1

General introduction and outline of the thesis

GENERAL INTRODUCTION

Venous thromboembolism (VTE) is a collective term for pathological thrombus formation and embolization in the venous system. Deep vein thrombosis of the leg and pulmonary embolism represent the main phenotypes of VTE. The overall incidence of VTE in developed countries is about 0.15% per year, varying from less than 0.005% in individuals younger than 15 years to as high as 0.5% at the age of 80.[1-3] More than a century ago, Virchow postulated three main causes of thrombosis: stasis of the blood, changes in the vessel wall, and changes in the composition of the blood.[4] Known risk factors for VTE fall in the first (stasis) and the third groups (blood composition), though nowadays a different classification is made into genetic and acquired risk factors.[5] Well established acquired risk factors for VTE include immobilization, surgery, major trauma, pregnancy, puerperium, malignancy, hormonal replacement therapy or oral contraceptives and long-distance travel (i.e., >4 hours).[5] VTE that occurs within 3 months from the abovementioned acquired risk factors is considered provoked or secondary VTE.[6] In absence of these risk factors VTE is considered idiopathic or unprovoked. Well known genetic risk factors for VTE include factor V Leiden, prothrombin G20210A mutation and hereditary deficiencies of natural anticoagulant proteins (i.e., antithrombin, protein C or protein S).[5] However, in as many as 50% of VTE cases, none of these known risk factors are present.[3] Therefore, search for new risk factors of VTE is a matter of intensive ongoing research.

It is widely acknowledged that arterial thromboembolism (ATE), comprising coronary heart disease, stroke or transient cerebral ischaemic attack, and peripheral artery disease is one of the leading causes of death and disability particularly in the developed countries.[7] ATE is mostly secondary to atherosclerosis, which is caused by various risk factors, such as hypertension, hyperlipidemia, diabetes, smoking, obesity, microalbuminuria and metabolic syndrome.[7] These and other less prevalent atherosclerosis risk factors are pathogenetically interrelated and frequently cluster in individuals.

Association between atherosclerosis risk factors and VTE:

Thromboembolic diseases of venous and arterial systems have been historically viewed as two different diseases with distinct risk factors.[8] This notion was challenged in the last decade since an increased incidence of atherosclerosis or ATE had been observed in subjects with VTE, especially in subjects with

idiopathic VTE.[9-18] Moreover, an increasing amount of data indicates that classic atherosclerosis risk factors (i.e., hypertension, hyperlipidemia, diabetes, obesity, and smoking) may also predispose individuals to VTE, though results are some times inconsistent.[19-32] A recent meta-analysis that included a total of 21 studies,[19] predominantly case-control studies, reported that obesity, hypertension, diabetes mellitus, high levels of triglycerides and low high-density lipoproteins were all significantly related to VTE. However, these results should be interpreted with caution as for most of the evaluated atherosclerosis risk factors there was considerable heterogeneity and the results of cohort studies were not adjusted for age. Since cardiovascular risk factors such as hypertension, lipid levels, and diabetes are strongly correlated with older age and older age in itself is a strong risk factor for VTE, crude associations between these atherosclerosis risk factors and VTE will be observed as epiphenomena or innocent bystanders, rather than as causative risk factors. Furthermore, smoking and elevated levels of total cholesterol did not reach statistical significance as predictors of VTE, in this meta-analysis,[19] although a weak positive trend with odds ratios of 1.18 and 1.16 were observed, respectively. Dyslipidemia is especially interesting as a potential VTE risk factor, considering the results from the JUPITER trial (**J**ustification for the **U**se of Statins in Primary **P**revention: An **I**ntervention **T**rial **E**valuating **R**osuvastatin) demonstrating significant reduction in VTE risk in the intervention-arm treated with rosuvastatin.[33] It has been hypothesized, however, that this risk reduction is not due to the cholesterol lowering effect of this drug, but secondary to pleiotropic effects of statins.[34] First, in the placebo-arm of JUPITER trial there was no clear evidence of higher VTE risk in subjects with higher lipid levels. Secondly, there was a lack of association between dyslipidemia and VTE in several recent large prospective cohort studies.[20,27] The association between smoking and VTE remains also controversial. Two recent large studies from Denmark showed a dose response relationship between smoking and VTE.[27,30] These studies concluded that the lack of association between smoking and VTE in other studies might be at least partially due to lack of precision by pooling ex-smokers with current smokers or non-smokers, and lack of distinction between heavy and light smokers. Finally, of the mentioned atherosclerosis risk factors, obesity as measured by body mass index or by waist-hip ratio is the only atherosclerosis risk factor that was consistently related with elevated risk of VTE.[19,24,25,27,29,31]

Albuminuria, estimated glomerular filtration rate and risk of VTE:
Microalbuminuria (urinary albumin excretion of 30-299 mg/24h), macroalbuminuria (urinary albumin excretion of ≥300 mg/24h) and decreased estimated glomerular filtration rate (eGFR) are known risk factors for ATE [35-39] and define chronic kidney disease (CKD) according to the 2002 Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines.[40] K/DOQI classified CKD in 5 stages as presented in the **Table** with the estimated prevalence in the general population in the USA and the Netherlands. Estimates in the Netherlands are based on data from the **P**revention of **RE**nal and **V**ascular **EN**d-stage **D**isease (PREVEND) study.

Table. K/DOQI staging system of chronic kidney disease

Stage	eGFR (ml/min/ 1.73m^2)	Albuminuria (>30 mg/24h)	Estimated prevalence USA	Estimated prevalence The Netherlands
1	> 90	Mandatory	1.8 %	1.3 %
2	60 - 89	Mandatory	3.2 %	3.8 %
3	30 - 59	Not mandatory	7.7 %	5.3 %
4	15 - 29	Not mandatory	0.4 %	0.1 %
5	< 15 or RRT	Not mandatory	0.2 %	0.1 %
Total			13.3 %	10.6 %

eGFR denotes estimated glomerular filtration rate assessed by the Modification of Diet in Renal Disease (MDRD) Study formula and RRT denotes renal replacement therapy. For prevalence estimates in the USA see *Coresh et al*[41]*. JAMA. 2007* and for the estimates in the Netherlands, which are based on the PREVEND study data see *De Zeeuw et al.*[42] *Kidney Int. 2005*.

Whereas all stages of CKD are well recognized risk factors for ATE, its association with VTE is new. Stage 5 CKD, which is also called end-stage renal disease, is accompanied by uremic thrombopathy for which reason it was historically thought that these patients may have lower risk of VTE. However, recent studies demonstrated an increased risk of VTE in stage 5 CKD patients.[43] The association between CKD stage 3-4 and VTE in the general population was first described by investigators of the Longitudinal Investigation of Thromboembolism Etiology (LITE) project,[44] using the Modification of Diet in Renal Disease (MDRD) equation.[45] The LITE project comprises the pooled data of the Atherosclerosis Risk

in Communities (ARIC) study and the Cardiovascular Health Study (CHS).[46,47] Recently the same group assessed the VTE risk in CKD 3-4 in the ARIC cohort only,[46] using a cystatin C based and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[48,49] Stage 3 to 4 CKD, based on eGFR cystatin C, but not eGFR CKD-EPI, was associated with an approximately 1.6-fold increased risk of VTE.[50] These findings contrast with early follow-up of the LITE project.[44] The authors ascribed this to potential unknown confounders and differences in creatinine measurements as creatinine measures of different follow-up periods were used. Stage 1 and 2 CKD are defined by normal or slightly decreased eGFR in the presence of additional signs of kidney damage. In the setting of epidemiological research these additional signs of kidney damage are based on the presence of albuminuria of ≥30 mg/24h. The association of stage 1 and 2 CKD with VTE has not been investigated previously and has been described in this thesis for the first time (**Table**). The pathophysiological mechanisms of the association of albuminuria of ≥30 mg/24h with VTE are unknown. In accordance with the Virchow triad, generalized endothelial dysfunction and the concomitant changes in the levels of several coagulation proteins including elevated levels of factor VIII and plasminogen activator inhibitor, may account for the higher risk of VTE in subjects with albuminuria of ≥30 mg/24h.[51-53] Moreover, given the new findings of a positive association of classic atherosclerosis risk factors (i.e. hypertension, hyperlipidemia, diabetes, obesity, and smoking) with VTE and the well known association of albuminuria of ≥30 mg/24h with the classic atherosclerosis risk factors, necessitates appropriate adjustment for classic atherosclerosis risk factors.

Nephrotic syndrome and risk of both VTE and ATE:

Overt proteinuria in patients with nephrotic syndrome is considered an established risk factor for VTE, whereas its association with ATE is relatively less well recognized.[54-56] Of note, in patients with diabetes, overt proteinuria is a well-known strong predictor for ATE.[57] The nephrotic syndrome is characterized by urinary protein losses in excess of 3.5 g/24h in association with hypoalbuminemia, hypercholesterolemia, and peripheral edema due to renal sodium retention. The nephrotic-range proteinuria (proteinuria ≥3.5 g/24h) is the main component of the nephrotic syndrome, and its association with the remaining components of nephrotic syndrome is variable. Diabetic nephropathy is the most common cause of nephrotic-range proteinuria.[55] In non-diabetics several primary glomerular diseases such as membranous glomerulopathy, minimal change disease, focal segmental

glomerulosclerosis and membranoproliferative glomerulonephritis account for the great majority of cases of the nephrotic-range proteinuria.[55] In the remaining cases the nephrotic-range proteinuria is caused by a wide range of diseases such as systemic diseases (e.g. lupus erythematosus), infectious diseases, heredofamilial syndromes (e.g. Alport syndrome) and certain drugs.[55] Although reliable data are lacking, it is likely that the risk of both VTE and ATE may also vary according to the underlying lesion accounting for the nephrotic-range proteinuria. Examples include nephrotic syndrome due to diabetic nephropathy predisposing for ATE and membranous glomerulopathy predisposing for VTE. Low levels of antithrombin due to urinary loss and alterations in plasma levels of various proteins involved in coagulation are considered to be the main predisposing factors for thromboembolism in patients with nephrotic-range proteinuria.[56,58,59] Enhanced platelet aggregation, decreased fibrinolysis, hyperviscosity and hyperlipidemia are other less often postulated mechanisms that may be responsible for the prothrombotic state in these patients.[56,60-62] As is true for microalbuminuria, nephrotic-range proteinuria is also associated with prominent endothelial dysfunction.[63] Experimental data support a role for hypoalbuminemia in the vascular dysfunction in nephrotic-range proteinuria.[64,65]

Hereditary thrombophilia and risk of both VTE and ATE:

Hereditary thrombophilic deficiencies of the natural anticoagulant proteins (i.e., antithrombin, protein C and protein S) are considered the strongest hereditary risk factors for VTE;[5,66,67] however, their association with ATE is controversial.[68,69] Whereas studies regarding these deficiencies and the risk of VTE are primarily conducted in thrombophilic families, the few available studies on ATE risk are mainly case-control studies in unrelated individuals. This study design may have led to inappropriate identification of hereditary deficiencies of protein S, protein C or antithrombin for the ATE end-point. Because acquired deficiencies of protein S, protein C or antithrombin are more prevalent than hereditary deficiencies of these proteins. Nevertheless, generally coagulation defects are considered more relevant for the pathogenesis of VTE as compared to ATE.[70,71]

Given the low prevalence of these thrombophilic defects (0.1–0.4% each in the general population), even the absolute risks of VTE in individuals with these deficiencies are mainly based on retrospective data.[6,66,67] Though search for new hereditary risk factors of VTE is a matter of ongoing research, some experts question the clinical implications of screening for known hereditary risk factors.[72]

Because in the general population long-term oral anticoagulant treatment is associated with a major bleeding risk of about 2.8% per year,[73] which outweighs the risk of VTE, there is reluctance to advocate long-term primary prophylaxis in asymptomatic subjects with protein S, protein C or antithrombin deficiencies.[74] As about 50% of VTE cases are provoked by acquired risk factors, transient thromboprophylaxis at exposure to acquired risk factors is nowadays the recommended approach for primary prevention of VTE, even in non-deficient subjects.[75] Thus, the effect of screening asymptomatic relatives of patients with protein S, protein C or antithrombin deficiencies and recommendation of subsequent preventative measures on VTE incidence has not been evaluated and was for the first time assessed in the current thesis.

In summary, whether VTE and ATE are two different entities, or two different phenotypes of the same disease, is a topic that currently is in the spotlight of cardiovascular research and this topic has been the main focus of research presented in the current thesis.

Outline of this thesis

In the current thesis the association between VTE and ATE is studied by evaluating the association of established ATE risk factors with VTE incidence and the association of deficiencies of the natural anticoagulant proteins with ATE incidence. Moreover, renal disease is considered as a potential shared risk factor for both VTE and ATE, and pathophysiological mechanisms of the prothrombotic state in proteinuric patients are assessed.

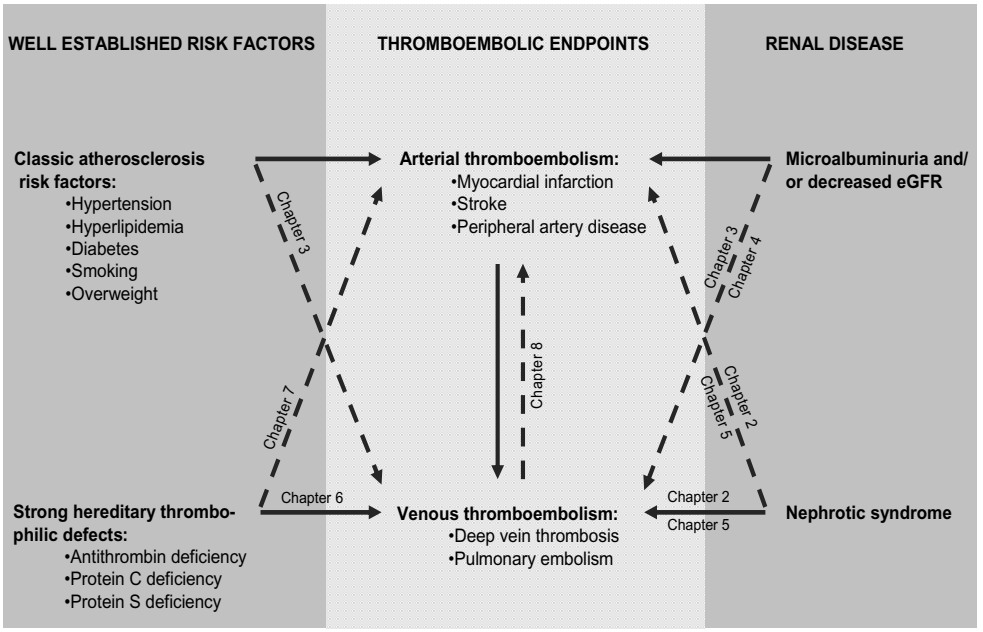

Figure. Outline of this thesis.

Solid arrows represent well-established association between the risk factors and the thromboembolic endpoints of interest. Dashed arrows represent controversial or new associations between the risk factors and the endpoints that are evaluated in current thesis. The corresponding chapters of this thesis that address the depicted associations are presented.

As depicted in the **Figure**, the association between nephrotic syndrome and ATE is relatively less well recognized as compared to the risk of VTE in non-diabetic patients with nephrotic-range proteinuria. Moreover, absolute risks of either VTE or ATE in patients with nephrotic-range proteinuria have not been assessed previously. In **chapter 2** we assess the absolute risks of both VTE and ATE in a

retrospective study investigating a large cohort of 298 patients with nephrotic-range proteinuria. Moreover, we attempt to identify predictive factors for incident ATE and VTE. The diagnosis of nephrotic-range proteinuria was defined as by proteinuria of ≥3.5 g/d, mainly derived from a 24-hour urine collection. Data on other components of the nephrotic syndrome, that is, hypoalbuminemia (serum albumin <3.4 g/dL), hypercholesterolemia, hypertriglyceridemia and edema were retrieved from medical records, but were not mandatory.

In contrast to the situation with nephrotic-range proteinuria, the association of microalbuminuria, classic atherosclerosis risk factors and decreased eGFR with ATE is well established; however, its association with VTE is not assessed, inconsistent or warrants confirmation, respectively (**Figure**). In **chapter 3** we evaluate the association of these risk factors with the risk of VTE in participants of the PREVEND study, which is an ongoing community-based prospective cohort study. The PREVEND study was designed to investigate prospectively the natural course of albuminuria and its relation to renal and cardiovascular disease. In brief, during 1997-1998, all 85,421 inhabitants of the city of Groningen, the Netherlands, between the ages of 28 and 75 years old were sent a 1-page postal questionnaire regarding demographics, cardiovascular morbidity, use of medication, and pregnancy, and a vial to collect a first morning void urine sample. A total of 40,856 (47.8%) individuals responded, of whom a sample of 8,592 subjects enriched for higher levels of albuminuria completed the screening protocol and formed the baseline PREVEND cohort. In **chapter 4** we assess the association of decreased eGFR with VTE incidence, using the K/DOQI classification for CKD, and study whether this association is independent of microalbuminuria status.

Since proteinuria ranging from microalbuminuria to nephrotic-range proteinuria turned out to be related to higher risk of thromboembolism, we opted to assess the exact coagulation disturbances in patients with overt proteinuria (**chapter 5**). Moreover, we also evaluated whether antiproteinuric treatment with losartan reverses the prothrombotic state in proteinuric patients. Of note, thus far the exact mechanism of the prothrombotic state in nephrotic syndrome is unknown. It is assumed that the decrease in serum albumin, due to urinary loss, is sensed by the liver that, in turn, up-regulates the production of all liver-synthesized proteins, including various coagulation factors. Studies evaluating the impact of intervention in the renin-angiotensin system, which is nowadays the cornerstone of

antiproteinuric treatment, on coagulation disturbances in patients with overt proteinuria have not been conducted previously.

Hereditary deficiencies of antithrombin, protein C and protein S are well established strong hereditary risk factors for VTE, though their association with ATE is controversial (**Figure**). Any reported association between deficiencies of these natural anticoagulant proteins and VTE is mainly based on retrospective studies. Thus, whether screening and subsequent preventative measures in asymptomatic relatives of these patients is effective in VTE risk reduction has yet to be addressed. Due to the low prevalence of hereditary protein S, protein C and antithrombin deficiencies in the general population, family-cohort studies will be most suitable for addressing the association between these deficiencies and thromboembolism. Therefore, we used the data from the **DE**ficiencies of protein **S**, protein **C** and **A**ntithrombin and the absolute **R**isk of **T**hrombo**E**mbolism **S**tudy (DESCARTES) that contained three cohorts of families with hereditary deficiencies of either protein S, protein C or antithrombin. Probands were consecutive patients with VTE who had one of these deficiencies. First-degree relatives >15 years of age were identified by pedigree analysis. Detailed data on previous episodes of VTE and ATE, risk factors for atherosclerosis and anticoagulant treatment, were collected by using a standardized questionnaire and reviewing medical records. Blood samples were taken after clinical data had been collected. In **chapter 6** we assess in a prospective analysis the risk of VTE and the impact of screening on VTE risk in relatives of antithrombin- protein C- or protein S-deficient patients. In **chapter 7** the association between these hereditary thrombophilic defects and incident ATE is assessed.

Finally, whereas the increased risk of VTE in patients with previous ATE, in particular in patients with stroke, is well recognized, a possibly higher risk of incident ATE in subjects with a history of VTE is new and studies addressing the absolute risks of ATE after VTE have not been conducted, previously. In **chapter 8** we report the absolute risk of subsequent ATE after a VTE in the more than 40.000 baseline participants of the PREVEND study.

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Chapter 2

High absolute risks and predictors of venous and arterial thromboembolic events in patients with nephrotic syndrome:

Results from a large retrospective cohort study

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ABSTRACT

Background: No data are available on the absolute risk of either venous thromboembolism (VTE) or arterial thromboembolism (ATE) in patients with nephrotic syndrome. Reported risks are based on multiple case reports and small studies with mostly short-term follow-up. We assessed the absolute risk of VTE and ATE in a large, single-center, retrospective cohort study and attempted to identify predictive factors in these patients.

Methods and Results: A total of 298 consecutive patients with nephrotic syndrome (59% men; mean age, 42±18 years) were enrolled. Mean follow-up was 10±9 years. Nephrotic syndrome was defined by proteinuria ≥3.5 g/d, and patients were classified according to underlying histological lesions accounting for nephrotic syndrome. Objectively verified symptomatic thromboembolic events were the primary study outcome. Annual incidences of VTE and ATE were 1.02% (95% confidence interval, 0.68 to 1.46) and 1.48% (95% confidence interval, 1.07 to 1.99), respectively. Over the first 6 months of follow-up, these rates were 9.85% and 5.52%, respectively. Proteinuria and serum albumin levels tended to be related to VTE; however, only the predictive value of the ratio of proteinuria to serum albumin was significant (hazard ratio, 5.6; 95% confidence interval, 1.2 to 26.2; P=0.03). In contrast, neither the degree of proteinuria nor serum albumin levels were related to ATE. Sex, age, hypertension, diabetes, smoking, prior ATE, and estimated glomerular filtration rate predicted ATE (P≤0.02).

Conclusions: This study verifies high absolute risks of symptomatic VTE and ATE that were remarkably elevated within the first 6 months. Whereas the ratio of proteinuria to serum albumin predicted VTE, estimated glomerular filtration rate and multiple classic risk factors for atherosclerosis were predictors of ATE.

INTRODUCTION

The first observations of an increased risk of venous (VTE) and arterial thromboembolism (ATE) in patients with nephrotic syndrome (NS) date back to >50 years ago.[1,2] In the ensuing half-century, VTE rates ranging from 2% in children to as high as 42% in adults[3–6] and a relative risk of ATE ranging from 1 to 5.5 have been reported in these patients.[7,8] Whereas NS resulting from membranous glomerulopathy has been correlated with an exceptionally high risk of VTE, especially renal vein thrombosis,[9,10] likewise correlations have not been described for ATE.[7,8]

The pathophysiological mechanisms of thromboembolism in patients with NS have yet to be unraveled. Nevertheless, alterations in plasma levels of proteins involved in coagulation and fibrinolysis, enhanced platelet aggregation, low plasma albumin, hyperviscosity, and hyperlipidemia, as well as treatment with corticosteroids and diuretics, are considered predisposing factors for the development of thromboembolic events.[5,6,10–16]

The reported risks of VTE and ATE in patients with NS are based on numerous case reports and small studies with mostly short-term follow-up and therefore are of limited accuracy. Data on the absolute risk of either VTE or ATE are not available. We conducted a single-center retrospective study to assess the absolute risk of symptomatic VTE and ATE in a large cohort of patients with NS. We also attempted to identify predictive factors.

METHODS

Study Patients

Consecutive patients with NS, seen between January 1995 and December 2004 at our outpatient nephrology clinic, were retrospectively identified from computer-stored hospital files. Patients who at time of study entry (February 2005) were ≥18 years of age, had not been diagnosed with acute life-threatening diseases, and had been followed up for at least 6 months at our center were enrolled. The diagnosis of NS was confirmed by proteinuria of ≥3.5 g/d, derived from a 24-hour urine collection, or a protein-to-creatinine ratio (7 patients) calculated from a single urine sample.[17] Hypoalbuminemia (serum albumin <3.4 g/dL), hypercholesterolemia, and hypertriglyceridemia were recorded but were not a requisite for the diagnosis of NS.[18] On the basis of histology of a percutaneous renal biopsy or the clinical context, patients were classified as having membranous glomerulopathy, minimal change disease, focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, diabetic nephropathy, and NS not otherwise specified.[18]

Medical records were reviewed for symptomatic thromboembolic events and exposure to risk factors for VTE and atherosclerosis, respectively. Risk factors for VTE included major surgery, trauma, malignancy, immobilization for >1 week, use of oral contraceptives, hormonal replacement therapy, and pregnancy. Risk factors for atherosclerosis, recorded at baseline (ie, diagnosis of NS), were hypertension, as defined by a systolic blood pressure of ≥140 mm Hg or ≥160 mm Hg in patients ≥60 years of age or a diastolic blood pressure of ≥90 mm Hg measured on at least 2 occasions or the use of antihypertensive drugs; diabetes mellitus[19,20]; cigarette smoking; and hyperlipidemia, defined by levels of total cholesterol >6.5 mmol/L (250 mg/dL) or triglycerides >2.5 mmol/L (220 mg/dL) or the use of lipid-lowering drugs. The diagnosis of a multisystem disease was documented. End-stage renal disease was defined by application of regular dialysis.

Proteinuria, serum albumin, cholesterol, triglycerides, and creatinine were recorded at baseline. In adults, the glomerular filtration rate (GFR) was estimated (eGFR) with a validated prediction formula[21]: $eGFR=30{,}849 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if black})$, where eGFR is measured in mL/min/1.73m^2 and age is in years. In children (<18 years of age), creatinine clearance was taken as measure for renal function.

Diagnosis of Thromboembolic Events

Only objectively verified symptomatic thromboembolic events were considered. Deep vein thrombosis (DVT) was confirmed by compression ultrasound; pulmonary embolism, by ventilation and perfusion lung scanning or spiral computed tomography; renal vein thrombosis, by venography or Doppler ultrasound; and mesenteric vein thrombosis, by computed tomography scanning. Unstable angina pectoris and Q-wave and non–Q-wave myocardial infarction were confirmed by typical ECG features, elevated levels of cardiac enzymes, radionuclide imaging techniques, or coronary angiography. Ischemic stroke was documented by computed tomography scanning or magnetic resonance imaging; peripheral artery disease was documented by intraarterial or magnetic resonance angiography. Cerebral transient ischemic attack required neurological symptoms and signs lasting <24 hours.[22] Amaurosis fugax was established when sudden monocular blindness lasted <24 hours.

Statistical Analysis

We calculated annual incidences of VTE and ATE by dividing the number of events by the total number of observation years. The observation time for each patient was defined as the period from the diagnosis of NS until the first episode of thromboembolism, a censoring event (end-stage renal disease or death), or end of study. When calculating the incidence rates of VTE, we ignored the occurrence of ATE and vice versa. The 95% confidence intervals (CIs) around the annual incidences were assessed with the Poisson distribution assumption.

Kaplan-Meier[23] methods were used for survival plots. To evaluate the effects of baseline characteristics on VTE- and ATE-free survival, we used the Cox proportional-hazards model with a single covariate.[24] Results were expressed as hazard ratios with 95% CIs and probability values.

Continuous variables are presented as mean±SD and categorical data as numbers and frequencies. For continuous data, differences were evaluated by the Student t test, Mann-Whitney U test, Kruskal-Wallis test, or univariate ANOVA, depending on the normality of the data and levels of the outcome variable. Categorical variables were compared with chi-square or Fisher's exact test. Statistical significance was considered at a 2-tailed value of $P<0.05$. Statistical analyses were

performed with SPSS software version 14.0 (SPSS Inc, Chicago, Ill, USA) and SAS software, version 9.1 (SAS Institute Inc, Cary, NC, USA).

RESULTS

Patients

Table 1 shows the clinical characteristics at diagnosis of NS of the total study population of 298 patients and subgroups according to the underlying nephropathies. Whereas all cases classified as membranous glomerulopathy, minimal change disease, focal segmental glomerulosclerosis, and membranoproliferative glomerulonephritis were confirmed by renal biopsy; the diagnoses of diabetic nephropathy and NS not otherwise specified were 28% and 46% based on renal biopsy, respectively. NS in the not-otherwise-specified group was due to systemic diseases (19%), infections (8%), heredofamilial diseases (8%), clinically suspected primary glomerular diseases that were not confirmed by biopsy (19%), biopsy-proven cases of IgA nephropathy (17%), and miscellaneous diseases (28%).

Overall, the mean±SD age was 42±18 years; 59% of patients were male; 10% were <18 years of age; 61% had hypertension, 92% had hyperlipidemia, 14% had diabetes, and 10% had a prior thromboembolic event at diagnosis of NS; and 50% reported ever having smoked. For individual patients, the mean observation period was 10±8 years for VTE and 10±9 years for ATE. The observation period differed among subgroups (P<0.001). Compared with patients with nondiabetic nephropathies, patients with diabetic nephropathy were older (P=0.005), had a higher prevalence of hypertension (P<0.001) and prior ATE (P=0.005), and had lower eGFR (P<0.001), less proteinuria (P=0.003), and higher serum albumin levels (P=0.02). Among patients with nondiabetic nephropathies, age, serum cholesterol, serum albumin, proteinuria, and eGFR varied significantly (P=0.003) according to the type of nephropathy.

Table 1. Characteristics of the Study Population

Variable	Total	MG	MCD	FSGS	MPGN	DN	NOS
Baseline characteristic							
Patients, n (%)	298 (100)	72 (24)	49 (16)	36 (12)	26 (9)	32 (11)	83 (28)
Male sex, n (%)	177 (59)	49 (68)	27 (55)	21 (58)	9 (35)	18 (56)	53 (64)
Age, y	42±18	45±17	36±18	39±18	31±16	50±13	43±19
Hypertension, n (%)	182 (61)	43 (60)	23 (47)	16 (44)	15 (58)	30 (94)	55 (66)
Diabetes, n (%)	42 (14)	3 (4)	0 (0)	3 (8)	1 (4)	32 (100)	3 (4)
Hyperlipidemia, n (%)*	221(92)	64 (98)	47 (100)	29 (97)	20 (95)	21 (91)	40 (73)
Prior VTE, n (%)	3 (1)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2)
Prior ATE, n (%)	28 (9)	9 (13)	2 (4)	2 (6)	0 (0)	8 (25)	7 (8)
Ever smoked, n (%)*	116 (50)	33 (59)	13 (37)	14 (50)	11 (44)	20 (67)	25 (42)
MSD, n (%)	28 (9)	2 (3)	2 (4)	0 (0)	5 (19)	0 (0)	19 (23)
Laboratory measurements†							
Proteinuria, g/day	8.1±5.2	9.7±6.5	9.2±5.4	10.2±5.9	6.5±2.6	6.0±2.5	6.4±3.6
eGFR, ml/min/1.73m^2	59±28	71±23	78±32	58±23	70±27	36±18	46±25
Albumin, g/L	28±8	26±7	24±7	29±10	28±9	33±8	31±9
Cholesterol, mmol/L	9.0±3.5	9.9±3.0	10.6±4.0	9.3±2.9	8.4±2.9	7.4±2.8	7.4±3.5
Triglycerides, mmol/L	3.0±2.1	3.3±2.3	2.3±1.3	3.1±1.8	2.4±1.4	4.1±4.1	3.0±1.2
Follow-up							
VTE observation period, y	10±8	10±8	13±11	10±7	15±10	5±5	7±6
ATE observation period, y	10±9	11±8	13±11	11±7	15±10	5±4	7±7
ESRD, n (%)	39 (13)	5 (7)	1 (2)	7 (19)	6 (23)	6 (19)	14 (17)
Death, n (%)	15 (5)	4 (6)	2 (4)	2 (6)	0 (0)	3 (9)	4 (5)

MG indicates membranous glomerulopathy; MCD, minimal change disease; FSGS, focal segmental glomerular sclerosis; MPGN, membranoproliferative glomerulonephritis; DN, diabetic nephropathy; NOS, nephrotic syndrome not otherwise specified; MSD, multisystem disease; and ESRD, end-stage renal disease. Values are mean±SD when appropriate. To convert values for cholesterol to milligrams per deciliters, divide by 0.0259; to convert values for triglycerides to milligrams per deciliters, divide by 0.0113.

*Hyperlipidemia and smoking status were unknown for 57 and 64 patients, respectively.

†Proteinuria, eGFR, serum albumin, serum cholesterol, and serum triglycerides measurements were available for 272, 251, 184, 142, and 117 patients, respectively.

Thromboembolic Events

Twenty-nine patients had at least 1 episode of VTE during the observation period, corresponding to an annual incidence of 1.02% (95% CI, 0.68 to 1.46) (**Table 2**). The median observation period until VTE was 0.9 years (interquartile range, 17.3 years). The most commonly encountered first VTE was pulmonary embolism (38%), followed by DVT (34%), combined pulmonary embolism and DVT (10%),

combined pulmonary embolism and renal vein thrombosis (10%), renal vein thrombosis (3%), and mesenteric vein thrombosis (3%). None of these patients had VTE before they received a diagnosis of NS. No fatal VTE was observed. At the onset of VTE, only 6 patients (21%) were exposed to another risk factor for VTE (immobilization, n=2; malignancy, n=2; surgery, n=1; and use of oral contraceptives, n=1]. The annual incidence of VTE, calculated over the first 6 months of observation time, was 9.85% (95% CI, 5.38 to 16.52).

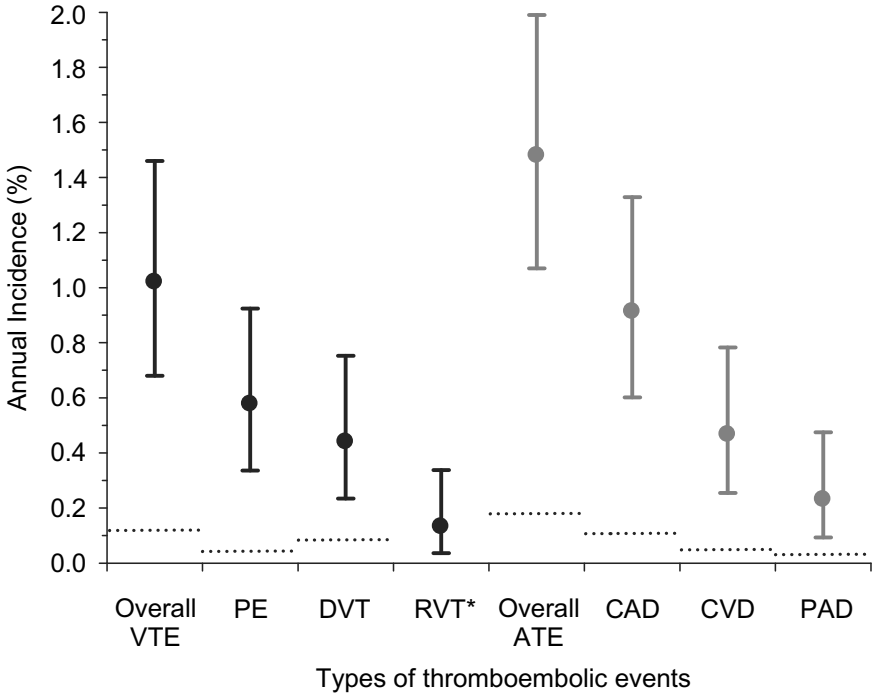
Table 2. Risk of thromboembolism by type of nephropathies

Variable	Total (n = 298)	MG (n = 72)	MCD (n = 49)	FSGS (n = 36)	MPGN (n = 26)	DN (n = 32)	NOS (n = 83)
VTE							
No. events	29	10	4	5	5	1	4
Observation period, y	2857	716	645	362	378	171	585
Annual Incidence (95%CI)-%	1.02 (0.68–1.46)	1.40 (0.67–2.57)	0.62 (0.17–1.59)	1.38 (0.45–3.22)	1.32 (0.43–3.09)	0.58 (0.01–3.26)	0.68 (0.19–1.75)
ATE							
No. events	43	10	2	6	2	11	12
Observation period, y	2904	762	656	390	396	148	551
Annual Incidence (95%CI)-%	1.48 (1.07–1.99)	1.31 (0.63–2.41)	0.30 (0.04–1.10)	1.54 (0.56–3.35)	0.51 (0.06–1.82)	7.43 (3.71–13.3)	2.18 (1.13–3.80)

Abbreviations as in Table 1

Forty-three patients had ≥1 ATE during the observation period, resulting in an annual incidence of 1.48% (95% CI, 1.07 to 1.99) (**Table 2**). The median observation period until ATE was 3.5 years (interquartile range, 7.3 years). The most commonly observed first ATE was myocardial infarction (44%), followed by unstable angina pectoris (14%), peripheral artery disease (14%), ischemic stroke (11.5%), cerebral transient ischemic attack (11.5%), amaurosis fugax (2%), and aorta thrombosis (2%). Three of these events (7%) were fatal. Fifteen of the 43 patients (35%) had already experienced ATE before the diagnosis of NS. The annual incidence of ATE, calculated over the first 6 months of observation time, was 5.52% (95% CI, 2.38 to 10.87). The annual incidence in nondiabetic patients without prior ATE was 0.82% (95% CI, 0.52 to 1.25) compared with 7.08% (95% CI, 3.77 to 12.10) in diabetics, including patients with prior ATE. Only 4 patients

had both ATE and VTE. The annual incidence of any thromboembolic event (ATE, VTE, or both) was 2.50% (95% CI, 1.94 to 3.18). Annual incidences for different types of thromboembolism are depicted in **Figure 1**.



No. patients	29	17	13	4	43	27	14	7
Observation period, y	2857	2944	2953	3034	2904	2957	3001	3037

Figure 1. Annual incidences per types of thromboembolism among the total cohort. Some patients are listed in 2 or 3 categories; therefore, the total of these categories exceeds 29 incidents of VTE and 43 of ATE. Solid circles denote annual incidences of VTE (black) and ATE risk (grey), with the corresponding 95% CIs represented by the vertical bars. Dotted lines represent the estimated age- and sex-weighted annual incidences in the general population (i.e., Worcester DVT study for VTE and the Framingham study for ATE).[25,26]

PE indicates pulmonary embolism; RVT, renal vein thrombosis; CAD, coronary artery disease (unstable angina pectoris or myocardial infarction); CVD, cerebrovascular disease (ischemic stroke or transient ischemic attack); and PAD, peripheral artery disease. *Annual incidence in the general population is unknown.

The probability of VTE-free survival was 69% and of ATE-free survival was 73% over 25 years of observation time (**Figure 2**). The highest risk of VTE was observed within the first 6 months and between 20 and 25 years. The likelihood of any event-free survival was 52%.

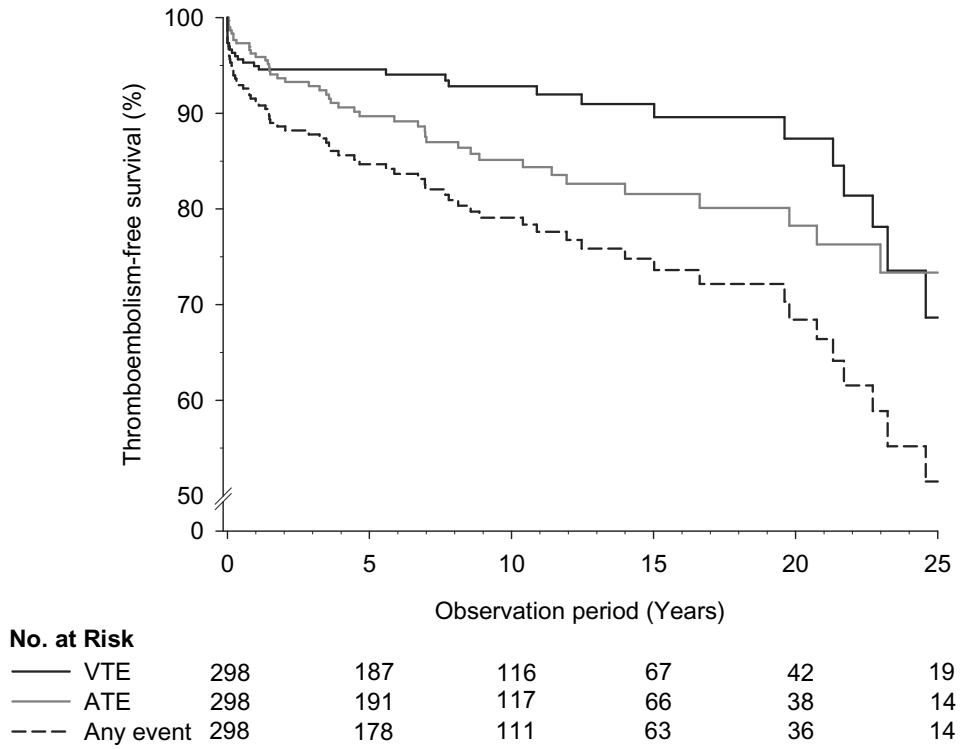


Figure 2. Kaplan-Meier estimates of the probability of VTE-, ATE-, and any (VTE, ATE, or both) event-free survival.

Predisposing Factors

Figure 3 shows the association of various variables at baseline with the risk of VTE and ATE. Overall, the extent of proteinuria tended to be associated with VTE. Statistical significance was reached at a level of ≥8.2 g/d (hazard ratio, 5.2; 95% CI, 1.1 to 23.0; P=0.03). Serum albumin showed an inverse relationship with VTE, although not significant (P=0.41). The ratio of proteinuria to serum albumin, analyzed as a continuous variable, was a more accurate predictor of VTE (hazard

ratio, 5.6; 95% CI, 1.2 to 26.2; P=0.03). The ratio of proteinuria to serum albumin was more sensitive and more specific than either proteinuria or serum albumin (data not shown). In contrast, neither the degree of proteinuria nor the level of serum albumin predicted ATE. Sex, age, hypertension, diabetes, smoking, prior ATE, and eGFR were significantly associated with ATE.

		VTE			ATE	
Variable	**no. patients/ no. studied**	**HR (95% CI)**	**P value**	**HR (95% CI)**	**HR (95% CI)**	**P value**
Sex, male	177/298	1.2 (0.6 - 2.6)	0.64		2.2 (1.1 - 4.5)	0.02
Age, y			0.84			< 0.001
< 35	117/298	1.0, reference			1.0, reference	
35 - 54	102/298	1.3 (0.5 - 3.0)			7.9 (2.7 - 23.5)	
≥ 55	79/298	1.1 (0.4 - 3.1)			14.2 (4.7 - 42.4)	
Hypertension	182/298	1.1 (0.5 - 2.4)	0.82		2.5 (1.2 - 5.1)	0.01
Hyperlipidemia	221/241	1.7 (0.2 - 12.6)	0.61		0.6 (0.2 - 1.7)	0.34
Diabetes	42/298	0.8 (0.2 - 3.3)	0.72		4.8 (2.4 - 9.5)	< 0.001
Smoking	116/234	2.0 (0.9 - 4.7)	0.10		2.8 (1.4 - 5.5)	0.003
Prior ATE	28/298	0.6 (0.1 - 4.2)	0.57		11.8 (6.0 -23.3)	< 0.001
Prior VTE	3/298	NE	-		5.3 (0.7 - 39.5)	0.10
MSD	28/298	1.1 (0.3 - 3.6)	0.90		0.7 (0.2 - 2.1)	0.47
Proteinuria, g/d			0.08			0.79
3.5 - 4.8	92/272	1.0, reference			1.0, reference	
4.9 - 8.1	91/272	3.1 (0.6 - 15.0)			0.8 (0.4 - 1.7)	
≥ 8.2	89/272	5.2 (1.1 - 23.0)			0.8 (0.4 - 1.8)	
eGFR, ml/min/1.73m^2			0.96			< 0.001
≥ 60	127/251	1.0, reference			1.0, reference	
30 - 59	85/251	0.9 (0.3 - 2.6)			4.9 (2.2 - 11.3)	
≤ 29	39/251	1.0 (0.2 - 4.9)			7.5 (2.8 - 20.0)	
Serum albumin, g/dl			0.41			0.25
≥ 3.4	50/184	1.0, reference			1.0, reference	
3.3 -2.5	67/184	1.6 (0.3 - 8.2)			0.5 (0.2 - 1.4)	
≤ 2.4	67/184	2.6 (0.5 - 12.4)			0.5 (0.2 - 1.2)	
P/A ratio	157/298	5.6 (1.2 - 26.2)	0.03		1.7 (0.4 - 6.5)	0.46

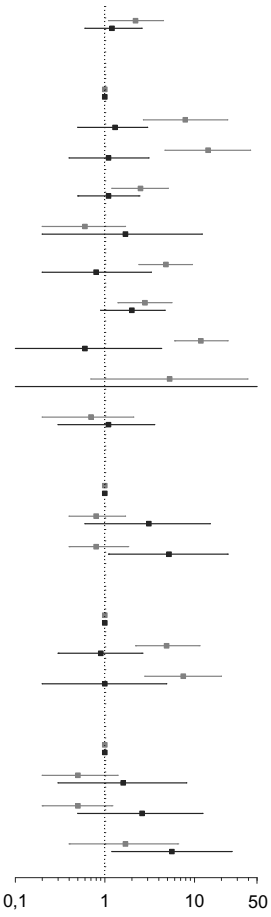


Figure 3. Proportional-hazards analysis of association with the time to the first VTE and ATE among the total cohort. Solid squares denote the hazard ratios of VTE (black) and ATE (red), with the corresponding 95% CIs represented by the horizontal bars. HR indicates hazard ratio; MSD, multisystem disease; NE, not estimatable; and P/A ratio, ratio of proteinuria to serum albumin.

DISCUSSION

This study shows a high risk of VTE and ATE in patients with NS. Absolute risks of VTE (1.02% per year) and ATE (1.48% per year) were each about 8 times higher in these patients than the estimated age- and sex-weighted annual incidences in the general population (the Worcester DVT study, 0.12%; and the Framingham study, 0.18%, respectively).[25,26] Risks of both VTE and ATE were particularly high within the first 6 months of NS (annual incidences, 9.85% and 5.52%, respectively). Survival analysis showed that over 25 years of observation, the probability of a thromboembolic event was 48%, with the risk of VTE being approximately equal to the risk of ATE. Whereas the risk of VTE was roughly similar among the different groups of NS, the risk of ATE was remarkably high in patients with diabetic nephropathy compared with patients with nondiabetic nephropathies. For the first time, we report that the ratio of proteinuria to serum albumin predicts VTE and that multiple classic risk factors for atherosclerosis are associated with ATE in patients with NS, including sex, age, hypertension, diabetes, smoking, prior ATE, and eGFR.

Our finding of the increased risk of VTE is in agreement with previous reports. In our study, 10% of patients experienced symptomatic VTE, confirmed by objective techniques, during a total observation period of 2857 years. Annual incidences were not provided in previous studies. Not considering the observation period, the incidence rate of VTE in adults with NS ranged from 8% to 42%, with an overall incidence of 23%.[5] However, risk estimates in those studies were based on small numbers of patients and asymptomatic cases of renal vein thrombosis in particular; it often was not clear whether thrombosis at other sites was objectively verified and whether consecutive patients were evaluated.[4,5,27,28] Renal vein thrombosis especially has received attention with an overall incidence rate of 28%, ranging from 22% to 42%.[10] However, the high rates of renal vein thrombosis in previous reports reflect the vigor of the investigators; 90% of renal vein thromboses were demonstrated in clinically asymptomatic patients.[9] Whereas the ratio of pulmonary embolism to DVT in the general population is approximately 1:2,[25] it was 1.3:1 in our study. Our finding of the high risk of pulmonary embolism could be explained by an apparently large proportion of asymptomatic renal vein thrombosis.

Although there has been controversy in the past about the association of NS and ATE,[29] an increased risk of coronary events has been documented in a retrospective, controlled study in 142 patients with NS.[8] After adjustment for age, sex, hypertension, and smoking, the relative risk of myocardial infarction in these patients was 5.5, and its annual incidence was 1.49%. Our absolute risk estimates were lower when we confined our analysis to nondiabetic patients without prior ATE (0.82% versus 1.49%), as Ordonez et al[8] did. This difference might reflect our longer follow-up because annual incidences of ATE over a comparable follow-up were 2.71% in the total cohort and 1.20% in nondiabetic patients without prior ATE in our study. The somewhat lower risk in our patients could be ascribed to application of statins (51% patients) and renin-angiotensin system inhibitors (81% patients) because these drugs were not available during the period in which patients in the study of Ordonez et al[8] were followed up. The beneficial effect of these drugs is supported by a 3-fold-lower annual incidence of end-stage renal disease in our patients (1.4% versus 4.3%). Furthermore, our patients were on average 6 years younger (38 versus 44 years). Given that the risk estimates by Ordonez et al[8] were based on only 11 episodes of myocardial infarction, their study size was less than half of ours, and the fact that they documented only coronary events, we presume that our estimates reflect a more accurate assessment of the absolute ATE risk conferred by NS. In addition, we established that compared with the general population,[26] the risks of cerebrovascular events and peripheral artery disease were elevated about the same extent as the risk of coronary artery disease in these patients.

The association between serum albumin levels and VTE has previously been suggested,[13] although others failed to confirm this finding.[4,9,10] We demonstrated that the ratio of proteinuria to serum albumin was a stronger predictor because it is probably a better reflector of the severity of NS than serum albumin levels or the magnitude of proteinuria alone. It is remarkable that ATE was not shown to be related to either proteinuria or serum albumin because an almost linear association with the occurrence of ATE has been established for non-nephrotic range proteinuria (<3.5 g/d) and even microalbuminuria.[30,31] Our finding could be ascribed to the imbalance of multiple other risk factors for ATE among different nephropathies. Whereas the incidence of ATE was shown to be higher in patients with diabetic nephropathy or not otherwise specified NS compared with other nephropathies, these patients had less proteinuria and higher serum albumin.

However, they were older; had higher prevalence of hypertension, prior ATE, and diabetes; and had lower eGFR. On the other hand, minimal change disease is considered a relatively mild disorder because it reacts more promptly to treatment.[32] Although the extent of proteinuria at presentation of this disorder was high, other risk factors were less prevalent.

The historical dichotomy of venous and arterial diseases as 2 different patho-physiological entities with distinct risk factors has recently been challenged because an increased risk of ATE was observed in patients with VTE.[33,34] Our data do not support such a relationship between VTE and ATE. Moreover, the findings that VTE was related to proteinuria and serum albumin (i.e., ratio of proteinuria to serum albumin) and that ATE but not VTE was associated with eGFR and multiple classic risk factors for atherosclerosis might indicate different pathophysiological mechanisms of VTE and ATE in these patients.

Over the first 6 months of observation time, we demonstrated a markedly elevated risk of about 140 times for VTE (annual incidence, 9.85%) and 50 times for ATE (annual incidence, 5.52%) compared with the general population (age- and sex-weighted annual incidences of 0.07% and 0.11%, respectively).[25,26] Therefore, one might consider primary thromboprophylaxis during this period. Further prospective studies are warranted to assess whether the decline in risk of thromboembolism during follow-up is related to the treatment of NS.

The main limitation of this study is its retrospective design. Consequently, baseline laboratory data were not available in all patients. It could be argued that asymptomatic VTE especially may have been missed because patients were not routinely screened and clinical diagnosis of VTE is unreliable. This would have resulted in an underestimated risk of VTE. However, the clinical relevance of asymptomatic VTE is a matter of debate. Referral bias may have been introduced by the setting of a university hospital. Selection bias seems less likely because consecutive patients were analyzed. Although our study is the largest evaluating thromboembolic events in patients with NS, CIs around the risk ratios were wide, indicating a limited power. We considered multivariable analysis for ATE. However, because of the large number of predictive factors and small number of events, the multivariable model was over-fitted and the power was severely limited to detect moderate effects. Consequently, these data were not included.

In summary, this study delineates high absolute risks of symptomatic VTE and ATE that were excessively elevated within the first 6 months. Whereas the magnitude of proteinuria and serum albumin levels were related to VTE, eGFR and multiple classic risk factors for atherosclerosis predicted ATE.

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Chapter 3

Microalbuminuria and risk of venous thromboembolism

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ABSTRACT

Context: Microalbuminuria (albuminuria 30-300 mg per 24-hour urine collection) is a well-known risk marker for arterial thromboembolism. It is assumed that microalbuminuria reflects generalized endothelial dysfunction. Hence, microalbuminuria may also predispose for venous thromboembolism (VTE).

Objective: To assess whether microalbuminuria is associated with VTE.

Design, Setting, and Participants: Prevention of Renal and Vascular End-stage Disease (PREVEND) study, an ongoing community-based prospective cohort study initiated in 1997. All inhabitants of Groningen, the Netherlands, aged 28 through 75 years (n=85 421) were sent a postal questionnaire and a vial to collect a first morning urine sample for measurement of urinary albumin concentration. Of those who responded (40 856), a cohort (8592 participants) including more participants with higher levels of urinary albumin concentration completed screening at an outpatient clinic. Screening data were collected on urinary albumin excretion (UAE) and risk factors for cardiovascular and renal disease.

Main Outcome Measure: Symptomatic and objectively verified VTE (ie, deep vein thrombosis, pulmonary embolism, or both) between study initiation and June 1, 2007.

Results: Of 8574 evaluable participants (mean [SD] age, 49 [13] years; 50% men), 129 experienced VTE during a mean (SD) follow-up period of 8.6 (1.8) years, corresponding to overall annual incidence of 0.14% (95% confidence interval [CI], 0.11%-0.19%). Annual incidences were 0.12%, 0.20%, 0.40%, and 0.56% in participants with UAE of less than 15 (n=6013), 15-29 (n=1283), 30-300 (n=1144), and greater than 300 (n=134) mg per 24-hour urine collection, respectively (*P* for trend <0.001). When adjusted for age, cancer, use of oral contraceptives, and atherosclerosis risk factors, hazard ratios associated with UAE levels of 15-29, 30-300, and greater than 300 mg/24 h were 1.40 (95% CI, 0.86-2.35), 2.20 (95% CI, 1.44-3.36), and 2.82 (95% CI, 1.21-6.61), respectively, compared with participants with UAE of less than 15 mg/24 h (global *P*=0.001). Adjusted hazard ratio for microalbuminuria vs normoalbuminuria (UAE<30 mg/24 h) was 2.00 (95% CI, 1.34-2.98; *P*<0.001). Microalbuminuria-related number needed to harm was 388 per year.

Conclusion: Microalbuminuria is independently associated with an increased risk for VTE.

INTRODUCTION

The overall incidence of venous thromboembolism (VTE) in developed countries is about 0.15% per year, varying from less than 0.005% in individuals younger than 15 years to as high as 0.5% at 80 years.[1-3] 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] Known risk factors for VTE fall in the first (stasis) and the third groups (blood composition).[5] However, in as many as 50% of VTE cases, none of the known risk factors are present.[1]

Arterial thromboembolism has historically been viewed as a different patho-physiological entity with distinct risk factors. However, this dichotomy between VTE and arterial thromboembolism has recently been questioned since an increased risk of arterial thromboembolism and atherosclerosis had been reported in patients with prior VTE.[6,7] Moreover, an increasing amount of data indicate that classic atherosclerosis risk factors (ie, hypertension, hyperlipidemia, diabetes, obesity, and smoking) may also predispose individuals to VTE.[8] This emerging concept may indicate the involvement of vessel wall changes in the pathogenesis of VTE.[9]

Classic atherosclerosis risk factors are also strongly correlated with microalbuminuria (albuminuria of 30-300 mg/d), which is itself an established risk marker for arterial thromboembolism.[10,11] Microalbuminuria is assumed to be a sensitive marker for generalized endothelial dysfunction that is, among others, associated with changes in the levels of several coagulation proteins.[12-19] The effect of coagulation disorders is more evident in the pathogenesis of VTE than in the pathogenesis of arterial thromboembolism. Hence, in theory, a link between micro-albuminuria and VTE is likely; however, research addressing this issue has yet to be conducted. We performed a study to assess whether microalbuminuria is associated with VTE in a population-based cohort study.

METHODS

Study Population and Design

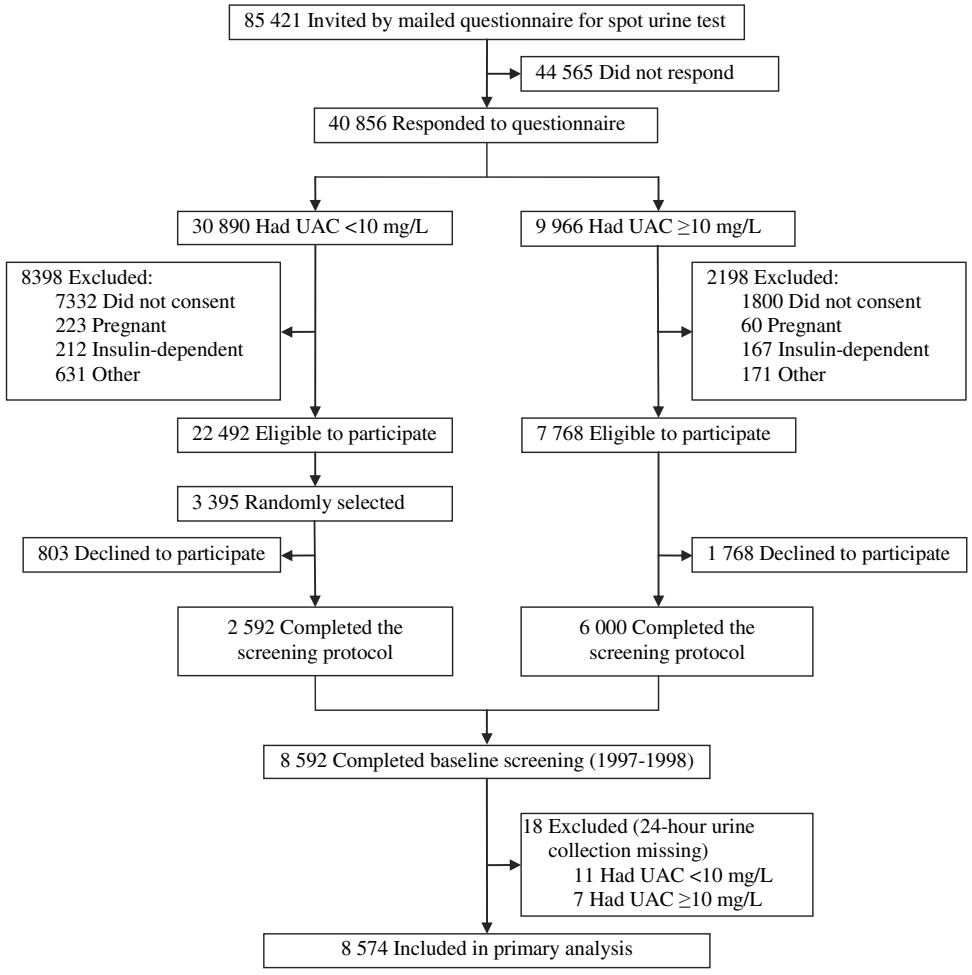
This study was conducted on participants in the Prevention of Renal and Vascular End-stage Disease (PREVEND) study. The PREVEND study was designed to investigate prospectively the natural course of albuminuria and its relation to renal and cardiovascular disease in a large cohort drawn from the general population. Details of the study protocol have been published elsewhere[10,20] and can be found at http://www.prevend.org. In brief, during 1997-1998, all 85 421 inhabitants of the city of Groningen, the Netherlands, between the ages of 28 and 75 years old were sent a 1-page postal questionnaire regarding demographics, cardiovascular morbidity, use of medication, and pregnancy, and a vial to collect a first morning void urine sample. A total of 40 856 (47.8%) individuals responded (**Figure 1**). Since the link between cardiovascular or renal disease and microalbuminuria in individuals with insulin-dependent diabetes mellitus was well established, and pregnant females may present with temporary microalbuminuria, these individuals were excluded from the PREVEND study. After the additional exclusion of individuals who were unable or unwilling to participate in the study, a total of 6000 individuals with a urinary albumin concentration of 10 mg/L or greater and a random control sample of individuals with a urinary albumin concentration of less than 10 mg/L (n=2592) completed the screening protocol and formed the baseline PREVEND cohort (n=8592). These participants twice visited an outpatient department where demographic, anthropometric, and cardiovascular risk factors were assessed. For the current analysis, 18 participants were excluded because of missing data on 24-hour urinary albumin excretion (UAE), leaving a total of 8574 participants. The PREVEND study has been approved by the local medical ethics committee and is conducted in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Laboratory Measurements and Definitions

Fasting blood samples were obtained during the morning in all participants. Serum creatinine, total cholesterol, and plasma glucose were measured by dry chemistry (Eastman Kodak, Rochester, New York). High-density lipoprotein cholesterol was measured with a homogeneous method (direct HDL, Aeroset TM System, Abbott Laboratories, Abbott Park, Illinois). Triglycerides were measured enzymatically. High-sensitivity C-reactive protein was determined by nephelometry (BN II, Dade Behring, Marburg, Germany). Plasma antigen levels of tissue plasminogen

activator and plasminogen activator inhibitor type-1 were measured using an ELISA kit from Technoclone Gmbh (Vienna, Austria). Participants collected two 24-hour urine samples, in which urinary albumin concentration was determined by nephelometry with a threshold of 2.3 mg/L and intra- and inter-assay coefficients of variation of less than 2.2% and less than 2.6%, respectively (Dade Behring Diagnostic, Marburg, Germany).

Figure 1. Participant Selection for the PREVEND Study Cohort



Urinary albumin concentration (UAC) was assessed in a first morning void urine sample and measured in milligrams per liter. The number of randomly selected participants was arbitrarily set at 3395 to obtain a total cohort size of approximately 10 000 accounting for a 15% nonparticipation rate.

Hypertension was defined as systolic blood pressure of 140 mm Hg or greater or diastolic blood pressure of 90 mm Hg or greater, or the use of antihypertensive drugs. Diabetes was defined as a fasting glucose level of 126 mg/dL or greater (≥7.0 mmol/L), nonfasting plasma glucose level of 200 mg/dL or greater (≥11.1 mmol/L), or the use of oral antidiabetic drugs. Hypercholesterolemia was defined as a total serum cholesterol concentration of 250 mg/dL or greater (≥6.5 mmol/L), or in the case of a previous myocardial infarction (MI) or stroke a concentration of 193 mg/dL or greater (≥5.0 mmol/L), or the use of lipid-lowering drugs. The metabolic syndrome was defined according to the Adult Treatment Panel III of the National Cholesterol Education Program.[21] Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. 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.[22] Low-density lipoprotein cholesterol was estimated using the Friedewald formula.[23] The UAE was measured as the mean of two 24-hour urine collections and was classified according to clinical classes: low-normal (<15 mg/24-hour urine collection), high-normal (15-29 mg/24-hour urine collection), microalbuminuria (30-300 mg/24-hour urine collection), and macroalbuminuria (>300 mg/24-hour urine collection).[10]

Identification and Validation of Venous Thromboembolic Events

We used the regional anticoagulation clinic database to identify participants of the PREVEND study who developed VTE between January 1, 1997, and June 1, 2007. This clinic monitors the anticoagulant therapy of all patients in the city of Groningen and in a well-defined geographical area proximal to the city. For fatal VTE cases, we searched the national registry of death certificates (Central Bureau of Statistics, The Hague/Heerlen, the Netherlands) and, as an additional check of the anticoagulant clinic database, we searched the database of the national registry of hospital discharge diagnoses (Prismant, Utrecht, the Netherlands). Patients' medical records were reviewed for all participants of the PREVEND study who had VTE according to any of the aforementioned databases. The investigators (B.K.M. and N.J.G.M.V.) who collected these clinical data were blinded for the UAE status of these participants. Only objectively verified symptomatic thromboembolic events were considered. Deep vein thrombosis was confirmed by compression ultrasound; and pulmonary embolism, by ventilation/perfusion lung scanning, spiral computed tomography, or at autopsy. VTE was considered provoked if it had occurred at or within 3 months after having acquired risk factors

including major surgery, trauma, immobilization for more than 7 days, oral contraceptives, hormone therapy, pregnancy, malignant disease, or miscellaneous (ie, long-distance travel for longer than 4 hours, active infectious disease, paresis/ paralysis of the leg, or heart failure). In the absence of these acquired risk factors, VTE was considered unprovoked.

Statistical Analysis

We assessed adjusted annual incidences of VTE for the enrichment of our cohort with participants with higher UAE, using survey probability weights.[24] The observation time for each patient was defined as the period from the testing of albuminuria (1997-1998) until the first episode of VTE or a censoring event (withdrawal from the study, moving out of the city, death, or end of the study). The 95% confidence intervals (CIs) were computed by a Jackknife approach assuming a Poisson distribution, and the *P* value for the test of trend was calculated via the Mantel-Haenszel method.

To evaluate the effects of baseline characteristics on VTE-free survival, we used univariate and sex- and age-adjusted Cox proportional hazards models. A multivariate model was developed that considered known VTE risk factors (i.e., age, malignancies, BMI, and use of oral contraceptives) as well as cardiovascular risk factors that yielded a $P<0.15$ from the univariate model. Results were expressed as hazard ratios (HRs) with 95% CIs and *P* values.

Continuous variables are presented as mean (SD) or as medians with the interquartile range (IQR) for skewed data. Categorical data are presented as counts and frequencies. For continuous data, differences were evaluated by Kruskal-Wallis test or 1-way analysis of variance, depending on the normality of the data. Categorical variables were compared with Chi-square test of association. Statistical significance was considered as a 2-tailed $P<0.05$. All statistical analyses were performed using STATA software version 10.0 (Stata-Corp LP, College Station, Texas).

RESULTS

Study Population

Individuals who responded (40 856) were more often women (54.4% vs 45.4%) and older (mean age 51.9 vs 46.4 years) than those who did not (**Figure 1**). The randomly selected group of 2592 participants with urinary albumin concentration of less than 10 mg/L was representative of the 30 890 eligible responding individuals with urinary albumin concentration of less than 10 mg/L, as previously reported.[25]

The **Table** represents baseline clinical characteristics of the analyzed study cohort of 8574 participants stratified into subgroups of UAE. Of the overall cohort, 70% (6013), 15% (1283), 13% (1144), and 1.6% (134) of participants had UAE of less than 15, 15-29, 30-300, and greater than 300 mg per 24 hour urine collection, respectively. The prevalence of male sex, hypertension, hyperlipidemia, current smoking status, diabetes, metabolic syndrome, history of myocaridal infarction, stroke, malignancy, and use of oral contraceptives were all higher in participants with increased levels of UAE (P<0.05). Similarly, age, BMI, total cholesterol, low-density lipoprotein cholesterol, triglycerides, C-reactive protein, tissue plasminogen activator, and plasminogen activator inhibitor-1 levels were positively associated with UAE (P<0.001). High-density lipoprotein cholesterol levels were inversely associated with UAE (P<0.001).

Urinary Albumin Excretion and Venous Thromboembolism

Overall, 129 participants developed at least 1 VTE during a mean (SD) observation period of 8.6 (1.8) years, corresponding to survey design–adjusted annual incidence of 0.14% (95% CI, 0.11%-0.19%), ranging from 0.12% (95% CI, 0.09%-0.17%) in participants with UAE of less than 15 mg/24 h to 0.56% (95% CI, 0.26%-1.47%) in participants with UAE of greater than 300 mg/24 h (**Figure 2**). These annual incidences were 0.40% (95% CI, 0.26%-0.65%) in microalbuminuric vs 0.12% (0.10%-0.17%) in normoalbuminuric participants (UAE<30 mg/24 hour urine collection). The drop-out rate due to study withdrawal and migration out of the city was 16% (1388) and was comparable between subgroups of UAE (P=.17). For these individuals, the available mean (SD) observation period was 6.5 (1.8) years.

Table 1. Baseline characteristics

Variables	Urinary albumin excretion, mg/24 h					P-value [a]
	Overall	<15	15 – 29	30 – 300	>300	
Participants, n	8,574	6,013	1,283	1,144	134	
Male sex, n (%)	4,282 (50)	2,737 (46)	727 (56)	732 (64)	89 (66)	<0.001
Age, yr	49±13	47±12	52±13	56±12	58±13	<0.001
Hypertension, n (%)	2,916 (34)	1,481 (25)	607 (48)	725 (64)	103 (77)	<0.001
Hyperlipidemia, n (%)	2,316 (32)	1,416 (28)	392 (35)	441 (43)	67 (58)	<0.001
Diabetes, n (%)	375 (4.4)	140 (2.4)	87 (6.9)	122 (10.8)	26 (19.9)	<0.001
Current smoker, n (%)	3,243 (38)	2,227 (37)	516 (40)	457 (40)	43 (33)	0.04
Body mass index, mean (SD)[b]	26.1±4.2	25.6±4.0	26.8±4.3	27.8±4.8	28.9±4.6	<0.001
Metabolic syndrome, n (%)	1,633 (21)	812 (15)	354 (30)	408 (39)	59 (48)	<0.001
History of MI, n (%)	270 (3.2)	100 (1.7)	61 (4.8)	93 (8.1)	16 (11.9)	<0.001
History of stroke, n (%)	80 (1.0)	39 (0.7)	13 (1.0)	24 (2.2)	4 (3.1)	<0.001
Malignancy, n (%)	134 (1.6)	79 (1.3)	28 (2.2)	24 (2.1)	3 (2.3)	0.048
Oral contraceptives use, n (%)[c]	889 (34)	695 (33)	104 (35)	84 (46)	6 (38)	0.003
Laboratory measurements						
eGFR, ml/min/1.73m^2	81±15	82±14	82±16	77±16	68±20	<0.001
Cholesterol, mg/dl	218±44	215±43	222±42	227±44	237±50	<0.001
HDL-C, mg/dl	51±15	53±15	48±15	47±15	44±12	<0.001
LDL-C, mg/dl	142±41	140±41	146±38	150±41	156±43	<0.001
Triglycerides, mg/dl	103 (75–149)	97 (72–139)	112 (79–167)	126 (88–190)	134 (96–199)	<0.001
CRP, mg/l	1.28 (0.56–2.98)	1.08 (0.48–2.59)	1.48 (0.67–3.30)	2.23 (1.03–4.66)	2.64 (1.27–5.50)	<0.001
t-PA, µg/l	3.13 (2.31–4.65)	3.03 (2.26–4.49)	3.29 (2.39–4.82)	3.51 (2.52–5.42)	4.02 (2.86–6.32)	<0.001
PAI-1, µg/l	72.2 (41.6–124.3)	66.4 (38.3–113.6)	85.9 (48.0–149.0)	92.8 (53.8–155.5)	96.6 (57.7–169.4)	<0.001

Abbreviations: BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate by the MDRD formula; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MI, myocardial infarction; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator. SI conversion factors: To convert values for cholesterol, HDL, or LDL to mmol/L, multiply by 0.0259; to convert values for triglycerides to mmol/L, multiply by 0.0113; to convert CRP to nmol/L multiply by 9.524; to convert PAI-1 to pmol/L, multiply by 19.231.

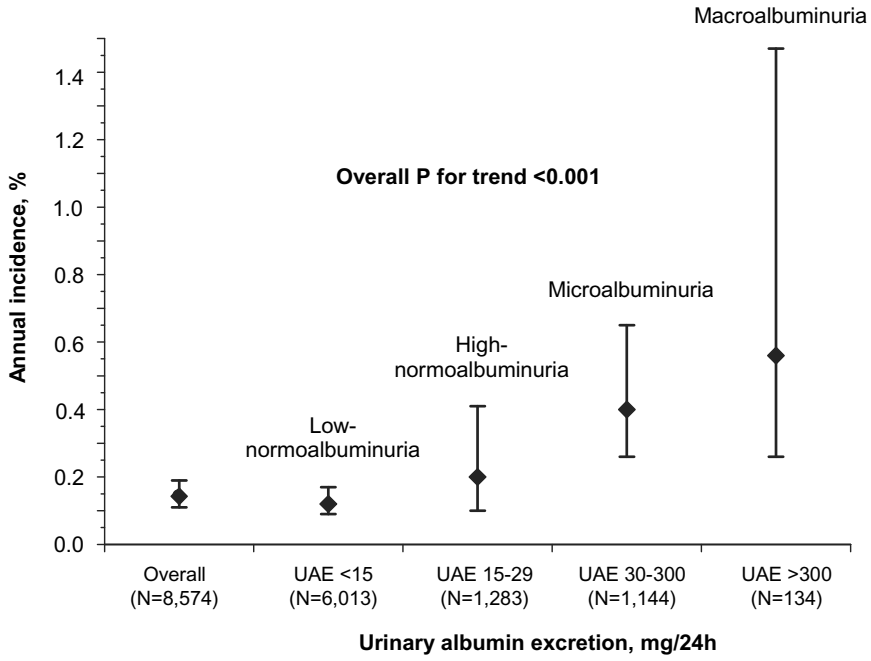
[a]*P* values are based on Chi-square test for categorical data and on Kruskal-Wallis test or 1-way analysis of varience for continuous data, depending on the normality of the data.

[b]BMI is calculated as weight in kilograms divided by height in meters squared.

[c]The percentages are the proportion of premenopausal women using oral contraceptives in the given groups of urinary albumin excretion.

The most commonly encountered first VTE was deep vein thrombosis (73, 57%), followed by pulmonary embolism (44, 34%), and combined deep vein thrombosis and pulmonary embolism (12, 9%). Types of VTE (pulmonary embolism vs deep vein thrombosis) did not differ among subgroups of UAE (*P*=.23). Of pulmonary embolism cases, 4 out of 56 (7%) were fatal. Of participants with VTE during follow-up, 24 (19%) had prior VTE and that rate was similar among different subgroups of UAE (*P*=.47). At onset of VTE, 63 participants (49%) were exposed to an acquired risk factor for VTE: 20 (32%) had a malignancy, 14 (22%) had surgery or trauma, 8 (13%) had a combined malignancy and surgery, 8 (13%) used oral contraceptives, 4 (6%) were immobilized, and 9 (14%) had other acquired risk factors.

Figure 2. Adjusted Annual Incidences of Venous Thromboembolism in Relation to Urinary Albumin Excretion by Clinical Class



VTE, n	129	63	23	37	6
Observation period, p-yrs	73,354	51,928	10,978	9,385	1,063

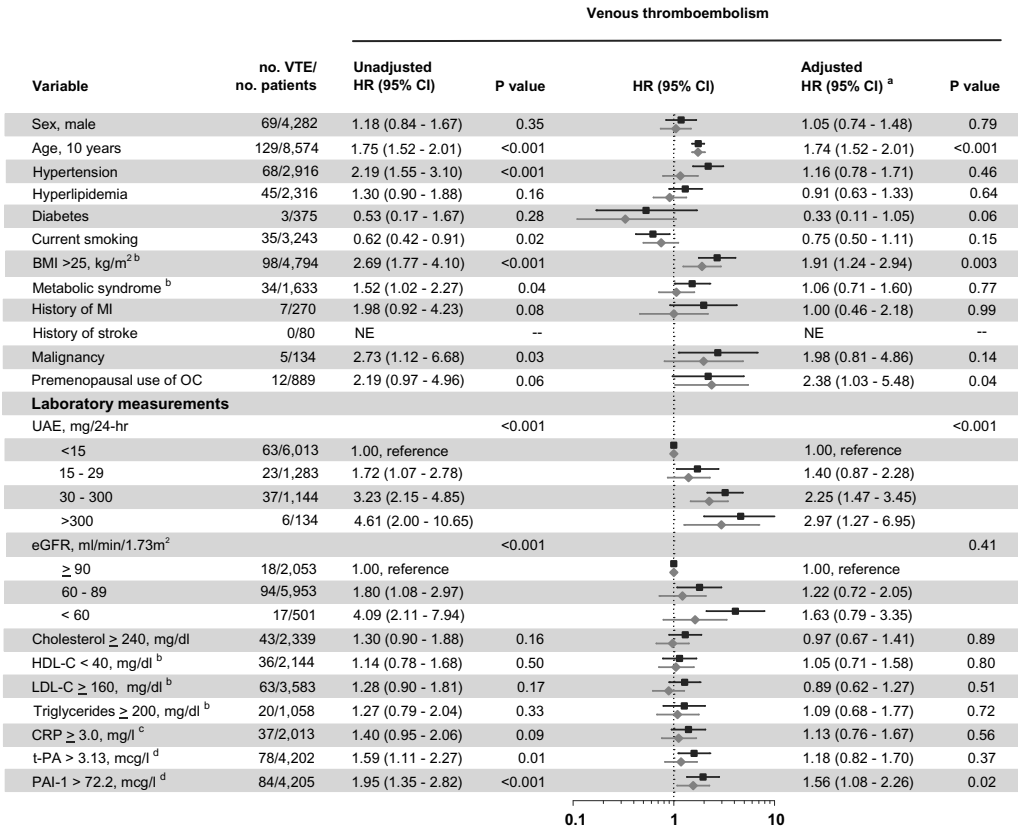
The annual incidences are adjusted for the enrichment of the cohort with participants with higher urinary albumin excretion using the survey probability weights.

Figure 3 shows the association of various variables at baseline with the unadjusted and sex- and age-adjusted risk of VTE. In the univariate analyses, multiple variables were associated with VTE. After adjustment for sex and age, only UAE, BMI, premenopausal use of oral contraceptives, and plasminogen activator inhibitor type-1 levels were significantly related to VTE. The multivariate Cox model included the following variables: UAE, established VTE risk factors (ie, age, malignancies, BMI, and use of oral contraceptives), hypertension, current smoking, history of myocardial infarction, eGFR, C-reactive protein,[26] and plasminogen activator inhibitor-1. In this model, UAE of 15-29, 30-300 and greater than 300 mg per 24-hour urine collection had HRs of 1.40 (95% CI, 0.86-2.35; *P*=.14), 2.20 (95% CI, 1.44-3.36; *P*<0.001) and 2.82 (95% CI, 1.21-6.61; *P*=.02), respectively, as compared with participants with UAE of less than 15 mg per 24 hour urine collection (global *P*=0.001). When UAE was entered as a dichotomous variable, that is, microalbuminuria vs normoalbuminuria (<30 mg/24 hour urine collection) in the multivariate Cox model, microalbuminuria conferred an HR of 2.00 (95% CI, 1.34-2.98; *P*<0.001). This adjusted HR conferred by microalbuminuria was 1.93 (95% CI, 1.24-3.03; *P*=0.004) if participants with prior VTE were excluded from the analysis. Of the mentioned variables in the multivariate model, age and eGFR were entered as continuous variables. Since metabolic syndrome is a cluster of other cardiovascular risk factors[21] and tissue plasminogen activator complexes with plasminogen activator inhibitor type-1, these 2 variables were not included in the multivariate model so as to minimize collinearity.

During 8 years of follow-up, 3% of microalbuminuric participants and 1% of normoalbuminuric participants developed VTE (**Figure 4**). As compared with participants with normoalbuminuria, the microalbuminuria related number needed to harm was 388 per year.

When we confined our analysis to participants with unprovoked VTE, UAE of 15-29, 30-300 and ≥300 mg per 24-hour urine collection conferred HRs (adjusted for age, malignancies, BMI, and use of oral contraceptives) of 1.07 (95% CI, 0.48-2.35), 3.03 (1.71-5.38), and 4.97 (1.87-13.18), respectively, as compared with participants with UAE of less than 15 mg per 24-hour urine collection (global *P*<0.001). Hazard ratios were 1.74 (95% CI, 0.94-3.24), 1.50 (0.77-2.92), and 0.98 (0.13-7.22), respectively, for provoked VTE (global *P*=0.31).

Figure 3. Univariate and Sex- and Age-Adjusted Proportional Hazards Analysis of Association With the First Venous Thromboembolism



eGFR indicates estimated glomerular filtration rate[22]; MI, myocardial infarction; UAE, urinary albumin excretion; VTE, venous thromboembolism. To convert values for cholesterol, HDL, and LDL to mmol/L, multiply by 0.0259; to convert values for triglycerides to mmol/L, multiply by 0.0113; to convert CRP to nmol/L multiply by 9.524.

Solid squares denote univariate hazard ratios of VTE (black) and solid diamonds sex- and age-adjusted hazard ratios of VTE (grey), with the corresponding 95% CIs represented by the horizontal bars.

[a] Sex- and age-adjusted hazard ratios (HRs) with corresponding 95% confidence intervals (95% CIs). Sex was adjusted for age only and age for sex only. Use of oral contraceptives was also adjusted for age only.

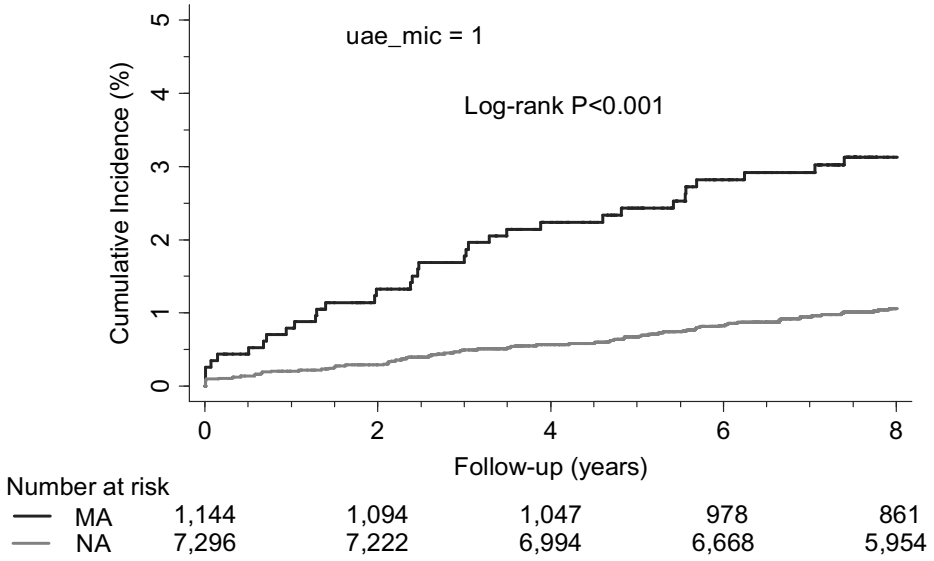
[b] Body mass index (BMI [calculated as weight in kilograms divided by height in meters squared]), metabolic syndrome, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides were classified according to the Adult Treatment Panel III of the National Cholesterol Education Program.[21]

[c] C-reactive protein (CRP) was classified according to the Centers for Disease Control and Prevention and the American Heart Association.[26]

[d] Since normal ranges of tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) in the general population are unknown, we dichotomized these variables using their medians as cutoffs.

When UAE measured in 24-hour urine collection was substituted by urinary albumin concentration measured in a spot urine sample, the adjusted HR for microalbuminuria (ie, urinary albumin concentration 20-200 mg/L) was the same, that is, 1.95 (95% CI, 1.34-2.83; $P<0.001$) as compared with normoalbuminuria (ie, urinary albumin concentration <20 mg/L). When the multivariate Cox model was also adjusted for the enrichment of the study cohort with participants with higher UAE, using survey probability weights,[27] the corresponding HR for microalbuminuria measured in 24-hour urine collection was 2.33 (95%CI, 1.34-4.05; $P = 0.003$), as compared with normoalbuminuria.

Figure 4. Cumulative Incidence of Venous Thromboembolism



MA denotes microalbuminuria (i.e., UAE 30-300 mg/24-hr); NA, normoalbuminuria (i.e., UAE<30 mg/24-hr).

As previously reported for UAE and arterial thromboembolism,[11,28] we found a gradual relationship between UAE and VTE in the normal ranges of UAE (<30 mg/24 h): adjusted HRs of UAE 10-19 and 20-29 mg per 24-hour urine collection were 1.31 (95% CI, 0.81-2.11) and 1.86 (95% CI, 1.00-3.43), as compared with UAE of less than 10 mg per 24-hour urine collection. Finally, there was no interaction between UAE and eGFR (*P*=0.67)

COMMENT

This study explored the relationship between microalbuminuria and VTE. A clear gradual relationship was found between levels of UAE and the incidence of VTE, even in the normal range of UAE. Besides UAE, multiple classic atherosclerosis risk factors were related to VTE in univariate analyses. However, after adjustment for sex and age, only UAE, BMI, premenopausal use of oral contraceptives, and plasminogen activator inhibitor type-1 levels were related to VTE. In a multivariate model, UAE remained an independent predictor of VTE. About half of the VTE cases were unprovoked. Moreover, higher levels of UAE were particularly associated with unprovoked VTE.

Several studies addressed the link between atherosclerosis risk factors and VTE.[8,29-33] Our results on atherosclerosis risk factors are consistent with a comparable community-based prospective cohort study[33] in which only BMI and diabetes were related to VTE; after adjustment for age, sex, and race. In our study, diabetes was not related to VTE; however, our results could not be generalized to all diabetics since individuals with insulin-dependent diabetes were excluded.[10,20] In a recent meta-analysis,[8] obesity, hypertension, diabetes, and higher triglyceride levels were positively associated with VTE, whereas higher high-density cholesterol levels were inversely related to VTE, and smoking and total cholesterol were not significantly related to increased risk of VTE. However, there was a significant heterogeneity among studies evaluated in this meta-analysis.[8] Moreover, most of the analyzed studies were not primarily conducted to assess the link between atherosclerosis risk factors and VTE, some were limited to only 1 sex, and the results from cohort studies were not adjusted for age. In our study, metabolic syndrome,[21] a cluster of cardiovascular risk factors, was not related to elevated risk of VTE after sex and age adjustment. In several studies, metabolic syndrome was associated with an approximately 2-fold increased risk of VTE.[34] However, this link might be due to the association between individual features of the metabolic syndrome and VTE.[35]

The value of microalbuminuria as an independent predictor of arterial thromboembolism has been demonstrated in individuals with diabetes as well as in those without.[10,11,36,37] In our previous publication,[10] microalbuminuria was related to an adjusted relative risk of 1.29 (95% CI, 1.04-1.60) and 1.58 (1.10-2.26) for MI

and stroke, respectively, as compared with participants with normoalbuminuria. In the HOPE study,[11] adjusted HRs for MI, stroke, or cerebrovascular death were 1.75 (95% CI,1.49-2.05) and 1.42 (1.18-1.71) in the placebo and intervention group, respectively. In comparison, in the current analysis microalbuminuria conferred an adjusted HR of 2.00 (95% CI, 1.34-2.98) for VTE, as compared with normoalbuminuria. Moreover, nephrotic-range proteinuria is a well-known risk factor for VTE and predisposes at least as often to VTE as arterial thromboembolism.[38] The high risk of VTE in individuals with nephrotic-range proteinuria is assumed to be secondary to loss of anticoagulant proteins. In individuals with microalbuminuria, this is unlikely to be a direct cause; more likely, the increased risk of VTE is secondary to endothelial injury and/or the related changes in the levels of procoagulant proteins.[9,12-19]

The fact that microalbuminuria has a high prevalence in the general population (7.2%) suggests that on the population level, microalbuminuria may be an important risk factor for VTE.[39] Moreover, in contrast to most of the established VTE risk factors, microalbuminuria could be treated by non-anticoagulant medication (eg, renin-angiotensin system inhibitors). Future studies are needed to evaluate the effect of these drugs on the risk of VTE.

Our study has some potential limitations that should be addressed. The incidence of VTE in our cohort may be underestimated as VTE cases were retrospectively identified. However, as compared with other prospective studies, the annual incidence of 0.14% is rather elevated given the very high incidence of VTE in individuals older than 75 years, whereas our cohort was confined to individuals aged 28 to 75 years. Furthermore, VTE was not adjudicated by an independent committee. Nevertheless, since only symptomatic and objectively verified events were considered, misclassification seems unlikely. Enrichment with participants with higher UAE is unlikely to have influenced our risk estimates (ie, HRs), as these estimates did not significantly change after accounting for the study design. Since we used predefined cutoff values for UAE, spectrum bias is unlikely despite the differences in sex and age between individuals who responded and those who did not. Due to lack of sufficient statistical power, as only 134 participants were known with malignant disease, malignancy was not associated with VTE after age and sex adjustment. Data on the use of oral contraceptives could not be generalized as participants younger than 28 years were not enrolled.

Despite these limitations, this is the first study assessing a link between microalbuminuria and VTE. Moreover, the PREVEND cohort is unique in its large population-based prospective setting in which UAE is assessed in two 24-hour urine samples, which is considered the criterion standard for measuring UAE. Although criterion standard is desirable for the proof of concept, in the clinical setting microalbuminuria is generally assessed in a spot urine sample. When we used the microalbuminuria definition for a spot urine sample (ie, urinary albumin concentration of 20-200 mg/L), results were the same.

In conclusion, microalbuminuria is an independent risk factor for VTE. The relative risk of VTE associated with microalbuminuria is comparable to previously reported risk of MI or stroke in individuals with microalbuminuria.

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Chapter 4

Chronic kidney disease stage 1-3 increases risk of venous thrombosis

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ABSTRACT

Background: End-stage renal disease has been associated with venous thrombosis (VT). However, the risk of VT in early stages of chronic kidney disease (CKD) has not yet been investigated. The aim of this study was to investigate whether CKD patients with stage 1-3 are at increased risk of VT.

Methods: 8 495 subjects were included in a prospective cohort study, in which renal function and albuminuria was assessed, starting in 1997-1998, and were followed for the occurrence of VT until June 1, 2007. CKD patients were staged according to the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines based on 24-hour urine albumin excretions and estimated glomerular filtration rates (GFR). Objectively verified symptomatic VT was considered as endpoint.

Results: Of the 8 495 subjects, 243 had stage 1 CKD, 856 stage 2, and 491 stage 3. During a median follow-up period of 9.2 years, 128 individuals developed VT. The hazard ratios (HRs) for CKD stages 1, 2, and 3 were respectively 2.2 (95% CI 0.9-5.1), 1.9 (95% CI 1.1-3.1), and 1.6 (95% CI 0.9-2.8) relative to those without CKD after adjustment for age, sex, BMI, hypertension, diabetes, malignancy, and hsCRP. Subjects with CKD stage 3 and albuminuria (≥30 mg per day) had an adjusted HR of 3.0 and subjects with stage 3 without albuminuria had an adjusted HR of 1.0.

Conclusions: CKD stage 1, 2, and CKD stage 3 in presence of albuminuria are risk factors for VT. The risk of VT is more related to albuminuria than to impaired GFR.

Introduction

Patients with severe chronic kidney disease (CKD) have both an increased risk of arterial cardiovascular disease as well as for venous thrombosis (VT). The Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines defined CKD as either kidney damage (albuminuria ≥30 mg per day) or decreased kidney function and categorized CKD in five stages [1;2]. The prevalence of CKD in the US is now 13% and is increasing, predominantly as a result of the type II diabetes epidemic [3].

The increased risk of arterial cardiovascular disease in CKD has been known for a long time and has been studied extensively for different CKD stages [4-8]. Recent studies have also shown an association between overt CKD and VT [9;10]. A study of the PREVEND cohort showed that the presence of micro-albuminuria (albuminuria 30-300 mg per day) was a risk factor for VT [9]. Another study of the LITE cohort showed that patients with a glomerular filtration rate (GFR) between 15 and 60 ml/min (CKD stage 3-4) had a two-fold increased risk of VT as compared to subjects with a normal kidney function (GFR >90 ml/min) [10]. However, information on albuminuria was not available in this study. To our knowledge, there is no study on the risk of VT in the different CKD stages taking into account albuminuria which is a prerequisite for staging CKD and for defining patients without CKD.

Therefore, we investigated whether patients with CKD stage 1, 2, and 3 had an increased risk of VT in a large population-based cohort, and set out to determine absolute and relative risks for various stages of CKD.

METHODS

Study design and population

For this study, we used data of the Prevention of Renal and Vascular Disease (PREVEND) study. The PREVEND study was designed to investigate the association between albuminuria and renal and cardiovascular outcomes in the general population. Details of the study have been published elsewhere [11-13] and can be found at http://www.prevend.org. The study outline is presented in **Figure 1**. In summary, all inhabitants of the city of Groningen, the Netherlands, aged 28-75 years (n= 85 421) were invited to send a morning urine sample to screen for albuminuria. Of these subjects, 40 856 responded. From these responders, the PREVEND cohort was selected aiming for a cohort enriched for the presence of albuminuria. Pregnant women and subjects with insulin-dependent diabetes mellitus were excluded. All participants with an urinary albumin concentration (UAC) of ≥10 mg/L were invited (N=9 966), of whom 6 000 subjects participated. Furthermore, a randomly selected cohort group of 2 592 subjects selected from 30 890 respondents with UAC of <10 mg/L participated. These 8 592 subjects formed the baseline PREVEND cohort. These participants twice visited an outpatient clinic for measurements concerning their health. For the current study, subjects were excluded because of missing data on 24-hour urinary albumin excretion or creatinine (n=86). Furthermore, subjects with CKD stage 4 (n=8) or stage 5 (n=3) were excluded of whom one had a VT event, leaving 8 495 subjects for the present analysis. The PREVEND study has been approved by the local medical ethics committee and is conducted in accordance with the guidelines of the Declaration of Helsinki.

Measurements and definitions

Serum creatinine, total cholesterol, and plasma glucose were measured by dry chemistry (Eastman Kodak, Rochester, New York). High-sensitivity C-reactive protein (hsCRP) was determined by nephelometry (BN II, Dade Behring, Marburg, Germany). Participants collected two 24-hour urine samples, in which UAC was determined by nephelometry (BN II, Dade Behring, Marburg, Germany). The amount of albuminuria was measured as the mean of the two 24-hour urine samples.

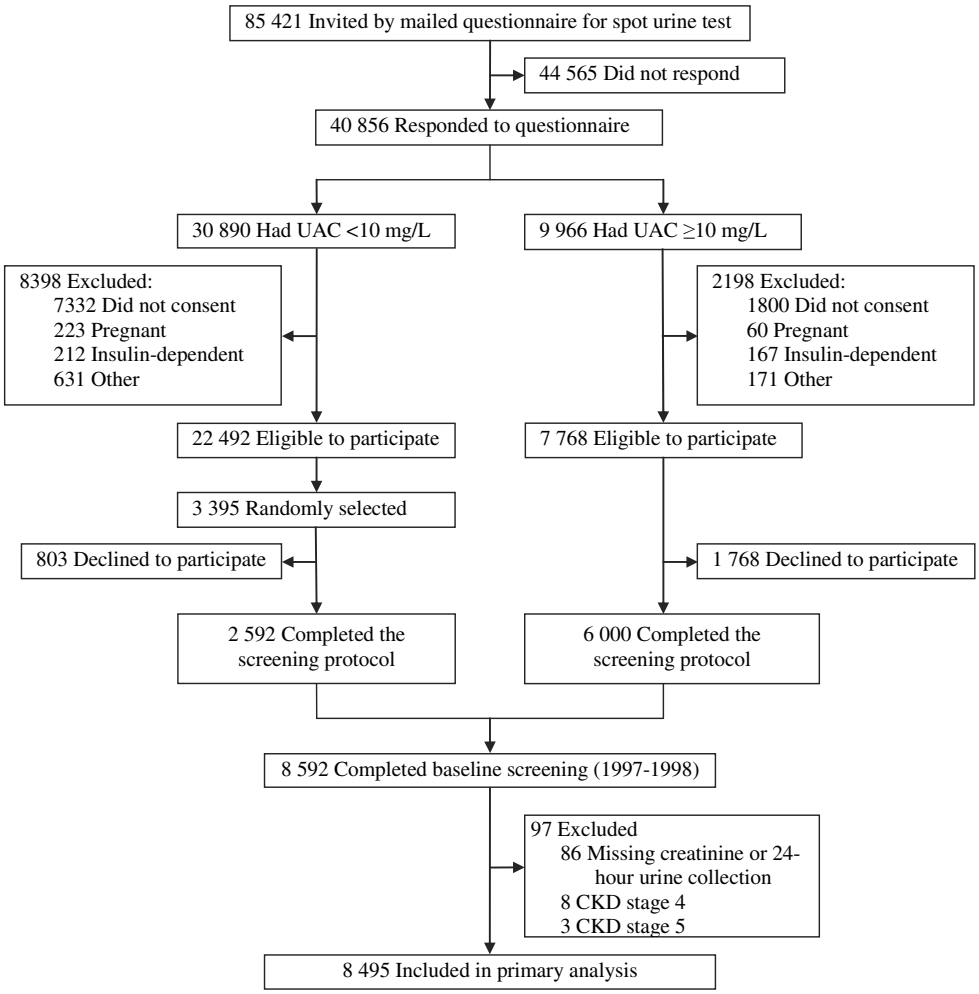

Figure 1. Outline of the PREVEND study.
CKD indicates chronic kidney disease; UAC, urinary albumin concentration.

Hypertension was defined as systolic blood pressure of ≥140 mm Hg, diastolic blood pressure of ≥90 mm Hg, or the use of antihypertensive drugs. Diabetes was defined as a fasting glucose level of ≥126 mg/dL, nonfasting plasma glucose levels of ≥200 mg/dL, or the use of oral antidiabetic drugs. Hypercholesterolemia was defined as a total serum cholesterol concentration ≥250 mg/dL, or in case of a previous myocardial infarction or stroke a concentration of ≥193 mg/dL, or the use of lipid-lowering drugs. Body mass index (BMI) was calculated as weight in

kilograms divided by height in meters squared. GFR was estimated by the Modification of Diet in Renal Disease (MDRD) study equation [14] taking into account sex, age, race, and serum creatinine level. In an additional analysis, the newly developed but less often used Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) study equation [15] was used to estimate eGFR to compare these results with the results of the MDRD-equation. The CKD-EPI equation has been shown to outperform the MDRD equation in estimating the eGFR above the 60 ml/min [15].

Chronic kidney disease

CKD was staged according to the K/DOQI guidelines [1;2]. CKD stage 1 was defined as eGFR >90 ml/min and albuminuria (urinary albumin excretion ≥30 mg per 24-hour urine collection), CKD stage 2 as eGFR between 60 and 90 ml/min and albuminuria, and CKD stage 3 as eGFR between 30 and 60 ml/min.

Venous thrombosis

The regional anticoagulation clinic database was used to identify participants who developed VT between January 1997 and June 2007. In the Netherlands, all outpatient treatment with vitamin K antagonists is monitored by regional anticoagulation clinics. Therefore, all VT events in treated outpatients are recorded by anticoagulation clinics. Moreover, as a secondary check for outpatient VT cases and identification of within hospital (fatal) cases, all study participants were searched for VT events in the national registry of death certificates and the national registry of hospital discharge diagnoses datasets. With the use of three independent sources, it is unlikely to miss VT events. The investigators who collected these data were blinded for CKD stages of the participants. In addition, all VT events according to the three sources were validated by reviewing medical records of these patients. Only objectively verified symptomatic VT events were considered. Deep vein thrombosis (DVT) was confirmed by compression ultrasound and pulmonary embolism (PE) by ventilation-perfusion lung scanning, spiral computed tomography, or at autopsy. The observation time of each participant was calculated as a time elapsed between the testing of albuminuria (1997-1998) and the first episode of VT or a censoring event (withdrawal from the study, moving out of the city, death, or June 2007), whichever occurred first. Incidence rates for VT were calculated by dividing the number of patients with a VT by the total observation time at risk. VT was considered unprovoked in the absence of major surgery,

trauma, immobilization for >7 days, oral contraceptives, hormone therapy, pregnancy, malignant disease, long-distance travel for >4 hours, active infectious disease, paresis/paralysis of the leg, or heart failure at or within three months before the development of VT. Medical records were viewed with a check-list including these well-defined and well-documented variables to categorize VT into provoked or unprovoked.

Statistical analyses

Baseline characteristics of the participants were compared between subjects without CKD and subjects with CKD stage 1-3. Continuous data were reported as medians with interquartile ranges. Kaplan-Meier life-tables were used to estimate cumulative survival for CKD stage 1-3 and no CKD. To investigate whether patients with CKD stage 1-3 had an increased risk of VT, proportional hazard regression was used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) as compared to participants without CKD (reference group). All analyses were performed for CKD stage 1, 2, and 3 combined and separately. In contrast to CKD stages 1 and 2, CKD stage 3 is only defined by decreased eGFR (between 30 and 60 ml/min) and not by the presence of albuminuria according to the K/DOQI guidelines. We also calculated HRs for CKD stage 3 stratified for the presence of albuminuria. We adjusted the HRs for age, sex, and BMI and additional for hypertension, diabetes, malignancy, and hsCRP. HRs were not adjusted for other cardiovascular risk factors such as hyperlipidemia and smoking, since these were not associated with VT in the PREVEND cohort.[9] We repeated the same analyses for provoked and unprovoked VT separately.

To investigate whether eGFR is a risk factor for VT apart from albuminuria, we calculated HRs with 95% CIs for eGFR adjusted for albuminuria and for albuminuria adjusted for eGFR to evaluate the associations of level of eGFR and albuminuria with risk of VT. Furthermore, we divided subjects in six categories based on albuminuria and eGFR (>90 ml/min, between 60 and 90 ml/min, and between 30 and 60 ml/min). HRs with 95% CIs were calculated for eGFR in absence or presence of albuminuria as compared to subjects with eGFR >90 ml/min without albuminuria (reference group).

Finally, we calculated HRs with 95% CIs for CKD stages 1-3 as compared to participants without CKD using the CKD-EPI formula for staging CKD. STATA

software version 10.1 (StataCorp LP, College Station, Tx) was used for the statistical analyses.

RESULTS

The baseline characteristics of the 8 495 subjects are shown in **Table 1**. Of the 6 905 subjects without CKD, 26.4% had a GFR >90 ml/min and 73.6% had a GFR between 60 and 90 ml/min. Of the 1 590 with CKD, 243 were in stage 1, 856 in stage 2, and 491 in stage 3. Of the 491 subjects with stage 3 CKD, 164 had albuminuria (≥30 mg per day). Subjects with CKD stage 1-3 were older, were more often male, had more often diabetes, hypertension and malignancy, and had a higher body mass index and higher CRP levels than subjects without CKD. The age of CKD patients increased with the CKD stage.

Table 1. Baseline characteristics

Characteristic	No CKD (n=6905)	CKD stage 1-3 (n=1590)	CKD stage 1 (n=243)	CKD stage 2 (n=856)	CKD stage 3 (n=491)
Age* (years)	46 (37-56)	59 (48-67)	47 (39-56)	58 (48-66)	65 (58-70)
Male, %	49	56	66	64	38
Caucasians, %	95	96	93	97	97
Diabetes, %	2.4	9.8	12.8	11.2	5.9
Hypertension, %	27	65	50	65	72
Hypercholesterolemia,%	28	46	36	44	54
BMI* (kg/m^2)	25 (23-28)	27 (24-30)	27 (24-30)	27 (25-30)	27 (25-30)
hsCRP* (mg/L)	1.1 (0.5-0.7)	2.2 (1.0-4.6)	2.1 (0.9-4.9)	2.3 (1.0-4.4)	2.2 (1.1-4.8)
Malignancy, %	1.4	2.3	1.6	2.2	2.8
eGFR* (ml/min)	81 (73-91)	72 (59-83)	97 (93-104)	76 (69-82)	55 (51-58)
UAE* (mg per day)	8 (6-12)	47 (33-93)	57 (39-101)	59 (39-107)	14 (7-47)

CKD indicates chronic kidney disease; BMI, body mass index; hsCRP, high-sensitivity c-reactive protein; eGFR, estimated glomerular filtration rate; UAE, urinary albumin excretion.
* median (interquartile range)

Overall, 128 subjects developed VT during a median observation period of 9.2 years (ranging from 0 to 10 years). Of the 128 patients with VT, 72 (56%) had DVT only, 44 had PE only (34%), and 12 (9%) had a combination of both. Of the 1590 subjects with CKD stage 1-3, 49 developed VT as compared with 79 of the 6905 subjects without CKD. Seven of the 243 patients with CKD stage 1, 26 of the 856 patients with CKD stage 2, and sixteen of 491 patients with CKD stage 3 developed VT. Four patients died because of a PE (three in CKD stage 3 and one without CKD). Furthermore, there was no significant difference in the distribution of PE and DVT in CKD stage 3 (63% of VT patients had a PE) as compared to

CKD stage 1-2 (36% had a PE) (P=0.09) or as compared to no CKD (43% had a PE) (P=0.16). The cumulative incidence for VT at eight years of follow-up were 3.2% for CKD stage 1, 3.0% for stage 2, 3.3% for stage 3, 3.1% for stage 1-3, and 1.1% for no CKD. The number needed to treat to prevent one VT event in patients with CKD stage 1-3 was approximately 400 patients per year. **Figure 2** shows the Kaplan-Meier risk curves for VT events for patients with CKD stage 1-3 versus subjects without CKD.

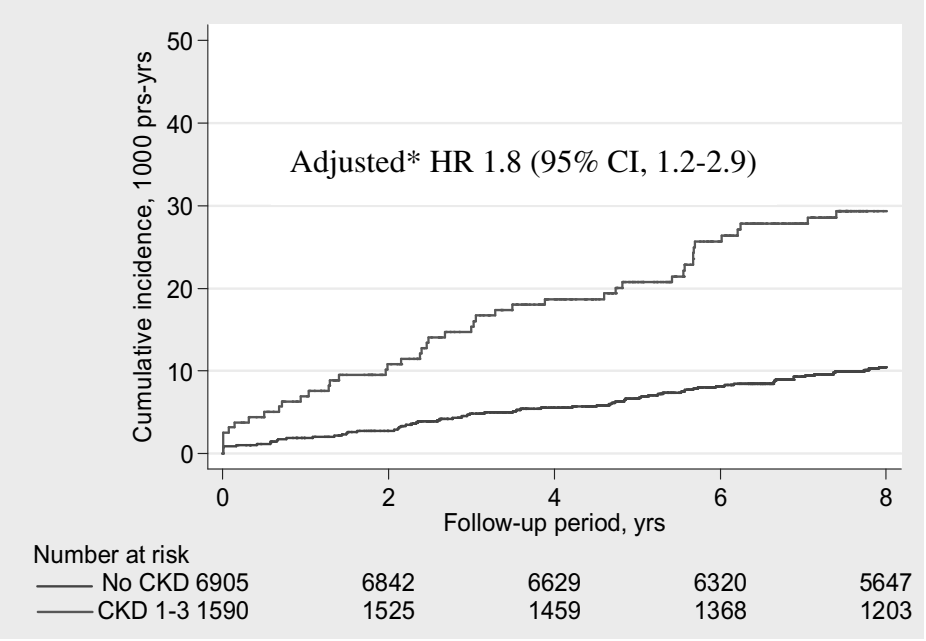

Figure 2. Kaplan-Meier estimates of the risk of venous thrombosis according to stages of chronic kidney disease.
CKD, chronic kidney disease; prs-yrs, person-years; yrs, years.
*Adjusted for age, sex, body mass index, hypertension, diabetes, malignancy, and hsCRP.

The incidence rate for VT in subjects without CKD was 1.3 (95% CI 1.1-1.7) per 1000 person-years and 3.7 (95% CI 2.8-4.0) for subjects with CKD stage 1-3 with a corresponding HR for VT of 2.8 (95% CI 2.0-7.3) for CKD stage 1-3 compared

to no CKD. The HR decreased to 1.8 (95% CI 1.2 -2.9) after adjustment for age, sex, BMI, hypertension, diabetes, malignancy, and hsCRP.

The crude HRs were 2.6 (95% CI 1.2-5.6), 2.8 (95% CI 1.8-4.3), and 3.0 (95% CI 1.8-5.2) for respectively CKD stage 1, 2, and 3. **Figure 3** shows adjusted HRs with 95% CIs for CKD stages 1, 2, and 3, the latter with or without the presence of albuminuria compared to no CKD. The HRs were 2.2 (95% CI 0.9 -5.1), 1.9 (95% CI 1.1 -3.1), and 1.6 (95% CI 0.9 -2.8). For CKD stage 3 with and without albuminuria, the HRs were respectively 5.5 (95% CI 2.8-11.0) and 1.9 (95% CI 0.9-4.2) without adjustment, and 3.0 (95% CI 1.4-6.5) and 1.0 (95% CI 0.4-2.4) after full adjustment.

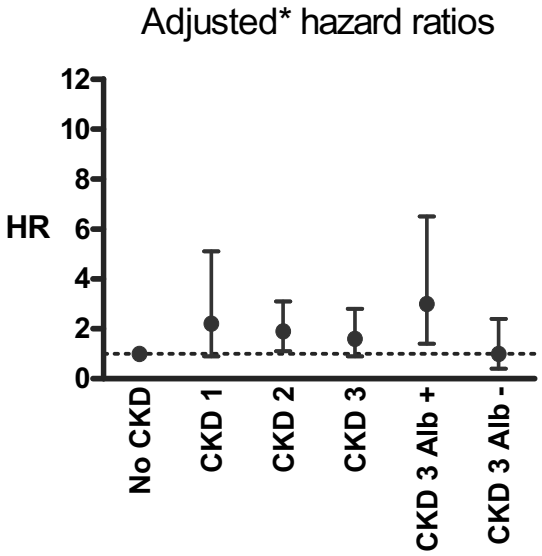

Figure 3. Adjusted hazard ratios for venous thrombosis by CKD stage.
CKD, chronic kidney disease; HR, hazard ratio; eGFR, estimated glomerular filtration rate; CKD 3 Alb +, CKD stage 3 and urinary albumin excretion ≥30 mg per day; CKD 3 Alb -, CKD stage 3 and urinary albumin excretion <30 mg per day.
*Adjusted for age, sex, body mass index, hypertension, diabetes, malignancy, and hsCRP.

Of the 128 VT events, 66 were unprovoked (51.6%) and 62 (48.4%) were provoked (**Table 2**). For unprovoked VT, the HRs after adjustment for age, sex, BMI, hypertension, diabetes, malignancy, and hsCRP.were 2.1 (95% CI 1.2-3.6)

for CKD stages 1-3, 2.5 (95% CI 0.8-7.4) for stage 1, 2.4 (95% CI 1.3-4.4) for stage 2, and 1.4 (95% CI 0.6-3.3) for stage 3. For provoked VT, the HRs after adjustment were 1.2 (95% CI 0.6-2.3) for CKD stages 1-3, 1.4 (95% CI 0.3-5.9) for stage 1, 0.8 (95% CI 0.3-2.2) for stage 2, and 1.7 (95% CI 0.8-3.9) for stage 3.

Table 2. Incidence rates and hazard ratios for provoked and unprovoked venous thrombosis

	No CKD (n=6905)	**CKD stage 1-3 (n=1590)**	**CKD stage 1 (n=243)**	**CKD stage 2 (n=856)**	**CKD stage 3 (n=491)**
Unprovoked VT					
No. of VT	35	31	5	19	7
Incidence rate per 1000 person-years	0.6 (0.4-0.8)	2.4 (1.7-3.4)	2.5 (1.0-5.9)	2.7 (1.7-4.2)	1.7 (0.8-3.7)
Crude hazard ratios (95% CI)	1.0	4.0 (2.5-6.5)	4.2 (1.6-10.6)	4.5 (2.6-7.9)	3.0 (1.3-6.7)
*Adjusted hazard ratios (95% CI)	1.0	2.1 (1.2-3.6)	2.5 (0.8-.7.4)	2.4 (1.3-4.4)	1.4 (0.6-3.3)
Provoked VT					
No. of VT	44	18	2	7	9
Incidence rate per 1000 person-years	0.7 (0.5-1.0)	1.4 (0.9-2.2)	1.0 (0.2-3.9)	1.0 (0.5-2.1)	2.3 (1.2-4.4)
Crude hazard ratios (95% CI)	1.0	1.9 (1.1-3.2)	1.3 (0.3-5.5)	1.3 (0.6-3.0)	3.1 (1.5-6.3)
*Adjusted hazard ratios (95% CI)	1.0	1.2 (0.6-2.3)	1.4 (0.3-5.9)	0.8 (0.3-2.2)	1.7 (0.8-3.9)

CKD indicates chronic kidney disease and VT indicates venous thrombosis
*Adjusted for age, sex, and body mass index, hypertension, diabetes, malignancy, and hsCRP

Albuminuria was associated with a 2.1-fold increased risk of VT after adjustment for age, sex, BMI, hypertension, diabetes, malignancy, hsCRP, and eGFR (**Table 3**). As compared to subjects with an eGFR >90 ml/min, subjects with an eGFR between 30 and 60 ml/min had 50% increased risk of VT after adjustment for after adjustment for age, sex, BMI, hypertension, diabetes, malignancy, hsCRP, and albuminuria.

Table 3. Association between eGFR, albuminuria, and risk for venous thrombosis

	*Adjusted hazard ratios
estimated glomerular filtration rate (eGFR)	
> 90 ml/min	1.0 (reference)
60-90 ml/min	1.3 (0.7-2.3)
30-60 ml/min	1.5 (0.7-3.3)

	†Adjusted hazard ratios
Albuminuria‡	
No	1.0 (reference)
Yes	2.1 (1.4-3.2)

* Adjusted for age, sex, body mass index, hypertension, diabetes, malignancy, and hsCRP, and albuminuria (continuous)

† Adjusted for age, sex, body mass index, hypertension, diabetes, malignancy, and hsCRP, and eGFR (continuous)

‡ Albuminuria defined as urinary albumin excretion ≥30 mg per day

Table 4 shows HRs for VT for decreased eGFR (between 60 and 90 ml/min and between 30 and 60 ml/min) in absence and presence of albuminuria as compared to subjects with eGFR >90ml/min without albuminuria. The adjusted HRs for subjects without albuminuria and an eGFR between 60 and 90 ml/min or an eGFR between 30 and 60 ml/min were respectively 1.5 (95% CI 0.7-3.1) and 1.4 (95% CI 0.5-4.1). HRs for VT were increased in the presence of albuminuria in all eGFR categories. The adjusted HRs were 3.1 (95% CI 1.1-8.9), 2.7 (95% CI 1.2-6.1), and 4.1 (95% CI 1.5-11.0) for subjects with albuminuria and respectively eGFR >90 ml/min, eGFR between 60 and 90 ml/min, and eGFR between 30 and 60 ml/min. and an eGFR between 60 and 90 ml/min or an eGFR between 30 and 60 ml/min were respectively 1.4 (95% CI 0.7-2.7) and 1.4 (95% CI 0.5-3.8). HRs for VT were increased in the presence of albuminuria in all eGFR categories. The age-, sex-, and BMI-adjusted HRs were 2.7 (95% CI 1.0-7.1), 2.2 (95% CI 1.1-4.7), and 3.2 (95% CI 1.3-8.3) for subjects with albuminuria and respectively eGFR >90 ml/min, eGFR between 60 and 90 ml/min, and eGFR between 30 and 60 ml/min.

Table 4. Hazard ratios for venous thrombosis by decreased glomerular filtration rates and albuminuria

	Crude hazard ratios		***Adjusted hazard ratios**	
	No albuminuria	†Albuminuria	No albuminuria	†Albuminuria
eGFR >90 ml/min/1.73 m^2	1.0 (reference)	4.8 (1.9-12.4)	1.0 (reference)	3.1 (1.1-8.9)
eGFR 60-90 ml/min/1.73 m^2	2.2 (1.2-4.1)	5.2 (2.6-10.5)	1.5 (0.7-3.1)	2.7 (1.2-6.1)
eGFR 30-60 ml/min/1.73 m^2	3.6 (1.4-9.3)	10.3 (4.2-24.7)	1.4 (0.5-4.1)	4.1 (1.5-11.0)

CKD indicates chronic kidney disease; eGFR, estimated glomerular filtration rate; UAE, urinary albumin excretion per day.
*Adjusted for age, sex, and body mass index, hypertension, diabetes, malignancy, and hsCRP
†Albuminuria defined as urinary albumin excretion ≥30 mg per day

The HRs for VT in CKD stage 1, 2, and 3 were respectively 1.6 (95% CI, 0.7-3.8), 1.9 (95% CI, 1.2-3.0), and 1.5 (95% CI, 0.9-2.7) using the CKD-EPI formula after adjustment for age, sex, and BMI. HRs for subjects with CKD stage 3 and albuminuria and subjects with CKD stage 3 without albuminuria were respectively 1.9 (95% CI 0.9-4.1) and 1.3 (95% CI 0.6-2.8) after adjustment.

DISCUSSION

In this study including 8495 subjects followed for over 8 years, we found a 2.2-fold (95% CI 0.9 -5.1) increased risk of VT in patients with CKD stage 1 and a 1.9-fold (95% CI 1.1 -3.1) increased risk of VT in patients with CKD stage 2 as compared to subjects without CKD according to the K/DOQI guidelines. CKD stage 3 patients with albuminuria had 3.0-fold (95% CI 1.4-6.5) increased risk of VT, while CKD stage 3 patients without albuminuria had a HR of 1.0 (95% CI 0.4-2.4). The risk of VT associated with CKD seemed related to albuminuria rather than to impaired eGFR. Furthermore, our findings showed that CKD stages 1-3 were mainly associated with unprovoked VT. Using the CKD-EPI formula instead of the MDRD formula for staging CKD did not result in large differences for any of the analyses.

Previous studies have investigated the association between MDRD based eGFR and VT [10;16]. In the study of the LITE cohort, HRs for VT were 1.3 (95% CI 1.0-1.6) for subjects with GFR between 60 and 90 ml/min and 2.1 (95% CI 1.5-3.0) for subjects with GFR between 15 and 60 ml/min (CKD stage 3-4) as compared to subjects with GFR >90 ml/min [10]. However, information on albuminuria was not available in this study and formal classification into CKD stages was therefore not possible. In our study, we found a HR of 1.5 for VT for CKD stage 3 after adjustment for age, sex, and BMI; we showed that the risk of VT was only increased in the presence of albuminuria. Recent findings from the LITE study group contrast their earlier findings: eGFR based on cystatin was associated with an approximately 1.6-fold increased risk of VTE, while eGFR based on creatinine was not associated with an increased risk of VT [16]. The authors, however, could not explain the discrepancy between the earlier and the current finding. Furthermore, albuminuria was not a risk factor for VT in their study in contrast to our study. An explanation for this discrepancy could be that the relatively low prevalence of albuminuria may have limited their power to detect an association between VT and albuminuria, while our cohort was enriched for the presence of albuminuria. Moreover, whereas in our study albuminuria was assessed in 24hr urine samples (gold standard) that were not frozen before assessment, albuminuria was assessed by albumin-creatinine ratio in frozen samples in their study. Frozen storage is known to induce a systematic decrease and more variability in albuminuria concentration [17]. Furthermore, subjects with albuminuria are

probably mainly diabetics in their study, while in PREVEND these are mainly non-diabetics, as per protocol insulin-using diabetics were excluded. This may have influenced the risk estimates, as diabetic subjects are usually on statin therapy and more frequently treated with anti-platelet medication for their cardiovascular morbidity. New findings indicate that statin use may reduce the risk of VT [18].

Although the seemingly higher risk of VT in CKD stage 1 and 2 as compared to stage 3 might be surprising, the same pattern in the association between CKD and cardiovascular disease was previously found in the PREVEND study [4]. CKD patients with stage 1 and 2 were at higher risk of cardiovascular disease as compared to CKD patients with stage 3. A plausible explanation for this might be the difference in staging of CKD stage 3 and CKD stage 1 and 2. Albuminuria is necessary to define CKD stage 1 and 2, whereas only GFR is needed to define CKD stage 3 to 5. Therefore, CKD stage 3 is a heterogeneous group with subjects with and without evident kidney damage (albuminuria). We found that CKD stage 3 patients with albuminuria were at higher risk of VT as compared to CKD patients with stage 3 without albuminuria. These findings are in line with several other studies suggesting a higher risk for CKD stage 3 subjects with albuminuria as compared to CKD stage 3 subjects without albuminuria for different adverse outcomes, such as cardiovascular disease and the development of end-stage renal disease [4;19-21]. These data taken together suggest that information on albuminuria could be added to CKD stage 3 in order to improve the value of CKD staging for risk prognosis.

Several mechanisms behind the increased risk of VT in CKD are possible. First, endothelial damage could explain the increased risk of VT. It is remarkable that the association between CKD stage 1-3 and VT was comparable to the previously reported association between CKD stages 1-3 and cardiovascular disease in the PREVEND study [4]. Therefore, it is tempting to hypothesize that a common risk factor for CKD leads to both VT and arterial cardiovascular disease. In our analysis, hypertension, BMI, and diabetes did not explain the increased risk of VT. Second, the increased risk of VT could be due to procoagulant changes in CKD patients which may be predominantly present in subgroups of CKD patients such as patients with nephrotic syndrome [22]. CKD and nephrotic syndrome have been associated with elevated levels of D-dimer, CRP, fibrinogen, factor VII, factor VIII, and von Willebrand factor [23;24], which are important proteins in the

development of VT. Third, inflammation may explain the increased risk of VT in CKD. It has been suggested that inflammation leads to VT [25]. However, additional adjustment of the HRs for hsCRP, which is currently the most widely used biomarker of inflammation [26], did not alter the HRs in our study.

This study has several limitations. First, the K/DOQI guidelines require impaired GFR or albuminuria for at least three months. Like most studies, repeated measurements for a period of at least three months were not available in our study and therefore some subjects may have been falsely classified as having CKD. Second, VT events were identified through anticoagulation clinic databases and registries for hospital discharge diagnoses and death certificates which could lead to an underestimation of the incidence rates of VT. However as compared to previous studies, incidence rates for VT in the PREVEND cohort (i.e. 1.4 per 1000 person-years) correspond well to those found in studies that had a complete case-finding procedure of objectively confirmed VT events [27]. Third, we may have underestimated renal function in subjects with a GFR >60 ml/min, because we used the MDRD study equation [28;29]. However, the use of the CKD-EPI formula did not result in large differences in the HRs. Fourth, there are studies suggesting that risk of adverse events increases when GFR drops below 45 ml/min [7;20]. Our study did not include enough subjects with a GFR <45 ml/min (n=52) to investigate this. Despite these limitations, PREVEND is a unique cohort in its large population-based prospective setting in which albuminuria was assessed in two 24-hour urine samples.

We showed that especially CKD stage 1, 2, and 3 in the presence of albuminuria are risk factors for VT. The relative risk of VT for CKD stage 1-3 was 1.8-fold increased relative to those without CKD. Although these relative risk estimates may be considered weak compared to for example relative risk estimates for venous thrombosis that have been reported for genetic thrombophilia [30], on a population level it may be an important contributor to VT because of the high prevalence of CKD, i.e. 12.7% for CKD stage 1-3 in the general population [3]. This is more than most well-known genetic risk factors for VT, such as prothrombin gene mutation [31]. Clinicians should be aware of the increased risk of VT in these patients. Further studies are needed to show whether VT prophylaxis in subgroups of these patients will be safe and cost-effective,

especially as the high risk of anticoagulant treatment-related major bleeding episodes applies to CKD stage 4 and 5, and not CKD stage 1-3 [32].

In conclusion, CKD stage 1, 2, and CKD stage 3 in presence of albuminuria were risk factors for VT. The risk of VT is more related to albuminuria than to impaired GFR.

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Chapter 5

The impact of antiproteinuric therapy on the prothrombotic state in patients with overt proteinuria

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ABSTRACT

Background: Overt proteinuria is a strong risk factor for thromboembolism due to changes in levels of various coagulation proteins and urinary antithrombin loss. The described coagulation disturbances in these patients are based on outdated studies conducted primarily in the 1970's and 80's. Whether these coagulation disturbances resolve with antiproteinuric therapy has yet to be studied.

Methods: A total of 32 adult patients with overt proteinuria (median 3.7 g/d; interquartile range 1.5-5.6) were matched for sex and age with 32 healthy volunteers. Patients were participants of an intervention trial designed to assess optimal antiproteinuric therapy with losartan and diuretics. Levels of various pro- and anticoagulant proteins, and two thrombin generation assays (i.e., Calibrated Automated Thrombogram [CAT] and prothrombin fragment 1+2) were performed at baseline and after anti-proteinuric treatment.

Results: Whereas levels of fibrinogen, factors V, VIII and Von Willebrand were higher in patients ($P<0.004$), levels of antithrombin were comparable in patients versus controls ($P=0.70$). Based on both thrombin generation assays, patients were substantially more prothrombotic than controls ($P<0.001$). Twenty-four weeks after start of antiproteinuric therapy, median proteinuria was reduced to 0.9 g/d (interquartile range 0.6-1.4). Similarly, levels of various liver-synthesized pro- and anticoagulant proteins as well as prothrombin fragment 1+2 levels were significantly reduced. However, of CAT parameters only moderate amelioration in activated protein C resistance was observed, and Von Willebrand and factor VIII levels remained elevated.

Conclusions: Proteinuric patients are in a more prothrombotic state with increased thrombin generation, as compared to non-proteinuric controls. Antiproteinuric therapy reverses the protrombotic state.

INTRODUCTION

Nephrotic syndrome is a known strong risk factor for both arterial and venous thromboembolism.[1-4] The nephrotic syndrome is characterized by urinary protein losses in excess of 3.5 g per 1.73 m^2 body surface area per day in association with hypoalbuminemia, hypercholesterolemia, and peripheral edema. The exact pathophysiological mechanisms of thromboembolism in patients with nephrotic syndrome have yet to be unraveled. Low levels of antithrombin due to urinary loss and alterations in plasma levels of various proteins involved in coagulation are considered to be the main predisposing factors.[4-6] Enhanced platelet aggregation, decreased fibrinolysis, hyperviscosity and hyperlipidemia are other less often postulated mechanisms that may be responsible for the prothrombotic state in these patients.[4,7-9] Finally, in the general population, generalized endothelial dysfunction and low-grade inflammation, as reflected by microalbuminuria and elevated levels of high sensitivity C-reactive protein, respectively, may be additional factors supporting a prothrombotic state.[10-12]

Although the association between the extent of proteinuria and changes in plasma levels of various coagulation proteins including low plasma antithrombin levels has been considered common textbook knowledge, solid evidence is lacking. The previously reported high risk of thromboembolism at presentation of nephrotic syndrome and the positive correlation between the extent of proteinuria and coagulation disturbances or venous thromboembolism suggest that antiproteinuric therapy may reduce the risk of thromboembolism in these patients.[1,5,13,14] Of note, the supposed beneficial effect of antiproteinuric therapy on the prothrombotic state rests on observational studies with immunosuppressive regimens, with major methodological shortcomings.[5,13,14] Studies evaluating the impact of intervention in the renin-angiotensin system, which is nowadays the cornerstone of antiproteinuric treatment, on coagulation disturbances in patients with overt proteinuria have yet to be conducted. Therefore, we set up this study in patients with overt proteinuria to assess the exact coagulation disturbances and the impact of renin-angiotensin system inhibiting antiproteinuric therapy on the prothrombotic state.

MATERIAL AND METHODS

Patients and study protocol

This study was conducted on patients who participated in a randomized, double-blind, placebo-controlled, crossover trial designed to investigate the effects of sodium restriction, hydrochlorothiazide, and their combination on proteinuria during losartan use in patients with overt proteinuria.[15] Details of the study protocol have been published elsewhere.[15] In brief, patients with overt proteinuria and stable renal function (i.e., creatinine clearance >30 ml/min), aged 18-70 years were considered eligible. Patients with uncontrolled hypertension (mean arterial pressure >100 mm Hg), serum potassium >5.5 mmol/L, cardiovascular disease, contraindication for losartan or hydrochlorothiazide use, and/or diabetes were excluded, as well as frequent users of nonsteroidal anti-inflammatory drugs (>2 doses/week). All enrolled patients were treated for 6-week periods with placebo, losartan, and losartan plus hydrochlorothiazide, in combination with either high or low sodium diet, in a random order.[15] In one out of 33 patients citrate plasma was not available; this patient was therefore excluded from the current analysis. The study was approved by the local medical ethics committee and was conducted in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all participants.

All participants were matched with control subjects for sex and age within 5 years. Controls were healthy volunteers who had no (family) history of thromboembolism, renal disease, were not pregnant, and had not used oral contraceptives for at least 3 months before testing.

Study Measures

Blood samples were obtained in the morning hours between 8:00 and 9:30 before study initiation (baseline) and at the end of the 36 weeks study period. Venous blood samples were anticoagulated with 1:10 volume of 0.109 mol/l trisodium citrate. Platelet-poor plasma was prepared by centrifugation at 2500 x g for 15 minutes, aliquoted and immediately frozen at -80^{o}C and analysed later after thawing at 37 ^{o}C for 15 minutes.

On the day before study visit, patients collected 24-h urine samples to determine proteinuria and creatinine excretion. Urinary protein was determined using the

pyrogallol red-molybdate method. Serum creatinine, cholesterol, triglycerides, total protein, and albumin levels were determined using an automated multi-analyzer (SMA-C; Technicon, Tarrytown, NY). Quantitative determination of fibrinogen was assessed with Siemens Thrombin Reagent (Siemens, Marburg, Germany) on a Sysmex CA-7000 automated coagulation analyzer (Siemens Diagnostics, Leusden, the Netherlands). Factor V:C and factor VIII:C levels were assessed by one-stage clotting assay (Siemens, Marburg, Germany). Total protein S antigen and Von Willebrand factor levels were measured with different enzyme-linked immunosorbent assays (ELISA) with reagents obtained from DAKO, Glostrup, Denmark. Free protein S antigen levels were assessed by ELISA after precipitation of protein S bound to C4-binding protein (C4BP) with 3.75% PEG 6000. Activity of protein C (Berichrom Protein C, Dade Behring, Liederbach, Germany), and antithrombin (Coatest, Chromogenix, Mölndal, Sweden) were assessed by chromogenic substrate assays. The thrombin generation was measured with the Calibrated Automated Thrombogram (CAT) method by Hemker et al[16] and commercialized by Thrombinoscope BV (Maastricht, The Netherlands). The CAT assay was performed in triplo, using a polypropylene round-bottomed microtiter plate (Greiner Bio-one Ltd, Stonehouse, UK) containing 20 µL trigger with 1 pM tissue factor and 4 µM phospholipids (Thrombinoscope BV, Maastricht, The Netherlands) in the absence and presence of 1.5 nM soluble thrombomodulin (TM; American Diagnostica, Stamford, CT, USA) that was added to 80 µL platelet poor plasma as previously described. After addition of 20 µL substrate-calcium reagent, the reaction was monitored in a Fluoroskan Ascent reader (Thermo Labsystems OY, Helsinki, Finland) equipped with a 390/460-nm filter set (excitation/emission) and a dispenser. Using a software program (Thrombinoscope BV, Maastricht, The Netherlands), the fluorescent signal was converted to a thrombin concentration by continuous comparison with the signal generated by a thrombin calibrator (obtained from Thrombinoscope BV, Maastricht, the Netherlands) added to a parallel sample of the test plasma.[16] Next, the thrombin concentration was calculated and displayed in time.[16] The thrombin generation curve is characterized by a lag phase, followed by a burst of thrombin which is finally completely terminated by plasma coagulation inhibitors. Five parameters were derived from the thrombin generation curves. The endogenous thrombin potential (ETP, mM.min) was calculated from the area under the thrombin generation curve corrected for alpha-macroglobulin–thrombin activity. The lag time (min) was the time to the start of thrombin formation and defined as the time to reach one-sixth of

the peak thrombin height, peak thrombin (nM) was the maximum concentration of thrombin formed, time to peak (min) was the time to reach the peak thrombin height and the velocity index (nmol/s) was the rate of thrombin formation per second (i.e. velocity index = peak thrombin / [peak time – lag time]). The ETP and peak thrombin are given as percentage to human normal pooled platelet poor plasma assayed in the same run, as described previously.[17] In the presence of TM the effects of the anticoagulant pathway by activated protein C and its cofactor protein S were additionally analyzed. Relative ETP reduction after TM addition was calculated as 100 – (ETP in presence of TM / ETP in absence of TM)*100%. Relative peak thrombin reduction after TM addition was calculated as 100 – (peak thrombin in presence of TM / peak thrombin in absence of TM)*100%.To assess the magnitude of *in vivo* thrombin generation we also measured prothrombin fragment 1+2, which are released from prothrombin during activation by factor Xa, using a commercially available ELISA assay system (Enzygnost F1+F2; Dade Behring, Marburg, Germany).

Laboratory technicians who measured the levels of (anti-)coagulant proteins and performed the CAT and prothrombin fragment 1+2 assays were not aware of the extent of proteinuria and the demographics of either study participants or controls.

Statistical analysis

The association between proteinuria and various coagulation proteins, CAT parameters and prothrombin fragment 1+2 were assessed by performing linear regression analysis. Both crude and age- and sex-adjusted standardized beta and the corresponding P values are presented.

Continuous variables are presented as medians with the interquartile range (IQR). Categorical data are presented as counts and frequencies. For continuous data, differences were evaluated by Wilcoxon rank-sum test or Wilcoxon matched-pairs signed-ranks test, depending on whether unpaired or paired analyses were performed. Statistical significance was considered as a 2-tailed $P < 0.05$. All statistical analyses were performed using STATA software version 10.1 (Stata-Corp LP, College Station, Texas).

RESULTS

Table 1 represents baseline characteristics of the 32 study participants and 32 sex and age-matched controls. Of the patients, ten participants had renal biopsy proven primary glomerulopathy, 19 had secondary glomerulopathies and in the remaining three cases renal biopsies were inconclusive. The median proteinuria was 3.7 mg/24 h (interquartile rage, 1.5 – 5.6) and median serum albumin levels were 39 g/l (interquartile range, 38 – 42). The levels of procoagulant fibrinogen, factor V, factor VIII and Von Willebrand factor were significantly higher in patients as compared to controls ($P \leq 0.004$). In contrast, levels of anticoagulant antithrombin, protein C and total protein S were comparable in patients versus controls ($P \geq 0.31$). However, levels of free proteins S were significantly higher in patients as compared to controls ($P < 0.001$). Except the lag time with and without thrombomodulin and time to peak in presence of thrombomodulin, all other CAT parameters including ETP, peak thrombin, and velocity index either with or without thrombomodulin were significantly higher in patients as compared to controls. In the presence of thrombomodulin, the differences between patients and controls in especially ETP levels were substantially accentuated from 1.7 fold to 3.3 fold. In patients, median prothrombin fragment 1+2 levels were 330 picomol/L (interquartile range, 209-419) versus 158 picomol/L (interquartile range, 125-197) in the controls ($P < 0.001$).

Table 2 describes crude and age- and sex-adjusted associations between proteinuria and various coagulation factors as well as CAT parameters and prothrombin fragment 1+2 levels. The extent of proteinuria was positively related to both pro- and anticoagulant proteins, except for antithrombin that was not associated with proteinuria at all. Parameters of CAT were also not related to the extent of proteinuria, whereas a positive trend was observed for the link between proteinuria and fragment 1+2 levels.

It is assumed that in response to reduction in levels of serum albumin, production of all liver-synthesized proteins is upregulated, including procoagulant and anticoagulant factors and lipoproteins.[8,13,18] If we substituted proteinuria by serum albumin, in Table 2 presented associations were almost the same, though in general somewhat weaker. When we introduced serum cholesterol in the regression

analysis instead of proteinuria, only factor V and protein C showed a positive association with serum cholesterol.

Table 1. Baseline characteristics.

Variable	Patients	Controls	P-value
Subjects, n	32	32	-
Male, n (%)	24 (75)	24 (75)	-
Age, yrs*	51 (43–59)	46 (32–52)	-
Laboratory measurements*			
Fibrinogen, g/l	3.9 (3.6–4.3)	2.7 (2.4–2.9)	<0.001
Factor V, %	137 (111–164)	104 (83–121)	0.004
Factor VIII, %	180 (140–213)	117 (94–136)	<0.001
Von Willebrand factor, %	191 (151–232)	100 (85–114)	<0.001
Antithrombin, %	98 (93–109)	99 (93–108)	0.70
Protein C, %	113 (103–131)	109 (100–126)	0.51
Total protein S, %	129 (111–140)	121 (104–135)	0.31
Free protein S, %	127 (101–147)	81 (66–101)	<0.001
CAT parameters†			
ETP, %	163 (146–191)	96 (76–110)	<0.001
With TM	147 (126–171)	44 (33–55)	<0.001
Lag time, min	3.6 (3.2–4.3)	3.3 (3.0–3.8)	0.24
With TM	3.6 (3.1–4.3)	3.1 (3.0–3.7)	0.18
Peak thrombin, %	280 (242–328)	90 (66–123)	<0.001
With TM	242 (203–294)	64 (49–84)	<0.001
Time to peak, min	6.8 (5.9–7.3)	8.3 (7.4–9.0)	<0.001
With TM	6.6 (5.9–7.0)	6.7 (6.3–7.3)	0.16
Velocity index, nmol/s	80 (66–113)	19 (14–36)	<0.001
With TM	85 (61–121)	22 (14–35)	<0.001
Prothrombin fragment 1+2, pmol/l	330 (209-419)	158 (123-197)	<0.001
Patients-specific variables			
Primary glomerulopathies, n (%)	10 (31)	-	-
Secondary glomerulopathies, n (%)	19 (59)	-	-
Urinary protein excretion, mg/24h*	3.7 (1.5–5.6)	-	-
Serum albumin, g/l*	39 (38–42)	-	-
Serum cholesterol, mmol/l*	6.1 (5.1–6.8)	-	-

CAT denotes Calibrated Automated Thrombogram; ETP, endogenous thrombin potential and TM, thrombomodulin.

* Values are medians and interquartile ranges.

†ETP was calculated from the area under the thrombin generation curve corrected for alpha-macroglobulin–thrombin activity. The lag time is the time to the first burst in thrombin formation, peak is the maximum concentration of thrombin formed and the velocity index is the rate of thrombin formation per second, that is, velocity index = peak thrombin / (peak time – lag time).

Table 2. Association between the extent of proteinuria and coagulation disturbances.				
Variable	**Crude std beta**	**P-value**	**Adjusted std beta***	**P-value**
Procoagulant proteins				
Fibrinogen	0.542	0.001	0.545	0.002
Factor V	0.467	0.007	0.404	0.03
Factor VIII	0.447	0.01	0.430	0.02
Von Willebrand factor	0.371	0.04	0.391	0.05
Anticoagulant proteins				
Antithrombin	0.199	0.27	0.085	0.66
Protein C	0.400	0.02	0.370	0.06
Protein S total	0.243	0.20	0.408	0.04
Protein S free	0.309	0.09	0.392	<0.05
Tests for prothrombotic state				
CAT parameters				
ETP	0.072	0.69	0.118	0.56
With TM	-0.045	0.81	-0.000	1.0
Lag time	-0.175	0.34	-0.340	0.08
With TM	-0.140	0.45	-0.289	0.15
Peak	-0.050	0.79	-0.030	0.88
With TM	-0.100	0.59	-0.095	0.64
Time to peak	-0.157	0.39	-0.289	0.14
With TM	-0.172	0.35	-0.315	0.11
Velocity index	-0.042	0.82	-0.063	0.76
With TM	-0.103	0.58	-0.130	0.53
Prothrombin fragment 1+2	0.380	0.04	0.330	0.10

Std beta denotes standardized beta; CAT, Calibrated Automated Thrombogram; ETP, endogenous thrombin potential and TM, thrombomodulin.
* adjusted for age and sex.

Figure 1 depicts the extent of proteinuria and levels of serum albumin, cholesterol, high sensitivity C-reactive protein, various pro- and anticoagulant proteins, CAT parameters and prothrombin fragment 1+2 at baseline versus after treatment. As we previously reported, sodium restriction, hydrochlorothiazide, and their combination during losartan use reduced the extent of proteinuria and serum cholesterol levels, and increased serum albumin levels (**Figure 1A**).[15] Of procoagulant proteins only factor V levels were reduced, and a negative trend was observed for fibrinogen at the end of treatment as compared to baseline (**Figure 1B**). Of anticoagulant proteins, protein C and free protein S levels were lower, total protein S were higher and antithrombin levels were unaltered (**Figure 1C**). Interestingly, CAT parameters in proteinuric patients were the same after treatment as compared to

baseline, whereas levels of prothrombin fragment 1+2 were significantly reduced after treatment (**Figure 1D**).

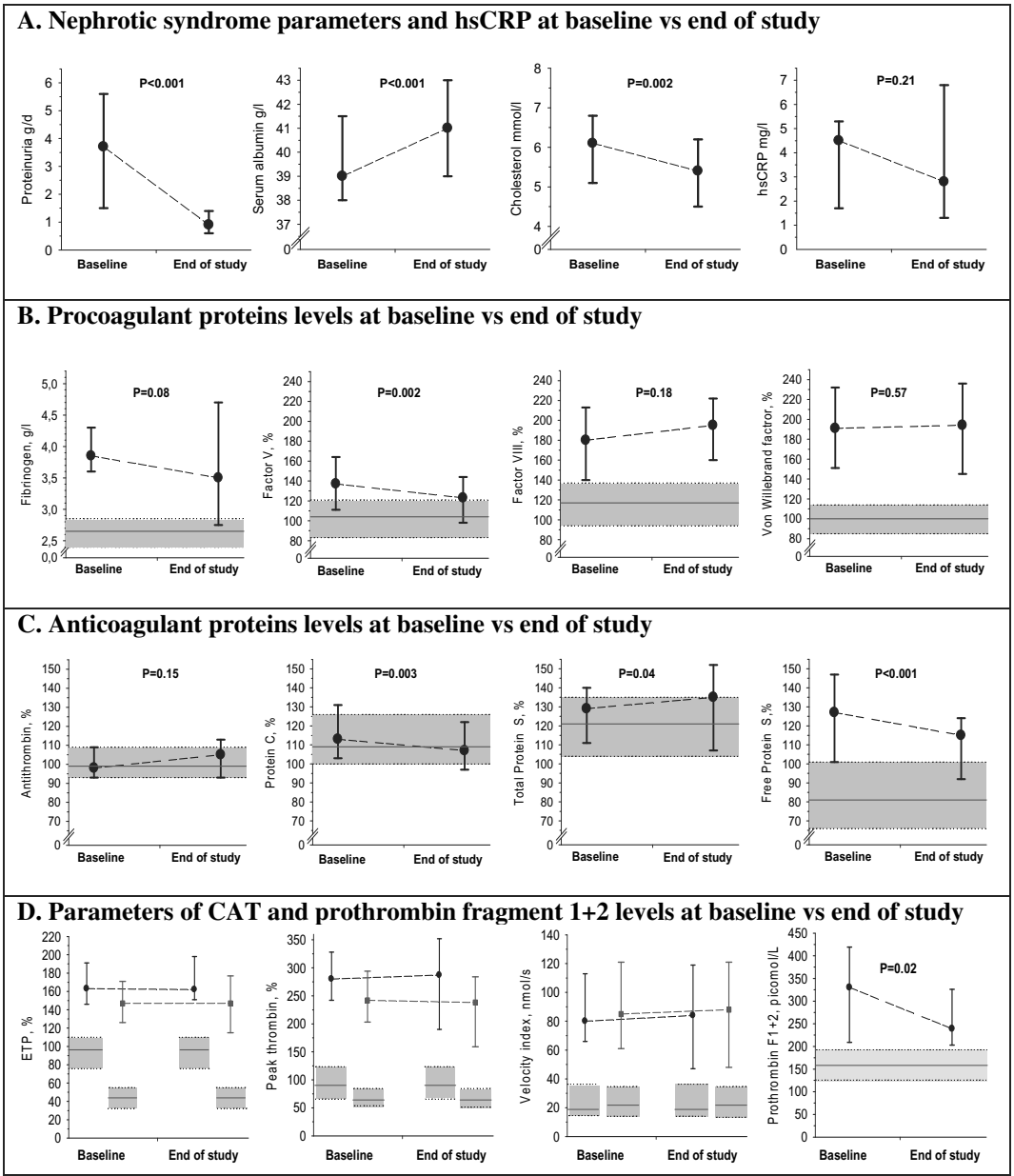

Figure 1. Components of nephrotic syndrome, high sensitivity C reactive protein, various pro- and anti-coagulation proteins and functional coagulation assays at baseline versus after antiproteinuric treatment.

Panel A: Error-bars depict the median values and interquartile ranges. **Panel B and C:** Error-bars depict the median values and interquartile ranges. The horizontal gray lines depict the median vales and the gray area's the interquartile ranges in the control group. The P values refer to the differences between baseline versus end of study, in patients. **Panel D:** The black error-bars represent the medians and interquartile ranges of the prothrombin fragment 1+2 levels and parameters of Calibrated Automated Thrombogram (CAT) without thrombomodulin addition. Medians and interquartile rages of CAT parameters with thrombomodulin addition are depicted by the gray error-bars. The horizontal gray lines represent the median vales and the gray area's the interquartile ranges in the control group. ETP denotes endogenous thrombin potential.

Next, we focussed on the relative reduction of ETP and relative peak thrombin reduction after thrombomodulin addition as these parameters appeared better predictors of activated protein C resistance.[17] The mean relative reduction in ETP after trombomodulin addition in our patients was 12.6% at baseline versus 15.9% after treatment (P=0.17) and the relative reduction in peak thrombin was 14.4% at baseline versus 19.1% after treatment (P=0.007), respectively. Finally, the higher levels of coagulation factors and inhibitors in these patients, at baseline as compared to after treatment could not be ascribed to hemo-concentration: the mean hematocrit at baseline was 44.1% versus 43.7% after treatment (P=0.81).

DISCUSSION

This study explored the relationship between overt proteinuria and coagulation disturbances, as well as the effect of renin-angiotensin system inhibiting-based antiproteinuric therapy on the coagulation disturbances in patients with non-diabetic nephropathies. As compared to age- and sex-matched controls, the patients were more prothrombotic, as indicated by markedly elevated thrombin generation levels, both by the CAT method and prothrombin fragment 1+2 measurements. This prothrombotic state was likely caused by the elevated levels of various procoagulant proteins, including fibrinogen, while the major inhibiting proteins antithrombin, protein C and total protein S were surprisingly similar in patients as compared to controls. Except antithrombin levels that had no association with the extent of proteinuria, levels of all other measured pro- and anti-coagulant factors were positively correlated with the extent of proteinuria. Antiproteinuric therapy reversed the prothrombotic state as reflected by reduction in prothrombin fragment 1+2 and amelioration in relative peak thrombin generation.

This is the first study addressing the effect of non-steroidal and non-immunosuppressive, symptomatic anti-proteinuric therapy on coagulation disturbances in patients with overt proteinuria. Despite effective reversal of proteinuria, factor V, protein C, free protein S and to a lesser extent fibrinogen levels were decreased after treatment, as compared to baseline. Other coagulation factors remained unchanged and total protein S levels were even increased after treatment. We speculate that lack of decrease in factor VIII, Von Willebrand factor and increase in total protein S levels could be explained by persistent endothelial damage. In previous studies mainly in children with nephrotic syndrome, remission of nephrotic syndrome with corticosteroid or immunosuppressive therapy reversed the high levels of especially fibrinogen and antithrombin.[5,13,14]

Our baseline results on levels of coagulation proteins in patients with overt proteinuria are comparable to previous studies, in patients with untreated nephrotic syndrome, that mainly date back to the seventies and eighties.[2,6,8,13,14] Based on these studies it is generally accepted that the prothrombotic state in nephrotic patients is due to elevated levels of various procoagulant proteins and low antithrombin levels. Low antithrombin levels are presumed to be due to urinary loss, though results are inconsistent.[4,5] Indeed, we also observed normal circulating levels of antithrombin in all patients. Because antithrombin was the only protein that had no positive association with the extent of proteinuria, we postulated that upregulation in antithrombin synthesis might be counterbalanced by urinary loss as antithrombin has relatively low molecular weight, which is comparable to the molecular weight of albumin. Indeed, in a small pilot study, we could confirm the presence of considerable amounts of antithrombin (6-21%) in urine of three patients with proteinuria ranging from 0.5 g/d to 1.5 g/d (data not shown).

The application of the CAT assay[16] for the assessment of the prothrombotic state in patients with overt proteinuria is new. Based on previous studies in non-proteinuric patients, this assay has been propagated as a suitable method for assessing the prothrombotic state.[19] Interestingly, though CAT parameters including peak thrombin, ETP and velocity index were substantially higher in patients as compared to controls, these were not correlated with the extent of proteinuria and remained elevated after proteinuria reduction with anti-proteinuric therapy. Lag time, which could be interpreted as a proxy of prothrombin time (PT) and in part as the activated partial thromboplastin time (APTT), was comparable between our

patients and controls. This is in line with a previous study that found no difference in PT or APTT in nephrotic patients as compared to controls.[20] In contrast, prothrombin fragment 1+2 levels that reflect *in vivo* thrombin generation showed a positive trend for the association with proteinuria and were significantly reduced after anti-proteinuric treatment. This suggests that *in vivo* thrombin generation is ameliorated by antiproteinuric therapy. Thus, despite the reduction in levels of liver-synthesized proteins, persistent high levels of endothelial derived proteins in particular factor VIII might be responsible for the lack of reduction in CAT parameters after antiproteinuric therapy. The effect of thrombomodulin addition in the CAT assay that boosts the protein C and S pathway was almost absent in patients, as compared to controls. This activated protein C resistance in our patients might be also caused by high factor VIII levels, as previously reported.[17,21] However, some degree of amelioration in activated protein C resistance was observed as reflected by the relative ETP and especially relative peak thrombin reductions after treatment as compared to baseline. The relative reduction in ETP and peak thrombin are considered more sensitive for assessment of activated protein C resistance than the absolute values of ETP and peak thrombin in the presence of thrombomodulin.

This study has several limitations that warrant attention. Firstly, we measured only a selection of coagulation proteins. However, this selection was performed with a predefined aim by assessing the role of antithrombin, the protein C and S pathway, endothelial damage (as measured by factor VIII and Von Willebrand factor), liver synthesis function (as measured by among others factor V and protein C) and thrombin generation (as measured by the CAT assay and prothrombin fragment 1+2). Secondly, the association between coagulation disturbances due to proteinuria might be better demonstrated in more extreme cases of proteinuria, whereas in our study almost half of the participants had non-nephrotic range proteinuria (i.e., <3.5g/day). Obviously, more extreme phenotypes of overt proteinuria are nowadays rare since the availability of effective anti-proteinuric medication. Despite these limitations, this is the first study that addressed the effect of renin-angiotensin system inhibiting anti-proteinuric medication on the coagulation disturbances and adds new insights into the role of endothelium in the pathogenesis of prothrombotic state in these patients with overt proteinuria.

In conclusion, patients with overt proteinuria are in a more prothrombotic state with increased thrombin generation, as compared to non-proteinuric controls. Antiproteinuric therapy reverses the protrombotic state as reflected by reduction in prothrombin fragment 1+2 and activated protein C resistance, though increased levels of endothelial derived factor VIII and von Willebrand factor persist.

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Chapter 6

A prospective cohort study on the absolute risks of venous thromboembolism and predictive value of screening asymptomatic relatives of patients with hereditary deficiencies of protein S, protein C or antithrombin

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ABSTRACT

Background: Absolute risks of venous thromboembolism (VTE) in protein S-, protein C-, or antithrombin-deficient subjects are mainly based on retrospective data. Screening asymptomatic relatives of these patients are disputed, though studies addressing this issue have yet to be conducted.

Methods: We prospectively followed 382 relatives of 84 probands. Participants were assessed for other thrombophilic defects and occurrence of exogenous risk factors (i.e., surgery, trauma, immobilization, malignancies, use of systemic estrogens, and pregnancy or puerperium). After screening, deficient subjects were advised to use thromboprophylaxis during exogenous risk factors; use of oral contraceptives was discouraged.

Results: Overall annual incidence of VTE was 1.53% (95%CI, 1.00-2.34) in deficient versus 0.29% (0.13-0.64) in non-deficient relatives; adjusted hazard ratio, 7.0 (95%CI, 2.7-18.0). Annual incidence of unprovoked VTE was 0.95% in deficient versus 0.05% in non-deficient subjects; age-adjusted hazard ratio, 22.3 (P=0.003). In contrast, annual incidence of provoked VTE was 0.58% versus 0.24%; age-adjusted hazard ratio, 2.8 (P=0.08). Fifty-five (37%) deficient and 80 (34%) non-deficient subjects experienced 91 and 143 exogenous risk factors, respectively, during which 6 versus 5 VTE (6.6% vs 3.5% per risk-period) occurred, despite the higher compliance with recommended thromboprophylaxis use in deficient (51%) versus non-deficient (22%) subjects. In deficient subjects all provoked VTE occurred when thromboprophylaxis was not used.

Conclusions: Protein S, protein C or antithrombin deficiencies confer high absolute risk of VTE. Screening and subsequent augmentation of thromboprophylaxis use may result in reduction of provoked VTE, whereas risk of unprovoked VTE could not be affected by screening.

INTRODUCTION

Several coagulation disorders are associated with an increased risk of venous thromboembolism (VTE). These thrombophilic disorders include hereditary deficiencies of protein S, protein C and antithrombin; factor V Leiden; the prothrombin G20210A mutation; high levels of clotting factor VIII; and antiphospholipid antibodies.[1] Hereditary deficiencies of protein S, protein C or antithrombin are rare (0.1% to 0.4% each in the general population), but the strongest hereditary risk factors for VTE.[2] In retrospective studies the incidence rate of VTE ranged from 0.5% to 3.1% per year.[3-6] Only three prospective studies addressed this issue, which reported incidence rates ranging from 0.7% to 4.0% per year.[7-9] The differences between studies may be explained by differences in study populations as these deficiencies interact with other genetic and acquired risk factors for VTE.[2,10]

Since in the general population long-term oral anticoagulant treatment is associated with a major bleeding risk of about 2.8% per year,[11] there is reluctance to advocate long-term primary prophylaxis in asymptomatic subjects with protein S, protein C or antithrombin deficiencies.[12] As about 50% of VTE cases are provoked by exogenous risk factors,[10] transient thromboprophylaxis at
exposure to exogenous risk factors is nowadays highly recommended even in non-deficient subjects.[13] Therefore, screening asymptomatic relatives of subjects with protein S, protein C or antithrombin deficiencies is a matter of debate,[14,15] even though studies addressing this issue have yet to be conducted.

We conducted a prospective follow-up study to assess the absolute risk of VTE in a large series of deficient versus non-deficient asymptomatic relatives of protein S-, protein C- or antithrombin-deficient patients. We also assessed the impact of screening, followed by preventative recommendations, on the VTE risk in deficient relatives of these patients.

METHODS

Study population and design

Details of the study protocol have been published elsewhere.[10,16] In brief, 1600 consecutive patients with VTE were screened over 12 years to identify 91 index subjects (probands) with either protein S, protein C or antithrombin deficiency. First-degree relatives, of these probands, older than 15 years of age were identified by pedigree analysis. As the number of antithrombin-deficient probands was small, second degree relatives from a deficient parent were also identified. For living relatives, response rates between 90% and 97% per cohort allowed us to identify 725 relatives. Subjects were enrolled after informed consent was obtained. Detailed information on previous episodes of VTE and anticoagulant treatment were collected, using a standardized questionnaire and reviewing medical records. Blood samples were taken after clinical data had been collected. Relatives were tested for other thrombophilic defects in addition to their index deficiencies, including deficiencies of protein S, protein C, and antithrombin; factor V Leiden; the prothrombin G20210A mutation; high levels of factor VIII; and lupus anticoagulant.

Asymptomatic relatives (i.e., without a history of VTE), irrespective of their deficiency status, were eligible for inclusion in the current prospective analysis (**Figure 1**). Subjects were excluded if they were on long-term (≥ 12 months) treatment with vitamin K antagonists. All subjects were instructed to seek medical attention when they encountered signs or symptoms of VTE. Both study subjects and their general physicians received written information concerning presence or absence of protein S, protein C or antithrombin deficiencies. In deficient subjects, additional information was provided concerning the implications of the observed deficiency and the advice to strongly consider anticoagulant thromboprophylaxis at exposition to exogenous risk factors (i.e., major surgery or trauma, immobilization for >7 days, pregnancy and puerperium). Nonetheless, the decision to use thromboprophylaxis was left to the discretion of the treating physician. Furthermore, use of oral contraceptives and hormonal replacement therapy were discouraged in deficient subjects. In nondeficient subjects no preventative recommendations were given and their treating physicians were expected to apply thromboprophylaxis in agreement with national guidelines that are based on the contemporary American College of Chest Physicians guidelines.[17]

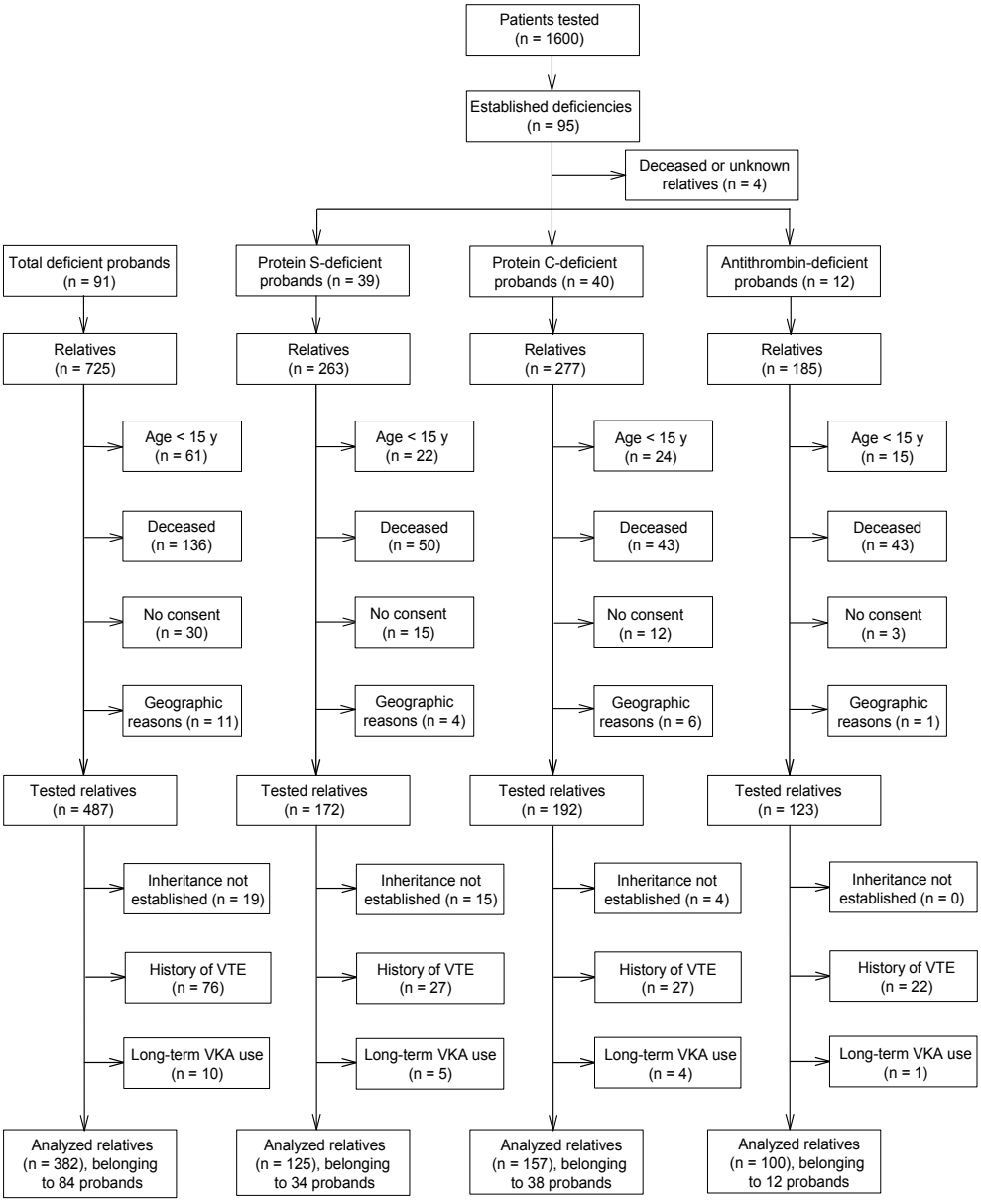

Figure 1. Recruitment of the family cohorts with hereditary deficiencies of protein S, protein C, or antithrombin.
VKA denotes vitamin K antagonists; VTE, venous thromboembolism.

Data from the participants were collected in family groups, and were seen at our outpatient clinic at start of the study and during follow-up that took place about

once every 3 years by phone or by visits to our outpatient clinic. In each case a standardized questionnaire was used to update information on the occurrence of VTE, exposition to exogenous risk factors and the use of thromboprophylaxis. Participants were last contacted in the period between September 2005 and December 2007. The study protocol was approved by the institutional review board of our hospital.

Diagnosis of venous thromboembolism

Only objectively verified symptomatic thromboembolic events were considered. Events were independently adjudicated and were classified using the following criteria: deep vein thrombosis had to be confirmed by compression ultrasound; and pulmonary embolism by ventilation/perfusion lung scanning or spiral computed tomography. Isolated calf vein thrombosis and superficial phlebitis were not classified as VTE. VTE was considered provoked if it had occurred at or within 3 months after exposure to exogenous risk factors, including major surgery or trauma, immobilization for >7 days, oral contraceptives, hormone replacement therapy, pregnancy, or malignant disease. In the absence of these risk factors, VTE was considered unprovoked.

Laboratory studies

Protein S and protein C antigen levels were measured by Enzyme Linked Immuno Sorbent Assay (ELISA) (reagents obtained from DAKO, Glostrup, Denmark), activity of protein C (Berichrom Protein C, Dade Behring, Liederbach, Germany), and antithrombin (Coatest, Chromogenix, Mölndal, Sweden) by chromogenic substrate assays. Normal ranges (mean±SD) were determined in 393 healthy blood donors, who had no (family) history of thromboembolism, were not pregnant, and had not used oral contraceptives for at least three months. Protein S deficiency type I was defined by lowered total (<68 IU/dl) and free (<65 IU/dL) protein S levels. Protein C deficiency type I and type II were defined by reduced levels of either protein C antigen (<63 IU/dl) and/or activity (<64 IU/dl). Antithrombin deficiency was defined by decreased levels of antithrombin activity (<74 IU/dl), using heparin co-factor assay which identifies both type I and type II antithrombin deficiencies.[18] Deficiencies were considered inherited if they were confirmed by measuring a second sample that was collected 3 months later and were found in at least 2 family members, while acquired conditions were excluded. If there was a discrepancy between the results of the 2 tests, a third sample was tested. A deficiency was considered acquired, through use of oral contraceptives or

pregnancy, unless it was confirmed at least 3 months after withdrawal of oral contraceptives or delivery, respectively. Factor V Leiden and the prothrombin G20210A mutation were demonstrated by polymerase chain reactions.[19,20] Factor VIII:C was measured by one-stage clotting assay and was considered increased at levels above 150 IU/dl.[21] Lupus anticoagulant was defined by abnormal values of dilute Russell viper venom time, activated partial thromboplastin time and/or tissue thromboplastin inhibition, which normalized by adding phospholipids to the subject's plasma.[22]

Statistical analysis

We calculated absolute risks of VTE in subjects with protein S, protein C or antithrombin deficiency, separately, and in the pooled cohorts of deficient and non-deficient subjects. Annual incidences were calculated by dividing the number of symptomatic subjects by the total number of follow-up years. Follow-up time was defined as the period from testing for the index deficiency until first VTE, death or end of study, whichever occurred first. The 95 percent confidence intervals (95% CI) around the annual incidences were assessed with the Poisson distribution assumption. Annual incidences in current prospective analysis were compared to our previously published retrospective analysis[10] by calculating incidence rate ratios with the corresponding 95% CI and P-values by using the immediate method in STATA software, version 10.1 (StataCorp LP, College Station, Texas, USA).[23] Annual incidences in previous retrospective analysis were calculated as the numbers of subjects with VTE that have occurred prior to screening divided by the total observation years. Observation period in the retrospective analysis was defined as the period from the age of 15 years until VTE, death or screening for the index deficiencies, whichever came first.[10]

In a multivariate Cox-model, hazard ratios conferred by these deficiencies were adjusted for age, sex, concomitant thrombophilic defects (i.e., factor V Leiden, the prothrombin G20210A mutation or factor VIII:C >150 IU/dl) and for clustering of VTE within families that used the robust sandwich method in STATA software. Results were expressed as hazard ratios, with 95% CIs and Pvalues.

Continuous variables were expressed as median values with the interquartile range (IQR) and categorical data as counts with frequencies. Differences between groups were evaluated by the Student's *t* test or Mann-Whitney U test, depending on the normality of data for continuous data, and by Fisher exact test for categorical data.

Statistical significance was considered as a 2-tailed probability <0.05. All statistical analyses were performed using STATA software, version 10.1 (StataCorp LP, College Station, Texas, USA).

RESULTS

Subjects

Ninety-one subjects with objectively verified VTE and either protein S, protein C or antithrombin deficiency served as index patients in this study (**Figure 1**). In 7 families, inheritance of the index deficiency was not established in at least two relatives, therefore, these families were excluded from analysis (19 relatives). After exclusion due to various reasons depicted in Figure 1, the current analysis was performed on 382 relatives. These subjects belonged to 84 different kindreds with protein S (n=34), protein C (n=38) or antithrombin (n=12) deficiency.

Table 1 shows the clinical characteristics of the pooled and separate study cohorts. Overall, 39% of subjects were deficient for either protein S, protein C or antithrombin. Deficiencies were equally distributed between men and women. Deficient subjects were on average 10 years younger than non-deficient subjects at testing for index deficiencies (P<0.001). If subjects with prior VTE and/or subjects using long-term vitamin K antagonists were not excluded from the analysis, the mean age at testing for these deficiencies in deficient subjects was 39 years versus 41 years in non-deficient relatives. Concomitance of other thrombophilic defects (i.e., factor VIII:C >150 IU/dl, prothrombin G20210A and factor V Leiden mutations) were comparable between deficient and non-deficient subjects.

Table 1. Baseline characteristics of the study population

Variable	Total cohort			Protein S cohort			Protein C cohort			Antithrombin cohort		
	Deficient	Non-deficient	P	Deficient	Non-deficient	P	Deficient	Non-deficient	P	Deficient	Non-deficient	P
Subjects, n	149	233		50	75		53	104		46	54	
Women, n (%)	73 (49)	121 (52)	0.60	26 (52)	39 (52)	1.00	25 (47)	52 (50)	0.87	22 (48)	30 (56)	0.55
Median age at testing for index deficiency (IQR), yr	31 (18-43)	41 (27-52)	<0.001	33 (18-42)	43 (25-55)	0.01	26 (18-44)	40 (30-52)	0.003	27 (17-47)	42(20-52)	0.05
Concomitance of, n (%)*												
FVIII:C >150 IU/dl	36 (30)	62 (32)	0.71	11 (28)	18 (26)	1.00	16 (35)	30 (38)	0.85	9 (25)	14 (30)	0.63
PT G20210A	4 (3)	15 (7)	0.15	1 (2)	4 (6)	0.65	3 (6)	6 (7)	1.00	0 (0)	5 (11)	0.06
Factor V Leiden	11 (8)	32 (15)	0.09	4 (10)	7 (10)	1.00	6 (12)	23 (24)	0.09	1 (3)	2 (4)	1.00

IQR denotes interquartile range; FVIII:C, factor VIII:C; PT G20210A, the prothrombin G20210A mutation.
* Of total study cohort 17%, 13% and 10% of subjects were not tested for FVIII:C, the prothrombin G20210A mutation and factor V Leiden, respectively.
None of these subjects were positive for lupus anticoagulant.

The total follow-up time was 1,375 years in deficient subjects (mean±SD, 9.2±5.9) and 2,097 years in non-deficient subjects (mean±SD, 9.0±5.9). Participating families were contacted on average once every 3 years. During follow-up, five (3.4%) deficient subjects and seven (3.0%) non-deficient subjects died; according to death certificates, none of the death causes were related to VTE.

Risk of venous thromboembolism

Twenty-one (14.1%) deficient and six (2.6%) non-deficient subjects developed VTE during follow-up, corresponding to an annual incidence of 1.53% (95%CI, 1.00 – 2.34) in deficient and 0.29% (95%CI, 0.13 – 0.64) in non-deficient subjects, respectively (**Table 2**). Nineteen (70%) of the 27 VTE were classified as deep-vein thrombosis in the leg and 8 (30%) as pulmonary embolism either alone or in combination with deep-vein thrombosis. There was no difference in types of VTE (i.e., deep-vein thrombosis and pulmonary embolism) between deficient and non-deficient subjects (P=0.82). Compared to the pooled cohort of non-deficient subjects, subjects with antithrombin deficiency had the highest risk for VTE, followed by protein S and protein C deficiencies. In a multivariate Cox-model that was adjusted for age, sex, concomitant thrombophilic defects (i.e., factor V Leiden, the prothrombin G20210A mutation or factor VIII:C >150 IU/dl) and clustering, any (i.e., protein S, protein C or antithrombin) deficiency conferred a hazard ratio of 7.0 (95% CI, 2.7 – 18.0; P<0.001), as compared to non-deficient relatives. Adjusted hazard ratios for separate deficiencies were 9.6 (95% CI, 3.0 – 30.1), 4.1 (95% CI, 1.2 – 13.9) and 10.2 (95% CI, 3.3 – 31.6) in subjects with protein S, protein C and antithrombin deficiencies, respectively.

A total of 13 deficient and only one non-deficient subject developed unprovoked VTE, corresponding to an annual incidence of 0.95% (95%CI, 0.55 – 1.63) in deficient versus 0.05% (95%CI, 0.01 – 0.34) in non-deficient subjects; age adjusted hazard ratio 22.3 (95% CI, 2.9 – 172.7) (**Table 2**). The risk of provoked VTE in deficient subjects was not significantly higher (P=0.08). Fifty-five (37%) deficient and 80 (34%) non-deficient subjects experienced a total of 91 and 143 high-risk periods, respectively (**Table 3**). At time of high-risk periods, thromboprophylaxis was used more often in deficient (51%) subjects as compared to non-deficient subjects (22%). The incidence of risk-period related VTE (provoked VTE) per risk-period was almost two-fold higher in deficient subjects (6.6% vs. 3.5%), despite more often used thromboprophylaxis. Furthermore, whereas no VTE were

encountered during 166 pill-years in non-deficient subjects, 2 VTE occurred during only 54 pill-years in deficient subjects.

Table 2. Annual incidence of Venous Thromboembolism

Variable	Total Follow-up, yrs	First VTE, n	Annual incidence, % (95% CI)	Hazard ratio (95% CI)*	P
Any VTE	3,472	27	0.78 (0.53 – 1.13)		
No deficiency	2,097	6	0.29 (0.13 – 0.64)	1.0, reference	
Any deficiency	1,375	21	1.53 (1.00 – 2.34)	7.0 (2.7 – 18.0)	<0.001
PSD	453	7	1.55 (0.74 – 3.24)	9.6 (3.0 – 30.8)	<0.001
PCD	528	5	0.95 (0.39 – 2.27)	4.1 (1.2 – 13.9)	0.02
ATD	394	9	2.29 (1.19 – 4.39)	10.2 (3.3 – 31.6)	<0.001
Unprovoked VTE	3,472	14	0.40 (0.24 – 0.68)		
No deficiency	2,097	1	0.05 (0.01 – 0.34)	1.0, reference	
Any deficiency	1,375	13	0.95 (0.55 – 1.63)	22.3 (2.9 – 172.7)	0.003
PSD	453	5	1.10 (0.46 – 2.65)	25.5 (2.9 – 221.3)	0.003
PCD	528	1	0.19 (0.03 – 1.34)	4.4 (0.3 – 71.7)	0.29
ATD	394	7	1.78 (0.85 – 3.73)	42.7 (5.2 – 350.7)	<0.001
Provoked VTE	3,472	13	0.37 (0.22 – 0.64)		
No deficiency	2,097	5	0.24 (0.10 – 0.57)	1.0, reference	
Any deficiency	1,375	8	0.58 (0.29 – 1.16)	2.8 (0.9 – 8.6)	0.08
PSD	453	2	0.44 (0.11 – 1.77)	2.0 (0.4 – 10.7)	0.40
PCD	528	4	0.76 (0.28 – 2.02)	3.6 (0.9 – 13.8)	0.06
ATD	394	2	0.51 (0.13 – 2.03)	2.5 (0.5 – 12.9)	0.28

VTE denotes venous thromboembolism; PSD, protein S deficiency; PCD, protein C deficiency and ATD, antithrombin deficiency.

* Hazard ratios for any VTE are adjusted for age, sex, concomitant thrombophilic defects (i.e., Factor V Leiden, prothrombin G20210A mutation or factor VIII levels >150 IU/dl) and clustering. Hazard ratios for unprovoked and provoked VTE were only adjusted for age due to the low numbers of events.

Table 3. Number of risk periods and correlated venous thromboembolic events.

Variable	Total cohort				
	Non-def (n=233)	Deficient (n=149)			
		Any def (n=149)	PS def (n=50)	PC def (n=53)	AT def (n=46)
Surgery, trauma or immobilization , n	116	60	22	27	11
With Prophylaxis, n (%)	24 (21)	27 (45)	11 (50)	6 (22)	10 (91)
VTE, n	5	5	1	4	0
Associated incidence of VTE/risk period, %	4.3	8.3	4.5	14.8	0.0
Pregnancy or puerperium, n	27	31	8	11	12
With Prophylaxis, n (%)	7 (26)	19 (61)	4 (50)	10 (91)	5 (42)
VTE, n	0	1	0	0	1
Associated incidence of VTE/risk period, %	0.0	3.2	0.0	0.0	8.3
Any risk moment , n*	143	91	30	38	23
With Prophylaxis, n (%)	31 (22)	46 (51)	15 (50)	16 (42)	15 (65)
VTE, n	5	6	1	4	1
Associated incidence of VTE/risk period, %	3.5	6.6	3.3	10.5	4.3
Oral contraceptive use, pill-yrs	166	54†	24	20	10
VTE, n	0	2	1	0	1
Associated incidence of VTE/pill-yrs, %	0.0	3.7	4.2	0.0	10.0

* Two subjects in non-deficient group and 3 subjects in deficient group were known with malignant disease. One subject with protein C deficiency and malignant disease developed VTE. Any risk moment included surgery, trauma, immobilization, pregnancy, puerperium and malignant disease.

† Fifty-four pill years were obtained in 6 deficient women.

We compared the incidence rates of VTE in the current prospective analysis to our previously published retrospective analysis in these same thrombophilic families (**Figure 2**).[10] In deficient subjects, the overall annual incidence of VTE during the prospective time period was comparable to annual incidence of VTE during the retrospective period (1.53% vs 1.65%; P=0.77). Compared to our retrospective

analysis,[10] the slight elevation in annual incidence of unprovoked VTE (0.77% vs 0.95%; P=0.58) and a decrease in the annual incidence of provoked VTE (0.84% vs 0.58%; P=0.32) were statistically not significant. In contrast, in the 46 of the 91 risk moments in which thromboprophylaxis was used, no VTE have occurred; underlining the effectiveness of primary thromboprophylaxis in VTE prevention in deficient subjects.

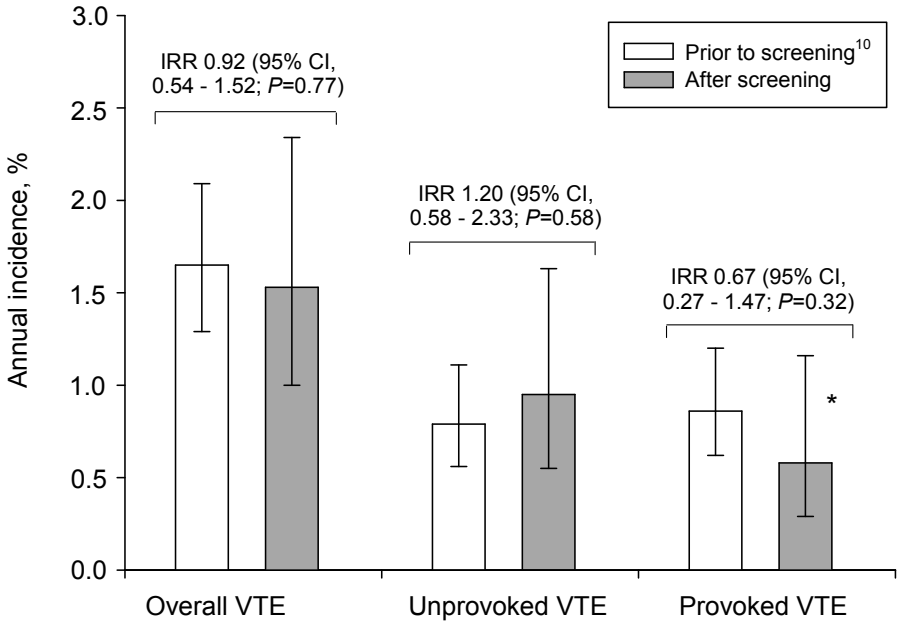

Figure 2. Incidence rates of VTE prior to screening versus after screening in the same thrombophilic families.

Histogram represents annual incidence of VTE in previously published retrospective (white)[10] versus current prospective analysis (gray), with the corresponding 95% CIs represented by the error-bars. IRR denotes incidence rate ratio.

* All provoked VTE during prospective follow-up have occurred when thrombo-prophylaxis was not used.

DISCUSSION

This prospective analysis confirmed the high risk of VTE in subjects with hereditary deficiencies of protein S, protein C or antithrombin. As compared to the pooled cohort of nondeficient subjects, antithrombin deficient subjects had the highest risk for VTE followed by protein S and protein C deficient subjects. Whereas the risk of unprovoked VTE was about 22-fold higher in subjects with any deficiency (i.e., protein S, protein C or antithrombin), the risk of provoked VTE was only 2 to 3-fold elevated, as compared to non-deficient subjects. The incidence of exogenous risk-periods was similar between deficient and non-deficient subjects, but primary thromboprophylaxis was used more frequently in deficient subjects, especially in subjects with antithrombin deficiency. Although the overall annual incidence of VTE in the current prospective analysis was comparable to our retrospective analysis,[10] the probability to capture a VTE is higher in a prospective than in a retrospective analysis. Therefore the two studies are not comparable.

Our results on the absolute risks are in line with previous prospective studies.[7-9] In a comparable family cohort study by Sanson et al,[7] the overall annual incidence of VTE was 0.7% (protein S), 1.0% (protein C) and 4.0% (antithrombin), in contrast to 1.6% (protein S), 1.0% (protein C) and 2.3% (antithrombin) in our study. The higher risk of VTE in protein S deficient subjects in our study could be attributed to the selection of severely affected subjects in our study (only type I protein S deficiency), whereas in the study of Sanson et al both type I and type III protein S deficient subjects were enrolled.[7] Though the risk of overall VTE in antithrombin deficient subjects was lower in our study, the risk of unprovoked VTE in our study was comparable to the study by Sanson et al (1.8% vs 1.6%),[7] Of note, the risk estimates in the study by Sanson et al were based on only 9 cases of VTE in 209 deficient subjects, reflecting a substantially shorter follow-up, as compared to our study.[7] In another study by Vossen et al,[8] annual incidences of 0.7% for each protein S and protein C deficiency, and 1.7% for antithrombin deficiency were reported. The observed differences might be attributed to difference in subjects' selection as we enrolled subjects from families with familial thrombophilia with proven inheritance, which was established if at least two relatives were tested positive for the index deficiency, whereas in the study of Vossen et al only one relative with the index deficiency was required.[8] Furthermore, both type I and type III protein S deficient subjects were included in

that study.[8] Finally, in a prospective study by Pabinger et al annual incidences of VTE were much higher than in our study, in both protein S (3.5% vs. 1.6%) and protein C deficient subjects (2.5% vs. 1.0%).[9] However, annual incidences in that study were based on only 3 VTE in each protein S (n=24) and protein C (n=20) deficient subjects with total follow-up of only 93 and 119 person-years, respectively.[9] Given that the risk estimates in previous prospective studies were based on very low number of VTE and/or small cohort with short follow-up, we presume that our estimates reflect the most accurate assessment of the absolute VTE risk conferred by these deficiencies.

In previous prospective studies as well as in our study, annual incidences in the three types of deficiencies varied widely, with antithrombin deficiency conferring the highest absolute risk for VTE.[8] It could be speculated that antithrombin deficiency is a stronger risk factor for VTE than are protein S or protein C deficiencies. Therefore, it could be questioned whether it is correct to pool these deficiencies. Nevertheless, annual incidences in protein S, protein C or antithrombin deficiencies fell within each other's 95% confidence intervals. Furthermore, in retrospective studies, differences in annual incidences among the three types of deficiencies were less evident and similar relative risks were reported.[3-6]

Though VTE risk estimates in current prospective analysis were nearly the same as the risk estimates prior to screening in these families (i.e., 1.53% vs 1.61%; **Figure 2**), these results may not give further credence against screening. This is especially important as the lack of overall VTE risk reduction, after screening and subsequent preventative recommendations, could be attributed to low compliance (51% of high risk-periods in deficient subjects). Moreover, all provoked VTE occurred when thromboprophylaxis was not used, underlining the effectiveness of thrombo-prophylaxis in these deficient subjects. Finally, two out of six deficient women using oral contraceptives developed VTE. Therefore, the relevance of discouraging oral contraceptive use in deficient women could be considered beneficial, but was hampered by small numbers. Taken together, since screening could be only considered beneficial for avoiding provoked VTE, it will be especially valuable in subjects with frequent external risk factors. Especially young women are more often exposed to external risk factors due to oral contraceptive use and pregnancy and may therefore benefit the most from screening.

For the first time we evaluated the impact of screening followed by preventative recommendations on VTE risk in asymptomatic deficient relatives of patients with protein S, protein C or antithrombin deficiency. Further strengths of the current study are its prospective nature, its relatively large size and long follow-up, and carefully established inheritance of protein S, protein C or antithrombin deficiencies. Moreover, in our study the control group consisted of non-deficient relatives, whereas in previous prospective studies controls were not available or consisted of unrelated subjects.[7,8] Selecting unrelated subjects as controls for subjects with these hereditary deficiencies may result in overestimated relative risk of VTE, as other thrombophilic defects also aggregate in these families.[10]

This study has some potential limitations. Subjects were contacted on average once every 3 years. This may have resulted in underreporting of exposure to exogenous risk factors and/or thromboprophylaxis use as this information was self-reported rather than additionally validated from medical records. However, it is likely that potential underreporting would have been similar in deficient versus non-deficient subjects, because data from the participants were collected in family groups, thereby avoiding bias in follow-up time between deficient versus non-deficient relatives. Moreover, the reasons for the low compliance in current study were not registered, nevertheless, the low compliance was in line with previous reports.[24,25] Selection bias seems less likely as consecutive subjects with VTE and either protein S, protein C or antithrombin deficiency served as probands. To limit ascertainment bias, we considered only symptomatic VTE. An appropriate comparison between the prospective and our previous retrospective analysis could be hampered by differences in study design, as it is possible that awareness of the deficiency status could have resulted in higher capturing of VTE incidence in the prospective analysis. Even though this is the largest prospective study on this issue, the results should be handled with caution as numbers were small, while use of thromboprophylaxis was not specifically evaluated in this study.

In conclusion, we confirmed the high absolute risk of VTE in subjects with hereditary protein S, protein C or antithrombin deficiencies in a large well-defined prospective cohort with long follow-up. As far as screening of asymptomatic carriers of these deficiencies is concerned, it is important to differentiate between provoked and unprovoked VTE cases. For the prevention of unprovoked VTE, the value of testing is obviously limited. However, most cases of provoked VTE will

be avoidable if appropriate thromboprophylaxis is applied, underlining the value of screening.

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Chapter 7

Hereditary deficiency of protein C or protein S confers increased risk of arterial thromboembolic events at a young age:
Results from a large family cohort study

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ABSTRACT

Background: Whether hereditary protein S, protein C, or antithrombin deficiency is associated with arterial thromboembolism (ATE) and whether history of venous thromboembolism in these subjects predisposes them to subsequent ATE have yet to be determined.

Methods and Results: On the basis of pedigree analysis, we enrolled a total of 552 subjects (52% women; mean age, 46±17 years), belonging to 84 different kindreds, in this retrospective family cohort study. Detailed information on previous episodes of venous thromboembolism, ATE, anticoagulant use, and atherosclerosis risk factors was collected. Primary study outcome was objectively verified symptomatic ATE. Of 552 subjects, 308 had protein S (35%), protein C (39%), or antithrombin (26%) deficiency. Overall, annual incidences of ATE were 0.34% (95% confidence interval [CI], 0.23 to 0.49) in deficient versus 0.17% (95% CI, 0.09 to 0.28) in nondeficient subjects; the hazard ratio was 2.3 (95% CI, 1.2 to 4.5). Because the risk hazards varied over lifetime, we performed a time-dependent analysis. After adjusting for atherosclerosis risk factors and clustering within families, we found that deficient subjects had a 4.7-fold (95% CI, 1.5 to 14.2; P=0.007) higher risk for ATE before 55 years of age versus 1.1 (95% CI, 0.5 to 2.6) thereafter compared with nondeficient family members. For separate deficiencies, the risks were 4.6- (95% CI, 1.1 to 18.3), 6.9- (95% CI, 2.1 to 22.2), and 1.1- (95% CI, 0.1 to 10.9) fold higher in protein S–, protein C–, and antithrombin-deficient subjects, respectively, before 55 years of age. History of venous thromboembolism was not related to subsequent ATE (hazard ratio, 1.1; 95% CI, 0.5 to 2.2).

Conclusions: Compared with nondeficient family members, subjects with protein S or protein C deficiency but not antithrombin deficiency have a higher risk for ATE before 55 years of age that is independent of prior venous thromboembolism.

INTRODUCTION

Several coagulation disorders are associated with an increased risk of venous thromboembolism (VTE). These thrombophilic conditions include hereditary deficiencies of protein S, protein C, and antithrombin; factor V Leiden; the prothrombin G20210A mutation; high levels of clotting factors VIII, IX, and XI; and antiphospholipid antibodies.[1] Hereditary deficiencies of protein S, protein C, and antithrombin have been recognized as the most potent thrombophilic conditions for VTE.[2–6] Recently, we demonstrated that the concomitance of other thrombophilic defects further enhances the risk of VTE associated with these deficiencies.[2] Whether hereditary protein S, protein C, or antithrombin deficiency also is involved in the development of arterial thromboembolism (ATE) has still to be elucidated. Evidence of such an association has been derived mainly from case reports.[7,8]

In 2003, a link between VTE and atherosclerosis was reported.[9] In the ensuing years, several other studies addressed this issue, most of these confirming this association.[10] This possible link has thus far been attributed to the sharing of common risk factors by the 2 conditions.[9,11] The contribution of thrombophilic defects to the link between VTE and ATE has yet to be defined.

We performed a retrospective follow-up study to assess the risk of ATE in a large series of protein S–, protein C–, or antithrombin-deficient subjects compared with nondeficient family members. Moreover, assuming that VTE and ATE share similar risk factors, we hypothesized that a relationship between VTE and subsequent ATE would be likely, especially in these subjects.

METHODS

Subjects

The study contained 3 cohorts of families with hereditary deficiency of protein S, protein C, or antithrombin. Probands were consecutive patients with VTE who had one of these deficiencies. First-degree relatives >15 years of age were identified by pedigree analysis. Because the number of antithrombin-deficient probands was small, second-degree relatives from a deficient parent also were identified. Subjects were enrolled after informed consent was obtained. Detailed data on previous episodes of VTE and ATE, risk factors for atherosclerosis, and anticoagulant treatment were collected by using a standardized questionnaire and reviewing medical records. Blood samples were taken after clinical data had been collected. Probands and relatives were tested for other thrombophilic defects in addition to their index deficiencies, including deficiencies of protein S, protein C, and antithrombin; factor V Leiden; the prothrombin G20210A mutation; high levels of factor VIII; and lupus anticoagulant.

Risk factors for atherosclerosis included hypertension, defined as a systolic blood pressure of ≥140 mm Hg or ≥160 mm Hg in patients ≥60 years of age, a diastolic blood pressure of ≥90 mm Hg measured on at least 2 occasions, or the use of antihypertensive drugs; diabetes mellitus[12]; cigarette smoking; and hyperlipidemia, defined as total cholesterol level >6.5 mmol/L (250 mg/dL), triglycerides >2.5 mmol/L (220 mg/dL), or use of lipid-lowering drugs. The study was approved by the institutional review board of our hospital.

Diagnosis of Thromboembolism

ATE was considered established if myocardial infarction, ischemic stroke, transient ischemic attack, or peripheral artery disease was symptomatic and objectively verified. Q-wave and non–Q-wave myocardial infarction was confirmed by typical ECG features, elevated levels of cardiac enzymes, radionuclide imaging techniques, or coronary angiography. Ischemic stroke was documented by computed tomography scanning or magnetic resonance imaging. Transient ischemic attack required neurological symptoms and signs lasting <24 hours. Peripheral artery disease was considered thromboembolic at acute signs and symptoms of ischemia and was documented by arteriography. VTE was considered established if deep vein thrombosis was confirmed by compression ultrasonography or venography, and pulmonary embolism was confirmed by ventilation-perfusion lung scanning, spiral computed tomography scanning, or

pulmonary angiography. Before these techniques were available, VTE was considered established when the patient had received full-dose unfractionated heparin and a vitamin K antagonist for at least 3 months.[2]

Laboratory Studies

Protein S and protein C antigen levels were measured by ELISA (reagents obtained from DAKO, Glostrup, Denmark); activity of protein C (Berichrom Protein C, Dade Behring, Liederbach, Germany) and antithrombin (Coatest, Chromogenix, Mölndal, Sweden) was measured by chromogenic substrate assays. Normal ranges (mean±SD) were determined in 393 healthy blood donors who had no (family) history of thromboembolism, were not pregnant, and had not used oral contraceptives for at least 3 months. Protein S deficiency type I was defined by lowered total protein S antigen levels (<68 IU/dL). Protein C deficiency types I and II were defined by reduced levels of protein C antigen (<63 IU/dL) and/or activity (<64 IU/dL); antithrombin deficiency was defined by decreased levels of antithrombin activity (<74 IU/dL). Deficiencies were considered inherited if they were confirmed by measurement of a second sample that was collected 3 months later and were found in at least 2 family members, whereas acquired conditions were excluded. If a discrepancy was found between the results of the 2 tests, a third sample was tested. A deficiency was considered acquired through use of oral contraceptives or pregnancy unless it was confirmed at least 3 months after withdrawal of oral contraceptives or delivery, respectively. Factor V Leiden and the prothrombin G20210A mutation were demonstrated by polymerase chain reactions.[13,14] Factor VIII:C was measured by 1-stage clotting assay and was considered increased at levels >150 IU/dL. Lupus anticoagulant was defined by abnormal values of dilute Russell viper venom time, activated partial thromboplastin time, and tissue thromboplastin inhibition, which was normalized by the addition of phospholipids to the subject's plasma.[15] In probands and symptomatic relatives, blood samples were collected at least 3 months after a thrombotic event. If they were on long-term treatment with acenocoumarol, a short-acting vitamin K antagonist, samples were taken after treatment had been interrupted for at least 2 weeks; meanwhile, nadroparin was given subcutaneously.

Statistical Analysis

We calculated overall and age-specific absolute risks of ATE in subjects with protein S, protein C, or antithrombin deficiency separately and in the pooled cohorts of deficient and nondeficient subjects. Because all probands were selected on the basis of VTE and the presence of protein S, protein C, or antithrombin deficiency, they were included in the current analysis for ATE. Annual incidences were calculated by dividing the number of symptomatic subjects by the total number of observation years. Observation time was defined as the period from 15 years of age until the first episode of ATE or the end of the study, considering that thrombosis is rare at younger ages. The 95% confidence intervals (CIs) around the annual incidences were assessed with the Poisson distribution assumption.

Kaplan-Meier methods were used for survival plots. On the basis of the observation that Kaplan-Meier curves in the pooled cohorts of deficient and nondeficient subjects diverged until approximately 55 years of age and then showed less divergence, the assumption for proportional hazards for the final model was not met over the entire observation period. Therefore, we chose a time-dependent Cox proportionalhazards model for the analyses of index deficiencies with a cutoff point set at 55 years of age.16 A multivariable model with additional adjustment for clustering of ATE within families that used the robust sandwich method in SAS version 9.1 (SAS Institute Inc, Cary, NC) was applied to all variables that yielded a value of $P<0.15$ from the univariable model.16 Results were expressed as hazard ratios with 95% CIs and P values.

Continuous variables were expressed as median values and ranges; categorical data, as numbers and frequencies. Differences between groups were evaluated by the Student t test or Mann-Whitney U test, depending on the normality of data for continuous data, and by the Fisher exact test for categorical data. Statistical significance was considered at a 2-tailed $P<0.05$. Statistical analyses were performed with SAS software, version 9.1.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

RESULTS

Subjects

Sixteen hundred consecutive patients with VTE were screened over 12 years to identify 91 probands with protein S, protein C, or antithrombin deficiency. For living relatives, response rates between 90% and 97% per cohort allowed us to identify 725 relatives who were the subjects in our study. Figure 1 details the reasons for exclusion of 257 relatives. Sixty-one relatives (8%) were <15 years of age; 136 (19%) died before enrollment; 30 (4%) refused or could not provide consent because of mental illness; and 11 (1.5%) were not enrolled for geographic reasons. Nineteen additional relatives and their 7 probands were excluded because inheritance of the index deficiency could not be established. The remaining 468 relatives and 84 probands were analyzed: 191 subjects in the protein S cohort, 226 in the protein C cohort, and 135 in the antithrombin cohort (**Figure 1**).

Characteristics of the study subjects are summarized in the Table. Deficiencies were demonstrated in 48% of relatives and were equally distributed among men and women. Age of enrollment in deficient and nondeficient subjects was similar. Overall, 50% of deficient subjects had a history of VTE in contrast to only 3% of nondeficient subjects. When probands were excluded from this analysis, 31% of deficient versus 3% of nondeficient relatives had a history of VTE (P<0.001). Moreover, 21% of overall deficient subjects received long-term (ie, ≥12 months) treatment with vitamin K antagonists compared with 2% of nondeficient subjects (median, 10 years; range, 1 to 42 years). Long-term use of antiplatelet agents was similar in deficient (2%) and nondeficient (1%) subjects. Although concomitance of the prothrombin G20210A mutation and factor V Leiden was equally distributed among deficient and nondeficient subjects, factor VIII levels >150 IU/dL were more prevalent in deficient subjects. Of risk factors for atherosclerosis, smoking history tended to be more frequent in deficient than nondeficient subjects (P=0.06), especially in protein C–deficient subjects, whereas diabetes mellitus tended to be more common in nondeficient subjects (P=0.06).

Risk of arterial thromboembolic events

ATE occurred in 11% (protein S), 11% (protein C), and 8% (antithrombin) of deficient subjects compared with 5%, 5%, and 7% of nondeficient subjects, respectively (the **Table**). Median age at onset of the first episode of ATE was 11 years lower in pooled cohorts of deficient subjects versus nondeficient subjects

(P=0.006). Compared with the pooled cohort of nondeficient subjects, this difference was most prominent in subjects with protein C deficiency (22 years; P<0.001), followed by protein S–deficient subjects (10 years; P=0.14) and antithrombin-deficient subjects (5 years; P=0.29).

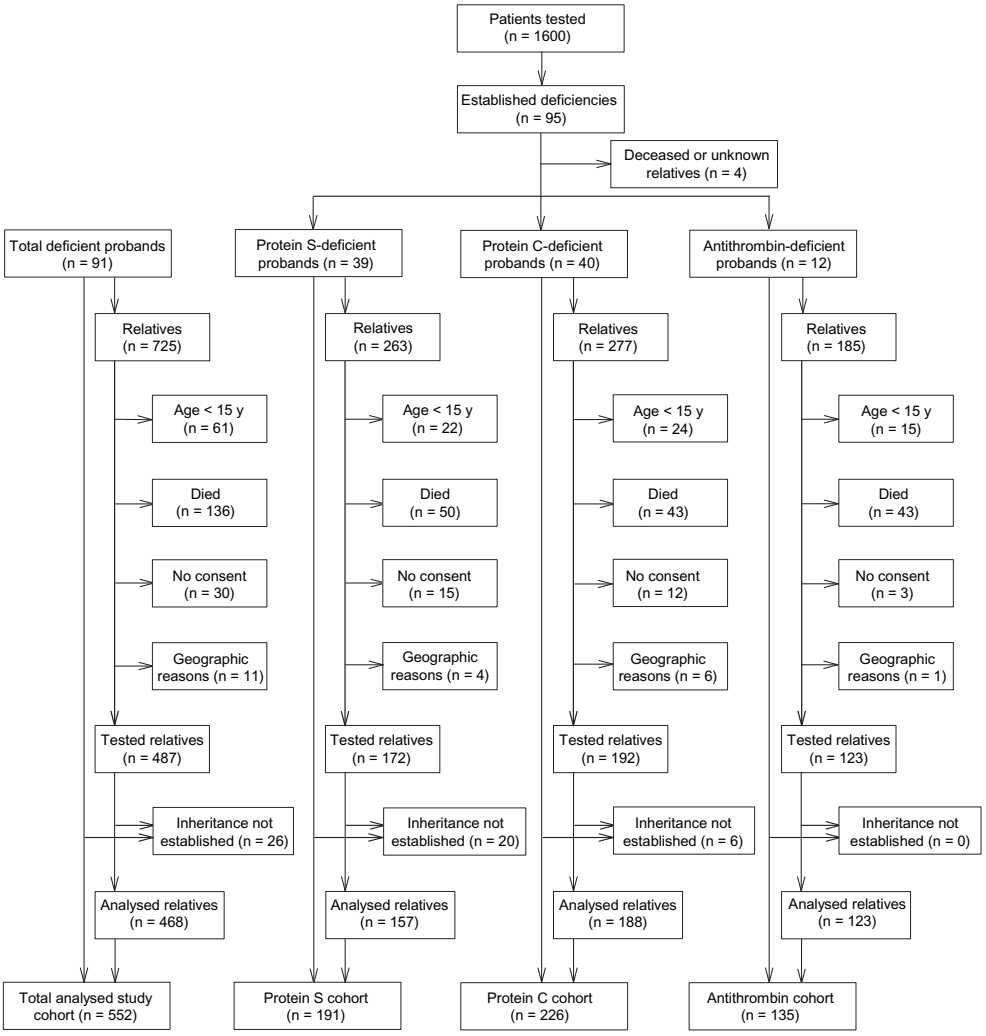


Figure 1. Recruitment of 3 family cohorts with hereditary protein S, protein C, or antithrombin deficiency.

Table 1. Characteristics of the study population.

Variable	Total cohort			Protein S cohort			Protein C cohort			Antithrombin cohort		
	Deficient	Non-deficient	P	Deficient	Non-deficient	P	Deficient	Non-deficient	P	Deficient	Non-deficient	P
Subjects, n	308	244		109	82		120	106		79	56	
Women, n (%)	159 (52)	128 (52)	0.93	57 (52)	44 (54)	0.88	61 (51)	54 (51)	1.00	41 (52)	30 (54)	0.86
Median age at enrolment, (range), yr	43 (15-89)	47 (15-92)	0.18	43 (15-84)	47 (15-84)	0.43	46 (15-89)	48 (15-86)	0.54	41 (15-84)	46 (16-92)	0.45
History of VTE, n (%)	153 (50)	7 (3)	<0.001	56 (51)	5 (6)	<0.001	63 (53)	2 (2)	<0.001	34 (43)	0 (0)	<0.001
Long-term VKA, n (%)	65 (21)	4 (2)	<0.001	22 (20)	1 (1)	<0.001	28 (23)	2 (2)	<0.001	15 (19)	1 (2)	0.002
Concomitance of, n (%)*												
FVIII:C >150 IU/dl	116 (43)	68 (34)	0.04	43 (45)	22 (29)	0.04	51 (49)	31 (39)	0.18	22 (32)	15 (32)	1.00
PT G20210A	19 (7)	19 (9)	0.50	5 (5)	6 (8)	0.54	9 (8)	7 (8)	1.00	5 (7)	6 (13)	0.35
Factor V Leiden	44 (15)	37 (17)	0.71	19 (19)	11 (14)	0.43	21 (19)	24 (25)	0.31	4 (6)	2 (4)	0.35
Diabetes, n (%)	6 (2)	12 (5)	0.06	3 (3)	5 (6)	0.29	2 (2)	5 (5)	0.26	1 (1)	2 (4)	0.57
Hyperlipidemia, n (%)	35 (11)	20 (8)	0.25	17 (16)	10 (12)	0.54	9 (8)	7 (7)	1.00	9 (11)	3 (5)	0.36
Hypertension, n (%)	41 (13)	29 (12)	0.70	12 (11)	14 (17)	0.29	20 (17)	9 (8)	0.08	9 (11)	6 (11)	1.00
Smoking history, n (%)	102 (33)	62 (26)	0.06	34 (31)	26 (32)	1.00	44 (37)	24 (23)	0.03	24 (30)	12 (21)	0.32
Overall ATE, n (%)	31 (10)	13 (5)†	0.06	12 (11)	4 (5)	0.19	13 (11)	5 (5)	0.14	6 (8)	4 (7)†	1.00
MI	12 (4)	4 (1.6)	0.13	5 (5)	1 (1)	0.24	4 (3)	2 (2)	0.69	3 (4)	1 (2)	0.64
Ischemic stroke	11 (4)	4 (1.6)	0.20	4 (4)	3 (4)	1.00	6 (5)	1 (1)	0.12	1 (1)	0 (0)	1.00
TIA	8 (3)	4 (1.6)	0.56	3 (3)	0 (0)	0.26	3 (3)	2 (2)	1.00	2 (3)	2 (4)	1.00
Median age at onset of ATE (range), yr	53 (30-80)	64 (41-78)	0.006	54 (31-80)	65 (41-72)	0.47	42 (30-61)	58 (52-70)	0.02	59 (55-65)	69 (63-78)	0.03

VTE denotes venous thromboembolism; Long-term VKA, use of vitamin K antagonists for ≥12 months; PT G20210A, the prothrombin G20210A mutation mutation; ATE arterial thromboembolism; MI, myocardial infarction; TIA, cerebral transient ischemic attack.

* Of total study cohort, 15%, 10% and 8% of subjects were not tested for FVIII:C, the prothrombin G20210A mutation and factor V Leiden, respectively. Of 416 tested subjects, 2 were positive for lupus anticoagulant.

† One subject in this group had peripheral artery disease.

The probability of ATE-free survival was 87% and 98% at 55 years of age and 71% and 74% at 73 years of age in deficient and nondeficient subjects, respectively (**Figure 2**). Overall, the annual incidences of ATE in the pooled cohorts were 0.34% (95% CI, 0.23 to 0.49) in deficient subjects versus 0.17% (95% CI, 0.09 to 0.28) in nondeficient subjects (hazard ratio, 2.3; 95% CI, 1.2 to 4.5; P=0.01). However, on the basis of the observation that the Kaplan-Meier curves in the pooled cohorts diverged until approximately 55 years of age and then showed less divergence (**Figure 2**), we opted for time-dependent analysis of the index deficiencies (age <55 versus ≥55 years).

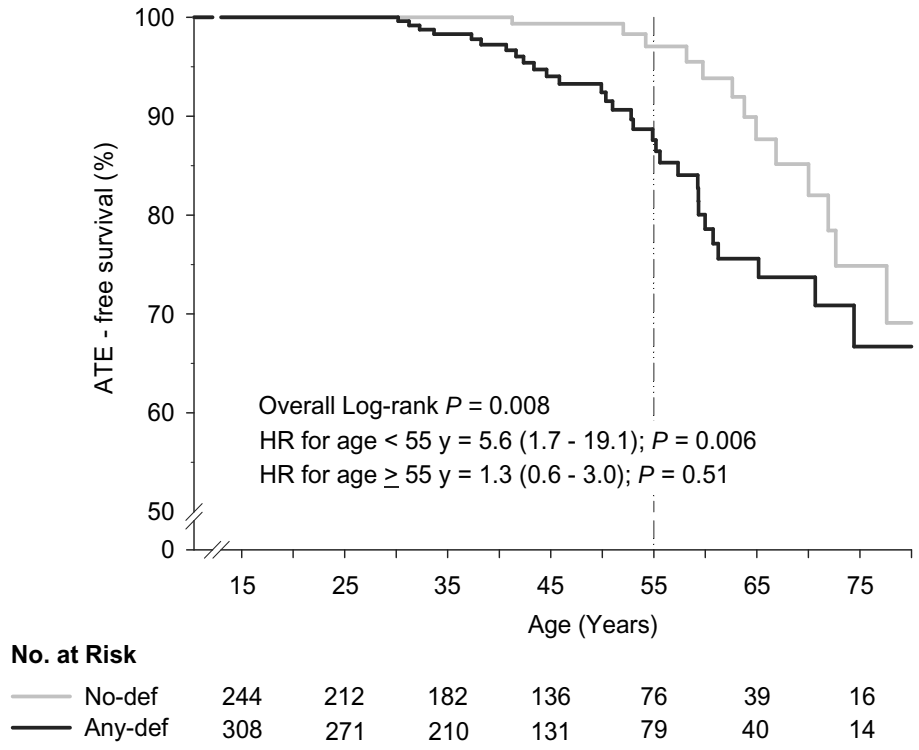
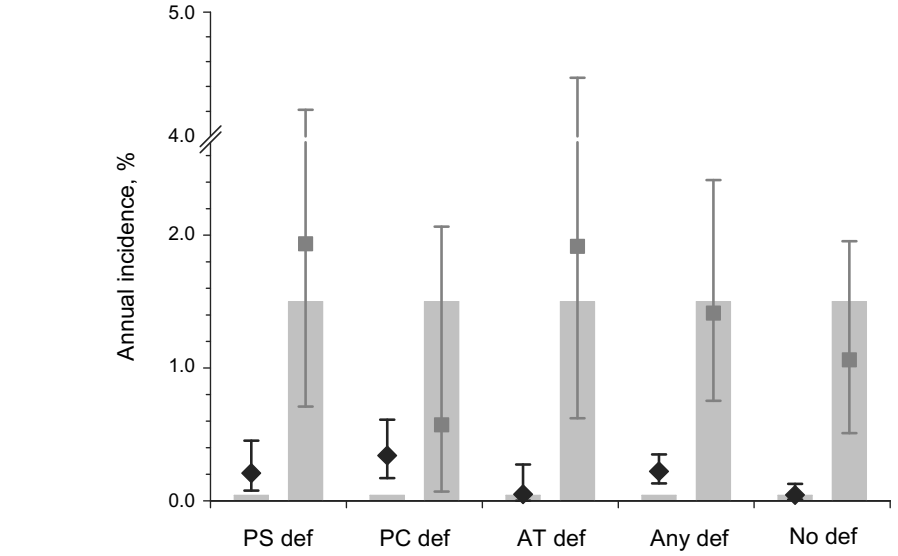


Figure 2. Event-free survival comparing subjects with and without any deficiency. HR indicates hazard ratio; No-def, no deficiency; and Any-def, any deficiency (ie, protein S, protein C, or antithrombin deficiency).

Figure 3 depicts the annual incidences of ATE in deficient and nondeficient subjects <55 and ≥55 years of age compared with age- and sex-weighted annual incidences in the general population.[17,18] Annual incidences in deficient subjects after 55 years of age were several-fold higher than before 55 years of age, but the former were similar to the age and sex-weighted annual incidence in the general population.[17,18] In contrast, annual incidences before 55 years of age were significantly higher in subjects with protein S, protein C, or any deficiency compared with the general population.[17,18]



Annual incidence, % (95% CI)	PS def	PC def	AT def	Any def	No def
◆ < 55 years of age	0.21 (0.08 - 0.45)	0.34 (0.17 - 0.61)	0.05 (0.001 - 0.27)	0.22 (0.13 - 0.35)	0.04 (0.01 - 0.13)
■ ≥ 55 years of age	1.94 (0.71 - 4.21)	0.57 (0.07 - 2.06)	1.92 (0.62 - 4.47)	1.41 (0.75 - 2.42)	1.06 (0.51 - 1.95)

Figure 3. Annual incidences of ATE in subjects with hereditary protein S, protein C, or antithrombin deficiency <55 and ≥55 years of age.
Solid diamonds (black) and squares (dark grey) indicate annual incidences of ATE <55 and ≥55 years of age, respectively. The corresponding 95% CIs are represented by vertical error bars. The vertical gray bars represent the age- and sex-weighted ATE in the general population (ARIC study for myocardial infarction[17] and Framingham study for cerebrovascular and peripheral artery disease[18]). PS def indicates protein S deficiency; PC def, protein C deficiency; AT def, antithrombin deficiency; Any def, any (ie, protein S, protein C, or antithrombin) deficiency; No def, no deficiency.

Figure 4 shows the univariable association of various variables with the risk for ATE. Compared with nondeficient subjects, the risk for ATE was 5.6-fold (95% CI, 1.7 to 19.1; P=0.006) higher in individuals with any deficiency (ie, protein S,

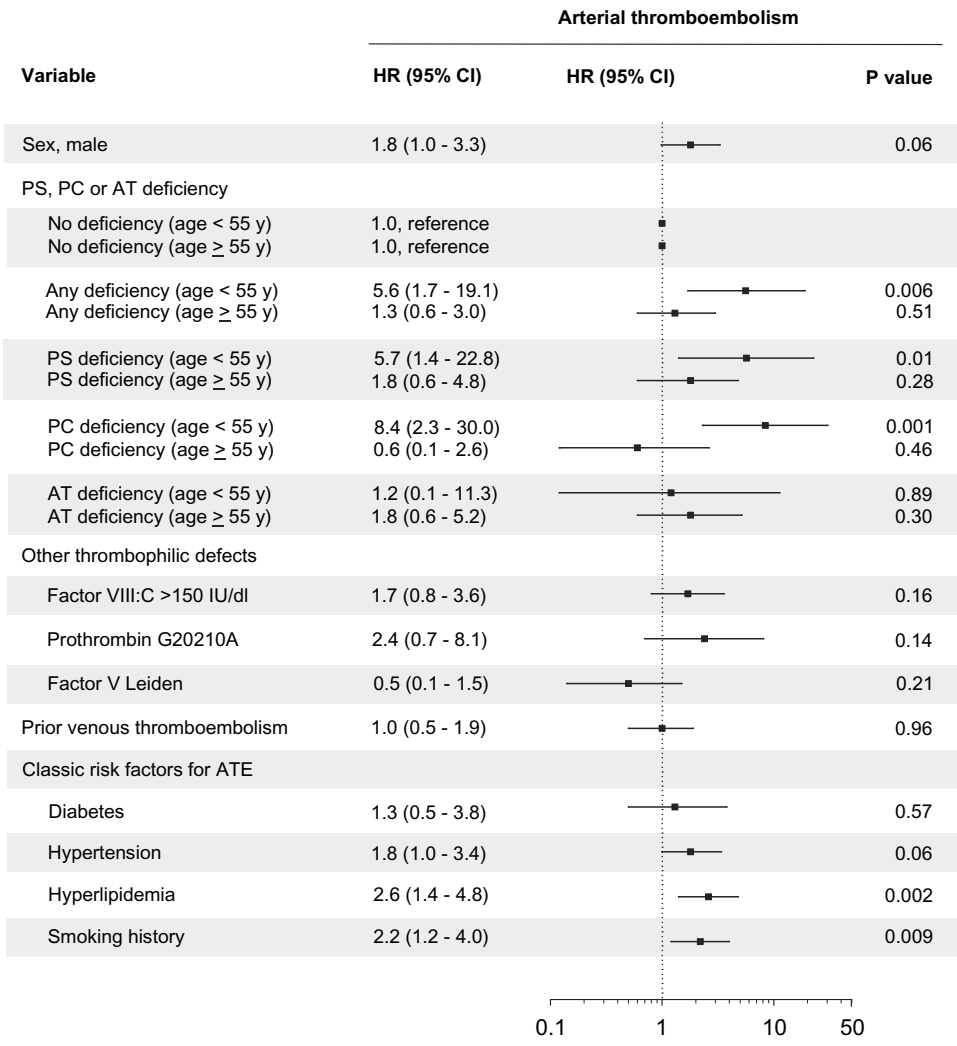


Figure 4. Univariable proportionalhazards analysis of association with the time to the first arterial thromboembolic event.

Solid squares indicate the hazard ratio of ATE with the corresponding 95% CIs represented by the horizontal error bars. Index deficiencies were analyzed as time-dependent variables, ie, age <55 and ≥55 years. HR indicates hazard ratio; PS, protein S; PC, protein C; and AT, antithrombin.

protein C, or antithrombin) before 55 years of age. It was 1.3-fold (95% CI, 0.6 to 3.0; P=0.51) higher thereafter. The high risk for ATE conferred by any deficiency before 55 years of age was confined to protein S– or protein C–deficient subjects. Although factor VIII:C levels >150 IU/dL (P=0.16) and the prothrombin G20210A mutation (P=0.14) tended to be positively related to ATE, factor V Leiden did not show a similar trend. However, subjects with factor V Leiden had significantly lower prevalence of hypertension and smoking compared with noncarriers (P≤0.02). Of 416 tested subjects, only 2 were positive for lupus anticoagulant. History of VTE was not related to subsequent ATE (hazard ratio, 1.0; 95% CI, 0.5 to 1.9; P=0.96). After the duration of anticoagulant treatment was subtracted from the observation period and 4 ATEs that occurred while these subjects were on anticoagulant therapy were excluded, the risk estimates remained unchanged (hazard ratio, 1.1; 95% CI, 0.5 to 2.2; P=0.86). Of the classic risk factors for atherosclerosis, hyperlipidemia and history of smoking were associated with ATE (P<0.01). Furthermore, hypertension and male sex tended to be associated with ATE (P=0.06).

Multivariable analysis with additional adjustment for clustering within families was applied to any deficiency, the prothrombin G20210A mutation, hypertension, hyperlipidemia, smoking history, and sex. Of these variables, any deficiency before 55 years of age, hypertension, hyperlipidemia, and smoking were independently associated with ATE (P≤0.03) (**Figure 5**). The adjusted hazard ratio for ATE conveyed by any deficiency before 55 years of age was 4.7 (95% CI, 1.5 to 14.2; P=0.007) versus 1.1 (95% CI, 0.5 to 2.6; P=0.84) thereafter. Adjusted hazard ratios conferred by hypertension, hyperlipidemia, and smoking were each about 2-fold elevated. Adjusted hazard ratios conferred by separate deficiencies were 4.6 (95% CI, 1.1 to 18.3; P=0.03), 6.9 (95% CI, 2.1 to 22.2; P=0.001), and 1.1 (95% CI, 0.1 to 10.9; P=0.94) in subjects with protein S, protein C, and antithrombin deficiencies, respectively, before 55 years of age (data not shown). No significant interaction was found between smoking and any deficiency or between other atherosclerosis risk factors or any of the concomitant thrombophilic defects (ie, elevated factor VIII:C, the prothrombin G20210A mutation, and factor V Leiden) and any deficiency (P≥0.37).

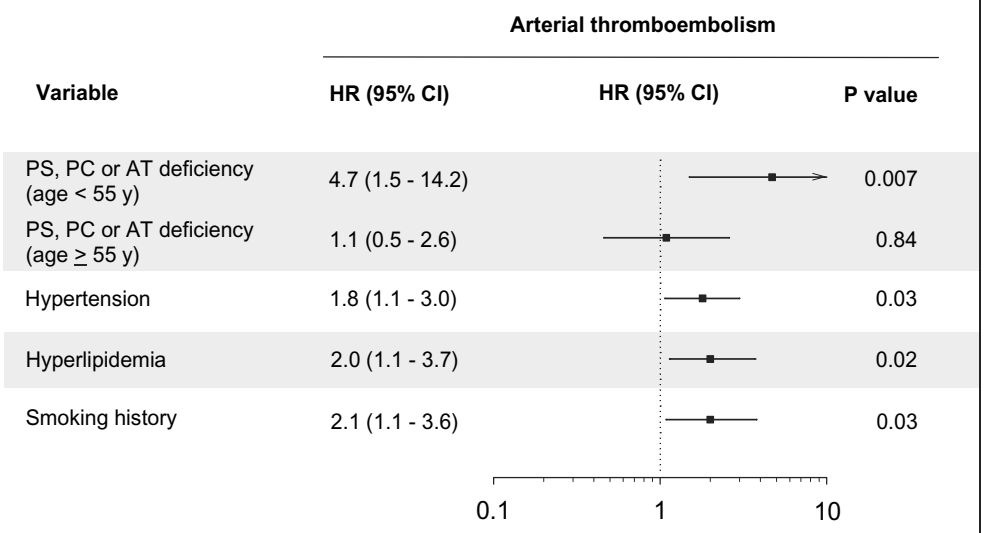


Figure 5. Multivariable proportionalhazards analysis.
Solid squares indicate the hazard ratio of ATE with the corresponding 95% CIs represented by the horizontal error bars. Any deficiency was analyzed as a time-dependent variable (ie, age <55 and ≥55 years). The hazard ratios (HR) are adjusted for clustering of ATE within families. PS indicates protein S; PC, protein C; and AT, antithrombin.

DISCUSSION

Overall, the lifetime risk of ATE was about 2-fold higher in subjects with any deficiency (ie, protein S, protein C, or antithrombin) compared with nondeficient subjects. However, the high risk of ATE conferred by any deficiency was evident only until approximately 55 years of age, a 5-fold risk increase. Moreover, subjects with any deficiency were on average 11 years younger at the onset of ATE compared with nondeficient subjects. Interestingly, only protein S and protein C deficiencies were related to ATE before 55 years of age. Antithrombin deficiency was not related to a significantly increased risk either before 55 years of age or thereafter. Subjects with both a history of VTE and any deficiency had a risk of subsequent ATE similar to that of subjects with any deficiency alone.

Only a few previous studies and several case reports have reported on this issue.[7,8] A comparison with our study is hampered by differences in design. In a family cohort study, arterial events were observed in 8% of 144 protein S– or protein C–deficient subjects and in 1% of 94 antithrombin-deficient subjects.[4] Annual incidences in that study were stratified into types of ATE, sex, and age according to the Framingham study.[18] However, because of the limited number of events, annual incidences were only estimated in a few strata, which hindered an appropriate comparison with our study. Furthermore, data on nondeficient relatives and conventional risk factors for atherosclerosis were not provided. In a large study in carriers of familial thrombophilia (the European Prospective Cohort on Thrombophilia [EPCOT] study),[19] overall annual incidences of myocardial infarction and/or ischemic stroke after 20 years of age were 0.15%, 0.18%, and 0.15% in subjects with protein S (n=111), protein C, (n=150), and antithrombin deficiency (n=92), respectively. When we confined our analysis to >20 years of age, annual incidences of myocardial infarction and/or ischemic stroke were 0.32% (protein S), 0.32% (protein C), and 0.21% (antithrombin) in deficient subjects and 0.19% in nondeficient subjects. It is likely that the risks of ATE in the EPCOT study[19] were underestimated because annual incidences in their control group also were remarkably low, only 0.03% in men (mean age, 58 years) and 0.01% in women (mean age, 58 years). Moreover, information on cardiovascular risk factors and on whether the recorded events were objectively verified was not available. In a case-control study, ATE was recorded more frequently in 88 cases with protein S, protein C, or antithrombin deficiency (19% ATE) compared with control subjects

with VTE without these deficiencies (1% ATE).[20] Although the control group in this study might not be appropriate because not all deficient subjects had history of VTE, no difference in ATE prevalence was found among protein S–, protein C–, or antithrombin-deficient subjects. Another case-control study reported significantly lower plasma levels of activated protein C in young patients with myocardial infarction (n=231) compared with healthy controls (n=231).[21] In a Japanese study, subjects with established inherited protein C deficiency and either myocardial infarction (n=10) or ischemic stroke (n=11) were on average 11 and 7 years younger at onset of myocardial infarction and ischemic stroke, respectively, than control subjects with myocardial infarction (n=42) or ischemic stroke (n=48) with normal protein C levels.[22] Finally, the prospective epidemiological Atherosclerosis Risk in Communities (ARIC) study reported that plasma protein C appeared protective against ischemic stroke but not myocardial infarction.[23]

On the other hand, in other case-control studies, prevalences of these deficiencies were similar between cases with ischemic stroke[7,24] or myocardial infarction[7,25,26] and matched controls, even young patients.[25,26] Furthermore, in the ARIC study, low levels of plasma protein C and antithrombin were not related to coronary heart disease.[27] However, the lack of association between protein S, protein C, or antithrombin deficiency and risk for ATE in these studies could be explained by the low prevalence of these hereditary deficiencies in the general population. This notion is given further credence by the finding that more common, but weaker, thrombophilic defects (ie, factor V Leiden, the prothrombin G20210A mutation) were more frequently related to myocardial infarction[25,26,28] and/or overall coronary disease (ie, myocardial infarction or coronary stenosis).[28] In addition, it could be speculated that protein S, protein C, or antithrombin deficiency in these studies might be acquired rather than hereditary, considering that acquired deficiencies are more prevalent. The latter is consistent with the exceptionally high prevalence of these deficiencies (up to 4%) in control subjects.[24]

Surprisingly, the increased risk for ATE was confined to subjects with protein S and protein C deficiencies. Subjects with antithrombin deficiency had a risk for ATE comparable to that for nondeficient subjects. That this difference is attributable by chance, for instance, as a result of the smaller cohort of subjects with antithrombin deficiency, could be argued because high risk for ATE conferred by protein S or protein C but not antithrombin deficiency also was reported earlier.[4,8] It could be speculated that the higher risk for ATE in subjects with

protein C deficiency could be ascribed to the potent cytoprotective effects of the protein C pathway.[29] Why protein S deficiency, rather than deficiency of antithrombin, was associated with ATE may be the synthesis of protein S by endothelial cells,[30] whereas antithrombin is synthesized by hepatocytes. Endothelial injury as a trigger of thrombosis may be enhanced by a preexisting defect in protein S synthesis at the site of injury. Furthermore, some cytoprotective effects also have been attributed to protein S.[30,31]

Since its first description,[9] the amount of data indicating a link between VTE and subsequent ATE has been increasing.[10] In our study, subjects with prior VTE had a risk for ATE similar to that of subjects without prior VTE. This lack of association could not be ascribed to use of vitamin K antagonists. On the basis of our estimated hazard risk, even in a larger sample, a link between VTE and subsequent ATE seems unlikely in these subjects. The potential link between VTE and subsequent ATE is believed to be due to the sharing of common pathophysiological mechanisms.[9] Because protein S and protein C deficiencies but not antithrombin deficiency were significantly related to ATE in our study, it could be speculated that endothelial dysfunction rather than coagulation disorders is the main actor in the link between VTE and subsequent ATE. Moreover, atherosclerosis risk factors are somewhat stronger risk factors for VTE than are the prothrombin G20210A mutation and factor V Leiden for ATE.[11,28] However, it remains unclear why subjects with both prior VTE and protein S or protein C deficiency had a risk for ATE similar to that of subjects with these deficiencies alone.

Concomitant thrombophilic events apparently aggregated in our families. The prevalence of factor V Leiden was 3 to 5 times higher in families with protein S or C deficiency than that reported in the general population (14% to 25% versus 5%).[5] The prothrombin G20210A mutation was more prevalent in families with any of the 3 deficiencies than in the general population (5% to 13% versus 2%),[5] as were increased factor VIII:C levels (29% to 49% versus 11%).[5] Aggregation of these concomitant thrombophilic defects was independent of the index deficiencies, except that factor VIII:C levels >150 IU/dL were more frequently observed in subjects with protein S or C deficiency. The latter might be secondary to these deficiencies per se because, in theory, low levels of activated protein C or protein S may result in less FVIII inactivation. In contrast to VTE,[2] the concomitance of factor VIII:C levels >150 IU/dL, the prothrombin G20210A mutation, or factor V

Leiden was not related to higher risk for ATE compared with subjects with the index deficiencies alone.

One may consider screening for protein S or protein C deficiency in young subjects with ATE if a family history is suspected or positive for thrombophilia because diagnosis of these deficiencies has clinical implications for the prevention of VTE. Further studies are needed to demonstrate clinical utility of thrombophilia screening for primary or secondary prevention of ATE.

The main limitation of this study is its retrospective design. Consequently, because patients were not routinely screened for atherosclerosis risk factors and the information on these factors was self-reported and/or derived from medical records, it is possible that the true incidence of these risk factors has been underestimated in asymptomatic subjects. This would have resulted in a slightly lower adjusted hazard ratio for ATE conferred by protein S, protein C, or antithrombin deficiency. Referral bias may have been introduced by the university hospital setting but was probably reduced by testing all consecutive patients with VTE for deficiencies. Because probands were consecutive patients with VTE and the response rate of eligible relatives was high, selection bias was probably limited. Although we cannot exclude the possibility that more deficient than nondeficient subjects died of VTE or ATE, this potential source of bias would have resulted in an underestimated risk for ATE in deficient subjects. Moreover, hereditary deficiencies were not associated with a reduced life expectancy in previous studies.[32,33] Despite these limitations, we believe that this is the first study to document the age-dependent elevated risk for ATE conveyed by hereditary protein S or protein C deficiency but not antithrombin deficiency in these thrombophilic families.

CONCLUSIONS

This study delineates an increased risk for ATE conferred by protein S or protein C deficiency before age 55 years compared with nondeficient family members. In contrast, antithrombin deficiency was not associated with a significantly elevated risk for ATE either before age 55 years or thereafter. Prior VTE was not related to subsequent ATE in these subjects.

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Chapter 8

Venous thromboembolism as a risk factor for subsequent arterial thromboembolism: Results from the Prevention of Renal and Vascular End-stage Disease (PREVEND) Study

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ABSTRACT

Context: Evidence on the relation between venous and arterial thromboembolism is inconsistent. Furthermore, age and other cardiovascular risk factors were not always taken into account.

Objective: To determine the risk of arterial thromboembolism after venous thromboembolism, independent of cardiovascular risk factors.

Design, Setting, and Participants: In 1997-1998, inhabitants of the city of Groningen, the Netherlands, aged 28-75 years (n=85,421), were sent a questionnaire on cardiovascular risk factors, and a vial to collect a urine sample. After entry, all responding subjects (n=40,856) were followed and monitored for venous and arterial thromboembolism. Median follow-up time was 7.8 years. Thromboembolism was verified with national registries of hospital discharge diagnoses and death certificates, the regional anticoagulation clinic and medical records.

Main Outcome measure: Arterial thromboembolism (ie, myocardial infarction, coronary artery disease, peripheral arterial occlusive disease, or ischemic stroke) between study initiation and December, 31, 2005.

Results: Of 40,856 responding subjects (46% male; median age at enrollment, 48 years), 410 developed venous thromboembolism (42% unprovoked), and 2314 developed arterial thromboembolism during a median follow-up period of 7.8 (interquartile range, 7.5-8.1) years. Annual incidence of arterial thromboembolism after venous thromboembolism was 2.36% (95% confidence interval [CI], 1.59-3.37), compared to 0.80% [95% CI, 0.77-0.83] in subjects without venous thromboembolism. Adjusted hazard ratio of arterial thromboembolism after venous thromboembolism was 1.81 [95% CI, 1.26-2.60]. This risk was highest within the first year after venous thromboembolism (annual incidence, 3.51% [95% CI, 1.81-6.13]; adjusted hazard ratio, 2.58 [95% CI, 1.46-4.55]) and after an unprovoked event (annual incidence, 2.78% [95% CI, 1.62-4.45]; adjusted hazard ratio, 1.94 [95% CI, 1.20-3.12]).

Conclusions: Subjects with venous thromboembolism are at a high risk to develop arterial thromboembolism, independent of age and other cardiovascular risk factors. This risk is particularly high in the first year after venous thromboembolism and after an unprovoked event.

INTRODUCTION

The concept that venous and arterial thromboembolism are separate pathophysiological entities has been challenged.[1] In 2003, Prandoni et al. were the first to report a twofold increased risk for unprovoked deep vein thrombosis in patients with atherosclerotic plaques.[2] More recently, a large case-control study showed a 2-3 fold increased relative risk of arterial thromboembolism after first venous thromboembolism, most predominantly in the first year following initial venous thromboembolism.[3] However, evidence on the relation between venous and arterial thromboembolism is inconsistent. Two observational studies could not identify an increased risk of overall or unprovoked venous thromboembolism in patients with atherosclerosis.[4,5] Furthermore, age effects were not fully taken into account in all studies that considered venous and arterial thromboembolism as two related diseases.[6-9] Age is a strong confounder to the risk of both venous and arterial thromboembolism, hence it is doubtful whether the high absolute risk of arterial thromboembolism after venous thromboembolism (reported to be as high as 5.5% per year),[8] is truly related to previous venous thrombotic disease or merely a result of ageing. In addition, previous studies did not always correct for the presence of cardiovascular risk factors.[3] This is remarkable as these factors (i.e. elevated albuminuria, hypertension, dyslipidemia, diabetes mellitus, and smoking) are also reported to be associated with venous thromboembolism.[6,10]

This study was conducted to advance our understanding of both venous and arterial thrombotic disease and to provide further insight into the clinical course of patients with venous thromboembolism. Our aims were to determine the absolute risk of arterial thromboembolism after venous thromboembolism, and to establish whether venous thromboembolism is a risk factor for subsequent arterial thromboembolism, independent of age, sex and other cardiovascular risk factors, in a population-based cohort of more than 40,000 subjects.

METHODS

Study population

This study was conducted on participants in 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 relation with renal and cardiovascular disease in a large cohort drawn from the general population. 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=85421) were sent a postal questionnaire and a vial to collect an early morning urine sample. A total of 40,856 subjects (47.8%) responded. The questionnaire provided information about the presence of established risk factors for cardiovascular disease. Subjects were classified as being diabetic when they positively answered the question whether they were diagnosed with diabetes by a physician, regardless of the type of antidiabetic treatment. Subjects were considered hypertensive or dyslipedemic when they positively answered the question 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 associated with a hospitalization for at least 3 days.

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

Morning urinary albumin concentration (UAC) 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).[11,12] First morning urine was used for analysis. Urine samples could be analyzed for 40,854 subjects. Albuminuria was considered elevated at a concentration of 20 mg/L or more.

Definition of thrombotic events

To identify subjects with thromboembolism between January 1997 and December 2005, 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 used. Previous reports have shown that the Prismant-database is of good quality, with 84-87% of the Prismant diagnoses matching the diagnoses found in the patient chart.[13,14] Venous events were verified with the database of the regional anticoagulation clinic, which monitors the anticoagulant therapy of all inhabitants of the city of Groningen and environs. For further corroboration, patients' medical records were examined for all subjects with venous thromboembolism according to any of the abovementioned databases. Arterial thromboembolism was defined as myocardial infarction, coronary artery disease, peripheral arterial occlusive disease, and ischemic stroke. Deep vein thrombosis had to be confirmed by compression ultrasound, and pulmonary embolism by ventilation/perfusion lung scanning, spiral computed tomography, or at autopsy. Venous thromboembolism was classified as being 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. Venous thromboembolism was classified as unprovoked when no such exogenous risk factor occurred.

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 number of years of follow-up. For subjects without venous thromboembolism, observation time started at time of enrollment and ended at time of arterial thromboembolism, a censoring event (intracranial arterial bleeding, death, moving out of the city) or end of study (December 2005). For subjects who had venous thromboembolism, observation time started at time of diagnosis and also ended at time of arterial thromboembolism, a censoring event or end of study. 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. Adjustments were made for age, sex, hypertension,

dyslipidemia, diabetes mellitus, smoking status, elevated albuminuria and history of arterial thromboembolism. Additional preplanned sensitivity analyses were performed for the first year of follow-up after venous thromboembolism and for the rest of follow-up, to investigate the persistence of venous thromboembolism as a risk factor through time. To exclude the possibility of hospitalization bias, causing patients with venous thromboembolism to have a spurious increased risk of subsequent arterial thromboembolism due to close monitoring in the period following the event, we also restricted these sensitivity analyses to hard arterial thrombotic endpoints (i.e. myocardial infarction, ischemic stroke or cardiovascular death).

Categorical data are presented as counts and percentages, continuous variables as medians with interquartile ranges (IQR). Statistical analyses were performed using SPSS version 16.0 (SPSS, Chicago, Illinois, United States) and SAS version 9.1 (SAS Institute, Inc, Cary, NC, USA).

RESULTS

Study Population

Clinical characteristics of the 40,856 participants are shown in the **Table**. Slightly more women responded than man. Median age at enrolment was 60 (IQR, 49-68) and 48 (IQR, 39-60) years for subjects with and without venous thromboembolism, respectively. Venous thromboembolism had occurred in 410 subjects at a median age of 64 years (IQR, 51-72). In 192 subjects the venous event was secondary to an external risk factor, 174 events were unprovoked. In 44 events the presence of an external risk factor was unknown. Thirty subjects with venous thromboembolism developed arterial thromboembolism at a median age of 71 years (IQR, 65-77). In the group without venous thromboembolism, 2284 subjects developed arterial thromboembolism at a median age of 68 years (IQR, 60-74). A total of 7181 subjects (107 subjects with venous thromboembolism, 7074 without) were censored at time of intracranial arterial bleeding (n=151, 0.37%), death (n=1770, 4.3%) or moving out of the city (n=5260, 12.9%). Median follow-up time was 7.8 (IQR, 7.5-8.1) years.

Table. Clinical Characteristics

	Venous thrombosis	**No venous thrombosis**
TOTAL	**410 (100)**	**40,446 (100)**
Baseline characteristics		
Male	200 (49)	18,425 (46)
Age at enrolment, y	60 (49-68)	48 (39-60)
Cardiovascular risk factors		
Hypertension	148 (36)	11,691 (29)
Dyslipidemia	65 (16)	5584 (14)
Diabetes Mellitus	12 (3)	1039 (3)
Current Smokers	151 (37)	16,997 (42)
Microalbuminuria (≥20 mg/L)	49 (12)	3151 (8)
History of arterial thromboembolism	26 (6)	1754 (4)
Characteristics during follow-up		
Thrombotic cardiovascular event	30 (7)	2284 (6)
Age at onset, y	71 (65-77)	68 (60-74)
Classification		
Myocardial infarction	14 (3)	813 (2)
Coronary artery disease	6 (1)	873 (2)
Peripheral arterial occlusive disease	4 (1)	170 (0)
Ischemic stroke	6 (2)	428 (1)

Continuous variables are presented as median (IQR), categorical variables as number (%)

Risk of arterial thromboembolism after venous thromboembolism

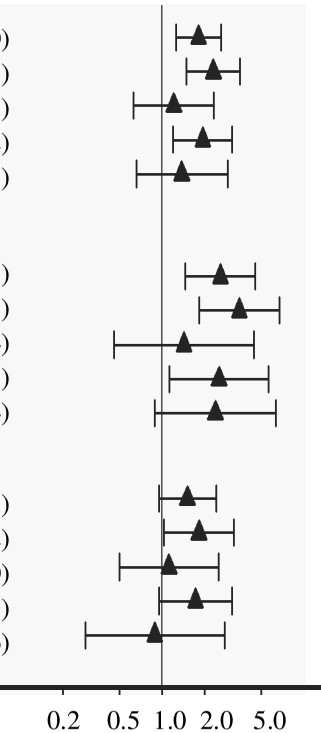
The **Figure** shows the risk of arterial thromboembolism after venous thromboembolism. The annual incidence of arterial thromboembolism after prior venous thromboembolism was 2.36% (95% CI, 1.59-3.37), compared to 0.80% (95% CI, 0.77-0.83) in subjects without venous thromboembolism. Crude hazard ratio of subsequent arterial thromboembolism was 2.90 (95% CI, 2.02-4.16) in subjects with venous thromboembolism, compared to subjects without. After adjustment for age, sex, cardiovascular risk factors and previous arterial thromboembolism, this risk was 1.81 (95% CI, 1.26-2.60). Within this model, age was a strong confounder as adjustment for age only, resulted is a hazard ratio of 1.91 (95% CI, 1.33-2.74).

When subgroups of venous thromboembolism were analyzed separately (i.e., deep vein thrombosis versus pulmonary embolism and unprovoked versus provoked venous thromboembolism), subjects with deep vein thrombosis or unprovoked venous thromboembolism had the highest risk of arterial thromboembolism, with adjusted hazard ratios of 2.30 (95% CI, 1.49-3.53) and 1.94 (95% CI, 1.20-3.12), respectively.

Risk of arterial thromboembolism was highest within the first year after venous thromboembolism with an annual incidence of 3.51% (95% CI, 1.81-6.13) and an adjusted hazard ratio of 2.58 (95% CI, 1.46-4.55). After 1 year of follow-up, the adjusted hazard ratio of arterial thromboembolism after venous thromboembolism decreased to 1.51 (95% CI, 0.95-2.41). When limited to myocardial infarction, ischemic stroke and cardiovascular death, overall adjusted hazard ratio after venous thromboembolism was 2.04 (95% CI, 1.31-3.19), and within the first year this was 2.88 (95% CI, 1.43-5.77).

Figure. Risk of arterial thromboembolism after venous thromboembolism

	Obs-yrs	No. ATE	Annual Incidence (95% CI)	Crude Hazard ratio[a] (95% CI)	Adjusted Hazard ratio[b] (95% CI)	Decreased risk for ATE	Increased risk for ATE	*P* value
OVERALL								
Venous thromboembolism (n=410)	1270	30	2.36 (1.59-3.37)	2.90 (2.02-4.16)	1.81 (1.26-2.60)			0.001
Deep vein thrombosis (n=252)	809	21	2.60 (1.61-3.97)	3.18 (2.07-4.89)	2.30 (1.49-3.53)			<0.001
Pulmonary embolism (n=158)	462	9	1.95 (0.89-3.70)	2.37 (1.23-4.57)	1.21 (0.63-2.33)			0.57
Unprovoked VTE (n=174)	612	17	2.78 (1.62-4.45)	3.40 (2.11-5.48)	1.94 (1.20-3.12)			0.01
Provoked VTE (n=192)	478	7	1.46 (0.59-3.02)	1.78 (0.85-3.73)	1.38 (0.66-2.91)			0.39
≤ 1 YEAR								
Venous thromboembolism	342	12	3.51 (1.81-6.13)	4.41 (2.50-7.78)	2.58 (1.46-4.55)			0.001
Deep vein thrombosis	209	9	4.31 (1.97-8.17)	5.41 (2.81-10.41)	3.50 (1.82-6.75)			<0.001
Pulmonary embolism	133	3	2.26 (0.47-6.59)	2.80 (0.90-8.70)	1.43 (0.46-4.44)			0.54
Unprovoked VTE	156	6	3.85 (1.41-8.37)	4.87 (2.19-10.83)	2.52 (1.13-5.63)			0.02
Provoked VTE	146	4	2.74 (0.75-7.01)	3.40 (1.28-9.05)	2.38 (0.89-6.34)			0.08
> 1 YEAR								
Venous thromboembolism	928	18	1.94 (1.15-3.07)	2.36 (1.48-3.75)	1.51 (0.95-2.41)			0.08
Deep vein thrombosis	599	12	2.00 (1.04-3.50)	2.43 (1.38-4.29)	1.82 (1.03-3.22)			0.04
Pulmonary embolism	329	6	1.82 (0.67-3.97)	2.20 (0.99-4.91)	1.12 (0.50-2.50)			0.78
Unprovoked VTE	456	11	2.41 (1.20-4.32)	2.92 (1.62-5.29)	1.72 (0.95-3.11)			0.07
Provoked VTE	332	3	0.90 (0.19-2.64)	1.09 (0.35-3.37)	0.89 (0.29-2.76)			0.84



VTE, venous thromboembolism; ATE, arterial thromboembolism; Obs-yrs, observation years

[a] Reference group are those without venous thromboembolism

[b] Reference group are those without venous thromboembolism, adjusted for age, sex, hypertension, dyslipidemia, diabetes mellitus, smoking status, elevated albuminuria and history of arterial thromboembolism

DISCUSSION

This large population-based cohort study shows that subjects with previous venous thromboembolism are at increased risk to develop arterial thromboembolism. Although age was a strong confounder to this risk, the relative risk was still 1.8-fold increased after adjustment for age, sex, cardiovascular risk factors and history of arterial thromboembolism. The overall absolute risk was as high as 2.4% per year and 3.5% within the first year after venous thromboembolism was diagnosed. These values approach the absolute risks of recurrent venous thromboembolism.[15-17] This implicates that clinicians should be aware of both arterial thromboembolism and recurrent venous thromboembolism, since both diseases are almost equally common in patients with previous venous thromboembolism. The risk of arterial thromboembolism was highest within the first year after venous thromboembolism. This early occurrence is in accordance with other studies.[3,9] Although the adjusted hazard ratio for subsequent arterial thromboembolism decreased from a statistically significant 2.6-fold (95% CI, 1.46-4.55; *P*-value, 0.001) increased risk in the first year of follow-up to a non-statistically significant 1.5-fold (95% CI, 0.95-2.41; *P*-value, 0.08) increased risk in the following years, a similar tendency was found in the study of Sørensen et al.[3]

The high risk of arterial thromboembolism in subjects with an unprovoked venous event compared to those with a provoked event suggests that a joint mechanism causes events in both venous and arterial systems. Our finding that the relation between arterial and venous thromboembolism remains persistent after adjustment for cardiovascular risk factors supports this idea. Obesity is related to a higher risk of arterial[18,19] and venous thromboembolism.[20-24] This might partly explain the relation between arterial and venous thromboembolism through endothelial damage and/or the related changes in the levels of procoagulant proteins.[25-32] Unfortunately, data on weight and height were not available for all the 40,856 participants in our cohort.

Our study has both strengths and limitations. Strengths are the large population-based cohort, the long follow-up time, the prospectively collected data on arterial events, the estimation of absolute risks, the possibility to analyze confounding cardiovascular risk factors, and the adjustments we made for age and sex in all analyses. A possible limitation of our study is that the data on cardiovascular risk factors were collected using self-reported histories. A certain degree of

misclassification might have occurred, which may have caused bias. Exact start- and stopping dates of anticoagulation were not available and hence not used in our analyses. However, since use of anticoagulation reduces the risk of arterial thromboembolism,[33] it is reasonable to have caused an underestimation of the strength of the association between arterial and venous thromboembolism in our study.

Interestingly, among the subjects with venous thromboembolism, subjects with pulmonary embolism seemed not to be at increased risk of arterial thromboembolism. A possible explanation for this finding could be that subjects with pulmonary embolism had a reduced life expectancy, and therefore did not have the opportunity to be at risk for arterial thromboembolism. However, compared to subjects with deep vein thrombosis, the life expectancy of patients with pulmonary embolism was not shorter in our cohort. Another explanation could be that pulmonary events were mainly provoked events, since provoked events did not increase the risk of arterial thromboembolism. Yet, the distribution of provoked versus unprovoked events were comparable in the subjects with deep vein thrombosis and those with pulmonary embolism. Klok *et al.* could not show a relation between pulmonary embolism and arterial thromboembolism either, but they did show an increased risk after unprovoked pulmonary embolism.[9] In our study, numbers were too small for this analysis.

The possibility that a higher risk of arterial thromboembolism after venous thromboembolism was spurious due to a hospitalization bias was ruled out as hazard ratios were not materially affected when we restricted the analysis to hard endpoints.

We conclude from this large cohort study that subjects with venous thromboembolism are at a high risk to develop arterial thromboembolism. This risk is especially high in the first year after venous thromboembolism and after an unprovoked event. The risk remains persistent after adjustment for age, sex, cardiovascular risk factors and previous arterial thromboembolism. Our findings implicate that, from now on, the clinical course of 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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Chapter 9

Summary

In the last decade the dichotomy between venous (VTE) and arterial thrombo-embolism (ATE) has been challenged. **Chapter 1** summarizes recent research on this issue and depicts the rationale and outline of this thesis.

In **chapter 2** the association between nephrotic syndrome and thromboembolism, including both VTE and ATE, was assessed. Though the association between nephrotic syndrome and especially VTE has been known for decades, for the first time we assessed the annual incidences of symptomatic and objectively verified thromboembolic endpoints in a large cohort of patients with nephrotic syndrome. Absolute risks of VTE (1.02% per year) and ATE (1.48% per year) were each about 8 times higher in these patients than the estimated age- and sex-weighted annual incidences in the general population. Risks of both VTE and ATE were particularly high within the first 6 months after the diagnosis of nephrotic syndrome (annual incidences 9.85% and 5.52%, respectively). Moreover, we reported for the first time that the ratio of proteinuria to serum albumin predicts VTE, and that various classic risk factors for atherosclerosis are associated with ATE, also in patients with nephrotic syndrome, including sex, age, hypertension, diabetes, smoking, prior ATE, and eGFR.

Chapter 3 describes the associations between various established atherosclerosis/ATE risk factors and incident VTE. These associations were studied in a population-based cohort study (the PREVEND study). Besides albuminuria, various classic atherosclerosis risk factors were related to VTE in univariate analyses. However, after adjustment for sex and age, only albuminuria, body mass index, premenopausal use of oral contraceptives, and plasminogen activator inhibitor type-1 levels were related to VTE incidence. In a multivariate model, albuminuria remained an independent predictor of VTE. A dose-response relationship between albuminuria and VTE was observed even in the range of albuminuria that is currently regarded as “normal” (i.e., <30 mg/day). Higher levels of urinary albumin excretion were particularly associated with unprovoked VTE, which formed about half of the VTE cases. In **chapter 4** the association of eGFR and albuminuria with the risk of VTE was studied using the Kidney Disease Outcome Quality Initiative (K/DOQI) classification for chronic kidney disease. Though a trend was observed for decreased eGFR predicting VTE, only in the presence of elevated levels of albuminuria the association between low eGFR and VTE was statistically significant.

In **chapter 2** through **4** urinary protein loss of various degrees of severity, ranging from microalbuminuria to nephrotic-range proteinuria, turned out to be associated with increased risk of thromboembolism. To assess the pathophysiological mechanisms that may explain this association, and to assess the impact of antiproteinuric therapy on the prothrombotic state in these patients, we evaluated in **chapter 5** thirty-two patients with overt proteinuria for coagulation disturbance at baseline and after treatment with losartan (an angiotensin II receptor blocker)**.** The 32 patients with proteinuria (median 3.7 g/d; interquartile range 1.5-5.6) were compared to 32 healthy controls. Patients were treated with losartan alone or in combination with diuretics for a period of 24 week with another 6 weeks of placebo before and at the end of losartan treatment. As compared to age- and sex-matched controls, patients with overt proteinuria were more prothrombotic, as indicated by markedly elevated thrombin generation. This prothrombotic state was likely caused by high levels of various procoagulant proteins, including fibrinogen, while the major inhibiting proteins antithrombin, protein C and total protein S were surprisingly similar in proteinuric patients as compared to controls. However, except for antithrombin levels that had no association with the extent of proteinuria, levels other anticoagulant as well as all measured procoagulant factors were positively correlated with the extent of proteinuria. Antiproteinuric therapy (partially) reversed the prothrombotic state, though increased levels of endothelial derived factor VIII and von Willebrand factor persisted.

Hereditary thrombophilia is another well known entity that leads to a prothrombotic state. Deficiencies of the natural anticoagulant proteins (i.e., protein S, protein C and antithrombin) are considered the strongest hereditary risk factors for VTE. This raises two questions. Firstly, the absolute risks in these subjects have mainly been based on retrospective studies due to the low prevalence of these deficiencies in the general population. Firstly, since retrospective studies are prone for bias, might it be that the true VTE risk conferred by these deficiencies is different than reported in the past? Secondly, how important is it to diagnose any of these deficiencies in asymptomatic subjects by screening? **Chapter 6** reports results of a prospective family cohort study on the absolute risks of VTE and the yield of screening asymptomatic relatives of patients with hereditary deficiencies of protein S, protein C or antithrombin. After screening, deficient subjects were advised to use thromboprophylaxis during exogenous risk factors and the use of oral contraceptives was discouraged. Whereas the risk of unprovoked VTE was about 22-fold higher in subjects with any deficiency (i.e., protein S, protein C or

antithrombin), the risk of provoked VTE was only 2- to 3-fold elevated, as compared with non-deficient subjects. Screening and subsequent augmentation of thromboprophylaxis may result in reduction of provoked VTE, whereas risk of unprovoked VTE could not be affected by screening.

Though hereditary deficiencies of protein S, protein C, and antithrombin are the strongest hereditary risk factors for VTE, their association with ATE has been controversial and well-designed large studies were not available. In **chapter 7** we describe the risk of ATE in a large cohort of families with these deficiencies. Overall, the lifetime risk of ATE was about 2-fold higher in subjects with any deficiency (i.e., protein S, protein C, or antithrombin) compared with non-deficient subjects. However, the high risk of ATE conferred by any deficiency was evident only until about 55 years of age, consisting of a 5-fold risk increase. Interestingly, only protein S and protein C deficiencies were related to ATE before 55 years of age. Antithrombin deficiency was not related to a significantly increased risk neither before 55 years of age, nor thereafter.

Finally, whereas the increased risk of VTE after ATE is well recognized, especially in stroke patients, a possibly higher risk of ATE after a VTE is new. In **chapter 8** this association was assessed in the baseline cohort of the PREVEND study. In this large population-based cohort study subjects with previous VTE were at increased risk to develop ATE. After adjustment for age, sex, cardiovascular risk factors and history of arterial thromboembolism the relative risk was still 1.8-fold increased. The overall absolute risk was as high as 2.4% per year, and was highest (3.5%) in the first year after VTE was diagnosed.

Chapter 10

Discussion & Future Perspectives

Thromboembolic diseases of venous and arterial systems have historically been viewed as two different diseases with distinct risk factors.[1] This notion was challenged in the last decade since an increased incidence of atherosclerosis and arterial thromboembolism (ATE) had been observed in subjects with venous thromboembolism (VTE).[2-12] Moreover, an increasing amount of data indicated that classic atherosclerosis risk factors (ie, hypertension, hyperlipidemia, diabetes, obesity, and smoking) may also predispose individuals to VTE, though results were sometimes inconsistent.[13-26] Moreover, not all data in literature support the hypothesis that VTE and ATE may be two different phenotypes of the same disease. For instance, hereditary thrombophilic deficiencies of the natural anticoagulant proteins (i.e., antithrombin, protein C and protein S) are considered strong risk factors for VTE; [27-29] while their association with ATE has been controversial.[30,31] Similarly, overt proteinuria in patients with nephrotic syndrome is considered an established risk factor for VTE, while its association with ATE in non-diabetics has been less well recognized.[32-34] Microalbuminuria on the other hand has been considered an established risk factor for ATE,[35-39] whereas its association with VTE was for the first time studied in the current thesis. Finally, decreased estimated glomerular filtration rate (eGFR) is also a long and well recognized risk factor for ATE,[35,40] whereas its possible link to increased risk of VTE has been studied only recently.[41] Thus whether VTE and ATE are two different entities or two different phenotypes of the same disease is a matter of intensive research in the field of cardiovascular research and has been the main topic of current thesis.

Classic atherosclerosis risk factors and risk of venous thromboembolism

The association of classic atherosclerosis risk factors (i.e., hypertension, dyslipidemia, smoking, obesity and diabetes) and incident VTE has been reported in various epidemiological studies.[13-26] Although the exact pathophysiological mechanisms accounting for this potential association have yet to be unraveled, endothelial dysfunction, which is strongly associated with atherosclerosis risk factors, is considered the main culprit. This also fits the Virchow triad, as endothelial dysfunction or damage is one of the three components of the Virchow triad that historically classified the pathophysiological mechanisms of factors that may lead to VTE. In **chapter 3** we assessed the association of various atherosclerosis risk factors and VTE. Whereas in univariate analysis various atherosclerosis risk factors were related to VTE, after age and sex adjustment only body mass index and albuminuria remained significant predictors of VTE. This

demonstrates that crude association of atherosclerosis risk factors and VTE that is observed may be due to aging alone, and that in epidemiological studies appropriate adjustment or matching should take place. The results of studies investigating the association of atherosclerosis risk factors and incident VTE are consistently positive for overweight or obesity, whereas for hypertension, dyslipidemia, and diabetes these are equivocal.[13-15,17-21,23-26] The differences between studies with respect to the predictive yield of these latter risk factors could be ascribed to differences in study populations with respect to presence of concomitant established and/or unknown VTE risk factors. For instance, subjects with diabetes may have a higher risk of VTE, but concomitant use of statins and/or antiplatelet agents in subjects with diabetes may neutralize this increased risk for VTE. Many of the studies that have been conducted thus far did not adjust for such potential confounders. In conclusion, at the moment there is insufficient data to consider hypertension, dyslipidemia, diabetes and smoking as established VTE risk factors. To assess whether there is an association of these variables with VTE, and whether these associations are causal, ideally clinical intervention studies should be conducted randomizing subjects to treatment or to no/less intensive treatment of these risk factors, with incident VTE serving as end-point. Of note, this may be less appropriate for dyslipidemia, because of the pleiotropic effects of statins.[42] For dyslipidemia and smoking, prospective observational cohort studies that adjust for potential confounders might be a better choice to study their relevance as VTE risk factors.

Thrombophilia and risk of venous and arterial thromboembolism

Thrombophilia is the clinical term for a hypercoagulable state that was originally used to define young patients with VTE mainly due to hereditary defects. The five best established hereditary thrombophilic defects include the two prevalent gain-of-function mutations (i.e., the factor V Leiden and the prothrombin G20210A mutation) and the relatively rare hereditary deficiencies of the natural anticoagulant proteins (i.e., protein S, protein C and antithrombin). Since these thrombophilic defects alone, or in combination with acquired risk factors, are responsible for only about 50-75% of VTE cases,[43] it is likely that other unknown acquired and genetic risk factors eventually will be identified. Considering the fact that literature indicates that the later the identification of a thrombophilic defect, the weaker the associated risk of VTE, potential new hereditary risk factors are expected to be even weaker risk factors for VTE than the presently known factors. An exception to this assumption would be if thrombophilic families that have tested negative for

the known hereditary defects are analyzed. However, any discovered new thrombophilic defects in such families will probably be family specific, be very rare and responsible for only a very low number of VTE on a population level. In contrast, if a risk factor is only weakly associated with VTE incidence, but highly prevalent in the general population, this may account for a considerable proportion of the overall VTE incidence at population level. At the same time the value of such a weak but prevalent risk factor for risk assessment at an individual patient level will be limited. Therefore, experts presently question the clinical relevance of screening for VTE prevention even for strong thrombophilic defects, such as the antithrombin, protein C and protein S deficiencies.[44] Their uncertainty is primarily based on two reasons: first, in the setting of continuous thromboprophylaxis, the risk of major bleeding outweighs the risk of VTE in the general population;[45] second, in the setting of temporary external risk factors for VTE, thromboprophylaxis is momentarily recommended even in non-deficient subjects.[46] Hence, screening asymptomatic relatives of patients with protein S, protein C or antithrombin deficiencies may be unnecessary. Of note, the risk of bleeding associated with long-term oral anticoagulants in subjects with protein S, protein C or antithrombin deficiency might be lower than is reported in the general population.[47] In **chapter 6** we therefore assessed the absolute risks of VTE and the effect of screening on the VTE risk, in asymptomatic relatives of patients with protein S, protein C or antithrombin deficiency, in a prospective analysis. The overall VTE risks were comparable to the risks of VTE in our previous retrospective analysis,[48] which was performed in these same thrombophilic families. Hence, screening and subsequent prophylactic recommendations may not affect the overall risk of VTE in relatives of protein S, protein C or antithrombin deficient patients. However, thromboprophylaxis was utilized in only half of all high-risk situations and still a trend for risk reduction of provoked VTE was observed. Moreover, all provoked VTE occurred when thromboprophylaxis was not used, underlining the effectiveness of thromboprophylaxis in these deficient subjects. On the other hand current guidelines strongly recommend thromboprophylaxis at time of high-risk situations such as surgery, immobilizations and trauma, even in non-deficient subjects.[46] Consequently, augmentation of thromboprophylaxis in these settings, in accordance with the prevailing guidelines, may make screening unnecessary as provoked VTE in deficient subjects would then also be reduced. Exceptions to this rule may be asymptomatic female relatives of patients with these deficiencies, as they are also exposed to higher VTE risk during pregnancy and oral contraceptive use. The

possible relevance of discouraging oral contraceptive was demonstrated by the observation that 2 out of 6 deficient women using oral contraceptives developed VTE during oral contraceptives use (**chapter 6**). Therefore, screening of asymptomatic female relatives of patients with protein S, protein C or antithrombin deficiencies may be considered beneficial, because, if proven positive for the index deficiency, use of oral contraceptives should be discouraged in these women.

Though the association of the known hereditary thrombophilic defects with VTE is well acknowledged, the risk of arterial thromboembolism (ATE) has been shown to be only slightly increased in subjects with Factor V Leiden or prothrombin G20210A mutation.[49] Data on the association of protein S, protein C or antithrombin deficiencies with ATE is based on case-series and case-control studies that reported inconsistent results.[30,31] As assessed in **chapter 7**, only protein S and protein C deficiencies, but not antithrombin deficiency, were risk factors for ATE before 55 years of age. That protein S and C deficiencies were found to be risk factors at young age could be ascribed to the fact that at higher age other ATE risk factors (i.e. classic atherosclerosis risk factors) overrule the risk of ATE conferred by these relatively weak risk factors (i.e., protein S and protein C deficiencies). Why only protein S and protein C deficiencies, but not antithrombin deficiency, were found to be associated with an increased risk of ATE might be due to non-anticoagulant effects of protein C and protein S.[50-52] It could be speculated that the higher risk for ATE in subjects with protein C deficiency could be ascribed to the potent cytoprotective effects of the protein C pathway.[51] The higher risk for ATE in subjects with protein S deficiency may be explained by the fact that protein S is synthesized by endothelial cells,[52] whereas antithrombin is synthesized by hepatocytes. Endothelial injury as a trigger of thrombosis may therefore be enhanced by a preexisting defect in protein S synthesis at the site of injury. Furthermore, some cytoprotective effects also have been attributed to protein S.[50,52]

In conclusion, even in thrombophilic families the risk of ATE is particularly driven by (classic) atherosclerosis risk factors. However, in young subjects without classic atherosclerosis risk factors, protein S or protein C deficiency increase the risk of ATE. In future studies, it will be interesting to assess any synergetic effects between thrombophilic defects and atherosclerosis risk factors for both venous and arterial thromboembolic events. Such studies, however, will be difficult to perform given the low prevalence of protein S, protein C and antithrombin deficiencies in

the general population as (very) large numbers of participants will be necessary for this question. For the more prevalent factor V Leiden and the prothrombin G20210A mutation, however, such studies could be very well feasible.

Risk of arterial thromboembolism in patients with prior venous thromboembolism

Several studies have consistently shown that the risk of ATE in subjects with prior VTE is higher than in matched controls.[2,3,5,10,11] In contrast, studies in subjects with VTE reported contradictory results with respect to the presence of asymptomatic atherosclerotic lesions.[4,6,8,9,12] If atherosclerosis is not or less associated with VTE, it may be postulated that the higher risk of ATE after VTE is probably due to medical conditions that predispose to both VTE and ATE. Examples of such predisposing conditions are cancer, antiphospholipid antibodies, infectious diseases, use of hormonal therapy and renal disease. On the other hand, thrombus formation in each the arterial and venous system is due to platelet aggregation and coagulation activation, which may also explain the stronger and more consistent association of VTE with subsequent ATE, as compared to the association of VTE with asymptomatic atherosclerosis. Moreover, the high risk of ATE especially after idiopathic VTE indicates that the association of VTE with subsequent ATE is not only due to aforementioned predisposing conditions. Another remarkable fact is that the risk of ATE after VTE is particularly high in the first year after diagnosis of VTE,[11] as we also observed in **chapter 8**. This is surprising because the standard treatment for VTE, which consists of 3 to 6 months of oral anticoagulant drugs, should have lowered the risk of ATE. A possible explanation includes inaccuracy in the exact dates of the two diagnoses, because data were based on hospital registries that may be prone to inaccurate date entry. Other possibilities include: 1) Failure to restart aspirin after cessation of anticoagulant therapy in patients who were on aspirin before VTE diagnosis. 2) In addition to the anticoagulant effects of vitamin K antagonists by blocking γ – carboxylation of various vitamin K dependent coagulation factors in the liver, these drugs may also promote arterial calcification by blocking γ – carboxylation of peripheral vitamin K dependent proteins.[53-55] In a recent small cross-sectional study long-term (>10 years) use of vitamin K antagonists was indeed associated with increased extracoronary arterial calcification.[53] Two potentially important peripheral vitamin K dependent proteins are Matrix Gla protein (MGP) and Growth Arrest Specific gene 6 protein (Gas-6).[54] These proteins have many diverse biologic functions. Produced by vascular smooth muscle cells, MGP functions primarily as a vascular calcification inhibitor and

Gas-6 affects vascular smooth muscle cell movement and apoptosis.[54] On the other hand, it should be mentioned that the vitamin K antagonists appeared effective in various trials in ATE prevention that could be ascribed to the anticoagulant effects of these drugs.[56,57] 3) Finally, patients with VTE might be diagnosed earlier with subsequent ATE in the first year due to enrollment in the medical circuit.

For clinical implications, an important issue that warrants attention is the effectiveness of statins in the prevention of VTE. It is assumed that the VTE risk reduction by these drugs is not due to their cholesterol-lowering effects, but rather to the pleiotropic effects of statins.[42] These include particularly endothelial stabilization that is accompanied by reduction of among others the tissue factor levels and reduction of thrombin generation.[42] The JUPITER trial indeed reported primary VTE risk reduction in the intervention arm with rosuvastatin as compared to the placebo-controlled arm.[58] Use of statin therapy in secondary preventions of VTE probably may have more clinical implications. Since patients with VTE have a high risk of recurrent VTE (about 5% in the first year)[59] and – as we know now – also an increased risk of ATE, prospective randomized trials are needed to address the effect of statin therapy on VTE recurrence and ATE incidence after a prior VTE. The Du Lac randomized trial will address this issue. In this trial, subjects with a VTE, after cessation of standard oral anticoagulants treatment of 6 months, will be randomized to a maintenance intervention-arm with rosuvastatin versus a control-arm with placebo. All patients will be followed for 2 years with recurrent VTE serving as the primary endpoint, and the development of ATE as one of the secondary endpoints.

Renal disease and risk of venous and arterial thromboembolism

As presented in **chapter 1**, renal disease with overt proteinuria predisposes to both VTE and ATE. Though this association was assumed for decades, the absolute risks were unknown, due to lack of data on large patient cohorts. In **chapter 2** we assessed the absolute risk of VTE (1.02% per year) and ATE (1.48% per year), in a large cohort of patients with nephrotic-range proteinuria. These risks were each about 8-fold higher than the age- and sex-weighted absolute risks in the general population. Compared with the general population, the risk of both VTE (about 140 fold) and ATE (about 50 fold) were particularly high in the first 6 months after diagnosis of nephrotic-range proteinuria. Therefore, one might consider primary thrombo-prophylaxis during this period. However, given the bleeding risk associated with the use of anticoagulants,[45] in the setting of proteinuria-related

hypercoagulability, safer therapies such as antiproteinuric medications and statins warrants evaluation first. Indeed, our unpublished retrospective observational data in this same cohort of patients indicated that statin therapy was associated with a 50% reduction of the VTE risk (data not shown). Furthermore, in **chapter 5** we assessed the effect of antiproteinuric therapy (i.e. losartan alone or in combination with diuretics and/or low sodium diet) on the procoagulant state in proteinuric patients. Antiproteinuric therapy indeed reversed the prothrombotic state in these patients, re-emphasizing the importance of proteinuria reduction as a treatment target. The pathophysiological mechanisms responsible for the increased risk of thromboembolism in patients with nephrotic-range proteinuria are unclear. Alterations in plasma levels of proteins involved in coagulation and fibrinolysis, urinary antithrombin loss, enhanced platelet aggregation, hyperviscosity, and hyperlipidemia are all considered to be predisposing factors.[34,60-62] Of these, changes in the levels of plasma coagulation proteins and loss of antithrombin in the urine have been historically considered the main predisposing factors, especially for VTE. In contrast with these historical assumptions, we observed in **chapter 5** that the levels of plasma antithrombin levels in patients with proteinuria were similar as in healthy controls, whereas all procoagulant proteins were elevated in these patients. Interestingly, except antithrombin, all pro- and other anticoagulant proteins were positively associated with the extent of proteinuria and inversely associated with serum albumin. The higher levels of various coagulation proteins in patients with proteinuria are assumed to be secondary to the decrease in levels of serum albumin, as in response to this decrease in serum albumin the liver upregulates the production of all liver-synthesized proteins, including coagulation proteins and lipoproteins.[63] We postulate that upregulation of antithrombin synthesis might be counterbalanced by urinary loss. Antithrombin has a relatively low molecular weight, comparable to the molecular weight of albumin, and will therefore easily leak via the glomeruli in (pre)urine. Indeed, in a small pilot study, including three patients with proteinuria ranging from 0.5 g/d to 1.5 g/d, we could confirm urinary loss of considerable amounts of antithrombin (6-21%).

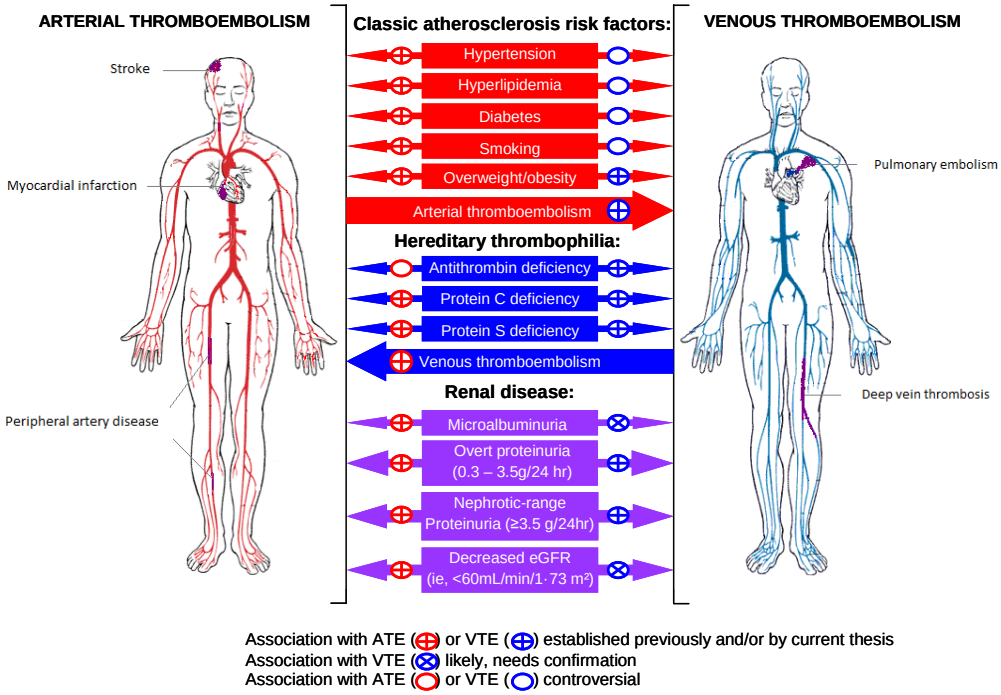
At the population level, the association of microalbuminuria or a mildly decreased eGFR (i.e. stage 1-3 chronic kidney disease, CKD) with thromboembolism is more relevant than that of nephrotic-range proteinuria, due to the higher prevalence of stage 1-3 CKD when compared to the prevalence of overt proteinuria (10-12% versus <0.1% of the general population, respectively).[64,65] Whereas the association of microalbuminuria and decreased eGFR with ATE is well-recognized,[35-40] we

described for the first time the association of microalbuminuria (i.e. mainly stage 1-2 CKD) with VTE in the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study (**chapter 3**). The association of decreased eGFR (i.e. CKD stage 3-4) with VTE was for the first time described by the investigators of the Longitudinal Investigation of Thromboembolism Etiology (LITE) project, which comprises the pooled data of the Atherosclerosis Risk in Communities (ARIC) study and the Cardiovascular Health Study (CHS).[41,66] As described in **chapter 4** the association of decreased eGFR and VTE in the PREVEND study was particularly driven by the presence of albuminuria of ≥30 mg/24h, while the ARIC study suggested that microalbuminuria had no significant association with VTE risk,[66] and that only eGFR showed an inverse association with VTE incidence. These discrepancies between the ARIC and the PREVEND studies could possibly be ascribed to differences in characteristics of the enrolled study participants. In the PREVEND study increased hazard ratios of VTE by eGFR level have been described that were comparable to those found in ARIC, but these did not reach statistical significance, which is likely due to limited power. In the PREVEND study a relatively limited number of subjects with more severely decreased eGFR participated. The PREVEND study is better powered to investigate the predictive value of albuminuria due to the enrichment of this study cohort with albuminuric subjects. The ARIC study on the other hand, is probably better powered for subjects with lower eGFR given the larger sample size and higher number of VTE events, but under-powered for albuminuria.[66] The weaker hazard ratio conferred by albuminuria in ARIC as compared to PREVEND, may be due to several reasons. In PREVEND albuminuria is assessed in 24hr urine samples, the gold standard, that were not frozen before assessment. Frozen storage is known to induce a systematic decrease and more variability in albuminuria concentration.[67] Furthermore, subjects with microalbuminuria in ARIC are mainly diabetics, while in PREVEND these are mainly non-diabetics, as per protocol insulin-using diabetics were excluded in PREVEND. This may have influenced the risk estimates, as diabetic subjects are usually on statin therapy and these subjects are more frequently treated for their cardiovascular morbidity with anti-platelet medication. To assess more definitively whether either eGFR or microalbuminuria or both are independent risk factors for VTE we are currently preparing a pooled individual patient level analysis of several databases, among others PREVEND and ARIC to increase the statistical power to address this issue.

The exact pathophysiological mechanisms linking decreased eGFR and increased albuminuria to VTE have yet to be identified. However, endothelial dysfunction might be the main culprit of this link that, in turn, is associated with mild coagulation disturbances, such as increased levels of factor VIII and plasminogen activator inhibitor-1.[68-70] This is in contrast to the situation with nephrotic-range proteinuria, in which coagulation disturbances probably play a more prominent role. However, these assumptions are merely based on literature, as we did not assess endothelial dysfunction in either microalbuminuric subjects or in patients with nephrotic-range proteinuria. In the near future we are planning to assess the exact coagulation disturbances in patients with microalbuminuria. Depending on the results, it will subsequently be interesting to assess the impact of antiproteinuric therapy with for example losartan and the effect of statins on the coagulation disturbances in subjects with microalbuminuria.

As compared to the risk of VTE, the risk of ATE in patients with CKD is relatively better established. In a recent meta-analysis by the CKD Prognosis Consortium the independent association of both eGFR and albuminuria with ATE was confirmed, using pooled data of 21 general population-based cohorts with over one million study participants.[40] Separate meta-analyses of the same consortium addressing the same issues in other populations, such as those at high risk of CKD and those with known CKD, are currently submitted. Notably, the classification of CKD according to the 2002 KDOQI classification has been increasingly criticized in the field of nephrology.[71] These criticisms ranged from terminology, methodology, definition, classification to prognosis. Based on the four meta-analyses by the CKD Prognosis Consortium a proposal for a new guideline to reclassify CKD is being developed. Of note, this consortium is led by the department of Nephrology of the University Medical Center Groningen and the department of Epidemiology and Clinical Research of the Johns Hopkins University in Baltimore, USA.

Figure. Association of risk factors with arterial and venous thromboembolism.



Conclusions

In conclusion, VTE and ATE share some, such as obesity and renal disease, but not all risk factors as summarized in the above **Figure**. Even for those risk factors that seem to be associated with both VTE and ATE the associations with each of both endpoints are in general not of similar strength. There may be at least one exception. Urinary protein loss, ranging from microalbuminuria to nephrotic-range proteinuria, turned out to be associated with similar relative risks for VTE and ATE. Based on the current available data, it seems therefore that VTE and ATE are probably not different phenotypes of the same disease, but rather different multifactorial diseases that share some risks factors. Nevertheless, a shared preventative intervention might be beneficial that needs to be addressed in future studies. Especially, the role of albuminuria lowering by intervention in the renin-angiotensin-aldosterone system and the use of statins are interesting and could be highly relevant for prevention of recurrent VTE and prevention of ATE in patients with a prior VTE.

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Nederlandse samenvatting

voor niet-medicus

(Lay summary in Dutch)

Hart- en vaatziektes vormen één van de meest voorkomende doodsoorzaken in de westerse landen. Hart- en vaatziekte zoals hartinfarct, herseninfarct en etalagebenen zijn meestal het gevolg van afsluiting of vernauwing van de slagaders (arteriën) ten gevolge van vaatwandverkalking (atherosclerosis). Wanneer een dergelijke vaatwandverkalking loslaat of doorbreekt in de vaatwand kan dit tot lokale stolselvorming leiden. Afsluiting van de aders (venen) veroorzaakt vooral diep veneuze trombose van de beenvaten, overigens zonder echt vaatwandafwijkingen en vernauwing. De stolsels hierin kunnen vervolgens loslaten en verstopping in de longen geven (longembolie). Het ontstaansmechanisme van trombose in arteriën is anders dan van venen en de risicofactoren zijn ook niet dezelfde. Althans, dat werd van oudsher verondersteld. In de laatste 10 jaar hebben verschillende onderzoeken echter uitgewezen dat sommige risicofactoren voor de arteriële en veneuze trombose overlap vertonen. Ook is in meerdere onderzoeken waargenomen dat mensen met veneuze trombose een hoger risico hebben op het krijgen van arteriële trombose. Dat bij elkaar suggereert dat veneuze en arteriële trombose mogelijk met elkaar verbonden zijn of zelfs twee verschillende uitingen van dezelfde ziekte zijn.

In het huidige proefschrift werd deze potentiële relatie tussen veneuze trombose en arteriële trombose onder de loep genomen. Dat werd bewerkstelligd door de associatie van arteriële risicofactoren met het optreden van veneuze trombose te onderzoeken en andersom de associatie van veneuze trombose risicofactoren met het optreden van arteriële trombose te onderzoeken. Nierziektes fungeren als rode draad in deze associatie tussen veneuze en arteriële trombose, waarbij de relatie van nierziektes met beide vormen van trombose (veneus en arteriëel) werd onderzocht. Daarnaast werd in het huidige proefschrift onderzocht wat het risico van arteriële trombose is bij patiënten met eerder doorgemaakte veneuze trombose.

Associatie tussen klassieke risicofactoren van arteriële trombose en het optreden van veneuze trombose

De associatie tussen klassieke risicofactoren van arteriële trombose zoals hoge bloeddruk, verhoogd cholesterol, suikerziekte en roken met het optreden van veneuze trombose werd de afgelopen decennia in meerdere onderzoeken beschreven, maar de resultaten zijn vaak inconsistent. Deze discrepantie is waarschijnlijk te wijten aan de geselecteerde onderzoekspopulaties, de kwaliteit van data en het wel of niet corrigeren voor potentiële vertroebelende factoren zoals leeftijd. Het exact verantwoordelijke mechanisme voor deze associatie is nog

onbekend maar wordt vooral toegeschreven aan de vaatwandschade, wat ook centraal staat in het optreden van arteriële trombose. In **hoofdstuk 3** hebben wij de associatie tussen de klassieke risicofactoren van arteriële trombose en het optreden van veneuze trombose in de PREVEND studie onderzocht. De PREVEND studie was oorspronkelijk in 1997 opgezet om de associatie tussen nierziektes en het optreden van met name arteriële trombose te onderzoeken in een grote groep individuen uit de algemene Groningse populatie. In totaal hebben ruim 8500 individuen uit Groningen in de leeftijd van 28 t/m 75 aan dit onderzoek meegedaan. Uit onze analyses in **hoofdstuk 3** bleek inderdaad dat meerdere risicofactoren voor arteriële trombose een verband lieten zien met het optreden van veneuze trombose. Echter, dat was alleen te zien in ongecorrigeerde analyses. Wanneer wij corrigeerden voor leeftijd en geslacht, bleken alleen (ernstig) overgewicht en eiwitverlies in de urine (albuminurie) gerelateerd te zijn aan het optreden van veneuze trombose. Deze resultaten verklaren een belangrijke deel van de discrepanties in de literatuur. Namelijk dat leeftijdcorrectie zeer essentieel is, omdat leeftijd een risicofactor is voor zowel veneuze als arteriële trombose. Zo zijn er andere, minder sterke vertroebelende factoren die in het ene onderzoek wel en in het andere niet meegenomen zijn, waardoor de resultaten discrepantie vertonen.

Op grond van het huidige proefschrift en de bestaande literatuur kan er geconcludeerd worden dat de klassieke risicofactoren voor arteriële trombose (hoge bloeddruk, verhoogd cholesterol, suikerziekte en roken) vooralsnog niet als bewezen risicofactoren voor veneuze trombose beschouwd kunnen worden (zie eventueel figuur aan het einde van dit hoofdstuk). Overgewicht daarentegen is in het huidige proefschrift en tevens in eerdere onderzoeken consistent een risicofactor voor veneuze trombose gebleken. Daarnaast werd in het huidige proefschrift voor het eerst gevonden dat albuminurie - een bekende risicofactor voor arteriële trombose - ook het risico op veneuze trombose in dezelfde mate verhoogt. Deze nieuwe bevinding nodigt uit tot evaluatie van deze bevinding in andere onderzoeksgroepen en wordt later in dit hoofdstuk verder toegelicht.

Erfelijke stollingsafwijkingen en het risico op arteriële en veneuze trombose

De risicofactoren van veneuze trombose worden verdeeld in erfelijke en verworven risicofactoren. De meest bekende verworven risicofactoren voor veneuze trombose zijn immobilisatie, operatie of trauma, gipsen van een been, kanker, pilgebruik en zwangerschap. Er zijn vijf relevante erfelijke risicofactoren voor veneuze trombose waarvan twee vaak voorkomen en de andere drie relatief zeldzaam zijn. De twee

meest voorkomende erfelijke risicofactoren zijn factor V Leiden dat bij ongeveer 5% van de Nederlandse populatie voorkomt en factor II mutatie dat bij ongeveer 2-3% van de Nederlandse populatie voorkomt. De drie minder vaak voorkomende risicofactoren zijn antitrombine-, proteïne C- en proteïne S deficiëntie, het vóórkomen waarvan tussen 0.1–0.4% varieert. Deze drie erfelijke risicofactoren voor veneuze trombose zijn in vergelijking met de twee vaak voorkomende risicofactoren, namelijk factor V Leiden en factor II mutatie, relatief sterke risicofactoren. Vanwege deze sterkere predispositie voor veneuze trombose door antitrombine, proteïne C en proteïne S deficiëntie wordt al jaren gediscussieerd door experts of gezonde familieleden van patiënten met deze erfelijke afwijkingen getest moeten worden om vroegtijdig preventieve maatregelen te nemen bij degenen die positief worden getest. Echter, de zeldzaamheid van deze afwijkingen maakt dat de discussies over het wel of niet testen van gezonde familieleden zijn gebaseerd op expertopinies en niet op goed uitgevoerd onderzoek.

In **hoofdstuk 6** hebben we in een grote studie 84 families met erfelijke antitrombine, proteïne C of proteïne S deficiënties onderzocht. In dit onderzoek werd gekeken naar het risico van veneuze trombose in familieleden die ten tijde van testen op deze afwijkingen nog geen veneuze trombose hadden. Familieleden die - wat deze erfelijke afwijkingen betreft - positief waren getest hebben vervolgens adviezen gekregen om tijdens verhoogde risicomomenten zoals operaties, trauma's, immobilisatie en gips antistollingsmedicatie te gebruiken; vrouwelijke familieleden kregen daarnaast het advies om geen hormonale anticonceptie (de pil) te gebruiken. Ondanks deze maatregelen hebben in de loop van gemiddeld 9 jaar observatie toch nog 21 van de 149 familieleden met deze afwijkingen een veneuze trombose ontwikkeld. In de 233 familieleden zonder deze afwijkingen hebben slechts 6 mensen veneuze trombose ontwikkeld. Ter vergelijking, in ons eerder gepubliceerd onderzoek in dezelfde families was het risico op veneuze trombose voordat ze getest waren net zo groot als in het huidige onderzoek. Op grond hiervan kan men concluderen dat testen op deze afwijkingen geen gunstige effect heeft op het voorkómen van veneuze trombose. Echter, bij nadere analyse bleek dat de voorzorgmaatregelen en preventieve adviezen in slechts 51% van de gevallen opgevolgd waren. Uit onze eerdere analyse in deze families bleek dat ongeveer de helft van het totaal aantal veneuze tromboses tijdens externe hoog risicomomenten optraden. Het risico op veneuze trombose in aanwezigheid van externe hoog risicomomenten was nihil in de huidige analyse als de voorzorgsmaatregelen goed opgevolgd waren. Dit geeft aan dat het testen van

deze individuen wel het risico op veneuze trombose kan verlagen bij positief geteste individuen, mits de voorzorgsmaatregelen goed opgevolgd worden. Echter, voorzorgsmaatregelen in de vorm van antistollingstherapie gedurende hoge risicomomenten zoals chirurgie, trauma, gips en immobilisatie worden tegenwoordig bij mensen zónder deze afwijkingen ook sterk geadviseerd, waardoor het testen op antitrombine, proteïne C en S deficiëntie mogelijk toch overbodig is. Vrouwen in de fertiele leeftijd die of de pil gebruiken of zwanger worden vormen een uitzondering want zij hebben waarschijnlijk baat bij staken van vooral het "Pil"-gebruik. Dit suggereert dat testen van jonge vrouwelijke familieleden van patiënten met deze afwijkingen overwogen moet worden. Om hierover solide uitspraken te kunnen doen is verder onderzoek nodig. Het uitvoeren van dergelijk onderzoek is echter zeer moeilijk vanwege de zeldzaamheid van beschikbare kandidaten voor dit soort onderzoek. Mogelijk kan een internationale samenwerking een oplossing bieden.

Het hoge risico op veneuze trombose in patiënten met de deficiënties van antitrombine, proteïne C en proteïne S is al lang bekend. De associatie van deze afwijkingen met het optreden van arteriële trombose is daarentegen veel minder bekend en goede onderzoeken daarnaar ontbraken tot op heden. In **hoofdstuk 7** hebben we de associatie tussen antitrombine, proteïne C en proteïne S deficiënties met het optreden van arteriële trombose in dezelfde 84 families onderzocht. Terwijl het risico op veneuze trombose het hoogst is bij individuen met antitrombine deficiëntie, bleken voor het optreden van arteriële trombose deficiënties van proteine C en S risicofactoren te zijn, terwijl antitrombinedeficiëntie geen risicofactor vormde voor arteriële trombose. Deze bevinding suggereert dat de gevonden associatie tussen proteïne C of proteïne S deficiënties met het optreden van arteriële trombose waarschijnlijk niet direct het gevolg is van verhoogde stollingsneiging maar eerder het gevolg is van vaatwandschade. Uit andere onderzoeken is gebleken dat deficiënties van proteïne C en proteïne S mogelijk een rol spelen bij vaatwandschade. Het is vermeldenswaardig dat het verhoogde risico op arteriële trombose bij individuen met proteïne C en proteïne S deficiënties vooral duidelijk was vóór de leeftijd van 55 jaar. Het risico op arteriële trombose nà 55 jaar werd vooral bepaald door de aanwezigheid van klassieke risicofactoren voor arteriële trombose, zoals hoge bloeddruk, verhoogd cholesterol, suikerziekte en roken. Voor antitrombinedeficiëntie was geen verschil voor de leeftijd van jonger of ouder dan 55 jaar. Concluderend, het risico op arteriële trombose speelt

in families met proteïne C of proteïne S deficiënties wel een (enige) rol op jonge leeftijd. Dat geldt niet voor families met antitrombinedeficiëntie.

Risico op arteriële trombose in patiënten met eerder doorgemaakte veneuze trombose

Het is bekend dat patiënten met een arteriële trombose vooral herseninfarct, een verhoogd risico op veneuze trombose hebben. Een omgekeerde relatie, namelijk een verhoogd risico op arteriële trombose in patiënten met veneuze trombose is nieuw. Dit laatste werd ook in **hoofdstuk 8** bevestigd. Er bleek dat vooral in het eerste jaar na veneuze trombose het risico op arteriële trombose het hoogst is. Dat is enigszins tegenstrijdig met de verwachtingen, want na veneuze trombose wordt standaard antistollingsmedicatie gebruikt voor de duur van meestal 6 maanden. Deze antistollingsmedicatie wordt ook dikwijls ingezet in de behandeling van arteriële trombose en is daarmee effectief in de preventie van arteriële trombose. Er zijn meerdere mogelijkheden die deze onverwachte bevinding zouden kunnen verklaren, zoals onbetrouwbare data omtrent de volgorde van veneuze en arteriële trombose als deze dicht bij elkaar zijn op getreden, of het niet herstarten van preventieve medicatie zoals aspirine na stoppen van antistolling, gegeven voor de veneuze trombose. Daarnaast is het gebruik van vitamine K antagonisten (volgens sommige recente experimentele onderzoeken) met progressie van vaatwandverkalking gerelateerd.

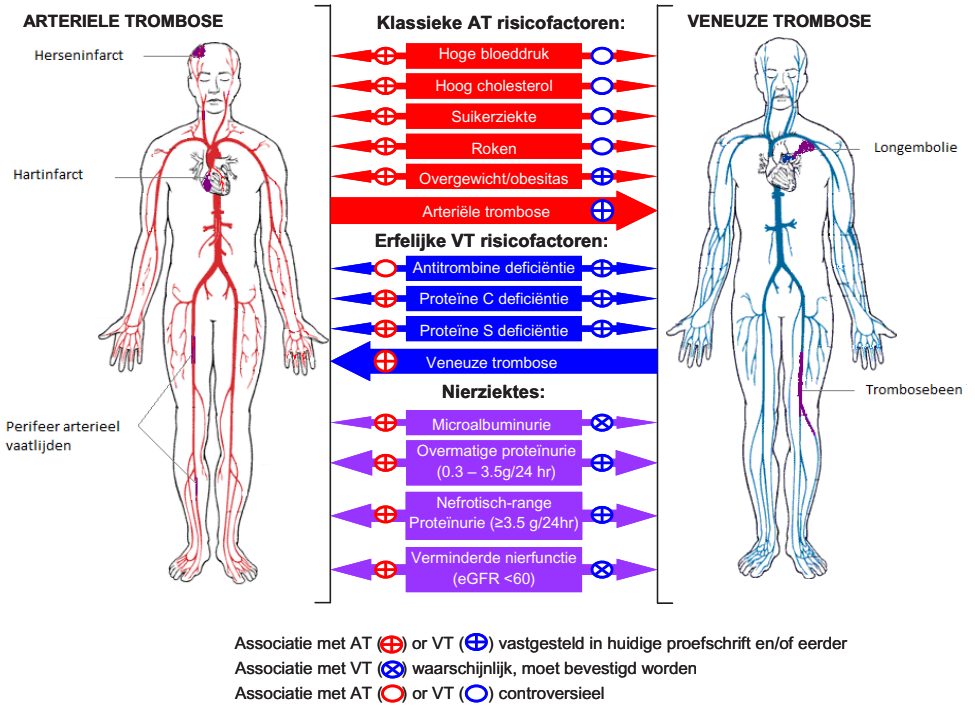
Een ander zeer relevant punt in dit opzicht is waarschijnlijk het effect van statinentherapie (cholesterolverlagende medicatie) op verlaging van het veneuze tromboserisico. Uit een aantal onderzoeken is gebleken dat het gebruik van statinen het risico niet alleen op arteriële trombose waarvoor deze medicamenten gebruikt worden verlaagt, maar ook het risico op veneuze trombose kan verlagen. Echter, de veneuze trombose risicoverlaging wordt niet toegeschreven aan de cholesterolverlagende effecten van deze medicatie, maar aan neveneffecten van statinen op de vaatwand en de stolling. Vanwege het zeer gunstige profiel van bijwerkingen van statinen, kunnen deze middelen vooral zeer goed ingezet worden in de preventie van recidief veneuze trombose nadat antistollingstherapie gestaakt wordt. Het risico op recidief veneuze trombose is vooral in het eerste jaar na staken van antistolling hoog (ongeveer 5%). Daarnaast zal statinegebruik preventief werken tegen het optreden van arteriële trombose, waarop het risico vooral in het eerste jaar na veneuze trombose het hoogst is

Nierziektes en het risico op veneuze en arteriële trombose

Sinds meer dan een halve eeuw is bekend dat overmatig eiwitverlies in de urine (proteïnurie) van meer dan 3.5 gram per 24 uur, ook wel nefrotisch-range proteïnurie genoemd, het risico op vooral veneuze trombose en waarschijnlijk ook op arteriële trombose verhoogt. Vanwege de zeldzaamheid van deze aandoening, zijn de beschikbare gegevens over dit onderwerp gebaseerd op kleine groepen patiënten en het is daarom niet bekend in hoeverre precies het risico op veneuze en arteriële trombose verhoogd is bij deze patiënten. In **hoofdstuk 2** hebben we het absolute risico op zowel veneuze als arteriële trombose in een relatief grote groep (n=298) patiënten onderzocht. De risico's op zowel veneuze als arteriële trombose waren in dezelfde mate verhoogd (ongeveer 8 keer) in vergelijking tot de algemene populatie van dezelfde leeftijd en geslacht. Het risico op zowel veneuze (ongeveer 140 keer) als arteriële trombose (ongeveer 50 keer) waren het hoogst in de eerste 6 maanden na diagnose van nefrotisch-range proteïnurie. Op grond van deze resultaten kan het gebruik van antistollingsmedicatie overwogen worden in de eerste 6 maanden na diagnose. Echter, gebruik van antistollingsmedicatie is geassocieerd met hoge risico's op ernstige bloedingen. Daarom zal er gezocht moeten worden naar meer veilige alternatieven. Te denken valt aan statinengebruik en behandeling van de proteïnurie zelf door middel van vooral renine-angiotensine-aldosteron systeem remmers. In dit opzicht hebben we in deze zelfde groep patiënten de invloed van statinengebruik op veneuze trombose onderzocht. Het bleek dat statinengebruik inderdaad ongeveer 50% veneuze trombose risicoreductie bewerkstelligde (data niet opgenomen in het huidige proefschrift). Verder hebben we in **hoofdstuk 5** de invloed van losartan (een renine-angiotensine-aldosteron systeem remmer) op de tromboseneiging (protrombotische staat) bij een groep van 32 patiënten met overmatige proteïnurie geëvalueerd. Zoals verwacht werd de protrombotische staat gedeeltelijk hersteld door reductie van proteïnurie middels losartan. Het pathofysiologische mechanisme van de protrombotische staat bij patiënten met overmatige proteïnurie wordt toegeschreven aan de daling van albuminespiegels in het bloed, waardoor de lever meer stollingseiwitten aanmaakt. Verder wordt één van de belangrijkste antistollingseiwitten, namelijk antitrombine, verondersteld verloren te gaan in de urine omdat dit eiwit even groot is als albumine. In dit kader hebben we urine van drie willekeurige patiënten onderzocht op verlies van antitrombine. Alle drie patiënten bleken inderdaad meetbaar antitrombineverlies te hebben in de urine.

In vergelijking tot overmatige proteïnurie of nefrotisch-range proteïnurie, hebben **micro**albuminurie (kleine hoeveelheid albumine in de urine) en/of verminderde nierfunctie een veel groter belang op populatieniveau omdat dit veel vaker voorkomt. Ongeveer 10-12% van de algemene populatie heeft microalbuminurie en/of een verminderde nierfunctie. De relatie van microalbuminurie en verminderde nierfunctie met arteriële trombose is relatief goed bekend, maar of deze ook met veneuze trombose gerelateerd zijn was tot recent onbekend. Zelf hebben wij dit voor het eerst onderzocht voor de microalbuminurie; eerder werd deze relatie voor het eerst door Amerikaanse onderzoekers onderzocht voor de rol van de verminderde nierfunctie. Zowel microalbuminurie in ons PREVEND onderzoek (**hoofdstuk 3**) als een verminderde nierfunctie in het Amerikaanse onderzoek waren gerelateerd aan het optreden van veneuze trombose. Wij hebben tevens gekeken naar de verminderde nierfunctie in ons onderzoek in **hoofdstuk 4**. Wij vonden vergelijkbare resultaten in ons onderzoek als in het Amerikaanse onderzoek over nierfunctie. Daarnaast vonden wij dat een verminderde nierfunctie vooral een risicofactor voor veneuze trombose was in aanwezigheid van microalbuminurie. Dat laatste was niet het geval in het Amerikaanse onderzoek. Deze verschillen kunnen meerdere oorzaken hebben. Het beste kan dit verklaard worden door verschillen in de onderzochte populatie. Aan het Amerikaanse onderzoek hebben bijvoorbeeld veel suikerziektepatiënten deelgenomen en deze patiënten hebben heel vaak microalbuminurie, maar worden behandeld met statinen. Zoals eerder beschreven kunnen statinen het risico op veneuze trombose reduceren en hierdoor zou het risico van microalbuminurie op veneuze trombose mogelijk tenietgedaan zijn. Daarentegen, in ons onderzoek waren suikerziektepatiënten die insuline gebruikten buiten de studie gebleven. Om deze nader te onderzoeken hebben we de Amerikaanse onderzoekers en een aantal andere onderzoekers die over dergelijke gegevens beschikken voor een samenwerking uitgenodigd. Na het samenvoegen van meerdere deelnemende onderzoeken zal het mogelijk zijn om de verschillen en de effecten van vooral suikerziektepatiënten nader te onderzoeken betreffende dit onderwerp. Tot slot, het pathofysiologische mechanisme van het verhoogde tromboserisico in patiënten met microalbuminurie en verminderde nierfunctie wordt toegeschreven aan gegeneraliseerde vaatwandschade.

Figuur. Associatie van verschillende risicofactoren met arteriële (AT) en veneuze trombose (VT).



Conclusies:

Concluderend, niet alle maar sommige risicofactoren zoals nierziektes en overgewicht lijden tot zowel veneuze als arteriële trombose welke samengevat zijn in bovenstaande **Figuur**. Op grond van het huidige proefschrift en eerder gepubliceerde gegevens, zijn veneuze trombose en arteriële trombose geen verschillende uitingen van dezelfde ziekte maar twee multifactoriële ziektes die sommige risicofactoren gemeenschappelijk hebben. Desalniettemin, een gemeenschappelijke interventie om zowel veneuze als arteriële trombose te voorkómen zal waarschijnlijk resulteren in effectieve preventie van beide soorten trombose in een geselecteerde groep patiënten. Dit zal vooral nierpatiënten en patiënten met eerder doorgemaakte veneuze trombose betreffen, waar het effect van statinen hopelijk veelbelovende resultaten zal geven in de preventie van zowel recidief veneuze trombose als het optreden van arteriële trombose.

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Curriculum vitae

Bakhtawar Khan Mahmoodi werd op 14 april 1983 in Khost, Afghanistan, geboren. Aldaar volgde hij het lager onderwijs en een deel van zijn middelbare schoolonderwijs. In 2001, op de leeftijd van 18 jaar emigreerde hij, samen met zijn ouders, broertjes en zusjes naar Nederland. In 2002 werd hij toegelaten tot het voorbereidend jaar van het Noorderpoortcollege te Groningen, om de 1-jarige voorbereiding voor het Nederlands hoger onderwijs, inclusief de Nederlandse taal te volgen. Na een succesvolle afronding van dit voorbereidend jaar werd hij ingeloot voor de studie geneeskunde aan de Rijksuniversiteit Groningen. Om de opleiding geneeskunde enigszins uitdagender te maken is hij vanaf zijn tweede studiejaar begonnen met wetenschappelijk onderzoek. In 2007 werd hij toegelaten tot het MD/PhD traject van de Junior Scientific Masterclass. In een dergelijke traject wordt de studie geneeskunde verlengd met 2 jaar, wat fulltime onderzoek inhoudt. In zijn geval werd de studie geneeskunde voor slechts 8 maanden verlengd. Hij heeft in mei 2010 zijn artsexamen met cum laude gehaald. Vanaf oktober 2010 is hij begonnen als post-doctoraal fellow bij de afdeling epidemiologie van de prestigieuze Johns Hopkins School of Public Health in Baltimore, VS. Na afloop van zijn fellowship in de VS, dat in totaal 2 jaar zal duren, is hij voornemens om terug te keren naar Nederland. Daar zal hij de opleiding tot medisch specialist gaan volgen. Hij is er nog niet helemaal uit wat betreft de keuze van de vervolgopleiding, maar interne geneeskunde, cardiologie en chirurgie staan op zijn lijst.

Bakhatwar Khan Mahmoodi was born on 14 april 1983 in Khost, Afghanistan. In 2001 at the age of 18 he immigrated together with his parents to the Netherlands, as a refugee. He went for about 3 years to secondary and 6 years to primary school. He did not attend secondary school in the Netherlands. After extensive self study and learning of the Dutch language, he passed the state admission exams for Medical School in 2003 and was admitted for Medicine at the State University of Groningen. From 2005, his second year of Medicine, he started research as a student-assistant and in October 2007 he was selected for the PhD program. The official MD/PhD program is 8 years in total, with 2 years of full-time research. In his case, he was able to combine the research for his PhD thesis with his training for the MD degree. Consequently only about 1/2 year of his PhD activities were conducted full-time. His remaining PhD activities were combined with internships/rotations. He graduated cum laude from Medical School in May 2010. Starting from October 2010 he will be doing a total of two years postdoctoral fellowships at the department of epidemiology of the prestigious Johns Hopkins school of Public Health. After his fellowships in Baltimore, he is planning to specialize in either internal medicine, cardiology, or surgery.