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
General introduction

idiopathic VTE.\textsuperscript{9-15} 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.\textsuperscript{19-32} A recent meta-analysis that included a total of 21 studies,\textsuperscript{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,\textsuperscript{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 (\textit{J}ustification for the \textit{U}se of \textit{S}tatins in \textit{P}rimary \textit{P}revention: An \textit{I}ntervention \textit{T}rial \textit{E}valuating \textit{R}osuvastatin) demonstrating significant reduction in VTE risk in the intervention-arm treated with rosuvastatin.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 \( \geq 300 \) 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 Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study.

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

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).\textsuperscript{46,47} Recently the same group assessed the VTE risk in CKD 3-4 in the ARIC cohort only,\textsuperscript{46} using a cystatin C based and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.\textsuperscript{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.\textsuperscript{50} These findings contrast with early follow-up of the LITE project.\textsuperscript{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 $\geq 30$ 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 $\geq 30$ 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 $\geq 30$ mg/24h.\textsuperscript{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 $\geq 30$ 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.\textsuperscript{54-56} Of note, in patients with diabetes, overt proteinuria is a well-known strong predictor for ATE.\textsuperscript{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 $\geq 3.5$ 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{56,60-62} As is true for microalbuminuria, nephrotic-range proteinuria is also associated with prominent endothelial dysfunction.\textsuperscript{63} Experimental data support a role for hypoalbuminemia in the vascular dysfunction in nephrotic-range proteinuria.\textsuperscript{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;\textsuperscript{5,66,67} however, their association with ATE is controversial.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{72}
Because in the general population long-term oral anticoagulant treatment is associated with a major bleeding risk of about 2.8% per year,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 $\geq 3.5$ 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 DEfficiencies of protein S, protein C and Antithrombin and the absolute Risk of ThromboEmbolism Study (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.
REFERENCES


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