Cover Page


Universiteit Leiden




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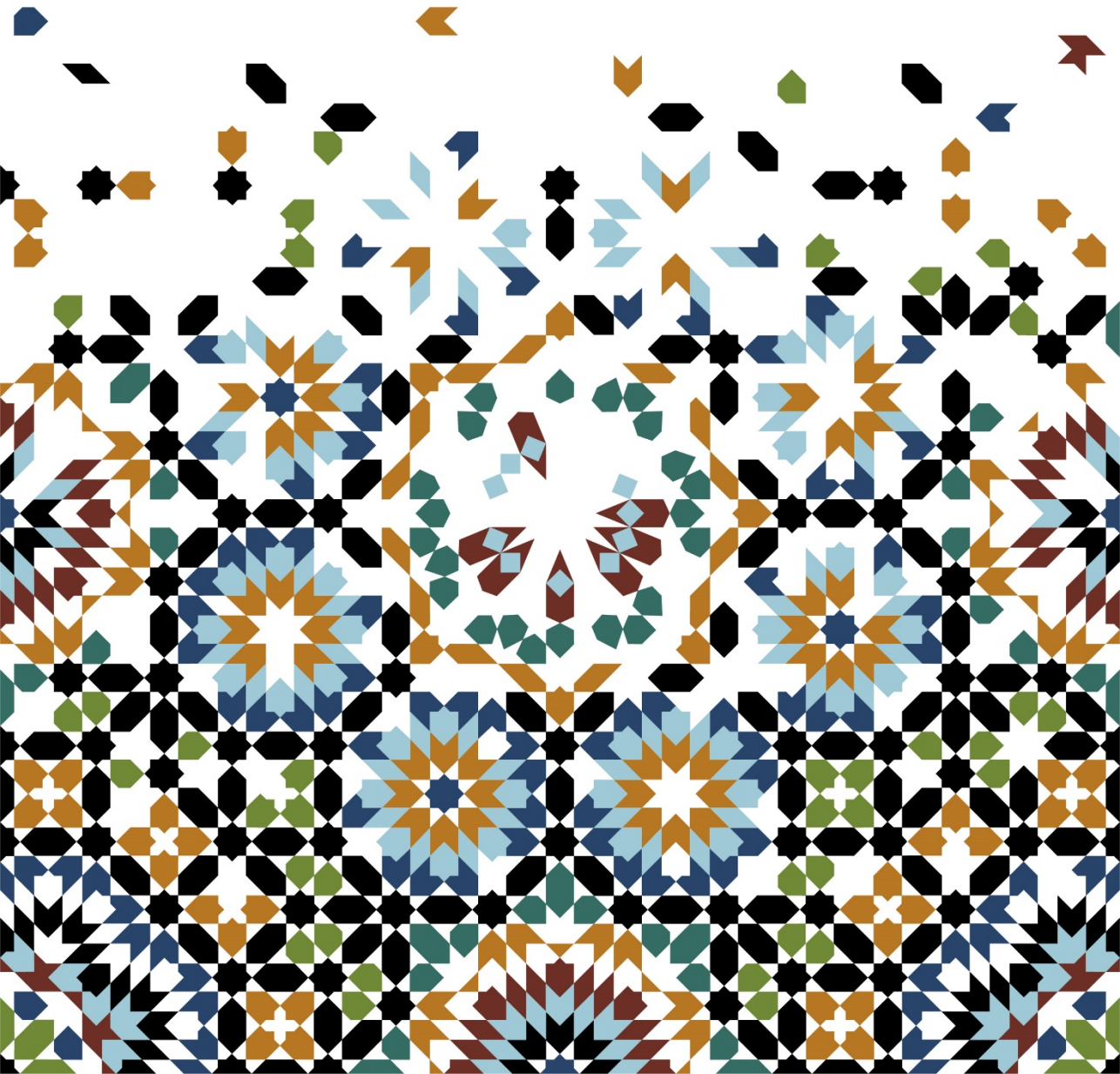
# Risk factors of venous thrombosis in the elderly

Alev Karasu



Risk factors of venous thrombosis in the elderly

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# Risk factors of venous thrombosis in the elderly

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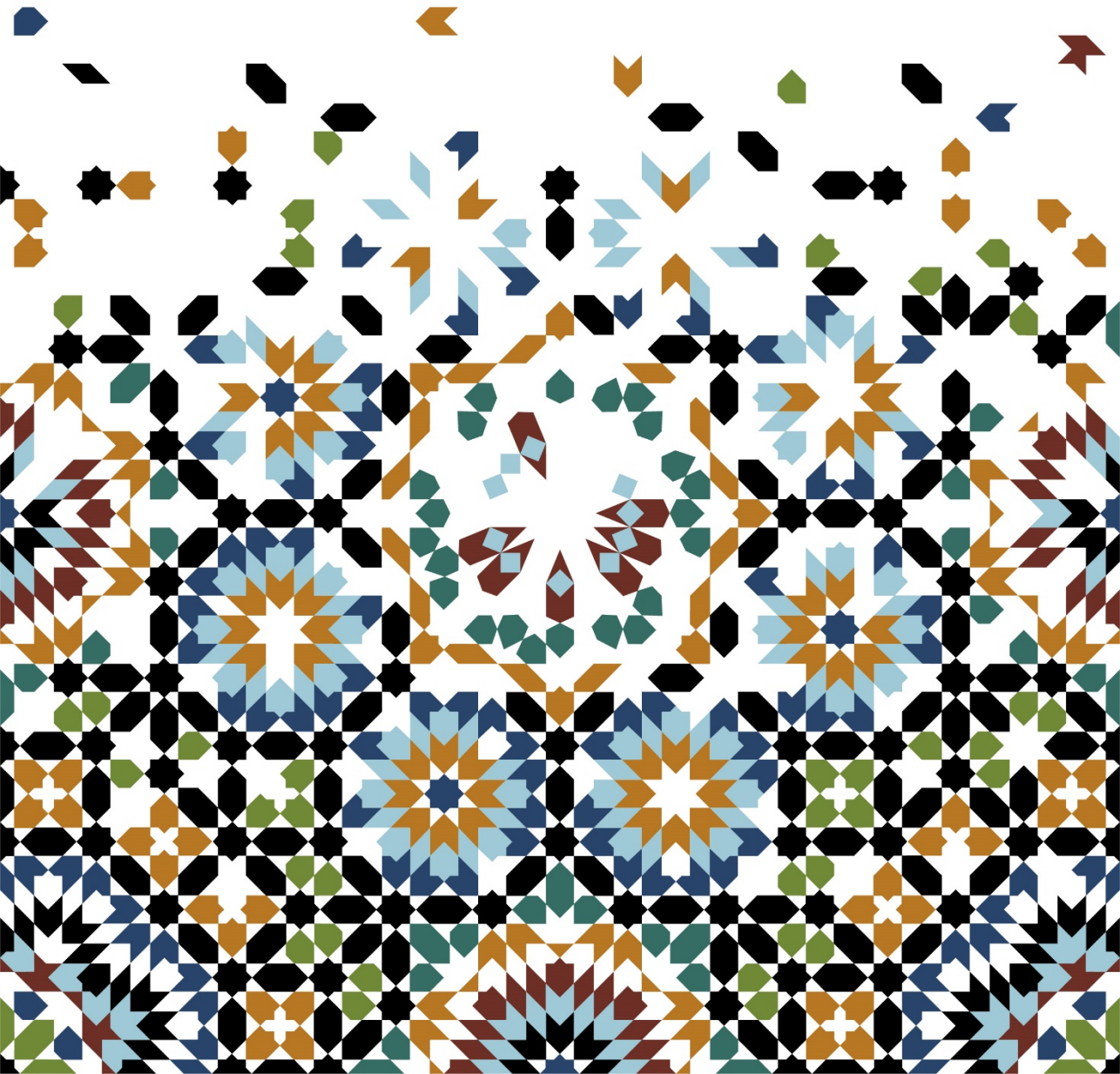
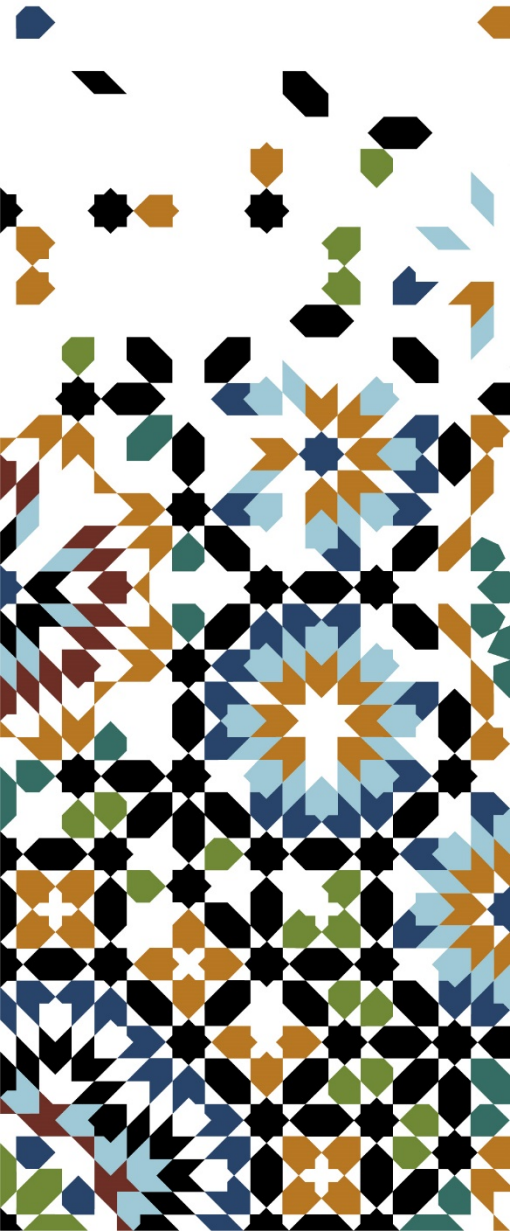
Voor mijn ouders

# Contents



# Chapter 1

## General Introduction and Outline of this Thesis



# Introduction

In this thesis the risk factors of venous thrombosis will be discussed in the general and particularly the elderly population. The goal of this thesis is to provide insights on risk factors of thrombosis in the elderly population, in order to advance our basic understanding of physiological age-related changes that increase the risk of venous thrombosis and which may ultimately lead to improved personalized interventions. In this chapter firstly background information will be provided on risk factors for venous thrombosis, focussing specifically on age as a risk factor. Secondly, the role of veins and venous valves in the development of venous thrombosis will be discussed and thirdly, global assays as a potential tool to identify patients at high risk for venous thrombosis will be considered. The study populations used in this thesis will discussed, and an outline of this thesis will be provided.

## Risk factors for venous thrombosis

The process of thrombus formation depends on the balance of the procoagulant, anticoagulant, and fibrinolytic systems. In normal physiological circumstances, this thrombohemorrhagic balance is maintained in the body by interactions between the three systems, platelets, and the vessel wall.[1] In individuals with venous thrombosis this balance is disrupted, leading to the formation of a blood clot in veins.[2] In 1856 Virchow theorized three main causes of thrombosis: stasis of the blood, changes in the vessel wall, and changes in the composition of the blood.[3] In the decades thereafter much effort has been put into identifying risk factors of venous thrombosis fitting in the three causes postulated by Virchow and this has resulted in an extensive list today, including environmental risk factors and genetic risk factors.[4]

### *Age as a risk factor of venous thrombosis*

The global proportion of the elderly, aged 60 years and older increased from 9.2% in 1990 to 11.7% in 2013 and will continue to grow, up to 21.1% by 2050.[5]

Studies addressing the prevalence and contribution of risk factors of thrombosis have been performed almost entirely in young and middle-aged patient cohorts, whereas the incidence of thrombosis in the elderly is much higher. The overall incidence of a first symptomatic venous thrombosis in the general population is 1-2 per 1000 person-years. In the age-group 25-30 years old, thrombosis occurs in approximately 1 per 10 000 person-years as compared with nearly 8 per 1000 person-years in the 85 years and older population, as studied in an European cohort.[6] In the Worcester Venous Thromboembolism Study, the incidence rates of venous thrombosis increased more than 10-fold in individuals aged ≥75 years, compared with patients aged <55 years.[7] Several explanations have been hypothesized to explain the increasing incidence of thrombosis with age. Ageing may be associated with an increased prevalence of conventional established risk factors, development of new age-specific risk factors, accumulation of risk factors with age, and risk factors having synergistic effects in the aged.[8] In this thesis we focus on conventional acquired (the airway diseases bronchitis, COPD, asthma, and venous insufficiency in chapters 2 and 4) and genetic risk factors of venous thrombosis (factor V Leiden rs6025 or prothrombin G20210A mutations rs1799963 in chapter 3), but we also explore new age-specific risk factors (thickened venous valves in chapter 6), in anatomical areas where venous disease occurs most (as described in chapters 4 and 5).

*Pulmonary embolism and deep vein thrombosis in the elderly*

Venous thrombosis manifests itself as two entities, i.e., pulmonary embolism and deep vein thrombosis. From several studies it appears that risk factors of the two entities are to a large extent similar but also sometimes differ, questioning the consideration of deep vein thrombosis and pulmonary embolism as a single disease. This observation is particularly evident with the factor V Leiden mutation.[9-11] The prevalence of factor V Leiden has been consistently higher in patients with deep vein thrombosis than in patients with pulmonary embolism, known as the factor V Leiden paradox.[12,13] In contrast, inflammatory lung diseases, such as COPD and sickle cell trait, have been associated with a higher risk of pulmonary

embolism than deep vein thrombosis.[14,15] We elaborate in this thesis on the differential effects of factor V Leiden and inflammatory lung diseases to pulmonary embolism and deep vein thrombosis.

## Veins and valves in the elderly

In 1851, Virchow observed a left-sided predominance of deep vein thrombosis of the legs and hypothesized that compression of the left common iliac vein by the right common iliac artery could be the underlying cause.[16] Over a hundred years later, May and Turner stated that venous webs or spurs had formed at the point of the compression,[17] causing the left-sided predominance. Pregnancies and hormone use have been related to left-sided thrombus distribution in women.[18,19] In the elderly, a population in which hormones and pregnancies do not play a role, the question arises whether the thrombus left-right distribution changes. In this thesis we aim to investigate this.

Venous valves in the veins of the lower extremities play a role in maintaining the blood flow back to the heart. Venous valves are usually bicuspid,[20,21] provide unidirectional flow,[22] and close as the valve leaflets move towards the centre of the vein.[23] Valves are located in a valve sinus, a local widening of the venous wall. The area between the valve leaflet and vessel wall, i.e., the valve pocket, is viewed as the predominant location of thrombus inititation (Figure 1) [24]. In the deepest part of the valve pockets, blood circulates with very low velocities creating a low shear field, allowing red cells to aggregate.[24] Stasis of blood in the pocket leads to hypoxia, causing endothelial activation and activation of the coagulation system. Age-related changes of venous valves have been described in several ex-vivo studies, showing thickening of valves with age in renal and in femoral veins.[25,26] The thickening of the valves was shown to be the result of atrophy of muscle fibers, hypertrophy of elastic fibers, and an increased number of collagen fiber bundles.[26] One in vivo study showed thickening of venous valves with age in healthy individuals, measured by ultrasound examinations.[27] Ageing of venous valves, defined as thickening of the valves, has not been studied in relation to

venous thrombosis. In chapter 6 we conduct a case-control study to examine the risk of venous thrombosis associated with thickened venous valves in the popliteal fossa. Furthermore, we investigate whether venous reflux, measured by the valve closure time, relates to the risk of thrombosis, as venous reflux contributes to stasis and might therefore add to the risk of thrombosis. For this reason, in chapter 4 we focus on clinical signs of venous insufficiency, such as history of varicose veins, leg ulcer, leg oedema and the risk of thrombosis.

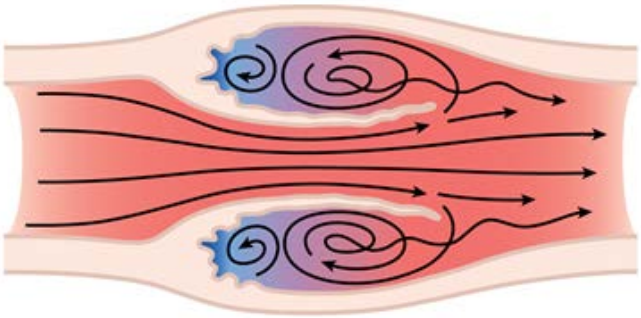


**Figure 1** Schematic drawing of the vortical flow in the deep venous system. The flow arrows define the genesis of the two counter rotating vortices within the valve sinus. The small vortex at the base of the valve sinus is isolated from the systemic circulation and is congruent with the region of most marked hypoxia, which is the usual site of valvular sinus thrombus initiation. Reprinted from Bovill et al. Annu Rev. Physiology 2011. 73:16.1-16.19

## Global assays

To minimize the global burden of venous thrombosis on health, timely diagnosis, risk stratification, and monitoring of treatment with haemostatic agents become crucial. From this background the need for global assays emerged and they advanced our understanding of blood coagulation dynamics in individuals.[28] The utility of global assays is still debated, but the thrombogram and thromboelastographic methodologies are two global assays that have proceeded into clinical evaluation.[28] Since it has been difficult to establish the risk of recurrent venous thrombosis using clinical factors, attempts have been made to identify global laboratory markers which are associated with an increased risk of a recurrent thrombosis.[29] In chapter 7 we investigate whether clot lysis time, which measures the plasma fibrinolytic potential,[30] is a candidate to identify patients with an increased risk of a first and recurrent venous thrombosis.

## Clinical studies used in this thesis

The analyses described in this thesis are performed in three different clinical studies: the **AT-AGE** (**A**ge and **T**hrombosis, **A**cquired and **G**enetic risk factors in the **E**lderly) study, the **Batavia** (**B**iology of **A**geing and **T**hrombosis: **A**ppraisal of **V**alve thickness and function, an **I**n vivo **A**ssessment) study, and the **THE-VTE** (**T**hrombophilia, **H**ypercoagulability and **E**nvironmental risks in **V**enous **T**hrombo**E**mbolism) study.

*The AT-AGE study*

The AT-AGE study is a two-center, population-based case-control study in Leiden and Vermont designed to study risk factors for venous thrombosis specifically in the elderly. Cases aged 70 years and older were included from anticoagulant clinics in Leiden and Haarlem in the Netherlands and in University of Vermont Medical Center in Burlington, Vermont. Controls were invited from several primary care practices in the same geographical area. As the elderly are often excluded from clinical studies due to age-related factors, such as immobility to reach the study

center, visual and cognitive impairments or comorbidities, home visits were conducted to reach high participation rate. During the home visits, interviews were performed and questionnaire data and blood samples were collected. 401 cases with a first-time thrombosis and 431 control subjects that never had experienced thrombosis were included and visited. Inclusion started in 2008 and was completed in 2011. Chapters 2 to 5 are based on data from the AT-AGE study.

*The Batavia study*

In the Batavia study we examined the relation between thickened venous valves in the popliteal vein and the risk of venous thrombosis. Age-related changes of the venous valves, have been described in several studies. Histology studies have shown an increased collagen deposition and thickening of the lamina elastic with age.[26] Furthermore, clinical studies related increasing age to thickening of venous valves as measured by ultrasound examination.[27] All previous studies were performed in healthy individuals, whereas in the Batavia study we examined the association between thickened venous valves and venous thrombotic disease. Cases with a deep vein thrombosis and controls from the AT-AGE study were approached to participate in the Batavia study. All participants were aged ≥70 years. Participants were invited to the LUMC for an ultrasound examination to visualize and measure the thickness of the valve in the popliteal vein, if present. The Batavia study is described in detail in chapter 6.

*THE-VTE study*

The aim of the THE-VTE study is to determine whether a global testing strategy enables the indentification ofan increased clotting tendency (hypercoagulability) and as such the identification of individuals at increased risk of a first and recurrent venous thrombosis. Cases in the Netherlands were identified at the anticoagulation clinic in Leiden and in the United Kingdom cases were identified at the Addenbrooke's Hospital in Cambridge. Partners were approached to participate as controls. All participants were aged 18-75 years. The association between the

clot lysis time and the risk of venous thrombosis, both first and recurrent, studied in the THE-VTE study, is discussed in chapter 7.

## Outline of this thesis

The aim of this thesis is to provide insight into risk factors for venous thrombosis in the elderly. We focussed on airway diseases in the elderly, and the risk of deep vein thrombosis and pulmonary embolism, both combined and separately in **chapter 2**. We hypothesized that since risk factors for deep vein thrombosis and pulmonary embolism differ in the middle-aged population, the effect of airway diseases will also be greater on pulmonary embolism than on deep vein thrombosis in the elderly. Next, **chapter 3** focusses on the question whether the most common genetic risk factors for venous thrombosis, i.e., the factor V Leiden and prothrombin G20210A variants, and non-O blood group, as well as a positive family history of VT, are risk factors for a first venous thrombosis at an older age. It is well known that these factors are associated with an increased risk of thrombosis in the middle-aged population, but the effect at older age has not been thoroughly studied. Clinical manifestations of venous insufficiency, such as a history of varicose veins, leg ulcer and leg oedema, are discussed in **chapter 4**. The anatomical thrombi distribution in the elderly is described in **chapter 5**. **Chapter 6** presents the data of the Batavia study, in which we investigate the role of venous valves in the popliteal vein on the risk of venous thrombosis.

Additional to an increased coagulation tendency, a decreased fibrinolytic capacity might also increase the risk of thrombosis in the general population. As an inspection of usage of global assays measuring decreased fibrinolysis in the middle-aged population, **chapter 7** was dedicated to the global assay clot lysis time. Analyses were performed in THE VTE study, which consists of participants aged 18-75 years.

Finally, **chapter 8** summarizes the findings of this thesis, places the main findings in a broader etiologic context, and provides suggestions for future research.

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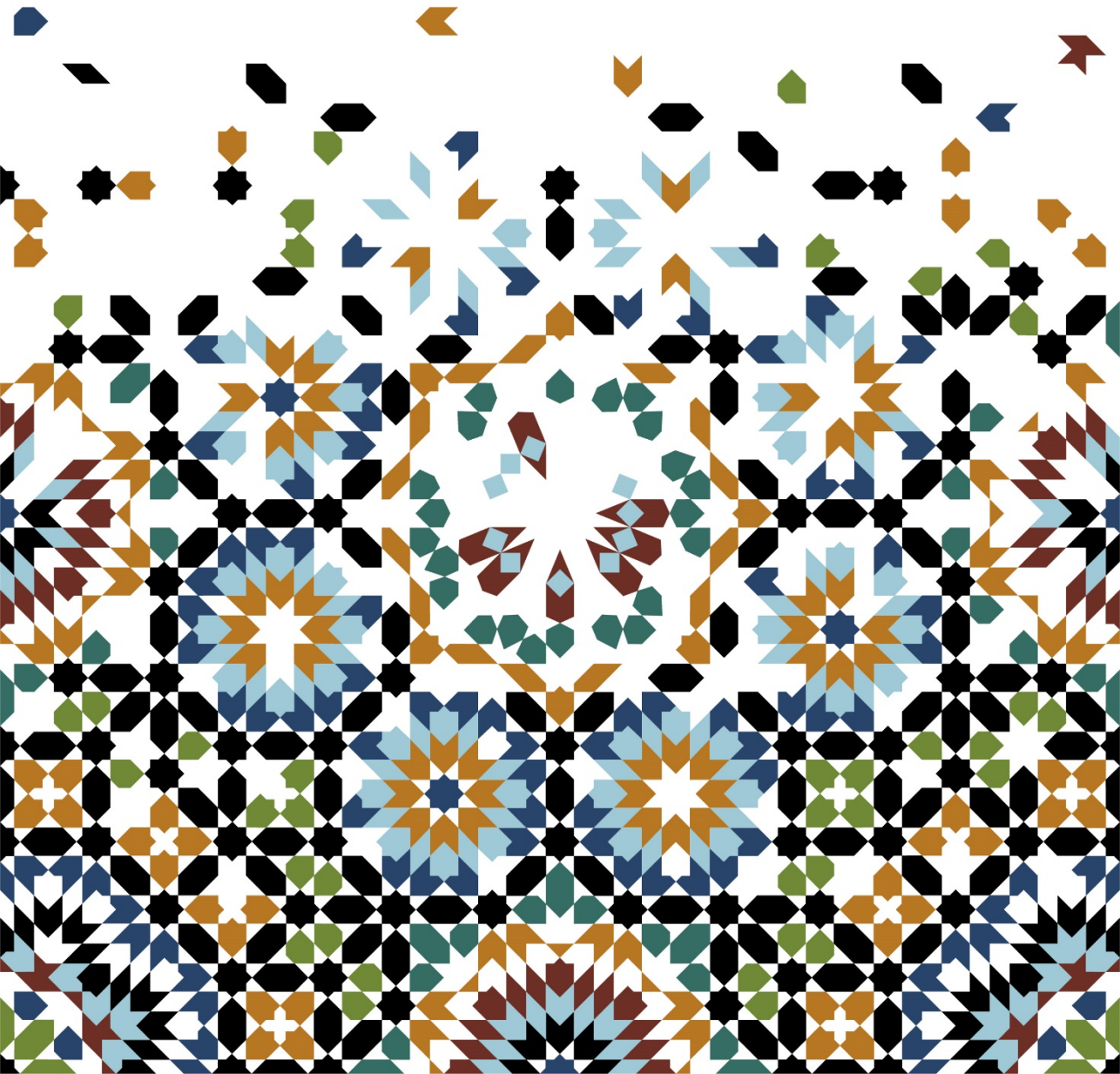
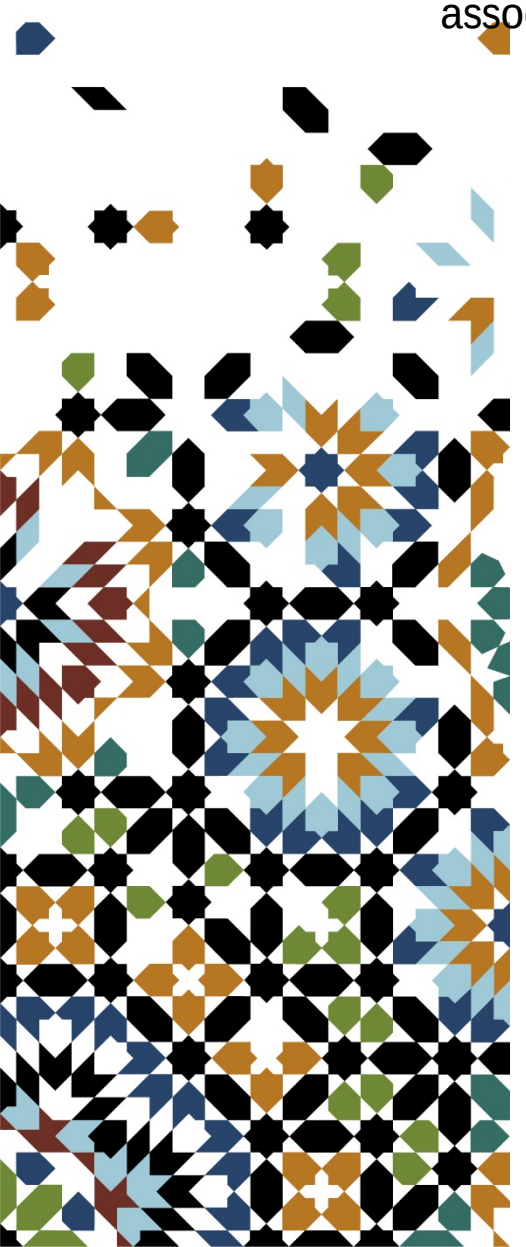


# Chapter 2

## Airway diseases in elderly people are associated with pulmonary embolism but not deep vein thrombosis



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## Abstract

Pulmonary embolism (PE) has long been thought to exclusively originate from a deep vein thrombosis (DVT), but recent studies suggest this may not always hold true in middle-aged populations with inflammatory airway diseases. We aimed to study the association between different airway diseases (chronic obstructive pulmonary disease – COPD, asthma and bronchitis) and venous thrombosis (VT) in a case-control study of elderly (>70 years) participants. 401 cases with a first-time VT and 431 controls without VT were included in the two-center AT-AGE study. Information on airway diseases was obtained from questionnaires during home visits. Bronchitis was associated with a 1.5-fold (95%CI: 0.9-2.4) increased VT risk, with similar risk estimates for DVT and PE. Individuals with COPD had a 1.8-fold increased risk of VT compared with individuals without COPD (95%CI: 1.1-3.0). The increased risk was limited to an increased risk of PE (OR 2.5, 95%CI: 1.4-4.4) while there was no association with DVT (OR 1.0, 95%CI: 0.4-2.1). Individuals with asthma only had a mildly increased risk of VT (OR 1.4, 95%CI: 0.7-2.8); again the risk was limited to PE: OR 1.9, 95%CI: 0.8-4.1; DVT: OR 0.8, 95%CI: 0.3-2.4. When analysing the risk of VT for all airway diseases combined, we found a 1.7-fold increased risk (95%CI: 1.2-2.4) for all VT; 2.2-fold (95%CI:1.5-3.3) for PE and 1.2-fold (95%CI: 0.7-1.9) for DVT. We conclude that inflammatory airway diseases in the elderly, i.e. bronchitis, COPD, asthma are associated with an increased risk of PE but not for DVT.

## Introduction

Venous thrombosis (VT) mainly manifests as pulmonary embolism (PE) and deep vein thrombosis (DVT). Many factors affect the risk of both DVT and PE, but some differ; those associated with increased APC-resistance, e.g. factor V Leiden and obesity, are more often seen in patients with DVT, whereas pulmonary inflammatory diseases, such as chronic obstructive pulmonary disease, pneumonia, and sickle cell disease are predominantly associated with increased risk of PE.[1,2] PE has long been thought to exclusively originate from a DVT, but recent studies suggest that this may not always hold true.[1] Other sources such as cardiac thrombi in patients with atrial fibrillation have been postulated. It has also been suggested that PE develops de novo in the lung.[3,4] Inflammatory processes in the respiratory tract can stimulate the coagulation system, as seen in a recent study on pneumonia and VT.[5] Pneumonia increases the risk of VT substantially, while it is yet unknown whether this is also the case for airway diseases such as bronchitis, chronic obstructive pulmonary disease (COPD) or asthma in an elderly population.

PE is an important cause of death in elderly people[6], and has a high incidence in this age group.[7,8] However, most studies on risk factors have focussed on young or middle-aged individuals. Considering the rising incidence of VT in the elderly and the high death rate from PE in this age group and in the general population[9], there is a need for studies focussing on risk factors specifically for DVT or PE in an older population.

The aim of this study was to evaluate the associations of three inflammatory airway diseases: bronchitis, COPD, asthma and risk of VT in a population-based case-control study of people aged 70 years and older. We also assessed the impact of factor V Leiden on these associations.

## Materials and methods

### *Study design and population*

The AT-AGE study (Age and Thrombosis: Acquired and Genetic risk factors in the Elderly) is a bicenter population-based case-control study performed in Leiden, The Netherlands and Burlington, Vermont, the United States. A detailed description of the study, including participant characteristics by center, was published previously.[10] In short, from June 2008 to August 2011 in Leiden and December 2008 to July 2011 in Vermont, all consecutive patients 70 years and older with DVT or PE were identified in anticoagulation clinics in Leiden and Haarlem (The Netherlands) and at the Vascular Laboratory and the Radiology department of the University of Vermont Medical Center (Burlington, Vermont, United States). We defined venous thrombosis as deep venous thrombosis of the leg (DVT) or pulmonary embolism (PE). Control subjects were identified in Leiden and Burlington in the same geographic area as the patients. Control subjects were randomly selected from five primary care practices in Leiden and four in Burlington. Individuals were excluded from participation if they reported that they had an active malignancy, defined as diagnosis of cancer within six months before the thrombotic event (or date of telephone call for the control subjects) or received chemotherapy or radiation therapy for cancer in the last six months. Furthermore, those with severe cognitive or psychiatric disorders or who reported a previous DVT or PE were also excluded. Approval for this study was obtained from the Medical Ethics Committee of the Leiden University Medical Center and by the Committee of Human Research of the University of Vermont. All participants provided written informed consent.

### *Risk factor assessment*

During home visits a detailed structured interview on risk factors for venous thrombosis was performed by trained personnel. The questions concerned self-reported comorbidities, including COPD, asthma and bronchitis, and a list of

current medications, confirmed by examining pill bottles. There were no missing data on bronchitis, COPD or asthma. Individuals were classified as having bronchitis, COPD or asthma if they answered yes to questions on the presence of these conditions or if they were taking any of the following medications used to treat these disorders: shortacting anticholinergics, shortacting beta$_2$ agonists, longacting beta$_2$ agonists, inhaled corticosteroids, or combinations. Smokers were divided into current, former, and never smokers. The index data was the date of the thrombotic event for VT patients and for the control subjects this was the date of filling in the questionnaire. Body mass index (BMI) was calculated by dividing body weight (kg) by squared height (m$^2$).

Blood samples were drawn into vacuum tubes containing 0.1-volume 0.106-mol/L trisodium citrate. The blood sample was separated into plasma and cells through centrifugation. DNA analysis for the factor V Leiden (G1691A) mutation was performed using a combined polymerase chain reaction method with use of the TaqMan assay. Heterozygotes and homozygotes were combined for analysis.

*Statistical analysis*

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the risk of VT with presence of bronchitis, COPD or asthma considered separately and in combination with presence of factor V Leiden. Risk estimates were adjusted for potential confounders, i.e., for age (continuous), sex, smoking status (current, former, never), BMI and study center (Leiden and Haarlem vs Burlington) by using multivariable logistic regression analysis. Separate analyses were performed for type of thrombosis, i.e., DVT and PE (±DVT). We carried out a sensitivity analysis classifying participants who did not report an airway disease as having an airway disease based on use of 1 or more medications used for airway diseases. Since systemic steroids are used as treatment for bronchitis, COPD and asthma and are associated with an increased risk of venous thrombosis, as reviewed by Van Zaane et al[11], we calculated ORs stratified on systemic steroid use. IBM SPSS Statistics 20.0 for Windows (SPSS Inc, Chicago, Ill) was used for data analysis.

## Results

The study included 401 patients of whom 166 had a DVT only and 235 had a PE with or without DVT, and 431 control subjects (Table 1). Patients were slightly older than control subjects, with a median age among patients of 78 years (range 70-101), and among control subjects 76 years (range 70-96). Patients were more often women than controls and had a similar BMI. In total, 106 (26%) patients and 78 (18%) control subjects reported a diagnosis of bronchitis, COPD or asthma before the index date. Of the patients, 48 (12%) reported bronchitis, 43 (11%) COPD and 19 (5%) asthma. In control subjects, these conditions were less common: 37 (9%) with bronchitis, 29 (7%) with COPD and 19 (4%) with asthma. Patients with PE ± DVT reported a history of airway diseases more often (N=71, 18%) than patients with DVT (N=35, 9%). Factor V Leiden data was available in 394 patients and 426 control subjects. Sensitivity analyses showed no difference between classifying individuals based only on their reported airway disease and classifying individuals based on their reported airway disease and usage of the indicated medication.

*Airway diseases and the risk of VT*

Individuals with bronchitis had a 1.5-fold increased risk of venous thrombosis compared with individuals without bronchitis (Table 2, OR 1.5, 95% CI 0.9-2.4). The increased risk was slightly higher for PE than DVT (ORs 1.6, 95% CI: 0.9-2.8 for PE and 1.4, 95% CI: 0.8-2.6 for DVT). Individuals with COPD had a 1.8-fold increased risk of venous thrombosis compared with individuals without COPD (OR 1.8, 95%CI: 1.1-3.0). The increased risk was limited to an increased risk of PE (OR 2.5, 95% CI: 1.4-4.4) while there was no association with DVT (OR 1.0, 95% CI 0.4-2.1). For asthma there was no overall increased risk of VT (OR 1.4, 95% CI 0.7-2.8), and a 1.9-fold increased risk of PE (95% CI: 0.8-4.1). When analysing the risk of VT for all airway diseases combined, there was a 1.7-fold increase in risk (OR 1.7, 95% CI 1.2-2.4), with a higher risk for PE (OR 2.2, 95% CI 1.5-3.3) than DVT (OR 1.2, 95% CI 0.7-1.9). Excluding individuals using systemic

corticosteroids, the risks were similar, individuals with any of the three airway diseases had an increased risk of PE (OR 1.8, 95% CI: 1.1-2.8) but not, or only weakly of DVT (OR 1.3, 95% CI: 0.8-2.2).

**Table 1** Baseline characteristics patients and controls

| | **Patients** | **Controls** |
|---|---|---|
| N | 401 | 431 |
| Men, n (%) | 166 (41) | 209 (49) |
| Age, years (range) | 79 (70-101) | 78 (70-96) |
| Type VT, N (%) | | |
| Deep vein thrombosis | 166 (41) | N.A. |
| Pulmonary embolism + deep vein thrombosis | 22 (5.5) | N.A. |
| Systemic corticosteroid use, N (%) | 11 (2.7) | 11 (2.6) |
| Factor V Leiden, N (%) | 34 (8.5) | 18 (4.2) |
| Smoking, N (%) | | |
| Never | 123 (31) | 120 (28) |
| Former | 234 (59) | 255 (59) |
| Current | 43 (10) | 55 (13) |

*Association of factor V Leiden and airway diseases on the risk of VT*

Factor V Leiden was present in 19 of 162 patients with DVT (11.7%) and 15 of 232 patients with PE with or without DVT (6.5%), while it was present in 18 of 426 control subjects (4.2%). Two patients had the homozygous mutation. The risk of DVT with factor V Leiden was 3-fold increased (OR 3.0, 95% CI: 1.5-6.0), while the risk of PE was only mildly increased (OR 1.5, 95% CI: 0.7-3.1).

We analysed the combined effect of having any of the three airway diseases (i.e., bronchitis, COPD or asthma) and factor V Leiden on the risk of VT, with results shown in table 3. With individuals without any airway disease and not carrying the factor V Leiden mutation as a reference category, individuals with any of the airway diseases had a 1.6-fold increased risk of VT. In absence of airway diseases, factor V Leiden was associated with 1.7-fold increased risk of VT. In patients with both an airway disease and factor V Leiden, the OR for VT was 13.2. Patterns of association were similar in those with DVT and PE, with the highest among those both an airway disease and factor V Leiden (OR for DVT 9.8 and for PE 14.6).

Table 2 Odds Ratios of VT with airway diseases

| Risk factor | Controls N (%) | Patients N (%) | VT OR*(95%CI) | VT OR†(95%CI) | DVT only OR*(95%CI) | DVT only OR†(95%CI) | PE only OR*(95%CI) | PE only OR†(95%CI) |
|---|---|---|---|---|---|---|---|---|
| Bronchitis | 37 | 48 | 1.5 (1.0-2.4) | 1.5 (0.9-2.4) | 1.4 (0.8-2.5) | 1.4 (0.8-2.6) | 1.7 (1.0-2.9) | 1.6 (0.9-2.8) |
| COPD‡ | 29 | 43 | 1.7 (1.0-2.8) | 1.8 (1.1-3.0) | 1.0 (0.5-2.1) | 1.0 (0.4-2.1) | 2.3 (1.3-3.9) | 2.5 (1.4-4.4) |
| Asthma§ | 19 | 19 | 1.4 (0.7-2.7) | 1.4 (0.7-2.8) | 1.0 (0.4-2.5) | 0.8 (0.3-2.4) | 1.9 (0.9-4.0) | 1.9 (0.8-4.1) |
| Any airway disease | 78 | 106 | 1.7 (1.2-2.4) | 1.7 (1.2-2.4) | 1.2 (0.8-2.0) | 1.2 (0.7-1.9) | 2.1 (1.5-3.1) | 2.2 (1.5-3.3) |

**adjusted for study center*

*†adjusted for smoking (current, former, never), sex, age, BMI (continuous), study center*

*‡8 patients and 5 controls also had bronchitis and 7 patients and 3 controls also had asthma*

*§7 patients and 5 controls also had bronchitis*

Table 3 Joint associations of the presence of airway diseases and factor V Leiden with VT, and stratified by type of VT

| Airway disease | FVL | Controls | Patients | VT | VT | DVT only | DVT only | PE only | PE only |
|---|---|---|---|---|---|---|---|---|---|
| | | N | N | OR*(95%CI) | OR†(95%CI) | OR*(95%CI) | OR†(95%CI) | OR*(95%CI) | OR†(95%CI) |
| Absent | Absent | 332 | 265 | 1 (ref) | 1 (ref) | 1 (ref) | 1 (ref) | 1 (ref) | 1 (ref) |
| Present | Absent | 76 | 95 | 1.6 (1.1-2.3) | 1.6 (1.1-2.3) | 1.3 (0.8-2.1) | 1.2 (0.7-2.0) | 1.9 (1.3-2.9) | 2.0 (1.3-3.0) |
| Absent | Present | 17 | 24 | 1.8 (0.9-3.4) | 1.7 (0.8-3.3) | 2.8 (1.4-5.8) | 2.7 (1.3-5.6) | 1.0 (0.4-2.3) | 0.8 (0.3-2.0) |
| Present | Present | 1 | 10 | 13 (1.6-104) | 13 (1.6-106) | 9.3 (0.9-92) | 9.8 (1.0-100) | 15 (1.8-127) | 15 (1.7-123) |

**adjusted for study center*

*†adjusted for smoking (current, former, never), sex, age, BMI (continuous), study center*

## Discussion

In this population-based case-control study among individuals aged 70 years and older the presence of either bronchitis, COPD or asthma was associated with the risk of PE (ORs ranging 1.6-2.5), and not or only weakly, with the risk of DVT (ORs ranging 1.0-1.4). The risk of VT associated with airway diseases was most pronounced in carriers of the factor V Leiden mutation (OR 13.2), with the combined association for airway diseases and the factor V Leiden being high for both PE (OR 14.8) and DVT (OR 9.8).

We are not aware of other studies that investigated the relationship between these airway diseases combined with the presence of factor V Leiden and risk of VT in an older population. Several studies have reported an association between COPD and PE.[12,13] The first study included only participants below age 70 years, the second study limited the analysis to a primary care setting and participants under age 79, and the third study also included patients who had a prior VT history, so these were not incident cases. In many studies an association between inflammation, such as seen in asthma, and enhanced coagulation was suggested.[14,15] A prospective study found a 3.5-fold increased risk of PE in patients with mild-to-moderate asthma [16], and IL-8 levels were associated with a 1.9-fold increased risk of VT in a case-control study.[17] While there was little association of asthma and PE in our study, power was limited by the relatively small numbers of participants with asthma. Our results show that patients with COPD, asthma or bronchitis are indeed at increased risk of PE.

The factor V Leiden paradox is the observation that this genetic variant mainly affects the risk of PE.[18,19] In our current study the 'factor V Leiden paradox' was also present in older people: individuals with FVL had a pronounced risk of DVT but not of PE (OR=3.0 vs OR=1.5), and this difference was still present when we excluded patients with airway diseases. While factor V Leiden affects the risk of PE only very mildly, we observed that in combination with presence of airway diseases the risk estimates increased. However, due to the low number of

individuals in this subgroup, definitive conclusions require confirmation by other studies.

Several causal explanations for our results may be hypothesized. Inflammatory airway diseases may predispose to a local prothrombotic state and therefore give rise to a local development of pulmonary thrombi which embolize within the pulmonary vasculature. Asthma is associated with a procoagulant state in the bronchoalveolar space in the lungs, via an increase in tissue factor activity.[14] The pulmonary arteries of COPD patients are characterised by endothelial cell dysfunction which in turn also leads to a procoagulant state.[20] Furthermore, COPD patients have increased FVIII, FIX, prothrombin and thrombin levels, leading to a prothrombotic tendency.[21] Similar mechanisms might hold for chronic bronchitis. The effects of C-reactive protein, a biomarker of inflammation, on haemostasis has been reviewed by Fay, demonstrating a link between inflammation and thrombosis.[22] These consistent results of associations between pulmonary inflammatory diseases and pulmonary thrombi make a local development of these thrombi plausible. The role of usage of corticosteroids should also be considered, as these are often prescribed for patients with airway diseases and their usage has been linked to a hypercoagulable state.[11,23] However, excluding the few participants using these medications did not alter our results.

Our study has several limitations. Firstly, data on airway diseases were self-reported. No functional tests were performed to diagnose the airway diseases and self-reports were not confirmed by medical record review. Participants could have misclassified their specific airway condition (bronchitis, COPD or asthma). However, as all investigated airway conditions are chronic, this misclassification is likely to be random and similar in patients and control subjects, which would at most result in an underestimation of the risks.[24] Moreover, COPD is usually underdiagnosed[25], again leading to underestimation of the risk. Secondly, we did not have information on the severity of COPD, asthma or bronchitis, which was previously shown to be of additional value to identify individuals who are at high

risk of PE.[16] Thirdly, the diagnosis of PE is difficult to establish in COPD patients with exacerbations. However, all PE diagnoses were confirmed by imaging and symptomatic PE is unlikely to remain undiagnosed. Fatal PE may have been missed since the presentation of PE can be as sudden death, and this would lead to an underestimation of the risks. Fourthly, due to the relatively low numbers of patients with airway diseases, particularly when analysed in subgroups of the three airway diseases and FVL, the confidence intervals of the odds ratios are wide and not discriminatory. Lastly, we could not distinguish between patients who had an isolated PE and those who had both PE and DVT, as un ultrasound examination of the lower extremities is not standard care when patients present with PE complaints. This would dilute our results. The strengths of our study include the large patient sample of individuals aged 70 years and older, the detailed assessment of risk factors for thrombosis, and the home visits for collection of measured risk factors.

The findings of our study might have important clinical implications. PE is a potentially life-threatening complication of VT which is also more prevalent in the older population than the middle-aged population.[26] The interaction of airway diseases and factor V Leiden that we observed, if it can be confirmed, suggests a high attributable risk for this combination of risk factors in this age group. For example, an individual with airway disease and factor V Leiden who is over age 80 may have as high as a 10% annual risk of PE. Physicians should therefore be more aware that patients with an airway disease are at risk of PE.

In conclusion, older individuals with airway disease had a higher risk of VT than individuals without an airway disease and the risk was most pronounced for PE than for DVT. The risk was highest when the factor V Leiden mutation was present.

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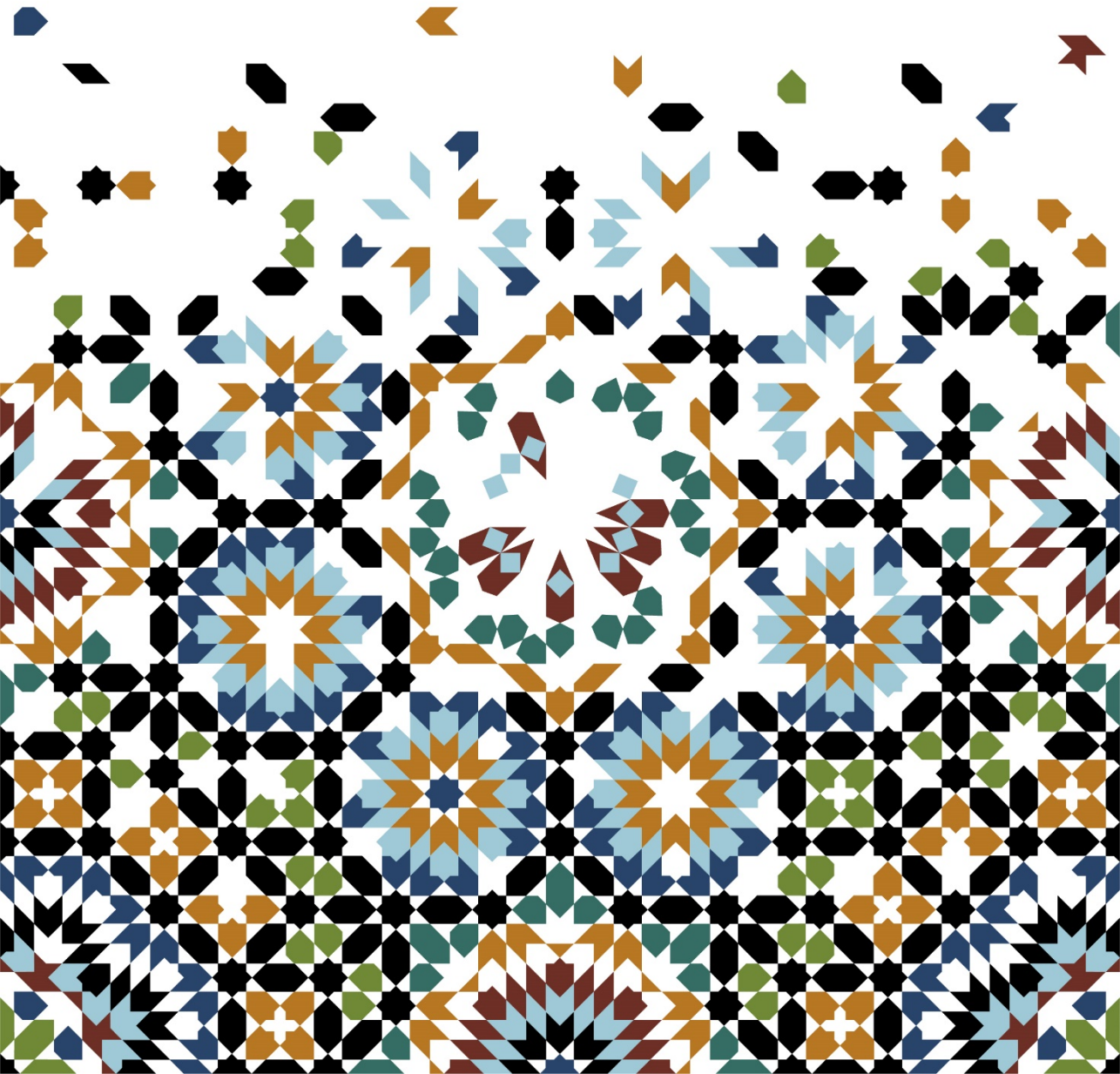
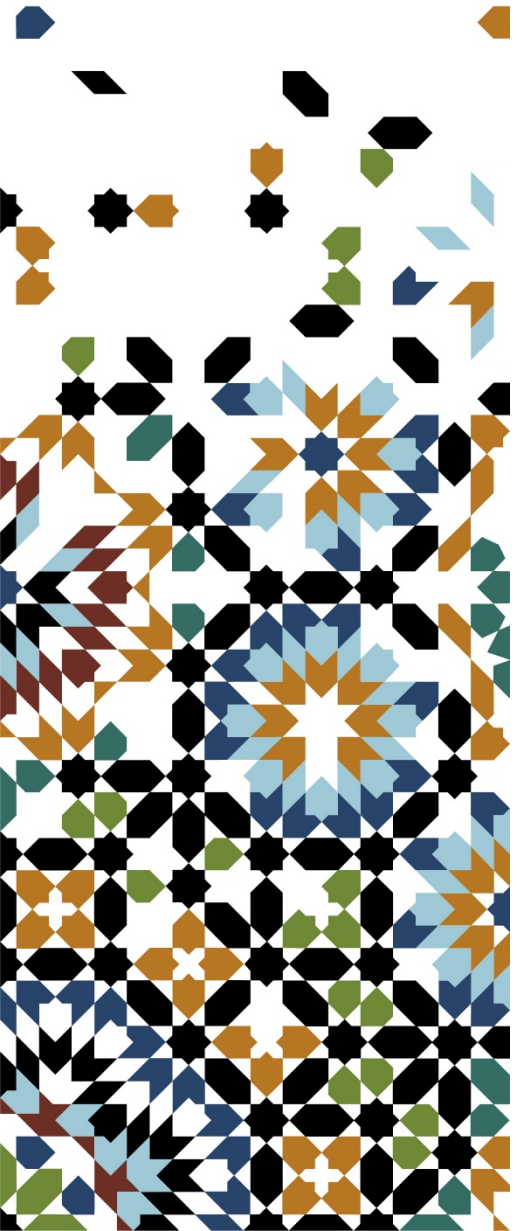


# Chapter 3



## Genetic risk factors for venous thrombosis in the elderly in a case-control study

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## Abstract

As the incidence of VT increases steeply with age and the number of elderly is on the rise, studies of VT in this age group are important. We aimed to study the associations of common genetic risk factors, i.e., the factor V Leiden and prothrombin G20210A mutations, non-O blood group, and family history of VT with risk of a first VT in older age (>70 years). 401 consecutive cases with a first-time thrombosis and 431 controls (all ≥70 years) were included in the AT-AGE case-control study. Information on risk factors for VT, including family history of VT in first degree relatives, was obtained by interview. Unprovoked VT was defined as thrombosis not related to surgery, fracture, plaster cast or immobility within three months prior to VT. The risk of VT was 2.2-fold increased in factor V Leiden carriers (95% CI 1.2-3.9), 1.4-fold increased in prothrombin mutation carriers (95% CI 0.5-3.9), and 1.3-fold increased in those with non-O blood group (95% CI 1.0-1.8). Positive family history of VT was associated with a 2.1-fold increased risk of VT (95% CI 1.5-3.1). The highest risk of VT was found in individuals who had both a positive family history and were carriers of one of the two prothrombotic mutations. Genetic factors clearly related to VT in younger populations were also risk factors in older age, and a positive family history was also important in this age group.

## Introduction

Venous thrombosis (VT) is a multicausal disease, associated with both environmental and genetic risk factors.[1] Factor V Leiden (rs6025) and the prothrombin G20210A mutation (rs1799963) are the most common prothrombotic variants (prevalence of 3-5%) in young and middle aged population and are associated with a 3-7-fold increase in the risk of VT compared with non-carriers.[2-4] Another genetically determined risk factor, i.e., the non-O blood group, is an important determinant of venous and arterial disease.[5-7] In the young and middle-aged population, blood group non-O is associated with a doubling in risk of VT.[8] Family history is a feasible and comprehensive genetic risk assessment method for VT, potentially useful to detect thrombophilia in patients, as reviewed by Zöller et al.[9] Aggregation of VT cases in a family may reflect the presence of known and unknown genetic risk factors. However, conflicting results are published regarding a positive family history as a predictor for the presence of inherited thrombophilia.[10-13]

Limited information is available regarding genetic risk factors for VT in the elderly. Elderly persons are often excluded from clinical studies into aetiology and management because of co-morbidities, short life expectancies, and logistical difficulties.[14, 15] Furthermore, it is unknown whether a positive family history of VT is predictive of a venous thrombotic event at an older age. As the incidence of VT increases strongly with age and the number of elderly is on the rise[16], studies of VT in the elderly becomes more relevant: VT is rare in young individuals (<1 per 10 000 per year under the age of 18) but increases to nearly 1% per year at very old age.[16]

This study aimed to assess whether common genetic risk factors, i.e., the factor V Leiden and prothrombin G20210A mutations, non-O blood group, as well as a positive family history of VT are risk factors for a first VT at an older age (>70 years). Additionally, we aimed to assess the predictive value of a positive family

history of VT for factor V Leiden, prothrombin G20210A and non-O blood group in this age group.

## Methods

### Study population and data collection

The Age and Thrombosis, Acquired and Genetic risk factors in the Elderly (AT-AGE) study is a two-center, population-based case-control study designed to study risk factors for VT in the elderly. The study design was described in detail previously.[17] From June 2008 to August 2011 in Leiden, the Netherlands and December 2008 to July 2011 in Burlington, Vermont, US, all consecutive patients 70 years and older with a first DVT or PE were identified. Cases were identified from the anticoagulation clinics in Haarlem and Leiden and from the Vascular Laboratory and the Radiology department of the University of Vermont Medical Center. Controls were randomly selected from five primary care practices in Leiden and four in Vermont. Subjects with an active malignancy or severe psychiatric or cognitive disorder were excluded. For all participants, a home visit took place, during which an extensive structured interview was completed by trained personnel and a blood sample or buccal swab was collected. The index date was defined as the date of diagnosis of the thrombosis for the cases and the date of the home visit for the controls. All participants provided written informed consent. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and by the Committee of Human Research of the University of Vermont.

### Risk factor assessment

Self-reported information on the presence of first-degree relatives (parent, sibling, or offspring) who experienced VT was obtained via the interview. Family history of VT was considered positive if at least one first degree relative experienced VT. Participants who indicated that they did not know whether a first degree relative had VT were classified as having a negative history.

Provoked VT was defined as thrombosis after hospitalisation, fracture, plaster cast, splint or transient immobility at home ≥4 consecutive days in the three months before the index date.

During the home visits, blood samples were drawn into vacuum tubes containing 0.1-volume 0.106-mol/L trisodium citrate or when no blood sample could be drawn a buccal swab was collected (N=28). The blood sample was separated into plasma and cells through centrifugation. DNA analysis for the factor V Leiden mutation (rs6025) and the prothrombin G20210A mutation (rs1799963) was performed using a combined polymerase chain reaction method with the TaqMan assay. The 20146G/- (rs8176719), 21463C/G (rs7853989), 21867A/G (rs8176749), and 21996C/- (rs8176750) blood group polymorphisms were determined by a 5'nuclease assay (Taqman; Applied Biosystems, Foster City, CA, USA) using a PCR reaction mix (Taqman Genotyping Master Mix, Applied Biosystems) and an allele-specific fluorescent probe equipped with a minor groove binding moiety (Applied Biosystems).

Statistical analysis

To estimate relative risks, we calculated odds ratios (ORs) with 95% confidence intervals (95% CI) using logistic regression. We determined associations of factor V Leiden, the prothrombin G20210A mutation, ABO blood group, and a positive family history with VT risk. All ORs were adjusted for age (continuous), sex (categorical), and study center (Leiden and Haarlem versus Vermont, categorical) using multivariable logistic regression. Additional to the individual associations, the combined associations of the risk factors were studied. Analyses were also stratified for provoked and unprovoked VT, and type of VT (DVT only or PE with or without DVT). Sensitivity analysis excluded individuals who did not know their family history status for VT. The risk of VT associated with a positive family history of VT was also studied in more detail by calculating ORs for having any affected first-degree relative, having a first-degree relative affected before age 50, and for having >1 affected first-degree relatives. We calculated the positive predictive

value of family history to identify factor V Leiden, prothrombin G20210A mutation or ABO blood group. The population attributable risk (PAR) is an estimate of the burden of a disease.[18] The PAR of factor V Leiden, prothrombin G20210A mutation, non-O blood group and a positive family history of VT was calculated using the formula: p x (OR – 1)/(OR), in which p is the proportion of cases exposed to the risk factor. IBM SPSS Statistics 20.0 for Windows (SPSS Inc, Chicago, Ill) was used for data analysis.

## Results

In this study, 401 patients and 431 controls were included (Table 1); 166 patients (41%) were diagnosed with an isolated DVT and 235 (59%) with PE with or without DVT. DNA analysis for factor V Leiden was available for 394 (98%) patients and 426 (99%) controls, for prothrombin G20210A mutation for 394 (98%) patients and 427 (99%) controls and for ABO blood group for 376 (94%) patients and 416 (97%) controls. Of all participants, 25 (6%) patients and 13 (3%) controls did not know whether one of their first degree relatives had VT. In the overall analyses, these individuals were classified as having a negative family history.

**Table 1** Baseline characteristics patients and controls

| | Controls | Patients |
|---|---|---|
| N | 431 | 401 |
| Men, N (%) | 209 (48.5) | 166 (41.4) |
| Age, mean (range) | 77.5 (70-96) | 78.7 (70-101) |
| Type VT, N (%) | | |
| Deep vein thrombosis (DVT) | N.A. | 166 (41.4) |
| Pulmonary embolism (PE) (±DVT) | N.A. | 235 (58.6) |
| Provoked VT*, N (%) | N.A. | 140 (34.9) |
| Factor V Leiden, N (%) | 18 (4.2) | 34 (8.6) |
| Prothrombin G20210A mutation, N (%) | 7 (1.6) | 9 (2.3) |
| Non-O blood group, N (%) | 232 (55.8) | 231 (61.4) |
| Positive family history for VT, N (%) | 54 (12.5) | 97 (24.2) |

** defined as thrombosis after hospitalisation, fracture, plaster cast, splint, or transient immobility at home in the three months before the index date.*
*N* = number, *VT* = venous thrombosis, *N.A.* = not applicable

*Prothrombotic variants, ABO blood group and risk of VT*

Out of 394 patients, 34 (8.6%) carried factor V Leiden (32 heterozygotes and 2 homozygotes). Of the controls, 18 (4.2%) were heterozygous for factor V Leiden and none were homozygous. Heterozygous plus homozygous carriers of factor V Leiden had a 2.2-fold increased risk of a first VT (Table 2, 95% CI 1.2-3.9), compared with participants who did not carry factor V Leiden. Factor V Leiden was present in 19 of 162 patients with DVT (11.7%) and 15 of 232 patients with PE with or without DVT (6.5%), leading to odds ratios of 3.0 for DVT (95% CI: 1.5-6.0), and 1.4 for PE (95% CI: 0.7-2.9). The OR for provoked VT was slightly higher than the OR for unprovoked VT with factor V Leiden (OR 1.6, 95% CI 0.7-3.7 for provoked VT and OR 2.4, 95% CI 1.2-4.5 for unprovoked VT). Nine patients (2.3%) and seven controls (1.6%) were heterozygous carriers of the prothrombin G20210A mutation, and none were homozygous. This led to an OR for prothrombin G20210A mutation of 1.4 (95% CI 0.5-3.9). The OR for unprovoked VT with prothrombin G20210A mutation was 1.2 (95% CI 0.3-4.9) while a similar result was observed for provoked VT (OR 1.5, 95% CI 0.5-4.7). 231 (61.4%) patients and 232 controls (55.8%) had blood group non-O, resulting in an OR of 1.3 (95% CI 1.0-1.8) for blood group non-O. Blood group non-O was not associated with the risk of provoked VT (OR 1.0, 95% CI 0.7-1.5), whereas the risk of unprovoked VT was 1.5-fold increased (95% CI 1.1-2.1).

We studied the combined effect of ABO blood group and the presence of either factor V Leiden or prothrombin G20210A mutation, with wildtype carriers of factor V Leiden and prothrombin G20210A mutation with blood group O as the reference category. Individuals carrying either prothrombotic variant and blood group O had a 2.3-fold increased risk of VT (95% CI 0.9-5.9) and wildtype carriers of factor V Leiden and prothrombin G20210A mutation with blood group non-O had a 1.3-fold increased risk of VT (95% CI 1.0-1.8). Those with both blood group non-O and a prothrombotic variant had a similar risk as those with blood group O and a prothrombotic variant (Table 3).

*Family history and risk of VT*

Family history of VT in a first degree relative was positive for 97 patients (24.2%) and 54 controls (12.5%). Individuals with a positive family history of VT had a more than 2.1-fold increased risk of VT compared with individuals without a positive family history of VT (95% CI 1.5-3.1). The association was similar when subjects who did not know their family history were excluded from the analysis (OR 2.2, 95% CI 1.5-3.2). The risk of both provoked and unprovoked VT was increased in the presence of a positive family history: OR provoked VT 1.7 (95% CI 1.0-2.8); OR unprovoked VT 2.4 (95% CI 1.6-3.6). The risk of VT was not increased when only family members who had VT before the age of 50 years were considered positive (OR 0.9, 95% CI 0.5-1.6). The number of affected relatives was also not associated with the risk of VT; having more than 1 positive family member versus only 1 positive family member resulted in an OR of 0.8 (OR 0.8, 95% CI 0.4-1.8).

**Table 2** Genetic risk factors of VT

| Risk factor | Absent or present | Controls | Patients (Nprovoked/ Nunprovoked) | ORoverall* (95%CI) | ORprovoked* (95%CI) | ORunprovoked* (95%CI) |
|---|---|---|---|---|---|---|
| Factor V Leiden | - | 408 | 128/232 | 1† | 1† | 1† |
| | + | 18 | 10/24 | 2.2 (1.2-3.9) | 1.6 (0.7-3.7) | 2.4 (1.2-4.5) |
| Prothrombin G20210A mutation | - | 420 | 135/250 | 1† | 1† | 1† |
| | + | 7 | 3/6 | 1.4 (0.5-3.9) | 1.2 (0.3-4.9) | 1.5 (0.5-4.7) |
| Blood group | O | 184 | 59/86 | 1† | 1† | 1† |
| | Non-O | 232 | 72/159 | 1.3 (1.0-1.8) | 1.0 (0.7-1.5) | 1.5 (1.1-2.1) |
| Family history | - | 377 | 111/193 | 1† | 1† | 1† |
| | + | 54 | 29/68 | 2.1 (1.5-3.1) | 1.7 (1.0-2.8) | 2.4 (1.6-3.6) |

**OR adjusted for age, sex, study center*
*†reference category*

**Table 3** Prothrombotic variants, blood group non-O and risk of VT

| Factor V Leiden or prothrombin mutation | Non-O blood group | Controls | Patients (N provoked/ N unprovoked) | ORoverall* (95%CI) | ORprovoked* (95%CI) | ORunprovoked* (95%CI) |
|---|---|---|---|---|---|---|
| - | - | 175 | 55/77 | 1† | 1† | 1† |
| + | - | 8 | 4/9 | 2.3 (0.9-5.9) | 1.6 (0.4-5.5) | 2.9 (1.0-8.1) |
| - | + | 216 | 63/141 | 1.3 (1.0-1.8) | 1.0 (0.6-1.5) | 1.6 (1.1-2.2) |
| + | + | 16 | 9/18 | 2.2 (1.1-4.3) | 1.7 (0.7-4.0) | 2.5 (1.2-5.3) |

**OR adjusted for age, sex, study center*
*†reference category*

To assess whether a positive family history was mainly determined by the presence of the factor V Leiden or the prothrombin G20210A mutations, we studied the association between a family history of thrombosis and these prothrombotic variants. Out of 97 patients with a positive family history, only 11 carried factor V Leiden or prothrombin G20210A mutation, yielding a positive predictive value of positive family history for factor V Leiden or prothrombin G20210A mutation of 11%. When including non-O blood group to the analyses, out of 93 patients with a positive family history, 59 carried factor V Leiden, prothrombin G20210A mutation, or non-O blood group. This results in a positive predictive value of positive family history for factor V Leiden, prothrombin G20210A mutation or non-O blood group of 63%.

In table 4 the associations of the combined risks of a positive family history of VT and carrying the factor V Leiden or the prothrombin G20210A mutation are shown. Excluding those who carried a prothrombotic variant, did not change the risk estimate of a positive family history: OR 2.1 (95% CI 1.4-3.1). Individuals with a positive family history of VT who also carried either a prothrombotic variant had a high risk of VT (OR 7.6, 95% CI 1.6-35.7). The PAR for factor V Leiden was 4.7%, for prothrombin G20210A mutation 0.7%, for non-O blood group 14.2%, and for a positive family history of VT 12.7%.

**Table 4** Prothrombotic variants, family history of VT and the risk of VT

| Factor V Leiden or prothrombin mutation | Family history | Controls | Patients (N provoked/N unprovoked) | ORoverall* (95%CI) | ORprovoked* (95%CI) | ORunprovoked* (95%CI) |
|---|---|---|---|---|---|---|
| - | - | 350 | 99/168 | 1† | 1† | 1† |
| - | + | 51 | 26/60 | 2.1 (1.4-3.1) | 1.7 (1.0-2.9) | 2.3 (1.5-3.6) |
| + | - | 23 | 10/20 | 1.7 (0.9-2.9) | 1.4 (0.7-3.2) | 1.7 (0.9-3.3) |
| + | + | 2 | 3/8 | 7.6 (1.6-35.7) | 4.1 (0.7-25.3) | 9.9 (2.0-48.9) |

**OR adjusted for age, sex, study center*
*†reference category*

## Discussion

In this population-based case-control study of 832 individuals, we show that factor V Leiden, the prothrombin G20210A mutation, and non-O blood group are risk factors for VT in the elderly (>70 years) as they are in younger individuals, increasing the risk of VT 2.2-, 1.4-, and 1.3-fold respectively. Furthermore, a positive family history of VT doubled the risk of VT, regardless of the number of affected family members and their age of onset. The highest risk of VT was found in individuals who had both a positive family history and were carriers of one of the two prothrombotic variants. Among the genetic factors analysed, non-O blood group had the highest PAR for VT and the prothrombin mutation the lowest.

Factor V Leiden and prothrombin G20210A mutation are well established risk factors for VT in young and middle-aged individuals. The risk is four- to sevenfold increased for factor V Leiden carriers and two- to threefold increased for the prothrombin G20210A.[3, 4, 19, 20] Our results indicate that factor V Leiden and prothrombin G20210A remain associated with the risk of VT in older age, as previously reported in several large studies [4, 20-22] and a small subgroup analysis.[23]

Compared with blood group O, non-O individuals had a 1.3-fold increased risk of VT. The increased risk can be partly explained by higher levels of FVIII and von Willebrand Factor in ABO blood group.[24, 25] High FVIII levels are associated with a lowered responsiveness to activated protein C. In carriers of factor V Leiden, this association is strengthened as observed in previous studies, which explains the interaction between non-O blood group and factor V Leiden.[24, 26]

We observed that the positive predictive value of family history of VT for a genetic risk factor for VT was low, as was also indicated in previous studies.[10, 13, 27, 28] This may indicate that unknown or unmeasured genetic risk factors are present in individuals with a positive family history. Taken together with our observation that family history was associated with a 2.1-fold increased risk of VT

among participants who were not carriers of factor V Leiden and prothrombin G20210A mutations, results support a strong hypothesis that unknown genetic factors or familial factors that are not genetic play a substantial role in VT aetiology.[29, 30]

The risk of VT was highest in the presence of multiple genetic risk factors, illustrating the multicausal character of VT[1], which remains so in the elderly. The results are in line with studies reporting that heterozygous factor V Leiden and prothrombin G20210A mutation are relatively weak risk factors for VT, unless another genetic or acquired risk factor is present.[31] We observed lower relative risks for VT for genetic factors here compared to other studies in younger people. There are several reasons relative risks for any risk factor might be lower in the elderly than in middle-aged individuals. Attrition of susceptibles, such that susceptible individuals with factor V Leiden or prothrombin G20210A mutations are more likely to develop VT earlier in life, would result in lower relative risks at an older age. The higher absolute risk of VT in the elderly lead to smaller relative effects of individual risk factors than in middle-aged individuals. In other words, whereas the relative risks are smaller than in the young, absolute risk differences for carriers versus non-carriers are substantial, given the high baseline risk in the elderly. Specifically, the PAR of non-O blood group of 14.2% in our study was lower than reported in a previous study conducted in a younger population (25.0%), and the PAR of factor V Leiden in our study was higher (4.7% compared to 2.8%).[7] Given their high prevalence, the PARs of both non-O blood group and a positive family history of VT (12.7%) were high.

The strengths of our study includes the specific focus on individuals aged 70 years and older. Home visits were performed in order to achieve a high participation rate (participation rate: cases 69%, controls 73%). It is one of the few studies on VT risk that evaluated family history of VT in the elderly.

Our study also has limitations. Controls with a positive family history of VT might be more willing to participate in a study of VT than those who do not have a

positive family history of VT. However, this selection bias would result in an underestimation of the true association. Patients with VT might be more likely to be aware of their family history after they have a VT, which could bias results toward overestimation of the association. This might have been mitigated by the short duration of time between the VT and the study interview (median was 5 weeks for patients). Moreover, as in any case-control study, recall bias might have occurred when obtaining information on risk factors used for the classification into provoked and unprovoked VT and family history. However, by using standardized interviews performed by trained personnel for both cases and controls, the risk of recall bias was minimized.

Our results may have clinical implications. A positive family history of VT doubled the risk of VT in the elderly. In clinical practice this information is easy to obtain, however it is not implemented in clinical decision rules of VT risk. In the elderly these clinical decision rules show a high failure rate.[32] Potentially, obtaining information on family history of VT in individuals aged 70 years or older could improve prediction of VT in the elderly.[33] Future studies should evaluate whether diagnostic tools improve when implementing family history.

In conclusion, this study demonstrated that factor V Leiden, prothrombin G20210A mutation, non-O blood group and a positive family history for VT are risk factors for VT in the elderly.

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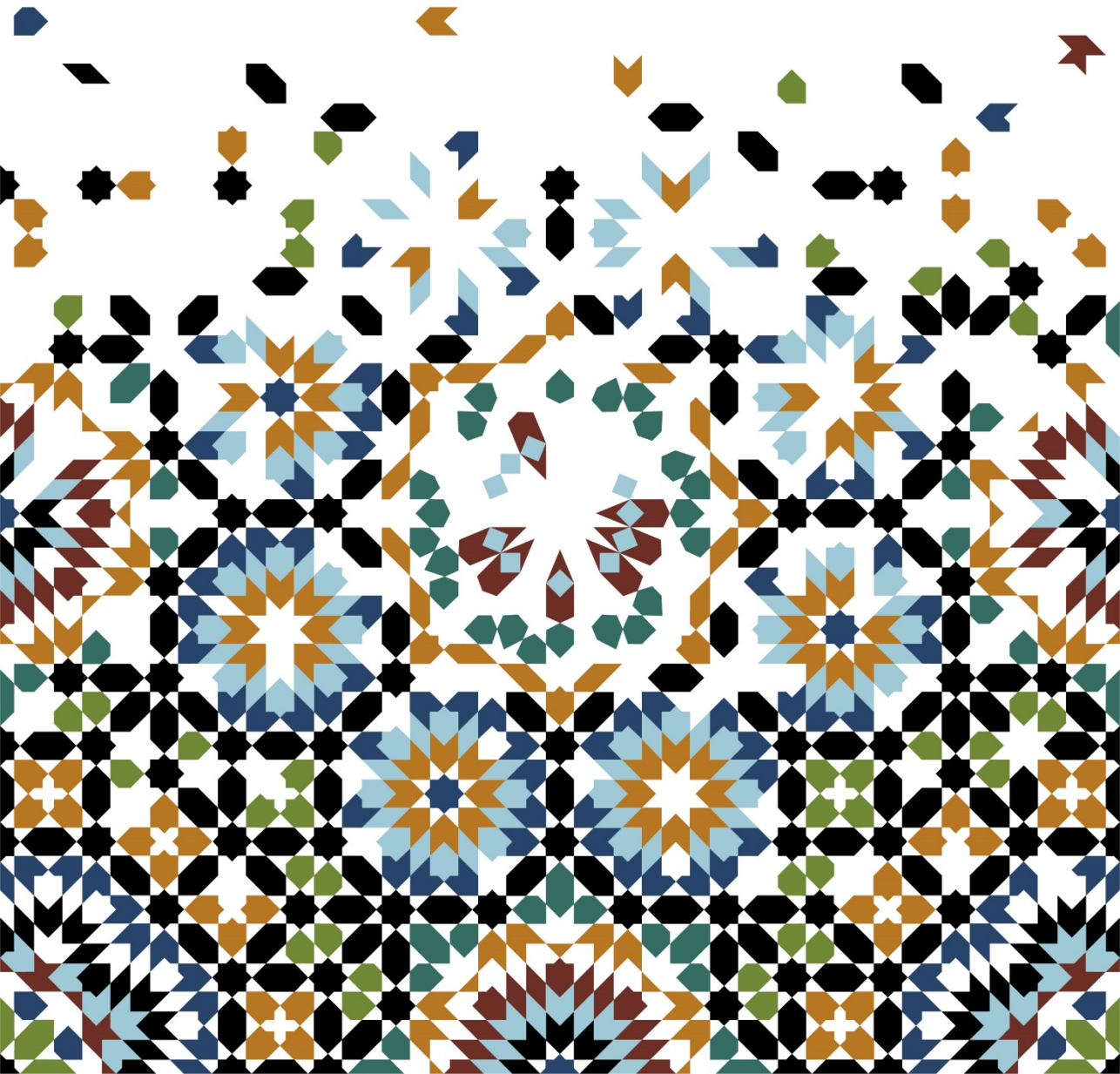
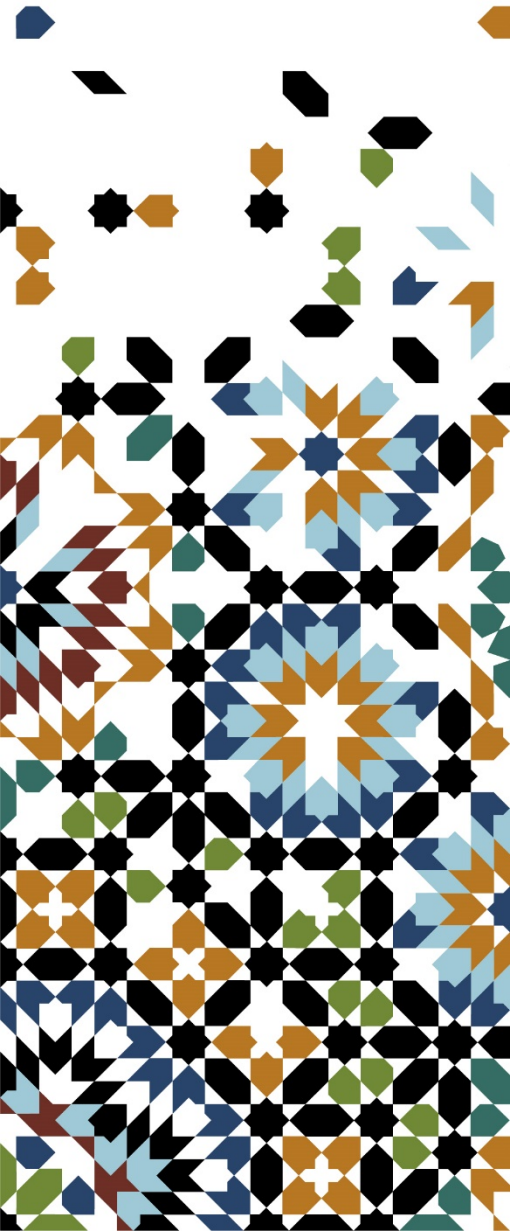


# Chapter 4



## Clinical features of venous insufficiency and the risk of venous thrombosis in older people

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## Abstract

Venous thrombosis is common in older age, with an incidence of 0.5–1% per year in those aged >70 years. Stasis of blood flow is an important contributor to the development of thrombosis and may be due to venous insufficiency in the legs. The risk of thrombosis associated with clinical features of venous insufficiency, i.e., varicose veins, leg ulcers and leg oedema, obtained with a standardized interview was assessed in the Age and Thrombosis Acquired and Genetic risk factors in the Elderly (AT-AGE) study. The AT-AGE study is a case–control study in individuals aged 70 years and older (401 cases with a first-time venous thrombosis and 431 control subjects). We calculated odds ratios (ORs) and corresponding 95% confidence intervals (CI) adjusted for age, sex and study centre. Varicose veins and leg ulcer were associated with a 1.6-fold (95% CI 1.2–2.3) and 3.3-fold increased risk of thrombosis (95% CI 1.6–6.7), respectively, while the risk was increased 3.0-fold (95% CI 2.1–4.5) in the presence of leg oedema. The risk of thrombosis was highest when all three risk factors occurred simultaneously (OR: 10.5; 95% CI 1.3–86.1). In conclusion, clinical features of venous insufficiency, i.e., varicose veins, leg ulcers and leg oedema, are risk factors for venous thrombosis in older people.

## Introduction

The incidence of venous thrombosis increases steeply with age, with an incidence of 0.5–1% per year in people aged over 70 years (Naess et al, 2007). More than 60% of all thrombotic events occur in this age group (Rosendaal et al, 2007). Stasis of blood flow is, together with hypercoagulability and endothelial injury, an important contributor to the development of venous thrombosis according to Virchow's triad (Virchow, 1856). Stasis can occur due to external pressure on the vein during, e.g., prolonged immobilization or pregnancy, or due to venous insufficiency in the legs. Venous insufficiency comprises a spectrum of clinical features, including varicose veins, venous ulcer and leg oedema (Duran et al, 2000; Mustoe, 2004; Langer et al, 2005). Like venous thrombosis, the prevalence of venous insufficiency increases steeply with age (Ruckley et al, 2002; Carpentier et al, 2004; Beebe-Dimmer et al, 2005). There is limited information on the role of venous insufficiency in the development of venous thrombosis. In young and middle-aged individuals, varicose veins strongly predispose to superficial thrombosis and are also associated with an increased risk of deep vein thrombosis (Heit et al, 2000; Marchiori et al,2006; Decousus et al, 2012). The contribution of venous insufficiency to the incidence of venous thrombosis in the older population is unknown. The aim of this study was to investigate whether clinical features of venous insufficiency, defined as a history of varicose veins, leg ulcer or leg oedema, are risk factors for venous thrombosis in individuals aged 70 years and older. In addition, the contribution of these factors to the incidence of deep venous thrombosis was evaluated.

## Methods

### *Participants*

The Age and Thrombosis Acquired and Genetic risk factors in the Elderly (AT-AGE) study is a two-centre population-based case–control study. The study has been described in detail previously (Engbers et al , 2014). In brief, between 2008 and 2011 all consecutive cases aged 70 years or older with an episode of deep venous

thrombosis in the leg (DVT) or a pulmonary embolism (PE) were identified in defined geographical areas in Leiden, the Netherlands and Burlington, Vermont, USA. In Leiden, cases were identified in two anti-coagulation clinics (Leiden and Haarlem). In Vermont, cases resided in a 45 km radius of Burlington, and were diagnosed at the University of Vermont Medical Center in Burlington, the only diagnostic centre in that area.

Presence of DVT required positive compression ultrasonography or Doppler ultrasound. PE was considered definite in case of a positive spiral computerized tomographic or high probability ventilation-perfusion lung scan. We defined venous thrombosis as DVT alone or PE with or without a proven DVT by ultra-sound (PE ± DVT). We invited the population-based control subjects randomly from primary care practices in the same geographical areas (five in Leiden and four in Vermont). We defined the index date for the cases as the date of diagnosis of the thrombotic event. For control subjects the index date was the date of completing the interview at home. Individuals with active malignancy, defined as diagnosis of cancer, or chemotherapy or radiation therapy for cancer within 6 months before the index date, and those with severe psychiatric or cognitive disorders were excluded from participation, as were individuals who self-reported previous DVT or PE within the past 10 years. Of 1187 identified cases, 689 (58%) were eligible. In Leiden, 398 (71%) of the 561 invited cases and 321 (76%) of the 422 invited control subjects participated. In Burlington, 128 cases were invited and 75 (59%) participated, while 140(67%) of the 209 invited control subjects participated. Detailed information about the reasons for exclusion or not participating in the study can be found elsewhere (Engbers et al, 2014). All participants provided written informed consent. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and by the Committee on Human Research of the University of Vermont.

*Data collection*

Trained personnel performed an extensive interview during a home visit. For the analyses on the aetiology of venous thrombosis we included the cases and control subjects without a history of venous thrombosis and who completed the interview at home (401 cases and 431 control subjects). For the cases, the median duration between the index date and the home visit was 5 weeks (range 1–44 weeks), 75% were visited within 7 weeks and 90% were visited within10 weeks. The interview obtained information about the presence of well-known risk factors, such as recent hospitalization, surgery and fracture and a blood sample or buccal swab was collected (Engbers et al, 2014). The participants were asked about any prior history of varicose veins or leg ulcer or whether they had a history of leg oedema that lasted until at least the 3 months before the index date. Leg oedema was classified by participants as 'daily oedema', 'intermittent oedema' or 'no oedema'. The first version of the interview questionnaire for the control subjects did not include the question of oedema, as this was incorporated after this. For this reason, this data could not be reported for 24 control subjects. We used self-assessed information on clinical features of venous insufficiency, a strategy that has been previously validated (Laurikka et al, 1995). In addition, cases were asked to provide self-assessed information on the type and duration (in days) of possible thrombotic complaints prior to the diagnosis of the thrombotic event. The thrombotic event was defined as provoked if, in the 3 months before the thrombosis, one of these conditions was positively identified: hospitalization, surgery, a fracture, plaster cast or splint use, a minor injury or immobilization in the home situation (Engbers et al, 2014). Physical measurements were performed including weight (measured with a calibrated scale) and height. Body mass index (BMI) was calculated by dividing bodyweight (kg) by squared height (m2).

*Statistical analysis*

To provide insight into the source population at the two study sites, we compared the characteristics of control subjects, in Leiden and in Vermont. We studied the

associations between potential confounders, i.e., sex and BMI and features of venous insufficiency in the complete control group. The risk of venous thrombosis associated with the presence of history of varicose veins, leg ulcer and leg oedema in the 3 months before the thrombosis was determined. We assessed a dose-response relationship for oedema across groups of those with none, intermittent or daily oedema. The risk of thrombosis associated with the presence of one, two or three of the symptoms of venous insufficiency was assessed. Odds ratios (OR) and 95% confidence intervals (CI) were calculated as an estimate of the relative risk of venous thrombosis. We used a multiple logistic regression model to calculate adjusted OR. All ORs reported here were adjusted for age (continuous), sex and study centre. Additionally we adjusted for BMI (continuous), smoking status and the presence of the F5 R506Q (factor V Leiden) or F2 (prothrombin)20210A mutation. Stratified analyses were performed by type of thrombosis (DVT and PE ± DVT) and the presence of provoking factors, i.e. provoked and unprovoked first venous thrombosis. We performed two analyses to address whether other causes of leg oedema than venous insufficiency explained our findings. Firstly, heart failure is associated with an increased risk of venous thrombosis and leg oedema can be present in individuals with heart failure (Breidthardt et al, 2012). To assess the risk of venous thrombosis associated with leg oedema as a symptom of venous insufficiency, we excluded participants with a self-reported history of heart failure. Secondly, to assess whether the presence of daily leg oedema might have been a (pre)clinical sign of the venous thrombosis (rather than due to venous insufficiency), we determined the risk of thrombosis associated with daily leg oedema in the individuals who reported a clinically asymptomatic event of venous thrombosis, e.g. individuals that did not reported any acute thrombotic complaints of the legs or lungs before the diagnosis. To assess the contribution of venous insufficiency to the incidence of venous thrombosis in this older population, we estimated the population attributable risk (PAR) of a history of varicose veins, leg ulcers and daily leg oedema and the number of these symptoms combined. The PAR was calculated as pd (OR - 1)/(OR), where pd is the prevalence of the risk factor among cases, and OR is the adjusted OR found in the study population

(Bruzzi et al, 1985). All statistical analyses were performed using SPSS 20 for Windows (SPSS Inc, Chicago, IL, USA).

## Results

Characteristics of the control subjects of the two AT-AGE study centres are shown in Table I. In both centres, one-third of the control subjects were 80 years or older. Median BMI was 25.9 kg/m2 (range 17.0–42.0 kg/m2) in Leiden, and 27.3 kg/m2 (19.0–49.7 kg/m2) in Vermont. The prevalence of intermittent and daily leg oedema was similar at the two study centres, while the prevalence of varicose veins and leg ulcers was higher in Leiden than Vermont. Symptoms of venous insufficiency were associated with sex and BMI. Women more often had varicose veins and leg oedema than men (varicose veins: women 28.4%, men 14.4%; oedema: women 17.1%, men 7.1%). Leg ulceration was rare and more common in men (3.3%) than women (1.8%). Obese individuals more often had leg oedema and leg ulceration than normal weight individuals but this association was less clear for varicose veins (BMI > 30 kg/m2 compared with <25 kg/m2: oedema: 38.3% vs. 16.1%, leg ulceration: 5.3% vs. 1.3%; varicose veins: 23.4% vs. 20.6%). Symptoms of venous insufficiency were not associated with smoking status.

**Tabel I** Characteristics of Control Subjects by study centre

| | **Leiden (NL)** | **Vermont (US)** |
|---|---|---|
| Control subjects, n | 306 | 125 |
| Median Age (range), years | 76 (70-94) | 76 (70-96) |
| 70-75 years, n (%) | 126 (41.2) | 49 (39.2) |
| 75-80 years, n (%) | 90 (29.4) | 39 (31.2) |
| 80-85 years, n (%) | 61 (19.9) | 24 (19.2) |
| >85 years, n (%) | 29 (9.5) | 13 (11.4) |
| Men, n (%) | 147 (48.0) | 62 (49.6) |
| Median BMI (kg/m$^2$) (range)* | 25.9 (17.0-42.0) | 27.3 (19.0-49.7) |
| Varicose veins, n (%) | 73 (23.9) | 20 (16.0) |
| Leg ulcer, n (%) | 10 (3.3) | 1 (0.8) |
| Leg oedema† | | |
| Never, n (%) | 216 (76.6) | 93 (74.4) |
| Intermittent, n (%) | 35 (12.4) | 13 (10.4) |
| Daily, n (%) | 31 (11.0) | 19 (15.2) |

*NL* = the Netherlands; *US* = United States; *n* = number; *BMI* = body mass index
** BMI: data missing for 8 controls*
*† Leg oedema: data missing for 24 controls*

Table II shows the risk of venous thrombosis associated with symptoms of venous insufficiency. Individuals with varicose veins had a 1.6-fold increased risk of venous thrombosis compared with individuals without varicose veins (OR 1.6; 95% CI 1.2–2.3). A history of a leg ulcer was associated with a threefold increased risk of thrombosis (OR 3.3; 95%CI 1.6–6.7). Leg oedema during the 3 months before thrombosis (daily and intermittent oedema combined) was associated with a threefold (95% CI 2.1–4.5) increased risk of venous thrombosis compared with no oedema. There was a dose-response relationship of severity of oedema and the risk of venous thrombosis. Compared with individuals without oedema, individuals with intermittent oedema had a 1.6-fold increased risk of venous thrombosis (95% CI 1.0–2.5) whereas this risk was 3.1-fold increased for individuals with daily oedema (95% CI 2.1–4.5). The associations between the clinical features of venous insufficiency and thrombosis remained present after adjustment for BMI and smoking (Table II). Adjustment for the presence of the F5R506Q and F2 20210A mutation showed similar risk estimates: varicose veins: OR 1.7 (95% CI 1.3–2.4), leg ulcer: OR 3.4 (95% CI 1.7–6.8), leg oedema: OR 3.1 (95% CI 2.1–4.6). Excluding individuals with heart failure (20 cases and 19 controls) did not change the risk associated with leg oedema, i.e., compared with no oedema, the risk of venous thrombosis was 1.5-fold increased for intermittent oedema (OR 1 .5, 95% CI 1.0–2.3) and 3.2-fold increased for daily oedema (OR 3.2, 95% CI 2.1–4.8).

Information on the presence or absence of thrombotic complaints prior to the diagnosis of venous thrombosis was available for 376 cases (93.8%). Of the cases, 322 (85.6%) reported leg complaints specifically attributable to the subsequent diagnosis of venous thrombosis, whereas 54 (14.4%) did not. Among the latter, daily leg oedema was associated with a 2.6-fold (95% CI 1.3–5.2) increased risk of venous thrombosis compared with no leg oedema. Of the cases, 219 (54.6%) had an unprovoked venous thrombotic event. Varicose veins were associated with a 1.3-fold (95% CI 0.9–2.0) increased risk of an unprovoked venous thrombosis. This risk was 3.2-fold increased (95% CI 1.4–6.9) for leg ulcer and 3.4-fold (95%

CI 2.1–5.3) for leg oedema (daily and intermittent oedema combined). For provoked venous thrombosis we found a 2.0-fold (95% CI 1.4–2.9) increased risk for varicose veins and a 3.4-fold increased risk (95% CI 1.5–7.4) for leg ulcer. Leg oedema was associated with a 2.8-fold (95% CI 1.7–4.4) increased risk of provoked thrombosis.

To assess the association of severity of venous insufficiency with risk of venous thrombosis, we determined the risk associated with number of venous insufficiency symptoms. The presence of one, two or three of the clinical features gradually increased the risk of thrombosis, with the highest risk of venous thrombosis associated with the presence of all three clinical features compared with none of the features (OR:10.5; 95% CI 1.3–86.8) (Table III). Of the cases, 166 (41%) were diagnosed with DVT only and 235 (59%) were diagnosed with PE ± DVT. The associations of venous insufficiency features with thrombosis risk were similar for both types of thrombosis except that the association of leg ulcer with DVT appeared stronger than the association with PE ± DVT (Table IV).

The PAR of the three manifestations of venous insufficiency was 12.3% for varicose veins, 6.0% for a leg ulcer and 22.0% for the presence of leg oedema. The PAR of presence of two symptoms of venous insufficiency was 22.7% and for the presence of three symptoms it was 4.6%.

**Table II** Clinical Features of Venous Insufficiency and the Risk of Venous Thrombosis

| | Cases n = 401 | Controls n = 431 | OR crude (95% CI) | OR adjusted* (95CI) | OR adjusted† (95CI) |
|---|---|---|---|---|---|
| Varicose Veins, n (%) | 131 (32.7) | 93 (21.6) | 1.8 (1.3-2.4) | 1.6 (1.2-2.3) | 1.6 (1.2-2.3) |
| Leg Ulcer, n (%) | 35 (8.7) | 11 (2.6) | 3.7 (1.8-7.3) | 3.3 (1.6-6.7) | 3.0 (1.5-6.2) |
| Leg Oedema, n (%)‡§ | 111 (33.0) | 50 (13.9) | 3.0 (2.1-4.4) | 3.0 (2.1-4.5) | 2.9 (1.9-4.3) |
| Leg Oedema‡§ | | | | | |
| Never, n (%) | 225 (56.8) | 309 (75.9) | 1 (ref) | 1 (ref) | 1 (ref) |
| Intermittent, n (%) | 60 (15.2) | 48 (11.8) | 1.7 (1.1-2.6) | 1.6 (1.0-2.5) | 1.4 (0.9-2.2) |
| Daily, n (%) | 111 (28.0) | 50 (12.3) | 3.0 (2.1-4.4) | 3.1 (2.1-4.5) | 2.9 (1.9-4.4) |

N, number; OR, odds ratio; CI, confidence interval; ref, reference group.

**Adjusted for age (continuous), sex, and study centre.*

*†Further adjustment: BMI (body mass index) (continuous) and smoking status.*

*‡ Leg oedema: no versus daily leg oedema.*

*§Leg oedema: data missing for 29 individuals.*

**Table III** Joint Associations of the presence of varicose veins, leg ulcer and leg oedema with thrombosis

| | Cases n=396 | Controls n=407 | OR crude (95CI) | OR adjusted* (95CI) |
|---|---|---|---|---|
| No risk factor | 149 (47.2) | 249 (66.9) | 1 (ref) | 1 (ref) |
| One risk factor, n (%) | 167 (52.8) | 123 (33.1) | 2.3 (1.7-3.1) | 2.2 (1.6-3.0) |
| Two risk factors, n (%) | 72 (32.6) | 34 (12.0) | 3.5 (2.2-5.6) | 3.3 (2.1-5.3) |
| Three risk factors, n (%) | 8 (5.1) | 1 (0.4) | 13.4 (1.7-108.0) | 10.5 (1.3-86.1) |

N, number; OR, odds ratio; CI, confidence interval; ref, reference group.
**Adjusted for age (continuous), sex, and study centre.*

**Table IV** Odds ratios of thrombosis with venous insufficiency stratified by type of thrombosis.

| | n, DVT/total VT (%) | DVT OR adjusted (CI% 95)* | PE±DVT OR adjusted (CI% 95)* |
|---|---|---|---|
| Varicose veins | 52/131 (39.7) | 1.7 (1.1-2.5) | 1.6 (1.1-2.3) |
| Leg ulcer | 18/35 (51.4) | 4.3 (2.0-9.4) | 2.4 (1.1-5.3) |
| Leg oedema† intermittent | 26/60 (43.3) | 1.7 (1.0-3.0) | 1.5 (0.9-2.5) |
| Leg oedema† daily | 42/111 (37.8) | 2.8 (1.8-4.6) | 3.2 (2.1-5.0) |

N, number; DVT, deep vein thrombosis of the leg; PE, pulmonary embolism; OR, odds ratio; CI,confidence interval. **Adjusted for age (continuous), sex and study centre. †Leg oedema: data missing for 29 cases.*

## Discussion

In this population-based case–control study among individuals aged 70 and older, symptoms of venous insufficiency, i.e., a history of varicose veins, leg ulcer and oedema, increased the risk of venous thrombosis (both DVT and PE), with ORs ranging from 1.6 to 3.0. The risk of venous thrombosis increased with severity of the venous insufficiency, defined as the number of clinical features. The risk was highest in individuals with all three clinical features combined (OR 10.5), although presence of all three features was uncommon.

In agreement with the literature, venous insufficiency was common, both in the cases and control subjects (Carpentier et al, 2004), leading to a high PAR of venous thrombosis associated with varicose veins and leg oedema. Adjustments for potential confounders did not influence the finding that clinical features of venous insufficiency are risk factors for thrombosis. Moreover, restricting the analysis to cases with unprovoked venous thrombosis demonstrated that the risks associated with symptoms of venous insufficiency are not explained by the presence of other major risk factors of thrombosis in the older population, such as recent hospitalization and surgery (Engbers et al, 2014).

Some, but not all, studies have reported that varicose veins are associated with an increased risk of DVT and PE in the young and middle-aged (Goldhaber et al, 1983; Heit et al, 2000; Huerta et al, 2007; Muller-Buhl et al, 2012; Zoller et al, 2014). Heit et al (2000) demonstrated a fourfold increased risk of venous thrombosis associated with varicose veins in middle-age up to age 60 years, but in contrast to the current study, they observed no increased risk in those 70 years and older. This difference in findings may be due to a difference in data collection: Heit et al (2000) used medical records to assess chronic venous insufficiency, whereas we collected data on clinical features of venous insufficiency by an interview. Mild disorders, such as varicose veins, may be documented less often in medical records of older individuals, particularly when other co-morbidities are present, potentially resulting in underestimation of relative risks.

To our knowledge, presence of leg oedema due to chronic venous insufficiency has not been investigated as a risk factor for venous thrombosis in the older population. Leg oedema could be a pre-clinical sign of thrombosis; however, we showed that, among individuals who did not report any thrombotic complaints prior to the diagnosis of venous thrombosis, leg oedema was still associated with a more than twofold increased risk of thrombosis compared with no oedema. This indicates that our findings are probably not explained by leg oedema being a symptom of deep vein thrombosis. Heart failure, as an underlying cause of leg oedema, may explain the association between leg oedema and venous thrombosis; however in our study excluding the individuals with heart failure did not change the results. We hypothesize several mechanisms by which venous insufficiency may increase the risk of venous thrombosis. Firstly, varicose veins, due to dysfunction of the venous valves, lead to low sheer stress in the veins and reduced blood flow, which subsequently lead to a pro-thrombotic state (Brooks et al, 2009; Atta, 2012). It has been hypothesized that damage to the valves results in hypoxia, especially in the valve pockets, which promotes thrombus formation at this location (Van Langevelde et al, 2010). Secondly, inflammation may play an important role. Leg oedema as a result of long-term venous insufficiency could lead to mediator release and inflammation in the veins (Bovill & van der Vliet, 2011; Cushman et al, 2014). Leg ulceration is also associated with local inflammatory processes (Chen & Rogers, 2007). We hypothesize that this inflammatory state could initiate local thrombus formation within the leg veins. A leg ulcer is the most severe expression of venous insufficiency of the three clinical features that we assessed in this study (Porter & Moneta, 1995). In concordance with this, among the venous insufficiency features evaluated, the strongest association with thrombosis risk was found for leg ulcer. Finally, it has been suggested that venous insufficiency in the absence of a prior history of DVT may be due in part to prior undiagnosed DVT, which might increase the risk of future clinically apparent DVT. (Cushman et al, 2010).

In the AT-AGE study we achieved high participation rates, which is challenging in older people (Bugeja et al,1997). As venous thrombosis and venous insufficiency are both associated with immobility, performing home visits to both cases and control subjects minimized selection bias due to selection of only mobile older individuals. Moreover, by performing interviews we were able to determine risk factors that are not reported regularly in medical reports. Limitations of the current study require consideration. Recall bias may have affected our results, although we minimized this by performing standardized interviews, in both the cases and the control subjects. Referral bias could play a role in our results, e.g., if individuals with varicose veins are more likely to be referred for ultrasound of the legs, resulting in an overestimation of the risks. However, as we also found an increased risk for PE with clinical features of venous insufficiency, we do not expect this to be a major influence. As the clinical features of venous insufficiency were all self-reported, over- or under-reporting of the clinical features cannot be ruled out. We would anticipate that the presence of any misclassification would be non-differential in the cases and the control subjects, which may have led to an underestimation of the risks. Ideally, clinical examination of the legs before the thrombotic event should have been performed. The AT-AGE study is a predominantly Caucasian study, thus we were not able to address racial differences in the associations.

In conclusion, clinical features of venous insufficiency(varicose veins, leg oedema, leg ulcer) were found to be risk factors for venous thrombosis in this older population. This gives further insight into the aetiology of venous thrombosis in older people. Physicians may be more alert for thrombosis when one of the clinical features of venous insufficiency is present as these contribute to the burden of venous thrombosis in this older patient group.

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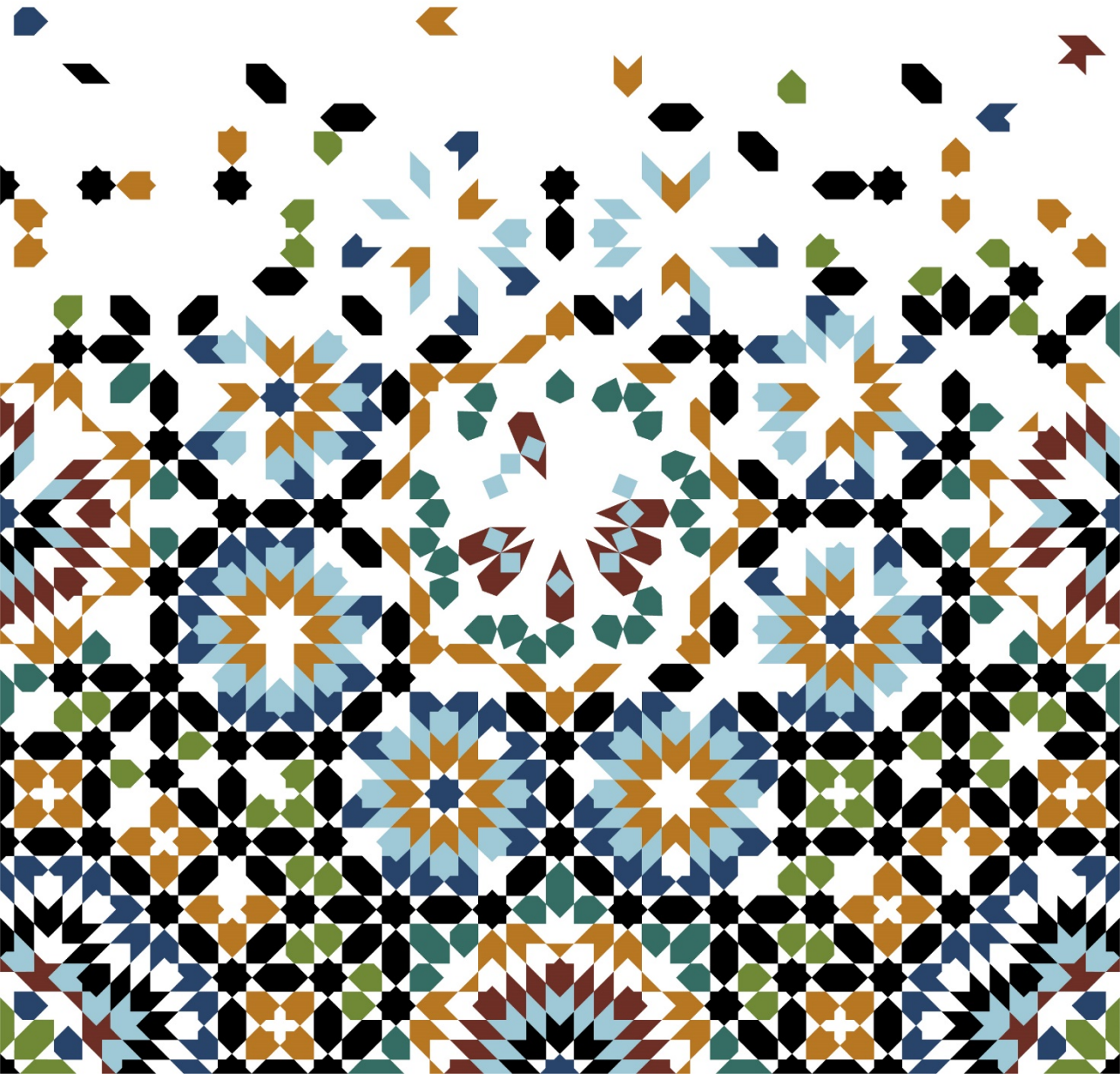
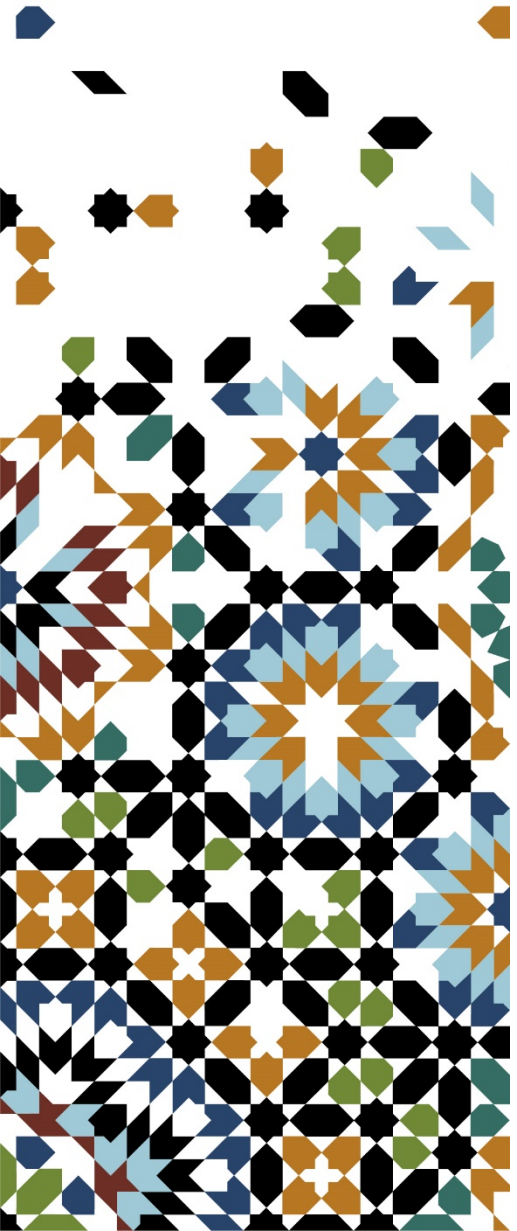


# Chapter 5

## Anatomic distribution of deep vein thrombosis in the elderly



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## Introduction

As early as 1851 Virchow observed a slight left-sided predominance of deep vein thrombosis. He explained this observation by relating it to compression of the left common iliac vein by the right common iliac artery.[1] In the 1950s researchers May and Thurner[2] found that the right iliac artery compressed the left common iliac vein against the fifth lumbar vertebra in 22% of 430 cadavers and that venous webs or spurs had formed at the point where the artery crosses the vein. It was hypothesised that venous spurs might predispose to deep vein thrombosis (DVT) at this location. When DVT occurs during pregnancy, the shift to the left becomes extreme (five times more often than right sided), and this might also be attributable to the compression of the left common iliac vein by the pregnant uterus.[3] However, in the middle aged non-pregnant population, obesity does not appear to cause left common iliac vein compression since there is no left-sided predominance for DVT associated with obesity.[4]

Ageing might affect the veins, vessel wall, and venous valves,[5, 6] and might lead to a change in the pattern of venous thrombi distribution. As the incidence of venous thrombosis increases markedly in the elderly,[7] mapping the distribution of venous thrombi in this age group is important. Therefore, the aim of our study was to examine how venous thrombi are anatomically distributed in patients aged 70 years and older.

## Methods

### *Study design and population*

The Age and Thrombosis: Acquired and Genetic risk factors in the Elderly (AT-AGE) study is a two-center population-based case-control study performed in Leiden and Haarlem, the Netherlands and Burlington, VT, the United States. The design of the AT-AGE has been described in detail previously.[8] All consecutive patients aged 70 years and older with deep venous thrombosis in the leg (DVT) or pulmonary embolism (PE) were identified (N=401). In the Netherlands cases were identified

from the anticoagulation clinic in Haarlem and Leiden and in Vermont from the Vascular Laboratory and the Radiology department the University of Vermont Medical Center (Burlington, Vermont, United States). Individuals with an active malignancy, chemotherapy, or radiation therapy for cancer in 6 months prior to the thrombotic event, individuals with severe psychiatric or cognitive disorders, and individuals who self-reported a previous DVT or PE, were excluded. All eligible cases who agreed to participate, were visited at home to perform an extensive structured interview. The index date was defined as the date of diagnosis of the thrombosis. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center and by the Committee of Human Research of the University of Vermont. All participants provided written informed consent.

*Outcome assessment*

Patients were identified from the anticoagulation clinics in Haarlem and Leiden and from the Vascular Laboratory and the Radiology department of the University of Vermont Medical Center. Presence of DVT required positive compression ultrasonography or Doppler ultrasound. PE was considered definite in case of a positive spiral computerized tomographic or high probability ventilation- perfusion lung scan. For the patients included in the Netherlands, discharge letters from treating physicians were obtained for 309 patients out of a total of 341 patients (90.6%). Information on affected veins was based on data from these discharge letters. For the other patients as well as for the patients included in Vermont, self-reported information on type of thrombosis (deep vein thrombosis or pulmonary embolism) and the side of the deep venous thrombosis of the leg was obtained from the interview. In Vermont no information on affected veins was available.

*Study definitions*

The proportions of left- and right-sided DVT were calculated. Descriptive statistics were used to analyse the anatomical distribution of venous thrombi, with the proximal location defined as involvement of inferior vena cava, iliac, femoral or

popliteal veins, and distal location defined as involvement of calf, tibial or gastrocnemius veins. Provoked DVT was defined as DVT within 3 months after hospitalisation, fracture, plaster cast, splint, or transient immobility at home ≥4 successive days. Body mass index (BMI) was calculated by dividing body weight (kg) by squared height ($m^2$). IBM SPSS Statistics 20.0 for Windows (SPSS Inc, Chicago, Ill) was used for data analysis.

## Results and discussion

401 patients with a first venous thrombosis were included, of whom 166 (41.4%) patients had DVT only, 22 (5.5%) DVT with PE and 213 (53.1%) PE without DVT. Of the 188 patients with a DVT of the lower extremities, 101 patients had a left-sided DVT, 4 patients had a bilateral DVT and for one patient the side of DVT was unknown (Table 1). The predominance of left-sided DVT did not differ between obese individuals (53.5% left) and non-obese individuals (59.4% left), or by whether the DVT was provoked (51.8% left) or idiopathic (56.7% left). There was a clear left-sided predominance within women (61.5% left, 38.5% right), while no clear predominance was observed in men (48.3% left, 51.7% right).

The most often affected vein was the popliteal vein (41%), followed by the femoral vein (39%), calf veins (23%), iliac vein (9%) and inferior vena cava (1%). In men, the popliteal and femoral veins were affected slightly more often than in women: 11% for the popliteal and the femoral vein in men compared with 8% for the popliteal vein and 7% for the femoral vein in women. There were 44 patients (23%) with involvement of both the popliteal vein and femoral vein. Only 18 patients (10%) had isolated calf DVT.

**Table 1** Distribution of risk factors for DVT in patients with left- and right-sided DVT

| | Left leg DVT* | Right leg DVT* |
|---|---|---|
| DVT, n (%, 95% CI) | 101 (55.2, 48.0-62.4) | 82 (44.8, 37.6-52.0) |
| Female sex, n (%) | 59 (58.4) | 37 (45.1) |
| Age, mean (95% CI) | 78.8 (77.6-79.9) | 78.7 (77.4-79.9) |
| BMI, mean (95% CI) | 27.7 (26.7-28.7) | 27.2 (26.3-28.2) |
| BMI ≥30, n† (%) | 23 (23.2) | 20 (27.8) |
| BMI <30, n (%) | 76 (76.8) | 52 (72.2) |
| Provoking factors, n (%) | 29 (28.7) | 27 (32.9) |
| Fracture, n left/right | 4/1 | 2/4 |
| Plaster cast, n left/right | 3/1 | 0/2 |
| Proximal vein involvement, n (%) | 57 (56.4) | 50 (61.0) |
| Distal vein involvement, n (%) | 26 (25.7) | 15 (18.3) |

**Bilateral DVT's were excluded*
*†BMI missing for 12 individuals*

The results of our study are consistent with those of previous studies conducted in younger individuals showing a predominance of left-sided DVT.[4, 9, 10] As in other studies, the popliteal and femoral veins were the most prevalent sites of thrombi.[11, 12] In our study, single occlusion of veins other than the calf vein was not uncommon (42 individuals, 22%). This was in contrast to a finding in another study, in which single occlusion of veins was unusual.[11] A limitation of our study is that we have not quantified abdominal fat with imaging techniques.

We conclude that in the elderly with DVT, there is a left-sided predominance of DVT and this observation did not vary between sexes, by age, BMI, provoking factors prior to the thrombotic event, or proximal and distal involvements of veins.

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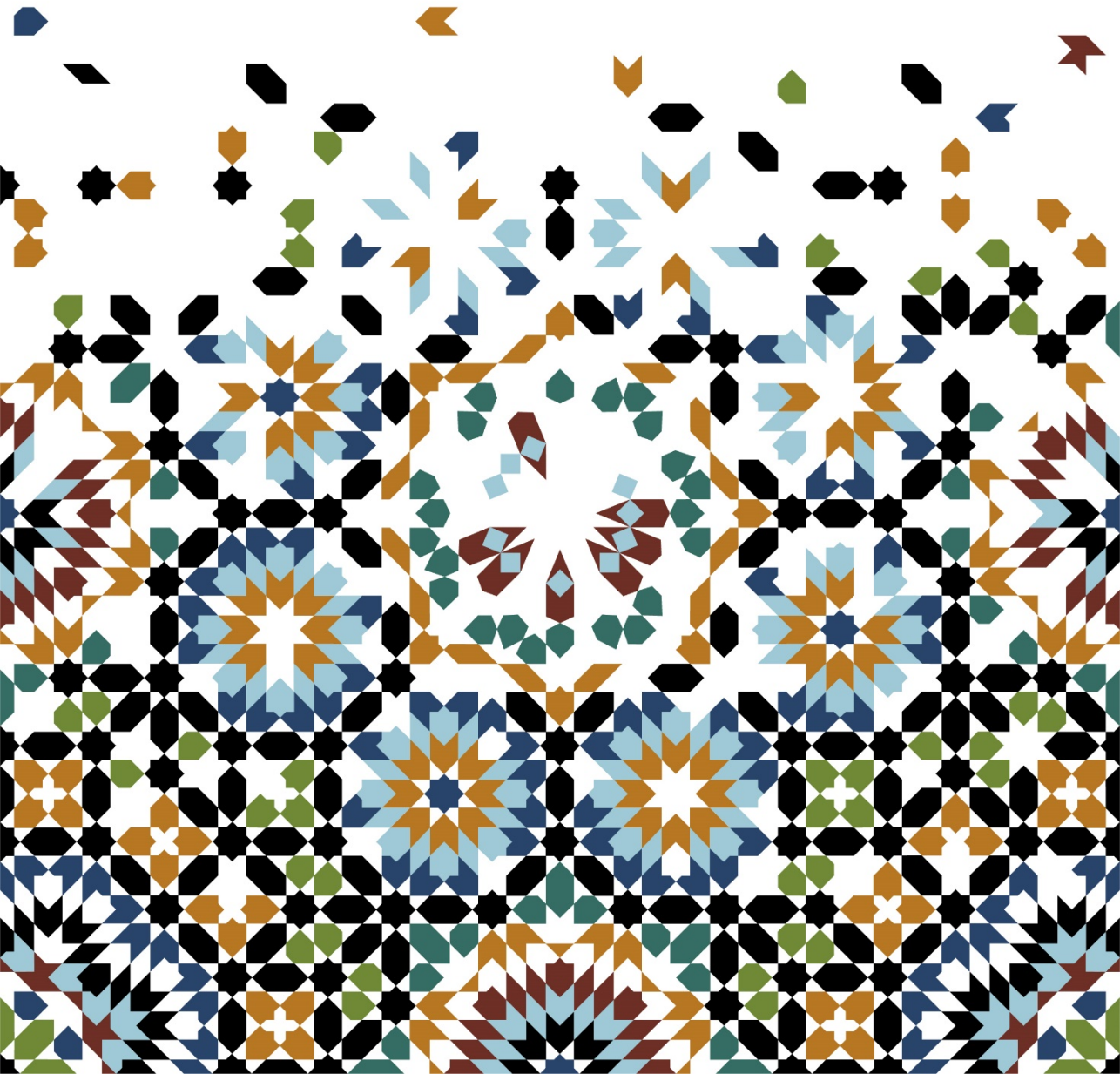
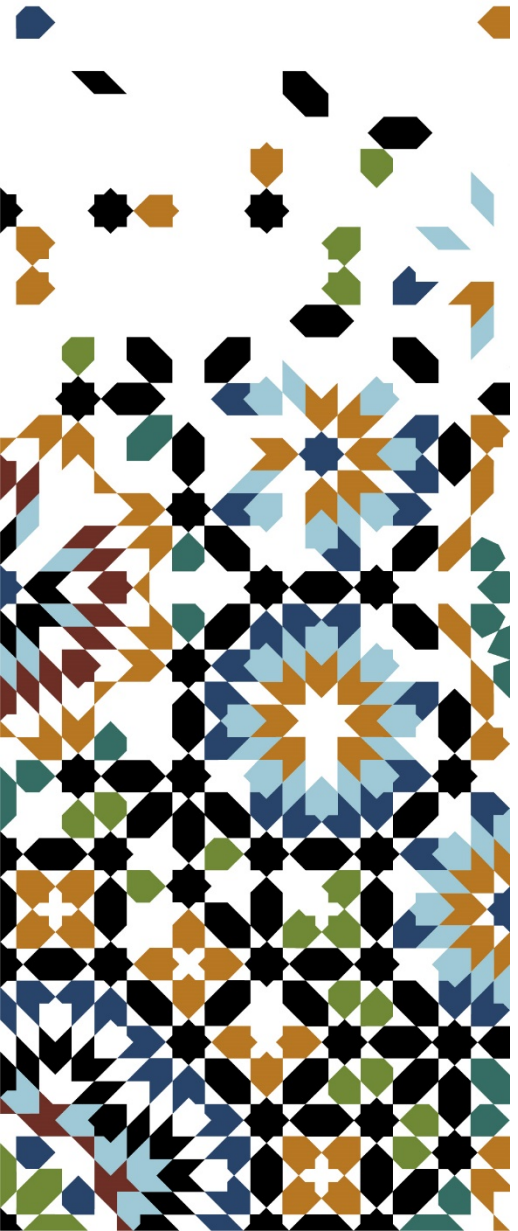


# Chapter 6

## Ageing of the venous valves as a new risk factor for venous thrombosis in the elderly – the BATAVIA study



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## Abstract

*Background* Increasing age is the strongest risk factor for venous thrombosis (VT). Increasing age has been related to a thickening of the venous valves and a decreased valvular function. The association between valve thickness and the risk of VT is not known.

*Objectives* To assess the association between increased valve thickness and valve closure time (VCT) and the risk of VT.

*Methods* Analyses were performed in the BATAVIA study, including 70 cases aged 70+ with a first VT and 96 controls. We performed an ultrasound examination of the valves in the popliteal veins. The valves were imaged with a 9 MHz linear probe using B-mode ultrasonography. VCT was measured as an indicator for valve function using an automatic inflatable cuff. To estimate the risk of VT, valve thickness was dichotomized at the 90$^{th}$ percentile as measured in controls and VCT was dichotomized at 1 s.

*Results* Mean valve thickness of controls was similar in the left (0.36 mm, 95%CI 0.34-0.37) and right (0.36 mm, 95%CI 0.35-0.38) leg. In 45 cases a valve was observed in the contralateral leg with a mean valve thickness of 0.39 mm (95%CI 0.36-0.42). Cases had an increased valve thickness compared with controls: mean difference 0.028 mm (95%CI 0.001-0.055). Valve thickness >90$^{th}$ percentile increased the risk of VT 2.9-fold. Mean VCT in controls was 0.38s, in contralateral leg of cases 0.58s. VCT>1 s increased the risk of VT 2.8-fold (95%CI 0.8-10.4).

*Conclusions* Risk of VT was associated with increased valve thickness and valvular reflux of >1s.

## Introduction

Several studies indicate that the majority of venous thrombi originate in the valve pockets. The valve pocket is the space between valve cusp and the vessel wall. In the deeper parts of the valve pockets, flow circulates at low velocity, creating two counter-rotating vortices[1], and becomes increasingly hypoxic.[2] Hypoxia leads to endothelial damage, creating a hypercoagulable environment.[3, 4]

Age-related changes of venous valves have been described in several ex vivo studies and in one in vivo study. The ex vivo studies demonstrated a thickening of the valves with age in renal[5] and in femoral veins[6]. The thickening of the valves in these studies was shown to be the result of atrophy of muscle fibers, hypertrophy of elastic fibers, and an increased number of collagen fiber bundles. We previously demonstrated that in vivo visualization of venous valves and measurement of valve thickness by ultrasound is possible and reproducible. It was shown that ageing is associated with thicker valves, as measured by ultrasound.[7] Valvular reflux can proceed from venous thrombosis (VT), leading to the post-thrombotic syndrome (PTS).[8, 9] However, reflux can also lead to an increased risk of VT by increasing stasis and subsequent local hypoxia, thereby contributing to a hypercoagulable environment.

VT is rare in young individuals (<1 per 10 000 per year) but increases to approximately 1% per year in old age.[10] As the incidence of VT increases strongly with age and the worldwide population of the elderly grows,[11] the focus on the aetiology and prevention of disease in the elderly becomes more relevant.

Ageing of venous valves, i.e. thickening of the valves and a decrease in valvular function has not been studied in relation to VT.

Therefore, the aim of our study was threefold: 1) to study the association between thickened valves in the popliteal vein and the risk of VT; 2) to study the association between valvular function, as measured by the valve closure time (VCT) and the

risk of VT, and 3) to study the association between valve thickness and valvular function.

## Methods

*Study participants*

The Biology of Ageing and Thrombosis: Appraisal of Valve thickness and function, an In Vivo Assessment (BATAVIA) Study is a population-based case-control study in Leiden, the Netherlands. Patients with deep venous thrombosis of the leg with or without pulmonary embolism, who were included in the AT AGE study, a case-control study on risk factors for venous thrombosis in the elderly, were invited to participate in the BATAVIA study.[12] In the AT AGE study, consecutive elderly patients (older than 70 years) who had a first deep vein thrombosis (DVT) with or without pulmonary embolism (PE) from June 2008 to July 2011 were identified at two regional Anticoagulation Clinics in Leiden and Haarlem as part of a large multicentre study on risk factors for venous thrombosis in the elderly. Diagnosis of DVT was objectively confirmed by compression ultrasonography or duplex ultrasound. Information on affected veins was based on the discharge letters collected from hospitals. Control subjects were identified in the same geographic area as the cases. Control subjects were randomly selected from five primary care practices in Leiden. Individuals with a history of VT, present or recent malignant disease (i.e. diagnosis of cancer, chemotherapy or radiation therapy for cancer within six months of VT diagnosis or date of telephone call for the control subjects), severe psychiatric or cognitive disorder were excluded. For the BATAVIA study, for practical reasons, individuals living in nursing homes were excluded.

Of the 160 identified cases from the ATE AGE study, 104 were eligible to participate in the BATAVIA study. To increase the sample size, additional patients with same inclusion and exclusion criteria were selected from the anticoagulation clinic in Leiden. Patients with a first DVT between August 2011 and July 2013 (n=61) were identified. Of these 61 newly identified cases, 32 were eligible. Of a total of 221 eligible cases, 136 (62%) were invited to participate in the BATAVIA

study (Figure 1). Reasons for not being invited were the following: 29 (34%) died before inclusion in the BATAVIA study was possible, 28 (11%) had developed a cognitive or psychiatric disorder or were currently living in nursing homes, 2 (2%) had active malignancy, and 30 (35%) had a prior history of VT. Of the 289 identified control subjects, 215 (74%) could be invited and of the 215 invited control subjects, 97 (45%) participated. Of those who could not be invited, 52 (70%) died before inclusion was possible, 15 (20%) had developed a cognitive or psychiatric disorder and were currently living in nursing homes, 2 (3%) had an active malignancy, and 5 (7%) had a history of VT. In the end 70 cases and 96 control subjects participated in our study.

All participants gave written informed consent. The study was approved by the Medical Ethics committee of the Leiden University Medical Center, Leiden, the Netherlands.

*Data collection*

All identified cases and control subjects received an invitation letter by mail, followed by a telephone call to discuss participation. Home visits for an extensive structured interview and LUMC visits for the ultrasound examinations were scheduled for all eligible cases and control subjects.

The interview included questions on presence of varicose veins, use of plaster cast of lower extremities, and surgery of lower extremities both at the time of the index date and ever in their lives. The index date was defined as the date of the diagnosis of the VT for the cases and the date of the interview for the control subjects. Physical measurements were performed including weight and height. Body mass index (BMI) was calculated by dividing body weight (kg) by height squared ($m^2$).

Ultrasounds examinations were performed using an adjustable examination table, set to an inclination of 45° anti-Trendelenburg, as described previously.[7] The incline was used to attain optimal filling of the venous system. A number of

participants were not able to stand on the examination table (n=18), they were asked to sit on the table, legs stretched. Imaging of the valves of the popliteal vein in both legs was done using 9-MHz linear probe using B-mode ultrasonography (z.one; Zonare® Medical Systems, Inc., Mountain View, CA, USA). In both legs, the transducer was first placed transversely in the popliteal fossa. Compression of the popliteal vein was done to confirm the absence of thrombosis. Then, the transducer was placed longitudinally following the vein over 10 to 12 centimeters in search of valves. When valves were visualized, multiple images were saved for offline processing.

Valvular function was assessed using an automatic inflatable cuff (Hokanson rapid cuff inflator, Bellevue, Wash). Time of reversed flow following valve closure was measured. We achieved valve closure by applying standardized calf compression of 100 mm Hg with the inflatable cuff. Valve closure time (VCT) is a validated method to discriminate between normal and incompetent venous valves.[13]

Valve thickness was measured offline using a dedicated in-house developed software program (VesselMass, Leiden University Medical Center, Leiden) [14]. For every participant, the presence or absence of valves in both legs was documented. Images of valve leaflets which had the best visibility, and sharpest contours and a clear attachment to the venous vessel wall, i.e., the so called valvular agger, were selected for further measurements. To standardize the measurement of the valve leaflet and because the attachment of the valve leaflet to the vessel wall is not always visible in the imaging procedure, we used a fixed measuring distance of the valve starting 2 mm from the vessel wall using the method previously described by van Langevelde et al. Figure 2 shows a schematic drawing of the valve measurement.

All ultrasonography examinations and offline measurements were done by one of the researchers (AK). The ultrasonography images were stored anonymously, and therefore the measurements were done without prior knowledge on whether individuals were case or control subject.

Blood samples were drawn into vacuum tubes containing 0.1-volume 0.106-mol/L trisodium citrate or when no blood sample could be drawn a buccal swab was collected. The blood sample was separated into plasma and cells through centrifugation. DNA analysis for the factor V Leiden mutation (rs6025) and the prothrombin G20210A mutation (rs1799963) was performed using a combined polymerase chain reaction method with the TaqMan assay. The 20146G/- (rs8176719), 21463C/G (rs7853989), 21867A/G (rs8176749), and 21996C/- (rs8176750) blood group polymorphisms were determined by a 5'nuclease assay (Taqman; Applied Biosystems, Foster City, CA, USA) using a PCR reaction mix (Taqman Genotyping Master Mix, Applied Biosystems) and an allele-specific fluorescent probe equipped with a minor groove binding moiety (Applied Biosystems).

*Statistical analysis*

Determinants of valve thickness were assessed in the control subjects as reflecting the general population of this age. Determinants were established by comparing means and using linear regression. Mean thickness of the imaged valve in the left and right leg in the control subjects was calculated separately. Since there was no difference between the left and right leg (mean difference 0.009 mm, 95% CI -0.016-0.034), we combined the thickness from both legs and calculated a mean valve thickness in control subjects. The mean value of both legs was used in all further analyses.

We studied the association between VT and an increased valve thickness in the total study population. The valves in the ipsilateral leg of the patients are often damaged by the thrombosis and not likely to provide a reliable assessment. Therefore, as an assessment of the ipsilateral leg, the contralateral leg was used for all analyses in patients. To assess whether the valve thickness in the contralateral leg was representative for the valve thickness in the ipsilateral leg, we compared the valve thickness in the ipsi- and contralateral leg in patients where imaging of the ipsilateral leg was possible using a paired t-test. To analyse whether

increased valve thickness increases the risk of VT, valve thickness was stratified at the 90$^{th}$ percentile as measured in control subjects. To study a dose-response relation, valve thickness was further stratified into quartiles. The risk of VT was assessed by calculating odds ratios (OR) with corresponding 95% confidence intervals (95% CI) after adjustment for age (continuous) and sex (dichotomous).

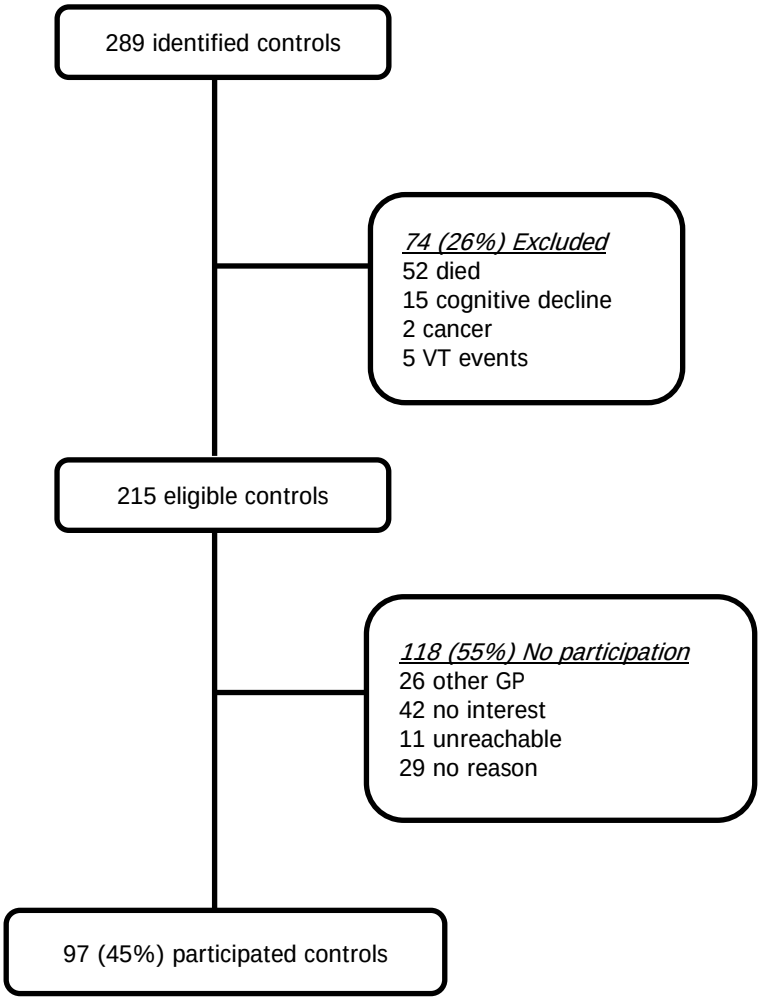
We analysed the association between VCT and VT. As the cut-off value for reflux in deep veins is defined as a VCT>1 s,[15] we stratified the VCT at 1 s and calculated the risk of VT associated with prolonged VCT. To study the relation between VCT and valve thickness, linear regression was used.

In patients where we were able to visualise the venous valve in the ipsilateral leg, we mapped the distribution of thrombi in the ipsilateral legs. Descriptive statistics have been used to explore the anatomical distribution of venous thrombi.

Two reproducibility studies were conducted to test our measurement techniques. In the first study, from 14 participants valve thickness of the same image from the saved ultrasound examination was measured again by the same researcher without knowledge of the outcome of the first measurement. As such, we measured the reproducibility of the program VesselMass. In the second study, 11 participants underwent a second ultrasound examination to test the reproducibility of the operator. Two Bland-Altman plots were made to visualize the results. In the first Bland-Altman plot the difference in valve thickness between measuring the same image twice with VesselMass (depicted on the y axis) and the mean valve thickness of the 2 measurements (depicted on the x axis) was shown. In the second Bland-Altman plot the difference in valve thickness between the first and second ultrasound examination (depicted on the y axis) and the mean valve thickness of the two measurements (also depicted on the x axis) was shown. Using the reproducibility method described by Bland and Altman, the 95% limits of agreement was calculated for repeated measurements. 95% of differences between the first and second measurement are expected to be less than 1.96 SD from the mean difference.[16]

All analyses were performed using IBM SPSS Statistics 20.0 for Windows (SPSS Inc, Chicago, Ill).

**Figure 1** Flowchart of the Batavia study.



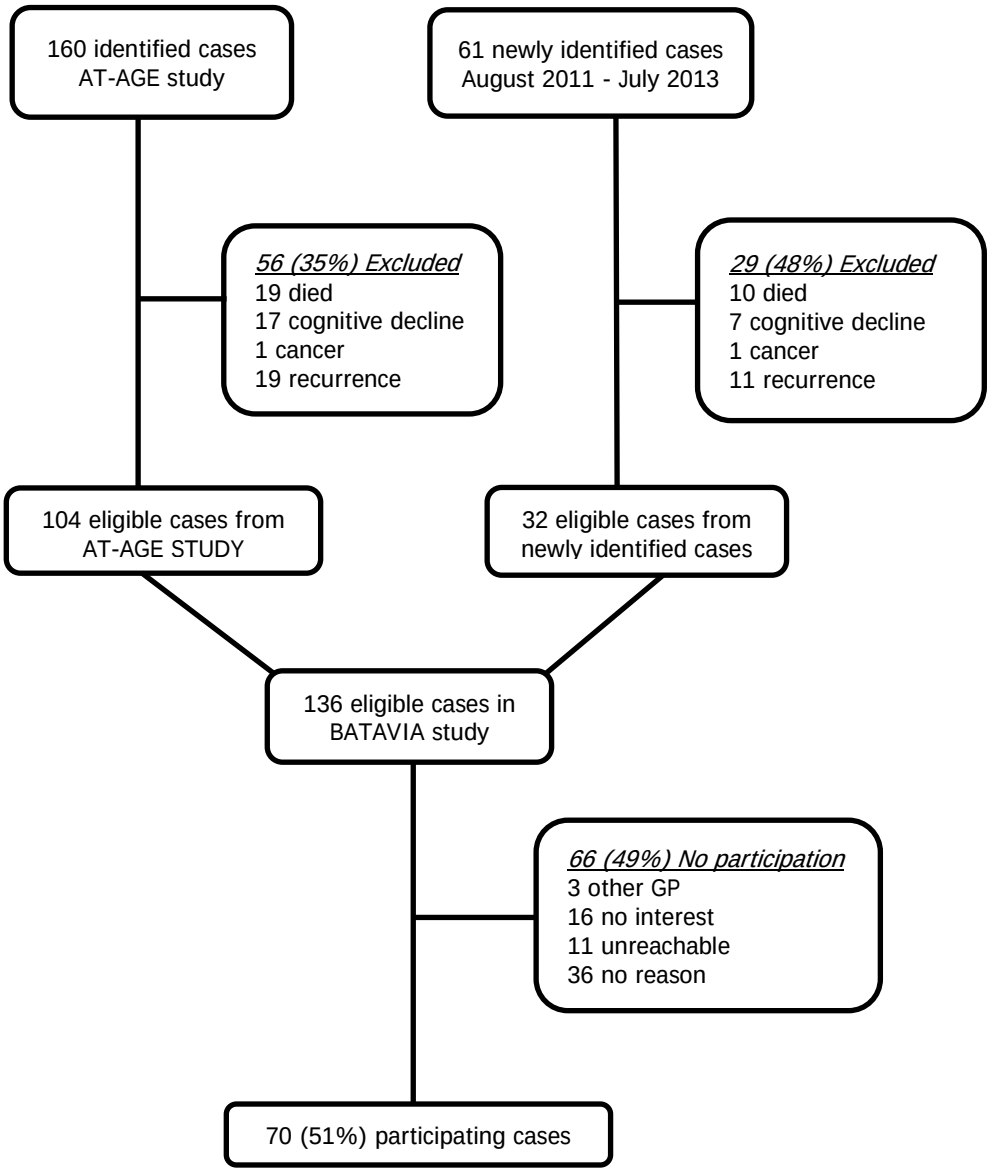
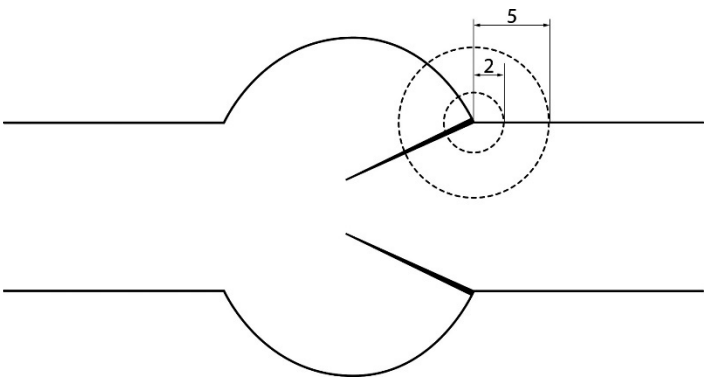

160 identified cases
AT-AGE study
61 newly identified cases
August 2011 - July 2013
56 (35%) Excluded
19 died
17 cognitive decline
1 cancer
19 recurrence
29 (48%) Excluded
10 died
7 cognitive decline
1 cancer
11 recurrence
104 eligible cases from
AT-AGE STUDY
32 eligible cases from
newly identified cases
136 eligible cases in
BATAVIA study
66 (49%) No participation
3 other GP
16 no interest
11 unreachable
36 no reason
70 (51%) participating cases

**Figure 2** Schematic drawing of the valve measurement. Adapted from van Langevelde et al [7]. To standardize the measurements, the distance between the 2 circles, which corresponds with 3 mm, was generated by VesselMass. Valve thickness over these 3 mm was measured.




## Results

General characteristics of the cases and control subjects are shown in Table 1. There were 31 (44%) women cases and 50 (52%) women control subjects. Mean age of cases and control subjects was 76 years. Among the 70 cases who participated, 69 (99%) underwent ultrasound examination and 69 (99%) completed the questionnaire; whereas all of the 96 participating controls (100%) completed both the ultrasound examination and questionnaire. Ultrasound examination took place after a mean duration of 31.9 months after VT diagnosis (range: 4-54 months). In 68 (97%) cases the side of the DVT was known. In the cases, in 45 (68%) individuals a valve was observed in the contralateral leg, and in 34 (51%) in the ipsilateral leg. In the control subjects in 75 (78%) individuals a valve was observed in the left leg, and in 62 (65%) in the right leg.

**Table 1** Study characteristics

| | Controls<br>N = 96 | Cases<br>N = 70 |
|---|---|---|
| Female, n (%) | 50 (52) | 31 (44) |
| Age, mean (95% CI) | 76.0 (75.1-77.0) | 76.1 (75.2-77.1) |
| BMI, mean (kg $m^{-2}$)† (range) | 26.4 (18.3-38.5) | 26.7 (19.6-38.1) |
| Lower extremity surgery | | |
| Ever, n (%) | 34 (35.4) | 30 (42.9) |
| 3 months prior to VT, n (%) | 1 (1.0) | 9 (12.9) |
| Fracture, n (%)* | 0 (0) | 4 (5.7) |
| Varicose veins, n (%) | 28 (29.2) | 24 (34.3) |
| Valve thickness, mean (mm) (95% CI) | | |
| Right leg | 0.36 (0.35-0.38) | |
| Left leg | 0.36 (0.34-0.37) | |
| Contralateral leg | | 0.39 (0.36-0.42) |
| VCT, mean (s) (range) | | |
| Right leg | 0.4 (0.1-5.3) | |
| Left leg | 0.4 (0.2-3.8) | |
| Contralateral leg | | 0.6 (0.1-5.6) |

**Three months prior to VT*
*†3 missings for BMI*

**Table 2** Valve thickness in healthy control subjects

| Control subjects | N = 96 | Mean valve thickness (95% CI) |
|---|---|---|
| Valve present, n (%) | 87* (91) | 0.36 (0.35-0.37) |
| Subgroup | | |
| Men | 44 | 0.37 (0.35-0.39) |
| Women | 43 | 0.35 (0.33-0.37) |
| Age | | |
| 70-75 | 44 | 0.35 (0.34-0.37) |
| 75-80 | 23 | 0.35 (0.33-0.38) |
| ≥80 | 20 | 0.38 (0.33-0.43) |
| BMI | | |
| ≤27 | 53 | 0.37 (0.35-0.39) |
| >27 | 33 | 0.35 (0.33-0.37) |
| Factor V Leiden | 4 | 0.41 (0.15-0.67) |
| Prothrombin 20210A mutation | 3 | 0.35 (0.33-0.38) |

** In 9 subjects no valve was present in either leg*

*Risk of VT associated with increased valve thickness*

Table 2 describes the mean valve thickness in different subgroups of control subjects. In 50 control subjects a valve was observed in both legs and there was no difference in valve thickness between the left (0.36 mm, 95% CI 0.34-0.37) and right (0.36 mm, 95% CI 0.35-0.38) leg in these control subjects. We found no difference in valve thickness between men and women, neither between a low (≤27) and high BMI (>27). Individuals below age of 80 had a mean valve thickness of 0.35 mm (95% CI 0.34-0.37), whereas those aged ≥80 years had a mean valve thickness of 0.38 mm (0.33-0.43), resulting in a mean difference of 0.029 mm (95% CI -0.004-0.061). Mean valve thickness in the contralateral leg of the cases was 0.39 mm (95% CI 0.36-0.42), leading to a mean difference of valve thickness between cases and control subjects of 0.028 mm (95% CI 0.001-0.055). This is a 8% difference between cases and control subjects. Valve thickness above the 90$^{th}$ percentile as measured in control subjects (>0.44 mm), was associated

with a 2.9-fold increased risk of VT (95% CI 1.0-8.0) compared with valve thickness ≤ 90$^{th}$ percentile. To study whether there was a dose-response relation between valve thickness and the risk of VT, we stratified valve thickness into quartiles. Using the first (lowest) quartile as the reference category, the OR remained similar for valve thicknesses in the second and third quartile with an OR of 0.9 (95% CI 0.3-2.7) and 0.7 (95% CI 0.2-2.3), respectively. The highest risk estimate was found in the fourth quartile (≥0.39): OR 2.3 (95% CI 0.8-6.5, Table 3).

**Table 3** Odds ratios of VT with valve thickness stratified into quartiles

| Percentile | Valve thickness at cut-off, mm | Controls, n | Cases, n | OR* (95% CI) |
|---|---|---|---|---|
| < 25 | < 0.32 | 19 | 8 | 1 (ref) |
| 25-50 | 0.32 – 0.35 | 24 | 9 | 0.9 (0.3-2.7) |
| 50-75 | 0.35 – 0.39 | 23 | 7 | 0.7 (0.2-2.3) |
| ≥ 75 | ≥ 0.39 | 21 | 21 | 2.3 (0.8-6.5) |

**adjusted for age and sex*

*Risk of VT associated with prolonged valvular reflux*

Mean VCT in the right leg of control subjects was 0.39 s (95% CI 0.26-0.51), in the left leg was 0.37 s (95% CI 0.26-0.48), generating a mean VCT in control subjects of 0.38 s (95% CI 0.30-0.46). Mean VCT in the contralateral leg of the cases was 0.58 s (95% CI 0.32-0.84). We stratified the VCT at 1 s, and 4 control subjects and 7 cases had a VCT ≥1 s, indicating that an VCT >1 s was associated with a 2.8 fold increased risk of VT compared with a VCT ≤1 s (OR: 2.8; 95% CI 0.8-10.4).Valve thickness was associated with VCT, i.e. for every 0.1 mm increase of valve thickness, VCT increased by 0.207 s (regression coefficient: 0.207).

*Characteristics of ipsilateral legs in cases*

In 19 patients a valve was observed in both legs and no difference was found between the contra- and ipsilateral leg in these individuals (mean difference of 0.015, 95% CI -0.027-0.058). Of the 68 cases in whom the side of DVT is known, in 32 (47%) individuals no valve was observed in the popliteal fossa of the affected leg. Involvement of veins was studied in these 32 cases (Table 4), showing vena poplitea involvement in 13 cases, vena femoralis involvement in 11 cases, vena iliaca involvement in 3 cases and calf veins involvement in 7 cases. Overlap existed between vein involvements: 1 individual had involvement of both calf veins and vena poplitea, 3 individuals had involvement of calf veins, vena poplitea and vena femoralis, 1 individual had involvement of calf veins, vena poplitea, vena femoralis, and vena iliaca, 3 individuals had involvement of both vena poplitea and vena femoralis, and 2 individuals had involvement of vena poplitea, vena femoralis, and vena iliaca. Of 32 ipsilateral legs in which no valve was observed, the vena poplitea was the most often affected vein (n=13, 41%). In 11 (46%) individuals with an affected popliteal vein (n=24), a valve was observed. Furthermore, in those whose popliteal vein was not affected (n=42), a valve was observed in 23 (55%) individuals.

**Table 4** Characteristics of ipsilateral legs in cases

| | Ipsilateral legs<br>N = 68 |
|---|---|
| Valves absent, n (%) | 32 (47) |
| Vein involvement known, n (%) | 41 (60) |
| Thrombus involvement of ipsilateral legs in absent of valves, n*: | |
| Vein involvement known, n (%) | 17 (53) |
| Vena poplitea | 13 |
| Vena femoralis | 11 |
| Vena iliaca | 3 |
| Calf vein | 7 |
| Vein combinations involved, n | |
| Popliteal + femoral + iliac + calf veins | 1 |
| Popliteal + femoral + calf veins | 3 |
| Popliteal + femoral + iliac | 2 |
| Popliteal + femoral | 3 |
| Popliteal + calf veins | 1 |

*Reproducibility studies*

To assess the reproducibility of the software VesselMass, we included 14 images of 14 participants and measured the valve thickness twice. The mean of the difference between the 2 measurements was 0.0143 mm (SD 0.06223). The 95% limits of agreement were -0.1077 to 0.1363, visualized by a Bland-Altman plot (Figure 3A). All of our measurements were within the 95% limits of agreement, except for one outlier.

To assess the reproducibility of the operator, 11 participants underwent an ultrasound examination a second time. The mean of the difference between the 2 measurements was 0.0045 mm (SD 0.07216) with 95% limits of agreement of -0.1369 to 0.1459, also visualized by a Bland-Altman plot (Figure 3B).

**Figure 3A** Bland-Altman plot of reproducibility of the software VesselMass. Valve thickness measurements were repeated for 14 images. The middle dashed line represents the mean difference in valve thickness. The upper and lower dashed lines represent the 95% limits of agreement.

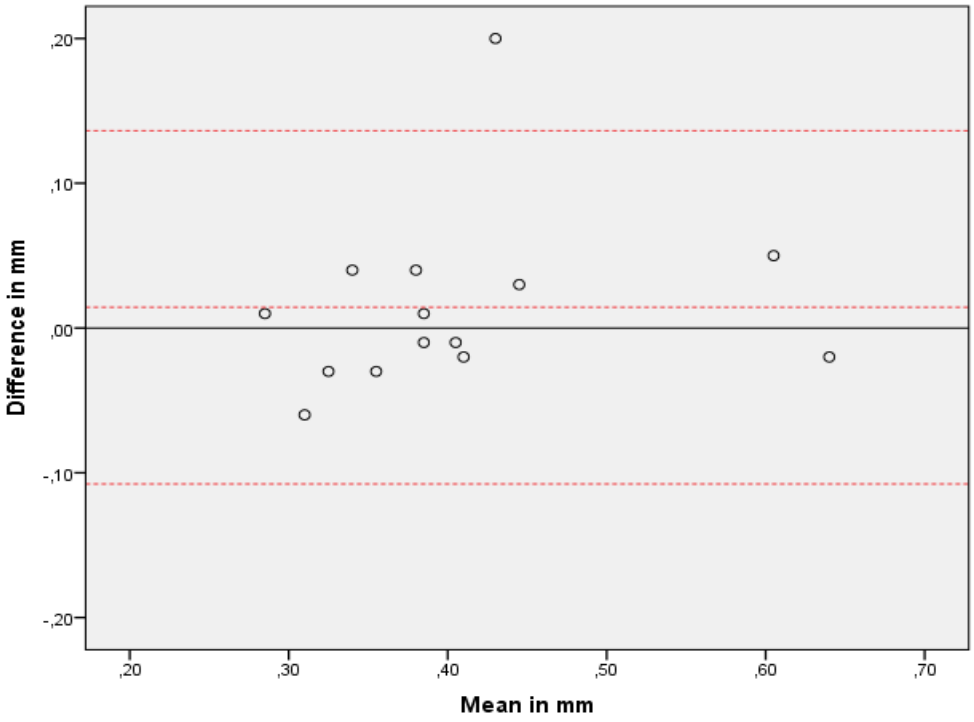
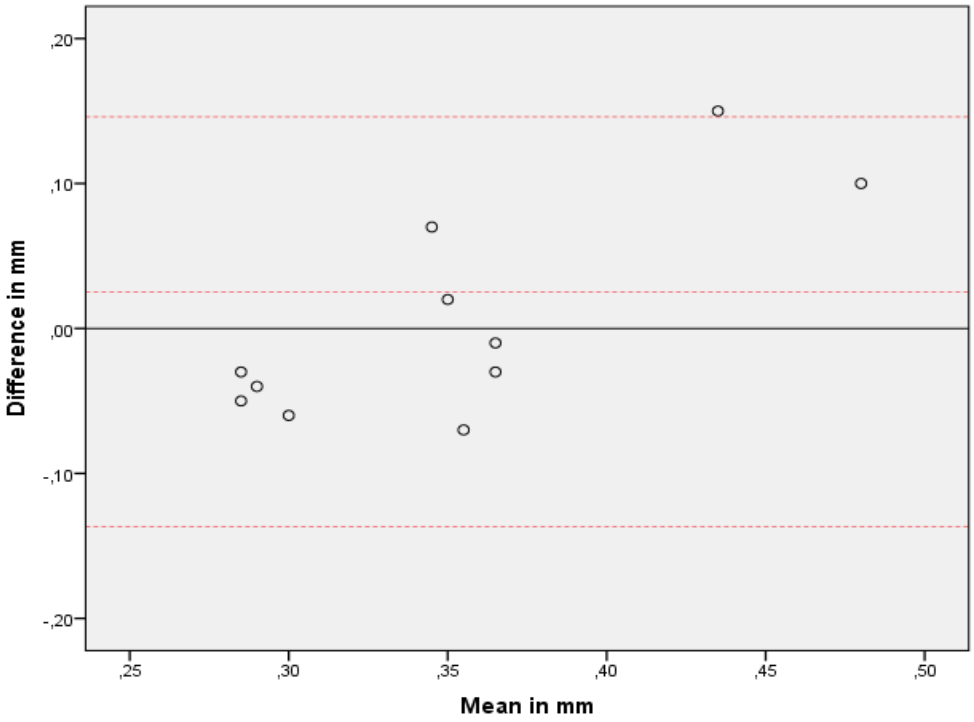


**Figure 3B** Bland-Altman plot of reproducibility of the operator. Valve thickness measurements were repeated for 11 participants. The middle dashed line represents the mean difference in valve thickness. The upper and lower dashed lines represent the 95% limits of agreement.



## Discussion

In this population-based case-control study among individuals aged 70 years and older, valve thickness of the popliteal vein above the 90$^{th}$ percentile, as measured in the control group, was associated with a 2.9-fold increased risk of VT. No clear dose-response effect was observed in relation to increased valve thickness, but those in the highest quartile of valve thickness had 2.3-fold increased risk of VT (OR 2.3, 95% CI 0.8-6.5) compared with those in the lowest quartile. Valvular reflux, as measured by the valve closure time, was associated with a 2.8-fold increased risk of VT. Furthermore, a relation between valve closure time and valve thickness was found. For every 0.1 mm increase of valve thickness, VCT increased by 0.2 s.

This is the first study to assess the risk of VT in relation to valve thickness and valve closure time in vivo in an elderly population. Previous studies were either human autopsy studies,[5, 6] or canine studies,[1] or not related to venous thrombotic disease[7].

Thickening of the valve leaflets has been assigned to increase of collagen fibre bundles in cadaver studies including individuals ranging in age from 1 day to 84 years.[5] Several studies have shown that a decreased compliance of vessel walls is also related to ageing.[17, 18] Together, they can disturb the normal blood flow and enhance stasis in the valve sinus, contributing to the development of thrombosis. Supporting this hypothesis is a previous study in which by using venography it was demonstrated that with increasing age valvular stasis progresses.[19] Also, a relation was found between frequency of VT and the number of valves in individuals.[20] The valvular sinus endothelium adapts to the hypercoagulable state by higher expression of protein C receptor (EPCR) and thrombomodulin (TM) and lower expression of von Willebrand factor (vWF), creating a thromboresistant phenotype compared to the luminal endothelium, as demonstrated in two studies.[21, 22]

In almost half of all ipsilateral legs, no valve was detected during the ultrasound examination (47%). We've mapped the vein involvements in the legs with absent veins, and noted that in most cases (41%), the vena poplitea was involved. This might be related to the mechanical destruction of potential present valves due to the prior thrombus. A valve was more often observed when the popliteal vein was not affected (55%) than when the popliteal vein was affected (46%), however due to the limited power in these subgroups definite conclusions should be cautiously made.

Our results regarding the reproducibility of the measurement software and operator showed that the reproducibility was good, as the mean difference between the measurements were small (0.0143 mm and 0.0045 mm) and the narrow limits of agreement are, in our opinion, an acceptable error level.

In our study, the thickest valves were found in those aged ≥80 years, in line with previous research.[7] Other than age, no other determinants were identified for valve thickness. It is currently unclear whether the process of ageing of the venous valves is confined to the location of the thrombosis or whether it is a generic process and therefore it is measurable in veins elsewhere in the body, e.g., in the jugular veins. The fact that there was no difference in valve thickness between the ipsi- and contralateral legs of patients supports the hypothesis that thickening of valves is a generic process.

The strengths of our study are the in vivo nature, the non-invasiveness of ultrasound, the specific attention to the elderly, and the ultrasonography information on both the contra- and ipsilateral leg. To our knowledge, presence of valves in contra- and ipsilateral legs have not been studied before.

Some aspects of our study warrant comment. First, quality of ultrasonography examinations is directly related to operator skill, training, and experience.[23] To minimize inter-individual bias all examinations were performed by one operator (AK). Second, in only 64% of contralateral legs of the cases, 46% of left legs and

39% of right legs of the control subjects we observed a valve in the popliteal fossa. Although this is not likely to substantially affect our data, the applicability and utility of this technique will be limited in clinical situations. In future studies, it may be beneficial to include the measurement of venous valves in multiple veins, e.g., including the vena femoralis to increase the number of potentially measurable valves. Third, due to the small number of participants, particularly when analysed in subgroups of valve thickness, the confidence intervals are wide indicating that larger studies to confirm our findings are warranted. Finally, valves in the contralateral legs in patients may not be a good representation for the valves in the ipsilateral legs. However, this is unlikely since no difference was observed between the valve thickness measured in the right and left leg of control subjects. Furthermore, in cases where valve thickness in both the ipsi- and contralateral leg could be measured, again, no difference was observed indicating that measuring the contralateral leg provides a good estimation of the valve thickness in the ipsilateral leg but increases the power of the study.

To conclude, our study demonstrates an increased risk of VT associated with increased valve thickness, and increased valvular reflux of >1 s. Furthermore, thickening in venous valves is associated with a decrease in valve function.

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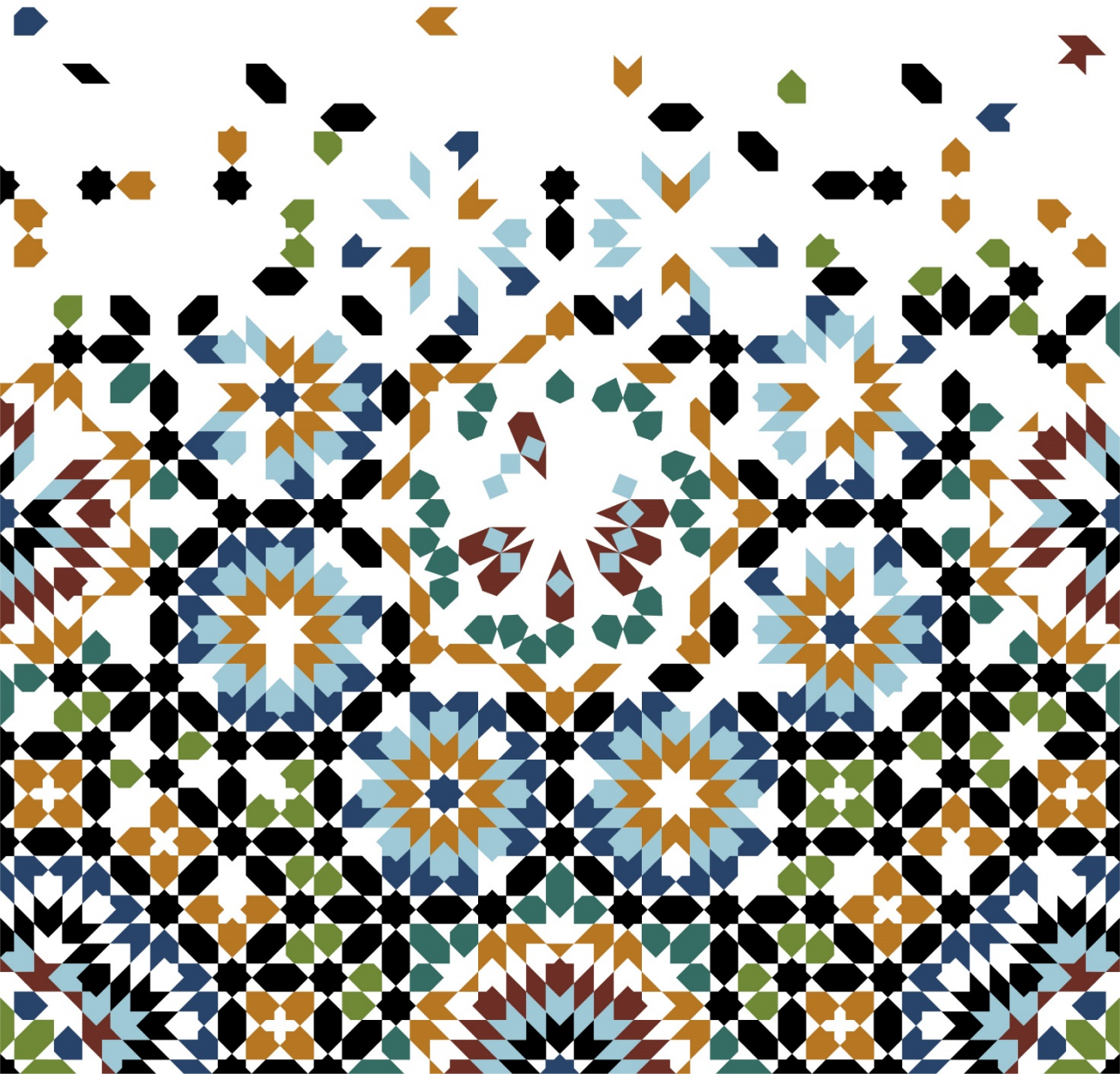
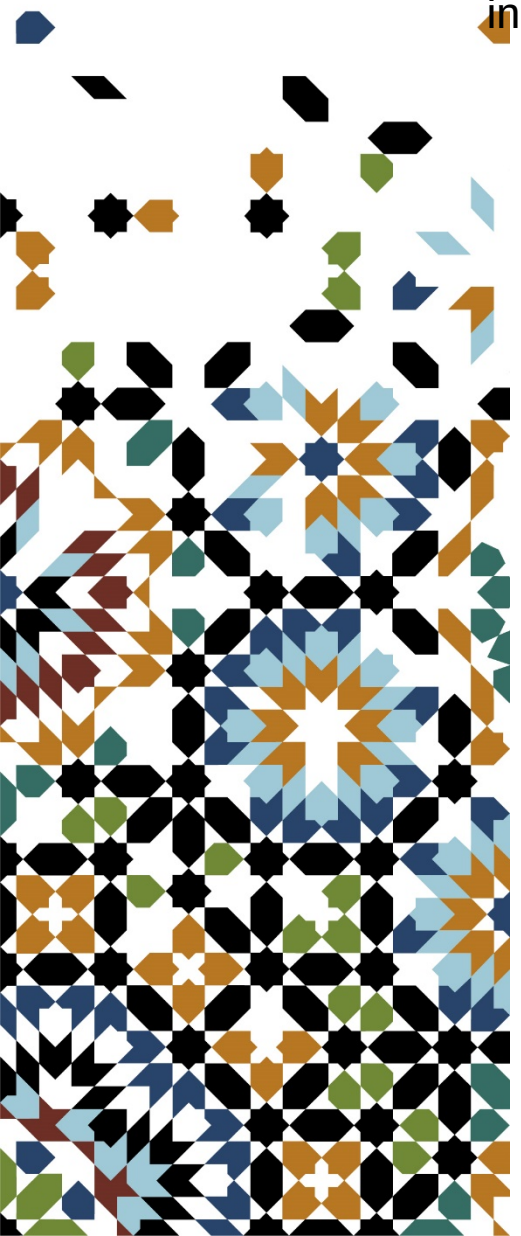


# Chapter 7

## Prolonged clot lysis time increases the risk of a first but not of recurrent venous thrombosis



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## Abstract

The role of the fibrinolytic system in the development of venous thrombosis (VT) is unclear. We studied the risk of first and recurrent VT associated with reduced fibrinolysis, as measured by clot lysis time (CLT). We also studied the relationship between CLT and thrombin generation to determine if any relationship between CLT and VT was affected by thrombin generation. Analyses were performed in the THE-VTE Study, a two-centre population-based case-control study, including 579 patients and 338 controls, with patients followed from the event to determine incidence of recurrent VT. Hypofibrinolysis was associated with a 1.8-fold increased risk of a first VT (95%CI 1.2-2.7). Adjustment for sex, age, study location and Endogenous Thrombin Potential (ETP) did not change the result. The risk of VT was 2.9-fold increased when the 90$^{th}$ percentiles of prolonged CLT and high ETP were combined, with the highest risk for unprovoked first events (OR=4.2, 95%CI 1.3-13.5). In the follow-up study the HR for a recurrent VT associated with hypofibrinolysis was 1.5 (95%CI 0.9-2.6). A weak dose response effect was observed in relation to prolongation of CLT and recurrent VT. Although hypofibrinolysis constitutes a risk factor for a first VT, an association with recurrence is at best weak.

## Introduction

The incidence of venous thrombosis (VT) is 1 to 3 per 1000 person-years with a one-year cumulative incidence of recurrent VT of 7.4% after unprovoked VT and 4.2% after a non-surgical trigger.(Baglin *et al*, 2003; Iorio *et al*, 2010) Risk of recurrence is very effectively decreased by anticoagulant treatment but this benefit must be balanced against the risk of anticoagulant therapy-associated major bleeding, which occurs in 2 to 3% of patients on treatment per year.(Bates *et al*, 2012) Estimating the personalised predicted risk on an individual basis would allow appropriate targeting of long-term anticoagulant therapy.

Since it has been difficult to establish the risk of recurrent venous thrombosis using clinical factors, attempts have been made to identify global laboratory markers which are associated with an increased risk of a recurrent VT.(Baglin, 2011) Most of these studies focussed on assays that provided a global assay for pro- and anticoagulant pathways.(Tosetto *et al*, 2012) Few studies have focussed on the association between fibrinolytic capacity and the risk of recurrent VT.(Meltzer *et al*, 2010a;Traby *et al*, 2012) The clot lysis time (CLT) is influenced by levels of several proteins involved in fibrinolysis, i.e., plasminogen activator inhibitor-1 (PAI-1), alpha2-antiplasmin and thrombin activatable fibrinolysis inhibitor (TAFI). Determinants of CLT have been described previously (Meltzer *et al*, 2010b;Lisman *et al*, 2005) and this assay can be considered as a measure of plasma fibrinolytic potential.(Meltzer *et al*, 2007) CLT is a potential candidate for predicting the risk of a recurrent VT.

Together with thrombomodulin thrombin forms a complex which activates TAFI.(Wang *et al*, 1998) Activated TAFI inhibits fibrinolysis by preventing plasminogen activation. Earlier studies have shown that increasing thrombin concentrations protect clots against lysis.(von dem Borne *et al*, 1995) This leads to the hypothesis that patients with a high thrombin generating capacity may also have a prolonged clot lysis time and that thrombin generation may be related to

venous thrombosis not only directly but also indirectly by influencing fibrinolytic activity.

The aim of this study was three-fold. First, to examine the association between CLT and the risk of a first and recurrent VT. Second, to examine the relationship between CLT and thrombin generation and third, to assess the composite association of CLT and thrombin potential with venous thrombosis.

## Materials and methods

*Study design and participants case-control study*

The Thrombophilia Hypercoagulability Environmental risk for Venous Thromboembolism study (THE-VTE) is a two center population-based case-control study. The design of THE-VTE has been described previously.(van, V *et al*, 2015) In short, from 2004 to 2008 consecutive patients aged 18 to 75 years with a first deep vein thrombosis (DVT) or pulmonary embolism (PE) were recruited from the anticoagulant clinic in Leiden, the Netherlands and the Addenbrooke's Hospital in Cambridge, the United Kingdom, covering a well-defined geographical area in which all VT patients are admitted to, both outpatients and hospitalized patients. Partners of patients were invited as control subjects. Subjects with an active malignant disease were excluded from the study. Presence of a thrombus in the deep veins of the legs demonstrated with compression ultrasonography or Doppler ultrasound was considered as definite deep venous thrombosis (DVT). Pulmonary embolism (PE) was considered definite in case of a positive spiral computed tomographic or ventilation-perfusion lung scan findings.

All participants gave written informed consent. The study was approved by the Medical Ethics committee of the Leiden University Medical Center, Leiden, the Netherlands and the NHS Research Ethics Committee in Cambridge, United Kingdom.

*Data collection*

Within a few weeks after their VT, patients were invited to participate in the study by mailed letter and subsequent telephone contact (Leiden) or directly at their visit to the thrombosis clinic (Cambridge).The index date in the patients was defined as the date of diagnosis of the thrombotic event, in the control subjects this was defined as the date venous thrombosis of their partner. All participants were asked to complete a standardized questionnaire on acquired risk factors for venous thrombosis, such as surgery, pregnancy, oral contraceptive use, plaster cast, injury, malignancy, and immobilisation. All items in the questionnaire referred to the period prior to the index date. Three months after discontinuation of the anticoagulant therapy, a blood sample was taken from patients and controls in the anticoagulation clinic.

In total, 796 patients and 531 control subjects were included. Of these, 626 patients and 361 control subjects had donated blood without using oral anticoagulants. 93% of the patients (N=579) and 94% of the control subjects (N=338) had a valid CLT measurement and these participants were included in the current analyses. Idiopathic venous thrombosis was defined as venous thrombosis in patients without malignant disease in the five years prior to the index date and without surgery, injury, plaster cast, or confinement to bed for more than 4 consecutive days in the three months prior to the thrombosis or oral contraceptive use or hormone replacement therapy at the time of the event. Body mass index (BMI) was calculated by dividing body weight (kg) by squared height ($m^2$).

*Study design and participants follow-up study*

The patients included in THE-VTE case-control study were subsequently followed for the risk of recurrent venous thrombosis. A detailed description was published previously.(van, V *et al*, 2015) In Leiden, information on recurrent events was obtained from the patients via a short questionnaire and via the anticoagulation clinics. For all potential recurrences, discharge letters were obtained from the

clinician who diagnosed the recurrence. In Cambridge, all potential recurrent events were seen and objectively diagnosed by one of the authors (TPB). This was done without prior knowledge regarding risk factors as measured in this study.

The end of follow-up was defined as the date of the recurrence. In Leiden, in the absence of a recurrence, the end date of follow-up was the date of filling in the short questionnaire (which was sent annually to all participating patients). If a patient did not fill in a questionnaire they were censored at the last date known to be recurrence free, i.e., the last visit to the anticoagulant clinic, date of death or emigration, or the last time the patient was known to be recurrence-free from information of the THE-VTE case-control study. In Cambridge, recurrence status was checked on the first of July 2013. In the absence of recurrence or death, this date was registered as the end of follow-up. The start of follow-up was the date of termination of anticoagulation after the first event. In total 52 (8.3%) patients did not complete follow-up either due to death (n=21) without recurrence or not replying to further queries after a last visit at the anticoagulation clinic (n=13) or at a later point in time during follow-up (n=18).

*Blood collection and laboratory analysis*

Blood was collected into 0.106 M trisodium citrate. Plasma was prepared by centrifugation for 15 minutes at 4000 rpm at room temperature and stored at -80ºC. All assays were performed without knowledge of whether the sample was from a patient or a control, and of whether a recurrent thrombosis had occurred. All assays were performed in one lab (Cambridge).

*Clot lysis time assay*

Lysis of a tissue factor-induced clot by exogenous tissue-type plasminogen activator (t-PA) was studied by monitoring changes in turbidity during clot formation and subsequent lysis as described previously.(Lisman *et al*, 2005) In short, 50µL of plasma was pipetted in a 96-well microtiter plate. Subsequently, a

50-µL mixture containing phospolipid vesicles, t-PA (final concentration 56 ng/mL), tissue factor, and $CaCl_2$ diluted in HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) was added by the use of a multichannel pipette. In a kinetic microplate the optical density was 405 nm and was monitored every 20 seconds, resulting in a clot-lysis turbidity profile. The CLT was derived from this clot-lysis profile and defined as the time from the midpoint of the clear to maximum turbid transition, representing clot formation, to the midpoint of the maximum turbid to clear transition, representing the lysis of the clot.

*Endogenous thrombin potential assay*

Thrombin generation was measured with the ThrombinoscopeTM Assay, (Thrombinoscope BV, Maastricht, the Netherlands). Tissue factor (TF) was mixed with phospholipids (PL) and added to polypropylene 96-well microtitre plates (Greiner Bio-one Ltd, Stonehouse, UK). Plasma was then added and thrombin generation was triggered and monitored by addition of premixed flurophore and calcium solution. For the calibrator wells the TF-PL mix was replaced with a thrombin solution (660nmol/l). Innovin tissue factor (TF) was at a final concentration of 5pmol/l (Dade Behring, Milton Keynes, UK). Tissue factor concentrations were confirmed with a Xa-based chromogenic assay (Actichrome TF, American Diagnostica). Phospholipids (PL) were at a final concentration of 4µmol/l (Avanti Polar Lipids (Alabama, USA) and consisted of 20mol% phosphatidylserine, 20mol% phosphatidylethanolamine and 60mol% phosphatidylcholine prepared by repeated extrusion (Avestin extruder, Mannheim, Germany). 80µl of platelet poor plasma were tested in triplicate in parallel with a calibrator. 20µl of Z-Gly-Gly-Arg-AMC reagent at a final concentration of 0.417 mmol/l per well (Bachem AG, Bubendorf, Switzerland) suspended in Buffer with 0.1 mol/l CaCl2, 20mmol/l HEPES, 60mg/ml Bovine Serum Albumin, pH 7.35 (all Sigma Inc, St.Louis,MO, USA) was added by the automatic dispenser. For the internal calibrator PL was mixed with 20µl of thrombin calibrator solution (alpha2-macroglobulin bound to thrombin (660 nmol/l), (Thrombinoscope BV, Maastricht,

Netherlands)). Fluorescence was measured by a Fluoroscan reader (Thermo Labsystems, Helsinki, Finland). Fluorescence filters used were an excitation filter at 390 nmol/l and an emission filter at 460 nmol/l as supplied by Thrombinoscope BV. The Thrombinoscope™ software was used to convert the fluorescence signal into nmol/l Thrombin activity with correction for the inner filter effect and thrombin bound to alpha-2 macroglobulin activity, as described by Hemker et al.(Hemker *et al*, 2003) Samples used for this study were not previously thawed.

*Statistical analysis*

We studied the association between venous thrombosis and a prolonged CLT in the total study population, and for provoked and unprovoked venous thrombosis separately. To analyse whether hypofibrinolysis increases the risk of VT, CLT was cut off at the 90$^{th}$ percentile as measured in control subjects. The risk of a first venous thrombosis was assessed by calculating odds ratio's (OR) with corresponding 95% confidence intervals (95% CI) according to Woolf's method after adjustment for age (continuous), sex (dichotomous), and study location (dichotomous). We additionally adjusted for ETP to assess the extent to which the association between prolonged CLT and venous thrombosis is modified by ETP. To analyse a dose-response relation, CLT was further stratified into quartiles.

We assessed the relation between endogenous thrombin potential and clot lysis time by performing a linear regression in control subjects. Lastly, we calculated the combined and separate effects of prolonged CLT and elevated ETP (both above the 90$^{th}$ percentile) on the risk of VT.

The cumulative incidence of recurrent venous thrombosis after 2 years was assessed and visualised by Kaplan–Meier survival analysis. The overall incidence rate of recurrent venous thrombosis with 95% confidence intervals (CI) was estimated. Hazard ratios for recurrent VT with 95% CI were estimated for the group with a prolonged CLT (i.e. above the 90$^{th}$ percentile as measured in the control subjects of the case-control study) compared with a normal CLT by using

multivariable Cox regression analysis adjusted for age, sex, and study location. Again, we additionally adjusted for ETP to assess the extent to which the association between prolonged CLT and VT is modified by ETP. To study the dose-response relation between CLT and recurrent VT, CLT was stratified into quartiles. We performed a sensitivity analysis in which we started the follow-up time from the moment of blood draw and additionally adjusted for the time between the VT event and blood draw. Two-year cumulative incidences of recurrent VT were estimated for quartiles of CLT. IBM SPSS Statistics 20.0 for Windows (SPSS Inc, Chicago, Ill) was used for data analysis.

## Results

Characteristics of the 579 patients and 338 controls are shown in Table 1. The mean age at time of blood sampling for patients was 53 (95% CI: 52-55) and mean age of the control subjects was 54 (95% CI 53-56). Of the patients, 306 (53%) were men compared with 128 (38%) of the control subjects. 357 (62%) Patients were diagnosed with DVT only, and 223 (38%) were diagnosed with PE with or without a DVT. The mean duration of anticoagulant therapy of the patients, i.e. vitamin K antagonists, was 5.7 months (95% CI 5.5-5.9).

Mean CLT in the control subjects was 91.6 min (95% CI: 89-94). There was no difference in CLT between men (91.3 min. 95% CI: 88-95) and women (91.8 min. 95% CI: 89-95) and the CLT increased by age (increase in CLT per year in controls: 0.1 min 95% CI: 0.07-0.48).

**Table 1** Baseline characteristics case-control and follow-up study

| | Patients N=579 | Controls N=338 |
|---|---|---|
| Case-control study | | |
| Men (%) | 306 (53) | 128 (38) |
| Age mean (95%CI) | 53.4 (52.3-54.6) | 54.2 (52.9-55.5) |
| BMI (kg/m$^2$) | | |
| <20 | 11 | 11 |
| 20-25 | 177 | 147 |
| >25 | 360 | 159 |
| Use of oral contraceptives in women (%) | | |
| Yes | 90 (33) | 30 (14) |
| No | 183 (67) | 179 (86) |
| Pregnancy or post-partum in women (%) | | |
| Yes | 14 (5) | 1 (1) |
| No | 255 (95) | 202 (99) |
| Smoking (%) | | |
| Current | 249 (44) | 141 (43) |
| Former | 201 (35) | 123 (37) |
| Never | 119 (21) | 68 (20) |
| Site of thrombosis (%) | | |
| DVT | 357 (62) | - |
| PE +/- DVT | 222 (38) | - |
| First event | | |
| Unprovoked | 270 | - |
| Provoked | 283 | - |
| CLT minutes mean (95%CI) | 100.0 (98-102) | 91.6 (89-94) |
| Follow-up study | | |
| Observation time, mean (SD), yr | 4.8 (1.8) | |
| Number of recurrences | 78 | |

*Prolonged CLT associated with a first VT*

Mean CLT in the patients was 100.0 min (95% CI: 98.0-102.1) and the mean CLT difference between patients and control subjects was 8.4 min (95% CI: 5.2-11.7) longer for patients. After adjustment for age, sex and study location, hypofibrinolysis, i.e., CLT above the 90$^{th}$ percentile as measured in control subjects (>122 minutes), was associated with a 1.8 fold increased risk of a first VT compared with a CLT ≤ 90$^{th}$ percentile (OR 1.8, 95% CI: 1.2-2.8). Additional adjustment for ETP did not change the results (OR 1.6, 95% CI: 1.0-2.5). Analyses by type of event showed a 1.5 fold increased risk of VT (95% CI: 0.9-2.6) in the presence of hypofibrinolysis for provoked VT. For unprovoked VT, hypofibrinolysis was associated with a 2.1 fold increased risk (95% CI: 1.3-3.5). There was a clear dose-response relation between CLT and the risk of venous thrombosis with the highest risk of thrombosis for those with a CLT in the highest quartile (>105 minutes) compared with the lowest quartile (<76 minutes): OR 2.7, 95%CI: 1.8-3.9 (Table 2). The thrombin potential was positively associated with the CLT in control subjects, i.e., for every 100 units increase in ETP levels, an increase of 1.12 minutes in CLT was found (95% CI: 0.397-1.839).

**Table 2** Dose-response relation CLT and risk of first VT

| Quartiles CLT | Controls | Patients | Lysis time at cut-off, min | OR* (95%CI) |
|---|---|---|---|---|
| 1 | 84 | 93 | 76.1 | 1 (ref) |
| 2 | 85 | 93 | 86.7 | 1.0 (0.7-1.6) |
| 3 | 85 | 153 | 104.5 | 1.7 (1.1-2.5) |
| 4 | 84 | 240 | | 2.7 (1.8-3.9) |

*CLT, clot lysis time; VT, venous thrombosis; OR, odds ratio; 95% CI, 95% confidence interval.*
**Adjusted for sex, age, study site.*

Relative to individuals with both a normal CLT and a normal ETP, we found that individuals with a prolonged CLT but a normal ETP had a 1.7 fold increased risk of venous thrombosis (OR 1.7, 95% CI 1.1-2.7, Table 3). The risk was also 1.7 fold increased for individuals with an elevated ETP only (OR 1.7, 95% CI: 1.0-2.7). The risk was highest for individuals with a prolonged CLT and an elevated ETP, i.e., a 2.9-fold increased risk of VT (OR 2.9, 95% CI: 1.1-8.0). The highest risk was found in individuals with unprovoked first VT (OR 4.2, 95% CI: 1.3-13.5). In patients with a provoked first event the risk was 2-fold increased (OR 2.1, 95% CI: 0.7-6.8).

*Prolonged CLT associated with recurrent VT*

After a mean follow-up period of 4.8 years, 78 out of 579 patients had a recurrent thrombotic event (13.5%, Table 1). The overall incidence rate was 27.9 per 1000 person years (95% CI: 21.7-34.1). The two-year cumulative incidence of recurrent VT was 7.4% (95% CI: 5.3-9.6). The two-year cumulative incidences of recurrent VT in quartiles of CLT are given in table 4 (Figure 1). After adjustment for age, sex and study location, the hazard ratio for recurrence in patients with hypofibrinolysis was 1.5 (95% CI: 0.9-2.6). Adjusting for ETP did not change the result (HR 1.5,

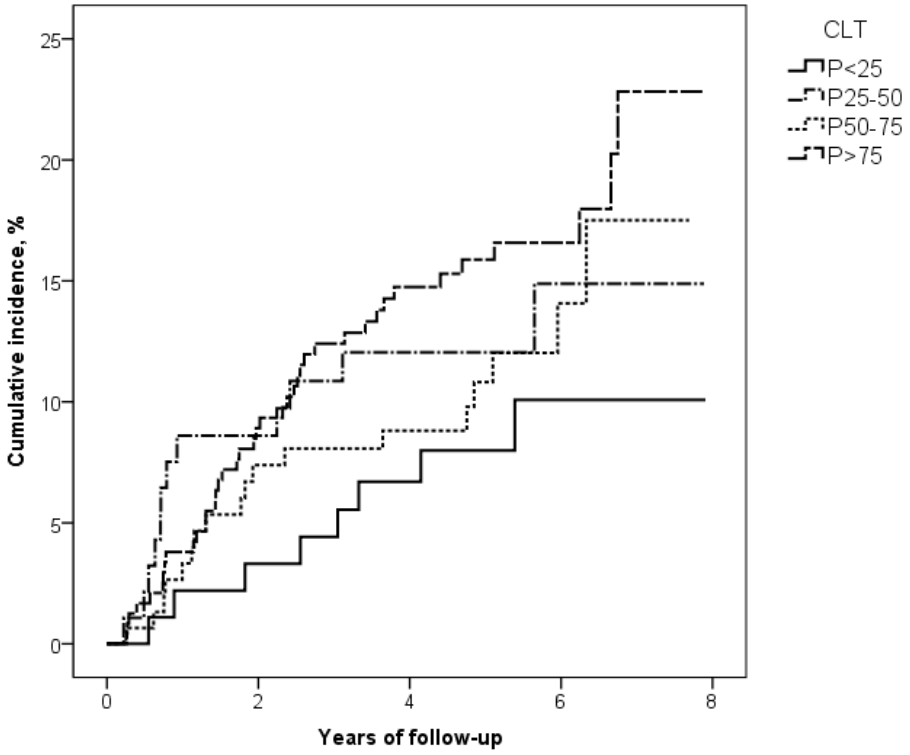
95% CI: 0.9-2.7). Subgroup analyses showed no difference in the risk of recurrence associated with hypofibrinolysis after a provoked VT or unprovoked VT (HR 2.0; 95% CI: 0.8-5.0 for provoked VT and HR 1.5; 95% CI: 0.7-3.2 for recurrence after an unprovoked VT). Relative to individuals with a CLT in the lowest quartile (as measured in the control subjects), the risk of a recurrence increased from 1.5-fold (95% CI: 0.6-3.7) in individuals with a CLT in the second quartile to a 1.9-fold (95% CI: 0.9-4.1) increased risk of a recurrent event for those with a CLT in the fourth quartile. Sensitivity analysis showed no differences in risk estimates when follow-up time was started from the moment of blood draw and when additional adjustment for the time between VT even and blood draw was made.

**Table 3** Interaction CLT and ETP in case-control study

| CLT 90th percentile | ETP 90th percentile | Patients | Controls | OR* overall (95% CI) | OR* provoked (95% CI) | OR* unprovoked (95% CI) |
|---|---|---|---|---|---|---|
| - | - | 414 | 275 | 1 (ref) | 1 (ref) | 1 (ref) |
| + | - | 75 | 28 | 1.7 (1.1-2.7) | 1.4 (0.8-2.5) | 2.0 (1.1-3.5) |
| - | + | 68 | 26 | 1.7 (1.0-2.7) | 1.4 (0.8-2.5) | 2.0 (1.1-3.6) |
| + | + | 18 | 5 | 2.9 (1.1-8.0) | 2.1 (0.7-6.8) | 4.2 (1.3-13.5) |

**adjusted for sex, age, study site*

**Figure 1** Cumulative incidence of recurrent thrombosis. Patients with CLT's in quartiles, cut-off as measured in control subjects of the case-control study.




| No. at risk | | | | |
|---|---|---|---|---|
| P<25 | 92 | 87 | 76 | 15 |
| P25-50 | 92 | 81 | 65 | 20 |
| P50-75 | 152 | 136 | 120 | 41 |
| P>75 | 239 | 212 | 171 | 77 |

**Table 4** Dose-response relation CLT and the risk of recurrent VT

| Quartiles CLT | Recurrence yes/no | 2-year cumulative incidence, % (95% CI) | HR* (95% CI) |
|---|---|---|---|
| 1 | 8/85 | 0.5 (-0.1-1.1) | 1 (ref) |
| 2 | 12/81 | 1.4 (0.4-2.3) | 1.5 (0.6-3.7) |
| 3 | 18/135 | 1.9 (0.8-3.0) | 1.3 (0.6-3.0) |
| 4 | 40/200 | 3.6 (2.1-5.1) | 1.9 (0.9-4.1) |

**adjusted for sex, age, study site*

## Discussion

In this study we aimed to investigate the association between CLT and a first and recurrent VT. Hypofibrinolysis, defined as CLT above the 90$^{th}$ percentile as measured in the control group, was associated with a 1.8-fold (95% CI: 1.2-2.8) increased risk of a first VT. A clear dose-response relationship was found between CLT and the risk of first VT: individuals with a CLT in the highest quartile had a 2.7-fold (95% CI: 1.8-3.9) increased risk of a first VT compared to individuals with a CLT in the lowest quartile. Additionally, the risk of first venous thrombosis was highest in individuals with both a prolonged CLT and a high ETP (both above 90$^{th}$ percentile), i.e., these individuals had a 2.9-fold (95% CI: 1.1-8.0) increased risk of a first thrombotic event compared to individuals with a normal CLT and ETP (i.e. under the 90$^{th}$ percentile). In the prospective cohort study formed from the cases in the case-control study there was a weak association of hypofibrinolysis with recurrent VT (HR 1.5, 95% CI: 0.9-2.6). A slight dose response effect was observed in relation to prolongation of CLT and recurrent VT with a 1.9-fold (95%

CI: 0.9-4.1) increased risk of a recurrent event in patients in the highest quartile for CLT compared to those in the lowest.

The findings from our case-control study are in line with previous studies reporting an increased risk of a first venous thrombosis associated with hypofibrinolysis, as shown in Table 5.(Lisman *et al*, 2005;Meltzer *et al*, 2008) Meltzer *et al* as well as Lisman *et al* reported a 2-fold increased risk of venous thrombosis associated with a prolonged clot lysis time. They also found a dose-response relation between hypofibrinolysis and venous thrombosis.

Because hypercoagulability and hypofibrinolysis are both risk factors for venous thrombosis and are related, we hypothesized that reduced fibrinolysis and increased thrombin generation may each increase the risk for a first venous thrombosis, and modify the effects. The CLT assay is a measure of overall fibrinolytic activity and the ETP assay a measure of the most important determinant of hypercoagulability: thrombin. We found, as expected, the highest risk of first venous thrombosis in patients with elevated ETP and prolonged CLT (>90$^{th}$ percentiles in controls for both assays). In addition, as might be anticipated, the association was strongest in patients with a first unprovoked event. These results again confirm previous reports of the risk of VT when both hypercoagulability and hypofibrinolysis are combined. Our novelty is that we measured ETP as an estimate of hypercoagulability, while other reports measured use of oral contraceptives in women.(Meltzer *et al*, 2008) Previous studies have shown that the thrombin/thrombomodulin complex activates TAFI and this inhibits fibrinolysis (Bouma & Meijers, 2003), thereby leading to prolonged clot lysis time. Based on these findings we studied the association between CLT and ETP. We demonstrated an association between hypofibrinolysis, as measured by CLT, and an increased thrombin generating potential. This supports the hypothesis that increased thrombin generation leads to reduced clot lysis, probably as a result of the thrombin/thrombomodulin complex generating TAFI,(Bouma & Meijers, 2003).

**Table 5** Overview literature

| Author | Study type | N patients<br>N controls | FU time for patients | Performed assays | $OR_{first\ VT}$ (95%CI) | $HR_{recurrence}$ (95%CI) | p-value |
|---|---|---|---|---|---|---|---|
| Crowther 2001 | Cohort | 25<br>105 | Mean=1.73 years<br>(SD 1.0) | Euglobulin CLT | - | - | 0.976 |
| Eichinger 2004 | Cohort | 600 | Mean=45 months | TAFI | - | 1.06 (1.0-1.16) | - |
| Lisman 2005 | Case-control | 421<br>469 | - | CLT | 2.0 (1.3-3.0)<br>(>90$^{th}$ percentile vs <90$^{th}$ percentile) | - | - |
| Meltzer 2008 | Case-control | 2090<br>2564 | - | CLT | 2.4 (2.0-2.8)<br>(4$^{th}$ quartile vs 1$^{st}$ quartile) | - | - |
| Meltzer 2009 | Cohort | 474 | Mean=7.3 years (SD 2.7) | CLT<br>TAFI | - | 1.1 (0.6-2.1)<br>1.0 (0.5-1.9) | -<br>- |
| Traby 2012 | Cohort | 704 | Mean=46 months (SD 30) | CLT | - | 1.08 (0.98-1.20) | 0.13 |

This result is in contast to only one previous report, in which CLT was not influenced by the thrombin-generating capacity of the plasma.(Lisman *et al*, 2005)

We found a weak association between CLT and recurrent VT. The magnitude of the association would not be strong enough to give measurement of CLT clinical utility in personalising decisions in individual patients regarding the duration of anticoagulant therapy. Conflicting results have been found in previous studies. No relationship between fibrinolysis and recurrent VT was found by Crowther et al (Crowther *et al*, 2001) or in the Leiden Thrombophilia Study.(Meltzer *et al*, 2010a). Eichinger et al reported an association with recurrent VT in patients with the highest TAFI levels (Eichinger *et al*, 2004) and a moderate increase in recurrence risk in association with prolongation of the CLT in women but not men.(Traby *et al*, 2012) Taken together with our study the results of previous studies do not indicate a clinical use for measurement of CLT in the prediction of VT recurrence risk. However, future studies are needed to determine the definite role of the fibrinolytic system in predicting recurrent VT.

The strength of our study is that all data were collected in the same manner for all participants and all venous thrombotic events were objectively diagnosed. Furthermore, all patients were followed-up for a mean period of nearly 5 years. Another strength of our study is that the same operator performed all CLT analyses to eradicate the bias that may be caused by the use of different techniques.

A potential weakness of our study, and case-control studies in general, is that blood was drawn after the thrombotic event. The associations found between laboratory parameters and the risk for venous thrombosis could be a consequence rather than cause of the disease. We investigated if this was the case this by analysing whether the CLT changed over time. After stratifying the time between the index date and the blood draw (≤6 months versus >6 months), we calculated the mean difference of CLT between ≤6 months and >6 months. The mean difference was 1.7 min (95% CI: -4.0-7.4), therefore, no relation was found between CLT and the time that had passed between the event and the blood draw.

Another limitation of our study is that levels of fibrinolytic proteins, such as plasminogen, plasmin-alpha2-antiplasmin complex, and PAI-1 have not been measured in THE-VTE population. As a result, potential determinants of the CLT could not be elucidated from this study.

In summary, our findings indicate that hypofibrinolysis is associated with venous thrombosis but measurement of CLT does not have clinical utility for the prediction and hence prevention of recurrent VT. The amount of thrombin generated is a determinant of clot lysis and so the amount of thrombin within a venous thrombosis may determine likelihood of embolisation and residual vein occlusion and post thrombotic syndrome by influencing subsequent fibrinolytic activity.

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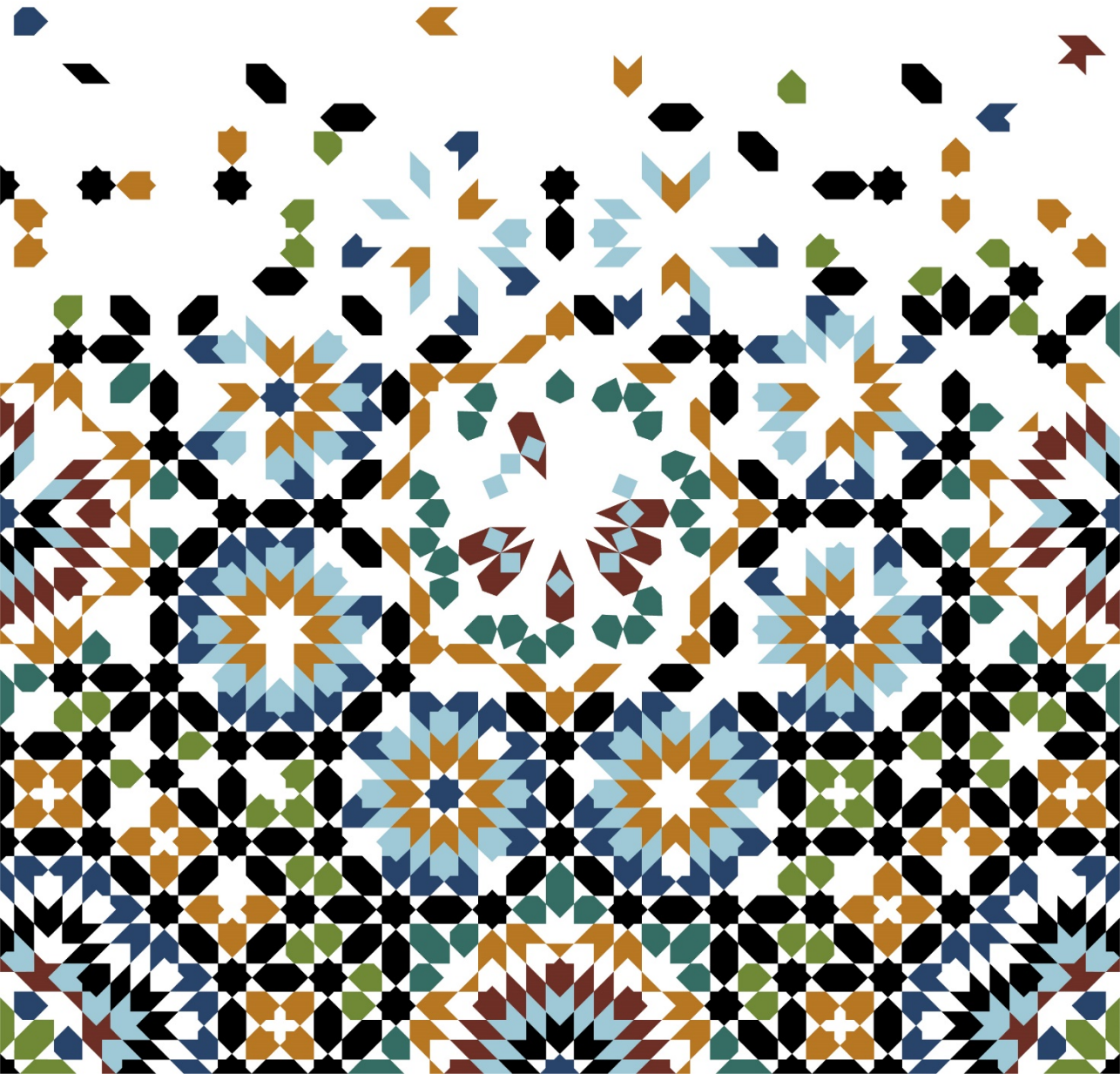
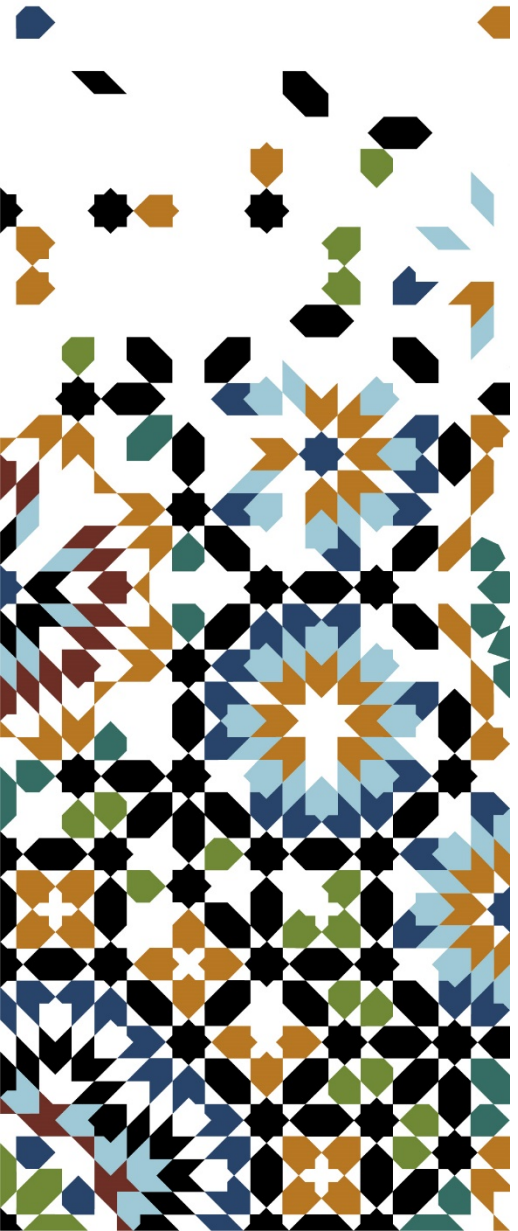


# Chapter 8

Summary and General discussion



# Summary and General discussion

The aim of this thesis was to provide more insight into environmental and genetic factors that influence the risk of a first venous thrombosis (VT) in the elderly, i.e. individuals aged ≥70 years. In addition, we aimed to investigate the utility of the global clot lysis time assay in assessing the risk of a first and recurrent VT, initially in a younger population (≤75 years). In this chapter we provide a summary of our main findings, put them in an etiologic context and give suggestions for future research.

## Main findings

*Conventional risk factors of VT in the elderly*

In **chapter 2** we explored the association between three inflammatory airway diseases, bronchitis, COPD, and asthma, and the risk of a first VT in the AT-AGE study. Using a detailed questionnaire on risk factors for a first venous thrombosis, we obtained information on comorbidities, including COPD, asthma and bronchitis. We confirmed the presence of COPD, asthma and bronchitis by reviewing the participants' medication use, which was validated by examining pill bottles during the home visits. Still, misdiagnoses are frequent, especially in the elderly. Asthma and COPD both overlap and converge in the elderly.[1-3] Our results show that for a number of patients diagnoses indeed overlapped. For this reason, we considered all diagnoses together, but we also calculated the risk of VT associated with the three inflammatory airway diseases separately. Presence of any airway disease increased the risk of VT 1.7-fold (95% CI 1.2-2.4), the risk of DVT alone 1.2-fold (95% CI 0.7-1.9), and the risk of PE 2.2-fold (95% CI 1.5-3.3). We observed that the presence of either bronchitis, COPD or asthma was more clearly associated with an increased risk of PE, and not or only weakly, with the risk of DVT. A possible explanatory mechanism is that inflammatory airway diseases predispose to local prothrombotic state and therefore give rise to a local development of pulmonary thrombi which embolize within the pulmonary vasculature. Several

studies suggest inflammation has a role in the pathogenesis of VT.[4-7] C-reactive protein (CRP) is an acute-phase protein whose levels rise following tissue injury or inflammation. CRP has therefore been associated with cardiovascular disease and measurements of CRP levels proved to be a valuable predictor of the risk of future cardiovascular events.[8] Therefore, the hypothesis that CRP might also be involved in the onset of VT has emerged, as reviewed by Lippi.[9]

Contrary to the effect of lung diseases on the risk of VT as specific for PE, as discussed above, several risk factors for VT have been shown to increase the risk of a DVT but not, or to a much lesser extent, the risk of a PE.[10] The 'factor V Leiden paradox' is the observation that the factor V Leiden G1691A mutation is at most a mild risk factor for isolated PE, whereas the risk of DVT is substantially increased.[11,12] We have shown that the 'factor V Leiden paradox' can also be observed in the elderly. Individuals with the factor V Leiden variant had a higher risk of DVT (OR 3.0, 95% CI 1.5-6.0) than of PE (OR 1.6, 95% CI 0.7-3.1), and this difference remained after exclusion of patients with airway diseases.

In **chapter 3**, we studied the risk of a first VT after the age of 70 years in association with several hereditary risk factors: factor V Leiden and prothrombin G20210A mutations, a positive family history of VT and non-O blood group. The most common prothrombotic variants are the factor V Leiden and prothrombin G20210A mutations, with an overall incidence of carriers of 2-5% among Caucasians.[13-16] We showed that the risk of VT was 2.2-fold increased in factor V Leiden carriers (95% CI 1.2-3.9), 1.4-fold increased in prothrombin G20210A mutation carriers (95% CI 0.5-3.9), and 1.3-fold increased in those with non-O blood group (95% CI 1.0-1.8). Positive family history of VT was associated with a 2.1-fold increased risk of VT (95% CI 1.5-3.1). A previous large cohort study on the association between the factor V Leiden and prothrombin G20210A mutations and the risk of VT, was consistent with our results: the mutations were associated with an increased risk of VT in the elderly.[16,17] Studies performed in younger individuals show us that factor V Leiden and prothrombin G20210A mutations increase the risk of VT 3- to 8-fold.[14,18] In our study, we found these risk estimates

to be lower, OR 2.2 and OR 1.4 respectively. In this thesis two probable suggestions are given for this observation. First, because the elderly have a higher absolute risk of VT, individual risk factors have smaller relative effects than in middle-aged individuals. Or, in other words, whereas the relative risks are smaller than in the young, absolute risk differences for carriers versus non-carriers are substantial, given the high baseline risk in the elderly. Second, attrition of susceptibles: susceptible individuals with the factor V Leiden or prothrombin G20210A mutations are likely to develop VT earlier in life, which may have led to lower relative risks at older age.

In **chapter 3** we also found that a positive family history of VT in first-degree relatives increased the risk of VT in the elderly 2.1-fold (95% CI 1.5-3.1). Family history is a feasible and global genetic risk assessment method for several clinical conditions.[19] Therefore not surprisingly, several other case-control studies have also examined the relationship between family history and VT risk in first-degree relatives. Two American[20,21] and five European studies[22-26] have found similar risk estimates, ranging from 2.2 to 3.0. These consistent results indicate a possible role of family history of VT in clinical decision rules of VT, not only in those younger than 70 years, but also in the elderly. Another important issue is the positive predictive value of family history of VT for a genetic risk factor for VT, specifically for the factor V Leiden and prothrombin G20210A mutations. We found in our study that 97 cases had a positive family history of VT. Among them 11 had either of the two mutations, which resulted in a positive predictive value of family history for carrying a prothrombotic variant of 11%. The low predictive value of family history for thrombophilias was also reported in previous studies[24,25,27,28] suggesting the presence of other, known or unknown, genetic factors influencing the risk of VT. The predictive value is determined by both the sensitivity and specificity of the predictor (i.e. assessment of a positive family history) and the prevalence of the predicted quality in the population being tested (i.e. factor V Leiden and prothrombin G20210A mutations). The interpretation of a positive or negative diagnostic test result varies from setting to setting, according to the

prevalence of the disease in that particular setting. Therefore, interpretation of the clinical validity of family history as a diagnostic tool to detect the factor V Leiden or prothrombin G20210A mutations should be made with caution.

ABO blood group is believed to contribute to VT risk through modifications of von Willebrand Factor (VWF) and factor VIII (FVIII) levels in plasma.[29-31] However, several studies have shown that ABO blood group remains associated with an increased risk of VT even after adjustment for VWF or FVIII levels.[32,33] These results suggest that ABO may contribute to VT risk by additional other mechanisms than just modifications of VWF and FVIII.[31] For example, genome-wide association studies have linked the ABO locus to different types of cancer, a situation apt to VT occurrence.[34,35]

*Veins and valves in the elderly*

In **chapter 4** we assessed the association between clinical features of venous insufficiency, such as history of varicose veins, leg ulcer or leg oedema, and the risk of VT in the AT-AGE study. We found that individuals who report a history of varicose veins or leg ulcer or who had leg oedema in the 3 months prior to the index date, were at an increased risk of VT (OR 1.6, 95% CI 1.2-2.3 for varicose veins, OR 3.0, 95% CI 1.5-6.2 for leg ulcer, OR 2.9, 95% CI 1.9-4.3). A leg ulcer is the most severe expression of venous insufficiency.[36] Leg oedema as a result of long-term venous insufficiency could lead to mediator release and inflammation in the veins.[37,38] Varicose veins are caused by a loss of vessel wall balance: venous hypertension leads to vein dilation, distortion, leakage, and inflammation, causing valve and wall disease and reflux.[39,40] The resulting valve dysfunction and reflux lead to a prothrombotic microenvironment, prone to VT.[41] In a previous study it was shown that valve thickness increased with age.[42] The authors hypothesized that the increased risk of VT in the elderly could partly be explained by thicker valves in the elderly. In **chapter 6** we investigated the risk of VT in presence of valve dysfunction, as a result of ageing, and reflux. We set up the BATAVIA study and we found on the one hand an association between thickened venous valves

and the risk of VT, and on the other hand an association between an increased valvular reflux and the risk of VT. Valve thickness of the popliteal vein above the 90th percentile, as measured in the control group, was associated with a 2.9-fold increased risk of VT. Valvular reflux, as measured by the valve closure time, was associated with a 2.8-fold increased risk of VT. Furthermore, a relation between valve closure time and valve thickness was found. For every 0.1 mm increase of valve thickness, valve closure time increased by 0.2 s. Recently, studies on mouse models have identified several mechanisms that regulate venous valve development and maintenance. Mice deficient in Connexin37 entirely lack venous valves.[43] Continuous integrin-α9 and ephrin-B2 signaling was found to be required for the maintenance of valves, since removal of either function results in regression of valve leaflets.[44] In another study conducted in individuals with and without a mutation in the FOXC2 gene, a strong association was observed between those with the mutation and the presence of primary venous valve failure.[45] In this study, as in ours, flow in the opposite direction – reflux – is used as a surrogate for valve failure. Further insight in these mechanisms could improve our understanding of failure and destruction of venous valves.

In **chapter 5** we investigated the distribution of thrombi in the AT-AGE study. Ageing may alter the venous circulation, resulting in a specific thrombi distribution pattern in the elderly.[46] We found in the AT-AGE study that PE (with or without DVT) occurs more often than DVT (58.6% versus 41.4%). This is in contrast to studies conducted in young and middle-aged individuals in which the incidence of DVT is always higher than the incidence of PE.[47-49] Furthermore, DVT occurred more often on the left side, and the popliteal and femoral veins were the most prevalent sites of thrombi. Left-sided predominance of DVT may be explained by the compression of the left common iliac vein by the right common iliac artery.[50]

*Global assays*

In **chapter 7** we found that hypofibrinolysis, defined as clot lysis time above the 90$^{th}$ percentile as measured in controls, increases the risk of a first VT 1.8-fold (95% CI 1.2-2.8) in THE-VTE study. There was a clear dose-response relationship between clot lysis time and the risk of a first VT, strengthening the notion of a causal relationship.[51] Additional adjusting for endogenous thrombin potential did not change the results. In the cohort study of patients followed after their first VT, we found a weak association between hypofibrinolysis and recurrent VT (HR 1.5, 95% CI 0.9-2.6). Additional adjustment for endogenous thrombin potential did not affect the risk of recurrent VT. The two assays used in this study link the coagulation and the fibrinolytic systems. The clot lysis time assay is a measure of overall fibrinolytic activity whereas the endogenous thrombin potential assay is a measure of the most important determinant of hypercoagulability: thrombin generation. When large amounts of thrombin are present, thrombin activatable fibrinolysis inhibitor will be activated. We hypothesized that increased thrombin generation would lead to decreased fibrinolysis and therefore to longer clot lysis time.[52] This hypothesis was supported by our observation of an association between hypofibrinolysis and an increased thrombin generating potential. Currently prediction models are being developed to identify individuals with an increased risk of recurrent VT. In chapter 7 we show that elevated clot lysis time is not associated with the risk of recurrent VT, which suggests that screening for hypofibrinolysis or including clot lysis time in prediction models of recurrent VT would be of no value in the prevention of a recurrent event.

## Future directions

The prevalence of obstructive airway diseases have increased in Western countries over the past decades, leading to a major health burden worldwide.[53] Future studies are needed to evaluate diagnostic strategies in elderly patients with airway diseases. In thrombosis research predictive biomarkers are needed that can identify low- and high-risk elderly individuals for VT. Evaluating markers such as CRP[9] could be valuable in determining which (hospitalized) patients would receive benefit from VT prophylaxis or prolongation oral anticoagulant therapy after a first VT. Furthermore, it's important to take the coexisting need for other medications into account, increasing the risks of side effects of oral anticoagulants in the elderly.

In the AT-AGE study we excluded individuals with cancer, receiving cancer therapy, and those who received cancer therapy in the six months prior to the VT. Future studies are needed to investigate whether the association between ABO and VT is related to cancer, changes in the haemostatic system by ageing, and if so, by what mechanisms. In clinical practice information on family history of VT is easy to obtain, however it is not implemented in clinical decision rules of VT risk. Possibly, obtaining information on family history of VT in individuals aged 70 years or older could improve prediction of VT in the elderly. Future studies could examine different candidate genes of venous insufficiency.

There is a lack of evidence associating genes with the development and severity of features of venous insufficiency. More studies are needed to confirm our results of the BATAVIA study, which to our knowledge is the first and only study examining the relation between VT risk and thickened venous valves and reflux in the elderly. Researchers should investigate whether valve thickness is related to post-thrombotic syndrome and whether valve thickness and function can predict post-thrombotic syndrome. Identifying patients at high risk of developing post-thrombotic syndrome would help improve the management of patients with VT.[54] Measures of D-dimer could allow for better distinction between elderly

patients with high or low risk of recurrent VT. Future studies should link clinical patient characteristics in the elderly, such as frailty, with laboratory testing to develop a scoring model that allow assessing the risk of recurrent VT.

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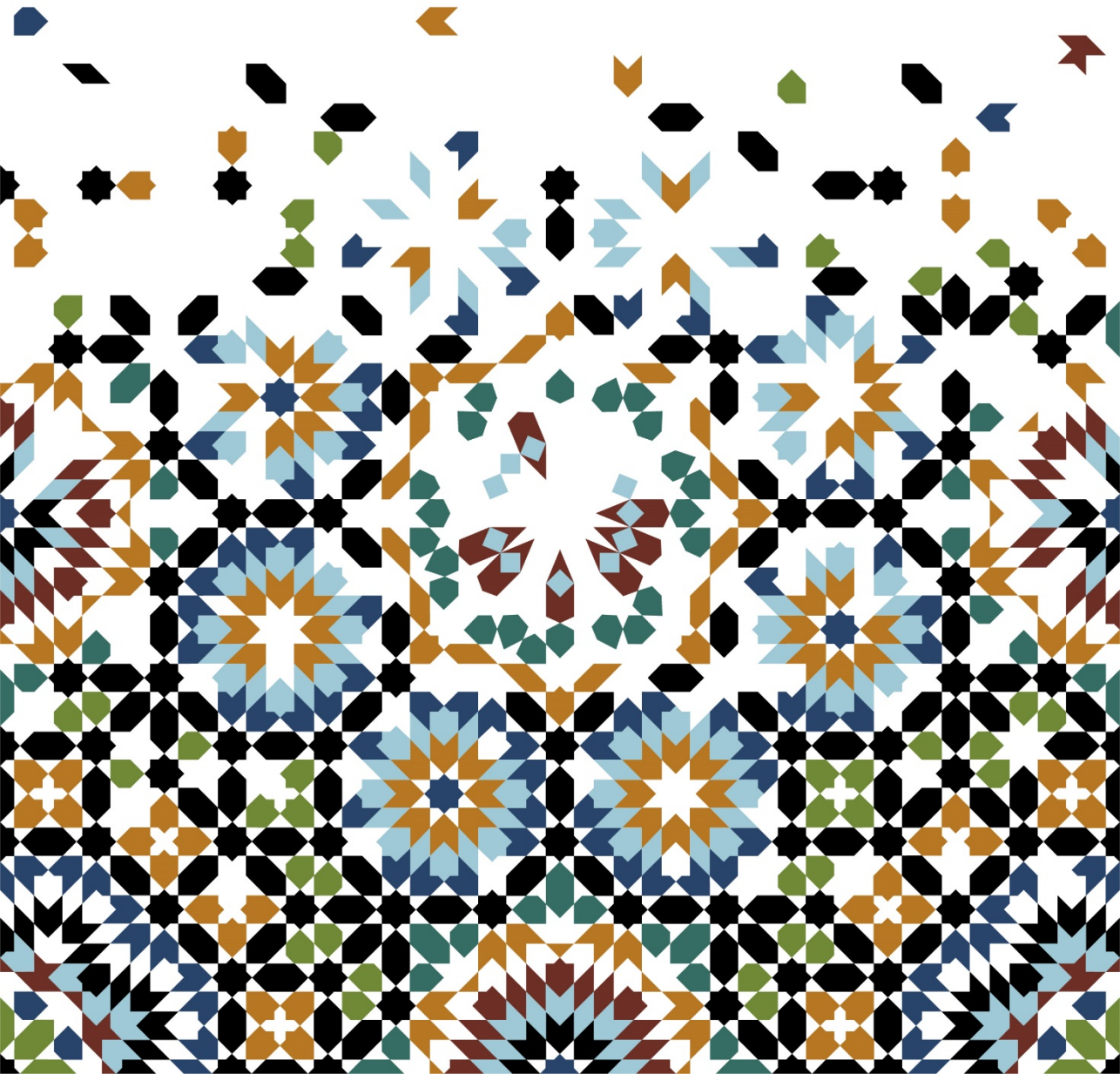
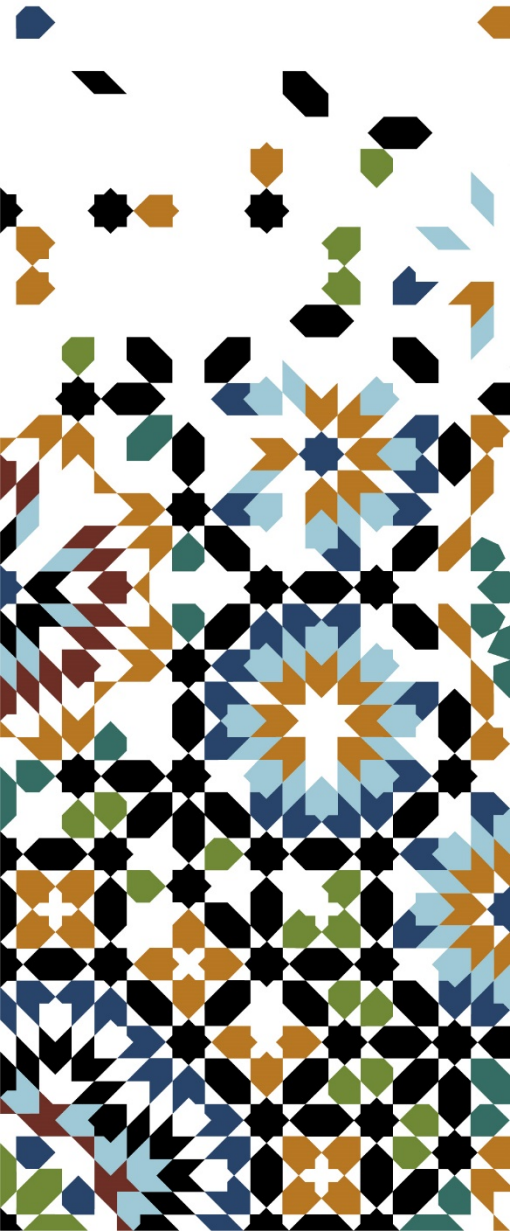


# Chapter 9





# Nederlandse samenvatting

Veneuze trombose is obstruerende stolselvorming in de veneuze bloedvaten, vaak veroorzaakt door een verstoring in de bloedstolling. Het bloedstolsel sluit het bloedvat geheel of gedeeltelijk af en dit geeft klachten van pijn, een rode kleur en zwelling van het been. Dit noemen we een trombosebeen. Als een deel van het bloedstolsel afbreekt en met de bloedstroom mee in de longen belandt, is er sprake van een longembolie. Een longembolie geeft kortademigheid en pijn op de borst en, afhankelijk van de grootte van de embolie, kan de bloedtoevoer naar de longen vanuit de rechter kamer van het hart geheel belemmerd raken, waardoor de patiënt in korte tijd kan overlijden. Leeftijd is een belangrijke risicofactor voor veneuze trombose. In de leeftijdscategorie 25-30 jaar komt veneuze trombose ongeveer bij 1 per 10 000 personen per jaar voor, terwijl bij 85+ers veneuze trombose bij 8 per 1000 per jaar voorkomt.

In dit proefschrift worden risicofactoren van veneuze trombose bij ouderen onderzocht. We onderzoeken verschillende verworven en genetische afwijkingen die in ouderen het risico op trombose kunnen beïnvloeden . Daarnaast bestuderen we de rol van verouderde veneuze kleppen en symptomen van veneuze insufficiëntie op het risico op trombose. Tot slot bekijken we de rol van de globale assay ‘clot lysis time’ om te zien of we met deze assay een recidief trombose kunnen voorspellen.

In dit proefschrift worden de resultaten van drie klinische onderzoeken gepresenteerd.

### Het AT-AGE onderzoek

‘AT-AGE’ staat voor Age and Thrombosis, Acquired and Genetic risk factors in the Elderly. Het AT-AGE onderzoek is een patiënt-controle onderzoek uitgevoerd in Leiden en Burlington, Vermont om risicofactoren voor veneuze trombose in specifiek de ouderen te onderzoeken. We hebben patiënten die voor het eerst een

veneuze trombose kregen na hun 70e levensjaar en controles die nog nooit een veneuze trombose hadden gekregen, gevraagd mee te doen. Ouderen worden vaak uitgesloten van wetenschappelijk onderzoek, vanwege moeilijkheden om het studiecentrum te bereiken, visuele en cognitieve comorbiditeiten, of allerlei andere leeftijdgerelateerde beperkingen. Daarom hebben we in het AT-AGE onderzoek alle participanten thuis bezocht en door middel van interviews de vragenlijsten ingevuld. We hebben 401 patiënten met een eerste trombose en 431 controles zonder trombose geïncludeerd en bezocht.

**Het Batavia onderzoek**

Leeftijd is een belangrijke risicofactor voor veneuze trombose. Veroudering van de vaatwand in bloedvaten leidt tot een trombogene omgeving. In de veneuze vaten zijn kleppen aanwezig om te assisteren bij de richting van de bloedstroom. In de onderbenen voorkomen veneuze kleppen terugstroom van het bloed naar lagere regionen. In eerder Leids onderzoek is naar voren gekomen dat veneuze kleppen dikker worden naarmate ze verouderen. Ook de functionaliteit van de kleppen wordt slechter.

In het Batavia onderzoek bestuderen we de associatie tussen verouderde veneuze kleppen en het toegenomen risico op veneuze trombose. 'Batavia' staat voor Biology of Ageing and Thrombosis: Appraisal of Valve thickness and function, an In vivo Assessment. Ook het Batavia onderzoek is een patiënt-controle onderzoek. In dit onderzoek hebben we enerzijds de associatie tussen klepdikte en het risico op trombose en anderzijds de associatie tussen klepfunctie en het risico op trombose bestudeerd.

De deelnemers van het AT-AGE onderzoek zijn gevraagd om mee te doen in het Batavia onderzoek. Alle participanten waren ≥70 jaar. De deelnemers werden uitgenodigd naar het LUMC voor een echo-onderzoek om de dikte en functionaliteit van de klep in de vena poplitea te meten.

## Het THE-VTE onderzoek

'THE-VTE' staat voor Thrombophilia, Hypercoagulability and Environmental risks in Venous ThromboEmbolism. Het THE-VTE onderzoek is uitgevoerd in Leiden en Cambridge, Verenigd Koninkrijk, om globale teststrategieën te ontwikkelen teneinde patiënten met een verhoogde stollingsneiging te identificeren. Globale assays zouden het mogelijk kunnen maken om patiënten met een verhoogd risico op een eerste en recidief trombose te herkennen. In Nederland zijn de patiënten geïdentificeerd uit de Trombosedienst in Leiden en in de Verenigd Koninkrijk zijn de patiënten geïdentificeerd in het Addenbrooke's ziekenhuis in Cambridge. De partners van patiënten zijn gevraagd om als controles mee te doen. Alle participanten waren 18-75 jaar. De globale assay om fibrinolyse te meten is onderzocht voor het risico op een eerste en recidief trombose.

In **hoofdstuk 2** laten we zien dat aandoeningen van de luchtwegen bij ouderen, namelijk bronchitis, COPD, en astma, vooral het risico op een longembolie vergroten (odds ratio [OR] 2.2, 95% betrouwbaarheidsinterval [BI] 1.5-3.3) en in mindere mate het risico op een trombosebeen (OR 1.2, 95% BI 0.7-1.9). We hebben een hypothese geformuleerd om dit verschil in risico's te verklaren. Lokale ontstekingen bij luchtwegaandoeningen zouden een aanleiding kunnen vormen om lokale trombose te veroorzaken. Toekomstig onderzoek zal moeten uitwijzen of lokale trombi, als gevolg van ontstekingen, longembolieën kunnen veroorzaken.

**Hoofdstuk 3** bevat de resultaten van genetische risicofactoren in ouderen (factor V Leiden mutatie, protrombine mutatie, non-O bloedgroep) alsmede een positieve familiegeschiedenis van trombose, en het risico op het krijgen van een eerste veneuze trombose. We zien dat de factor V Leiden mutatie het risico op trombose 2.2-keer verhoogt, protrombine mutatie 1.4-keer, non-O bloedgroep 1.3-keer, en een positieve familiegeschiedenis van trombose 2.1-keer. We concluderen dat ook in ouderen genetische risicofactoren een rol blijven spelen bij het ontstaan van trombose. Het vragen naar een positieve familiegeschiedenis is makkelijk en

goedkoop, en we stellen voor dat toekomstig onderzoek zich richt op het includeren van familiegeschiedenis in predictiemodellen voor trombose.

In **hoofdstuk 4** bekijken we klinische uitingen van veneuze insufficiëntie, namelijk oedeem, spataderen en ulcus cruris in de AT-AGE studie. We hebben het risico op veneuze trombose berekend bij aanwezigheid van deze klinische beelden. Het risico op trombose wordt 2.9-keer (95% BI 1.9-4.3), 1.6-keer (95% BI 1.2-2.3), en 3.0-keer (95% BI 1.5-6.2) verhoogd bij aanwezigheid van respectievelijk oedeem in de onderbenen, spataderen en ulcus cruris.

**Hoofdstuk 5** wordt de anatomische verdeling gerapporteerd van veneuze trombose in patiënten ≥70 jaar. We zien dat bij ouderen longembolieën vaker voorkomen dan trombosebenen (53.7% vs. 43.6%), terwijl onderzoek uitgevoerd in jongere mensen ons leert dat het omgekeerde het geval is bij hen. Ook zien we dat een trombose in het linkerbeen vaker voorkomt (53.7%) dan trombose in het rechterbeen (43.6%).

Resultaten van het Batavia onderzoek worden besproken in **hoofdstuk 6**. In verschillende post-mortem onderzoeken komt naar voren dat trombose vaak ontstaat rondom veneuze kleppen. Veneuze kleppen worden dikker naarmate men ouder wordt. Tussen de klep en de vaatwand circuleert de bloedstroom op een zeer lage snelheid, waardoor stase en een lage zuurstofspanning ontstaat. Dit leidt tot nog meer klepschade, en mogelijk tot klepdysfunctie die zich uit in reflux. Wij hebben gevonden dat een dikke klep en veneuze reflux risicofactoren vormen voor veneuze trombose in ouderen. Een verdikte klep deed het risico op trombose 2.9-keer verhogen (95% BI 1.0-8.0), veneuze reflux langer dan 1 s deed het risico 2.8-keer verhogen (95% BI 0.8-10.4).

In **hoofdstuk 7** wordt de relatie van een maat van het fibrinolytisch systeem, de ‘clot lysis time’ (CLT), en het risico op veneuze trombose besproken. Naast een ongeremde stollingsneiging, kan ook een gestoorde fibrinolyse, de capaciteit om bloedstolsels op te ruimen en te remmen, het risico op trombose verhogen. Dit

onderzoek is uitgevoerd in patiënten en controles ≤75 jaar van het THE-VTE onderzoek en laat zien dat een lange CLT een risico vormt voor een eerste trombose. In de vervolgstudie van THE-VTE zijn patiënten met een eerste veneuze trombose gemiddeld 4.8 jaar gevolgd om te bepalen of zij een recidief kregen. Een verlengde CLT bleek geen voorspeller te zijn voor een recidief trombose. Toekomstig onderzoek zal moeten uitwijzen of deze resultaten ook gelden voor de oudste ouderen (>75 jaar). Vooralsnog is er gering onderzoek aanwezig dat recidief trombose bij de ouderen kan voorspellen.

**Tot slot**

In dit proefschrift hebben wij risicofactoren voor veneuze trombose bij ouderen onderzocht. Onze resultaten laten zien dat luchtwegaandoeningen bij ouderen vooral het risico op longembolieën vergroten. Bij de etiologie van veneuze trombose spelen bij ouderen ook genetische risicofactoren nog steeds een rol. Verder laten we zien dat tekenen van veneuze insufficiëntie het risico op trombose bij ouderen vergroot, dat longembolieën vaker dan trombosebenen voorkomen in deze populatie, en dat verouderde veneuze kleppen een risicofactor vormen voor veneuze trombose. Toekomstig onderzoek is nodig om onze resultaten te bevestigen en om hoog-risico groepen te identificeren binnen de ouderen die in aanmerking kunnen komen voor preventief tromboseprofylaxe.

# Curriculum vitae

Alev Karasu werd op 28 november 1987 geboren te 's-Gravenhage. In 2006 behaalde zij haar gymnasiumdiploma aan het Atlas College te Rijswijk, waarna zij Geneeskunde studeerde aan de Universiteit Leiden. Tijdens haar studie nam zij deel aan de zomerstudies 'Infectious Diseases and Tropical Medicine' en 'Disaster Medicine and Management' in Egypte en Indonesië. Na haar derde jaar deed zij een onderzoeksstage bij Centre Hospitalier Universitaire Sainte-Justine in Montréal, Canada. In haar vierde jaar in 2011 verbleef ze gedurende 5 maanden in Cambridge voor haar wetenschappelijk stage naar vertraagde fibrinolyse en het risico op veneuze trombose, onder begeleiding van dr. Trevor P. Baglin en dr. Astrid van Hylckama Vlieg. Dit leidde tot een promotietraject van gedurende 4 jaar op de afdeling Klinische Epidemiologie van het Leids Universitair Medisch Centrum onder supervisie van dr. Astrid van Hylckama Vlieg en Prof. dr. Frits R. Rosendaal, waarvan de resultaten in dit proefschrift zijn beschreven. Zij heeft de Batavia studie opgezet en aan de AT-AGE en MEGA studies gewerkt. Daarnaast heeft zij de opleiding tot epidemioloog B gevolgd en onderwijs gegeven aan (bio)medische studenten. Alev presenteerde de resultaten van haar werk op nationale en internationale congressen, en ontving tweemaal de Young Investigator Award van de International Society on Thrombosis and Haemostasis (2012 en 2015). Begin 2016 startte ze met haar coschappen en in 2017 behaalde ze haar artsexamen. Sinds januari 2018 werkt zij als basisarts bij WoonZorgcentra Haaglanden.

# Dankwoord

Ik wil graag iedereen bedanken die op enigerlei wijze heeft bijgedragen aan de totstandkoming van dit proefschrift. Allereerst uiteraard de deelnemers van de THE-VTE, AT-AGE en Batavia onderzoeken. Het contact met de bijzondere groep mensen die op hun oude dag nog enthousiast mee wilden doen met wetenschappelijk onderzoek maakt mij bescheiden.

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