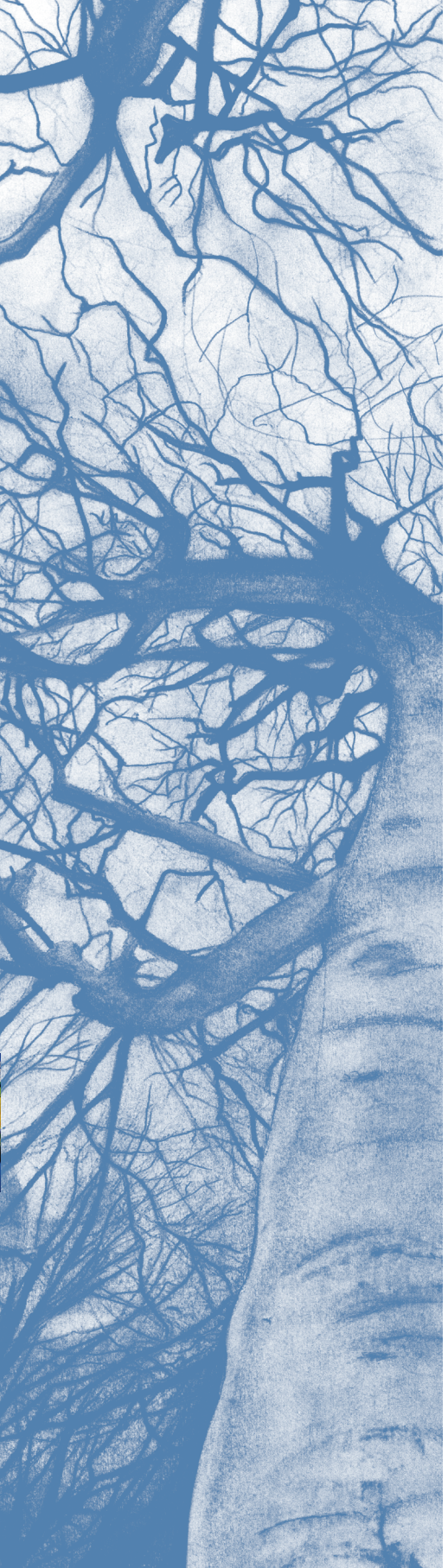

MARISSA ENGBERS

Conventional and age-specific risk factors for venous thrombosis in older people

The AT-AGE study

CONVENTIONAL AND AGE-SPECIFIC RISK FACTORS FOR VENOUS THROMBOSIS IN OLDER PEOPLE

The AT-AGE study

Marissa Josephina Engbers

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Promotor:
Prof dr. F.R. Rosendaal

Copromotores:
Dr. A. van Hylckama Vlieg
Dr. J.W. Blom

Leden promotiecommissie:
Prof. dr. J. Gussekloo
Prof. dr. S. Middeldorp, *Academische Medisch Centrum, Amsterdam*
Prof. dr. J.P. Mackenbach, *Erasmus Medisch Centrum, Rotterdam*

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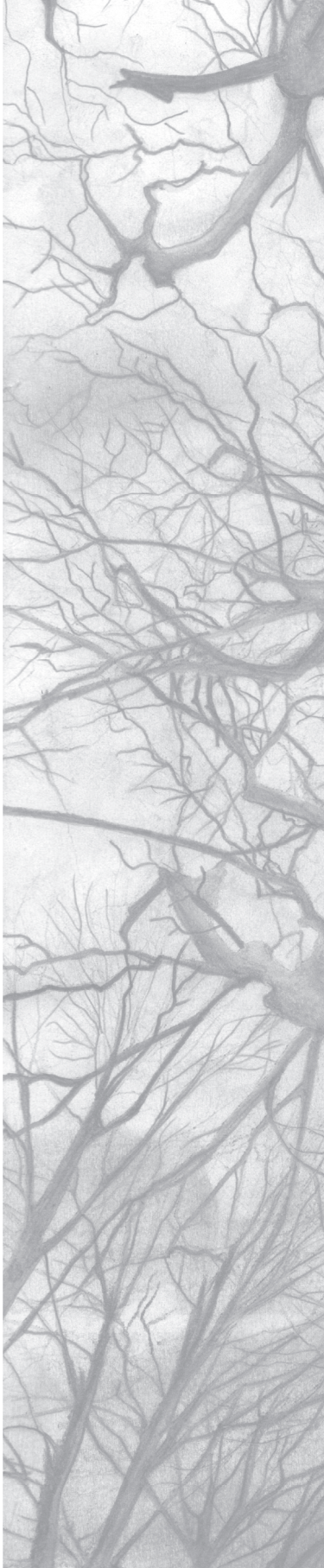

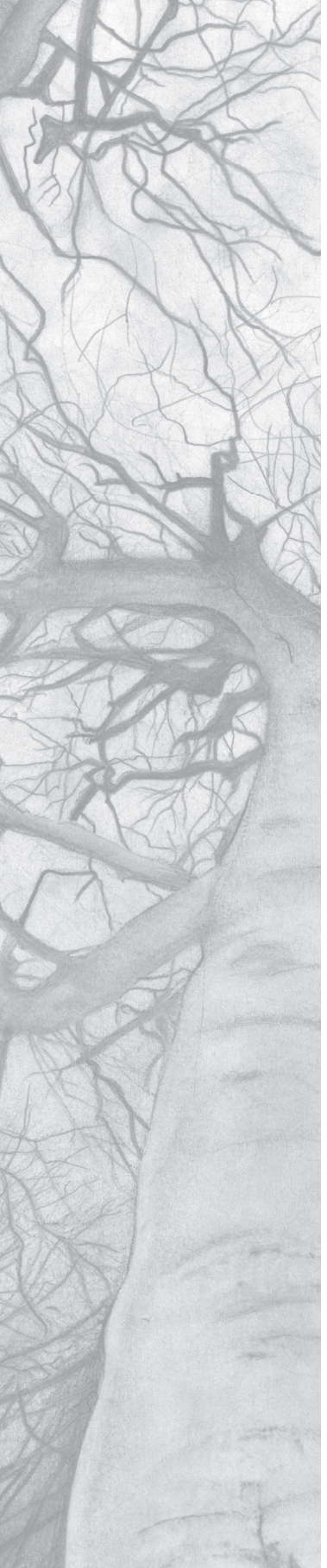

CHAPTER 1

General introduction and outline of thesis

VENOUS THROMBOSIS

Clot formation is a natural and vital mechanism in quickly sealing disruption of vessel walls, thereby preventing excessive bleeding. Vessel wall disruption leads to tissue factor exposure to the blood which induces factor X conversion into factor Xa, subsequent activation of prothrombin into thrombin, and finally activation of fibrinogen into insoluble fibrin. Platelets aggregate at the site of the injury and together with fibrin a blood clot is formed. Once in place, a clot is not a passive entity but the result of a delicate balance between pro- and anticoagulant activity. When the vessel wall is sufficiently repaired, procoagulant activity is reduced again by plasmin, which degrades fibrin clots. This will reduce and eventually eliminate the clot. However, if coagulation is overactivated or if inhibition is incomplete or fails, excessive clot formation occurs. [1] Situations in which this excessive thrombus formation blocks the vein and hinders blood flow, are called venous thrombosis. The two main clinical manifestations of venous thrombosis are deep vein thrombosis of the lower extremities (deep venous thrombosis of the leg, DVT) and embolisation of a dislodged clot to the pulmonary arteries (pulmonary embolism, PE).

Venous thrombosis is a common disease of which the annual incidence is approximately 1 per 1000 persons. [2] In young and middle-aged individuals many risk factors have been established such as immobilisation, oral contraceptives, surgery, fractures, and the use of plaster casts. Furthermore, genetic risk factors such as the factor V Leiden mutation are known to increase the risk of venous thrombosis. [3] With age, the incidence of venous thrombosis increases sharply. [4] E.g., the incidence of venous thrombosis is more than 10-fold higher in octogenarians than in middle-aged individuals (40-50 years). Venous thrombosis will pose an increasing medical and economic burden in the future, given the worldwide growth of the older population. In developed countries, in the upcoming five decades the number of people of 70 years and older will quadruple. [5] In the Netherlands, 15% of the population is 65 years and older, which will increase to 25% in de next 30 years. [6] This shift in the age distribution in the population will lead to an increase in the prevalence and incidence of diseases such as venous thrombosis, which will be challenging for policy makers and healthcare professionals. Due to the increased incidence of venous thrombosis in older age, an improved understanding of the etiology of this disease is specifically needed in the older population in order to target adequate preventive measures.

Virchow has described three mechanisms that contribute to thrombus formation. Stasis of blood flow, damage to the vessel wall (e.g., by hypoxia) and changes in blood composition. [7] It is unknown what causes the increase of the incidence of venous thrombosis in the older people, i.e., what is the role in older individuals of the well-known ''conventional' risk factors of thrombosis, such as immobilisation due to hospital admission and surgery, and whether we can distinguish age-specific risk factors for thrombosis.

In general, there is a lack of studies involving older people. This phenomenon, referred to as "ageism", has occurred in several fields of medical research. This may have practical reasons, e.g., when researchers anticipate mobility problems for older people to reach the study centre, or when excluding older people is inherent to the study question, e.g., researchers expect genetic risk factors to stand out most in young individuals. The difficulties of recruiting older people for research are illustrated by the 'Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis' (MEGA) study [8], a large case-control study on risk factors of venous thrombosis in 18-80 years old individuals performed in the Netherlands (1999-2004). This study discontinued the recruitment of individuals aged 70 to 80 after one year due to a low participation rate (*MEGA-study, data not published*). In 2007, our study group reported that few risk factors have been studied in older people. [9] Knowledge about conventional and age-specific risk factors for venous thrombosis in the older population is insufficient. This was the rationale behind the "Age and Thrombosis, Acquired and Genetic risk factors in the Elderly", or AT-AGE study, a case-control study among older individuals.

THE AT-AGE STUDY

The AT-AGE study is a two-centre, population based case-control study in Leiden, the Netherlands and Burlington, Vermont, US. From June 2008 to August 2011 in Leiden and December 2008 to July 2011 in Vermont, all consecutive patients aged 70 years and older with deep venous thrombosis (DVT) in the leg or a pulmonary embolism (PE), were identified. In Leiden, cases were identified from two anticoagulation clinics in a defined geographical area in the western part of the Netherlands. In Vermont, cases were identified in the Vascular Laboratory and the Radiology department of University of Vermont Health Centre, Burlington. Control subjects were randomly selected from primary care practices in Leiden and Vermont. The AT-AGE study was specifically designed to optimise the participation-rate in the older population. Therefore, we performed home visits to all participants. A home visit enabled us to perform an interview, rather than sending a questionnaire, since the latter is often difficult for older individuals leading to a decrease in the participation rate. The interview assessed conventional thrombosis risk factors that have been established in young and middle-aged people and putative age-specific risk factors, e.g., the presence of varicose veins and functional status. The interview was conducted by trained research assistants, in order to minimise missing data and potential misinterpretation of questions by the participant. In addition, during the home visits a blood sample was drawn and we performed multiple anthropometric measurements and physical tests relevant in the older population, e.g., hand grip strength and gait speed.

OUTLINE OF THIS THESIS

The main objective of this thesis is to determine the risk of venous thrombosis associated with conventional and age-specific risk factors in older people. Moreover, the contribution of these risk factors to the increased incidence of venous thrombosis in older people is assessed. To this aim, we review, in **chapter 2**, the current literature (until 2010) on the effects of conventional risk factors as well as age-specific risk factors for venous thrombosis in older people. In **chapter 3** we elucidate to what extent immobility is a risk factor for thrombosis in older people. We assess the risk of venous thrombosis associated with hospitalisation, surgery, fracture, plaster cast (or splint) use, minor injuries, and transient immobility at home. In **chapter 4** we assess whether the two common genetic risk factors in younger people, i.e. factor V Leiden (FVL, rs6025) and prothrombin 20210A mutation (PT20210, rs1799963) are risk factors for a first venous thrombosis at older age. Moreover, the association of non-O blood type, and a positive family history of thrombosis and thrombosis is determined. In **chapter 5 and 6** we focus on "age-specific" risk factors. In **chapter 5** we determine the risk of venous thrombosis associated with clinical features of venous insufficiency, specifically a history of varicose veins, leg ulcer, and the presence of leg oedema. In **chapter 6** we focus on the presence of functional impairment in older individuals and the risk of venous thrombosis. In **chapter 7** we study the participation rate of older patients in research studies with a focus on practical aspects of the study design (i.e., home visits instead of inviting participants to a study centre). Because in the AT-AGE study blood samples are obtained at home, these could not be processed directly. In **chapter 8**, we study whether the time between venipuncture and blood processing and difference in storage temperature affected the levels fibrinogen, prothrombin, factors VIII, IX, XI and anti-thrombin. Finally, in **chapter 9**, we summarise the main results of this thesis and reflect on the strength and limitations of our findings and we translate our findings into implications and recommendations for future research.

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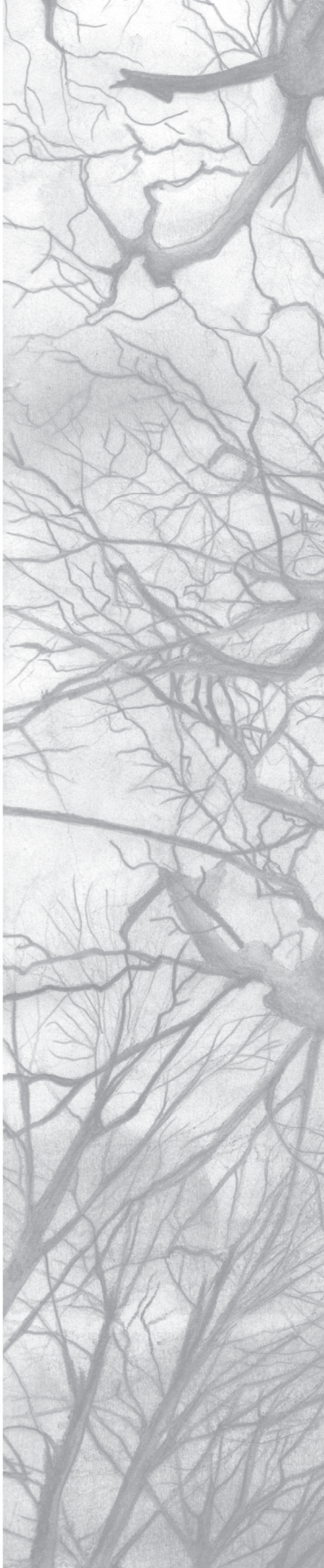

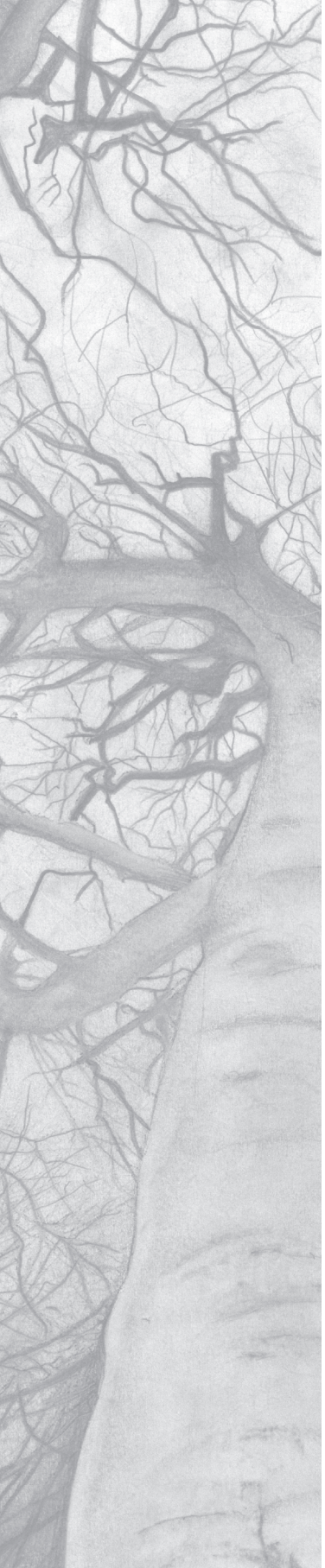

CHAPTER 2

Venous thrombosis in the elderly: incidence, risk factors and risk groups

M.J. Engbers
A. van Hylckama Vlieg
F.R. Rosendaal

J Thromb Haemost. 2010; 10: 2105-12

ABSTRACT

Ageing is the strongest risk factor for venous thrombosis. The cause of this steep age-gradient is as yet, unexplained. The aim of this review was to provide an overview of studies on the effect of conventional risk factors as well as age-specific risk factors for venous thrombosis in the elderly.

Limited data are available on risk factors for venous thrombosis in the elderly, i.e., all results are based on small study groups. Results indicate that, of the conventional risk factors, malignant disease, the presence of co-morbidities and the genetic risk factors factor V Leiden and the prothrombin mutation seem to be associated with an increased risk of venous thrombosis in the elderly. Age-specific risk factors of thrombosis, i.e., endothelial dysfunction, diminished muscle strength, and frailty may be important in the explanation of the increased incidence of venous thrombosis in the elderly.

In conclusion, since ageing is the strongest risk factor for venous thrombosis, further identification of the risk factors for thrombosis in the elderly is needed to elucidate the age-gradient of the incidence of venous thrombosis and to target preventive measures.

INTRODUCTION

Thrombosis in the venous system, i.e., deep venous thrombosis or pulmonary embolism is a multicausal disease. The well-established or "conventional" risk factors are mostly identified in young and middle-aged populations. Conventional risk factors can be acquired, e.g., immobility, surgery, malignant disease, and hormone use, whereas the most well-known genetic risk factors are deficiencies of natural anticoagulants protein C, protein S, and antithrombin and the Factor V Leiden and prothrombin 20210A mutations [1-3].

The overall incidence of a first symptomatic venous thrombosis in the general population is 1-2 per 1000 person-years (py). In the age-group 25 to 30 years old, venous thrombosis occurs in ~1 per 10,000 py as compared to nearly 8 per 1000 py in the 85 years and older population [4]. The risk of developing venous thrombosis is therefore 80-fold increased in the older population, leading to a high attributable risk. The population attributable risk is more than 90%, indicating that 90% of the total incidence of thrombosis in the population can be ascribed to ageing. The life-time risk (cumulative incidence) of venous thrombosis is up to 15% in those aged 90, and ~60% of all venous thrombosis events occur in those aged 70 years and older [4].

It is therefore clear that ageing is by far the strongest risk factor for venous thrombotic disease, resulting in a high incidence of venous thrombosis in the elderly population (figure 1) [1-3]. The strong age-gradient can, at least in part, be explained

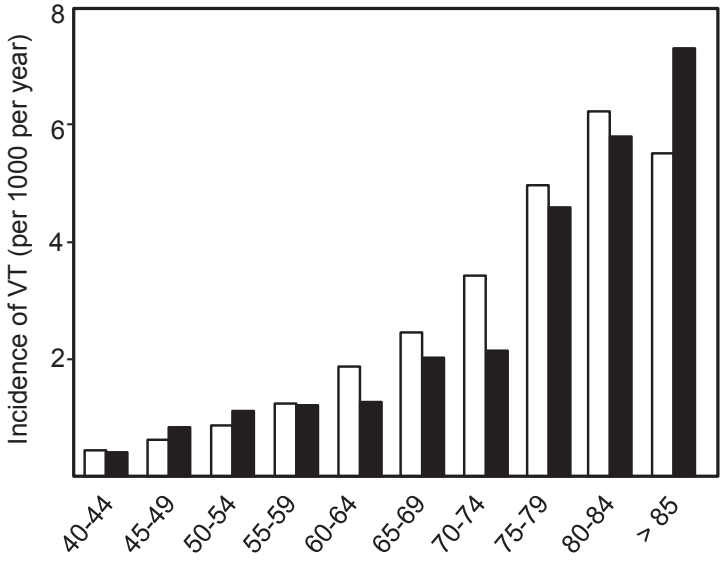

Figure 1. Incidence of first venous thrombosis (VT) [deep vein thrombosis (DVT) and pulmonary embolism (PE)] stratified by age group and gender. Rates are shown by 1000 per year, for man in open bars , for women in closed bars. *Adapted from Naess et al. (2007).

by an increased prevalence of conventional risk factors and the presence of other, age-specific, risk factors. The incidence of venous thrombosis is similar for men and women indicating that, given the excess of women in the old age groups, most elderly patients with venous thrombosis are women [5]. Furthermore, a difference in the distribution of deep venous thrombosis and pulmonary embolism between young and old people has been described. In one study in middle-aged individuals (40 to 44 years old) deep vein thrombosis (incidence of 0.3/1000 py) was three times more frequent than pulmonary embolism (incidence of 0.1/1000 py) [6]. In patients of 80 to 84 years old, pulmonary embolism (incidence of 5.5/1000 py) was more frequently diagnosed than deep vein thrombosis (incidence of 3.0/1000 py) [6]. These findings suggest that also an increase in the embolisation of clots may contribute to the age-gradient. However, in contrast, a large European registry found that deep vein thrombosis was more often diagnosed than pulmonary embolism in the 80 to 84 year old individuals (incidences of 3.8/1000 py for deep vein thrombosis compared to 2.2/1000 py for pulmonary emboli) [4].

In general, people over 60 years are considered to be 'the elderly' [5]. Why does thrombosis incidence increase with age? Several explanations can be postulated. Ageing may be associated with an increased prevalence of conventional risk factors or development of new, age-specific risk factors. In addition, risk factors may have synergistic effects conditional on age, i.e., the effect of a factor on the risk of thrombosis may be different in young and middle-aged populations compared with the elderly.

The interpretation of the effect of a risk factor for venous thrombosis in elderly populations is difficult as new risk factors may develop and accumulate during life. In addition, age-related changes of the association between a risk factors and venous thrombosis could be explained by the phenomenon: "attrition of susceptibles", i.e., individuals highly vulnerable to a risk factor are likely to develop thrombosis early in life, resulting in an elderly population that is less affected by this risk factor, leading to a weakened association between this risk factor and the occurrence of a first venous thrombosis in the elderly.

Several statistical measures can be used to express the importance of a risk factor in the development of disease. The rate difference ($I_e - I_0$), i.e. the difference of the incidence in the presence (I_e) or absence of the risk factor (I_0) indicates the excess number of cases due to the presence of a risk factor in a certain time period. The proportion of disease among those with the risk factor which can be attributed to that risk factor is expressed as the attributable risk (AR: $I_e - I_0$)/ I_e. A population attributable risk (PAR= (I - I_0)/ I) indicates what proportion of the total incidence of a disease in the general population can be attributed to a certain risk factor. The latter is dependent on the prevalence of the risk factor in the general population. While the AR is also called 'aetiologic fraction', the PAR is the 'preventable fraction', for it indicates for a specific population which proportion of the burden of a certain disease could be removed by complete removal of the risk factor.

The role of many conventional and age specific risk factors in the explanation of the strong age-gradient of venous thrombosis has not before been summarised. This review will provide an overview of the prevalence and effect of conventional risk factors as well as age-specific risk factors for venous thrombosis in the older population. We will, wherever possible, calculate (population) attributable risks, to quantify the impact of risk factors on venous thrombosis and to compare the risk in young and the elderly populations. Furthermore, using available information on risk factors for venous thrombosis in the elderly, we will attempt to identify high-risk groups within the elderly population.

CONVENTIONAL RISK FACTORS

Table 1 shows conventional risk factors of venous thrombosis and attributable and population attributable risks in the young and older population are provided.

Immobility

Immobility, leading to rheologic changes by increasing blood viscosity and stasis, is associated with an increased risk of venous thrombosis. [7,8] There is no single definition of immobility. Differences in the definition for immobilisation, and the presence of underlying conditions while immobilised, such as hospitalisation because of surgery, make it difficult to provide one estimate of the strength of the association with thrombosis.

In a meta-analysis by Pottier *et al.*, a pooled odds ratio (OR) of more than two was reported for immobilised compared with non-immobilised medical patients, not taking into account the age of the patients. [9] In hospitalised patients aged >65 years,

Table 1. Conventional risk factors of venous thrombosis: attributable and population attributable risks in the young and older population

Conventional risk factors VT	Young AR (%)	Old AR (%)	Young Prev (%)	Old Prev (%)	Young PAR (%)	Old PAR (%)
Immobilisation*	50–90	66–83	10	25	9–47	33–56
Malignancy†	86	86	3	10	15	35
CHF‡	60–71	33–60	5	22	7–11	10–25
COPD§	50-80	33	1	11	1–4	5
DM	50	0–50	6	16	6	0–14
HRT use**	50	50	4	1	4	1
Genetic factors††	67–86	50–80	7	7	12–30	7–22

VT, venous thrombosis; Young, young and middle-aged population (< 65 years old); Old, older population (≥ 65 years old); AR, attributable risk; Prev, prevalence; PAR, population attributable risk; CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HRT, hormone replacement therapy. *Ref. [7–13,18]. †Ref. [11,19–23]. ‡Ref. [10,24–28]. §Ref. [32–34]. ¶Ref. [35–40]. **Ref. [51–62]. ††Ref. [70–76].

short-term bed rest (up to 14 days) was associated with an almost six-fold increase in thrombotic risk compared with patients without bed rest. [10] Furthermore in young and middle-aged populations, hospitalisation was found to contribute to venous thrombosis in more than half of all cases in the general population (PAR: 50%), and a 8 to 10-fold increased risk of venous thrombosis during hospitalisation has been reported. [11] Surgery leads to a six-fold increased risk of venous thrombosis [12] and approximately three percent of individuals undergoing lower limb arthroplasty (whose average age is over 65) develop venous thrombosis even when they receive prophylactic medication. [13] The thrombotic risk associated with hospitalisation has not been studied specifically in the elderly population; however, since the prevalence of hospitalisation doubles with age (from ~ 10% in the population of 45 to 64 years to 25% in individuals of 65 years and older), the population attributable risks will be highest in the elderly. (PAR young ~10%, PAR old: ~40%). [9-15]

Numerous other causes for immobility can be identified, e.g., plaster cast and prolonged travel. Lower extremity injury and plaster casts often lead to immobilisation, and both have been identified as strong risk factors for venous thrombosis in the young and middle-aged population [14]; however, they have not been studied in the elderly.

Furthermore, long-haul travel leads to short-term immobility and was associated with a two-fold increased risk of venous thrombosis in the young and middle aged population in the MEGA study. [15,16] Air travel was found to affect the thrombotic risk also in the eldest subgroup of the study (50-71 years), but their absolute risk of thrombosis was lower than in the younger group. [17] The frequency of air travel is four-fold higher among young and middle aged than in the older population. Therefore, the population attributable risk will be lower in the elderly population than in the young and middle-aged indicating that air travel contributes mainly to the overall burden of thrombosis in the younger population. [18]

Long-term reduced mobility predominantly affects the elderly. In the Netherlands, ~10% of the people aged 45 to 64 years have disabilities affecting mobility, increasing to more than one third of the 75 years and older individuals. [19] Long-term immobility appears to be associated with the risk of venous thrombosis, although the risk appears to be strongest in the first four weeks of bed rest (3 to 4-fold increased). Similar results have been reported for arm chair immobilisation in a 65 years and older population. [10,20]

Malignancy

The overall risk of venous thrombosis in cancer patients is seven-fold increased compared with individuals without cancer. [11,21] This risk differs according to the type and location of the primary tumor, the extensiveness of the disease, and the cancer therapy. [22] Little information is available on the estimates of the risk of venous thrombosis

associated with malignancy in specifically the elderly patients. The absolute risk of venous thrombosis for ovarian cancer patients (treated with surgery and chemotherapy) of 61 years and older was reported to be ~250 per 1000 patients years. [23] Similar to the young and middle-aged population, pancreatic, ovarial and brain tumors are described to be associated most strongly with venous thrombosis in the elderly cancer patient. [4,22,24] Furthermore, cancer associated venous thrombosis is more prevalent in the population of 70 years and over than in a younger population. [25] This can be explained by the increase of the incidence of malignancy with age (i.e., three-fold increase of incidence for those over 65 years compared with those younger than 65 years). [19] As the present literature suggests that a seven-fold increased risk of development of thrombosis in cancer patients exists, we can conclude that the PAR is 15% in the younger population and 35% in the elderly.

Co-Morbidity

Diseases that have been identified to affect the risk of thrombosis are, e.g., heart failure, stroke, chronic obstructive pulmonary disease (COPD) and diabetes mellitus. Congestive heart failure has been associated with a 2.5 to 3.5-fold increase of the risk of venous thrombosis. [26,27] This risk was mainly increased for pulmonary emboli. These risk estimates were reported in studies among patients with a mean age of 60 years and older. In studies in which only patients older than 65 years (median 82 and 85 years) were included, risk estimate of ~1.5 to 2.5 were reported [10,28]. As congestive heart failure is > four times more prevalent in individuals of 75 years and older compared with those of 55 to 74 years old (prevalence ~22% versus ~5%), it contributes more to the incidence of thrombosis in the elderly than in the middle aged population. [29-32]

Stroke increases the risk of venous thrombosis 1.3-3.5-fold in elderly inpatients (>65 years old). The severity of stroke is positively associated with the risk of thrombosis. [28,33,34] The risk of venous thrombosis in younger stroke patients has not been studied. However, it is likely, as stroke is a disease of the elderly, [34,35] that the population attributable risk will be higher in older than in middle-aged populations.

Chronic obstructive pulmonary disease (COPD) is a risk factor for pulmonary embolism and deep venous thrombosis. In individuals aged over 60, the presence of COPD was associated with a 1.2-1.4 increased risk of pulmonary embolism. [36,37] In a younger population (aged 40 to 59 years) the relative risk was higher, i.e., 2 to 5. As COPD is ten times more prevalent in the elderly than in the younger population (75 to 79 years: prevalence ~11%; 45 to 49 years 1%), population attributable risks are less than 5% for the overall COPD population. In the elderly population with thrombosis the highest contribution of COPD is expected. [19,38]

In a meta-analysis it was shown that individuals with diabetes mellitus have an almost 50% increased risk of venous thrombosis compared with individuals without

diabetes. [39] However, individual studies report contradictory results on the risk of thrombosis associated with diabetes, i.e., risk estimates range between no effect (RR=1) to a twofold increased risk for those with diabetes compared with those without the disease. [40-42] The risk of venous thrombosis associated with diabetes was reported to be higher in the younger (RR = ~2) than in the older population (RR= 1.5). [40] Since diabetes mellitus is more prevalent in the elderly (16% vs. 6%) than in young and middle-aged individuals, this will indicate that the contribution of diabetes to the thrombosis incidence is approximately equivalent in the young and elderly population. [43,44]

Increasing age is positively associated with the prevalence of various chronic disorders. The prevalence of two or more chronic diseases has been estimated at 35% in 40 to 59 years old, and increases to almost 80% in people aged 80 and older. [45,46] Elderly with multi-morbidity are likely to be especially vulnerable to develop thrombosis as interaction of the separate effects of these risk factors may exist.

Hormone replacement therapy

Hormone replacement therapy (HRT) leads to a procoagulable state, with elevated levels of factors VII, IX, X, XII, and FXIII and decreased levels of the anticoagulant proteins antithrombin and protein S. [47,48] Hormone therapy is associated with a 2 to 3-fold increased risk of thrombosis in the middle-aged and elderly population. [49-54] Combined use of estrogen and progestin was reported to be associated with a higher thrombotic risk than with estrogen use only. [54] The relative risk of venous thrombosis associated with hormone use was similar for middle aged and elderly women, i.e., within age strata of 50-59 years, 60-69 years, and 70-79 years, the relative risk of venous thrombosis for hormone users was two-fold increased compared with non-use [55,56]. The publication of studies reporting an increased risk of complications associated with hormone use, e.g. breast cancer, led to a more than 50% decrease in the use of post-menopausal hormones. [57] In the Netherlands, HRT is used predominantly by middle aged women, and less so in the elderly, i.e., HRT use in 2004 was 4% in women aged 50 to 54 years and 1% in women aged 70 to 74 years. [58] Therefore, while HRT use is associated with a similar relative risk of thrombosis in the elderly compared with middle aged women, it is associated with a lower population attributable risk in the elderly due to its scarce use.

Haemostasis factors

Elevated levels of D-dimer, homocysteine, von Willebrand Factor (vWF) and coagulation factors, FVIII, FIX, FXI, fibrinogen, and prothrombin are all associated with increased risks of venous thrombosis, with roughly a doubling of the risk for those with a coagulation factor level in the upper 10 percent (P90) of the population distribution of levels. [59-63] With increasing age plasma levels of many haemostatic factors are also increasing e.g., fibrinogen, FVIII, FVII, d-dimer and homocysteine. [60,64]

Scarce data are present comparing high and low levels of haemostatic factors in the elderly and their associated risk of venous thrombosis. Thus far, for high FVIII and VWF levels an increased risk of thrombosis in the elderly has been reported (2 to 3-fold) [62]. However, the association between high fibrinogen levels and the risk of venous thrombosis is still controversial. [62,65]

Cut-off points used in young and middle-aged populations for low and high levels in young individuals are difficult to interpret within the older population. Therefore, population attributable risks are not provided.

Genetic Risk factors

The most common genetic risk factors for venous thrombosis are the prothrombotic mutations factor V Leiden (FVL, rs6025) and the prothrombin 20210A mutation (PT20210, rs1799963). The prevalence of these genetic variants are similar in the young and elderly. [66-68] However, they vary widely between ethnic groups The factor V Leiden mutation increases the risk of venous thrombosis three to seven-fold in individuals younger than 70 years compared with non-carriers. [69-71] This mutation has also been associated with an increased risk of thrombosis in the elderly, i.e., in individuals over 60 years a five-fold increased risk of thrombosis has been reported. [72] Studies in the elderly population found a 1.5 to 4.5-fold increased risk of thrombosis for carriers of the PT20210A mutation as compared with wild type carriers. [73,74] Thus, the factor V Leiden and the PT20210A mutation increase the risk of thrombosis 2 to 5-fold, and their prevalence is 7%. This implies that these two mutations are responsible for ~25% of all thromboses (PAR), regardless of age.

AGE-SPECIFIC RISK FACTORS

Age-specific factors are factors that are almost exclusively present in the older population. Although not studied in great detail yet, we can speculate that these factors are likely to explain at least partly, the steep age gradient in the risk of venous thrombosis.

Muscle strength

The overall muscle strength declines with age starting from the age of 50 years. [75] It is likely that this also affects the calf muscle pump. Indeed, it has been reported that both the calf compliance and the capacitance response, a marker for the redistribution of peripheral venous blood to the central circulation, decline over time. [76] Although the role of muscle strength of the lower limbs and the concomitant deterioration in the venous hemodynamics is not yet clarified, diminished function or efficacy of the calf

muscle pump could lead to reflux and stasis, which subsequently may lead to thrombus formation.

Endothelial dysfunction

Endothelial dysfunction is the alteration of the actions of the endothelium toward reduced vasodilation, and more prothrombic properties. Endothelial dysfunction is an important age-associated cardiovascular phenomenon. [77] With increasing age the anatomy of the venous vessel wall is modified, e.g., muscle fibers atrophy and valves thicken by an increase the number of collagen fibers. [78] Moreover, ageing has been associated with as shift towards less anticoagulant properties of the endothelium, i.e., it has been suggested that endothelial thrombo-resistance in the valves diminishes with age. [79] In addition, changes in laminar shear stress that for example occurs in varicose veins could diminish the release of anti-inflammatory and anticoagulant factors. [80] Hence, remodeling of the venous vessel wall with increasing age may contribute to the age gradient in the risk of thrombosis. The finding of an increased risk of venous thrombosis associated with microalbuminuria which may be a reflection of endothelial dysfunction strengthens this hypothesis. [81]

Venous insufficiency

The pathophysiology of chronic venous insufficiency consists of failure of valves through dilation of the venous wall or remodelling of the valve leaflets, which subsequently can lead to stasis and elevation of distal venous blood pressure. The prevalence of this functional disease increases with age. [82,83] Histological findings associated with venous insufficiency are increased collagen content of the vessel wall and disruption of the smooth muscle cells and elastic fibers. Currently it is unknown whether venous insufficiency is associated with an increased risk of thrombosis in the elderly.

AGE SPECIFIC RISK GROUPS

Venous thrombosis is a multicausal disease, i.e., more then one risk factor needs to be present simultaneously to cause the disease. Especially in the elderly risk factors are likely to accumulate. Therefore, high risk-profiles may often be present in the elderly, which may be specifically of interest when considering preventive measures, e.g., prophylactic treatment with anticoagulants.

Female sex

Women have a higher life expectancy than men, leading to a sex-ratio in favour of women in the elderly population. [5] As no sex-difference is reported for the risk of

thrombosis in the general population, this will lead to a higher proportion of women among elderly patients with venous thrombosis. Interest should therefore focus on risk factors for venous thrombosis with a high prevalence in women. These are likely to be of great importance in the explanation of the age gradient of the risk of venous thrombosis.

Nursing home

In the Netherlands, approximately 6% of the people of 65 years and older are institutionalised and half of the residents of these institutions are 85 years and older. [84] It was reported that living in a nursing home increased the risk of venous thrombosis eight-fold as compared with similarly aged individuals not institutionalised. [85] An incidence of venous thrombosis of 13 events per 1000 py was reported in nursing home residents. [86] However risk estimates are not yet reported for this specific group. The potential high risk of venous thrombosis in nursing homes would likely be the result of a high prevalence of other risk factors discussed before, e.g., immobilisation and co-morbidities.

Frailty

Buchner and Wagner defined the concept of frailty as "losses of physiologic reserve that increase the risk of disability" [87]. Many indices are used to objectify frailty. Most commonly deficits in health including restricted activity, disability in activities of daily living (ADL), and impairments in general cognition and physical performance are taken into account. Additionally, co-morbidity and self-rated health are often measured. It was shown that the percentage of individuals with frailty increases with age, from less than 4% in the 65-74 years old group to 25% in the 85 years and older age group, and the frail group appeared to have a 30% increased risk to develop venous thrombosis compared with the no-frailty. [88]

CONCLUSION

Despite the clear age gradient in the risk of venous thrombosis, limited data are available on risk factors for venous thrombosis in the age group that is mostly affected by the disease, i.e., the elderly population. Therefore, the risk estimates provided in this review are mostly based on small study groups and subgroup analyses.

Many conventional risk factors for venous thrombosis established in the young and middle-aged population are likely to also increase the risk of thrombosis in the elderly. Immobility, malignant disease, co-morbidities, and increased levels of coagulation factors remain associated with an increased risk of thrombosis in the elderly population. Furthermore, common genetic risk factors, e.g., the factor V Leiden and the prothrombin

20210A mutation are also associated with thrombotic risk in the elderly, although lower risk estimates as compared with the younger population were reported.

We found that many of the conventional risk factors such as, immobilisation, malignant diseases and co-morbidities are more prevalent in the elderly than in young and middle-aged individuals. The risk factors that contribute highly to the increased incidence of venous thrombosis in the elderly (i.e., with the highest PAR), are immobilisation and the genetic risk factors. We found that immobilisation contributes to more than 40% off all venous thrombotic events, and genetic factors explain between 7 to 22 % of the thrombotic events. Thrombotic risk factors that appear to contribute less in the incidence of thrombosis in the elderly are long haul travelling and the use of hormone replacement therapy.

We hypothesise that age-specific risk factors of venous thrombosis such as endothelial dysfunction and venous insufficiency in the elderly could in part clarify the strong age gradient of thrombosis. This also includes factors as frailty and institutionalised living.

The strong age-gradient in the risk of venous thrombosis has led to numerous reports on the association between risk factors and thrombosis in the elderly, however only in very small subgroups. Subsequently, only limited conclusions can be drawn. We speculated about the different factors that may play a role in the development of venous thrombosis and their role in the incidence gradient with ageing, though research specified on the most affected, older population is necessary to draw firm conclusions. Moreover, high risk groups need to be identified, in order to be able to target preventive measures such as prophylactic treatment with anticoagulants to high risk groups. This may result in the prevention of life-threatening side effects of this treatment such as major bleeding. In addition, complications and morbidities after a venous thrombotic event need to be evaluated in this potentially more vulnerable population.

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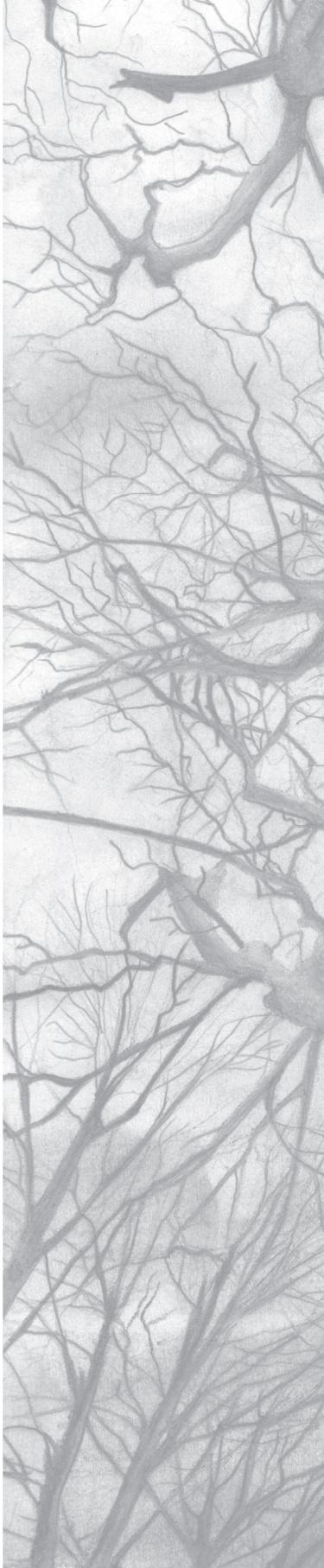

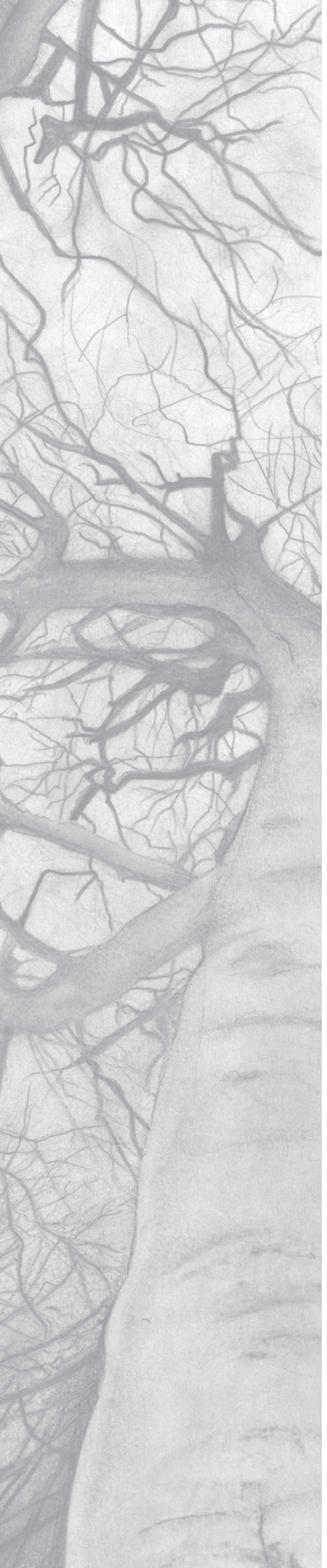

CHAPTER 3

The contribution of immobility risk factors to the incidence of venous thrombosis in an older population

M.J. Engbers
J.W. Blom
M. Cushman
F.R. Rosendaal
A. van Hylckama Vlieg

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ABSTRACT

Background

Venous thrombosis is common in the older population. Assessment of risk factors is necessary in order to implement preventive measures.

Objectives

We studied the associations between immobility-related risk factors and thrombosis, specifically, hospitalisation, surgery, fractures, plaster cast use, minor injuries, and transient immobility at home in an older population.

Methods

Analyses were performed in the Age and Thrombosis, Acquired and Genetic risk factors in the Elderly (AT-AGE) study, a two-centre population-based case-control study. Consecutive cases aged ≥70 years with a first-time thrombosis (n= 401) and control subjects ≥70 years old without a history of thrombosis were included (n= 431). Exclusion criteria were active malignancy and severe cognitive disorders. We calculated odds ratios (OR) with 95% confidence intervals (CI95) after adjustment for age, sex, body mass index and study centre, and population attributable risks (PAR).

Results

There was a 15-fold (OR 14.8; CI95 4.4-50.4) increased risk of thrombosis within two weeks after hospital discharge. Surgery (OR 6.6; CI95 3.7-11.6), fractures (OR 12.7; CI95 3.7-43.7), plaster cast (OR 6.2; CI95 2.0-18.9), minor leg injuries (OR 1.9; CI95 1.1-3.3), and transient immobility at home (OR 5.0; CI95 2.3-11.2) were all associated with thrombosis risk over three months. The PAR for in-hospital immobility was 27%, and for out-of-hospital immobility 15%.

Conclusions

In those over 70 years of age, in-hospital and out-of hospital immobility are strong risk factors for thrombosis. Additional studies on preventive measures during immobilisation in this age group should not focus solely on hospital settings.

INTRODUCTION

Venous thrombosis presents mainly as deep venous thrombosis of the leg (DVT) and pulmonary embolism (PE). The incidence of thrombosis increases sharply with age, being rare in young individuals (<1 per 10 000 per year) and increasing to approximately 1% per year in very old age. [1] More than two thirds of all patients with venous thrombosis are aged 60 years and older, and 25% are older than 80 years. [2] So, increasing age is one of the most important risk factors. As venous thrombosis is a potentially lethal disease, morbidity (e.g. the post thrombotic syndrome) is common, and treatment has frequent side effects, prevention efforts will have large effects in older individuals. [3] However, the risk factors for thrombosis in the older population are not well characterised since studies to date mainly included young and middle aged individuals. [4]

Immobility is associated with reduced venous blood flow, particularly in the pockets of the venous valves, leading to inflammation and hypercoagulability. [5,6] In young and middle-aged individuals, immobility, for example due to hospitalisation or minor injuries, is an established risk factor for thrombosis with relative risk estimates ranging from 3 to 11. [7,8] However, it is unknown to what extent immobilisation increases the risk of venous thrombosis in older individuals. We hypothesised that immobility-related risk factors would be strong risk factors in this population.

The aim of this study was to assess the risk of venous thrombosis associated with hospitalisation, surgery, use of a plaster cast, minor injury, and transient immobility at home in a case-control study of people aged 70 years and older.

METHODS

Identification of 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 in Leiden, the Netherlands and Burlington, Vermont, US, designed to study risk factors for venous thrombosis in the older population. 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 Leiden, cases were identified from two anticoagulation clinics in a defined geographical area in the western part of the Netherlands. In Vermont, cases were identified in the Vascular Laboratory and the Radiology department of the University of Vermont Medical Centre in Burlington, Vermont, which are the only diagnostic centres in that geographic area. We defined venous thrombosis as DVT alone or PE with or without a proven DVT by ultrasound (PE±DVT). We were unable to accurately define isolated

PE without DVT since diagnostic measures of thrombosis of the legs are not routinely performed in all PE patients. Control subjects were identified in Leiden and Vermont in the same geographical area as the cases. Control subjects were randomly selected from five primary care practices in Leiden and four in Vermont.

All identified cases and control subjects were mailed an invitation letter, followed by a telephone call to discuss participation. Individuals were excluded from participation if they responded affirmatively 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 chemotherapy or radiation therapy for cancer in the last six months. Potential participants with severe psychiatric or cognitive disorder, as judged by the telephone contact, were excluded. We also excluded individuals who self-reported previous DVT or PE within the past 10 years.

Of the 1187 identified cases, 689 (58%) were eligible and 498 (42%) were excluded. (figure 1) Of those excluded, 55 (11%) died before inclusion was possible, 159 (32%) had active malignancy, 108 (22%) had an apparent severe cognitive or psychiatric disorder, and 171 (34%) had a history of venous thrombosis within the last 10 years. Of the 723 identified control subjects, 631 (87%) were eligible and 92 (13%) were excluded: 15 (16%) died before inclusion was possible, 19 (21%) had active malignancy, 34 (37%) had an apparent severe cognitive or psychiatric disorder and 10 (11%) had a history of venous thrombosis within the last 10 years (see figure 2 for participation flowchart by study centre).

All participants provided written informed consent in accordance with the Declaration of Helsinki and gave permission to obtain information about their medical history. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and by the Committee of Human Research of the University of Vermont.

Data collection

In Leiden, 398 (71%) of the 561 invited cases and 321 (76%) of the 422 invited control subjects participated. In Vermont, 128 cases were invited and 75 (59%) participated, while 140 (67%) of the 209 invited control subjects participated. For all eligible cases and controls subjects who agreed to participate, home visits were scheduled. During this home visit, an extensive structured interview and blood collection was completed by trained personnel. The index date was defined as the date of diagnosis of the thrombosis for the cases and the date of the in home interview for the control subjects.

The interview assessed thrombosis risk factors that have been established in the young and middle-aged as well as other putative age-specific risk factors that were present within 3 months of the index date. Questions queried hospitalisations, surgery during hospitalisation, fractures and use of plaster cast (or splint), minor injuries of the lower extremities and transient immobility at home, including dates and location.

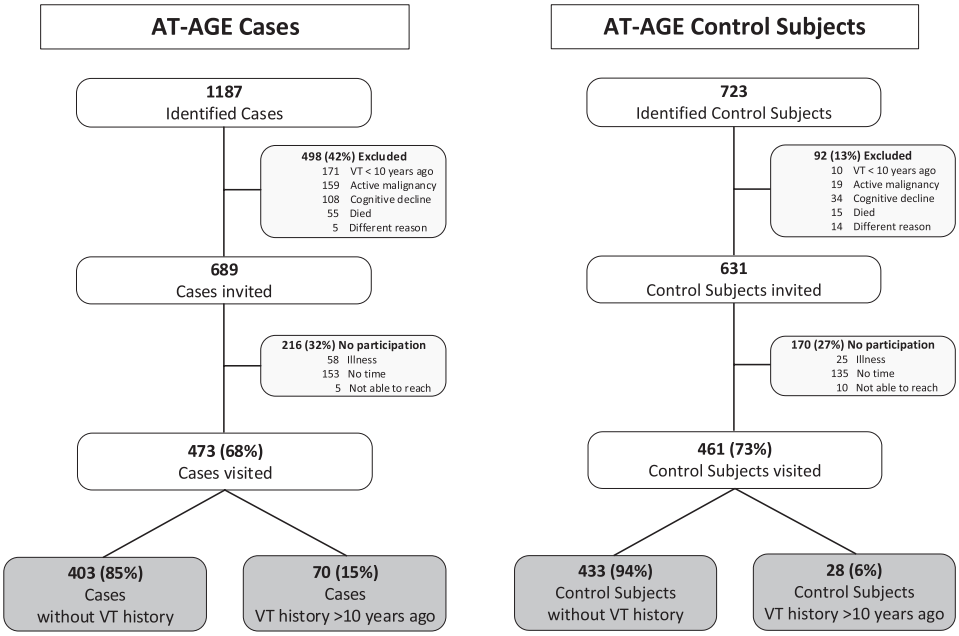

Figure 1. Flowchart of AT-AGE study

Physical measurements were performed including weight (measured with a calibrated scale) and height. Body mass index (BMI) was calculated by dividing body weight (kg) by squared height (m2).

Analyses

For these analyses on the etiology of thrombosis we included only cases and control subjects without a history of venous thrombosis (403 cases and 433 control subjects) who had complete interview data (401 cases and 431 control subjects). Characteristics of the control subjects included in Leiden and in Vermont were analysed separately to provide insight into the source populations. For all further analyses, we combined data from the two sites. We determined associations between transient immobility-related risk factors and venous thrombosis. Transient immobility was defined as a status of immobility that is shortly present in one's life. As estimates of relative risk, we calculated odds ratios (OR) and their 95% confidence intervals (CI95) using logistic regression models. All reported ORs were adjusted for age (continuous), sex, BMI (continuous) and study centre using multivariable logistic regression analysis. Stratified analyses were performed for DVT and for PE±DVT.

Hospitalisation was defined as present when the participant was hospitalised at the index date or the discharge date was within the three months window previous to the

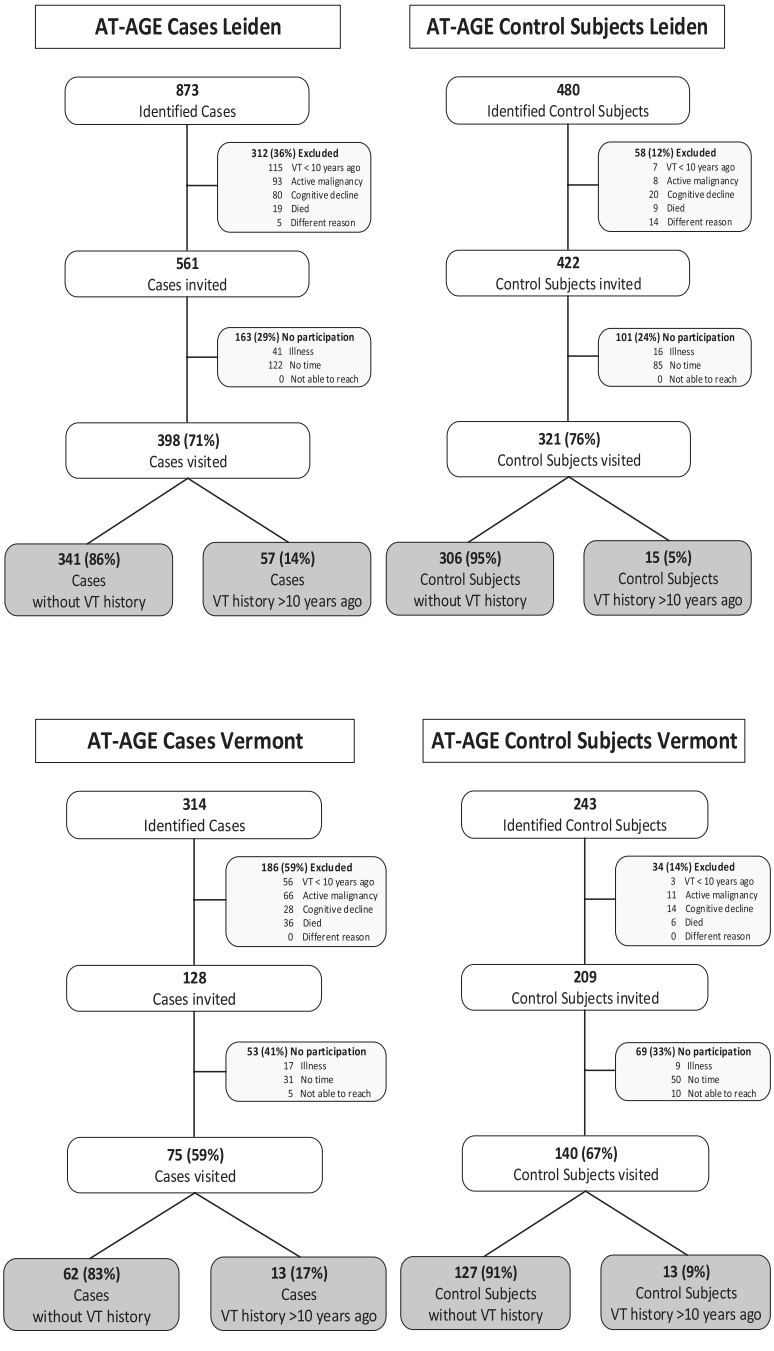

Figure 2. Flowchart of AT-AGE study per center

Table 1. Characteristics of control subjects by center

	Controls NL	Controls VT
	306	125
Median Age, n (Range)	76 (70-94)	76 (70-96)
70-75 years, n (%)	126 (41)	49 (39)
75-80 years, n (%)	90 (29)	39 (31)
80-85 years, n (%)	61 (20)	24 (19)
>85 years, n (%)	29 (10)	13 (11)
Men, n (%)	147 (48)	62 (50)
Ethnicity White, n (%)*	284 (93)	124 (99)
Smoking status*		
Never, n (%)	88 (29)	32 (26)
Former, n (%)	168 (55)	87 (69)
Current, n (%)	49 (16)	6 (5)
Median BMI (kg.m-2) (Range) *	25.9 (17.0-42.0)	27.3 (19.0-49.7)
Hospitalisation, n (%)†	16 (5)	13 (10)
Surgery, n (%)†	12 (4)	4 (3)
Fracture, n (%)†	1 (0.3)	2 (2)
Plaster cast (splint), n (%)†	2 (1)	2 (2)
Minor injury, n (%)*†	18 (6)	8 (7)
Transient Immobility at home, n (%)*†	5 (2)	3 (2)

NL= the Netherlands, VT= Vermont, n = number, BMI = Body Mass Index.

*ethnicity 5 missings, smoking 1 missings, BMI 8 missings, minor injury 1 missing, transient immobility at home 1 missing

† < three months before index date

index date. Hospital admission for both in-patients and day patients were taken into account. Hospitalisation for surgical and non-surgical indications was analysed separately. The presence of a fracture or plaster cast (or splint) in the three months before the index date were analysed as putative risk factors as were minor injuries of the lower extremities and transient immobility at home. A minor injury was defined as an injury of the lower extremities (hip, knee, ankle or foot) such as a sprained ankle or contusion of the lower leg that started within the three months window. A period of transient immobility at home was defined as a period of four or more consecutive days of immobility, such as being bedridden or continuously sitting in a chair, that started within the three months before the index date.

If participants were bed- or chair-ridden for the entire three months prior to the index date they were classified as chronically immobilised and not included in these analyses. To study duration of risk of venous thrombosis after the transient risk factor, we dichotomised the time between the risk factor and venous thrombosis by the median time from the end of the risk period (for hospitalisation) or the start of the risk period (for minor injury or transient immobility at home) in the control subjects. Since the group of

Table 2. Association of transient immobility related risk factors with venous thrombosis

	Cases n = 401	Controls n = 431	OR crude (CI95)	OR adjusted* (CI95)
Hospitalisation, n (%)[†]	126 (31.4)	29 (6.7)	6.4 (4.1-9.8)	7.2 (4.5-11.4)
Surgery, n (%)[†]	79 (19.7)	16 (3.7)	6.4 (3.6-11.1)	6.6 (3.7-11.6)
Thrombosis after discharge, n (%)	84 (67)			
Time after discharge[‡]				
< 2 weeks (%)	28 (9.3)	3 (0.7)	13.6 (4.1-45.3)	14.8 (4.4-50.4)
2-4 weeks (%)	17 (5.9)	3 (0.7)	8.3 (2.4-28.5)	8.8 (2.5-31.5)
>4 weeks - 3 months (%)	38 (12.1)	22 (5.2)	2.5 (1.5-4.4)	2.9 (1.6-5.1)
Fracture, n (%)[†]	27 (6.7)	3 (0.7)	10.3 (3.1-34.2)	12.7 (3.7-43.7)
Plaster cast (splint), n (%)[†]	21 (5.2)	4 (0.9)	5.9 (2.0-17.3)	6.2 (2.0-18.9)
Minor injury, n (%)[†‡]	41 (10.5)	26 (6.1)	1.8 (1.1-3.0)	1.9 (1.1-3.3)
Start of minor injury~				
< 4 weeks (%)	15 (4.1)	15 (3.6)	1.1 (0.6-2.4)	1.3 (0.6-2.7)
> 4 weeks-3 months (%)	26 (6.9)	11 (2.7)	2.7 (1.3-5.6)	2.8 (1.3-5.8)
Transient immobility at home, n (%)[†‡]	34 (8.8)	8 (1.9)	5.1 (2.3-11.1)	5.0 (2.3-11.2)
Start of transient immobility[‡]				
< 9 weeks (%)	25 (6.6)	4 (0.9)	7.5 (2.6-21.7)	7.7 (2.6-22.9)
> 9 weeks - 3 months (%)	9 (2.5)	4 (0.9)	2.7 (0.8-8.8)	2.5 (0.8-8.5)

n = number, OR = oddsratio, CI = confidence interval.

*adjusted for age (continuous), sex, BMI: body mass index (continuous) and study center.

[†] < three months before index date

[‡]time after discharge: cases: 1 missings, controls 1 missings; minor injury 6 missings, transient immobility at home: 6 missings

participants with hospitalisation in the three months prior to the index date was large enough for further stratification, to study the time trend in risk of venous thrombosis in more detail, the time after hospital discharge was divided into three periods (< two weeks, two-four weeks, four weeks-three months). The small number of control subjects with fractures or plaster cast prohibited a detailed analysis of the risk by time from immobilisation.

In addition, sensitivity analyses were performed. Since the index date was defined as the date of the home visit for the control subjects and therefore, per definition, none of the controls was hospitalised on the index date, assessment of the risk of thrombosis during hospitalisation was not directly possible. To estimate the risk of venous thrombosis during hospitalisation, the index date of the controls was moved back by five weeks, i.e, by the median time (in weeks) of the cases between diagnosis of thrombosis and home visit.

We calculated population attributable risk (PAR) as: pd (OR-1)/(OR); in which pd is the proportion of cases exposed to the risk factor of interest. In this case the PAR indicates the proportion of the total incidence of venous thrombosis in those 70 and older who were eligible for this study that can be attributed to the risk factor of interest. [9,10] We calculated the PAR for all immobility related risk factors combined, and for in-hospital

Table 3. Odds ratios of thrombosis over three months with transient immobility risk factors stratified by type of thrombosis

Exposure	N, DVT/total VT (%)	DVT OR (CI95)*	PE±DVT OR (CI95)*
Hospitalisation	43/126 (34)	5.6 (3.2-9-8)	9.1 (5.5-15.2)
Surgery	27/79 (34)	5.3 (2.7-10.4)	7.9 (4.2-14.6)
Fracture	11/27 (41)	14.2 (3.7-55.3)	10.9 (2.9-40.5)
Plaster cast (splint)	6/21 (29)	4.2 (1.1-16.5)	7.6 (2.3-24.9)
Minor injury	24/41 (59)	2.6 (1.4-4.9)	1.4 (0.7-2.6)
Transient immobility at home	7/34 (21)	2.4 (0.8-6.8)	7.4 (3.2-17.2)

N= number, VT = venous thrombosis, DVT= deep venous thrombosis of the leg, PE = pulmonary embolism, OR = odds ratio, CI = confidence interval.
*adjusted for age (continuous), sex, BMI: body mass index (continuous) and study center.

and out-of-hospital immobility, separately. Out-of-hospital immobility was defined as the presence of fractures, plaster cast (or splint), minor injuries, and transient immobility at home within the non-hospitalised population.

RESULTS

For the cases, the median duration between the index date and the home visit was five weeks (range 1-44 weeks), 75% were visited within seven weeks, and 90% within 10 weeks. General characteristics of the control subjects in Leiden and Vermont are shown in table 1. In both centres, ~30% of the control subjects were 80 years and older. Median BMI was slightly higher in Vermont than Leiden. Of the 401 cases, in Leiden, 134 (39%) of the cases had DVT, and 207 (61%) had PE±DVT, and in Vermont 32 (53%) had DVT and 28 (47%) PE±DVT. In 155 of the 166 DVT cases (93%), and in 220/235 of the PE cases (94%) we were able to obtain the diagnostic report of the thrombotic event, and was the thrombosis thus objectively confirmed by ultrasound and PE was confirmed by spiral computed tomography or ventilation-perfusion lung scan.

Table 2 shows the risk of venous thrombosis associated with immobility-related risk factors. Overall, hospitalisation was associated with a more than 7-fold increased risk of venous thrombosis (OR = 7.2, CI95 4.5-11.4). Among cases and controls with hospitalisation, the median duration of hospital stay in the cases was 10 days (range 2-55) and in the control subjects 3 days (range 1-22). Dichotomisation of the time between discharge from hospital and the index date, based on the median time of hospitalisation until the index date in the control subject (48 days, range 4-89) showed that the risk of venous thrombosis was 7.9-fold increased in the first seven weeks after discharge (OR 7.9; CI95 4.2-14.7) and 2.1-fold increased after seven weeks (7 weeks- 3 months, OR 2.1; CI95 1.0-4.4). Further stratification of the time between hospital discharge and the

index date showed a 14.8-fold increased risk of thrombosis within the first two weeks after discharge from the hospital (OR 14.8; CI95 4.4-50.4) and gradually decreasing risk to a 3-fold increased risk between four weeks and three months after discharge (table 2). Performing a sensitivity analysis using the recalculated index date for the controls, 41 (10.1%) cases and 1 (0.2%) control subject was hospitalised during the index date indicating that the thrombotic risk was highest during hospitalisation although the confidence interval was wide (OR 48.7; CI95 6.6-361.0).

Among the cases hospitalised within the three months prior to the index date, 79 of the 126 (63%) had surgery during the hospital admission. When compared with individuals without hospitalisation, the risk of venous thrombosis associated with surgery-related hospitalisations (OR 6.6, CI95 3.7-11.6) was similar to non-surgery hospitalisations (OR 5.5 CI95 2.7-10.4) (OR for surgical versus non-surgical admission = 1.1, CI95 0.4-2.7). Thirty-one (7.8%) of the cases and 4 (0.9%) of the control subjects underwent lower extremity surgery, indicating that lower extremity surgery was associated with an almost 9-fold increased risk of thrombosis (OR = 8.6, CI95 3.0-25.1).

Fracture was associated with a nearly 13-fold increased risk of thrombosis (OR 12.7, CI95 3.7-43.7). In the cases, two-thirds of fractures (n=17) were of the lower extremities of which 8 (47%) presented with a DVT. In 87% of these cases the DVT was diagnosed on the ipsilateral side as the fracture. Use of a plaster cast or a splint was associated with a 6-fold increased risk of thrombosis (OR= 6.2; CI95 2.0- 18.9).

Minor leg injury was associated with a 1.9-fold increased risk of thrombosis (OR = 1.9; CI95 1.1- 3.3). The median time of occurrence of the minor injury until the index date was 43 days (range 1-92) for the cases and 27 days (range 4-93) for the controls. Compared with individuals without a minor injury in the three months prior to the index date, the risk of venous thrombosis was 1.3-fold (CI95 0.6-2.7) increased in the first 4 weeks after start of the minor injury and remained 2.8-fold (CI95 1.3-5.8) increased between four weeks and three months after the start of the immobility. The risk of thrombosis was increased in individuals with sprains of the ankle or knee (OR 1.9; CI95 0.6-6.1) and a contusion of the leg (OR 1.5; CI95 0.7-3.1). In 24 of the 41 cases (59%) with a minor injury a DVT was diagnosed, while 17 cases (41%) had PE±DVT. In 22 of these 24 cases (92%) the DVT was diagnosed on the ipsilateral side as the minor injury.

Transient immobilisation was associated with a 5-fold increased risk of thrombosis (OR = 5.0, CI95 2.3- 11.2). Median duration of transient immobilisation at home in the cases was 8 days (range 4-77 days) and 10 days (range 4-30 days) in control subjects. The median time of the start of transient immobility until the index date, was 27 days (range 2-81) for the cases, and 63 days (range 38-86) for the control subjects. The risk of thrombosis was 7.7-fold increased (CI95 2.6-22.9) within the first 9 weeks (63 days) after the transient immobility, whereas as an OR of 2.5 (CI95 0.8-8.5) was found if transient im-

mobility was more than 9 weeks up to 3 months previous. In 42% of the cases the reason for transient immobilisation at home was an infection, 23% had generalised weakness or "malaise", 17% had fracture, and 9% each had back pain or a minor injury. Of the cases, 5 (1.2%) were chronically immobilised, whereas none of the control subjects were.

All immobility risk factors were similarly associated with both DVT and PE±DVT. (Table 3) Overall, immobilisation from any cause had a PAR of 39%. In-hospital immobility and out-of-hospital immobility had population attributable risks of 27% and 15%, respectively.

DISCUSSION

In the AT-AGE study, a case-control study on venous thrombosis risk in people aged 70 years and older, we determined that immobility-related risk factors, i.e., hospitalisation, surgery, fractures, plaster cast (or splint), minor injuries of the legs, and transient immobility at home were strongly associated with the risk of venous thrombosis (both DVT and PE±DVT) in the 3 months after the start of the immobility (ORs ranging between 2 and 13). The highest risk of thrombosis was for found for immobilisation during hospitalisation (OR 48.7; CI95 6.6-361.0), and the risk of thrombosis out-of hospital was 15-fold increased within the two weeks after hospital discharge, and the risk remained increased for 3 months after hospital discharge. Predefined potential confounders of the risk factors, i.e, age, sex, BMI, and study centre, did not alter any of the associations. Previous studies on immobility and the risk of thrombosis in older populations reported similar risk estimates, ranging from 1.5 up to more than 8-fold increased risks. [4] Based on the PARs we observed, the overall contribution of immobility to thrombotic risk (both in and out of hospital) in this study population was 40%. A PAR of 27% was found for in-hospital related immobility. This contrasts with data previously reported for younger people, where the PAR was only 15% for hospital-related immobility. [4] Importantly, the PAR was 15% for out-of-hospital immobility in the last three months in this older population. These findings indicate that immobility explains part of the age gradient in the incidence of venous thrombosis.

Findings illustrate the large impact of immobility, a common occurrence in the older population. The prevalence of immobility related risk factors in the three months prior to the index date for our control group ranged from 2-8% for the different exposures.

Hospitalisation causes immobilisation. [11] In line with this we found that cases were hospitalised for a longer period than the control subjects. One should take into account that severity and disease entity during hospitalisation can influence the risk of thrombosis, as can the duration of hospitalisation. [12]

As in a younger population, we found that minor injuries were associated with a higher risk of thrombosis over three months. [8] For minor injury the time of highest risk differed than for other types of immobility, with a higher risk after four weeks compared to shortly after the minor injury. For the other studied factors the risk was highest shortly after the exposure of immobility. It is possible that the seriousness of the minor injury, and the long term consequences, leading to more or less immobility, increase the thrombotic risk, rather than the minor injury itself. It is also possible this finding was a chance finding. Transient immobilisation at home increased the risk of thrombosis 5-fold, and this risk was highest in the first two months after immobilisation. Transient immobilisation at home was most frequently due to infection, an important trigger for thrombosis. [13]

The increased risk of thrombosis associated with out-of-hospital immobility indicates that prophylaxis may be beneficial. Home treatment with prophylaxis has effectively been implemented in other high risk groups, such as orthopedic surgical patients. [14] The EXCLAIM trial showed a beneficial effect of a longer duration of treatment within the older population (>75 years). [15] However, in two clinical trials including inpatients, extended thromboprophylaxis after discharge reduced thrombosis rates at the cost of higher bleeding rates (30 days event rate: 0.5-0.8%). [16,17] Other preventive measures that might be considered in this high risk group include the use of graded elastic compression stockings or aspirin. [18,19]

Recruiting older individuals in research is challenging. [20] We overcame this by performing home visits to assess the presence of risk factors. This enabled us to recruit less mobile individuals and achieve a high participation rate (participation rate: cases 68%, control subjects 73%). As in any case-control study, recall bias might have occurred. However, both cases and controls were interviewed by trained personnel using a standardised interview, which minimises the risk of bias. Using an interview for assessment of risk factors for thrombosis within three months before the index date enabled us to determine putative risk factors, such as transient immobility at home, that might be challenging to determine, e.g., as these are not mentioned regularly in medical reports and they might be difficult to recall precisely after a longer period. Unfortunately, data on preventive measures in the hospital (e.g., low molecular heparin injections) were not collected. However, individuals with in-hospital immobilisation were most likely more often treated with thromboprophylaxis as their risk of venous thrombosis is thought to be increased. More frequent treatment with thromboprophylaxis in immobilised individuals compared with individuals who are not immobilised, leads to an underestimation of the true relative risk of venous thrombosis associated with in-hospital immobilisation.

In a case-control study, associations may be biased if the willingness to participate is affected by the presence of the risk factor. We minimised this bias by performing home visits, and achieving a high participation.

Moreover, the sensitivity analysis in which we recalculated the index date of the control subjects, did not alter interpretations of our results. We excluded cancer patients so our results are not generalisable to these individuals. Finally, a number of potential participants died before they could be invited to participate. The impact on our results is difficult to determine, but these participants were more likely immobilised, resulting in an underestimation of the true risk.

In conclusion, the contribution of immobility-related risk factors, defined as hospitalisation, fracture, plaster cast (or splint), minor injury of the leg, and transient immobilisation at home to the risk of venous thrombosis in the older population is high. Studies regarding preventive measures during immobilisation should focus on both in-hospital and out-of-hospital patients.

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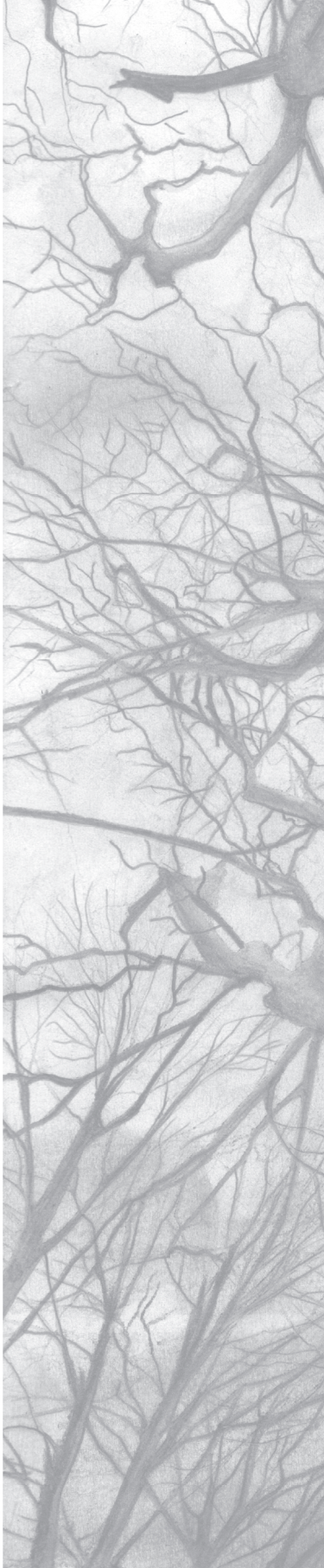

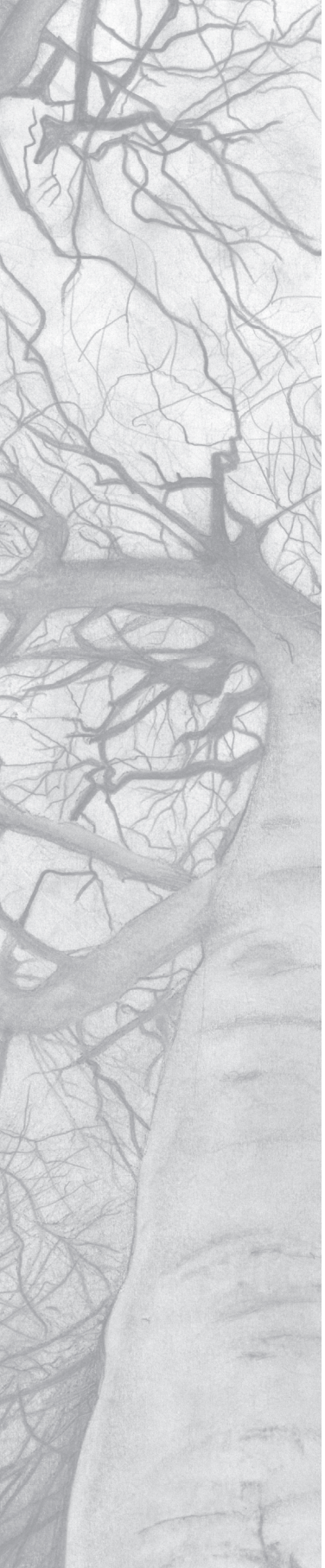

CHAPTER 4

Genetic risk factors for venous thrombosis in older people

A. Karasu
M.J. Engbers
M. Cushman
F.R. Rosendaal
A. van Hylckama Vlieg

To be submitted

ABSTRACT

Introduction

As the incidence of venous thrombosis increases strongly with age and the number of older people is on the rise, the focus on the older people becomes more relevant. We aimed to assess whether common genetic risk factors, i.e., the factor V Leiden (FVL) and prothrombin G20210A variants (PT), and non-O blood group, as well as a positive family history of venous thrombosis are risk factors for a first venous thrombosis at an older age (≥70 years).

Methods

401 consecutive cases with a first-time thrombosis and 431 control subjects were included in the AT-AGE case-control study. All subjects were ≥70 years. Information on risk factors for thrombosis, including family history, was obtained from questionnaires. Unprovoked thrombosis was defined as thrombosis not related to surgery, fracture, plaster cast, minor injuries or immobility within three months prior to the venous thrombotic event. FVL and PT were determined in 394 cases and 426 control subjects. The risk of thrombosis was assessed by calculating odds ratios (OR) with 95% confidence intervals (CI95) after adjustment for age, sex, and study centre.

Results

The risk of venous thrombosis was 2.2-fold increased in FVL carriers (CI95 1.2-3.9). This risk was 1.4-fold increased in PT mutation carriers (CI95 0.5-3.9). A positive family history was associated with a 2.1-fold increased risk of thrombosis (CI95 1.5-3.1). The OR for non-O blood group was 1.3 (CI95 1.0-1.8). The highest risk of thrombosis was found in individuals who had both a positive family history and were carriers of one of the two prothrombotic variants.

Conclusion

Factor V Leiden, prothrombin G20210A mutation, non-O blood group and a positive family history for venous thrombosis were risk factors of venous thrombosis in older people.

INTRODUCTION

Venous thrombosis 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 venous thrombosis compared with non-carriers. [2,3] Another genetically determined risk factor, i.e., the non-O blood group, is an important determinant of venous and arterial disease. [4,5] In the young and middle aged population, blood group non-O is associated with a doubling in risk of thrombosis. [6] Aggregation of venous thrombosis 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. [7-10]

Most epidemiological studies include only young and middle-aged individuals. Older people are often excluded from clinical studies into aetiology and management because of co-morbidities, short life expectancies, and logistical difficulties. [11,12] Limited information is available regarding genetic risk factors for venous thrombosis in older people. Furthermore, it is unknown whether a positive family history of venous thrombosis is predictive of a venous thrombotic event at an older age. As the incidence of venous thrombosis increases strongly with age and the number of older people is on the rise, the focus on older people becomes more relevant. Venous thrombosis 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. [13-14]

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

METHODS

Study population and data collection

The Age and Thrombosis, Acquired and Genetic risk factors in the Elderly (AT-AGE) study is a two-centre, population-based case-control study designed to study risk factors for venous thrombosis in older people. The design of the AT-AGE study was described in detail previously. [15] From June 2008 to August 2011 in Leiden, the Netherlands and December 2008 to July 2011 in Vermont, US, all consecutive cases 70 years and older with a first deep venous thrombosis of the leg (DVT) or pulmonary embolism (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 Centre (Burlington, Vermont, United States). Control subjects 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 control subjects. 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 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 venous thrombosis was obtained via the interview. Family history of venous thrombosis was considered positive if at least one first degree relative experienced thrombosis. Participants who indicated that they did not know whether a first degree relative has had venous thrombosis were classified as having a negative history.

Provoked venous thrombosis was defined as thrombosis after hospitalisation, fracture, plaster cast, splint, minor injuries of the lower extremities (such as a sprained ankle or contusion of the lower leg) or transient immobility at home ≥4 successive 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. Blood group was determined by a 5'nuclease assay (Taqman; Applied Biosystems, Foster City, CA) using a standard PCR reaction mix (Eurogentec, Seraing, B) and an allele specific fluorescent probe equipped with a minor grove binding moiety (applied Biosystems, Foster City, CA).

Statistical analysis

As estimates of relative risks, we calculated odds ratios (ORs) with 95% confidence intervals (CI95) using logistic regression. We determined associations between venous thrombosis risk and factor V Leiden (FVL), the prothrombin G20210A mutation (PT), ABO blood group, and a positive family history. All reported ORs were adjusted for age (continuous), sex (categorical), and study centre (Leiden and Haarlem versus Vermont,

categorical) using multivariable logistic regression. Additional to the individual effect, the combined effect of the risk factors was studied. Furthermore, analyses were stratified for provoked and unprovoked thrombosis, and type of thrombosis (DVT only or PE with or without DVT).

Of all participants, 25 (6%) cases and 13 (3%) control subjects did not know whether one of their first degree family members had experienced a venous thrombotic event. In the overall analyses, these individuals were classified as having a negative family history, however, we performed a sensitivity analysis in which the risk of thrombosis associated with a positive family history was calculated after exclusion of these individuals. The risk of thrombosis associated with a positive family history of thrombosis was studied in more detail by calculating ORs for having any affected first-degree relative, having a first-degree relative affected before the age of 50 years, and for having multiple affected first-degree relatives. We calculated the positive predictive value of family history to identify FVL and PT. IBM SPSS Statistics 20.0 for Windows (SPSS Inc, Chicago, Ill) was used for data analysis.

RESULTS

In this study, 401 cases and 431 control subjects were included (Table 1). 166 Cases (41%) were diagnosed with an isolated DVT and 235 (59%) were diagnosed with PE with or without DVT. DNA analysis for factor V Leiden was available for 394 (98%) cases and 426 (99%) control subjects, for prothrombin G20210A mutation for 394 (98%) cases and 427 (99%) control subjects and for ABO blood group for 376 (94%) cases and 416 (97%) control subjects.

Table 1. Baseline characteristics of control subjects and cases

	Controls	Cases
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)
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)
Provoking factors, N (%)	67 (15.5)	170 (42.4)

N = number, VT = venous thrombosis

Prothrombotic variants, ABO blood group and risk of venous thrombosis

Out of 394 cases, 34 (8.6%) cases carried the FVL mutation (32 heterozygotes and 2 homozygotes). Of the control subjects, 18 (4.2%) were heterozygous for the FVL mutation and none of the control subjects were homozygous. Carriers of the FVL mutation (heterozygous and homozygous carriers combined) had a 2-fold increased risk of a first thrombosis (Table 2, OR 2.2, CI95 1.2-3.9), compared with participants who did not carry the FVL mutation. The FVL mutation was present in 19 of 162 cases with DVT (11.7%) and 15 of 232 cases with PE with or without DVT (6.5%), leading to odds ratios of 3.0 for DVT (CI95: 1.5-6.0), and 1.5 for PE (CI95 0.7-3.1). The odds ratios for provoked thrombosis and unprovoked thrombosis in the presence of FVL were similar (OR 2.2, CI95 1.0-4.4 for provoked thrombosis and OR 2.0, CI95 1.0-4.0 for unprovoked thrombosis). Nine cases (2.3%) and seven control subjects (1.6%) were heterozygous carriers of the prothrombin G20210A mutation, and none were homozygous. This led to an OR for prothrombin G20210A of 1.4 (OR 1.4, CI95 0.5-3.9). In presence of the PT mutation, the odds ratio for unprovoked thrombosis was 1.8 (CI95 0.6-5.4) while no association was observed for provoked thrombosis (OR 1.0, CI95 0.3-4.0). 231 (61.4%) cases had blood group non-O and 232 control subjects (55.8%), resulting in an OR of 1.3 (CI95 1.0-1.8) for blood group non-O. Blood group non-O was not associated with the risk of provoked thrombosis (OR 1.0, CI95 0.7-1.5), whereas the risk of unprovoked thrombosis was 1.6-fold increased (CI95 1.1-2.3).

We studied the combined effect of ABO blood group and the presence of either the two variants (i.e., FVL or PT mutation), with wildtype carriers of the FVL and PT variants with blood group O as the reference category. Individuals carrying either prothrombotic

Table 2. Genetic risk factors of venous thrombosis

Risk factor	Absent or present	Controls N	Cases (Nprovoked / Nunprovoked)	ORoverall* (CI95)	ORprovoked* (CI95)	ORunprovoked* (CI95)
Factor V Leiden	-	408	153/207	1†	1†	1†
	+	18	15/19	2.2 (1.2-3.9)	2.2 (1.0-4.4)	2.0 (1.0-4.0)
Prothrombin G20210A mutation	-	420	165/220	1†	1†	1†
	+	7	3/6	1.4 (0.5-3.9)	1.0 (0.3-4.0)	1.8 (0.6-5.4)
Blood group	O	184	71/74	1†	1†	1†
	Non-O	232	89/142	1.3 (1.0-1.8)	1.0 (0.7-1.5)	1.6 (1.1-2.3)
Family history VT	-	377	127/177	1†	1†	1†
	+	54	43/54	2.1 (1.5-3.1)	2.3 (1.4-3.6)	2.0 (1.3-3.1)

*OR adjusted for age, sex, study center
†reference category
N = number, VT = venous thrombosis

variant and blood group non-O had a 2.3-fold increased risk of venous thrombosis (CI95 0.9-5.9) and wildtype carriers of FVL and PT with blood group non-O had a 1.3-fold increased risk of venous thrombosis (CI95 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).

Table 3. Prothrombotic variants, blood group non-O and risk of venous thrombosis

FVL/PT	Non-O blood group	Controls N	Cases (N provoked/N unprovoked)	ORoverall* (CI95)	ORprovoked* (CI95)	ORunprovoked* (CI95)
–	–	175	64/68	1†	1†	1†
+	–	8	7/6	2.3 (0.9-5.9)	2.5 (0.8-7.4)	2.1 (0.7-6.6)
–	+	216	78/126	1.3 (1.0-1.8)	1.0 (0.7-1.5)	1.6 (1.1-2.3)
+	+	16	11/16	2.2 (1.1-4.3)	1.8 (0.8-4.1)	2.5 (1.2-5.4)

*OR adjusted for age, sex, study center
†reference category
N = number, FVL = Factor V Leiden, PT= Prothrombin G20210A mutation

Family history and risk of venous thrombosis

Family history of thrombosis was positive for 97 cases (24.2%) and 54 control subjects (12.5%). Individuals with a positive family history of thrombosis had a more than two-fold increased risk of venous thrombosis compared with individuals without a positive family history of thrombosis (OR 2.1, CI95 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, CI95 1.5-3.2). The risk of both provoked and unprovoked thrombosis was increased in the presence of a positive family history, i.e., OR provoked thrombosis: 2.3 (CI95 1.4-3.6); OR unprovoked thrombosis: 2.0 (CI95 1.3-3.1). The risk of venous thrombosis was not increased when only family members who had thrombosis before the age of 50 years were considered positive (OR 0.8, CI95 0.5-1.4), taking individuals without a positive family history as a reference. The number of affected relatives was also not associated with the risk of thrombosis, having more than 1 positive family member versus only 1 positive family member resulted in an OR of 0.8 (CI95 0.4-1.8). 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 cases with a positive family history, 11 carried the FVL or PT variant. This results in a positive predictive value of positive family history for the FVL or PT variant of 11%.

In table 4 the associations of the combined risks of a positive family history of thrombosis and carrying the factor V Leiden or the prothrombin G20210A mutation are shown.

Table 4. Prothrombotic variants, family history of thrombosis and the risk of venous thrombosis

FVL/PT	Family history	Controls N	Cases (Nprovoked/N unprovoked)	ORoverall* (CI95)	ORprovoked* (CI95)	ORunprovoked* (CI95)
-	-	350	112/155	1†	1†	1†
-	+	51	38/48	2.1 (1.4-3.1)	2.2 (1.4-3.6)	2.0 (1.3-3.2)
+	-	23	13/17	1.7 (0.9-2.9)	1.7 (0.8-3.5)	1.6 (0.8-3.1)
+	+	2	5/6	7.6 (1.6-35.7)	7.1 (1.3-37.6)	7.7 (1.5-40.1)

*OR adjusted for age, sex, study center
†reference category
N = number, FVL = Factor V Leiden, PT= Prothrombin G20210A mutation

Individuals with a positive family history of venous thrombosis who also carried either a prothrombotic variant had a high risk of thrombosis (OR 7.6, CI95 1.6-35.7).

DISCUSSION

In this population-based case-control study of 832 individuals aged 70 years and older, we show that factor V Leiden, the prothrombin G20210A mutation, and non-O blood group are risk factors for venous thrombosis in older individuals (≥70 years) as they are in younger individuals, increasing the risk of venous thrombosis 2.2-, 1.4-, and 1.3-fold respectively. Furthermore, a positive family history of thrombosis increased the risk of thrombosis 2.1-fold, without an effect of the number of affected family members and their age of onset. The highest risk of thrombosis was found in individuals who had both a positive family history and were carriers of one of the two prothrombotic variants.

The FVL and PT variants are well established risk factors in young and middle-aged individuals. The risk is four- to sevenfold increased for FVL carriers and two- to threefold increased for the PT variant. [3,16] Our results indicate that the FVL and PT variants remain associated with the risk of thrombosis in older age, as previous small subgroup analyses showed. [17]

The risk of thrombosis was highest in the presence of multiple genetic risk factors. These results illustrate the multicausal character of thrombosis, which is still present in older people. [1] Furthermore, the results are in line with studies explaining that the heterozygous mutations of the FVL and PT mutations are relatively weak risk factors for venous thrombosis, unless another genetic or acquired risk factor is present. [18] The finding of lower relative risks in older people than in middle-aged individuals may be partly explained by the higher absolute risk of venous thrombosis in older people which leads to smaller relative effects of individual risk factors in older people 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 older people. Furthermore, our results may be due to attrition of susceptibles, indicating that susceptible individuals with FVL or PT are more likely to develop venous thrombosis earlier in life, resulting in lower relative risks at an older age.

Compared with blood group O, non-O individuals had a 1.3-fold increased risk of thrombosis. The increased risk can be partly explained by higher levels of FVIII and VWF in ABO blood group. [6,19,20] High FVIII levels are associated with a lowered responsiveness to activated protein C (APC). In carriers of factor V Leiden, this associated is strengthened, which explains the interaction between non-O blood group and factor V Leiden carriers. [6,21]

Family history and the two prothrombotic variants were poorly associated in this study, as was also indicated in previous studies. [7,10] The positive predictive value of family history as a test for genetic risk factors, i.e. FVL and PT, is low. This may indicate that unknown or unmeasured genetic risk factors are present in individuals with a positive family history. This hypothesis is supported by the finding of a 2.3-fold increased risk of thrombosis in persons with a positive family history and non-carriers of factor V Leiden and prothrombin G20210A variants.

The strength of our study is 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%, control subjects 73%).

Our study has a number of limitations. Control subjects with a positive family history of venous thrombosis might be more willing to participate in a study of venous thrombosis than those who do not have a positive family history of thrombosis. However, this selection bias would only result in an underestimation of the true effect. 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 thrombosis and family history. However, by using standardised interviews performed by trained personnel for both cases and control subjects, the risk of recall bias was minimised.

Our results may have clinical implications. A positive family history of venous thrombosis doubled the risk of venous thrombosis in older people. In clinical practice this information is easy to obtain, however it is not implemented in clinical decision rules of venous thrombosis. In older people these clinical decision rules show a high failure rate. [22] Potentially, obtaining information on family history of thrombosis in individuals aged 70 years or older could improve prediction of thrombosis in older people. [23]

In conclusion, factor V Leiden, prothrombin G20210A mutation, non-O blood group and a positive family history for thrombosis were risk factors of venous thrombosis in older people.

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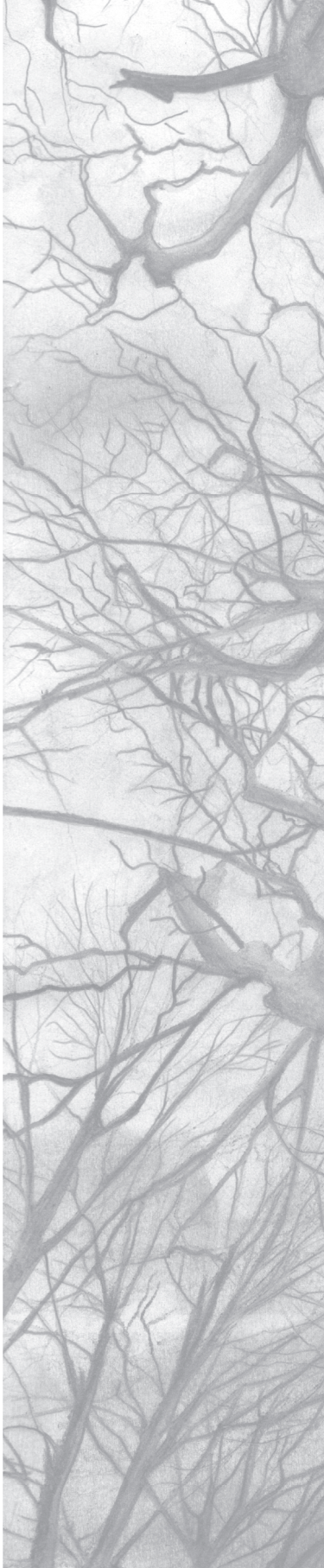

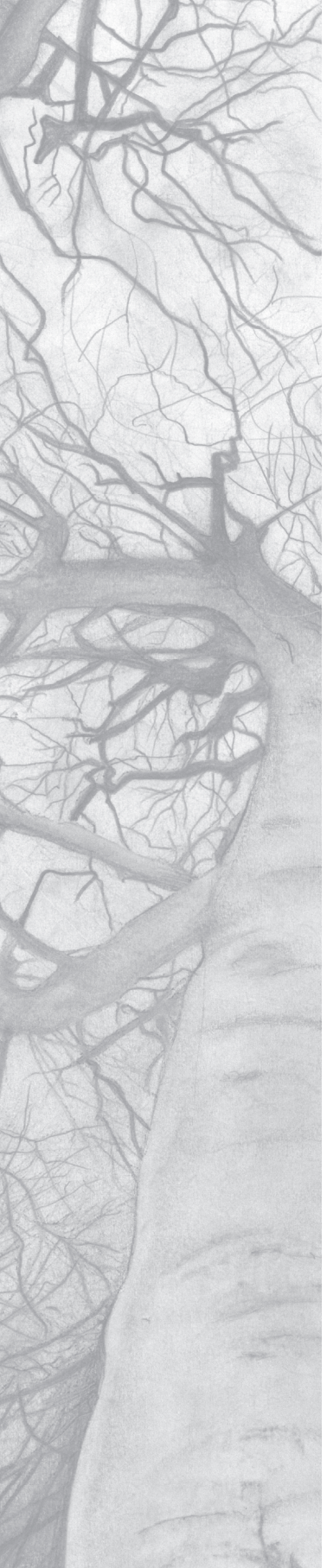

CHAPTER 5

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

M.J. Engbers
A. Karasu
J.W. Blom
M. Cushman
F.R. Rosendaal
A. van Hylckama Vlieg

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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 ulcer, and leg oedema obtained with a standardised interview was assessed in the AT-AGE study. The AT-AGE study is a case-control study in 70 years and older individuals (401 cases with a first-time venous thrombosis and 431 control subjects). We calculated odds ratios (ORs) and corresponding 95% confidence intervals (CI95) adjusted for age, sex, and study centre. Varicose veins were associated with a 1.6-fold (CI95 1.2-2.3) and a leg ulcer with a 3.3-fold increased risk of thrombosis (CI95 1.6-6.7), while the risk was 3.0-fold (CI95 2.1-4.5) increased in the presence of leg oedema. The risk of thrombosis was highest when all three risk factors occurred simultaneously (OR: 10.5; CI95 1.3-86.1). In conclusion, clinical features of venous insufficiency, i.e., varicose veins, leg ulcer, 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. [1] More than 60% of all thrombotic events occur in this age group. [2]

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. [3] Stasis can occur due to external pressure on the vein during, e.g., prolonged immobilisation 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. [4-6] Like venous thrombosis, the prevalence of venous insufficiency increases steeply with age. [7-9] 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. [10-12] 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. [13] 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, US. In Leiden, cases were identified in two anticoagulation clinics (Leiden and Haarlem). In Vermont, cases were resided in a 45 km radius of Burlington, and were diagnosed at the University of Vermont Medical Centre 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 computed tomographic or high probability ventilation-perfusion lung scan. We defined venous thrombosis as DVT alone or PE with or without a proven DVT by ultrasound (PE±DVT). We invited the population-based control subjects randomly from primary care practices in the same geographic 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 six 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. [13] 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

During a home visit an extensive interview was performed by trained personnel. 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 within 10 weeks.

In the interview information about the presence of well-known risk factors, such as recent hospitalisation, surgery, and fracture was obtained and a blood sample or buccal swab was collected. [13] 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 three months before the index date. Leg oedema was classified by participants as "daily oedema,""intermittent oedema", or "no oedema". In the first version of the interview questionnaire of the control subjects the question of oedema was not incorporated yet. For this reason, for 24 control subjects we could not report this data.

We used self-assessed information on clinical features of venous insufficiency, a strategy that has been previously validated. [14] Additionally, among cases we obtained 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 three months before the thrombosis one of these conditions was present: hospitalisation, surgery, a fracture, plaster cast or splint use, a minor injury or immobilisation in the home situation. [13] Physical measurements were performed including weight (measured with a calibrated scale) and height. Body mass index (BMI) was calculated by dividing body weight (kg) by squared height (m^2).

Statistical Analysis

To provide insight into the source population at the two study sites, we compared participant 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 three months before the thrombosis was determined. We assessed a dose-response relation 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 (95CI) were calculated as an estimate of the relative risk of venous thrombosis. We used a multiple logistic regression model to calculate adjusted odds ratios. All reported ORs in the manuscript were adjusted for age (continuous), sex and study centre. Additionally, we adjusted for BMI (continuous), smoking status and the presence of the factor V Leiden or 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. [15] 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), in which the *pd* is the prevalence of the risk factor among cases, and OR is the adjusted OR found in the study population. [16] All statistical analyses were performed using SPSS 20 for Windows (SPSS Inc, Chicago, Ill).

RESULTS

Characteristics of the control subjects of the two AT-AGE study centres are shown in table 1. 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.

Tabel 1. Characteristics of control subjects by study centre

	Leiden (NL)	Vermont (US)
Controls, n	**306**	**125**
Median Age (Range)	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 8 missings, †Leg oedema 24 missings

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%). Individuals with obesity more often had leg oedema and leg ulceration than normal weight individuals but this association was less clear for varicose veins (BMI>30 compared with <25 kg/m^2: oedema: 38.3% versus 16.1%, leg ulceration: 5.3% versus 1.3 %; varicose veins: 23.4% versus 20.6%). Symptoms of venous insufficiency were not associated with smoking status.

In table 2 the risk of venous thrombosis associated with symptoms of venous insufficiency are shown. Individuals with varicose veins had a 1.6-fold increased risk of venous thrombosis compared with individuals without varicose veins (OR 1.6; CI95 1.2-2.3). A history of a leg ulcer was associated with a 3-fold increased risk of thrombosis (OR 3.3; CI95 1.6-6.7). Leg oedema during the three months before thrombosis (daily and inter-

Table 2. Clinical features of venous insufficiency and the risk of venous thrombosis

	Cases n = 401	Controls n = 431	OR crude (95CI)	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: 29 missings.

mittent oedema combined) was associated with a 3.0 fold (CI95 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 (CI95 1.0 -2.5) while this risk was 3.1-fold increased for individuals with daily oedema (CI95 2.1-4.5). The associations between the clinical features of venous insufficiency and thrombosis remained present after adjustment for BMI and smoking (Table 2). Adjustment for the presence of the factor V Leiden and prothrombin 20210A mutation showed similar risk estimates: varicose veins: OR 1.7 (CI95 1.3-2.4), leg ulcer: OR 3.4 (CI95 1.7-6.8), leg oedema: OR 3.1 (CI95 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; CI95 1.0-2.3) and 3.2-fold increased for daily oedema (OR 3.2; CI95 2.1-4.8). For 376 cases (93.8%), information on the presence or absence of thrombotic complaints prior to the diagnosis of venous thrombosis was available. 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 (CI95 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 (CI95 0.9-2.0) increased risk of an unprovoked venous thrombosis. For leg ulcer this risk was 3.2-fold increased (CI95 1.4-6.9) and for leg oedema (daily and intermittent oedema combined) 3.4-fold (CI95 2.1-5.3). For provoked venous thrombosis we found a 2.0-fold (CI95 1.4-2.9) increased risk for varicose veins, and a 3.4-fold increased risk (CI95 1.5-7.4) for leg ulcer. Leg oedema was associated with a 2.8-fold (CI95 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; CI95 1.3-86.8) (Table 3). Of the cases, 166 (41%) were diagnosed with DVT only, and 235 (59%) 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 4).

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 presence of three symptoms, 4.6%.

Table 3. 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 4. Odds ratios of thrombosis with venous insufficiency stratified by type of thrombosis

	N, DVT/total VT (%)	**DVT OR adjusted (CI95)***	**PE±DVT OR adjusted (CI95)***
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: 29 missings.

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, [8] 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 hospitalisation and surgery. [13]

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. [12, 17-20] Heit et al. demonstrated a 4-fold increased risk of venous thrombosis associated with varicose veins in middle-aged up to age 60 years, but in contrast to the current study, they observed no increased risk in those 70 years and older. [12] This difference in findings may be due to a difference in data collection. Heit et al. used medical record to assess chronic venous insufficiency, and 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 two-fold increased risk of thrombosis compared with no oedema. This indicates that our findings are not likely 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 hypothesise 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. [21,22] It has been hy-

pothesised that damage to the valves results in hypoxia especially in the valve pockets, which promotes thrombus formation at this location. [23] 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. [24,25] Leg ulceration is also associated with local inflammatory processes. [26] We hypothesise 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. [27] 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. [28]

In the AT-AGE study we achieved high participation rates, which is challenging in older people. [29] As venous thrombosis and venous insufficiency are both associated with immobility, performing home visits to both cases and control subjects, minimised 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 minimised this by performing standardised 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 ultra sound of the legs, resulting in an overestimation of the risks. However, as we also found an increased risk for pulmonary embolism with clinical features of venous insufficiency, we do not expect 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. If misclassification is present, we would expect that the misclassification would be non-differential in the cases and the control subjects. This 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 were 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 on 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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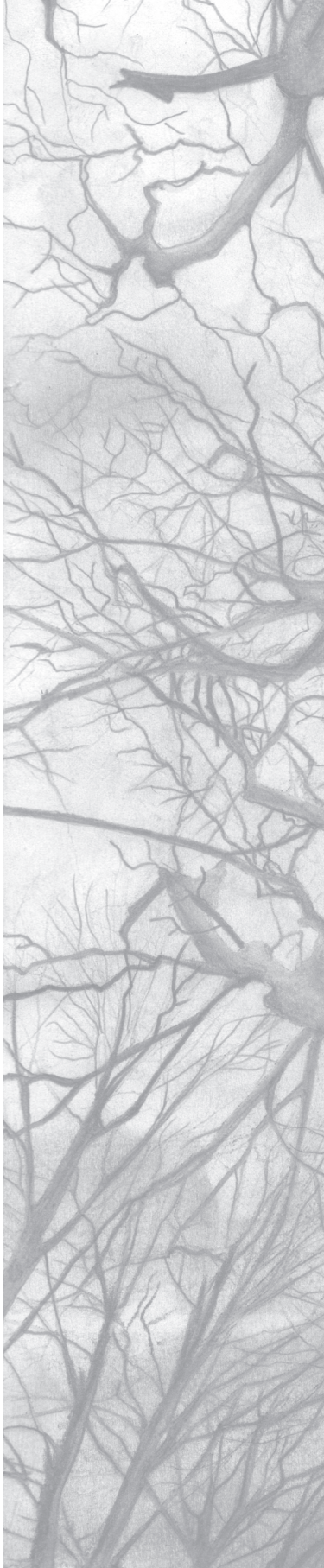

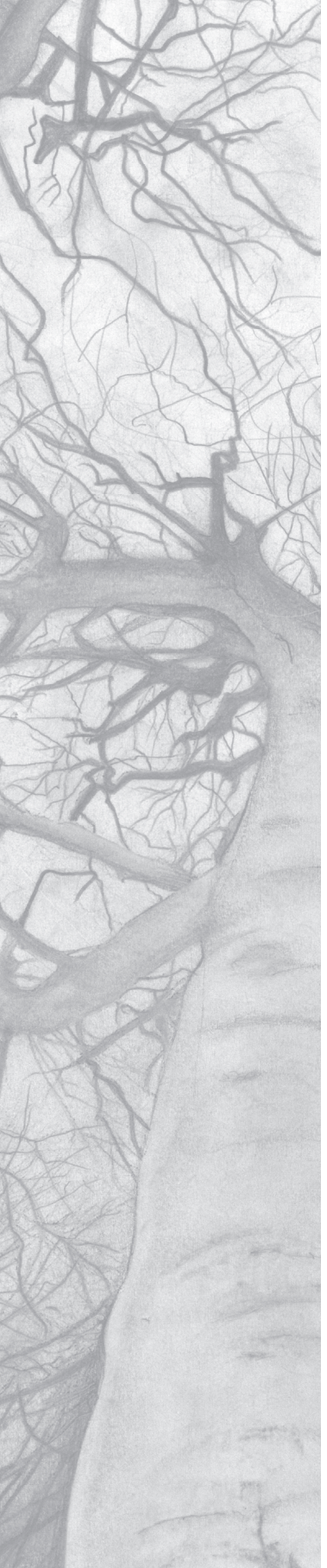

CHAPTER 6

Functional impairment and risk of venous thrombosis in older people

M.J. Engbers
J.W. Blom
M. Cushman
F.R. Rosendaal
A. van Hylckama Vlieg

Submitted

ABSTRACT

Background

Venous thrombosis incidence increases with age. The role of age-specific risk factors in thrombosis etiology at older age, such as functional impairment, is unclear.

Objective

Assess the thrombotic risk associated with functional impairment, defined as impaired activities of daily living (ADL), impaired mobility, sedentary lifestyle, and low handgrip strength.

Methods

The AT-AGE study is a two-centre case-control study conducted in the Netherlands and the USA (2008-2011). Consecutive cases (n=401) aged 70 years and older with a first-time deep venous thrombosis in the leg or pulmonary embolism and control subjects ≥70 years (n=431) without a history of thrombosis were included. Exclusion criteria were active malignancy and severe cognitive disorders. Estimations of the risk of thrombosis associated with two or more impaired Katz ADL items, inability to walk outside for 15 minutes, sedentary life style (>20 hours per day) and low handgrip strength (<15%) were performed. Odds ratios (ORs) adjusted for age, sex, and study centre with 95% confidence intervals (CI95) and population attributable risks (PAR) were calculated.

Results

Impaired ADL was associated with a 2.9-fold (CI95 1.6-5.3) increased risk of venous thrombosis, impaired mobility with a 3-fold (OR 3.0 (CI95 1.9-4.7)), a sedentary lifestyle with a 4-fold (OR 4.0 (CI95 2.5-6.3)) and low handgrip strength with a 2.3-fold (CI95 1.5-3.4) increased risk of thrombosis. The PARs for ADL disability, inability to walk outside for 15 minutes, sedentary lifestyle, and low hand grip strength were 8%, 13% 29%, and 13%, respectively.

Conclusion

In those over 70 years of age, functional impairments are major risk factors for venous thrombosis. Findings may have important implications for awareness of venous thrombosis risk by providers caring for older people.

INTRODUCTION

The incidence of venous thrombosis increases steeply with age. [1] The oldest old have a 100-fold increased risk of venous thrombosis compared with young people, thus age is one of the most important risk factors. It is unclear why ageing leads to an increased incidence of thrombosis, and it is remarkable that few data are available on risk factors that are almost exclusively present in older individuals, i.e., age-specific risk factors. [2] With ageing a decline in physiological functioning occurs with an increased susceptibility to functional impairments. Functional impairment predisposes to adverse health outcomes including death. [3-5] Functional impairment is associated with an inflammatory state, which promotes pro-coagulation [6], so functional impairment associated with ageing could result in an increased risk of venous thrombosis. Functional impairment is also associated with reduced mobility [3] An increased risk of thrombosis with mobility impairment would be expected based on the detrimental effects on muscular pump function and subsequent stasis of blood flow in the lower extremities. [6]

To study whether impaired functional status is an age-specific risk factor for venous thrombosis, we evaluated the associations between impaired functional status, expressed as disability of activities of daily living (ADL), impairment of mobility, sedentary lifestyle, and impaired hand grip muscle strength, and the occurrence of venous thrombosis in individuals aged 70 years and older.

METHODS

A detailed description of the Age and Thrombosis - Acquired and Genetic risk factors in the Elderly (AT-AGE) study has been published previously. [7] In brief, AT-AGE is a two-centre population-based case-control study among individuals aged 70 years and older to determine risk factors for venous thrombosis in the older population. Individuals aged 70 years and older with a first occurrence of deep vein thrombosis of the leg (DVT) and/or pulmonary embolism (PE) were enrolled in the Leiden area (the Netherlands) and Burlington (Vermont, United States). Control subjects were randomly selected from several primary care practices in the same geographical area as the cases. For both the cases and the control subjects, exclusion criteria were the presence of an active malignancy and psychiatric or cognitive disorders that hindered communication during the first contact. All participants provided written informed consent and gave permission to obtain information about their medical history. The study was approved by the Medical Ethical Committee of the Leiden University Medical Centre and by the Committee of Human Research of the University of Vermont. In Leiden, 504 cases were invited and 341 (68%) participated. In Burlington, 115 cases were invited and 62 (54%) participated. Of

the 407 invited control subjects in Leiden, 306 (75%) participated, while in Burlington 127 (65%) of the 196 invited control subjects participated. [7] The index date for the cases was defined as the date of diagnosis of the thrombotic event and for control subjects, this was the date of completing the interview. At enrollment, a structured detailed interview took place at the participant's home. The median duration between the index date and the home visit for the cases was 5 weeks (range 1–44 weeks), 75% were visited within 7 weeks, and 90% were visited within 10 weeks.

We used four instruments during the home visit to ascertain functional status prior to the index date. For ADLs we used the Katz Index [8] which includes questions on whether the participant was independent in six daily activities by yes/no answers: bathing, dressing, toilet use, transferring in and out of bed, eating, and presence of urinary or bowel incontinence. The range of the impairment score can be 0 of 6 (no ADL impairment) to 6 of 6 (fully ADL impaired). We used two items of the Barthel index to assess impairment of mobility; ability to walk outside for 15 minutes and ability to climb stairs. [9] We also estimated sedentary lifestyle by querying participants about the percentage of time spent sleeping and sitting per day during the two weeks before the index date. [10,11] Measurement of hand grip strength was performed twice in the dominant hand using a Jamar dynamometer, with the highest result used. [12] Weight was measured with a calibrated scale and height was measured. Body-mass index was calculated in kg per m^2. We also obtained information about the presence of other diseases such as a history of myocardial infarction and chronic obstructive pulmonary disease, and about other risk factors for venous thrombosis including recent hospitalisation, surgery, fracture, plaster cast or splint use, and minor injury or immobilisation. [7] A provoked event was defined when one of the following conditions was present in the three months before the thrombotic event: recent hospitalisation, surgery, fracture, plaster cast or splint use, minor injury or immobilisation in the home situation.

Analyses

We included cases and control subjects who completed the interview (401 of the 403 cases and 431 of the 433 control subjects). Characteristics of the control subjects were tabulated by study centre. We estimated the risk of venous thrombosis associated with the following expressions of the Katz ADL instrument: 'yes' versus 'no' to each of the 6 items of the Katz score, ADL disability present, defined as ≥ two impaired Katz ADL items compared to no impairment and for ≥ 3 impairments versus none. Impairment of mobility was present if an individual was not able to walk outside for 15 minutes. [9] Impairment of mobility was also separately analysed measured by the ability to climb stairs. A sedentary lifestyle was analysed by comparing individuals who spent 14 hours or less sleeping or sitting (20^{th} percentile in the control subjects) with individuals who spent 20 hours or more sleeping or sitting (80^{th} percentile in the control subjects). We

also considered tertiles of the hours of sedentary time, with the lowest tertile as the reference category. As handgrip strength is sex dependent, we assessed risk by comparing participants below versus above the sex-specific 15th percentile hand grip strength in the control group (<26 kg in men, and <16 kg in women). [13,14] We also assessed handgrip strength in sex specific tertiles based on the distribution in control subjects.

For all analyses, multivariable logistic regression models were used to estimate the odds ratios (ORs) and their 95% confidence intervals (CI95s) as estimates of the relative risk. All odds ratios were adjusted for the pre- defined potential confounders, age (continuous), sex and study centre. As weight loss is seen as an important marker of functional status, [3] further adjustments were made for BMI in three categories, <25kg/m2, 25-30 kg/m2 and >30kg/m2). Also adjustments were performed for the presence of co-morbidities (a history of myocardial infarction, transient ischemic attack, stroke, heart failure, or chronic obstructive pulmonary disease). To assess whether the association of functional impairment and thrombosis varied within the three BMI groups, we performed stratified analyses for all four functional impairments within the three BMI groups. The risk associated with an impaired functional state was calculated for provoked and unprovoked venous thrombosis and DVT and PE separately. To investigate whether an accumulation of functional impairments would influence the risk of thrombosis, we calculated the risk of thrombosis associated with the number of impairments present (1-4).

To obtain insight into the contribution of impaired functional state to the incidence of venous thrombosis in the older population we estimated the population attributable risk (PAR) for each of the four functional state entities. The PAR was calculated by *pd* (OR-1)/(OR). In which *pd* is the prevalence of the risk factor among cases, and OR is the adjusted OR. All statistical analyses were performed using SPSS 20 for Windows (SPSS Inc, Chicago, Ill).

RESULTS

Median age was similar for control subjects in the Netherlands (76 years (range 70-94) and the United States (76 years, range 70-96). Distributions of sex and BMI (kg.m^{-2}) were also similar between the two centres (Table 1). The prevalence of impaired functional status by each of the four measures was low among controls, with sedentary lifestyle being the most prevalent. Since no major differences in other variables were observed, we combined data from the two centres for all analyses.

The separate Katz ADL items were each associated with venous thrombosis. The adjusted ORs for impairment of bathing, getting dressed, toilet use, eating, transferring in and out of bed (or chair) and the presence of urinary or bowel incontinence were respectively: 2.0 (CI95 1.2-3.2), 3.9 (CI95 1.9-8.2), 16.1 (CI95 2.1-124.9), 5.7 (CI95 0.6-51.1)

Table 1. Characteristics of control subjects by center

	Controls NL	Controls US
Number of Participants	306	125
Male sex (%)	147 (48)	62 (50)
Median Age (Range)	76 (70-94)	76 (70-96)
70-75 years (%)	126 (41)	49 (39)
75-80 years (%)	90 (29)	39 (31)
80-85 years (%)	61 (20)	24 (19)
>85 years (%)	29 (10)	13 (11)
Median BMI* (range)	26 (17-42)	27 (19-49)
Number of KATZ ADL disability		
0 (%)	253 (86.9)	117 (95.1)
1 (%)	38 (13)	6 (6)
2 (%)	8 (3)	2 (2)
≥ 3 (%)	6 (2)	0 (0)
Inability to walk outside 15 min (%)	23 (8)	9 (7)
Sedentary lifestyle (>20 h/24 h) (%)	138 (12)	18 (14)
Impaired hand grip strength (%)	25 (8)	19 (15)

NL= Netherlands, US= United States
* BMI = Body Mass Index kg.m-2;

13.9 (CI95 1.8-109.5), and 1.5 (CI95 1.0-2.3). In table 2, the associations of four functional impairment entities with venous thrombosis are presented. The risk of thrombosis increased 3-fold (OR 2.9; CI95 1.6-5.3) when two or more impairments were present on the ADL score compared with no impairments. Impairment of mobility was associated with a 3-fold (CI95 1.9-4.7) increased risk. The risk of thrombosis was up to 4-fold higher in individuals with a sedentary lifestyle defined as ≥20 hours of sitting/sleeping than in the group of ≤14 hours (OR 4.0; CI95 2.5-6.3). A gradually increasing risk of thrombosis was also found across tertiles of hours of sedentary time. Low handgrip strength was associated with a 2.3-fold (CI95 1.5-3.4) increased risk of thrombosis.

Functional impairment entities were associated with BMI in the control subjects. Impairment on the ADL score was lowest in the middle BMI group (1.7%) and 3.9% in the low, 4.3% in the high BMI group. Impairment of mobility was highest (10.6%) in the high BMI group. Impairment of mobility was present in 4.5% in the low BMI group, and 6.9% in the middle BMI group. A sedentary lifestyle was present in 17.2% in the low BMI group, 17.5% in the middle BMI group, and 29.0% in the high BMI group. There was no association between BMI and low hand grip strength (low: 13.5%, middle: 10.9%, high: 11.6%).

Additional adjustment for BMI did not alter the associations of any of the four functional impairment measures with thrombosis risk. Stratified analyses by BMI group for all four functional impairment entities showed that functional impairment was associated with thrombosis in each group. In the lowest BMI group, impairment of the ADL score was associated with a 1.9 fold (CI95 0.6-5.4) increased risk, in the middle BMI group and

the high BMI group, a risk of 5.9 (CI95 1.7-20.8) and 2.4 (CI95 0.7-8.1) was found. Corresponding ORs for impairment of mobility for the three BMI groups were 3.7 (CI95 1.5-9.1), 3.1 (CI95 1.5-6.5) and 2.2 (CI95 0.9-5.4). For a sedentary lifestyle these ORs were 3.7 (CI95 2.0-6.6), 3.6 (CI95 2.1-6.2) and 2.8 (CI95 0.9-3.3). A low handgrip strength increased the risk of thrombosis in the low BMI group 2.2 fold (CI95 1.0-4.5), 1.6 fold (CI95 0.8-3.3) in the middle group, and 2.9 (CI95 1.2-7.0) in the high BMI group.

Associations between the impairments and thrombosis risk were similarly present after adjustments for the presence of co-morbidities. (table 2) Functional impairment was associated with an increased risk of both DVT and PE. Presence of two or more im-

Table 2. Associations of functional impairment with venous thrombosis

Functional Impairment Type□	Cases	Controls	OR crude (CI95)	OR adj* (CI95)	OR adj** (CI95)	OR adj*** (CI95)
ADL disability						
0 vs ≥ 2 disabilities	50 (14.6)	16 (4.1)	4.0 (2.2-7.1)	2.9 (1.6-5.3)	2.9 (1.5-5.6)	2.7 (1.5-5.2)
Number of disabilities						
0 (%)	292 (84.1)	370 (89.4)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
1 (%)	55 (15.9)	44 (10.6)	1.6 (1.0-2.4)	1.3 (0.8-2.0)	1.3 (0.8-2.1)	1.2 (0.7-1.9)
2 (%)	23 (7.3)	10 (2.6)	2.9 (1.4-6.2)	2.1 (1.0-4.7)	2.6 (1.1-6.1)	1.7 (0.7-3.9)
≥ 3 (%)	27 (8.5)	6 (1.6)	5.7 (2.3-14.0)	3.9 (1.5-9.8)	3.1(1.1-8.7)	3.9 (1.5-10.3)
Impairment of mobility						
Walk outside, not able (%)	78 (19.5)	32 (7.4)	3.0 (2.0-4.7)	3.0 (1.9-4.7)	2.9 (1.8-4.7)	2.8 (1.7-4.6)
Climbing stairs, not able (%)	72 (18.0)	29 (6.7)	3.0 (1.9-4.8)	2.4 (1.5-3.9)	2.2 (1.3-3.7)	2.1 (1.3-3.5)
Sedentary lifestyle						
20th percentile (≤14 h)	42 (10.6)	92 (23.2)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
80th percentile (≥20h)	173 (43.6)	90 (22.7)	4.2 (2.7-6.6)	4.0 (2.5-6.3)	4.4 (2.7-7.2)	4.0 (2.4-6.8)
Tertiles						
< 16 h	68 (17.4)	133 (31.5)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
16-18 h	115 (29.5)	156 (37.0)	1.4 (1.0-2.1)	1.4 (0.9-2.0)	1.3 (0.9-2.0)	1.3 (0.9-2.0)
>18 h	207 (53.1)	133 (31.5)	3.0 (2.1-4.4)	2.8 (1.9-4.1)	2.8 (1.9-4.1)	2.7 (1.8-4.0)
Low hand grip strength						
<15%	92 (23.2)	50 (11.6)	2.3 (1.6-3.3)	2.3 (1.5-3.4)	2.1 (1.4-3.2)	1.7 (1.1-2.6)
>15%	305 (76.8)	380 (88.4)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Tertiles						
<33%	222 (55.9)	161 (37.4)	2.1 (1.5-2.9)	2.2 (1.5-3.2)	2.0 (1.4-3.0)	2.1 (1.4-3.1)
33-66%	90 (22.7)	142 (33.0)	1.0 (0.7-1.4)	1.0 (0.7-1.4)	1.6 (0.7-1.5)	1.0 (0.7-1.5)
> 66%	85 (21.4)	127 (29.5)	1 (ref)	1 (ref)	1 (ref)	1 (ref)

*adj for age, sex and study center **adj for age, sex, study center and BMI ***adj for age, sex, study center and co-morbidities

□ ADL missing cases 4; missing control subjects 1; Impairment of mobility: missing cases 1 missing control subjects 1; Sedentary lifestyle: missing cases: 11 missing control subjects 9; Low handgrip strength: missing cases: 4 missing control subjects: 1

pairments on the ADL score was associated with a 3.8 fold (CI95 1.9-7.6), increased risk of DVT and of 2.3 fold (CI95 1.1-4.5) increased risk of PE. Impairment of mobility had an odds ratio for DVT of 3.2 (CI95 1.9-5.4), and of PE of 2.6 (CI95 1.6-4.4). Sedentary lifestyle increased the risk for both DVT and PE (OR DVT 2.6; CI95 1.8-3.9, OR PE 3.1; CI95 2.2-4.5). Unlike the other measures, low handgrip strength was mainly associated with DVT; OR 3.1 (CI95 1.8-5.3) for DVT and 1.7 (CI95 1.1-2.8) for PE. Of the 401 cases, 182 (45.4%) had a provoked venous thrombotic event. In general, the functional measures were more strongly associated with provoked events. For unprovoked thrombosis, two or more impairments on the ADL score was associated with a 1.8 fold (CI95 0.9-3.7) increased risk, impaired mobility was associated with an odds ratio of 2.4 (CI95 1.4-4.1), sedentary life style had an odds ratio of 1.7 (CI95 1.1-2.4), and low handgrip strength had an odds ratio of 1.8 (CI95 1.1-2.9). For the provoked events the odds ratio of thrombosis was 4.9 (CI95 2.3-8.7) when two or more impairments on the ADL score were present, while this was 3.6 (CI95 2.1-5.9) for impaired mobility, 5.4 (CI95 3.6-8.1) for sedentary lifestyle and 2.8 (CI95 1.7-4.7) for impaired handgrip strength.

Table 3 shows that the number of functional impairment entities was positively associated with risk of venous thrombosis. The prevalence of at least one functional impairment was 56.5% in the cases (221 of 391) and 28.7% the control subjects (121 of 422). Compared with those with no impairments, the OR of thrombosis increased from 3.0 (CI95 2.1-4.3) when one impairment was present, up to 25.1 (CI95 3.2-195.0) when four impairments were present.

Population attributable risk estimates the proportion of a disease attributable to a risk factor under the assumption of causality. The PARs for sedentary lifestyle, ADL disability, inability to walk outside for 15 minutes and low hand grip strength were 29%, 8%, 13% and 13%, respectively.

Table 3. Association of accumulation of functional impairments with venous thrombosis

Functional Impairment	Cases	Controls	OR crude (95%CI)	OR adj* (95%CI)	OR adj** (95%CI)	ORadj*** (95%CI)
n= 0	170 (43.5)	301 (71.3)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
n= 1	121 (30.9)	72 (17.1)	3.0 (2.1-4.2)	3.0 (2.1-4.3)	3.2 (2.2-4.6)	2.9 (2.0-4.3)
n= 2	50 (12.8)	34 (8.1)	2.6 (1.6-4.2)	2.8 (1.7-4.5)	2.8 (1.7-4.7)	2.4 (1.4-4.0)
n= 3	34 (8.7)	14 (3.3)	4.3 (2.2-8.2)	4.4 (2.2-8.6)	4.1 (2.0-8.3)	3.0 (1.5-6.3)
n= 4	16 (4.1)	1 (0.2)	28.3 (3.7-215.5)	25.1 (3.2-195.0)	18.8 (2.4-148.1)	22.9 (2.8-186.1)

*adj for age, sex and study center

**adj for age, sex, study center and BMI

***adj for age, sex, study center and co-morbidities

DISCUSSION

In a two-centre population-based study of people over age 70, comprising 401 patients with thrombosis and 431 control subjects, we showed that functional impairment defined as an impaired ADL, impaired mobility, sedentary life style, and low handgrip strength were associated with an increased risk of venous thrombosis. These four manifestations of functional impairment each were associated with a 2- to 4-fold increased risk of thrombosis. Further, an increasing number of functional impairments was also associated with risk of thrombosis. The overall relative contribution of each functional impairment entity to the thrombotic risk, based on the PAR, varied from 8% to 29%. Furthermore, all functional impairment entities were also associated with unprovoked thrombosis, which suggests that the associations were not explained by the provoking factors for venous thrombosis. The associations of functional impairment with venous thrombosis were also present in the provoked thrombosis group, indicating that when other major risk factors are present in this older age group, functional impairment is also important.

Our findings are in line with previous reports that showed that functional impairment is associated with the risk of thrombosis. A cross-sectional analysis of older in-hospital patients illustrated that a decreased Katz ADL score was associated with 2-fold increased risk of asymptomatic thrombosis. [15] Furthermore, Folsom et al reported a 1.5- to 2-fold increased risk of future thrombosis with frailty, also a measure of functional status, in those 65 years and older. [16] Having a sedentary lifestyle or transient immobility have been previously reported as risk factors in both young and older populations. [7,17,18] In this study, the number of functional impairment markers was positively associated with the risk of venous thrombosis, indicating that impairment on several aspects, which likely reflects the severity, influences the risk of thrombosis.

Various causal mechanisms regarding the association of impaired functional status and venous thrombosis can be hypothesised. [16,19] Biological age is related with the functional status of an individual. [20] Age-related alterations of the venous vessel wall are postulated to provoke thrombus formation. [21,22] Low hand grip strength reflects loss of overall muscle mass and strength. A decline of leg muscle strength, and specifically calf muscles could result into stasis of the blood flow in the legs, and subsequently venous hypertension could induce a pro-thrombotic environment. [6] Furthermore, functional impairment with ageing is associated with inflammation and procoagulation. [23,24] Thrombosis might occur more easily in older, impaired individuals due to deregulation of the blood coagulation system by increased inflammation and higher levels of D-dimer, factor VIII and von Willebrand factor [25], all of which are related to risk of venous thrombosis. [16,26]

Strengths and limitations of this study require discussion. We performed home-visits, thereby allowing functionally impaired individuals to participate. This resulted in a high participation rate and minimised selection bias. [7] However, we cannot completely rule out that participation was related to the presence of functional impairment which would, if different in cases and controls, lead to biased estimates. If at all present, this would most likely have affected controls, with those with impairments participating less readily than those with. If this bias was present, the reported risks are an overestimation. Recall bias may have been present for the self-reported measures, but not for the handgrip assessment. Handgrip strength could have been influenced by conditions associated with the venous thrombosis event, such as recent hospitalisation or surgery, but presence of associations in both unprovoked and provoked thrombosis suggests a minimal impact on interpretation of results. Despite the use of standardised questionnaires for both the cases and control subjects, we cannot rule out differential recall of functional status by case control status.

Functional impairment may be the result of disorders affecting the risk of thrombosis, leading to confounding. However, the risk of thrombosis remained clearly elevated also after adjustment for several diseases, such as myocardial infarction and pulmonary disorders. BMI was not a confounder in the association of functional impairment and venous thrombosis, although weight loss is seen as an important marker of functional status [3], and higher BMI is a risk factor of thrombosis in the middle aged population.

In conclusion, functional impairment is a risk factor for venous thrombosis in older people and the contribution of functional impairment to the overall incidence of venous thrombosis is high. The risk of thrombosis is increased in older individuals with functional impairments, also when other major risk factors are present. Moreover, accumulation of multiple functional impairments signified substantial risk. Our findings have important implications for awareness of venous thrombosis risk by providers caring for older people.

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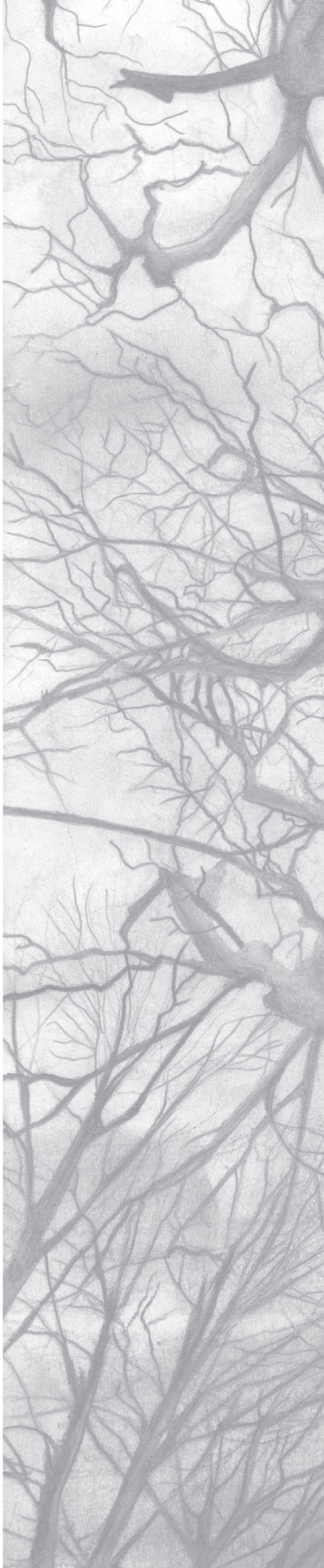

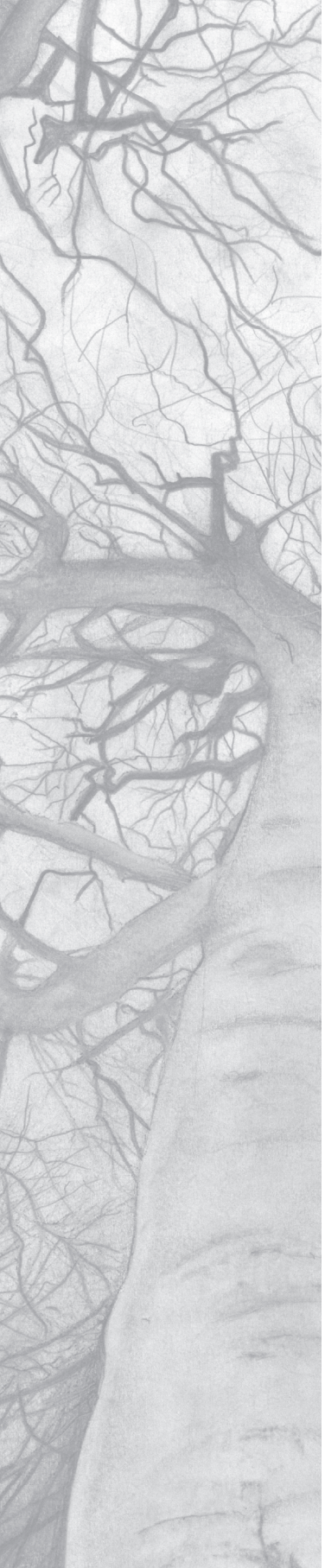

CHAPTER 7

Participation of older people in research studies

M.J. Engbers
A. Karasu
J.W. Blom
M. Cushman
F.R. Rosendaal
A. van Hylckama Vlieg

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INTRODUCTION

At advanced age, individuals are increasingly prone to develop diseases and multi morbidity is more frequently present. [1] Since life expectancy in developed countries is increasing, [2] research into causes and prevention of disease in the elderly is necessary. Nevertheless, older people are underrepresented in research studies; Bugeja et al reported that one third of research articles in the *BMJ*, *Gut*, the *Lancet*, and *Thorax* published between June 1996 and June 1997, excluded older adults without justification. [3] McMurdo et al repeated this analysis in 2005, and found that nearly 15% of articles still excluded older adults and that fewer than 5% of published articles focused specifically on older adults. [4] Several explanations for the scarcity of studies among older people may be postulated. [5-7] First, researchers may anticipate barriers to participation such as mobility problems to reach their study centre. Second, impairments (e.g., visual and cognitive) of older people may make it difficult to participate. [8-10] Third, co-morbidities and competing risks among older people may play a role in influencing study outcomes. Because of the lack of inclusion of older persons in key trials, external validation of findings from studies of younger persons to an older population may be questioned. [4,11,12] Furthermore, research studies that are conducted among older people often include the most vital individuals, i.e., without co-morbidities, recent surgery, or hospitalisation, which can make the generalizability of the study results questionable. Therefore, there is a need for research studies among the older people.

The incidence of venous thrombosis increases steeply with age and ~60% of all venous thrombosis events occur in those aged 70 years and older. [13] However, vast majority of studies into etiology, treatment and prevention of venous thrombosis are restricted to younger people. [14] Here we report on factors that affecting the participation of older patients in a population-based case-control studies on the etiology of venous thrombosis among older people (70-80 years). We compared the participation in older patients in the Multiple Environmental and Genetic risk factors for venous thrombosis (MEGA) and the Age and Thrombosis, Acquired and Genetic risk factors in the Elderly (AT-AGE) study. [15]

METHODS

The designs of the MEGA and the AT-AGE study have been described in detail previously. [15,16]. Both studies are population-based case-control studies designed to investigate acquired and genetic risk factors for venous thrombosis. From March 1999 onwards, In the MEGA study, all consecutive patients aged 18-80 years old with a first episode of venous thrombosis (deep venous thrombosis of the leg (DVT), the arm, or a pulmonary

embolism (PE)) were identified via the anticoagulation clinics in the Netherlands (6 clinics: Amersfoort, Amsterdam, Den Haag, Leiden, Rotterdam, Utrecht). From March 2001 until the end of the study in 2004, patients in age 70-80 years were no longer invited due to low agreement rates to participate. In the AT-AGE study, consecutive patients aged 70 and older with a first DVT of the leg or PE between 2008 and 2011, were identified via the anticoagulation clinics in Leiden and Haarlem, the Netherlands and the Vascular Laboratory and the Radiology department of the University of Vermont Medical Centre, Burlington, Vermont, USA. In both the MEGA study and the AT-AGE study, patients with severe psychiatric problems or an inability to speak Dutch or English were excluded. In the AT-AGE study, patients with an active malignancy were also excluded.

For this manuscript we analysed the patients age 70-80 years included in the MEGA study and the AT-AGE study in the Netherlands. Patients were invited in a similar way in both studies, i.e., by means of a personalised invitation letter sent by the anticoagulation clinic followed by telephone contact by a dedicated data manager or trial nurse from the study centre. To allow comparisons in the MEGA and the AT-AGE study, MEGA study participants with active malignancy were excluded from this analysis.

Subsequent data collection differed for the two studies. In the MEGA study a detailed questionnaire on acquired risk factors for venous thrombosis was sent by mail to all patients. Patients who were unable or did not want to fill in the mailed questionnaire were approached by telephone and a short questionnaire was completed during the telephone interview. Blood samples were obtained approximately three months after discontinuation of anticoagulation treatment. If treatment duration was longer than one year, blood was sampled during treatment. For this blood draw all patients were invited to come to the anticoagulation clinic in their region. In the AT-AGE study all participants were visited at home twice. During the first home visit an interview was conducted by a trained research assistant and a detailed questionnaire on acquired risk factors for venous thrombosis was completed. During this home visit, a blood sample was drawn (to obtain DNA and non-vitamin K dependent coagulation factors). One year after the venous thrombosis (when most patients had discontinued anticoagulation therapy), a second home visit was conducted and, another blood sample obtained.

Analyses

For this study we calculated the participation rate for filling in a questionnaire (questionnaire participation rate) and for both filling in a questionnaire and donating a blood sample (overall participation rate). In the MEGA study the questionnaire participation rate was determined as the percentage of patients for whom there was a returned mailed questionnaire or for whom a short telephone administered questionnaire was completed. In the AT-AGE study, this was the percentage of patients for whom a first home visit was conducted, during which the study interview was completed. The overall

Table 1. Characteristics of patients of the MEGA and the AT-AGE study

	MEGA study (70-80 years) N(%)	AT-AGE study (70-80 years) N (%)
Questionnaire participation	**251**	**224**
Men, N (%)	130 (51.8)	100 (45.0)
DVT without PE, N (%)	148 (59.0)	87 (38.8)
Median BMI kg.m-2 (range)	25.9 (17.1-53.0)	26.8 (18.0-43.3)
Hospitalisation(%)	23 (9.2)	72 (32.1)
Surgery(%)	20 (8.0)	50 (22.3)

N = number, DVT= deep venous thrombosis, BMI = Body Mass Index
MEGA: Multiple Environmental and Genetic risk factors for venous thrombosis
AT-AGE: Age and Thrombosis, Acquired and Genetic risk factors in the Elderly

participation rate was calculated as the percentage of patients that finished the full research study including filling in the questionnaire, and participating for blood sampling (for the AT-AGE study the second blood sample). To investigate whether the participation rate differed with age, we stratified the analyses by age groups 70-75 and 75-80 years old. We also assessed the characteristics of the participating patients in the two studies, such as sex, BMI, and the presence of recent surgery.

RESULTS

The participation rate in the two studies is shown in figure 1. In the MEGA study, 446 patients and in the AT-AGE study, 309 patients aged 70-80 years old were eligible to participate. In the MEGA study, 251 patients provided information on the questionnaire (56%) as compared with 224 patients in AT-AGE (72%). In the MEGA study the median time between the venous thrombotic event and the blood draw was 10 months (range 3-25 months) and in the AT-AGE study this was 12 months (range 11-16 months). Of the patients 22 (8.8%) patients died in the MEGA study before the blood draw was performed compared with 11 patients (4.9%) in the AT-AGE study. In the MEGA study, a blood sample was provided by 53% of the patients (122 of 229 patients). In the AT-AGE study this was 89% of the patients (189 of 213 patients). The overall participation (questionnaire participation plus blood draw)) was 27% (122/446) in the MEGA study and 61% in the AT-AGE study (189/309) (figure 1). Age-stratification demonstrated that the questionnaire participation rate was similar across the 5-year age-groups within the studies. (MEGA study 70-75 years: 56% (125/223) of which 51% men, 76-80 years: 57% (126/223) of which 52% men. In the AT-AGE study: 70-75 years: 74% (131/178), 76-80 years: 71% (93/131). In the AT-AGE study 48% were men in the 70-75 years old group, and 40% in the 75-80 years old group.

Characteristics of the participating patients in both studies are shown in Table 1. Patients included in the MEGA study were diagnosed more frequently with DVT only (59%) than patients in the AT-AGE study (39%). Hospitalisation and surgery three months prior to the thrombosis were more frequently present in the AT-AGE study than the MEGA study.

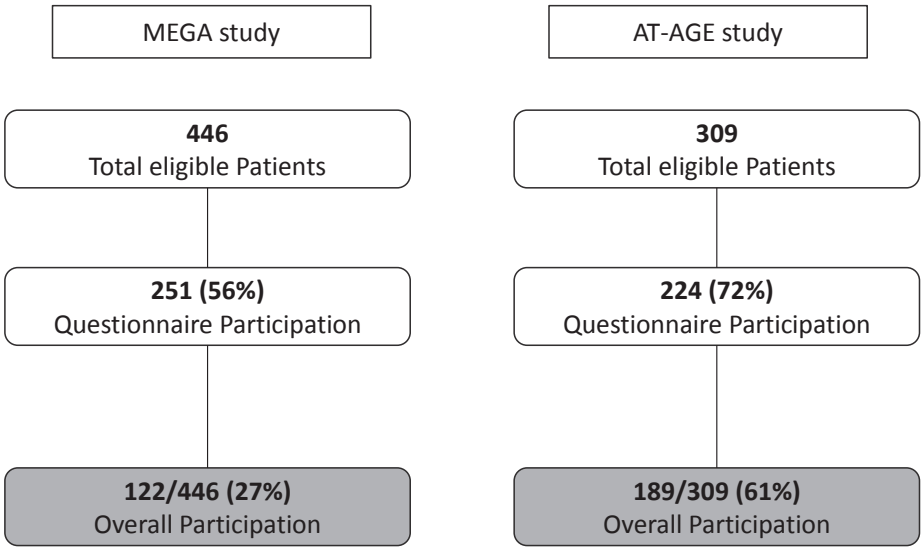

Figure 1. Participation rates in the MEGA and the AT-AGE study

DISCUSSION

In this study we show that participation rate of older patients in research is substantially increased (from 27% up to 61%) when home visits were used. Questionnaire participation via a structured interview during a home visit is superior to asking participants to complete a mailed questionnaire (72% versus 56% completion). Participation did not differ among those aged 70-75 compared to 75-80.

Reasons for higher participation via an in-home visit in old patients with thrombosis are likely related to mobility and social aspects, i.e., already having frequent hospital trips, reluctance to ask assistance of caregivers to participate in research, and a lack of personal contact with investigators for a mailed questionnaire. Visual or cognitive impairment are also more likely to deter participation in a questionnaire as compared to a home visit as participants may gain confidence when a research co-worker is assisting them to answer the questions. Davies et al. previously reported on the role of the distance between study site and the person's residence and the importance of

minimising the participant reluctance to participate in the New Castle 85+ study. [9] Moreover, it was previously recognised that the personal contact of a researcher during a home visit encourages older adults to be more dedicated to the research study, and thus to participate for a longer period. [17] Comparing the percentages of older patients with hospitalisation and surgery, we found higher prevalences in AT-AGE than in the MEGA study. This indicates that home visits increased participation rates also in the most vulnerable group. Unfortunately, we do not have reliable data on characteristics of the non-responders and the reasons why patients were not able or willing to participate. We cannot rule out that other study differences apart from the ones described here may explain a difference in the participation rate, e.g., in the AT-AGE study it was emphasised to the patients that this study was specifically focused on the older adults and this might have increased willingness to participate.

In conclusion, as the MEGA and the AT-AGE study were both performed in the same region (the Netherlands), study the same disease (venous thrombosis), and were performed by the same investigators, we had the opportunity to compare the participation rates among older patients when using different approaches for inclusion. Home visits were an effective approach to increase the participation rate in this age group.

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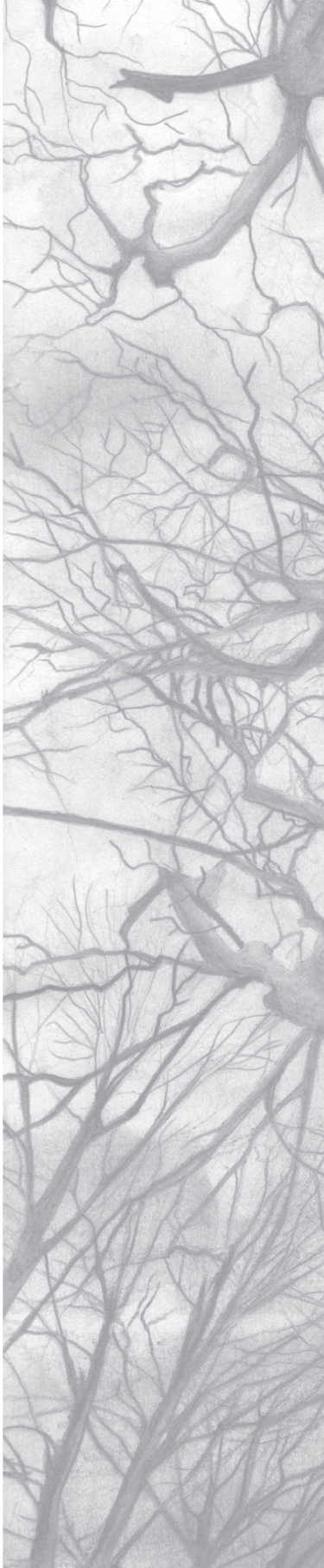

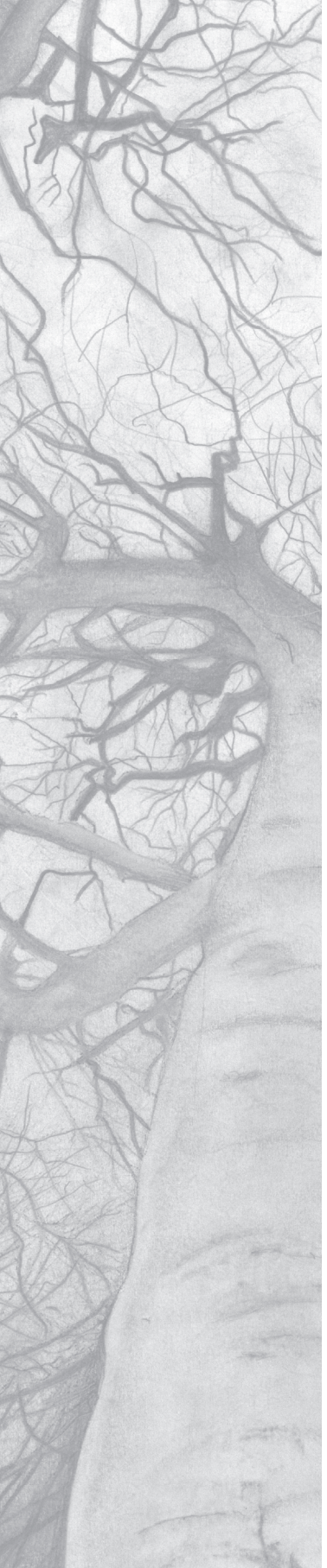

CHAPTER 8

The effect of time between venipuncture, processing and freezing on the measurement of coagulation factor levels

M.J. Engbers
M. Cushman
F.R. Rosendaal
A. van Hylckama Vlieg

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INTRODUCTION

Coagulation factor levels are commonly measured in research studies evaluating etiology of diseases, in particular vascular diseases. Levels in the upper 10 percent (>P90) of the population distribution of procoagulant factors prothrombin, FVIII, FIX, FXI, and low levels of anticoagulation proteins are associated with an increased risk of venous thrombosis. [1-5] Many pre-analytical variables may affect the accuracy of these assays, including the duration of time from venipuncture to specimen processing and storage environment. [6,7] Clinic-based research settings are able to minimise the time from venipuncture to blood processing. However, immediate processing of blood samples is not feasible with some study designs, e.g., when samples are drawn in participant homes. In these settings, optimal sample handling should minimise pre-analytical factors that could impact coagulation factor level measurements.

Factor VII, factor VIII, and von Willebrand factors levels may be affected by a cold environment prior to centrifugation. [8-10] Pre-centrifugation storage temperature and time exposed to cold temperature prior to centrifugation did not appear to influence measured levels of factors prothrombin, V, VII, IX, XI and XII in the study of Favaloro. [8] Recommendations regarding the optimal temperature for storage prior to centrifugation of whole blood for performing coagulation factor assays are given in the fifth edition of the guidelines of the *Clinical and Laboratory Standards Institute*. [11] Cold storage prior to centrifugation of samples for factor VIII and von Willebrand factor is not advised in this guideline; however, no strict advice is given regarding the optimal sample handling conditions for other coagulation factors. This is due to the limited available evidence. The aim of this study was to assess the effect of differences in the time-interval between venipuncture and processing of blood on the levels of fibrinogen, prothrombin, factors VIII, IX, XI and antithrombin. Furthermore, the effect of storage of blood samples at different temperatures prior to centrifugation was assessed.

METHODS

A blood sample was obtained from 10 healthy volunteers; 4 males and 6 females with a mean age of 28 years (range 24–41 years). Venous blood was collected from the antecubital vein into three tubes of 3.8% sodium citrate (*Starstedt®*). Venous access was achieved at first attempt in all participants and a tourniquet was placed for a maximum of 25 seconds. All volunteers gave informed consent and the study was approved by the Medical Ethical Committee of the Leiden University Medical Centre in the Netherlands. The three citrate tubes were processed separately. Tube A, the reference sample, was processed directly after blood sampling. The sample was centrifuged at 18°C for 10 min

at 2500g and the plasma was immediately stored at -80°C. Tube B was stored for 2.5 hours at room temperature (~21˚C) while tube C was put on ice for 2.5 hours immediately after the venipuncture (~4˚C). After 2.5 hours both tube B and C were processed and stored similarly as tube A.

Analyses of the coagulation factor levels were all performed according to the instructions of the manufacturer of the STA-R analyser (Diagnostica Stago, Asnières, France). Fibrinogen (g/L) was determined according to methods of Clauss. [12] Levels of prothrombin, factors VIII, IX, XI, (*one-stage clotting assays*) and antithrombin (*amidolytic assay*) were measured as activity assays with mechanical clot detection methods. Mean coagulation factor levels were compared between the different methods of sample handling using a Student's paired t-test. Mean differences with 95% confidence intervals (CI) were calculated.

In addition, Bland-Altman plots were used to visualise the differences in coagulation factor levels between different storage conditions (on the *y*-axis) versus the mean coagulation factor level of the two storage conditions (*x*-axis). [13] The 95% limits of agreement of the three methods of storage were assessed to evaluate if the magnitude of the measurements affected the mean and the standard deviation of the difference.

RESULTS

The mean levels of the measured coagulation factor levels in tube A (immediately processed), tube B (processed after 2.5 hours storage at room temperature), and tube C (processed after 2.5 hours storage on ice) are shown in table 1. The table also shows the mean differences in coagulation factor levels among the three methods of sample handling. None of the coagulation factor levels (fibrinogen, prothrombin, factor VIII, factor IX, factor XI, and antithrombin) were affected by varying the time between venipuncture and centrifugation as shown by the mean differences between tubes A and B. Varying storage temperature, i.e., room temperature (tube B) versus cold storage on ice (tube C), only affected the measured levels of fibrinogen and coagulation factor VIII. For fibrinogen the mean difference was -0.014 g/L. By contrast, measured factor VIII levels were 16% *lower* after 2.5 hours of storage on ice compared with immediate centrifugation. Bland-Altman plots showed that the differences of the mean levels and their standard deviations were not influenced by the mean levels of fibrinogen, prothrombin, factor VIII, factor IX, factor XI and antithrombin, i.e., the difference between measured levels after different storage conditions was similar for individuals with high or low coagulation factor levels (Data not shown).

Table 1. Mean levels of coagulation factors in different storage conditions

N=10				Mean difference (95% confidence interval)		
Coagulation factor (range in adult population)	**Mean tube A**	**Mean tube B**	**Mean tube C**	**Tube A vs. tube B**	**Tube A vs. tube C**	**Tube B vs. tube C**
Fibrinogen (2–4 g L^{-1})	2.69	2.55	2.83	0.14 (−0.07 to 0.4)	−0.14 (−0.34 to 0.06)	−0.28 (−0.54 to −0.02)
Prothrombin (70–120%)	94.0	91.8	93.8	2.2 (−3.3 to 7.7)	0.1 (−4.2 to 4.5)	−2.1 (−7.0 to 2.9)
Factor VIII (60–150%)	86.6	91.7	70.6	−5.1 (−19.3 to 9.1)	16.0 (−1.6 to 33.7)	21.2 (13.1 to 29.2)
Factor IX (60–150%)	96.5	92.7	99.5	3.8 (−4.1 to 11.6)	−3.1 (−9.3 to 3.2)	−6.9 (−14.1 to 0.4)
Factor XI (60–150%)	100.3	99.4	98.0	0.9 (−3.1 to 4.9)	2.4 (−1.6 to 6.3)	1.49 (−2.4 to 5.4)
Antithrombin (80–120%)	106.8	105.3	107.2	1.5 (−0.08 to 3.1)	−0.4 (−2.3 to 1.5)	−1.9 (−4.9 to 1.1)

Tube A: direct processing. Tube B: room temperature (approximately 21°C), processing after 2.5 h. Tube C: cold environment (approximately 4°C), processing after 2.5 h. Mean differences with the 95% confidence intervals were calculated with Student's paired *t*-test.

DISCUSSION

Guidelines for blood sample storage time and temperature prior to centrifugation for coagulation factors are only available for von Willebrand factor and factor VIII, and are based on only a few studies. [8,9] We demonstrate here that time to centrifugation of blood samples up to 2.5 hours does not affect the levels of prothrombin, antithrombin and factors VIII, IX and XI. These findings are in accordance with the results of Favaloro et al. who reported no effect of time to centrifugation up to 3.5 hours, or differences in storage temperature (room temperature versus cold storage) for measurement of coagulation factors II, V, VII, IX, X, XI, XII. [8]

We also observed that sample temperature prior to centrifugation influenced fibrinogen and FVIII levels. The lower level of factor VIII in our study after exposure to a cold environment supports the idea that cold-activation of factor VIII may occur, so levels are less accurate after storage in a cold environment. [8,9] Differences for fibrinogen were too small to be of relevance.

In conclusion, we demonstrated that storage of blood samples on ice resulted in lower levels of coagulation factor VIII, and did not alter the levels of prothrombin, factor IX, factor XI and antithrombin. Moreover, somewhat higher fibrinogen levels were found after storage of the tubes on ice. Potentially, the effect of storage temperature may differ among different subgroups of the study population, e.g., a different coagulation factor activation in men and women, however the small study size did not allow us to perform subgroup analyses. Storage of the blood samples at room temperature for up to 2.5

hours prior to processing did not affect the measurement of the coagulation factor levels. This indicates that research studies where samples are drawn in participant homes or other non-laboratory settings, can obtain reliable coagulation factor level results when storing the samples at room temperature rather than on ice.

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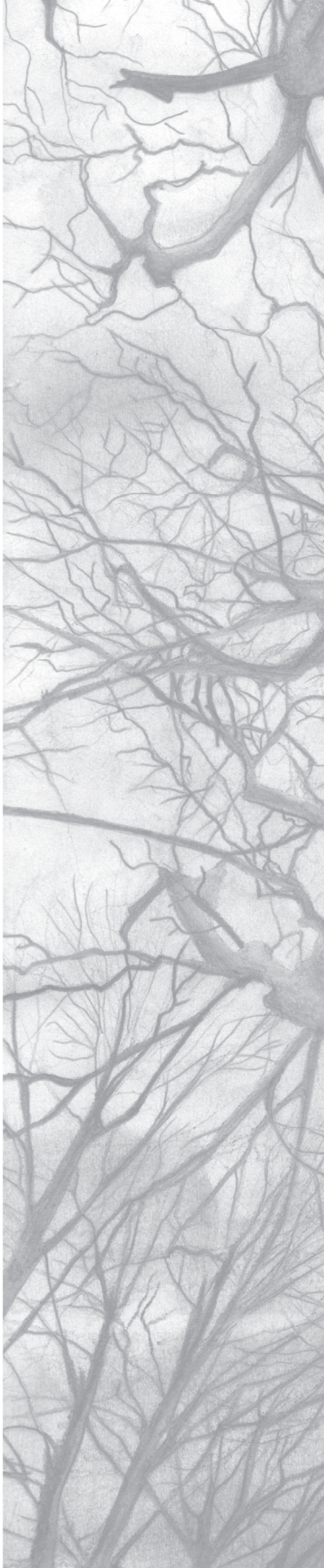

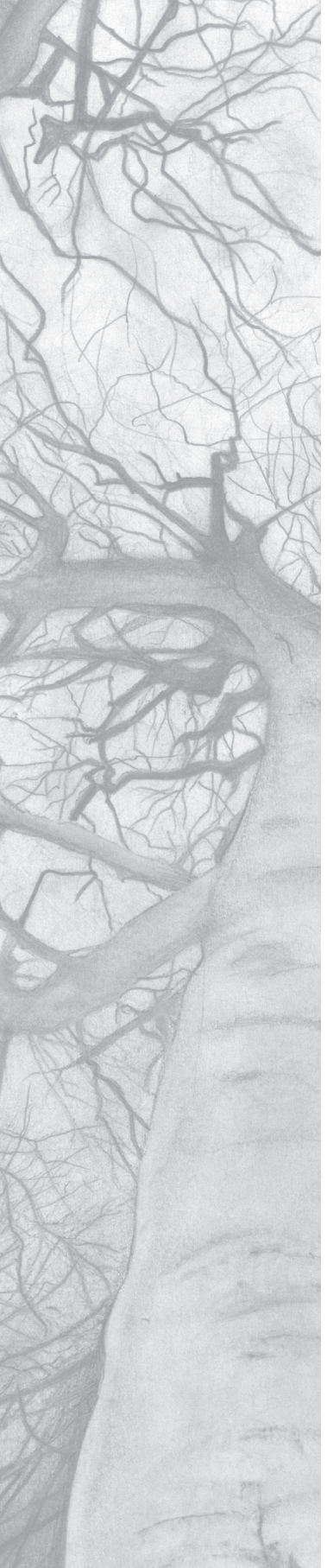

CHAPTER 9

Summary and general discussion

In this discussion section we will summarise the main results of this thesis and possible implications of the findings, discuss methodological considerations and focus on future perspectives in the AT-AGE study.

In **chapter 2** we provided an overview of the available publications (up to 2010) on the effect of conventional risk factors as well as age-specific risk factors for venous thrombosis in the older population. Many conventional risk factors for venous thrombosis established in the young and middle-aged population appeared to increase the risk of thrombosis in the older population as well. Previous reports showed that immobilisation by hospital admission, malignant diseases, heart failure and genetic mutations ((factor V Leiden (FVL, rs6025) and prothrombin 20210A mutation (PT20210, rs1799963)) are risk factors for venous thrombosis in older individuals. As the prevalence of acquired risk factors is higher in the older than in the younger population we found higher population attributable risks (PAR). However, it must be noted that most risk estimates in the older population were based on studies with a small sample size or on subgroup analyses. Furthermore, only a limited number of studies concerning age-specific risk factors such as functional decline and endothelial dysfunction have been published. So, there was limited knowledge regarding risk factors for venous thrombosis in the older population. In order to study the effect of conventional and age-specific risk factors for venous thrombosis in the older population, the "Age and Thrombosis, Acquired and Genetic risk factors in the Elderly" (AT-AGE) study was performed. The AT-AGE study is a population based case-control study among individuals aged 70 years and older for whom home visits were performed. [1]

In **chapter 3** we demonstrated that short-term immobility at home, e.g., due to minor injuries or infections, is a risk factor for venous thrombosis in the older population (2- to 5-fold increased risk). Furthermore, we illustrated that also hospitalisation is a major risk factor in older individuals, similar as in the young and middle aged individuals. Recent hospital discharge was identified as a risk factor, as the risk of venous thrombosis within two weeks after hospitalisation was still increased up to 15-fold. Surgery and plaster cast increased the risk of thrombosis up to 7-fold and fractures increased the risk up to 13-fold. We demonstrated that the relative contribution of in-hospital immobility and out-hospital immobility to the incidence of venous thrombosis is high. Preventive drugs, i.e., thrombosis prophylaxis with low-molecular weight heparin is frequently given to older individuals during hospitalisation. [2] Our results indicate that extended thromboprophylaxis after discharge may be beneficial. This notion is supported by results from the EXCLAIM trial, which included individuals aged 75 years and older. [3] Prolonged duration of treatment after discharge reduced thrombosis rates (absolute difference in the 30 days thrombosis rate −4.2% [95% CI: −6.5-2.0]) but this came at the cost of increased bleeding rates (30 days event rate: 0.5-0.8%). Prolonged use of thromboprophylaxis may

be justified in older individuals who are at particularly high risk of venous thrombosis in whom the benefit of treatment will outweigh the risk of major haemorrhage. Future studies need to carefully examine for which patients and risk circumstances the balance of treatment would be beneficial.

In **chapter 4** we showed that genetic risk factors also play a role in the risk of venous thrombosis in older individuals. Factor V Leiden (FVL, rs6025) and the prothrombin 20210A mutation (PT20210, rs1799963) increased the risk of thrombosis 1.5 to 2-fold in the older population. Also a positive family history of venous thrombosis remained associated with an increased risk of venous thrombosis in 70 years and older individuals (OR 2.3, 95% CI 1.6-3.3) These results indicate that genetics play a role in the etiology of venous thrombosis in older people, and that the effect is not limited to the young.

In **chapter 5 and 6** we showed that age-specific risk factors are relevant in the development of thrombosis. Venous stasis, a risk factor for thrombosis described already by Virchow, can be present due to diminished venous function or a damaged venous wall, causing venous insufficiency. Clinical features of venous insufficiency increased the risk of thrombosis in individuals aged 70 years and older. Varicose veins were associated with a 1.6-fold increased risk of venous thrombosis, and both a leg ulcer and leg oedema increased the risk of thrombosis 3-fold. The combination of these three clinical features showed a 10-fold increased risk of thrombosis compared to none of the clinical features. These increased risks could not be explained by the presence of other major risk factors of thrombosis in the older population, such as recent hospitalisation and surgery. We showed in **chapter 6** that an impaired functional status, more specifically the disability to perform activities of daily living, having an impaired mobility, having a sedentary life, or an impaired hand grip strength were associated with a 2- to 4-fold increased risk of thrombosis. Therefore, in the older population not only short term immobility due to hospitalisation and minor injuries, (**chapter 2**), but also a long term state of decreased mobility and functioning was shown to increase the risk of thrombosis.

METHODOLOGICAL CONSIDERATIONS OF THE AT-AGE STUDY

In the AT-AGE study we achieved high participation rates in cases (69%) and control subjects (73%) for questionnaires combined with blood sampling at first visit (**chapter 3**). In **chapter 7** we demonstrate that performing home visits rather than inviting participants to a study centre is an effective approach to increase the participation rate of older patients in research studies. Home visits enabled us to conduct an extensive, structured interview and to obtain a blood sample. We were able to visit less mobile individuals which diminished the threshold for participating. However, immediate processing of blood samples in the laboratory is not feasible when conducting home visits.

For this reason we determined optimal sample handling, i.e., blood sample storage time and temperature prior to centrifugation with regard to the measurement of coagulation factor levels at a later stage in the study (**chapter 8**). We concluded that determination of coagulation factor levels was reliable when blood samples were stored at room temperature for up to 2.5 hours.

In the AT-AGE study, individuals with an active malignancy were excluded. Exclusion of individuals with an active malignancy preserved internal validity, as we anticipated that control subjects with an active malignancy would be less likely to participate in a research study than cases who suffered from a malignant disease. Moreover, selection bias was a potential problem due to recruitment of cases in the Netherlands via anticoagulation clinics, which only treat patients with vitamin K antagonists. Since 2007, patients with venous thrombosis and a malignancy are increasingly using low-molecular weight heparin injections instead of vitamin K antagonists, which would have led to an undersampling of venous thrombosis patients with a malignancy. [2]

In each case-control study there is a chance of recall bias. The risk of recall bias is not similar for all risk factors in our study. A risk factor such as a history of oedema of the legs in DVT patients probably has a higher chance to be affected by recall bias than the presence of more severe diseases such as leg ulcera. We performed standardised interviews by trained personnel, instead of a questionnaire by mail, which enabled us to clarify the questions for each participant, and determine more precisely the presence of risk factors in cases and control subjects. With this we reduced the chance of recall bias occurring in the AT-AGE study.

Relative risks found in etiological research within the older population tend to be lower than relative risks found in a younger population, due to the higher baseline risk of diseases in the older population. To be able to compare the relative influence of a risk factor on the incidence of venous thrombosis between the younger and the older population, we assessed the population attributable risk (PAR). This enabled us to identify risk factors for venous thrombosis that exhibit major consequences on a population level, albeit with a mild relative risk of thrombosis.

FUTURE PERSPECTIVES

Multimorbidity is frequently present in the older population, and therefore it would be useful to evaluate within the AT-AGE study population, the role of the medical history of diseases in the older individuals. For example, it could be hypothesised that arterial disease, lung disorders such as chronic obstructive pulmonary diseases (COPD), chronic kidney disease, or hypothyroidism influence the risk of thrombosis in older individuals. Recent analyses in the AT-AGE study show that individuals with COPD have a 1.8-fold

increased risk of thrombosis compared with individuals without COPD (CI95 1.1-2.9: 1.1-2.9). (*Karasu et al, manuscript in preparation*) Furthermore, it is currently not known what the effect is of multimorbidity on the risk of thrombosis, i.e., is interaction between these risk factors present? More insight into medication use as a potential risk factor or as a preventive factor for thrombosis in the older population is needed, as polypharmacy is frequently present in the older population. For instance, the potential protective effect of statin use on thrombosis risk in the older population could be determined. With age, plasma levels of many hemostatic factors are increasing, such as of fibrinogen, FVIII, FVII, D-dimer and homocysteine. [4,5] With the AT-AGE study we will gain insight in the role of these elevated levels of coagulation factors and the risk of venous thrombosis in the older population. In the younger population a venous thrombotic event most frequently presents itself as a DVT, whereas in the older population PE is more frequently diagnosed. [6] PE and DVT have long been thought to have the same etiology. However, recently it was shown that risk factors for DVT and PE sometimes differ. [7] The Factor V Leiden paradox is the well-established notion that this genetic variant affects predominantly the risk of DVT and not of PE. Lung disorders such as pneumonia and COPD, however, are risk factors for PE, but have little or no effect on DVT in the middle-aged population. A similar difference was recently demonstrated in the 70 years and older population of the AT-AGE study (COPD: OR_{PE} 2.5, 95%CI: 1.4-4.3; OR_{DVT} 1.0, 95%CI: 0.5-2.1). (*Karasu et al, manuscript in preparation*) In older individuals it would be interesting to define more risk factors specifically for PE, as PE is more common and is associated with higher mortality rates as compared to DVT. [6]

Follow up of the participating patients in the AT-AGE study has taken place one year after the thrombotic event when we revisited the patients and obtained data from questionnaires and a blood sample. With these data, we will able to evaluate the prevalence and severity of the post-thrombotic syndrome in patients aged 70 years and older. Furthermore, the effect of the occurrence of venous thrombosis on the quality of life within older individuals can be evaluated. It will be interesting to perform additional follow-up of the AT-AGE study patients and focus on the recurrence risk of thrombosis, the development of co-morbidities, and the mortality risk after thrombosis at higher age.

In a separate project, patients with deep venous thrombosis of the leg (n= 76) and control subjects (n=97) of the AT-AGE study are included to study the process of ageing of the venous valves (valve thickness and function) and the risk of venous thrombosis (BATAVIA study). (*Karasu et al, manuscript in preparation*) These subjects underwent an ultrasound examination of the venous valves in the popliteal veins.

In general, future research should focus on the use of preventive measures of venous thrombosis within the highest risk groups in the older people. In this thesis we indicate that extended thromboprophylaxis might be beneficial in older individuals who are at

high risk of thrombosis. However, the risk of major hemorrhage needs to be taken into account. The role of direct oral anticoagulant (DOAC) drugs within specific risk groups can be explored. These DOACs are used as thromboprophylaxis in orthopedic surgery. However, beneficial effects on prevention of venous thrombosis in the older population are currently unknown. The ADOPT trial showed that apixaban was associated with more major bleeding events than enoxaparin in middle aged and older people (>40 years). [8] Moreover, an antidote is not yet available. Other medications that have been suggested to prevent thrombosis are aspirin and statins. Aspirin may be a good option for prevention in the older population. [9] The preventive effect is lower than that of low molecular weight heparin and oral anticoagulants; however, the bleeding risk is low. If further research confirms the protective effect of statins, this would be, if well-tolerated by the patients, an elegant alternative in the prevention of thrombosis in the older population. [10,11] The effect of preventive measures for thrombosis other than medication, such as compression stockings, frequent ambulation, or the use of electrical calf muscle stimulation are important targets of future research in the older population. For now, physicians could advise patients with in and out-of-hospital immobility to walk a few times per day, or to perform exercises of the calf muscles even while being bedridden, to prevent stasis. [12]

Currently,' the primary care rule', a clinical decision rule, is used to rule out DVT in primary care together with the point of care D-dimer assay. [13] Recently, it was found that in older patients the primary care rule showed a higher failure rate than in younger patients. [14] This might indicate that other predictors of thrombosis are present and needed in the older individuals. It would be interesting to investigate the addition of age-specific risk factors, such as functional impairment, short-term immobility at home, or minor injuries in order to improve the clinical decision rule in the older population.

CONCLUSIONS

Venous thrombosis will lead to increasing medical and economic burdens in the near future, given the worldwide growth of the older population. Determining risk factors of thrombosis specific in older individuals provides insight in opportunities to prevent thrombosis in this age group. Research studies in older individuals are challenging; however, we showed that performing home visits rather than inviting participants to a study centre can be an effective approach to increase the participation rate of older patients. In this thesis we showed that conventional risk factors such as immobilisation due to hospital admission and also immobility at home, due to for instance infection and minor injury, increase the risk of venous thrombosis also in 70 years and older individuals. We report the presence of age-specific risk factors: venous insufficiency and

functional impairment. The relative contribution of these risk factors to the incidence of venous thrombosis in this population is high. We identified new high risk groups in older people, e.g., recent hospital discharge in which preventive measures could be of special interest. In the upcoming years the AT-AGE study will provide information on additional risk factors for venous thrombosis in the older population. With further identification of risk factors and high-risk groups within the older population we can put effort into research focusing on adaptation of diagnostic rules and the safe use of preventive measures.

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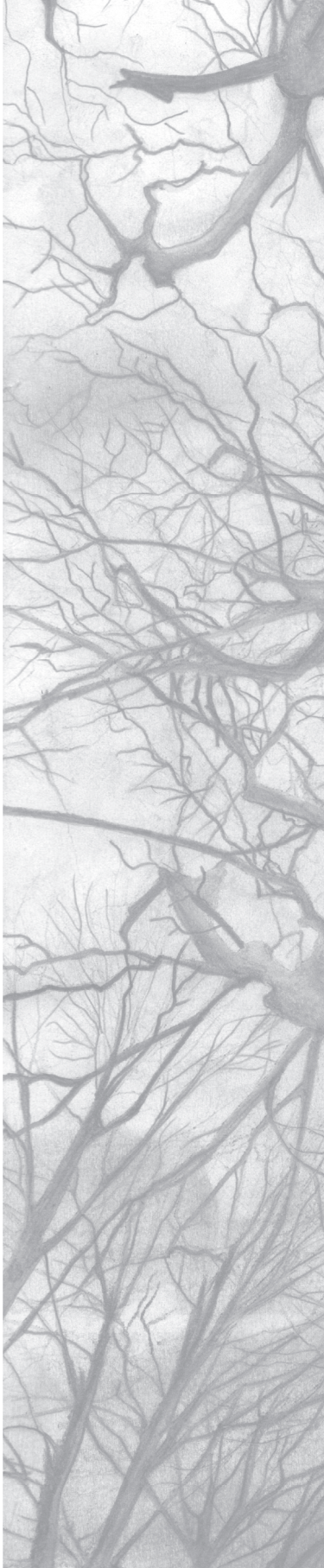

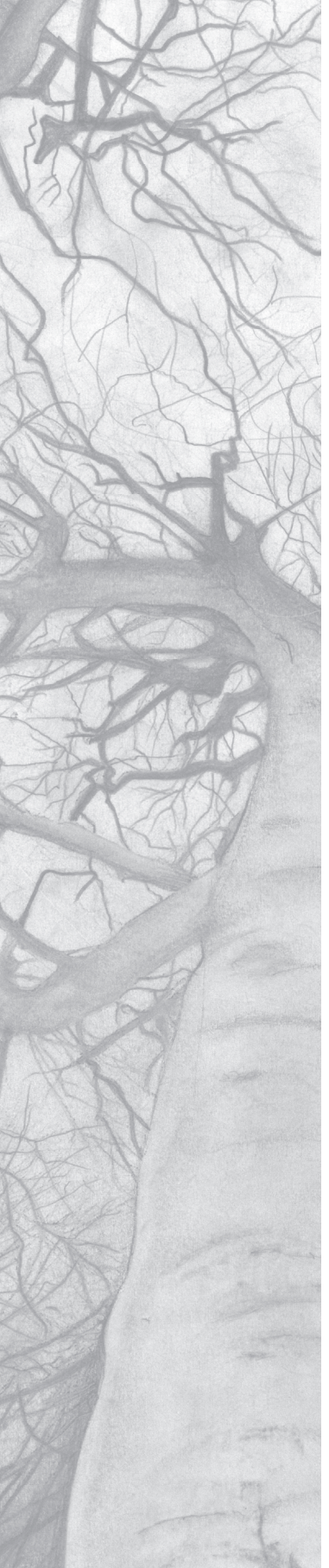

CHAPTER 10

Nederlandse samenvatting, list of publications, curriculum vitae, dankwoord

NEDERLANDSE SAMENVATTING

Stolselvorming is een natuurlijk en onmisbaar mechanisme dat ervoor zorgt dat schade aan een bloedvat hersteld wordt, zodat er geen grote hoeveelheid bloedverlies optreedt. Schade aan het bloedvat leidt tot vrijkomen van weefselfactor in het bloed. Dit induceert de omzetting van factor X in actief factor X (factor Xa), waarna activatie van protrombine naar trombine optreedt. Trombine zorgt voor de omzetting van fibrinogeen tot fibrine. Bloedplaatjes aggregeren bij de plek van de bloedvatschade en vormen samen met fibrine een bloedstolsel. Dit bloedstolsel is het resultaat van een delicate balans tussen pro- en antistollende processen. Als het bloedvat voldoende is gerepareerd wordt de stollende activiteit verminderd door plasmine, waardoor het fibrinestolsel oplost. Echter, indien het stollingsproces overactief is, of indien er geen vermindering van de stollingsactiviteit plaatsvindt, ontstaat overmatige bloedstolling. Deze overmatige stolselvorming kan de aders blokkeren ten koste van de normale bloeddoorsstroming. [1] Het blokkeren van aders door een stolsel wordt veneuze trombose genoemd. De twee meest voorkomende vormen van veneuze trombose zijn diep veneuze trombose van het been (DVT) en embolisatie van een of meerdere stolsels naar de longarteriën, ofwel een longembolie (LE).

Veneuze trombose komt jaarlijks bij 1 per 1000 personen per jaar. [2] Bij jongeren en mensen van middelbare leeftijd zijn in de afgelopen decennia veel risicofactoren van veneuze trombose vastgesteld, zoals immobilisatie, orale anticonceptie, operatie, fracturen en gips. Ook genetische factoren, zoals de factor V Leiden mutatie, verhogen het risico op veneuze trombose. [3]

Leeftijd is een belangrijke risicofactor voor veneuze trombose: ten opzichte van 40-50 jarigen hebben 80 jarigen een 10x verhoogd risico op het ontwikkelen van trombose. [4] In de gehele westerse wereld, neemt het aantal ouderen toe. In de aankomende 50 jaar zal de 70+ populatie verviervoudigen. [5] In Nederland is 15% van de bevolking 65 jaar en ouder en dit zal toenemen tot 25% in de aankomende 30 jaar. [6] Deze verandering in leeftijdsverdeling zal leiden tot een toename van zowel de prevalentie als de incidentie van ziekten zoals veneuze trombose, leidend tot een hogere medische en economische last in de toekomst. Door de stijging van de incidentie van trombose met de leeftijd is het van belang om de etiologie van veneuze trombose bij ouderen op te helderen, zodat adequate preventieve maatregelen ingezet kunnen worden.

In de 19^{e} eeuw zijn drie mechanismen beschreven die tot stolselvorming kunnen leiden en bekend zijn geworden als de trias van Virchow: (1) stase van de bloedstroom, (2) schade aan het bloedvat (b.v. door hypoxie) en (3) veranderingen in de bloedsamenstelling. [7] Risicofactoren van veneuze trombose zijn veelal terug te voeren op 1 of een combinatie van deze drie elementen. Vooralsnog is er onvoldoende kennis over

de risicofactoren van veneuze trombose bij de meest aangedane groep, de ouderen. Dit wordt uiteengezet in hoofdstuk 2.

In **hoodstuk 2** presenteren we een overzicht van de tot dus ver (tot 2010) verschenen artikelen die risicofactoren van veneuze trombose bij ouderen en jongeren vergelijkt. Veel conventionele risicofacteren voor veneuze trombose, oftewel risicofactoren die geïdentificeerd zijn in jongeren en patiënten van middelbare leeftijd, lijken ook van toepassing in de oudere populatie. De gepubliceerde onderzoeken geven aan dat immobilisatie door ziekenhuisopname, maligniteiten, hartfalen en genetische mutaties (factor V Leiden (FVL, rs6025) and prothrombin 20210A mutatie (PT20210, rs1799963), risicofactoren zijn voor trombose bij de ouderen. Omdat de prevalentie van de "verkregen" risicofactoren bij ouderen hoger is dan bij jongeren werd er een hoger populatie attributief risico (PAR) gevonden bij de ouderen. Echter, een belangrijk nadeel van deze onderzoeken is dat ze vaak uitgevoerd zijn in kleine groepen. Bovendien, waren slechts een aantal onderzoeken gericht op leeftijdsspecifieke risicofactoren, zoals functionele achteruitgang en endotheel disfunctie. We concluderen dat er beperkte kennis was van de risicofactoren voor veneuze trombose bij de ouderen. Met deze reden is het Age and Thrombosis, Acquired and Genetic risk factors in the Elderly studie (AT-AGE-studie) opgezet, een patiënt-controle onderzoek in twee centra (in Leiden, Nederland en Burlington, Vermont, Verenigde Staten). In Leiden werden van juni 2008 tot augustus 2011 en in Vermont werden van december 2008 tot juli 2011, alle opeenvolgende patiënten ouder dan 70 jaar met een DVT in het been of een LE geïdentificeerd. In Nederland werden patiënten die werden aangemeld bij de trombosedienst (Leiden of Haarlem) met een DVT of LE benaderd. In Vermont werden patiënten benaderd in het vasculair laboratorium en de radiologie afdeling van de University of Vermont Health Centre in Burlington. De controlepopulatie bestaat uit deelnemers die willekeurig zijn geselecteerd uit huisartsenpraktijken in Leiden en Vermont. De AT-AGE studie is specifiek ontworpen om inclusie van de oudere populatie te faciliteren. Om het deelnemerspercentage te optimaliseren zijn bijvoorbeeld huisbezoeken afgelegd. Er is een uitgebreid interview afgenomen, waarin werd gevraagd naar conventionele en potientiële leeftijdsspecifieke risicofactoren voor veneuze trombose. Tevens werd gedurende deze huisbezoeken een bloedafname gedaan en zijn er fysieke testen uitgevoerd zoals de handkrachtmeting. Veel analysen in dit proefschrift zijn gebaseerd op dit onderzoek, waarin 401 opeenvolgende patiënten ≥70 jaar oud met een eerste trombose en 431 controle deelnemers ≥70 jaar oud zonder een trombose in de voorgeschiedenis, geincludeerd zijn.

In **hoofdstuk 3** beschrijven we de studiepopulatie en het onderzoeksdesign van de AT-AGE studie. Bovendien laten we zien dat kortdurende immobiliteit in de thuissituatie, bijvoorbeeld door een blessure of een infectie een risicofactor is voor veneuze trombose in de ouderen (2 tot 5 x verhoogd risico). Het blijkt dat ziekenhuisopname, net als bij jongeren en bij mensen van middelbare leeftijd, een belangrijke risicofactor is voor

trombose. Tevens hebben we gevonden dat een recent ontslag uit het ziekenhuis een risicofactor is. Het risico op trombose was twee weken na thuiskomst uit het ziekenhuis tot 15 keer verhoogd ten opzichte van de groep zonder een recente ziekenhuisopname. Een operatie en gips verhoogde de kans op veneuze trombose 7 keer, en botbreuken 13 keer in vergelijking met het niet hebben van deze risicofactoren. Omdat de prevalentie van deze immobiliteit in een oudere populatie hoog is, vonden we een hoog populatie attributief risico (PAR) voor zowel ziekenhuisgerelateerde immobiliteit (27%) en voor immobiliteit in de thuissituatie (15%). Preventieve maatregelen, zoals trombose profylaxe met laagmoleculair-gewicht heparine, wordt frequent gegeven aan ouderen tijdens een ziekenhuisopname. [8] Onze resultaten geven aan dat het langer geven van zulke tromboseprofylaxe na ontslag uit het ziekenhuis mogelijk gunstig zou kunnen zijn. Dit wordt bevestigd door de resultaten van de EXCLAIM trial uitgevoerd in individuen van 75 jaar en ouder. [9] In deze studie leidde verlenging van de duur van tromboprofylaxe na ziekenhuisopname tot een vermindering van het optreden van trombose (30 dagen absolute verschil in trombose: −4.2% [95% CI: −6.5-2.0%]). Echter, het zorgde wel voor een verhoging van het aantal bloedingen (in 30 dagen: 0.5-0.8% bloedingsrisico). Wij stellen dat langduriger gebruik van tromboseprofylaxe mogelijk gegrond is bij ouderen die in het bijzonder een verhoogd risico hebben op veneuze trombose, waarbij de behandeling opweegt tegen het risico op bloedingen.

In **hoofdstuk 4** laten we zien dat genetische risicofactoren een rol spelen bij veneuze trombose in ouderen. Factor V Leiden (FVL, rs6025) en de prothrombine 20210A mutatie (PT20210, rs1799963) verhogen het risico op trombose 1.5 tot 2-keer in de oudere populatie. Een positieve familie anamnese is ook geassocieerd met een verhoogd risico op veneuze trombose in ouderen boven de 70 jaar (OR 2.3, 95%BI 1.6-3.3). Deze resultaten geven aan dat genetica een rol blijft spelen in de etiologie voor veneuze trombose, ook in ouderen.

In **hoofdstuk 5 en 6** worden leeftijd-specifieke risicofactoren geïdentificeerd die relevant zijn bij de ontwikkeling van trombose. Virchow beschreef al dat veneuze stase een risicofactor voor trombose is. [7] Stase van bloed kan ontstaan door een verminderde functie of beschadiging van de aderen, leidend tot veneuze insufficientie. Wij laten zien dat klinische kenmerken van veneuze insufficientie het risico op trombose in ouderen boven de 70 jaar verhogen. Spataderen zijn geassocieerd met een 1.6 keer verhoogd risico op veneuze trombose. Een voorgeschiedenis van een ulcus cruris verhoogde het risico 3 keer, evenals het hebben van oedemen in de benen. De combinatie van deze drie klinische factoren liet een 10 keer verhoogd risico op trombose zien vergeleken met het niet hebben van deze factoren. Deze risicoschattingen werden niet verklaard door de aanwezigheid van andere belangrijke risicofactoren van de oudere populatie zoals recente ziekenhuisopname en operatie.

We laten in **hoofdstuk 6** zien dat een verminderde functionele status leidt tot een verhoogd risico op trombose. We hebben gekeken naar: verminderde validiteit bij de algemene dagelijkse levensverrichttingen (ADL), verminderde mobiliteit, een zittende leefstijl en een verminderde handknijpkracht. Al deze vier manifestaties verhoogden afzonderlijk het risico op veneuze trombose 2 tot 4 keer. Hoe meer manifestaties er aanwezig waren hoe hoger het risico op trombose. Concluderend, in de oudere populatie leidt niet alleen kortdurende immobilteit door bijvoorbeeld ziekenhuisopname of een blessure tot een hoger trombose risico (**hoofdstuk 2**), maar ook een langdurige staat van verminderde mobiliteit en functie verhoogd dit risico.

METHODOLOGISCHE OVERWEGINGEN BIJ DE AT-AGE STUDIE

De deelnemerspercentages in de AT-AGE studie zijn hoog, 69% van de patiënten en 73% van de controles deden mee met het eerste huisbezoek bestaande uit een interview met vragenlijst en een bloedafname (**hoofdstuk 3**). In **hoofdstuk 7** laten we zien dat het doen van huisbezoeken in vergelijking met het uitnodigen van deelnemers naar een onderzoekscentrum, een effectieve manier is om het deelnemerspercentage in ouderen te verhogen. Een huisbezoek heeft het mogelijk gemaakt om een uitgebreid en gestructureerd interview uit te voeren en tegelijkertijd een bloedafname te doen. Het verlaagt de drempel tot deelname aan onderzoek voor minder mobiele patiënten. Echter, door het uitvoeren van huisbezoeken was het niet mogelijk de bloedafname direct in het laboratorium te bewerken. Daarom hebben we bekeken wat de meest optimale werkwijze is voor de bloedafname ten aanzien van de duur en de temperatuur van deze bloedafnameopslag, met als doel de stollingsfactoren te bepalen in een latere fase van het onderzoek (**hoofdstuk 8**). We concluderen dat de bepaling van coagulatiefactoren betrouwbaar is indien het afgenomen bloed opgeslagen wordt op kamertemperatuur tot 2 ½ uur. Dit is geïmplementeerd in de AT-AGE studie.

In de AT-AGE studie zijn individuen met een actieve maligniteit geëxludeerd van deelname. Met deze exclusie hebben we de interne validiteit behouden, aangezien we hebben geanticipeerd op het feit dat controles met een actieve maligniteit mogelijk minder zouden deelnemen aan het onderzoek dan patiënten die een trombose doormaken en een maligniteit hebben. Bovendien, omdat we in Nederland de patiënten via de trombosediensten hebben gerecruteerd is er kans op selectiebias, aangezien sinds 2007 patiënten met een veneuze trombose en een maligniteit vaak behandeld worden met een laagmoleculair-gewicht heparine injecties in plaats van belandeling met vitamine K antagonisten in een trombosedienst. [8] Dit zou kunnen leiden tot lagere steekproef van patiënten met een maligniteit bij recruteren bij de trombosedienst.

In elke case-control studie is er kans op recall bias. Het risico op recall bias is niet gelijk voor alle bestudeerde risico factoren in dit proefschrift. Bij een risicofactor zoals een voorgeschiedenis van oedemen in de benen bij een patiënt met een DVT is het risico op bias hoger dan bij ernstigere ziekten zoals een ulcus cruris. Gedurende de huisbezoeken hebben we gestandaardiseerde interviews gehouden door getraind personeel, in plaats van een vragenlijst per post. Dit maakte het mogelijk om alle vragen duidelijk uit te leggen aan alle deelnemers, en de aanwezigheid van risicofactoren bij patiënten en controles goed te documenteren. Hiermee is gepoogd de kans op recall bias zo laag mogelijk te maken in de AT-AGE studie.

Relatieve risico's die in etiologische onderzoeken worden gevonden in de oudere populatie hebben de neiging lager te zijn dan in de jongere populatie, door een hoger basisrisico op ziekten in de oudere populatie. Om de relatieve invloed van een risicofactor op de incidentie van een veneuze trombose te vergelijken in de jongeren en ouderen hebben we de PAR berekend. Dit maakt het mogelijk om risicofactoren voor veneuze trombose te identificeren die grote consequenties op populatie niveau hebben, ofschoon er een mild relatief risico op trombose is.

CONCLUSIES EN IMPLICATIES

Met een wereldwijde groei van de oudere populatie zal veneuze trombose, in toenemende mate, een hogere medische en economische last in de toekomst worden. Het determineren van risicofactoren voor veneuze trombose specifiek in ouderen geeft mogelijkheden voor preventieve maatregelen in deze leeftijdsgroep. Het uitvoeren van onderzoek in ouderen is uitdagend, echter, met de AT-AGE studie laten we zien dat het uitvoeren van huisbezoeken een effectieve mogelijkheid is om het deelnemerspercentage te verhogen. In dit proefschrift laten we zien dat conventionele risicofactoren voor veneuze trombose, zoals immobilisatie door ziekenhuisopname en ook immobiliteit in de thuissituatie, het risico op veneuze trombose bij individuen van 70 jaar en ouder verhoogt. We hebben leeftijdsspecifieke risicofactoren voor veneuze trombose geïdentificeerd: veneuze insufficientie en vermindering in de functionele status leiden tot een hoger risico op veneuze trombose. De prevalentie van deze risicofactoren is hoog waardoor de relatieve bijdrage van deze risicofactoren op de incidentie van veneuze trombose in de ouderen hoog is. Door deze kennis zijn hoog-risicogroepen voor veneuze trombose bij ouderen geindentificeerd. Preventieve maatregelen voor veneuze trombose bij ouderen die recentelijk ontslagen zijn uit het ziekenhuis verdient extra aandacht. Het langer geven van tromboseprofylaxe, nu vaak in de vorm van laagmoleculair-gewicht heparine, is mogelijk gunstig voor de ouderen die een hoog risico hebben op trombose, hoewel het tegen het risico op bloedingen moet worden afgewogen.

Mogelijk is hier een rol weggelegd voor de directe orale anticoagulantia (de DOACs). Een nadeel hierbij kan zijn dat een antidotum nog niet aanwezig is. Het gebruik van andere medicatie die mogelijk het risico op trombose verlagen zijn aspirine en statines. Aspirine zou een goede optie zijn in de oudere populatie. [10] Het preventieve effect is lager dan bij gebruik van laagmoleculair-gewicht heparine en orale anticoagulantia, maar het bloedingsrisico is laag. Als verder onderzoek laat zien dat statines het risico op trombose daadwerkelijk kan verlagen, zou dit een elegant alternatief zijn. [11,12] Andere preventieve, niet medicamenteuze maatregelen zijn belangrijk om te onderzoeken in de oudere populatie, bijvoorbeeld het gebruik van steunkousen, elektrische kuitspierstimulatie en het stimuleren van systematisch bewegen van de benen. Op dit moment kunnen artsen de patiënten met immobiliteit in het ziekenhuis, of in de thuissituatie adviseren om een aantal keren per dag te lopen, en om kuitspier oefeningen te verrichten. Ook als de patiënt hele dagen in bed ligt zou het goed zijn om dit uit te voeren, zodat stase van de bloedstroom voorkomen wordt. [13]

Ons onderzoek naar risicofactoren voor veneuze trombose bij ouderen is van belang om nieuwe risicofactoren te detecteren binnen de meest aangedane groep. Deze kennis kan bijdragen aan het determineren van voorspellende factoren van trombose en voor het verder optimaliseren van klinische beslisregels voor DVT en LE. Er bestaat een "eerste lijnsbeslisregel", dit is een klinische beslisregel die gebruikt wordt om DVT uit te sluiten in de huisartspraktijk samen met een point of care D-dimeer test. [14] Recentelijk is gebleken dat deze beslisregel in ouderen niet optimaal werkt. [15] Het determineren van andere voorspellers voor trombose in ouderen is dus nodig. Het zou interessant zijn om de rol van de leeftijds-specifieke risicofactoren in deze beslisregels te onderzoeken.

In de aankomende jaren zal de AT-AGE studie nog aanvullende informatie gaan brengen over risicofactoren in ouderen. Met de verdere identificatie en kwantificatie van het effect van de conventionele en de leeftijdsspecifieke risicofactoren in ouderen en het determineren van hoog-risicogroepen, kan verder onderzoek zich richten op aanpassingen van diagnostische beslisregels voor veneuze trombose en het veilig gebruik van preventieve maatregelen in ouderen.

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CURRICULUM VITAE

Marissa Josephina Engbers is geboren op 1 mei 1982 te Leiderdorp. In 2000 behaalde zij haar VWO diploma aan het Esdal College te Emmen, waarna zij in 2000 begon met de opleiding Geneeskunde aan de Universiteit Leiden. In 2004 heeft ze gedurende 6 maanden haar wetenschapsstage in de Longgeneeskunde in het SHRU in Montpellier, Frankrijk uitgevoerd, onder begeleiding van Prof. dr. P. Chanez. Gedurende haar co-schappen heeft ze meegewerkt aan onderzoek over bronchopulmonaire dysplasie bij neonaten onder begeleiding van Dr. A.B. Te Pas, LUMC. Na het behalen van haar arts-examen in 2007 heeft zij gewerkt als arts niet-in-opleiding Interne Geneeskunde in het Diaconessenhuis, Leiden. Eind 2007 is zij begonnen met promotieonderzoek op de afdeling Klinische Epidemiologie in het LUMC, waar zij de AT-AGE studie heeft opgezet en uitgevoerd in Nederland en in de Verenigde Staten onder directe begeleiding van Dr. A. Hylckama Vlieg, Prof. dr. M. Cushman en Prof. dr. F.R. Rosendaal. Tevens heeft zij de opleiding tot epidemioloog B gevolgd. Vanaf 2010 is het promotieonderzoek en de opleiding Huisartsgeneeskunde in Leiden gecombineerd via een AIOTHO-traject. Van maart 2012 tot december 2015 is zij redactielid geweest bij Huisarts en Wetenschap, het wetenschappelijk tijdschrift van het Nederlands Huisartsen Genootschap. Sinds september 2015 werkt zij als waarnemend huisarts en als redacteur bij de huisartsgeneeskundige "Kleine Kwalen" boeken. Marissa Scherptong-Engbers is getrouwd met Roderick Scherptong en is moeder van Oliver (2012) en Babette (2014).

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