Risk Factors and Predictors for Recurrent Venous Thrombosis

Building blocks for a prognostic model

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Chapter 1

General introduction and Outline of this thesis

General introduction

Haemostasis, the highly regulated process of blood clotting after vascular damage, depends on a delicate interplay between bleeding and clotting. A shift of the haemostatic balance towards a prothrombotic state contributes to the development of obstructive clot formation in the venous system, and venous thrombosis.

The incidence of venous thrombosis is estimated to be around 1-2 per 1000 persons per year and increases exponentially with age up to about 5 per 1000 persons per year in the elderly.[1,2] Venous thrombosis commonly manifests as deep vein thrombosis of the leg, where it can cause symptoms of pain, redness and swelling. In about one third of the patients embolization occurs and parts of a clot lodges in the vasculature of the lung, termed pulmonary embolism. Pulmonary emboli present with symptoms of shortness of breath and chest pain on inspiration and are lethal in up to 20% of the cases.[1,3] Thrombosis rarely occurs, in about 10% of the total cases of venous thrombosis, in other veins of, e.g. the arms, retina, mesentery and portal vein or the cerebral sinus.[4]

It was in the 13th century that the first case of venous thrombosis that we know of was described.[5] Deep vein thrombosis was reported in the right leg of a young man in France. Several hypotheses were suggested through the centuries for understanding the mechanism underlying venous thrombosis. It was only in 1856 that Virchow proposed the modern pathogenesis of thrombosis, now known as Virchow's triad. [6] This triad explains thrombosis as a result of changes in blood flow, damage of the vessel wall and changes in blood composition. Over the years a long list of risk factors for venous thrombosis has arisen, all of which can be fitted under at least one of the three components of the triad.[7]

Recurrent venous thrombosis

After a *first* event *recurrent* venous thrombosis is common, which is associated with considerable comorbidity, mortality and health-care costs. Five-year cumulative incidence of recurrences is reported to be around 25%.[8-10] Case fatality rates of recurrent venous thrombosis are estimated at 11% during the first three months of anticoagulant treatment and the rate of fatal recurrent venous thrombosis is 0.3-0.5% per year after discontinuation of anticoagulant treatment.[11,12]

Secondary prevention of recurrent venous thrombosis could greatly reduce the number of events. Secondary prevention can be achieved in two ways, either by elimination of modifiable risk factors or by extending the anticoagulant treatment period in patients at high risk of recurrence. For this we need knowledge of risk factors and/ or predictors of recurrent venous thrombosis.

The difference between a risk factor and a predictor is that risk factors are causally related to the outcome of interest, in this case recurrent venous thrombosis, while predictors are associated with the outcome, but are not a causal factor for the outcome per se. For example, carrying a lighter in your pocket is not causally associated with an outcome such as lung cancer. However it will be able to predict an increased risk of lung cancer, since people carrying a lighter in their pockets are more often smokers than people who do not carry a lighter. Smoking is the risk factor for lung cancer.

To prevent recurrent venous thrombosis we need knowledge of both predictors ánd risk factors for recurrence. Ideally, we find information on modifiable risk factors. Modifiable risk factors are factors we can advise patients to refrain from or factors we can intervene on, and in that way decrease the patient's risk of recurrence. This is in contrast to genetic factors that cannot be readily intervened on. The focus of this thesis will therefore not be on genetics. In this thesis the association between a modifiable risk factor and recurrence is described in both Chapters 7 and 8. The second option for prevention of recurrent venous thrombosis is by extending the anticoagulant treatment period. However, such life-long treatment is not feasible in all patients, considering the substantial risk of major haemorrhage (1-2% per year)[13,14], and should be targeted at high risk patients only. Estimating the risk of recurrent venous thrombosis has proven to be challenging while knowledge of good predictors is much needed.[15] These predictors can be either factors purely predictive of recurrences (Chapter 6) or risk factors for recurrence (Chapters 2, 4, 9).

Despite that risk factors for a *first* venous thrombotic event are well known, for *recurrent* venous thrombosis this is not the case. It appeared that the risk profile for a first event cannot be directly translated to recurrent events. For example, age is strongly associated with first events[3], while it is not, or at most very weakly, associated with recurrent venous thrombosis.[8,16,17] The same is true for the presence of genetic thrombophilia.[18]

Some risk factors for recurrent venous thrombosis have been described in the literature, of which the most important ones are the absence of a transient provoking risk factor at time of the first event and the presence of active cancer.[19] However, only a proportion of the patients can be classified as such. Additionally, male sex has proven to be a moderately strong risk factor for recurrent venous thrombosis.[20-22] Some other factors have been positively associated with recurrences as well (see Table for short overview).

Factor	*Relation with recurrent venous thrombosis**
Unprovoked vs provoked 1st event	Strong[23]
Presence of active cancer	Strong[8,9,24]
Proximal vs distal deep vein thrombosis	Strong[25,26]
D-dimer levels (measured after discontinuation anticoagulant treatment)	Strong[28-30]
Male sex	Moderately strong[20,22]
Antiphospholipid syndrome	Moderately strong[27]
Residual thrombosis in proximal veins	Moderately strong[31,32]
Hereditary thrombophilia	Weak, controversial[18,33-35]
Overweight/ obesity	Weak[36]

* Strong denotes: relative risk >2; Moderately strong: relative risk ~2; Weak: relative risk <1.5

The aim of this thesis is to identify additional modifiable risk factors for as well as factors that might be able to predict recurrent venous thrombotic events.

Study populations

The MEGA study

The MEGA (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis) study is a large case-control study into risk factors for venous thrombosis.[37] Between March 1999 and August 2004, 4956 consecutive patients with an objectively diagnosed first deep vein thrombosis of the leg or pulmonary embolism were included. Patients were aged 18-70 years and were enrolled from six anticoagulation clinics in the Netherlands. Anticoagulation clinics monitor all patients taking vitamin K antagonists in a well-defined geographical area. Control subjects, without a history of venous thrombosis, were partners of the patients (n=3297) or collected via random digit dialing (n=3000). All participants filled in a detailed questionnaire on their medical history and the presence of possible risk factors for venous thrombosis. Additionally, blood was collected from cases three months after discontinuation of anticoagulant treatment or one year after the event if cases continued anticoagulant treatment for more than one year. Controls who were partners of the cases provided blood at the same time as the case. Controls from the random digit dialing group provided blood within a few weeks after the questionnaire was sent.

The MEGA follow-up study

Of 4956 patients included in the MEGA study, 4731 gave written informed consent for future follow-up on recurrent venous thrombosis (MEGA follow-up study).[38] The aim of the MEGA follow-up study was to assess the incidence of recurrent events and to identify new risk factors and predictors of recurrences. The MEGA follow-up study is to date the largest non-register based study on recurrent venous thrombosis.

In diagnosing recurrent venous thrombosis it is sometimes challenging to distinguish between new thrombosis and extensions of a previous lesion (residual thrombosis). We aimed to make a clear distinction between the two and collected as many data as possible on recurrences during follow-up from different sources of information. Between June 2008 and July 2009 patients were asked whether they had developed a recurrent venous thrombotic event by means of a short answer form. Furthermore, between 2007 and 2009 the vital status of all MEGA follow-up patients was obtained from the Dutch population register and causes of death from the national registry of death certificates. Data from the answering forms, causes of death, anticoagulation clinics and discharge letters from treating physicians were combined to make a classification of certain and uncertain recurrences.

Data on the presence of risk factors or predictors for recurrent venous thrombosis were additionally obtained via different sources of information. First, all patients were asked to complete a questionnaire on potential risk factors after their first event. Second, our data were linked to the Dutch hospital data register which covers complete, nationwide data on hospital admissions since 1986. Third, all patient records were linked to the SFK register (the Dutch Foundation for Pharmaceutical Statistics).[39]

Outline of this thesis

In **Chapter 2** results from the MEGA follow-up study are reported and an accurately determined incidence rate of *recurrent* venous thrombosis using a strict definition of recurrence is presented. Additionally the influence of the previously described risk factors male sex and type of the *first* event (provoked or unprovoked) on risk of recurrence was studied.

Cancer has been shown to be one of the strongest risk factors for venous thrombosis. Of all first venous thrombotic events about 20-30% are cancer-related.[40-43] Furthermore, cancer increases the risk of a first venous thrombotic event four- to seven-fold.[9,37,44,45] To obtain a better insight in this relationship and to obtain an idea of current knowledge with regard to the risk of recurrent venous thrombosis in patients with cancer, **Chapter 3** presents an extensive review of the literature on this topic.

Few studies have investigated the risk of recurrent venous thrombosis in patients with cancer. However, all of these studies report an increased recurrence risk.[8,9,24,46] The relation between cancer, diagnosed either before or after the first venous thrombotic event, and recurrent venous thrombosis in the MEGA follow-up study is presented in **Chapter 4**. Furthermore, recurrence risks were studied separately for different types of cancer and for different time periods after cancer diagnosis, which is helpful information in the clinic in case a decision on thromboprophylaxis has to be made.

Despite a lot of research on the topic the pathophysiology underlying the relation between cancer and venous thrombosis is largely unknown. The relation between cancer and venous thrombosis is strong; however, not every patient with cancer develops thrombosis. To obtain a better insight in this relation plasma levels of coagulation factors, both procoagulant and anticoagulant, were studied in patients with and without cancer and patients with and without venous thrombosis **(Chapter 5)**.

Levels of coagulation factor VIII have been shown to be strongly related to first venous thrombotic events.[47] Only a few studies, mostly with rather small sample sizes, studied the relation between factor VIII and recurrent venous thrombosis and showed contradictory results.[18,48,49] In **Chapter 6** the predictive value of factor VIII levels for recurrent venous thrombosis in the MEGA follow-up study is described. Additionally, the effect of adding factor VIII to an existing prediction model for recurrent thrombosis was studied.

An important risk factor for a first venous thrombotic event is the use of oral contraceptives because of its high prevalence.[50-52] In **Chapter 7** the aim was to study the risk of recurrent venous thrombosis in women who continue or start using hormonal contraceptives after a first venous thrombotic event and to see whether taking away this risk factor could reduce recurrence risk.

Another modifiable risk factor for venous thrombosis is seated immobility. It has been shown that for a first venous thrombotic event the risk is increased by immobility, such as during a long-haul flight, other types of travel or a sedentary lifestyle.[53-56] **Chapter 8** discusses whether the risk of recurrent venous thrombosis is additionally increased after such periods of seated immobility and whether prophylactic measures could potentially decrease the recurrence risk.

Infections are currently not considered provoking risk factors for a first venous thrombotic event. However, a relation between infectious and inflammatory diseases and thrombosis has been shown before.[57-59] The risk of both first and recurrent venous thrombosis during periods of antibiotic use, as a proxy for infectious diseases, is presented in **Chapter 9**. Additionally the joint effect of both antibiotic use and genetic thrombophilia on the risk of venous thrombosis is discussed.

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Chapter 2

Incidence and characteristics of recurrent venous thrombosis in a large cohort of patients with a first venous thrombosis

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Submitted for publication

Abstract

Background
The reported incidence of recurrent venous thrombosis (VT) varies widely.

Objectives
The aim of this study was to estimate the incidence of a recurrent event and the effect of location, age, and sex in a large cohort of patients with a first VT.

Patients
We followed 4731 patients with a first VT between 1999 and 2004 (MEGA study) until 2008-2009. Recurrences were adjudicated from self-reported information in questionnaires, anticoagulation clinics, and discharge letters. We calculated incidence rates and hazard ratios (HR) to estimate the effect of various factors on recurrence.

Results
673 patients (14.2%) had a recurrent event. The overall incidence rate was 27.9 per 1000 person years (95%CI, 25.8-30.0). The cumulative incidence at 5 years was 11.3%. An idiopathic first thrombosis was a risk factor for recurrence in men and women. Men had a higher risk of recurrence than women regardless of location or the presence of a provoking factor (HR overall: 2.2 (95%CI, 1.9-2.6). Age did not affect recurrence risk.

Conclusions
This study provides precise and valid estimates of recurrence risk, which is substantial at about 3% per year. For duration of treatment, sex, type of first event and location of first event may need to be taken into account.

Introduction

Venous thrombosis is a multi-causal disease that occurs in 1-3 per 1000 persons per year.[1,2] It is associated with substantial mortality and morbidity including recurrence. The cumulative incidence of recurrent venous thrombosis is much higher than that of a first event and varies between studies from 4-11% within the first year to 12-30% within five years after the first event.[3-7] Incidence rates of recurrence also vary between studies, from 25 to 46 per 1000 person-years.[5,7] Sources of this variation include definition of recurrence, setting and size of study (clinical versus research setting) and starting point of follow-up.

In contrast to a first event, only few risk factors are known to be associated with the risk of recurrent thrombosis, such as male sex, the presence of a malignancy, and an idiopathic first venous event.[3,5,7-14] Age, which is the strongest risk factor for a first event does not, or only slightly, increase the risk of recurrence.[5,9-13] However, the separate effects of age, male sex, and an idiopathic first venous thrombosis are not well established, mainly as a result of small sample sizes of the studies reported so far, different cut-off points for age, and different definitions for idiopathic venous thrombosis.

The best way to prevent recurrence is by anticoagulant treatment. However, this has the drawback of a major bleeding risk, which, if it were to be given indiscriminately, is not outweighed by the prevention of thrombosis. Therefore, duration of anticoagulant treatment is limited, and the optimal duration is not well known, despite several trials into this.[15-18]

A recent study showed that recurrent events do not occur at random sites.[19] The ability to predict the location of recurrence may influence the duration of treatment especially when a pulmonary embolism (PE) as recurrent event is more likely than a deep vein thrombosis (DVT) of the leg.

To address all questions mentioned above, we performed a large follow-up study of almost 5000 patients with a first venous thrombosis. In this study, we estimated risk of recurrence, the separate associations of age, sex, and an idiopathic first thrombosis with the risk of recurrence, the effect of different durations of anticoagulation, and the relation between site of first and recurrent events.

Methods

Study population

Patients were included from the MEGA study[20,21], a large population-based case-control study into risk factors for a first venous thrombosis, which included consecutive patients at six anticoagulation clinics in the Netherlands between March 1999 and September 2004. In total, 5182 cases and 6297 controls were included. From the cases, patients with a deep venous thrombosis of the leg, pulmonary embolism (PE), or both were included and patients with a venous thrombosis of the upper extremity were excluded from this analysis (follow-up data reported previously [20]). Of 4956 patients eligible for follow-up 225 indicated that they did not want to participate in a follow-up study and were therefore excluded (Figure 1) leaving 4731 patients for the follow-up study. This study was approved by the Medical Ethics Committee of the Leiden University Medical Center and all participants gave written informed consent.

Information about recurrences was retrieved in two ways, i.e., from the patients themselves via a short questionnaire and from the anticoagulation clinics, which monitor all outpatients' anticoagulant treatment with vitamin K antagonists. The short questionnaire consisted of two questions: 1)"Did you have a recurrent event?" and 2)"By which doctor was it diagnosed?" Questionnaires were sent by mail between June 2008 and July 2009. When questionnaires were not returned, questions were asked by telephone interview. During the same period, information on possible recurrences of all patients was obtained from the anticoagulation clinic where they were included for their first event and, in case they moved house, at the clinic near their new address. Information on duration of anticoagulant treatment was also obtained from the anticoagulation clinics.

For all potential recurrences found by the questionnaire, anticoagulation clinic, or both, discharge letters were requested from the clinician who diagnosed the recurrence according to the patient or the clinic.

Definition of recurrence

A decision rule regarding certainty of diagnosis was made according to the information collected for each patient. Reported recurrences were classified into certain and uncertain recurrences. In this study certain recurrences were used as endpoint and patients with an uncertain recurrence were censored at time of their uncertain recurrence, since they were definitely recurrence-free until that time.

To be classified as a certain recurrence, a reported recurrence should fulfil one of the following criteria.

1. A discharge letter was present concluding a diagnosis of recurrence, based on available clinical and radiological data. This recurrence should be in a different vein or in a different part of the body than the first event. The discharge letter had to contain information about instrumental diagnostic procedures. If location of either first or second thrombosis was not known, an event was still classified as certain if at least three months had passed since the first thrombosis.

2. A discharge letter was not available (e.g., when the treating physician was unknown) but both the anticoagulation clinic and the patient reported a recurrence at a clearly different location than the first event (contralateral leg, DVT after PE or vice versa) or a time period of more than a year had passed between the two events (Figure 2).

3. A registered cause of death from PE or DVT at least six months after the first event.

Uncertain recurrences were defined by four criteria, one of which had to apply:

4. A diagnosis of a possible recurrence in the discharge letter, where clinical and radiological data could not distinguish between an extension of the first and a new thrombotic event.
5. A discharge letter was not available but both the patient and the anticoagulation clinic reported a recurrence within a year after the first event.
6. Information was only available from either the patient or the anticoagulation clinic.
7. A registered cause of death from PE or DVT within six months after the first event.

Statistical analysis

End of follow-up was defined as the date of a recurrent event and, in the absence of a recurrence, the date of filling in the short questionnaire. If patients did not fill in a questionnaire they were censored at the last date we knew them to be recurrence free. This could be either the last visit to the anticoagulant clinic, date of death or emigration, or the last moment the patient was known to be recurrence-free from information of the MEGA case-control study (Figure 1). Duration of follow-up was calculated in two ways, i.e., by starting follow-up at 1) the date of the first event or 2) the date of discontinuation of anticoagulant therapy. Both incident rates and cumulative incidences of recurrence were calculated from these two starting points.

In order to find a range of incidences which includes the true incidence of recurrence, we calculated, as a sensitivity analysis, incidences for all possible recurrences combined (certain and uncertain) and separately for certain recurrences with both starting points of follow-up. Additionally we refined our incidence estimation by a multiple imputation analysis in patients with an uncertain recurrence. With the multiple imputation analysis the recurrence status of the uncertain recurrences was estimated using information on all comorbidities and risk factors present at time of first thrombosis.[14,19]

Idiopathic thrombosis was defined as venous thrombosis without surgery, trauma, plaster cast, hormone use (oral contraceptives and hormone therapy) or pregnancy,

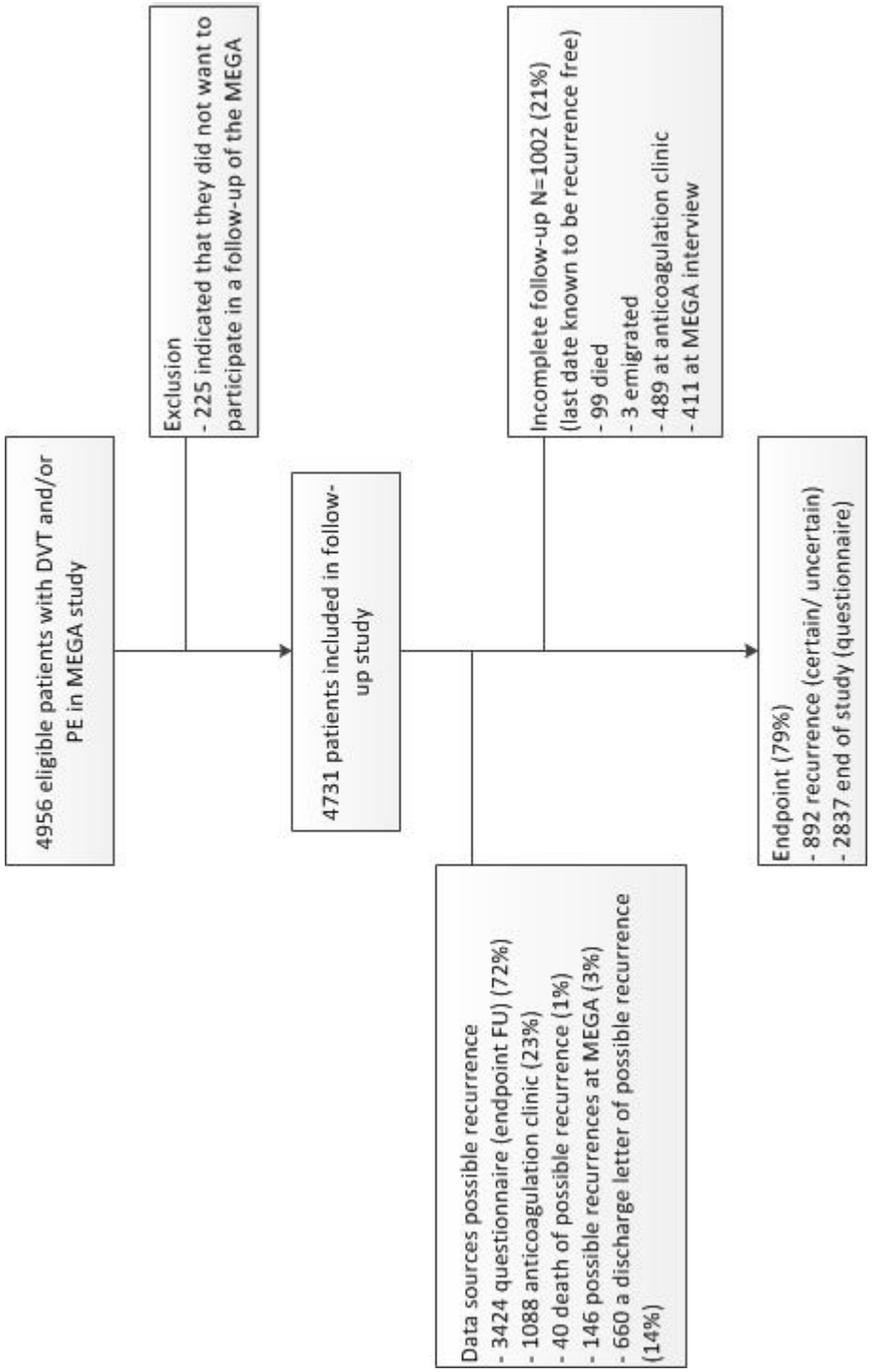

Figure 1. Flowchart of follow-up

all within three months before the event, or puerperium or active malignancy at the time of the event. Kaplan-Meier curves adjusted for competing risks were estimated for men, women, and patients with an idiopathic and provoked first venous thrombosis separately. Incidence rates were calculated for men, women, and idiopathic and provoked cases separately in different age categories to study risk patterns for these three factors.

For the analysis on risk factors and optimal duration of anticoagulation, only certain recurrences were used and uncertain recurrences were censored at the time of reported recurrence. Hazard ratios (HR) were estimated for all potential risk factors, and all HRs were adjusted for age and sex when applicable. All HRs were estimated with follow-up starting at time of first venous thrombosis. The effect of male sex was also studied in a restricted analysis excluding women who used hormones at time of first thrombotic event as well as women who were or had recently been pregnant at that time. Age was studied both as a continuous and a categorical variable with 10-year age categories. These effects were studied in all patients and in patients without cancer and life-long anticoagulants separately. These restrictions were done to determine the effect of these risk factors in those without another strong risk factor of thrombosis (cancer) and in those who cannot be treated longer than they already were (lifelong treatment).

First and recurrent events were compared for location (lungs or leg). Left versus right-sided first and recurrent thrombosis of the leg were analysed. Observed numbers versus expected numbers when locations and sides would be random were calculated as well as HRs for site of recurrence per site of first thrombosis.

For the analysis of the effect of duration of anticoagulation therapy, patients with malignancies at the time of thrombosis were excluded. Duration of anticoagulation therapy was calculated in months. Survival curves were made for patients who used anticoagulation therapy for 3, 4-6, 7-12, and >12 months. Survival curves were adjusted for competing risks due to death and included only recurrences that occurred after discontinuation of anticoagulant therapy.

Analyses were performed with SPSS version 21.0 (Chicago, Ill) and STATA SE version 12 (Stata Corporation, College Station, Texas) for Windows.

Results

Population

Mean age of patients at time of first venous thrombosis was 48 years and 54% of patients were women. Mean duration of follow-up was 5.1 years when follow-up started at time of venous thrombosis and 5.0 years when follow-up started after discontinuation of anticoagulation. Total volumes of follow-up were 24 124 and 20 031 person-years respectively. In total 79% of patients (n=3729) had a complete follow-up of whom 2837 filled in the questionnaire and 892 were followed until recurrence. 1002 (21%) patients did not complete follow-up either due to death (n=99) or emigration (n=3) without recurrence, or did not reply to further queries after a last visit at the anticoagulation clinic (n=489) or at a later point in time during follow-up (n=411) (Figure 1).

Recurrences

We obtained information about recurrence status from 3757 patients. 972 possible recurrences were found that needed to be confirmed. From the information obtained from clinicians, we concluded that 80 of these were not recurrences but were either post-thrombotic syndrome or suspected recurrences that were subsequently excluded by ultrasound or CT-scan. Therefore, 892 recurrences could be further classified. Of these, 673 patients were classified to have a certain recurrence according to the criteria listed in the Methods section. 593 patients fulfilled criterion 1) for certain recurrence. Fifty-eight patients were identified as a certain recurrence with criterion 2) and 22 patients with criterion 3). 219 patients had an uncertain recurrence of which 32 fulfilled criterion 4), 19 criterion 5), 159 criterion 6), and 9 criterion 7).

Incidence of recurrence

When follow-up started at time of first venous thrombosis, we found an incidence of 27.9 per 1000 person-years (95%CI, 25.8-30.0) when only certain recurrences were taken into account. When certain and uncertain recurrences were counted as recurrent events, as a sensitivity analysis, we found an incidence of 37.0 per 1000 person-years (95%CI, 34.6-39.4) (Table 1). These incidence rates corresponded to a 5-year cumulative incidence of 11% and 15%, respectively.

Of the 673 certain recurrences, 61 (9%) occurred during anticoagulation therapy prescribed after the first thrombotic event, whereas 53 of the 219 uncertain recurrences (24%) occurred during treatment. When follow-up was started after discontinuation of anticoagulation treatment, the incidence of recurrence was 30.6 per 1000 person-years (95%CI, 28.1-33.0) when only certain recurrences were taken into account and 38.8 (95%CI, 36.1-41.6) per 1000 person-years when both certain and uncertain recurrences were taken into account. When recurrence status was imputed in the group who had uncertain recurrences the incidence of recurrence became 29.4 (95%CI, 27.4-31.7) per 1000 person-years when follow-up started at time of first venous thrombosis and 32.0 (95%CI, 29.6-34.6) when follow-up started after discontinuation of treatment (Table 1).

Recurrence rate was highest during the first 1.5 years, i.e., 54 per 1000 (95%CI, 45-65) person-years at 1 year and 42 per 1000 (95%CI, 33-52) person-years at 1.5 years, and decreased to 25 per 1000 (95%CI, 18-34) person-years after 4 years. After this time the incidence of recurrence remained stable at 25 per 1000 person-years.

Risk factors

Table 2 shows incidences of recurrence stratified by age, sex, and whether the first venous thrombosis was idiopathic or not. Incidence rates were in all instances higher for men than women. No clear effect of age was seen in any of the categories.

Men had a 2.2-fold (95%CI, 1.9-2.6) increased rate of recurrence compared with women (Figure 3, Table 3). After exclusion of women who used hormones, or were pregnant at time of first thrombosis the relative rate increased to a 2.8-fold (95%CI, 2.2-3.6) increased rate in men. Age at time of first venous thrombosis was not associated with an increased risk of recurrence (Table 3).

Patients with a first idiopathic thrombosis had a 2.0-fold (95%CI, 1.7-2.3) increased rate of recurrence compared with patients with a provoked first thrombosis. However, after adjustment for sex this rate ratio diminished to 1.4 (95%CI, 1.2-1.7).

Incidence of recurrent thrombosis was higher in men than in women, regardless whether the first event was provoked or idiopathic, i.e., the incidence after a provoked first event in men: 35.5 per 1000 person-year (95%CI, 29.4-39.5) and in women: 16.5 per 100 person-years (95%CI, 14.2-18.8) and the incidence after an idiopathic first event in men: 47.6 per 1000 person-years (95%CI, 41.7-53.5) and in women: 28.2 per 1000 person-years (95%CI, 20.1-36.3). The increased rate of recurrent thrombosis after an idiopathic first event was present in both men (HR 1.4 (95%CI, 1.1-1.6)) and women (HR 1.7 (95%CI, 1.3-2.5))(Figure 2). Exclusion of patients with cancer and life-long treatment did not lead to more than trivial changes in these estimates (Table 3).

Location of recurrent and first thrombosis

Sixty-two percent of recurrences were DVTs, 31% were PEs, 5% had DVT+PE, and 1% of recurrences were in a different location (upper extremity, portal vein, intestines or sinus) (Table 4a). Recurrences occurred more than expected at the same location as the first event (Table 4a). Patients with DVT were 1.5-fold (95%CI, 1.1-1.9) more likely to have a DVT as second event than patients with a PE. Patients with a first PE were 1.9-fold (95%CI, 1.4-2.8) more likely to suffer a recurrent PE than those with a first DVT or with a DVT+PE.

In patients who had a first DVT in their left leg, the side of the recurrent DVT appeared to be equally distributed whereas in patients who had a first DVT in their right leg, the chance of a recurrent event in the right leg was slightly higher (60%, 95%CI, 50%-66%) than a recurrent event in the left leg (Table 4b).

Anticoagulation therapy for the initial event

For this analysis, 575 patients with malignancy were excluded as these patients often

Table 1. Recurrence rates

Start follow-up after 1st thrombosis								
Type of recurrence	**N**	**Person years**	**Incidence (CI95)**	**5 years cum incidence**	**N men**	**Incidence men (CI95)**	**N women**	**Incidence women (CI95)**
Certain	673	24124	27.9 (25.8-30.0)	11.3%	427	41.1 (37.2-45.0)	246	17.9 (15.7-20.1)
Uncertain	219	24124	9.1 (7.9-10.3)	3.9%	119	11.4 (9.4-13.5)	100	7.3 (5.9-8.7)
Certain & Uncertain	892	24124	37.0 (34.6-39.4)	15.2%	546	52.5 (48.1-56.9)	346	25.2 (22.5-27.9)
Imputed recurrences	711	24124	29.4 (27.4-31.7)	12.0%	454	43.7 (39.8-47.8)	257	18.7 (16.5-21.1)
Start follow-up after discontinuation of treatment								
Certain	612	20031	30.6 (28.1-33.0)	15.5%	395	46.5 (41.9-51.1)	217	18.8 (16.3-21.3)
Uncertain	166	20031	8.3 (7.0-9.5)	4.2%	89	10.5 (8.3-12.7)	77	6.7 (5.2-8.2)
Certain & Uncertain	778	20031	38.4 (35.6-41.1)	19.7%	484	57.0 (51.9-62.1)	294	25.5 (22.6-28.4)
Imputed recurrences	641	20031	32.0 (29.6-34.6)	14.2%	415	46.6 (42.3-51.3)	226	19.6 (17.1-22.3)

Table 2. Incidences of recurrence in several subgroups

	Men all			Men, first Idiopathic VT			Women all			Women, first idiopathic VT		
Age (years)	N, total	N, rec/FUy	IR (CI95)	N, total	N, rec/FUy	IR (CI95)	N, total	N, rec/FUy	IR (CI95)	N, total	N, rec/FUy	IR (CI95)
18-30	93	17/415	41.0 (21.5-60.5)	34	6/174	34.5 (6.9-62.1)	402	41/2030	20.2 (14.0-26.4)	12	1/69	14.5 (0.0-43.0)
30-40	290	48/1459	32.9 (23.6-42.2)	138	26/680	38.2 (23.5-52.9)	530	46/2985	15.4 (10.9-19.9)	18	3/118	25.4 (0.0-54.2)
40-50	446	102/2158	47.3 (38.3-56.5)	206	51/1011	50.4 (36.6-64.2)	652	55/3612	15.2 (11.2-19.2)	47	8/261	30.7 (9.5-51.9)
50-60	656	128/3529	36.3 (30.0-42.6)	330	82/1693	48.4 (37.9-58.9)	551	49/2998	16.3 (11.7-20.9)	80	10/565	17.7 (6.7-28.7)
60-70	679	132/3103	42.5 (35.2-49.8)	331	83/1646	50.4 (39.6-61.2)	432	55/2103	26.2 (19.3-33.1)	108	24/622	38.6 (23.2-54.0)
Total	2164	427/10394	41.1 (37.2-45.0)	1039	248/5205	47.6 (41.7-53.5)	2567	246/13729	17.9 (15.7-20.1)	265	46/1634	28.2 (20.1-36.3)

- Idiopathic thrombosis was defined as venous thrombosis without surgery, trauma, plastercast or hormone use (oral contraceptives and hormone replacement therapy), and pregnancy within three months before the event, and without active malignancies or puerperium

Table 3. Risk factors for recurrent venous thrombosis

Risk factor	HR (CI95)	Adjusted HR (CI95)*	HR (CI95) excl cancer and lifelong treatment	Adjusted HR (CI95)* excl cancer and lifelong treatment
Men vs women	2.3 (1.9-2.7)	2.2 (1.9-2.6)	2.5 (2.1-3.0)	2.4 (2.0-2.9)
Men vs women without hormones	3.0 (2.4-3.7)	2.8 (2.2-3.6)	3.6 (2.7-4.6)	3.5 (2.7-4.5)
Age (per year)	1.01 (1.01-1.02)	1.00 (1.00-1.01)	1.00 (1.00-1.01)	1.00 (1.00-1.01)
Age categories				
Age 18-30	1 (ref)	1 (ref)	1 (ref)	1(ref)
Age 30-40	0.9 (0.6-1.2)	0.8 (0.6-1.1)	0.9 (0.6-1.3)	0.7 (0.5-1.1)
Age 40-50	1.1 (0.8-1.5)	0.9 (0.7-1.3)	1.1 (0.8-1.6)	0.9 (0.6-1.2)
Age 50-60	1.2 (0.9-1.6)	0.9 (0.6-1.2)	1.3 (0.9-1.7)	0.9 (0.6-1.2)
Age 60-70	1.5 (1.1-2.0)	1.0 (0.8-1.4)	1.5 (1.1-2.1)	1.0 (0.7-1.4)
Idiopathic 1st VTE	2.0 (1.7-2.3)	1.4 (1.2-1.7)	2.2 (1.9-2.6)	1.6 (1.3-1.9)

- Idiopathic thrombosis was defined as venous thrombosis without surgery, trauma, plastercast or hormone use (oral contraceptives and hormone replacement therapy), and pregnancy within three months before the event, and without active malignancies or puerperium.
- Women without hormones were those without pregnancy, puerperium, oral contraceptive use and use of hormone replacement therapy.
*Adjusted for age and sex when applicable.

have treatment for prolonged periods of time or for life. Of the 4156 patients without malignancy, duration of anticoagulation therapy for the initial event was obtained for 4053 (98%) patients. Most patients received 4-6 months of anticoagulation treatment after the first venous thrombosis (Figure 3). Patients with a clear provoking factor were slightly more likely to have received less than 4 months of treatment than those with a first idiopathic venous thrombosis (30% vs. 25%). Figure 3 shows the cumulative incidence of recurrence over time for four different duration periods of anticoagulation. This Figure shows that the curves for the different durations of anticoagulation therapy run parallel indicating that the risk of recurrence is equal after discontinuation of anticoagulation regardless of duration.

Table 4a Location first versus recurrent thrombosis, observed versus expected.

	Recurrence				
1st event	DVT	PE	DVT+PE	Other	Total
DVT	319 (267)	71 (99)	23 (58)	4 (0)	424
PE	44 (99)	105 (37)	4 (21)	1 (0)	157
DVT + PE	51 (58)	29 (21)	9 (13)	3 (0)	92
Total	414 (424)	205 (157)	36 (92)	8 (0)	673

N observed (N expected)

Table 4b. Side of DVT.

	Recurrence			
1st event	Left	Right	Both
Left	79 (50%, CI95 42%-57%)	78 (49%, CI95 42%-57%)	2 (1%)
Right	54 (40%, CI95 32%-48%)	81 (60%, CI95 50%-66%)	0
Both	1	0	0

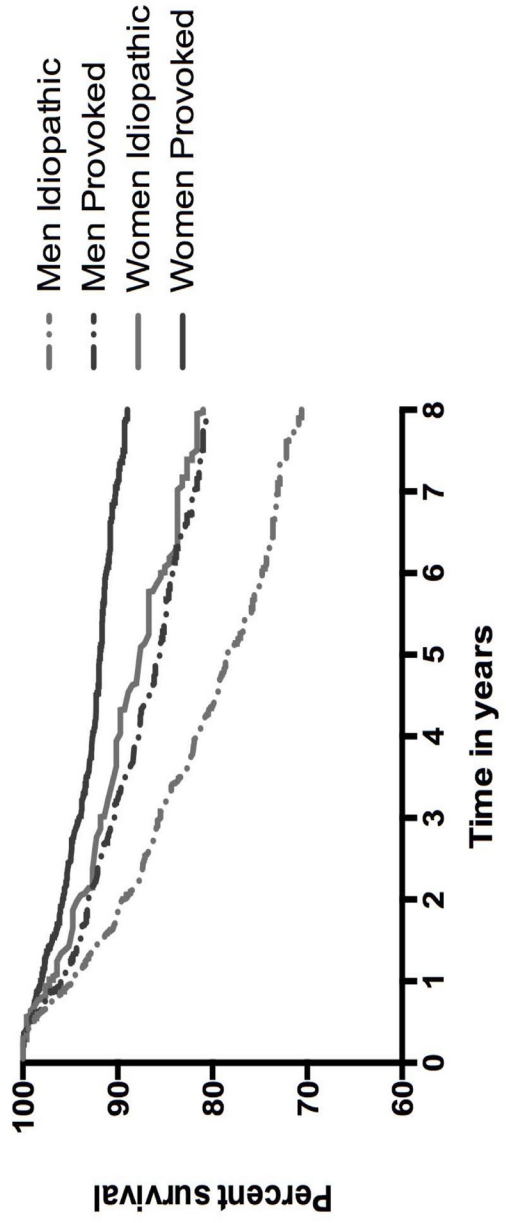

Figure 2. Risk of recurrence for idiopathic versus provoked first venous thrombosis, stratified for sex.

Duration of follow-up in years

- Idiopathic thrombosis was defined as venous thrombosis without surgery, trauma, plaster cast or hormone use (oral contraceptives and hormone replacement therapy), and pregnancy within three months before the event, and without active malignancies or puerperium.
- Provoked venous thrombosis was defined as thrombosis due to surgery, plaster caster or minor injuries, oral contraceptive use, hormone replacement therapy use, pregnancy and puerperium.

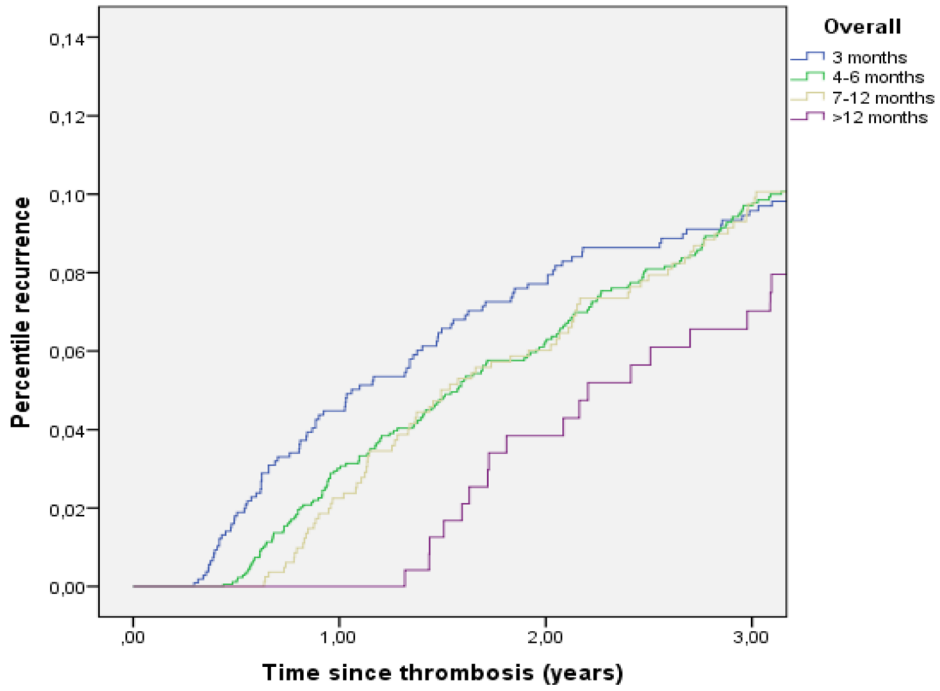

Figure 3. Cumulative risk of recurrence for different durations of treatment when follow-up started at time of first venous thrombosis.

Total N	1-3 months oac N (%)	4-6 months oac N (%)	7-12 months oac N (%)	>12 months oac N (%)
3603	1027 (29)	1637 (45)	724 (20)	215 (6)

- Idiopathic thrombosis was defined as venous thrombosis without surgery, trauma, plaster cast or hormone use (oral contraceptives and hormone replacement therapy), and pregnancy within three months before the event, and without active malignancies or puerperium.
- Provoked venous thrombosis was defined as thrombosis due to surgery, plaster caster or minor injuries.

Discussion

In this large follow-up study of 4731 patients with a first venous thrombosis followed for a total follow-up time of 24 124 person-years we found 683 certain recurrent events for an incidence rate of 27.9 per 1000 person-years and a cumulative incidence of 3% after one year. We found male sex to be a risk factor for recurrence with a 2.2-fold increased rate (95%CI, 1.9-2.6). Overall, an idiopathic first thrombosis was associated with a 1.4-fold increased rate (95%CI, 1.2-1.7). Increasing age was not associated with recurrence risk. When we studied different durations of anticoagulation therapy we found no difference in recurrence risk after discontinuation.

To establish the true incidence of recurrence we performed a sensitivity analysis. We found an incidence of 27.9 per 1000 person-years when only certain recurrences were taken into account and 37.0 per 1000 person-years when both certain and uncertain recurrences were taken into account. The range of incidences of recurrent thrombosis we report is similar but more precise than incidences previously reported.[3-6,11,21,22] Additional to small study sizes, the variation in incidence rates found in the literature may be explained by different definitions of start of follow-up (starting at time of thrombosis or at discontinuation of treatment). Both methods of defining start of follow-up are justifiable, but lead to results that should be interpreted differently. To start follow-up at date of first event has the advantage that recurrences during anticoagulation are taken into account. Furthermore, previous studies generally did not take (un)certainty of recurrences into account. In our study we showed incidences of recurrence both starting follow-up after the date of thrombosis and after the date of discontinuation of treatment. We chose to show the main results of only those with a certain recurrence, as these are most likely to truly have had a recurrent event. However, we also present results where uncertain diagnoses were counted as recurrence, and in which recurrence status of patients with an uncertain recurrence was imputed. By showing all possible ways of estimating incidence rates, our results can easily be compared with those of other studies.

Recurrent events occurred more often than expected just by chance at the same site as the first thrombosis, i.e., patients with a first PE were more likely to have a PE as recurrent event, and patients with a first DVT had more DVT as recurrence. This may be explained by damage to the veins or by a higher awareness of thrombotic symptoms at the location of the first event. However, when a patient had had a first venous thrombosis of the left leg with a recurrence in the leg, the side of recurrence was random. These results suggest that most recurrences are not due to vascular damage or residual thrombosis but may be the result of a more general hypercoagulable state.

When studying the effect of treatment duration, we observed parallel running survival curves. The curves ran parallel because we considered for this analysis only recurrences that occurred after discontinuation of anticoagulation therapy. Obviously, with

increasing duration of anticoagulation, discontinuation occurred later during follow-up. This finding implies that the risk of recurrence is not higher after three months of treatment than with longer periods, which is in line with findings from a recent meta-analysis by Boutitie et al.[23]

As has been consistently shown in other studies, we found a two-fold higher risk of recurrence in men than women. For women more modifiable provoked risk factors are known and therefore they are at lower risk of recurrence than men. These risk factors are not present in idiopathic patients. However, men with an idiopathic thrombosis were still at higher risk of recurrence (incidence: 47.6 per 100 person-years; 95%CI, 41.7-53.5) than women with an idiopathic first event (incidence: 28.2 per 1000 person-years; 95%CI, 20.1-36.3), indicating that men have a higher intrinsic risk of thrombosis. Such an intrinsic higher risk in men has also recently been demonstrated by a study of our group where we showed that the risk of a first event is also twice as high in men when hormonal risk factors are taken into account.[23] Most previous studies did not stratify by sex in the analysis of idiopathic versus provoked first venous thrombosis and the risk of recurrence.[5,9,10,13] Increasing age did not increase the risk of a recurrent event after adjustment for sex. Similar results were obtained from previous studies, including our own.[5,17,18]

The MEGA follow-up study is the largest single study on risk of recurrent venous thrombosis. While varying estimates of recurrence risk have been reported in literature, the large number of patients and the long duration of follow-up resulting in the identification of almost 700 recurrences, allowed us to estimate the risk of recurrence with great precision, overall and in several subgroups.

A limitation of this study is that it was based in a clinical setting. We did not perform CUS for all patients after the first event to better evaluate a subsequent recurrence. However, we tracked all possible recurrences and had access to three sources of information to decide on the likelihood of a true recurrence. The sensitivity analysis showed little difference between the minimum and maximum recurrence rate possible (27.9-37.0) per 1000 person-years as described in Table 1. However, an advantage of this clinical setting is that our study gives the optimal estimate of true effects in clinical practice. A second limitation is that we included only patients with a first event who were younger than 70 years of age. Therefore, our results are not generalizable to patients with a first venous thrombosis above 70 years. A third limitation is that for 21% of patients limited follow-up was available. Some of these patients were lost to follow-up due to death. However, from some of them we still knew their recurrence status up to death through registries of the causes of death and information from anticoagulation clinics. Therefore, in the end we did not know the recurrence status of 8% of patients who died, which at most would have led to a slight underestimation of the incidence of recurrent thrombosis. The majority of patients were lost to follow-up due to reasons that are unlikely to be related to the recurrence risk (non-availability of contact details).

Currently, most guidelines indicate that patients with a provoked first thrombosis may be treated with three months of anticoagulation while those with an unprovoked first event benefit from a longer treatment duration.[24,25] The results of the current study indicate that sex of the patients should be included in the guidelines as all men are at increased risk of recurrence regardless of the type of thrombosis or presence of a provoking factor. Therefore, while women with a provoked event may be treated with anticoagulants for a duration of three months, a longer duration of treatment should be considered in men and in women with an unprovoked first event. Furthermore, as the likelihood of a same type of recurrent event is higher, patients with a first PE may need to be distinguished from those with a first DVT with respect to duration and intensity of treatment, considering that a recurrent PE is a more severe event than a DVT. Future studies should examine this.

In conclusion, in this large study of patients with a first venous thrombosis we found an overall recurrence rate between three and four percent per year. The recurrence rate was highest during the first year after the first event (i.e., 54 per 1000 person-years) and decreased until it became stable at 25 per 1000 person-years at 4 years after the first venous thrombosis. Age did not affect recurrence risk. An idiopathic first thrombosis is a risk factor for recurrence in men and women, and men had an overall higher risk of recurrence than women. For duration of treatment, sex, type of first event (idiopathic or provoked), and location of first event (DVT or PE) may need to be taken into account. Clinical trials are indicated to test such a strategy.

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Chapter 3

Epidemiology of cancer-associated venous thrombosis

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Abstract

Cancer-associated venous thrombosis is a common condition, though the reported incidence varies widely between studies depending on patient population, start and duration of follow-up and the method of detecting and reporting thrombotic events. Furthermore, as cancer is a heterogeneous disease, the risk of venous thrombosis depends on cancer types and stages, treatment measures and patient-related factors. In general, cancer patients with venous thrombosis do not fare well and have an increased mortality compared with cancer patients without. This may be explained by the more aggressive type of malignancies associated with this condition. It is hypothesized that thromboprophylaxis in cancer patients might improve prognosis and quality of life by preventing thrombotic events. However, anticoagulant treatment leads to increased bleeding, particularly in this patient group, so in case of proven benefit of thromboprophylaxis only patients with a high risk of venous thrombosis should be considered. This review describes the literature on incidence of and risk factors for cancer-associated venous thrombosis, with the aim to provide a basis for identification of high-risk patients, and for further development and refinement of prediction models. Furthermore, knowledge on risk factors for cancer-related venous thrombosis may enhance understanding of the pathophysiology of thrombosis in these patients.

Introduction

In 1865, Armand Trousseau, a French physician, was one of the first to describe an association between thrombosis and cancer. Not many know the association had already been reported earlier in 1823 by Jean Baptiste Bouillaud.[1,2] Perhaps because of the irony of Trousseau diagnosing the condition on himself and dying from it in 1867, the condition was later called Trousseau's syndrome. Since then, many studies have confirmed the association between cancer and venous thrombosis and demonstrated that the incidence of venous thrombosis in cancer patients is high, that it has risen over the past decades and that cancer patients with venous thrombosis do not fare well. It is hypothesized that thromboprophylaxis targeted at cancer patients with a particular high risk of thrombosis might improve their prognosis. Therefore a need exists to identify such patients, which is not easy since cancer is a heterogeneous disease and the risk of venous thrombosis depends on the interaction between tumor cells, the hemostatic system and characteristics of the patient. Furthermore, identification of risk factors for cancer-related venous thrombosis will help to improve understanding of the pathophysiology of thrombosis in cancer patients. Thus, even 150 years after Trousseau died, there is still a need to study the epidemiology of venous thrombosis and cancer in detail.

Incidence of venous thrombosis in cancer patients

It is estimated consistently that about 20 to 30% of all first venous thromboembolic events are cancer-associated (Table 1).[3-9] In a population-based, nested case-control study within the Olmsted County population (Minnesota), 625 residents with an incident deep vein thrombosis (DVT) or pulmonary embolism (PE) were matched on age and sex to 625 unaffected residents. A population attributable risk (PAR; the percentage of all cases of a disease in a population that can be attributed to a risk factor) was calculated and reported to be 18% (95%CI; 13.4-22.6) for an active malignancy.[5] White and coworkers used the California discharge data set to identify a cohort of 21 002 patients hospitalized with incident venous thrombosis in 1996. Of these patients again about 20% (4368) were reported to have cancer-associated venous thrombosis.[9] In a third study, medical records of residents from the Worcester metropolitan area were obtained for a total of 1399 subjects with a confirmed episode of venous thrombosis. Of these patients 29% had a recent or active malignant neoplasm.[8] In a more recent registry, the RIETE registry, which included over 35 000 consecutive symptomatic VT patients from 2001 up to 2011, active cancer was reported in 6075 patients (17%).[4] Lastly, the Tromsø study is a population-based prospective follow-up study of over 26 000 subjects. Participants were followed for venous thrombosis from 1994 to 2007. Of 462 patients with a first-ever VT event, 106 had an active cancer (23%).[3]

Cancer patients have a several fold increased risk of venous thrombosis as compared with the general population or patients without cancer, with relative risks ranging from 4 to 7 (Table 1).[10-13] Frequently cited is the Olmsted County population study. In this

Table 1: Incidences and risk factors for venous thrombosis as discussed in the review

	Study population	*Study Design*	*Number of patients*	*Effect estimate*	*Reference*
Proportion of cancer-associated VT cases	Olmsted county population	Nested case-control	625/625	18% (PAR)	5
	California Discharge DataSet	Cohort	21 002	21%	9
	Worcester metropolitan area, outpatient setting	Cohort	1399	29%	8
	RIETE Registry	Cohort	35 539	17%	4
	Tromsø Study	Cohort	462	23%	3
Relative risk of VT for cancer vs no cancer	MEGA study	Case-control	2131/3220	OR 6.7 (95%CI; 5.2-8.6)	10
	Olmsted county population	Nested case-control	625/625	OR 4.1 (95%CI; 1.9-8.5)	12
	Linked United Kingdom databases	Cohort	82 203/577 207	HR 4.7 (95%CI; 4.5-4.9)	13
	Danish population-based registries	Cohort	57 591/287 476	HR 4.7 (95%CI; 4.3-5.1)	11
Absolute risk of VT in cancer patients	Linkage of California Cancer Registry and California Discharge Dataset	Cohort	235,149	1.6% within two years	14
	Referred patients with solid tumors	Cohort	1041	7.8% (median follow-up 26 months)	15
	Vienna Cancer and Thrombosis Study (CATS)	Cohort	840	8% within one year	16
	38 Papers on cohorts with cancer patients	Meta-analysis	NA	13/1000 PY (95%CI; 7-23) for average risk patients	17
				68/1000 PY (95%CI; 48-96) for high risk patients	17
	Linked United Kingdom databases	Cohort	82 203	14/1000 PY (95%CI; 13-14)	13
Incidence of VT in cancer patients over time	US National Hospital Discharge Survey	Cohort	40 787 000	1.5% in 1989; 3.5% in 1999	19
	Discharge Database from University HealthSystem Consortium	Cohort	1 015 598	~3.5% in 1995; ~4.5% in 2002	18
	Linked United Kingdom databases	Cohort	82 203	10.3/1000 PY in 1997; 19/1000 PY in 2006	13
Risk factors for VT in cancer patients					
Type of cancer	38 Papers on cohorts with cancer patients	Meta-analysis	NA	Pancreatic cancer: ~110/1000 PY	17
				Brain cancer: ~80/1000 PY	
				Lung cancer: ~45/1000 PY	
				Haematologic cancer: ~40/1000 PY	
				Colorectal cancer: ~30/1000 PY	
				Bone cancer: ~30/1000 PY	
				Prostate cancer: ~10/1000 PY	
				Breast cancer: ~10/1000 PY	
Stage of cancer	Danish population-based registries	Cohort	40994/204970	HRs 2.9, 2.9, 7.5 and 17.1 for stage I, II, III and IV cancer patients respectively, vs general population	11
	Linkage of California Cancer Registry and California Discharge Dataset	Cohort	235149	HRs ranging from 1.1-21.5 for different types of cancer, metastatic vs localized cancer	14
	Vienna Cancer and Thrombosis Study (CATS)	Cohort	740	HR 2.0 (95%CI; 1.1-3.5) for (solid) tumor grade G3+G4 vs G1+G2	24
Time since cancer diagnosis	MEGA study	Case-control	2131/3220	OR 53.5 (95%CI; 8.6-334.3) in first 3 months after cancer diagnosis	10
				OR 14.3 (95%CI; 5.8-35.2) in 3-12 months after cancer diagnosis	
				OR 1.1 (95%CI; 0.6-2.2) >15 years after cancer diagnosis	
	Linkage of California Cancer Registry and California Discharge Dataset, colorectal cancer patients	Cohort	68142	5.0/100 PY 0-6 months after cancer diagnosis	25
				1.4/100 PY 6-12 months after cancer diagnosis	
				0.6/100 PY 12-24 months after cancer diagnosis	
	Linked United Kingdom databases	Cohort	82,20	Median ratio 3.2 for VT risk in first 3 monhts after diagnosis vs whole follow-up period, for cancer types separately	13
Treatment	Olmsted county population	Nested case-control	625/625	OR 4.1 vs OR 6.5 for treatment with and without chemotherapy	12
	Node-positive primary operable breast cancer patients	RCT	353/352	Cum. inc. of VT: 13.6% vs 2.6% for 2 years tamoxifen with vs without 6 months additional chemotherapy	33
	Advanced gastroesophageal cancer patients	RCT	490/474	Cum. inc. of VT during and 30 days after chemotherapy: 12.2% for cisplatin vs 6.5% for oxaliplatin containing regimens	34
	35 Papers on trials with cancer patients	Meta-analysis	6769	RR 1.7 (95%CI; 1.4-2.1) for VT in cancer patients treated with red blood cell transfusions with vs without ESAs	35
	38 Papers on phase 3 trials with cancer patients	Meta-analysis	8172	RR 1.6 (95%CI; 1.3-1.9) for VT in cancer patients treated with red blood cell transfusions with vs without ESAs	36

	15 Papers on trials with patients with solid tumors	Meta-analysis	7956	RR 1.3 (95%CI; 1.1-1.6) for VT in cancer patients treated with standard antineoplastic therapy with vs without bevacizumab	37
Patient-related	Linkage of California Cancer Registry and California Discharge Dataset, colorectal cancer patients	Cohort	68142	HR 2.0 (95%CI; 1.7-2.3) for three or more comorbid conditions vs no comorbidities HR 0.4 (95%CI; 0.3-0.5) for Asian/Pacific Islanders vs Caucasians	25
	Discharge data base of University Healthsystem Consortium	Cohort	1015598	ORs ranging from 1.4 to 1.8 for cancer patients with a comorbidity vs cancer patients without comorbidities OR 1.2 and 0.7 for patients with black and asian ethnicity respectively vs white	18
	MEGA study	Case-control	2131/3220	OR 2.2 (95%CI; 0.3-17.8) for VT in cancer patients with vs without factor V Leiden	10

VT denotes venous thrombosis; PAR, population attributable risk; OR, odds ratio; CI, confidence interval; NA, not applicable; PY, person years; HR, hazard ratio; RCT, randomized controlled trial; Cum. inc., cumulative incidence; ESAs, erythropoiesis-stimulating agents

study malignant neoplasm was shown to increase the risk of venous thrombosis four-fold (OR 4.1 (95%CI; 1.9-8.5).[12] However patients were included between 1976 up to 1990, which might outdate the findings. In a Dutch population-based case-control study (the MEGA study), over 3000 consecutive patients with venous thrombosis were included between 1999 and 2004, together with over 2100 partner controls. [10] The risk of venous thrombosis was increased seven-fold in patients with cancer compared with patients without (OR 6.7 (95%CI; 5.2-8.6)). By linkage of four United Kingdom databases Walker and coworkers estimated the relative risk of VT in cancer versus age-matched non-cancer controls from the general population to be 4.7 (HR 4.7 (95%CI; 4.5-4.9)).[13] Surprisingly similar results were reported from a Danish population-based cohort of 57 591 incident cancer cases that were followed in time for venous thrombosis, together with 287 476 individuals without cancer from the general population. Non-cancer controls were matched on age, sex and county of residence. After adjustment for comorbid conditions the risk of venous thrombosis was also 4.7 times higher in cancer patients compared with the non-cancer participants (RR 4.7 (95%CI; 4.3-5.1)).[11] Although these relative risks demonstrate a strong association between cancer and venous thrombosis, absolute risks are clinically more meaningful, for example to communicate a patient's risk of venous thrombosis or to decide whether a patient needs prophylactic treatment with anticoagulants or not, for which it needs to be balanced with the risk of unwanted side-effects (minor or major bleeding) of the anticoagulant treatment. Cohort studies are best suited for this purpose because they provide absolute risks.

The reported absolute risk (cumulative incidence) of venous thrombosis in cancer patients varies widely (1% - 8%) depending on patient population, duration of follow-up, calendar period and the method of detecting and reporting venous thrombotic events (Table 1). The heterogeneity of the studies makes it difficult to compare rates of venous thrombosis between these studies. Some follow-up studies include cancer patients with a diagnosis long before start of follow-up, in others follow-up is started at the beginning of cancer treatment. When comparing studies and generalizing results to other populations, follow-up should start at the same time, preferably at time of cancer diagnosis. When follow-up starts at a later time, some patients may have died and are therefore missing in the analyses. By linkage of the California Cancer Registry to the California Patient Discharge Data Set, Chew and colleagues followed 235 149 cancer patients from time of cancer diagnosis. Within 2 years 5032 patients developed a venous thrombotic event (1.6%).[14] The cumulative incidence reported in populations of such cancer registries or hospital discharge data is generally lower compared with rates reported in, for example, patients admitted to an inpatient oncology service. This is indeed observed in data from Sallah et al. who reported a cumulative incidence of venous thrombosis of 7.8% in 26 months in cancer patients referred to hematology/oncology services.[15] In the CATS study, a prospective follow-up of 840 cancer patients admitted to the Medical University in Vienna, 8% of the cancer patients developed a venous thrombotic event within one year after diagnosis or progression of disease.[16]

A recent meta-analysis by Horsted et al described incidence rates of venous thrombosis in cancer patients, stratified by 'background risk' of venous thrombosis.[17] Among cohorts with average-risk patients, defined as cancer patients representative of all patients with cancer, the incidence rate of venous thrombosis was estimated to be 13 per 1000 person-years (95%CI; 7-23). Among cohorts with high-risk patients, defined as cancer patients with high-grade or metastatic disease or treated with therapeutic strategies that increase thromboembolic risk, the overall incidence rate was 68 per 1000 person-years (95%CI; 48-96). In the abovementioned study with linkage of four United Kingdom databases, over 82 000 cancer patients and over 577 000 age-matched control participants were followed in time for venous thrombotic events. The incidence rate of VT in all cancers was 13.9 per 1000 person-years (95%CI; 13.4-14.4).[13]

Over the years the incidence of venous thrombosis in cancer patients has increased (Table 1).[18,19] Among patients hospitalized with cancer between 1979 and 1999 the cumulative incidence of venous thrombosis was reported by Stein and coworkers. Data was obtained from the US National Hospital Discharge Survey. The cumulative incidence of venous thrombosis increased from the late 1980s onward (1.5% in 1989) and this trend continued to the late 1990s (3.5% in 1999).[19] A similar trend was seen in another study of hospital discharge data. In this study the cumulative incidence of venous thrombosis was 3.6% in 1995-1996 and 4.6% in 2002-2003 (28% increase).[18] A similar rise in VT incidence over time in cancer patients, but not in non-cancer controls, is seen in the study with linkage of four United Kingdom databases by Walker (Figure 1).[13] In this study the rise in VT incidence is reported for different cancer types. Several factors could explain this finding, including a greater awareness of the association between cancer and venous thrombosis and improvements in diagnostic tests. Also, due to improved treatment strategies patients with cancer currently survive longer, leading to more aged patients undergoing more cancer treatments, which in themselves also increase thrombosis risk. For these reasons, the incidence is expected to rise further in the future.

Risk factors for venous thrombosis in cancer patients

Cancer is a heterogeneous disease and its different types and stages should be taken into account when determining the risk of venous thrombosis. Also several patient-associated and treatment-associated factors are known to increase the risk of thrombosis.

Extensive work has been published on type of malignancy and subsequent risk of venous thrombosis (Table 1). Overall, pancreas, brain, lung and ovarian cancer are reported to induce highest risks.[11,13,17,20] In the literature high risks are additionally reported for lymphomas, myeloma and kidney, stomach and bone cancer.[11,14,18,21] Relatively low risks are generally seen in patients with breast or prostate cancer. Horsted and colleagues summarized in their meta-analysis incidence rates of venous thrombosis for eight different types of malignancy (Figure 2).[17] For the absolute risks presented in this figure only cohort studies with start of follow-up at

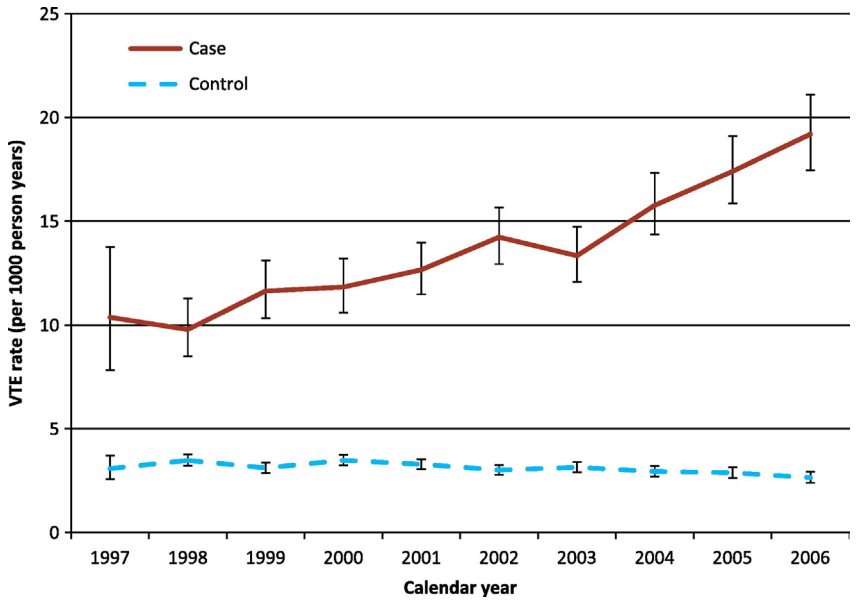

Figure 1. Absolute rates of venous thrombosis (per 1000 person years) for individual calendar years between 1997 and 2006. Cases are cancer patients and controls are age-matched non-cancer controls from the general population. Figure from Walker European Journal of Cancer 2013, with permission from Elsevier.[13]

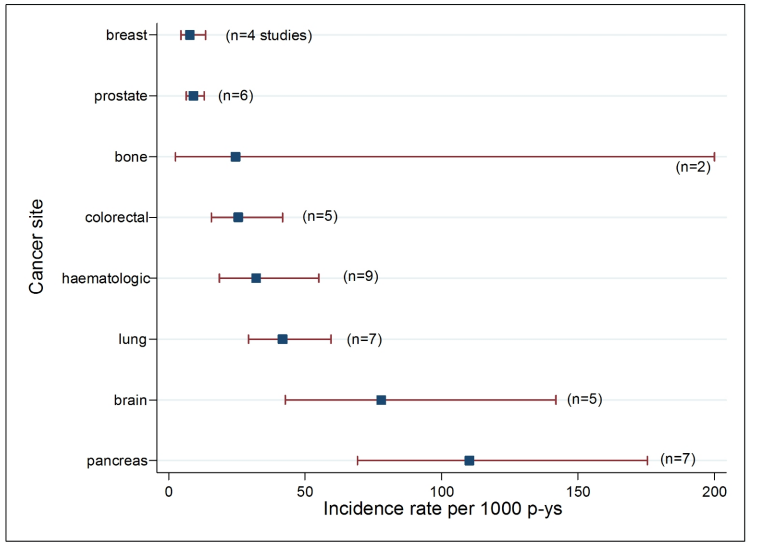

Figure 2. Pooled incidence rates (per 1000 person years) of venous thrombosis per type of cancer. Only studies with start of follow-up at time of cancer diagnosis were included. Numbers in brackets refer to the number of studies that contributed to the pooled estimate. Figure from Horsted Plos Med 2012.[17]

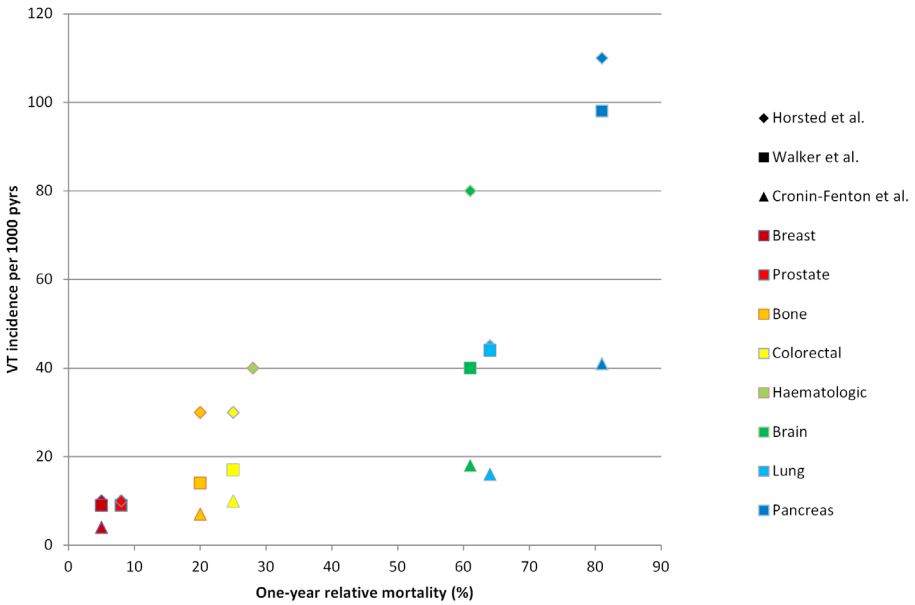

Figure 3. Incidence rates of venous thrombosis (VT) (per 1000 person years) per type of cancer (according to Horsted[17], Walker[13] and Cronin-Fenton[11]) plotted against the one-year relative mortality for each cancer type . One-year relative mortality was calculated by (1 – One-year relative survival) according to Eurocare.it.[23] For haematologic cancer VT incidence is exclusively shown for results of Horsted et al., since Walker et al. and Cronin-Fenton et al. did not present VT incidence rates for haematologic cancer as a combined group.

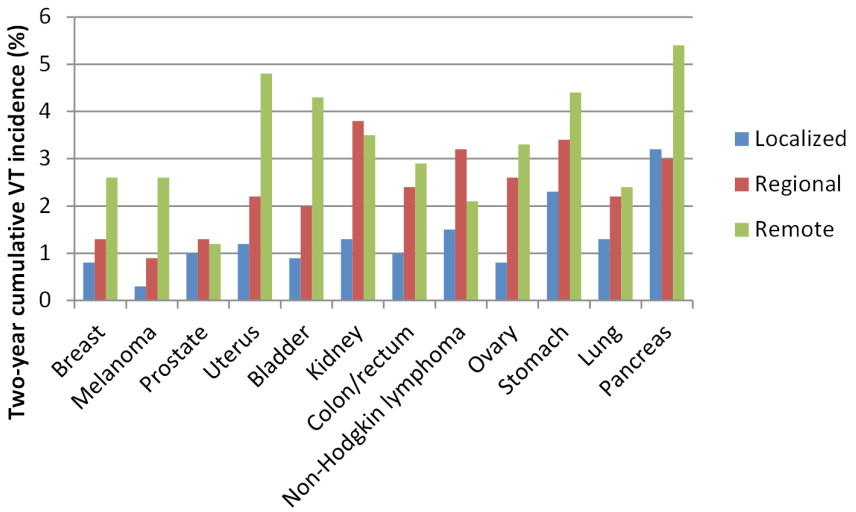

Figure 4. Two-year cumulative incidence (%) of venous thrombosis per type and stage of cancer. Types of cancer were ordered by their respective one-year mortality rates, according to Eurocare.it.[23] Data from Chew Arch Intern Med 2006.[14]

time of cancer diagnosis were included. It appears that especially the cancer types that are biologically aggressive, as evidenced by short survival time and early metastatic spread, are correlated with a high incidence of venous thrombosis.[22] Figure 3 shows VT incidence rates for different types of cancer (according to results of Horsted[17], Walker[13] and Cronin-Fenton[11]) grouped and plotted against the one-year relative mortality for each cancer type. One-year relative mortality rates were derived from Eurocare.it.[23] Although VT incidence per type of cancer varies for the different studies, a clear positive association can be observed with one-year relative mortality of the cancer type, as a measure of biological aggressiveness of the cancer, and an associated thrombogenic potential.

Such an association between aggressiveness of cancer and thrombogenic potential can also be observed when taking stage of cancer into account, which is highly correlated with risk of venous thrombosis (Table 1).[10,11,14,17] In the Danish follow-up study, mentioned above, where 55 000 cancer patients and over 285 000 matched non-cancer controls from the general population were followed in time, the risk of venous thrombosis in cancer patients appeared to be strongly dependent on stage of the cancer, with adjusted relative risks of 2.9, 2.9, 7.5 and 17.1 among patients with stage I, II, III, and IV disease.[11] Also in the California Cancer Registry study, increased relative risks of venous thromboembolic events in metastatic cancer patients compared with patients with localized disease were reported for 12 different types of cancer (range of hazard ratios 1.1-21.5).[14] In this study metastatic disease at time of cancer diagnosis was found to be the strongest predictor of subsequent venous thrombosis. Figure 4 shows two-year cumulative incidence rates of venous thrombosis per type and stage of cancer, according to data from this California Cancer Registry.[14] For every type of cancer presented, VT incidence increases from localized, to regional to remote cancer. Lastly, in the Vienna Cancer and Thrombosis study (CATS), which included 740 patients with newly diagnosed (or progressed after remission) patients with solid tumors, tumor grade (G3+G4 vs G1+G2) was also significantly associated with risk of venous thrombosis (HR 2.0; 95%CI; 1.1-3.5).[24] This was after correction for age, sex, tumor histology, types and stage.

The incidence of venous thrombosis is clearly highest in the first few months after cancer diagnosis and decreases thereafter (Table 1). In the MEGA-study, the risk of venous thrombosis was highest in the first three months after cancer diagnosis (OR 54 (95%CI; 8.6-334.3)), was decreased but still high in the period between three and twelve months (OR 14.3 (95%CI; 5.8-35.2)) and decreased to almost no elevated risk ten years after cancer diagnosis.[10] In a retrospective analysis of over 68 000 colorectal cancer patients from the California Cancer Registry, incidence rates of symptomatic venous thrombosis were calculated.[25] The incidence was reported to decrease over time from 5.0/100 person years in the first 6 months after cancer diagnosis, 1.4/100 person years 6-12 months after cancer diagnosis to 0.6/100 person years 12-24 months after cancer diagnosis. This phenomenon has been shown for all types of cancer in the large

follow-up study by linkage of four United Kingdom databases.[13] This change in risk over time again illustrates why follow-up studies into incidence of venous thrombosis in cancer patients need to start at time of cancer diagnosis. If follow-up is started at a later point in time, the incidence will be lower and studies can not be compared directly. There are several possible explanations for a higher risk of venous thrombosis in the first few months after diagnosis compared with the period thereafter. First, several cancer treatment modalities increase the risk of venous thrombosis (see below), inducing a high risk directly after diagnosis and start of treatment. Second, a proportion of treated cancer patients will go into remission, leading to a reduced thrombotic risk thereafter. A third explanation is that over time a considerable proportion of the cancer patients will succumb to the disease. The occurrence of such a competing event (death) will prevent thrombotic events from being observed.

In addition to type and staging of cancer, cancer treatment modalities also substantially increase the thrombotic potential (Table 1). Surgery, chemotherapy, hormonal therapy, anti-angiogenic drugs, immunomodulatory agents, erythropoiesis stimulating agents, blood transfusions and central venous catheters are all reported to be associated with an increased risk.[26,27] Surgery is a well-known risk factor for venous thrombosis, also in non-cancer patients. In cancer patients, risk of 90-day post-operative venous thrombosis is reported to be twice as high as in non-cancer patients.[28] Incidence rates in patients treated with chemotherapy are high, with an annual incidence of 11%-20%.[29] Also, other new systemic cancer treatments and supportive therapies are reported to predispose to venous thrombosis.[29] An important caveat, however, in interpreting these risks is that most studies on this topic are observational studies. In observational studies the decision on (type of) treatment is made by the treating physician, depending on several patient's characteristics, such as stage of disease and prognosis. Therefore treated and untreated patients are not directly comparable and it cannot be discerned whether increased risk of venous thromboembolism is due to the treatment, the cancer or the patients' prognosis. This phenomenon is called confounding by indication and plays a role in all observational studies. In randomized clinical trials exposure (treatment) is assigned in a random fashion, for which reason patients are directly comparable with respect to their thrombotic risk. A direct comparison of different treatment modalities is even more difficult when thrombosis prophylaxis is indicated for specific types of treatment. For example, the risk in patients who underwent surgery can not be directly compared with the risk in patients treated with chemotherapy as thromboprophylaxis is common practice after surgery, but not during chemotherapy. A disadvantage of clinical trials is the highly selected patient population, limiting the generalizability of the results.

Out of the large literature on this topic, we will present some examples of randomized clinical trials as an illustration of increased risk induced by several types of treatment. Several randomized clinical trials in women with breast cancer have shown a clear link between chemotherapy and/or hormone therapy and venous thrombosis risk. [30-33] In a randomized trial in postmenopausal women with node-positive primary

operable breast cancer (with positive estrogen and progesterone receptor status), the cumulative incidence of thromboembolic events was assessed for women randomized to 2 years of tamoxifen or to tamoxifen (2 years) plus chemotherapy for 6 months. [33] The cumulative incidence in the tamoxifen only group was 2.6% versus 13.6% in the combined treatment group. Similarly, results from a clinical trial in advanced gastro-esophageal cancer patients showed varying rates of venous thrombosis for either one of four epirubicin/platinum/fluoropyrimidine combination regimens during treatment until 30 days after the last treatment cycle. A higher cumulative incidence of venous thrombosis was observed in patients receiving a cisplatin-containing combination regimen (12.2%) as compared with oxaliplatin (6.5%).[34] A systematic review of randomized controlled trials demonstrated that cancer patients treated with erythropoiesis-stimulating agents (ESAs) in addition to red blood cell transfusions had an increased risk of thromboembolic events over patients not additionally treated with ESAs (relative risk 1.7).[35] These results are supported by a systematic review from Bennett et al.[36] In another large meta-analysis of clinical trials, patients with cancer receiving the angiogenesis inhibitor bevacizumab, had a somewhat increased risk of venous thrombosis (relative risk 1.3 95%CI; 1.1-1.6).[37]

Apart from cancer-related factors, patient-related factors play a role in the development of thrombosis in cancer patients (Table 1). Several traditional risk factors for thrombosis are additionally present in many cancer patients like older age, prolonged immobility, prior history of venous thrombosis and comorbidities. In the California Cancer Registry study in colorectal cancer patients, a significant predictor of venous thrombosis during the first year after diagnosis was the presence of three or more comorbid conditions (HR 2.0 (95%CI; 1.7-2.3)).[25] In a retrospective cohort study using discharge databases of all cancer patients admitted to US academic medical centers, over 1 000 000 cancer patients were followed for venous thrombosis. [18] Variables associated with VT in a clinically significant way were ethnicity and the presence of comorbidities. Such comorbidities included arterial thromboembolism, pulmonary disease, renal disease, infection and anemia which all increased the risk of venous thrombosis (ORs 1.5, 1.4, 1.5, 1.8 and 1.4 respectively). Patients with black ethnicity seemed to be at increased risk (OR 1.2 (95%CI; 1.1-1.2)), while patients with Asian ethnicity had a decreased risk of venous thrombosis when compared with Caucasians (OR 0.7 (95%CI; 0.7-0.8)). Similarly, in colorectal cancer patients from the abovementioned California Cancer Registry, the risk of venous thrombosis was significantly reduced among Asians/Pacific Islanders (HR 0.4 (95%CI; 0.3-0.4)) compared with Caucasian patients.[25] This is probably explained by an overall lower risk of venous thrombosis in Asians/ Pacific Islanders.[9] Prothrombotic mutations are additionally reported to influence risk of thrombosis in cancer patients.[10,38] For example, the Factor V Leiden mutation seems to interact with cancer with respect to VT risk. Cancer patients with Factor V Leiden were reported to have a 2-fold increased risk of venous thrombosis compared with non-carriers with cancer (adjusted OR 2.2 (95%CI; 0.3-17.8).[10]

Clinical presentation

A limited number of studies have looked at differences in the clinical presentation of venous thrombosis between patients with and without cancer. Bilateral DVT seems to be more common among cancer patients than in non-cancer patients.[39,41] A recent study by Imberti showed that rates of symptomatic bilateral lower limb DVT, symptomatic iliocaval thrombosis and upper limb DVT were higher in cancer patients compared with patients free from cancer (8.5% vs 4.6%), (22.6% vs 14.0%) and (9.9% vs 4.8%); respectively.[6] In this study rates of PE and symptomatic proximal DVT were similar. The relatively high incidence of upper limb DVT in cancer patients is at least partly explained by the frequent use of a central venous catheter.[42] Furthermore, cancer is reported to be common in rare forms of thrombosis such as Budd-Chiari syndrome, extrahepatic portal vein obstruction and mesenteric vein thrombosis.[43]

Prognosis

In general, cancer patients with venous thrombosis do not fare well. Thrombotic events are reported to be the second leading cause of death in cancer patients.[44] Patients with cancer-associated venous thrombosis have higher risks of bleeding complications during anticoagulant treatment and of recurrent venous thrombosis than patients with venous thrombosis but without cancer.[4,45,46] In a Norwegian study of 740 patients with a first venous thrombotic event, the one-year case fatality rates (the proportion of deaths within one year after the venous thrombotic event) were 5-fold higher in patients with cancer-associated venous thrombosis (63.4% (95%CI; 54.5-71.8)) than in venous thrombosis patients without cancer (12.6% (95%CI; 10.1-15.5)).[7] In the RIETE registry, a large prospective cohort of over 35 000 VT patients, three month mortality was much higher in the patients with cancer-related VT as compared with VT patients without cancer (26% vs 4% respectively).[4]

Furthermore, cancer patients who develop a venous thrombotic event have a lower survival rate than cancer patients without venous thrombosis.[14,47-50] In a large, Danish, population-based study, patients diagnosed with cancer at the time of venous thrombosis were matched to control cancer patients without venous thrombosis, based on age, sex, type of cancer and year of diagnosis.[50] The one-year survival rate for the group with cancer and venous thrombosis was 12%, as compared with 36% in the control group. Chew and colleagues investigated the survival of over 235 000 cancer patients and compared these survival rates between cancer patients with and without a subsequent diagnosis of venous thrombosis.[14] In a multivariate analysis with adjustment for age, race and stage of cancer, a diagnosis of venous thrombosis was a significant predictor of decreased survival within one year for all cancer types (hazard ratios ranging from 1.6 to 4.2). We studied mortality rates in participants of the Tromsø study, a large Norwegian follow-up study in participants free of cancer and venous thrombosis at baseline in 1994-1995.[3] In total, 25,983 subjects were followed until September 1, 2007, of whom 1751 subjects developed cancer and 417 developed venous thrombosis (109 of which cancer-related). By means of a time-dependent

analysis mortality rates and hazard ratios for death were estimated for disease free subjects, subjects with cancer only, subjects with venous thrombosis only and subjects with cancer-related venous thrombosis (Table 2). Subjects with cancer-related venous thrombosis had a 30-fold increased risk of death during follow-up as compared with disease-free subjects (HR 31.2 (95%CI; 24.6-39.6)), while subjects with cancer only or venous thrombosis only had a 7-fold and 3-fold increased risk respectively. An explanation for the difference in mortality rates could be the more aggressive course of the malignancies associated with high thrombosis risk (Figure 3). It is unknown to what extent the high mortality rates in patients with cancer and venous thrombosis can be attributed to the thrombotic events themselves. In a study in 4466 cancer patients in the US starting with chemotherapy and followed for a median of 75 days, thrombosis (including both venous and arterial events) was the second leading cause of death (n=13; 9%) after cancer progression (n=100; 71%).[44] In this study causes of death were assigned by the treating physicians, mainly based on clinical data, rather than autopsies. Among patients from a large database comprised of Multiple-Cause Mortality Files from 1979 to 1998 in whom pulmonary embolism was reported on the death certificates, 23% were reported to have cancer.[51] Causes of death according to the treating physician or death certificate may not be that reliable and autopsy studies should be used to answer this question. In two autopsy studies from Sweden and the US, the incidence of pulmonary embolism in cancer patients was 26% and 17%, respectively, of which 8% and 14% were fatal pulmonary emboli.[52]

Table 2. Crude mortality rates and age- and sex-adjusted hazard ratios of death in participants without cancer and without venous thrombosis, with venous thrombosis only, with cancer only and with cancer-related venous thrombosis, The Tromsø study 1994-2007

Exposure	Person years	Deaths (n)	MR per 100 pyrs (95%CI)	HR (95%CI)
None	277713	1750	0.63 (0.60-0.66)	1.0 (reference)
VT only	1317	67	5.1 (4.0-6.4)	2.6 (2.0-3.3)
Cancer only	5650	721	12.7 (11.9-13.7)	7.4 (6.8-8.2)
Cancer-related VT	131	72	55.0 (43.6-69.3)	31.2 (24.6-39.6)

MR denotes mortality rate; pyrs, person-years; CI, confidence interval; HR: hazard ratio; VT, venous thrombosis.
Hazard ratios were calculated by means of a time-dependent Cox regression analysis.

Thromboprophylaxis

It is hypothesized that anticoagulant treatment for the prevention of venous thrombotic events in cancer patients might improve prognosis and quality of life. However, such treatment comes with a disadvantage of an increased risk of bleeding, which is especially pronounced in cancer patients.[46,53,54] In a prospective follow-up of 842 DVT patients, Prandoni et al. investigated bleeding rates during anticoagulant treatment. The 12-month cumulative incidence of major bleeding was about two-

fold higher in patients with active cancer (12.4% (95%CI; 6.5-18.2%)) than in patients without cancer (4.9% (95%CI; 2.5-7.4%)).[46] Several randomized clinical trials have investigated the effects of thromboprophylaxis in ambulatory cancer patients receiving chemotherapy. A recent Cochrane review summarized results of 9 of those trials.[55] Thromboprophylaxis was reported to significantly reduce the incidence of symptomatic VT (RR 0.62 (95%CI; 0.41-0.93). However, this treatment was also associated with an increase in bleeding events. The number needed to treat to prevent one venous thrombotic event, was 60. Thromboprophylaxis should therefore be targeted only at cancer patients with a high risk of venous thrombosis, which outweighs the risk of bleeding events. Several biomarkers have been associated with risk of venous thrombosis in cancer patients, like P-selectin, D-dimer, tissue factor-bearing microparticles, pre-chemotherapy hemoglobin, platelet and leukocyte counts, factor VIII and C-reactive protein.[16,56-63] A recent clinical trial randomized advanced cancer patients with higher levels (> 3.5 x 10^4 microparticles/μL) of circulating tissue factor-bearing microparticles (TFMP) to either enoxaparin for two months (n=23) or observation without any treatment (n=11).[64] Advanced cancer patients with lower levels of TFMP were followed without treatment (n=32). Patients with higher TFMP levels, not randomized to enoxaparin, had a significantly higher two-month cumulative incidence of venous thrombosis (27%) as compared with patients with lower TFMP levels (7%). Patients with high TFMP levels randomized to enoxaparin had the lowest cumulative incidence of venous thrombosis (6%). Median survival was 17.8 months in patients treated with enoxaparin as compared with 11.8 months in untreated patients with higher levels of TFMP.

Although this clinical trial using risk stratification based on one biomarker shows promising results, prediction models incorporating several risk factors, instead of one, are probably more useful for guiding decisions on prophylaxis in individual patients. Such a risk assessment model has been developed by Khorana et al.[59] In a randomly selected development cohort of 2701 cancer patients initiating a new chemotherapy regimen, baseline clinical and laboratory risk factors for venous thrombosis were included in a risk model, which was validated in an independent cohort of 1365 cancer patients from the same population. Patients were followed for symptomatic venous thromboembolic events for a median of 73 days. Five predictive variables present before initiation of chemotherapy were identified in the final multivariate analysis and used for a risk score model: primary site of cancer, platelet count ≥350 000/μL, hemoglobin less than 10 g/dL and/or use of red cell growth factors, leukocyte count more than 11 000/ μL and body mass index ≥35 kg/m^2 (Table 3). Rates of venous thrombosis in the development and validation cohort were 0.8% and 0.3% in low-risk (score=0), 1.8% and 2% in intermediate-risk (score=1-2) and 7.1% and 6.7% in high-risk patients (score≥3), respectively. Ay and colleagues applied this risk model to their prospective observational cohort study of patients with newly diagnosed cancer or with progression of disease after complete or partial remission who had not recently received chemotherapy, surgery and/or radiotherapy (CATS study).[65] Additionally,

they expanded the model by adding two predictive biomarkers, i.e. soluble P-selectin (≥53.1 ng/mL) and D-dimer levels (≥1.44μg/mL) and they added additional types of cancer to the high and very high risk groups. In the expanded risk model the cumulative probabilities of VT after six months of follow-up were 35% in patients with a score ≥5, 10.3% in patients with score 3 and 1.0% in patients with score 0. The disadvantage of this expanded risk model is that additional laboratory tests have to be performed since D-dimer and P-selectin levels are not routinely measured in the clinic. Intervention trials based on risk assessment models are necessary to demonstrate the effectiveness and safety of prophylactic anticoagulant treatment in high-risk patients. In an ongoing study, the use of thromboprophylaxis in patients deemed high-risk, based on the original prediction model by Khorana, is currently being tested (www.clinicaltrials.gov No. NCT00876915).

Table 3. Predictive model for chemotherapy-associated venous thrombosis

Patient characteristic	*Risk score*
Site of cancer	
very high risk (stomach, pancreas)	2
high risk (lung, lymphoma, gynecologic, bladder, testicular)	1
Prechemotherapy platelet count 350 x 10^9/L or more	1
Hemoglobin level less than 100 g/L or use of red cell growth factors	1
Prechemotherapy leukocyte count more than 11 x 10^9/L	1
BMI 35 kg/m^2 or more	1

From Khorana Blood 2008.[59]

Recurrent venous thrombosis and cancer

The overall risk of recurrent venous thrombosis in patients who suffered once from VT is high, with a five to ten year cumulative incidence ranging from 25% to 30%. [66-68] Cancer patients are at an approximately two to three-fold increased risk of recurrent venous thrombosis compared with non-cancer patients.[46,67-69] Prandoni and coworkers followed 355 consecutive patients with a first episode of DVT for eight years and found a two-fold risk of recurrent venous thrombosis in cancer patients compared with non-cancer patients (hazard ratio 1.7 95%CI; 1.3-2.3).[68] The same group of investigators found a 12-month cumulative incidence of recurrent venous thrombosis of 20.7% in cancer patients on conventional anticoagulant treatment versus 6.8% in patients without cancer on anticoagulant treatment in a prospective cohort study including 842 DVT patients.[46] Recurrence appeared to be related to extent of disease, classified according to the tumor-node-metastasis (TNM) classification, with highest recurrence rates in patients with extensive vs moderately or less extensive cancer. This again reflects the apparent relation between agressiveness of cancer and thrombogenic potential. In the RIETE study patients with symptomatic, acute venous thrombosis were enrolled and three-month outcomes of the participants were studied.

Of 18 883 participants, 3805 had been diagnosed with active cancer. A relative risk for recurrent PE of 2.0 and for recurrent DVT of 2.4 was found for patients with a cancer diagnosis less than three months before their first venous thrombosis.[69] Not much is known about the risk of recurrent venous thrombosis for different types of cancer and results from previous studies are contradictory.[46,69] A clinical prediction rule (Ottawa prognostic score) has been developed for recurrent venous thrombosis during the first six months of anticoagulant treatment in a retrospective cohort study of 543 patients with a cancer-associated venous thrombotic event.[70] The final model included four predictors (sex, primary tumor site, stage and number of prior venous thrombotic events) leading to a score sum that ranged between -3 and +3 points. Patients with a score ≤0 had a low risk of recurrence (4%) while patients with a score ≥1 had a relatively high recurrence risk (16%). The prediction rule was validated by the investigators in an independent set of patients from two randomized clinical trials and results appeared to be consistent. Another group of investigators from the Netherlands assessed the reproducibility of the Ottawa score in an independent sample of 419 patients with cancer-associated venous thrombosis.[71] Their results were similar to those reported by Louzada and coworkers in their validation sample. Recently the Ottawa score was additionally validated in an independent patient population in a tertiary hospital in Korea.[72] In 546 patients with cancer associated-VT the model was less discriminatory compared with the derivation study. Of patients in the low-risk group (score<=0) 13.2% were identified with recurrent venous thrombosis, while 22.4% of patients in the high-risk group (score >=1) were identified with a recurrence. Thrombosis risk as well as cancer predominance is known to be different in the Asian population, which may be an explanation for the different findings. Furthermore differences in study design, like different durations of follow-up or definition of recurrences may explain these findings.

Screening

Acute venous thrombosis can be the first manifestation of an occult cancer. Rates of occult cancer detection at the time or shortly after diagnosis of venous thrombosis vary in the literature, depending on patient population, duration of follow-up and detection methods. While some articles published in the eighties contradict each other as to whether there is an association between venous thrombosis and an increased risk of subsequent cancer diagnosis[73-75], recent articles show a clear association between the two. In a nationwide, retrospective cohort study in Scotland almost 60 000 patients with DVT or PE diagnosed between 1982 and 2000 were followed for the occurrence of cancer until the end of 2000.[76] The ratio of the observed cases of cancer and the number of cases expected based on national cancer incidence rates was calculated, which gives a standardized incidence ratio (SIR). For all malignancies combined there was an excess risk of being diagnosed with cancer in VT patients which remained up to 2 years after diagnosis of VTE. Especially in the first one to six months after diagnosis of venous thrombosis the risk was high (SIR 4.2 (CI 3.9-4.5)). Two other follow-up studies, quite alike in design, showed similar results with respect to risks and

types of cancer (liver, pancreas, ovary, brain and lymphoma) for which the association was most pronounced.[77,78] In a recent systematic review by Carrier and colleagues, data from 34 studies that reported prevalence of undiagnosed cancer at the time of an acute, first thromboembolic event were combined.[79] In 4.1% (95%CI; 3.6-4.6%) of the included patients, a previously undiagnosed cancer was detected within a month after the venous thrombotic event. Within a year after the event 6.3% (95%CI; 5.6-6.9%) of the patients were diagnosed with cancer.

Patients with an idiopathic venous thrombosis have a higher risk of detection of an occult cancer than patients with a venous thrombotic event secondary to a provoking risk factor.[79,80] In the abovementioned study by Carrier the period prevalence of previously undiagnosed cancer between baseline (venous thrombotic event) and 12 months was 10.0% (95%CI; 8.6-11.3%) for patients with unprovoked venous thrombosis versus 2.6% (95%CI; 1.6-3.6%) for patients with a secondary event. This raises the question whether only patients with an idiopathic venous thrombosis should be screened for occult cancer. Van Doormaal and colleagues prospectively followed 630 idiopathic venous thrombosis patients who underwent either baseline cancer screening (consisting of history, physical examination, basic laboratory tests and chest X-ray) or extensive cancer screening (consisting of additional abdominal and chest CT scan and mammography), based on the center in which patients were treated.[81] After baseline screening 7 out of 288 patients (2.4%) were diagnosed with cancer versus 12 out of 342 patients (3.5%) after extensive screening methods. Survival did not differ between the groups, which led the authors to conclude to not support extensive routine screening for cancer in patients with a first episode of idiopathic venous thrombosis. In one randomized clinical trial by Piccioli and colleagues,[82] acute idiopathic venous thrombosis patients were randomized to either an extensive screening for occult cancer or to no further testing. Unfortunately the trial was terminated prematurely due to a lower than anticipated number of participating centers and an increasing tendency among physicians to perform screenings tests for occult cancer in control patients. Extensive screening was found to be able to detect hidden malignancies and to lead to identification of malignancies at an earlier stage. However, due to the limited sample size, effects on prognosis of patients remained again unclear. Cancer related mortality during the 2-year follow-up period did not significantly differ between both groups (absolute difference 1.9% (95%CI; -5.5-10.9%)). The effect of extensive screening in idiopathic venous thrombosis patients on prognosis remains elusive.[83,84] Further studies are needed to investigate whether screening procedures are cost-effective and affect cancer-related mortality.

Superficial venous thrombosis and cancer

Superficial vein thrombosis (SVT), or superficial thrombophlebitis, is a common condition of which the incidence in general has so far not been properly assessed, possibly because in the past SVT was considered a benign, self-limiting, disease. However, it is thought to occur at least as often as deep vein thrombosis. Interest in

the disease was renewed when more and more studies in the past decade described an association between SVT and deep venous thrombosis.[12,85,86] Many conditions have been reported to predispose to SVT, mostly also well-known risk factors for deep venous thrombosis. For this reason it would be reasonable to suspect an association between cancer and SVT.[87-90] The incidence of SVT in cancer patients has not been studied. Whether SVT should be seen as a marker of occult cancer is also controversial. In a sub-study of the Calisto trial, a trial in which ~3000 SVT patients with isolated SVT were randomized to either fondaparinux or placebo, Prandoni and coworkers compared 737 SVT patients with 1438 control patients with regard to cancer diagnoses during an average of 26 months of follow-up.[91] They concluded that occurrence of SVT in the legs does not represent a risk factor for subsequent malignancies. The same conclusion was drawn in a small study performed in the Netherlands.[92] However, Sorensen et al. did find a relation between a diagnosis of SVT and a subsequent cancer diagnosis in the Danish population.[93] The occurrence of cancer in 7663 SVT patients was compared with the expected number of cancer diagnoses based on national incidence rates and a SIR of 2.5 (95%CI; 2.1-2.9) for the first year of follow-up was reported. A possible explanation for the difference in findings is that in the study by Sørensen unrecognized concomitant deep vein thrombosis was possibly present, which increased the risk of a cancer diagnosis. Prandoni and colleagues excluded cases with a concomitant venous thrombotic event confirmed by ultrasonography. Future epidemiologic studies are needed to study the strength of the relationship between SVT and cancer and the incidence of SVT in cancer patients.

Concluding remarks

Despite the fact that the strong association between cancer and venous thrombosis has been known for more than 150 years, cancer-associated thrombosis is still a topic of extensive (epidemiologic) research from which there is much to gain for patients. Future studies need to be targeted at development and validation of prediction models to categorize cancer patients into high or low risk of venous thrombosis. Randomized trials should study the benefit of thromboprophylaxis in patients deemed at high risk based on these models. Furthermore, studies are needed to investigate whether cancer screening procedures in idiopathic venous thrombosis patients are cost-effective and affect cancer-related mortality.

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Chapter 4

Cancer before and after venous thrombosis and the risk of recurrent venous thrombosis: Results from the MEGA follow-up study

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Abstract

Background
The magnitude of the risk of recurrent venous thrombosis (VT) in patients with cancer is not well described and results for different types of cancer are not consistent. We aimed to evaluate the risk of recurrent VT in relation to time of diagnosis and for several types of cancer.

Patients and methods
Patients with a first deep vein thrombosis of the leg or pulmonary embolism were followed for recurrence from time of VT-diagnosis (MEGA follow-up study). Incidence rates (IR) of recurrence per 1000 person-years (py) were estimated for patients with cancer as well as (time-dependent) hazard ratios (HR) adjusted for sex and age comparing recurrence in patients with cancer with those without. Cancer diagnoses were self-reported and complemented with data from the Dutch Hospital Data Register.

Results
4643 Patients were included with a median follow-up of 5.9 years (IQR 1.7-7.8). Participants with a history of cancer within five years *before* first VT (n=423) did not have an increased risk of recurrence (HR 1.1; 95%CI,0.8-1.6), except for patients with a malignancy that was still active during follow-up. Their recurrence-risk was about two-fold increased (HR 2.3; 95%CI,1.5-3.6). Participants who developed cancer *after* the first thrombosis (n=161) also had an increased recurrence-risk (HR 2.2; 95%CI,1.5-3.4), which was especially high in the first three months after cancer diagnosis (HR 5.2; 95%CI,2.3-11.6; cumulative incidence 4%). Risk of recurrence was high for patients who developed non-Hodgkin lymphoma, cancer of the gastro-intestinal tract, prostate or testis.

Conclusion
VT patients with a history of cancer do not have an increased risk of recurrent VT compared with patients without cancer, except when their cancer is still active after the first VT. Patients who develop cancer *after* a first VT also have an increased recurrence-risk, which varies for different types of cancer and for different time periods after cancer diagnosis.

Introduction

A strong relation between cancer and venous thrombosis has been known for a long time, since its first notion in the early 19th century.[1,2] Venous thrombosis, encompassing both deep vein thrombosis (DVT) and pulmonary embolism (PE), is a multicausal disease which affects about 1-2 per 1000 persons per year.[3] A long list of risk factors, both genetic and acquired has arisen over the past decades, of which the presence of active cancer is reported to be one of the strongest.[4] About 20-30% of all first venous thrombotic events are related to cancer and cancer increases the risk of a first thrombosis about 4- to 7-fold.[5-11]

After a first venous thrombotic event recurrence is common. The five-year cumulative incidence is reported to range from 12 to 25%.[12-14]Few studies have investigated the risk of recurrent venous thrombosis in patients with cancer.[9,13-19] Furthermore, most of these previous studies were small and heterogeneous with regard to duration of follow-up (either during anticoagulant treatment or after discontinuation), selection of patients (either DVT and PE or DVT only) and definition of recurrent venous thrombosis.[9,13-19]

The risk of a first event depends strongly on type and stage of cancer and the time point after cancer diagnosis.[5,20-22] Furthermore, certain cancer treatment modalities, such as chemotherapy, substantially increase the thrombotic potential.[9,23,24] It is likely that the risk of recurrent venous thrombosis also varies between patients with different types and stages of cancer, different treatment strategies and for different time periods after diagnosis. However, results of previous studies are contradictory with regard to recurrence risk for different types of cancer.[19,25-27] Prandoni and colleagues reported increased risks of recurrence of similar magnitude for patients with cancer at various sites as compared with patients without cancer.[19] The exception was with patients with breast cancer, for whom a much lower recurrence risk was found than for other cancer patients. Findings from the RIETE register showed that only age and time since cancer diagnosis were associated with recurrent DVT and only age, time since cancer diagnosis and type of first event with recurrent PE.[27] Although patients with either recurrent PE or DVT more often had lung or pancreatic cancer than patients who did not develop recurrent thrombosis, on multivariate analyses no association was found between type of cancer and risk of recurrence. Two other studies, however, showed considerable variation in recurrence risk for patients with different types of cancer, with high recurrence rates seen in patients with lung, brain and ovarian cancer.[25,26]

Although cancer is a heterogeneous disease all patients with cancer and a venous thrombotic event are currently treated the same way and for the same duration of time.[28,29] Knowledge on characteristics that influence risk of recurrent venous

thrombosis in these patients is needed, so that targeted and prolonged therapy may be offered only to patients with a high recurrence risk and that such prolonged therapy is withheld in patients with a low recurrence risk to prevent them being exposed to an unnecessary risk of bleeding.

We aimed to evaluate the risk of recurrent venous thrombosis in relation to the presence of cancer in a large prospective cohort of venous thrombosis patients (MEGA follow-up study) with a strict definition of objectively identified recurrent events, both during and for a prolonged period after discontinuation of anticoagulant treatment. Our secondary aim was to study recurrence risk according to different types of cancer and according to different time periods after cancer diagnosis.

Methods

Patients

This study includes patients who took part in the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study. Details of the MEGA study have been described previously.[5] In brief, between March 1999 and August 2004, 4956 consecutive patients with an objectively diagnosed first DVT of the leg or PE were included. Patients were aged 18-70 years and were enrolled from six anticoagulation clinics in the Netherlands. Anticoagulation clinics monitor all patients taking vitamin K antagonists in a well-defined geographical area. All patients filled in a detailed questionnaire ("Questionnaire CC") on the presence of possible risk factors for venous thrombosis before their first venous thrombotic event.

Of 4956 patients included in the MEGA study, 4731 gave written informed consent for future follow-up on recurrent venous thrombosis (MEGA follow-up study). Between June 2008 and July 2009 these participants were asked whether they had developed a recurrent venous thrombotic event by means of a short answer form with one yes/no question. Furthermore, all participants were asked to complete a second questionnaire on the presence of risk factors for venous thrombosis after their first event (" Questionnaire FU"). Duration of follow-up was estimated as the time at risk from the date of the first thrombotic event to the end of follow-up. The end of follow-up was defined as the date of a recurrent event and in the absence of a recurrence, the date of filling in the short answer form. If a patient did not fill in this form, they were censored at the last date we knew them to be recurrence free. This could be date of death (n=99), date of emigration (n=3), date of the last visit to the anticoagulation clinic (n=489) or the last moment known to be recurrence free from information of the MEGA case-control study (n=411). Details of assessment of end of follow-up have been described previously.[30] Data on anticoagulant treatment during follow-up, both starting dates and dates of discontinuation of treatment, were retrieved for all

participants from the anticoagulation clinics where patients were treated for their first event. All participants gave informed consent and gave written permission to obtain information about their medical history. The study was approved by the ethics committee of the Leiden University Medical Center, the Netherlands.

Adjudication of cancer diagnoses

In Questionnaires CC and FU, participants were asked to self-report on the presence of cancer, either before or after the first venous thrombotic event, and if present, on date of diagnosis, type of cancer and presence or absence of metastases. Data from Questionnaire CC, i.e., cancer diagnosed before the first venous thrombosis, have been verified earlier by Blom et al.[5] For Questionnaire FU the response rate was 60% (2827/4731). Our data were linked to the Dutch hospital data register, which allowed us to verify the cancer diagnoses. The Dutch hospital data register covers complete, nationwide data on hospital admissions since 1986. The data from the follow-up were linked to discharge diagnosis data from 1995 up to 2010 from this register by the Dutch Central Bureau of Statistics (CBS). Discharge diagnosis data are collected in practically all general and university hospitals and most specialized clinics, such as cancer clinics. Diagnosis at discharge is determined by the treating physician and subsequently coded by trained hospital staff according to International Classification of diseases, ninth version clinical modification (ICD-9-CM). 92% (4350/4731) of our participants could be individually linked to records of the hospital data register. Furthermore, between 2007 and 2009 the vital status of all follow-up participants was acquired from the Dutch population register, as has been described previously.[31] For the patients who died during follow-up, the cause of death was obtained from the national register of death certificates. The causes of death were encoded according to the International Classification of Diseases, tenth revision, clinical modification (ICD-10-CM).

Data on cancer diagnoses from questionnaires CC and FU, the Dutch hospital data register and cause of death statistics were combined. From these 4 different sources, a decision rule regarding certainty of the cancer diagnosis was made using the information collected per patient. Participants were classified as having no cancer, probably no cancer, a probable cancer diagnosis, a certain cancer diagnosis or as having missing data regarding cancer (see Supplement for decision rule).

As date of a certain or probable cancer diagnosis we used the first date reported in either Questionnaire CC or FU, the hospital discharge diagnoses or cause of death statistics. If a date of cancer diagnosis was not available from any source of information, we classified date of diagnosis as the date of the first thrombosis in case of a cancer diagnosis before the first thrombotic event (n=9) and as the date halfway between date of the first thrombotic event and end of follow-up in case of a cancer diagnosis during follow-up (n=1). In case a malignancy was reported in the cause of death statistics only (n=22), the date of cancer diagnosis was set at the date halfway between the first thrombotic event and date of death.

Patients could have a cancer diagnosis before or after the first thrombotic event. See Figure 1 for a timeline of events and overview of cancer exposure categories. We classified participants with a cancer diagnosis before the first thrombotic event as participants with a cancer diagnosis within five years before first venous thrombosis. Participants who reported a cancer diagnosis date of more than five years before the first thrombotic event and for whom no other data on subsequent cancer progression from any of our sources was registered (and who were therefore assumed to be relapse free) (n=66), were excluded from all analyses. Furthermore, we decided to exclude participants with a missing cancer diagnosis (n=22). This left 4643 participants to be included for analyses, out of 4731 participants eligible for follow-up.

Cancer that was diagnosed after the end of follow-up was not taken into account. Types of skin cancer other than melanoma were not registered as a cancer diagnosis.

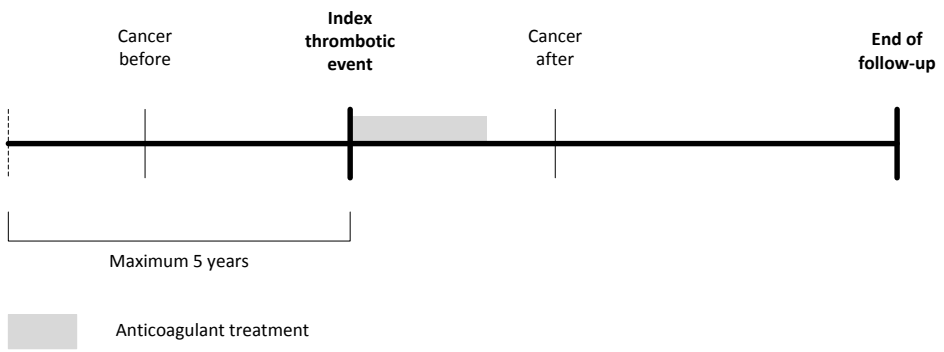

Figure 1. Timeline of exposure to cancer and outcome. Some of the patients had two diagnoses of cancer, which could be either; 1) both before the first thrombotic event or 2) both after the first thrombotic event or 3) one before and one after the first thrombotic event.

Adjudication of recurrent venous thrombotic events

During the same period when participants were asked to self-report on any recurrent thrombotic events during follow-up, information about recurrences was additionally retrieved from the anticoagulation clinics where patients were initially included for their first event and in case they moved house, at the clinic nearest to their new address. Death due to venous thrombosis was also included. For recurrent events reported by either the patient or the clinic discharge letters from the treating physician were requested. A decision rule regarding certainty of the diagnosis was made according to the information collected per patient. Possible recurrences were classified into certain recurrences and uncertain recurrences, with as a main purpose to distinguish extensions of a first event from truly new thromboses. Details of this decision rule have been described previously.[30] For this study, we considered certain recurrences as outcome event only (n=664). In short, reported recurrences were classified into certain recurrences when 1) there was a discharge letter stating a diagnosis of a recurrent

event based on clinical and radiological data, or when 2) both the anticoagulation clinic and the patient reported a recurrent event at either a clearly different location than the first event or that occurred more than one year since the first event, or when 3) a registered death from a recurrent event at least six months after the first event was found. Participants with uncertain recurrent events (n=212) were censored from this uncertain recurrent event onward.

Statistical analyses

All statistical analyses were performed separately for: 1) patients with cancer diagnosed *before* the first thrombotic event and 2) patients with cancer diagnosed *after* the first thrombotic event. This was done since selection into the study was different for both patient groups. Patients with a cancer diagnosis *before* first venous thrombosis only represent subjects who survived long enough to develop venous thrombosis, while we identified all participants with a cancer diagnosis *after* the first thrombosis.

Incidence rates of recurrent venous thrombosis were first estimated separately for participants without cancer, with probably no cancer, with probable cancer and with certain cancer. Next, we further classified the probable groups based on the recurrence rates we found in these groups (Supplementary Table 1). For analyses on cancer diagnosed *before* the first thrombotic event, patients with a probable diagnosis of cancer were further excluded, considering their low recurrence rate. For analyses on cancer diagnosed *after* the first thrombotic event participants with a probable cancer diagnosis were grouped with the certain cancers. Participants with probably no cancer were reclassified in the group without cancer.

After these classifications, incidence rates of recurrent venous thrombosis were estimated as the number of events over the accumulated follow-up time with person time split and divided over persons with and without cancer. The Cox-proportional hazards model was used to evaluate risks of recurrent venous thrombosis between groups. Hazard ratios and corresponding 95% confidence intervals were estimated by means of a time-dependent Cox regression analysis using anticoagulant treatment and cancer diagnosis as time-dependent variables. Hazard ratios were adjusted for age, sex and anticoagulant treatment.

Analyses for cancer before the first thrombotic event

Incidence rates of recurrence for patients with a diagnosis of cancer *before* the first thrombotic event and for participants without cancer were estimated. Incidences were further split for participants with and without cancer during and after the initial anticoagulant treatment period. Participants with a cancer diagnosis within five years *before* the first thrombosis probably represent a mix of patients who have gone into remission and patients whose cancer continued to be active after the first event. For this reason, in a subgroup analysis, we classified patients separately as active during follow-up and not active. In the ‘active’ group we entered subjects with metastases at time of the first venous thrombosis or during follow-up and those who died of cancer.

Data on whether cancer was metastasized or not came from either Questionnaire CC or FU and the hospital discharge data.

Analyses for cancer after the first thrombotic event

Incidence rates of recurrence and corresponding hazard ratios for patients with a cancer diagnosis *after* the first thrombotic event and for participants without cancer were estimated. This was additionally done for different time periods after cancer diagnosis (three months, one year, two years and five years). Furthermore cumulative incidences were estimated for the different time frames. These were corrected for competing events, since cumulative incidences derived from standard life-table methods are biased in studies on cancer-associated venous thrombosis in which patients with cancer are both at risk for venous thrombosis and death.[32] For this competing risk approach, cumulative incidence functions were generated using Stata's user-contributed *stcompet* suite. Risks of recurrence were additionally estimated for different types of cancer.

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS), version 20.0 (IBM Corp., Armonk, NY) and Stata version 12 (Stata Corp., College Station, Texas).

Results

Clinical characteristics

4643 Patients with a first episode of venous thrombosis were followed for recurrent events. Mean age of participants was 48 years and 46% were men. Most of the first events were deep vein thrombosis (67%). Median duration of follow-up was 5.9 years (IQR 1.7-7.8). During 23 650 person-years of follow-up 664 certain recurrent venous thrombotic events were identified for a total incidence rate of 28.1 per 1000 person-years (95%CI 26.0-30.3). We identified 575 patients with certain cancer and 81 patients with probable cancer.

Analyses for cancer diagnosed* BEFORE *the first thrombotic event:

423 Patients with a cancer diagnosis before the first thrombotic event were identified. The mean time between cancer diagnosis and thrombosis diagnosis was 2.8 years and most of the diagnoses were cancer of the colon (18%), breast (15%), lung (12%), prostate (9%) or a gynaecological type of cancer (8%). 25 Patients were identified with both a cancer diagnosis before and after the first thrombotic event, at different sites. Table 1 shows incidence rates and hazard ratios of recurrence for participants with or without cancer. Participants with cancer did not have a clearly increased risk of recurrence as compared with participants without cancer, with an incidence rate of recurrence of 35.7 per 1000 person-years (95%CI; 26.4-48.3) and a corresponding hazard ratio of 1.1

Table 1. Risk of recurrent venous thrombosis for patients without or with cancer, diagnosed before the 1st event

Group	N	Observation years	Recurrent events	IR/ 1000 pyrs (95% CI)	HR (95% CI)	HR* (95% CI)
No cancer	3987	21336	588	27.6 (25.4-29.9)	1 (reference)	1 (reference)
Cancer before 1st event	423	1176	42	35.7 (26.4-48.3)	1.3 (0.9-1.7)	1.1 (0.8-1.6)
Two cancer types before 1st event	12	14	1	72.0 (10.1-511.3)	2.7 (0.4-18.9)	2.7 (0.4-20.7)
Cancer before and after 1st event	25	84	5	59.7 (24.8-143.3)	2.1 (0.9-5.0)	1.7 (0.7-4.1)

IR denotes incidence rate; pyrs, person-years; CI, confidence interval; HR, hazard ratio.
*Hazard ratio adjusted for age and sex.

Table 2. Risk of recurrent venous thrombosis for patients without or with cancer, diagnosed before 1st event, according to anticoagulant treatment

Group	N	Observation years	Recurrent events	IR/ 1000 pyrs (95% CI)	HR (95% CI)	HR* (95% CI)
During anticoagulant treatment						
No cancer	3987	3331	23	6.9 (4.6-10.4)	1 (reference)	1 (reference)
Cancer before 1st event	423	343	9	26.2 (13.6-50.4)	4.6 (2.1-10.2)	4.2 (1.8-9.7)
After discontinuation treatment						
No cancer	3550	18194	565	31.1 (28.6-33.7)	1 (reference)	1 (reference)
Cancer before 1st event	217	857	33	38.5 (27.4-54.2)	1.2 (0.8-1.7)	1.0 (0.7-1.5)

IR denotes incidence rate; pyrs, person-years; CI, confidence interval; HR, hazard ratio.
*Hazard ratio adjusted for age and sex

(95%CI; 0.8-1.6) after correction for age and sex. Participants with a diagnosis of cancer both before and after the first thrombotic event had a recurrence rate of 59.7 per 1000 person-years (95%CI; 24.8-143.3). Additional correction for anticoagulant treatment (as a time-dependent variable) in the abovementioned analyses did not materially affect results (data not shown).

Incidence of recurrent venous thrombosis during and after the initial anticoagulant treatment period

Overall, in the current study population 33 recurrent thrombotic events were identified during anticoagulant treatment, for an incidence rate of 8.8 per 1000 person-years (95%CI; 6.3-12.4). Recurrence rate after discontinuation of anticoagulant treatment was much higher (31.3 per 1000 person-years (95%CI; 28.9-33.9)). During anticoagulant treatment participants with a cancer diagnosis before the first thrombotic event had an approximately four-fold higher recurrence risk than patients without cancer (HR 4.2 (95%CI; 1.8-9.7)) (Table 2). However, after discontinuation of anticoagulant treatment recurrence risk in this group of patients with cancer was not increased compared with patients without cancer (HR 1.0 (95%CI; 0.7-1.5)).

Incidence of recurrent venous thrombosis, according to activity of cancer

Participants with a cancer diagnosis within five years before the first thrombosis probably represent a mix of patients who have gone into remission and patients whose cancer was still active during follow-up after the first thrombotic event. The risk of recurrence for participants with cancer which we considered active during follow-up was two-fold increased as compared with participants without cancer (HR 2.3; 95%CI, 1.5-3.6 for participants who died of cancer and HR 1.7; 95%CI, 1.0-2.7 for participants with metastasized cancer (Table 3)). Participants with cancer without metastases, which might have gone into remission before thrombosis, did not seem to have an increased risk of recurrent thrombosis compared with participants without cancer (HR 0.8; 95%CI, 0.5-1.2). After additional correction for anticoagulant treatment (as a time-dependent variable) results remained similar (data not shown).

Analyses for cancer diagnosed AFTER the first thrombotic event:

161 Patients were identified who developed cancer *after* the first thrombotic event. The mean time between cancer diagnosis and thrombosis date was 2.9 years for these 161 patients and most of the diagnoses were cancer of the lung (16%), breast (15%), colon (14%) or prostate (11%). Table 4 shows incidence rates and hazard ratios of recurrence for participants with and without cancer. Participants with cancer had an increased risk of recurrence compared with participants without cancer, with an incidence rate of recurrence of 64.5 per 1000 person-years (95%CI; 43.9-94.7) and a corresponding adjusted hazard ratio of 2.2 (95%CI; 1.5-3.4). Additional correction for anticoagulant treatment (as a time-dependent variable) did not change results (data not shown).

Table 3. Risk of recurrent venous thrombosis for patients with cancer diagnosis before 1st event, according to cancer severity

Group	N	Observation years	Recurrent events	IR/ 1000 pyrs (95% CI)	HR (95% CI)	HR† (95% CI)
No cancer	3987	21336	588	27.6 (25.4-29.9)	1 (reference)	1 (reference)
Cancer before 1st event, without metastasis	187	718	18	25.0 (15.9-39.8)	0.9 (0.6-1.4)	0.8 (0.5-1.2)
Cancer before 1st event, with metastasis	189	351	17	48.5 (32.0-80.6)	1.7 (1.1-2.8)	1.7 (1.0-2.7)
Cancer before 1st event, who died from cancer during follow-up	266	287	21	73.2 (47.8-112.3)	2.5 (1.6-3.9)	2.3 (1.5-3.6)

IR denotes incidence rate; pyrs, person-years; CI, confidence interval; HR, hazard ratio.
*Groups are not mutually exclusive.
†Hazard ratio adjusted for age and sex.

Table 4. Risk of recurrent venous thrombosis for patients with or without cancer, diagnosed after the 1st event

Group	N	Observation years	Recurrent events	IR/ 1000 pyrs (95% CI)	HR (95% CI)	HR* (95% CI)
No cancer	3987	21797	588	27.0 (24.9-29.2)	1 (reference)	1 (reference)
Cancer after 1st event	161	403	26	64.5 (43.9-94.7)	2.6 (1.8-3.9)	2.2 (1.5-3.4)
Two cancer types after 1st event	<10	15	0	0 (0-245.9)	NA	NA

IR denotes incidence rate; pyrs, person-years; CI, confidence interval; HR, hazard ratio.
*Hazard ratio adjusted for age and sex.

Table 5. Risk of recurrent venous thrombosis for patients with cancer diagnosis after 1st event, according to time periods after diagnosis

Group	N	Observation years	Recurrent events	IR/ 1000 pyrs (95% CI)	HR (95% CI)	HR* (95% CI)
No cancer	3987	21797	588	27.0 (24.9-29.2)	1 (reference)	1 (reference)
Cancer after 1st event	161	403	26	64.5 (43.9-94.7)	2.6 (1.8-3.9)	2.2 (1.5-3.4)
0-3 months after diagnosis	161	36	6	164.5 (74.0-366.5)	6.2 (2.8-13.9)	5.2 (2.3-11.6)
3 months to 1 year after diagnosis	136	83	9	108.0 (56.2-207.5)	3.6 (1.9-7.0)	3.1 (1.6-6.0)
1 year to 2 years after diagnosis	94	83	3	36.2 (11.7-112.4)	1.4 (0.5-4.4)	1.2 (0.4-3.8)
2 years to 5 years after diagnosis	74	152	6	39.7 (17.8-88.1)	1.8 (0.8-4.0)	1.5 (0.7-3.3)
>5 years after diagnosis	33	49	2	40.6 (10.2-162.3)	2.1 (0.5-6.8)	1.9 (0.5-7.6)

IR denotes incidence rate; pyrs, person-years; CI, confidence interval; HR, hazard ratio.
*Hazard ratio adjusted for age and sex.

Incidence of recurrent venous thrombosis according to time after cancer diagnosis

For participants with a cancer diagnosis during follow-up, i.e. *after* their first thrombosis, recurrence rates and corresponding hazard ratios were estimated for different time periods after their cancer diagnosis (Table 5). The risk of recurrent venous thrombosis was especially high in the first three months after cancer diagnosis, with an incidence rate of 164.5 per 1000 person-years (95%CI; 74.0-366.5), and a five-fold increased risk of recurrence compared with participants without cancer (HR 5.2 (95%CI; 2.3-11.6)). Recurrence risks steadily decreased after the first three months up until the first year after diagnosis, and was about 40 per 1000 person-years in the years thereafter. After correction for anticoagulant treatment results remained similar (data not shown).

Cumulative incidences of recurrence, corrected for the competing risk of death, were 4% (95%CI; 2-8) in the first three months after diagnosis, 10% (95%CI; 4-15) in the first year, 13% (95%CI; 8-19) in the first two years and 20% (95%CI; 13-28) in the five years following cancer diagnosis.

Incidence of recurrent venous thrombosis for different types of cancer

Table 6 shows incidence rates and corresponding hazard ratios of recurrent venous thrombosis for participants with different types of cancer diagnosed during follow-up. High recurrence risks were seen for patients with lung cancer, certain types of gastrointestinal cancer, prostate cancer, urinary tract cancer, non-Hodgkin lymphoma and testicular cancer.

Discussion

In this follow-up study with over 4500 participants with a first venous thrombosis we studied risk of recurrent thrombosis for participants with and without cancer. In a time-dependent analysis we found participants with cancer diagnosed *after* the first thrombotic event, to have a two-fold increased risk of recurrence (HR 2.2; 95%CI, 1.5-3.4). We found a high rate of recurrence in the first three months after these cancer diagnoses (IR 165 per 1000 pyrs; 95%CI, 74-367 and cumulative incidence 4%), which corresponds with a five-fold increased risk compared with participants without cancer. Recurrence risks were different for different types of cancer, with high rates observed in participants with gastrointestinal cancer, lung cancer, prostate and urinary tract cancer, non-Hodgkin lymphoma and testicular cancer. For participants with a cancer diagnosis *before* the first venous thrombosis the risk of recurrence was not increased compared with patients without cancer (HR 1.1; 95%CI, 0.8-1.6). However, in a selection of these participants, i.e. with cancer which was active during follow-up, we did find an increased recurrence risk, which was of similar size as in those patients who developed cancer *after* venous thrombosis (doubled).

Table 6. Risk of recurrent venous thrombosis for patients with cancer diagnosis after 1st event, according to type of cancer

Group	N†	Observation years	Recurrent events	IR/ 1000 pyrs (95% CI)	HR (95% CI)	HR* (95% CI)
No cancer	3987	21797	588	27.0 (24.9-29.2)	1 (reference)	1 (reference)
Lung	26	28	2	71.0 (17.8-283.9)	2.8 (0.7-11.3)	2.1 (0.5-8.3)
Gastrointestinal	33	94	4	42.3 (15.9-112.8)	1.8 (0.7-4.7)	1.2 (0.4-3.2)
Esophagus	<10	6	1	180.4 (25.4-1280.5)	8.9 (1.2-63.8)	5.6 (0.8-40.3)
Stomach	<10	8	1	132.0 (18.6-937.1)	5.6 (0.8-39.6)	3.6 (0.5-25.4)
Colon	23	81	1	12.4 (1.7-88.1)	0.5 (0.1-3.7)	0.3 (0.0-2.5)
Pancreas	<10	1	1	1250.9 (176.2-8879.9)	39.7 (5.5-285.3)	25.2 (3.5-181.6)
Breast	24	72	2	28.0 (7.0-111.8)	1.2 (0.3-4.9)	1.6 (0.4-6.6)
Gynaecological	13	45	1	22.0 (3.1-156.3)	0.9 (0.1-6.2)	1.3 (0.2-9.4)
Prostate	18	43	6	140.0 (62.9-311.7)	5.4 (2.4-12.0)	3.4 (1.5-7.7)
Urinary	17	53	4	75.7 (28.4-201.7)	2.9 (1.1-7.7)	2.4 (0.9-6.4)
Brain	<10	1	0	0 (0-3689.9)	NA	NA
Hematological	10	30	3	100.4 (32.4-311.4)	3.6 (1.1-11.1)	3.0 (1.0-9.3)
Leukemia	<10	12	0	0 (0-307.4)	NA	NA
Hodgkin	0	0	0	NA	NA	NA
Non-hodgkin	<10	16	3	183.3 (59.1-568.3)	6.6 (2.1-20.6)	5.2 (1.7-16.3)
Kahler	<10	1	0	0 (0-3689.9)	NA	NA
Testis	<10	2	2	976.6 (244.2-3904.9)	25.3 (6.3-102.0)	18.2 (4.5-73.8)
Melanoma	<10	12	0	0 (0-307.4)	NA	NA
Other‡	15	31	2	64.3 (16.1-257.0)	2.8 (0.7-11.4)	2.4 (0.6-9.5)

IR denotes incidence rate; pyrs, person-years; CI, confidence interval; HR, hazard ratio; NA, not applicable.
*Hazard ratio adjusted for age and sex.
†Number of patients per type of cancer do not add up to 161, since patients with several types of cancer were counted more than once.
‡ Patients diagnosed with metastasized cancer, without information on primary tumor site, were included in this group.

The few studies that have so far investigated the risk of recurrent venous thrombosis in patients with cancer described a two- to nine-fold increased risk compared with patients without.[9,13-19] However, these studies differed substantially with regard to patient characteristics, duration of follow-up, type of analysis and data collection. Two studies, which included DVT patients only, with long duration of follow-up (3-9 years), reported similar hazard ratios of 1.97 (95%CI; 1.20-3.23) and 1.72 (95%CI; 1.31-2.25) for recurrence in patients with known cancer at time of the first thrombosis compared with patients without cancer.[13,14] One study, based on data from the Olmsted County population, with a similarly long duration of follow-up, that included both patients with DVT and PE, reported hazard ratios of 2.2 and 4.2 for patients treated with and without chemotherapy, respectively.[9] Another study reported a high relative risk of 9.2 (HR 9.2 (95%CI; 2.0-41.7)).[15] This result might be explained by a small number of recurrent events or the design of the study in which thrombotic events were identified by hospital discharge records only. Four studies reported relative risks of recurrence during anticoagulant treatment.[16-19] They all reported that patients with active cancer at time of thrombosis had an approximately three-fold increased risk of recurrent venous thrombosis as compared with patients without cancer during anticoagulant treatment (OR 2.7; HR 2.6; RR 3.0; HR 3.2). Some of the abovementioned relative risks were adjusted for potential confounders, while others were not.

In this large follow-up study with long duration of follow-up we were able to study the risk of recurrent venous thrombosis for patients with different cancer characteristics, such as type of cancer or metastasized cancer and to study recurrence risk for different time points after cancer diagnosis. The risk of recurrence in patients who developed cancer *after* the first thrombotic event has not been studied before, since all of abovementioned previous studies were in patients with cancer known or active at time of the first venous thrombotic event only. We included diagnoses of cancer both *before* and *after* the first thrombotic event. Additionally, we were able to study the risk of recurrent venous thrombosis both during and after discontinuation of anticoagulant treatment and to show results with and without adjustments for age and sex. This increases comparability with other studies. Recurrent events reported in this study were objectively defined and only certain recurrent events were taken into account. Diagnoses of cancer were considered based on four different sources of information and we took care to use only those in whom we were certain of a correct diagnosis.

We did not find an increased risk of recurrent venous thrombosis in participants with a cancer diagnosis *before* the first thrombotic event. This finding is probably explained by participants with a cancer diagnosis within five years before the first thrombosis representing a mix of patients who had gone into remission and patients whose cancer was still active. Additionally, we could have had a selection of patients with cancer with a relatively good prognosis, because patients with a worse prognosis may not have wanted to participate in our MEGA study. When we stratified results for patients

whose cancer was still active during follow-up and patients who might have gone into remission we found increased recurrence risks for these participants with active cancer.

During anticoagulant treatment of the first venous thrombosis we found participants with a cancer diagnosis *before* this event to have an almost four-fold increased recurrence risk compared with participants without cancer. After discontinuation of anticoagulant treatment, however, recurrence risk was similar between participants with and without cancer. This finding is largely explained by the much increased recurrence rate in participants without cancer after discontinuation of treatment (31.1 vs 6.9 per 1000 person-years during treatment). Patients with cancer had a less strong increase in absolute risk (38.5 vs 26.2 per 1000 py after discontinuation of anticoagulant treatment). Possibly, the risk of recurrent venous thrombosis in patients with cancer is increased to such an extent that it outweighs the anticoagulant effect of treatment. An additional explanation might be that anticoagulant treatment in patients with cancer is usually provided as long as the cancer is active or as long as patients receive antineoplastic treatment. If a patient is in remission, and anticoagulant treatment is discontinued, these patients may not have an increased risk of recurrent venous thrombosis anymore as compared with participants without cancer.

Currently, guidelines provide treatment recommendations for the group of patients with cancer-associated venous thrombosis as a whole and recommend long-term treatment with low molecular weight heparins (LMWHs) for as long as cancer is active.[28,29] However, the risk of recurrent venous thrombosis in these patients may well vary and be influenced by tumour characteristics, such as tumour site, histology and stage. If this is the case it might be worthwhile to adjust treatment regimens accordingly. Obviously, risk of bleeding should additionally be taken into account.

It was suggested in a recent meta-analysis that metastatic malignancy, adenocarcinoma or lung malignancy confers a higher risk of recurrence than localized malignancy, non-adenocarcinoma or breast cancer.[33] The main finding according to the authors was, however, that “no definitive conclusions can be drawn from the published literature because reporting of malignancy characteristics in patients with cancer and recurrent venous thrombosis during the anticoagulation period is scarce”. A large register study recently reported on an increased recurrence risk for patients with brain, lung and ovarian cancer, for patients with myeloproliferative or myelodysplastic disorders and for patients with advanced stage of cancer.[25] In the Ottawa prognostic score lung cancer was reported to increase recurrence risk, while patients with breast cancer or localized disease were reported to have lower risks of recurrent venous thrombosis.[26]

Our study supports current thought that risk of recurrent venous thrombosis is not the same for all patients with cancer and that stratification of patients with cancer-associated venous thrombosis according to their recurrence risk is of relevance to offer these patients a better tailored treatment approach. Our results show that patients

with advanced cancer have a higher risk than patients with more localized or less active disease. Our results for different types of cancer diagnosed after a first event are based on small numbers, but we could still observe differences in recurrence risk for the different types of cancer. We also show an increased risk of recurrent venous thrombosis in patients diagnosed with cancer *after* a first thrombotic event. The risk was especially high in the first three months after cancer diagnosis and steadily decreased thereafter. Physicians should be aware of this in case a diagnosis with cancer is made in a patient with a history of venous thrombosis. To give definite answers to which patients would benefit from long-term anticoagulant treatment and which patients should not, larger studies are required. For this, meta-analyses of individual patient data could prove useful.

Some limitations of this study warrant comment. First, 8% of all MEGA study participants could not be individually linked to the Dutch hospital data register. Furthermore some diagnoses of cancer from the hospital data register might have been missed because of incomplete recording. This possible underreporting of cancer diagnoses might have led to a slight underestimation of our incidence rates. However, since data from the hospital data register were combined with two questionnaires filled in by the participants at two points in time and with causes of death, underreporting of cancer diagnoses was probably limited. Second, before a cancer diagnosis is made the malignancy has been present for some time. This implies that person-time may occasionally have been misclassified as unexposed in participants with a malignancy that was present but not yet diagnosed. The recurrence rate for patients without cancer may therefore have been somewhat overestimated and the hazard ratios therefore somewhat underestimated. Third, our classification of patients with a history of cancer within five years before the first thrombotic event into patients whose cancer was still active during follow-up and patients whose cancer had gone into remission, may have been somewhat crude. However, our finding of similarly increased recurrence risks in patients we classified as still active during follow-up, and patients with a cancer diagnosis *after* venous thrombosis suggests that misclassification has been limited. The same applies to the patients we classified as having cancer that had gone into remission, as we found a similar recurrence risk in this group of patients as in participants without cancer. Fourth, we had information on anticoagulant treatment from the anticoagulation clinics, that register outpatient use only. Participants (with cancer) may have received anticoagulant treatment in the hospital. Data on this use of anticoagulant treatment lack in our study, possibly inducing an additional underestimation of the recurrence risk. However, after adjustment for anticoagulant treatment hazard ratios did not change, which suggests that this did not play a major role. Fifth, we had not enough data to take cancer treatment regimens into account in our study. Cancer treatment regimens affect cancer activity and risk of venous thrombosis and it would have been interesting to study recurrence risks during and after treatment. However, we found increased recurrence rates in patients with a cancer diagnosis *after* their first thrombotic event,

especially during the first three months after diagnosis. Cancer treatment might have played a role in this highly increased recurrence risk we found shortly after diagnosis.

To conclude, patients with venous thrombosis and cancer had an increased risk of recurrent venous thrombosis compared with patients without cancer. Participants with a cancer diagnosis *before* the first venous thrombotic event whose malignancy was still active after thrombosis had a two- to three-fold increased risk of recurrence compared with patients without cancer. Participants who developed cancer *after* the first thrombosis had an increased recurrence risk, which was especially high in the first three months after cancer diagnosis (about five-fold compared with patients without cancer, cumulative incidence 4%). Risk of recurrent venous thrombosis varied for different types, stages and for different time periods after cancer diagnosis. Stratification of patients with cancer-associated venous thrombosis according to their recurrence risk is of relevance to offer these patients a better tailored treatment approach.

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SUPPLEMENTAL DATA

Supplemental Methods

Decision rule regarding certainty of cancer diagnosis

Participants were classified as having a certain cancer diagnosis either when their cause of death listed cancer, when any hospital discharge diagnose contained a cancer diagnosis or when a cancer diagnosis was mentioned at both questionnaires CC and FU (n=575). Participants were classified as having a probable cancer diagnosis when they responded on either questionnaire CC or questionnaire FU that they had cancer and when hospital discharge register data did not report a cancer diagnosis or were missing (n=81). Participants were classified as having no cancer diagnosis when, in questionnaire FU, they responded, "No, I have never had a cancer diagnosis" and when hospital discharge register data did not contain a cancer diagnosis or were missing (n=2504). Participants who responded in questionnaire CC "No, I did not have a cancer diagnosis before my 1st thrombotic event", who did not fill in questionnaire FU, and for whom hospital discharge registry data did not contain a cancer diagnosis or were missing were classified as 'probably no cancer diagnosis' (n=1483). When no information was obtained from either questionnaire regarding cancer and when hospital discharge register and cause of death statistics data did not report a cancer diagnosis, data regarding cancer diagnoses were considered to be missing (n=22).

Classification of types of cancer

All cancer diagnoses were classified into one the following types; lung, gastrointestinal (esophagus, stomach, colon, pancreas), breast, gynaecological, prostate, urinary (bladder, kidney, urinary tract), brain, hematological (leukaemia, Hodgkin, non-hodgkin, Kahler), testis, melanoma, thyroid or other. When patients with a certain or probable cancer diagnosis appeared to have been diagnosed with several types of cancer (n=244) we checked for every patient individually, whether the second diagnosis with cancer was most probably a metastasized tumour of the first cancer type or a new malignancy. When this was the case only the first type of cancer was taken into account. For (n=43) patients we decided that the second reported cancer type was probably a second primary tumour, rather than a metastasis of the first.

Supplementary Table 1.

Table 1. Incidence rates of recurrent venous thrombosis in patients with or without cancer

Groups	N	Observation years	Recurrent events	IR (95%CI)
*Cancer before 1st event**				
No cancer	2504	15459	429	27.8 (25.2-30.5)
Probable cancer	45	173	3	17.4 (5.6-53.9)
Certain cancer	423	1176	42	35.7 (26.4-48.3))
Missing data with regard to cancer	22	77	1	12.9 (1.8-91.9)
Probably no cancer	1483	5877	159	27.1 (23.2-31.6)
Cancer after 1st event†				
No cancer	2504	15920	429	26.9 (24.5-29.6)
Probable cancer	34	106	5	47.3 (19.7-113.7)
Certain cancer	127	298	21	70.5 (45.0-108.2)
Missing data with regard to cancer	22	77	1	12.9 (1.8-91.9)
Probably no cancer	1483	5877	159	27.1 (23.2-31.6)

IR denotes: incidence rate per 1000 person years; CI: confidence interval.
*Patients with a cancer diagnosis after the first thrombotic event excluded from analyses
†Patients with a cancer diagnosis before the first thrombotic event excluded from analyses

Chapter 5

Coagulation factor levels in relation to venous thrombosis and cancer: Results from the MEGA study

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Abstract

The pathophysiology underlying the association between cancer and subsequent venous thrombosis (VT) is not well known. Furthermore, it is not known in what way patients with cancer who develop VT are different from those who do not.

We aimed to study plasma coagulation factor levels (procoagulant, anticoagulant, fibrinolytic) in four groups of individuals with and without cancer and with and without VT.

From the MEGA case-control study (n=11253) four groups of participants with or without cancer (CA+ or CA-) and with or without VT (VT+ or VT-) were included, and plasma coagulation factors measured after the initial anticoagulant treatment for the thrombotic event. Cancer diagnoses were objectively verified. Median levels of coagulation factors, with 95% confidence intervals, were estimated, as well as geometric mean differences in factor levels over the groups of participants with the VT-CA- group as the reference.

Median levels of coagulation factors were generally lowest in the VT-CA- group (n=2825). Compared with this group, levels of fibrinogen, factor VIII, von Willebrand factor and factor XI were increased in the VT+CA- participants (n=2166) and highest in the VT+CA+ participants (n=147). Results were most pronounced for factor VIII and von Willebrand factor. Levels of factor V, IX, total and free protein S and TFPI were increased only in the VT+CA+ participants.

To conclude, increased levels of procoagulant coagulation factors in participants with both VT and cancer suggest a generalized role of procoagulant pathways in patients with cancer and suggest the importance of a procoagulant state in cancer-associated VT.

Introduction

An association between cancer and venous thrombosis was first described by Bouillaud and Trousseau in the 19th century already.[1,2] Since then, the strong relation between cancer and venous thrombosis has been confirmed in various studies. It is estimated that a fifth of all venous thrombotic events are cancer associated.[3-5] Cancer is reported to increase the risk of venous thrombosis about four- to seven-fold[3,6], and venous thrombotic events are a major cause of morbidity and mortality in patients with cancer.[7]

The pathophysiology underlying the association between cancer and venous thrombosis is largely unknown. It is likely to be multifaceted and to involve interactions of tumor cells, the hemostatic system, cancer treatment measures and characteristics of the patient. General procoagulant effects are exerted by the host response to cancer (acute-phase reaction, paraprotein production, inflammation, necrosis and hemodynamic disorders) and by anticancer therapies.[8] In addition, several substances released by and activities directly associated with tumor cells (including tissue factor, tumor derived cytokines, inhibitors of fibrinolysis and cell adhesion molecules) play a prominent role.[8-10]

Progression of cancer is accompanied by the development of a hypercoagulable state. It is cited in literature that about 50% of all patients with cancer and up to 90% of patients with metastasised cancer exhibit abnormalities in one or more routine coagulation parameters.[11-16] The most commonly described hemostatic changes in patients with cancer are an increase in plasma levels of clotting factors I (fibrinogen), V, VIII, IX and XI as well as in fibrinogen degradation products and platelet count.[17] Most of these studies were, however, conducted a long time ago.

Cancer treatment strategies, and therefore the prognosis of patients, have changed considerably. Furthermore, it is not well known how patients with cancer who develop venous thrombotic events differ from those patients with cancer without thrombosis. Few studies have linked the coagulation profile in patients with cancer with the clinical occurrence of venous thrombosis.[18-20] Neither has this, as far as we know, been done for a wide range of procoagulant and anticoagulant factors.

We aimed to study several plasma coagulation factor levels (procoagulant, anticoagulant and fibrinolytic) in four groups of individuals with and without cancer and with and without venous thrombosis to determine to what extent the coagulation profile differs between these groups. For this purpose, we used data from the MEGA case-control study (n>10 000) in which for more than half of the participants blood was sampled and factors of the hemostatic system were measured.

Methods

Participants

This study was performed within the MEGA- (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis) study, which is a large case-control study aimed at identifying risk factors for venous thrombosis. Details of this study have been described previously.[6] In brief, from March 1999 until September 2004, 4956 consecutive patients with a first objectively diagnosed venous thrombotic event were included from six anticoagulation clinics in the Netherlands. Anticoagulation clinics monitor all patients taking vitamin K antagonists in a well-defined geographical area. Detailed diagnostic information was obtained from hospital discharge reports and general practitioners. 3297 Partner controls of the patients, who had no history of venous thrombosis, were included in the study. Additionally from January 2002 to September 2004, 3000 random-digit-dialing controls (RDD), with no history of venous thrombosis, were included. All participants were 18 to 70 years of age. All participants completed the informed consent process prior to enrolment, and the study was approved by the ethics committee of the Leiden University Medical Center.

Between 2007 and 2009 the vital status of all MEGA participants was acquired from the Dutch population register, as has been described previously.[21] For the participants who died, a cause of death (encoded according to International Classification of Diseases ICD-10-CM) was obtained from the national register of death certificates.

Cancer diagnosis

All participants were asked to complete a detailed questionnaire on acquired risk factors for venous thrombosis. All items in the questionnaire referred to the period before the index date. We used the date of diagnosis of venous thrombosis as the index date for patients as well as their partner controls. For the RDD controls the index date was the date of completing the questionnaire. Participants were asked to report on the presence of acquired risk factors, amongst others any type of diagnosed malignancy, date of diagnosis and type of malignancy diagnosed. Approximately three months after discontinuation of anticoagulant treatment, or one year after the event in case anticoagulant treatment was continued for more than a year, cases and their partner controls were interviewed. The RDD group was invited for the interview at time of returning the questionnaire. In the interview, participants were again asked to report on any malignancies diagnosed after inclusion in the study, date of diagnosis and type of cancer diagnosed. Self-reported cancer diagnoses were verified by means of discharge letters from the primary physician or hospital where patients were treated. Details of this verification process were described previously.[6] For the current study, participants with a cancer diagnosis within five years before the index date, or a cancer diagnosis within six months after the index date, were included. Participants with a cancer diagnosis outside this period were excluded from analyses.

Blood collection and laboratory analyses

At the time of the interview blood was sampled from both cases and controls. Details of collection and processing of blood samples have been described previously.[22,23] For logistic reasons, blood sampling for measurement of coagulation proteins was done in patients diagnosed with venous thrombosis before June 1, 2002. Blood samples were drawn at least three months after discontinuation of oral anticoagulant therapy, or during anticoagulant therapy in patients who continued this therapy for more than one year. Partner controls visited the anticoagulation clinic for blood sampling at the same time as their partner and therefore blood samples were available only for partner controls recruited before June 2002. The additional group of controls recruited via RDD were invited for a blood sample irrespective of their time of enrolment. Participants who were unable or unwilling to provide blood samples or were patients and partner controls recruited after June 1, 2002, were sent buccal swabs to collect DNA for genetic profiling.

The levels of natural anticoagulants (antithrombin, protein S, protein C levels and TFPI), procoagulant factors (fibrinogen, factor II, factor V, factor VII, factor VIII, von Willebrand factor, factor IX, factor X, and factor XI), and the fibrinolytic marker D-dimer were assessed in the blood samples. All assays were performed in automated machines by laboratory technicians who were unaware of the case–control status of the samples. For details on the measurements of coagulation factors, see the Supplement.

Statistical analyses

Blood samples were available for 2377 participants with venous thrombosis (48% (2377/4956)) and 2939 controls (47% (2939/6297)). 123 Participants (64 cases, 59 controls) were excluded from analyses because of a cancer diagnosis more than five years before inclusion in the study, because of a cancer diagnosis more than six months after the index date or because information regarding a possible cancer diagnosis or cancer diagnosis date was missing. In total, 2313 participants with venous thrombosis and 2880 controls without venous thrombosis were included for the current analyses.

Median coagulation factor levels with corresponding 95% confidence intervals were estimated for participants with neither venous thrombosis nor cancer (VT-CA- ; n=2825), participants with cancer but without venous thrombosis (VT-CA+ ; n=55), participants with venous thrombosis but no cancer (VT+CA- ; n=2166) and for participants with both cancer and venous thrombosis (VT+CA+ group; n=147). Boxplots with medians and corresponding 95% confidence intervals were constructed for every coagulation factor separately to visually show the spread of coagulation factor levels over the groups of participants (Supplementary Figure 1). Levels of D-dimer were plotted on the 10log scale, because of the wide range of D-dimer measurements.

Mean differences in coagulation factor levels (and corresponding 95% confidence intervals) were estimated between the groups of participants, with the VT-CA- group as the reference category. Mean differences were adjusted for age and sex by means of multivariate linear regression analysis. Mean differences between the groups

of participants were estimated with a natural logarithmic transformation, thus providing geometric mean differences, since mean differences without a logarithmic transformation are substantially influenced by extremely high or low values of coagulation factors for some of the participants. The interpretation of a geometric mean difference is different from the interpretation of a mean difference. Instead of an absolute difference in factor levels it represents a relative difference with in our case the VT-CA- group as the reference. For example, a geometric mean difference for factor VIII of 1.10 in the VT+CA+ group means that on average factor VIII levels are 1.10 times higher (10% higher) in the VT+CA+ group than in the VT-CA- group. We chose a cut-off of five percent to define levels of coagulation factors as increased.

Participants with a cancer diagnosis within five years before their thrombotic event could have gone into remission in the meantime and might have a different coagulation profile than participants whose malignancy was still active at time of blood sampling. For this reason, we estimated median coagulation factor levels in the VT+CA+ group separately for participants who died of cancer in the years after the index date (as registered by the national register of death certificates) and for participants who survived the years following the index event. The first group of participants was classified as having 'active cancer' while the second group of participants was classified as 'unknown activity'. Geometric mean differences, adjusted for age and sex, in coagulation factor levels between the two groups of participants with cancer (active cancer vs activity unknown) were estimated.

At the time of blood collection 304 participants (275 individuals with venous thrombosis; 29 controls without venous thrombosis) were on anticoagulant treatment. These participants were excluded from all analyses concerning vitamin K dependent coagulation factors (factor II, VII, IX and X, protein C activity, total protein S antigen, free protein S antigen) and factors that are otherwise affected by anticoagulant treatment (D-dimer).

Results

The clinical characteristics of the participants are shown in Table 1, for all participants included and for the four groups of participants separately. Mean age of all participants was 48 years. Mean age was higher in participants with cancer, i.e. 55 years in the VT+CA+ group and 58 years in the VT-CA+ group. The most common types of cancer were breast, prostate, colorectal and a hematological type of cancer.

Median levels of coagulation factors differed considerably across the four groups of participants and were generally lowest in the group of participants without venous thrombosis and without cancer (VT-CA-) (Table 2). Differences in median levels over the groups of participants were most pronounced for levels of factor VIII (activity and antigen), von Willebrand factor and D-dimer. Levels were lowest in the VT-CA- group, higher in the other groups of participants and highest in the VT+CA+ group. Levels of factor V, factor VII, protein C activity and total protein S antigen were increased in both cancer groups (VT-CA+ and VT+CA+) as compared with the groups of participants without cancer (VT-CA- and (VT+CA-).

Geometric mean differences in coagulation factor levels, adjusted for age and sex, between the VT-CA- group and the VT-CA+, VT+CA- and VT+CA+ groups are presented in Table 3. The VT-CA- group was used as the reference category and the geometric mean differences for the other groups of participants represent the relative increase in levels of the coagulation factors. Following a cut-off of five percent to define an increase in coagulation factor level, we identified four patterns for the coagulation factors over the four groups. For the first pattern, levels were increased by at least 5% for the VT+CA+ participants only, and not for the other groups. We identified pattern 1 for factor V, factor IX, total and free protein S and TFPI. Fibrinogen, factor VIII activity and antigen, von Willebrand factor, factor XI and D-dimer levels were increased by at least 5% in the VT+CA- group and highest in the VT+CA+ group (pattern 2). Only D-dimer levels were increased to the same extent both in the VT+CA- and VT+CA+ groups (by aproximately 40%). Levels of factor VII were increased to about the same extent in both groups of participants with cancer (VT-CA+ and VT+CA+) (pattern 3). The fourth pattern showed no clear difference in factor levels over the groups of participants, which was the case for factor II, factor X, antithrombin and protein C. In all of abovementioned analyses adjustment for age had a larger effect on mean differences in coagulation factor levels than adjustment for sex (results not shown).

In Table 4, the median coagulation factor levels are shown for participants who died of cancer in the years following their thrombotic event (n=39) and for participants who survived in the years following thrombosis (n=97) ('active cancer' vs 'unknown activity'). The geometric mean differences in coagulation factor levels, adjusted for age and sex, between the two groups are additionally shown. After adjustments, levels of factor VIII activity, factor VIII antigen and von Willebrand factor were increased by at least 20% in the active cancer group as compared with the group of participants with cancer with unknown activity. Levels of both TFPI and D-dimer were increased in the active cancer patients as compared with the cancer patients with unknown activity by approximately 15%.

Table 1. Clinical characteristics of the study population

	VT* - Cancer -	VT – Cancer +	VT + Cancer -	VT + Cancer +	Total
General characteristics					
Number of participants	2825	55	2166	147	5193
Male sex n, (%)	1351 (48%)	26 (47%)	986 (46%)	72 (49%)	2435 (47%)
Mean age (range)	48 (18-70)	58 (31-70)	47 (18-70)	55 (18-70)	48 (18-70)
Types of cancer					
Breast n, (%)	0	12 (22%)	0	23 (16%)	35 (1%)
Prostate n, (%)	0	15 (27%)	0	15 (10%)	30 (1%)
Colorectal n, (%)	0	3 (5%)	0	21 (14%)	24 (0%)
Haematologic n, (%)	0	3 (5%)	0	20 (14%)	23 (0%)
Lung n, (%)	0	1 (2%)	0	7 (5%)	8 (0%)
Other n, (%)	0	21 (38%)	0	61 (42%)	82 (2%)

*VT denotes: venous thrombosis

Table 2. Median coagulation factor levels for participants with or without cancer and with or without venous thrombosis

Coagulation factor	*VT* - Cancer -* median (95%CI)	*VT - Cancer +* median (95%CI)	*VT + Cancer -* median (95%CI)	*VT + Cancer +* median (95%CI)
Procoagulant				
Fibrinogen activity (g/L)	3.2 (3.2-3.3)	3.5 (3.2-3.7)	3.4 (3.4-3.4)	3.7 (3.5-3.8)
Factor II activity (IU/dL)†	109 (109-110)	113 (107-119)	112 (111-112)	111 (109-115)
Factor V (IU/dL)	92 (91-93)	97 (91-103)	93 (92-94)	101 (96-104)
Factor VII acitivity (IU/dL)†	109 (108-110)	124 (109-130)	112 (110-113)	119 (116-128)
Factor VIII activity (IU/dL)	106 (104-107)	110 (103-132)	134 (131-136)	148 (139-152)
Factor VIII antigen (IU/dL)	108 (107-110)	123 (115-132)	146 (143-148)	162 (152-175)
Von Willebrand factor antigen (IU/dL)	105 (103-105)	110 (102-123)	138 (136-140)	158 (148-164)
Factor IX antigen (IU/dL)†	103 (102-104)	109 (101-117)	107 (106-109)	113 (109-117)
Factor X activity (IU/dL)†	116 (115-117)	126 (115-129)	118 (117-119)	116 (111-120)
Factor XI activity (IU/dL)	98 (97-99)	106 (101-112)	104 (102-105)	105 (103-111)
Anticoagulant				
Antithrombin (IU/dL)	105 (105-106)	105 (101-109)	105 (105-106)	107 (105-109)
Protein C activity (IU/dL)†	116 (115-117)	124 (115-131)	115 (113-116)	122 (117-127)
Total protein S antigen (IU/dL)†	101 (100-102)	113 (100-117)	102 (101-103)	109 (105-112)
Free protein S antigen (IU/dL)†	90 (89-91)	88 (82-105)	92 (91-94)	97 (92-102)
TFPI (U/mL)	1.7 (1.7-1.7)	1.9 (1.7-2.0)	1.7 (1.7-1.7)	1.9 (1.9-2.0)
Fibrinolytic				
D-dimer (ng/mL)†	236 (230-240)	263 (230-312)	327 (315-339)	347 (303-400)

*VT denotes: venous thrombosis, CI: confidence interval
†Participants on anticoagulant treatment during blood sampling were excluded

Table 3. Geometric mean differences in coagulation factor levels between groups of participants with and without cancer and with and without venous thrombosis

Coagulation factor	*VT - Cancer -*	*VT - Cancer +* GMD (95%CI)†	*VT + Cancer -* GMD (95%CI)†	*VT + Cancer +* GMD (95%CI)†
Procoagulant				
Fibrinogen activity (g/L)	reference	1.00 (0.95-1.06)	1.05 (1.04-1.06)	1.11 (1.07-1.14)
Factor II activity (IU/dL)‡	reference	1.01 (0.97-1.05)	1.01 (1.00-1.02)	1.03 (1.00-1.05)
Factor V (IU/dL)	reference	1.02 (0.97-1.07)	1.02 (1.01-1.03)	1.06 (1.03-1.09)
Factor VII activity (IU/dL)‡	reference	1.05 (0.99-1.12)	1.02 (1.01-1.04)	1.08 (1.04-1.13)
Factor VIII activity (IU/dL)	reference	1.04 (0.95-1.13)	1.25 (1.23-1.27)	1.30 (1.23-1.37)
Factor VIII antigen (IU/dL)	reference	1.04 (0.95-1.14)	1.34 (1.32-1.37)	1.42 (1.34-1.50)
Von Willebrand Factor antigen (IU/dL)	reference	1.02 (0.93-1.12)	1.33 (1.30-1.36)	1.43 (1.35-1.52)
Factor IX antigen (IU/dL)‡	reference	1.03 (0.98-1.08)	1.04 (1.03-1.05)	1.09 (1.06-1.12)
Factor X activity (IU/dL)‡	reference	1.04 (0.99-1.09)	1.02 (1.01-1.03)	1.01 (0.98-1.04)
Factor XI activity (IU/dL)	reference	1.00 (0.05-1.06)	1.05 (1.04-1.06)	1.09 (1.05-1.12)
Anticoagulant				
Antithrombin (IU/dL)	reference	1.00 (0.97-1.03)	1.00 (0.99-1.00)	1.03 (1.01-1.05)
Protein C activity (IU/dL)‡	reference	1.02 (0.97-1.07)	0.99 (0.98-1.01)	1.03 (1.00-1.07)
Total protein S antigen (IU/dL)‡	reference	1.04 (0.99-1.09)	1.01 (1.00-1.02)	1.05 (1.01-1.08)
Free protein S antigen (IU/dL)‡	reference	1.01 (0.93-1.10)	1.04 (1.03-1.06)	1.05 (1.01-1.10)
TFPI (U/mL)	reference	1.03 (0.95-1.10)	1.00 (0.99-1.02)	1.08 (1.03-1.13)
Fibrinolytic				
D-dimer (ng/mL)‡	reference	0.97 (0.81-1.15)	1.42 (1.37-1.47)	1.41 (1.25-1.58)

*VT denotes: venous thrombosis, GMD: geometric mean difference, CI: confidence interval
†Adjusted for age and sex
‡Participants on anticoagulant treatment during blood sampling were excluded

Table 4. Median coagulation factor levels and geometric mean differences according to activity of cancer

Coagulation factor	Median (95%CI)		GMD (95%CI)
	VT patients with cancer with unknown activity (n=97)	VT patients with active cancer (n=39)	Adjusted†
Procoagulant			
Fibrinogen activity (g/L)	3.7 (3.5-3.8)	3.7 (3.4-3.9)	1.03 (0.95-1.12)
Factor II activity (IU/dL)‡	111 (107-116)	112 (109-116)	1.02 (0.97-1.07)
factor V (IU/dL)	97 (93-102)	106 (97-111)	1.05 (0.98-1.14)
Factor VII acitivity (IU/dL)‡	120 (116-128)	119 (107-138)	0.98 (0.90-1.07)
Factor VIII activity (IU/dL)	141 (132-152)	151 (147-195)	1.23 (1.03-1.46)
Factor VIII antigen (IU/dL)	155 (143-166)	198 (157-222)	1.22 (1.07-1.38)
Von Willebrand factor antigen (IU/dL)	152 (136-162)	192 (154-226)	1.27 (1.09-1.48)
Factor IX antigen (IU/dL)‡	113 (108-120)	112 (102-123)	0.99 (0.92-1.07)
Factor X activity (IU/dL)‡	115 (110-121)	120 (110-128)	1.05 (0.99-1.11)
Factor XI activity (IU/dL)	107 (103-112)	105 (94-115)	0.98 (0.91-1.05)
Anticoagulant			
Antithrombin (IU/dL)	106 (105-110)	108 (103-110)	1.00 (0.96-1.05)
Protein C activity (IU/dL)‡	123 (117-127)	116 (111-137)	1.00 (0.94-1.07)
Total protein S antigen (IU/dL)‡	108 (103-113)	110 (105-120)	1.01 (0.94-1.08)
Free protein S antigen (IU/dL)‡	97 (92-102)	103 (92-107)	1.03 (0.94-1.12)
TFPI (U/mL)	1.9 (1.7-2.0)	2.2 (1.9-2.3)	1.14 (1.03-1.27)
Fibrinolytic			
D-dimer (ng/mL)‡	345 (297-400)	317 (275-536)	1.15 (0.81-1.64)

*CI denotes: confidence interval, VT: venous thrombosis, GMD: geometric mean difference, for which the patients with cancer with unknown activity were set as reference.
†Adjusted for age and sex
‡Participants on anticoagulant treatment during blood sampling excluded

Discussion

In this study, in which we studied plasma coagulation factor levels in participants with and without cancer and with and without venous thrombosis, we found that all coagulation factor levels (procoagulant, anticoagulant and fibrinolytic) were lowest in individuals without venous thrombosis and without cancer. Compared with this group of participants, levels of fibrinogen, factor VIII activity and antigen, von Willebrand factor and factor XI were increased in participants with venous thrombosis without cancer and were highest in participants with both venous thrombosis ánd cancer. These findings were most pronounced for factor VIII and von Willebrand factor (30-40% increase). Levels of factor V, factor IX, total and free protein S and TFPI were increased only in the group of participants with both venous thrombosis and cancer. Levels of factor VII were increased in participants with cancer and were unaffected by the presence or absence of venous thrombosis.

Our findings of increased levels of procoagulant coagulation factors in participants with venous thrombosis without cancer and even higher levels of these factors in participants with both venous thrombosis and cancer support prior observations of a generalized role of procoagulant pathways in patients with cancer and thrombosis and emphazise the importance of the coagulation system in cancer-associated venous thrombosis. These findings were most pronounced for levels of factor VIII and von Willebrand factor. Our finding of slightly increased levels of anticoagulant proteins, free protein S and TFPI, in participants with cancer and venous thrombosis is suggestive of an additional effect of cancer on anticoagulant pathways.

Although previous studies have compared coagulation profiles for individuals with and without cancer[11,16,19,24], few studies have linked these profiles with venous thrombotic events in patients with cancer.[18,19] Johnson et al compared coagulation profiles between 98 (hospice) patients with advanced cancer either with or without deep vein thrombosis (identified on screening) with a group of control participants without cancer.[19] Goldenberg et al studied coagulation factor levels in 36 patients with cancer-only, 58 patients with venous thrombosis-only and 32 patients with both cancer and venous thrombosis.[18] Some of our findings are in accordance with these studies, while others are not. Similar to our observations, Johnson et al found increased levels of fibrinogen, factor VIII and D-dimer in cancer patients as compared with healthy controls. However, cancer patients with DVT had somewhat lower levels of fibrinogen and factor VIII than cancer patients without DVT, which is contrary to our findings. Goldenberg et al reported an increased level of von Willebrand factor in the group of cancer patients with DVT as compared with the group of patients with cancer or DVT alone. This is in line with our findings. These studies were, however, not comparable with ours with respect to patient selection and study design. For example, Johnson et al only included hospice-in patients with advanced cancer. Furthermore, none of the results were adjusted for age and sex.

We found somewhat increased levels of the anticoagulant proteins total and free protein S and TFPI in the group of participants with cancer and venous thrombosis. In

general, levels of anticoagulant proteins, such as antithrombin, protein C and protein S are assumed to be lower in patients with cancer than in non-cancer individuals due to a decreased hepatic synthesis of such anticoagulant proteins.[17] Decreased levels of both protein C and protein S in patients with cancer as compared with healthy controls have indeed been shown in several studies.[25-27] A possible explanation for these conflicting results is that previous studies included patients with more advanced disease. In patients with advanced disease, consumption of these proteins (as seen in sepsis)[28] or active liver disease may have led to decreased levels of these proteins.

We found increased levels of factor VII in patients with cancer, independent of the presence of VT, which is in line with a study by Kakkar et al.[29] In this study in over 100 patients with solid tumors and a comparison group of healthy volunteers, plasma levels of factor VII were found to be 46% higher in patients with cancer. These results were, however, not corrected for age and sex.

In our study, factor VIII, von Willebrand factor and D-dimer showed the highest rise in levels in participants with cancer. High factor VIII and von Willebrand factor levels have been described before in different types of cancer patients.[30-32] Levels of these factors were largely determined by age in our study, which is in accordance with studies showing progressive increase in plasma coagulation factors with age.[33] Levels of factor VIII (activity and antigen) and von Willebrand factor were substantially increased in participants with both cancer and venous thrombosis but were not increased in VT-CA+ participants after adjustment for age and sex. An explanation for these findings could be that the more aggressive types of cancer and advanced stages of cancer are associated with venous thrombosis.[6,34-37] Perhaps these types and advanced stages of cancer induce higher levels of coagulation factors, which subsequently induce a higher risk of venous thrombosis than other types of cancer. Indeed, Vormittag and colleagues observed a significant difference in factor VIII levels according to tumor site,[38] which were highest in patients with tumor sites associated with a high risk of venous thrombosis. Auwerda et al. reported an association between factor VIII and von Willebrand factor levels and disease stage, with highest levels in patients with stage III disease (vs stage I or stage II disease).[30] The same can be concluded from our analysis in which we found that venous thrombosis patients who died from cancer had much higher factor VIII and von Willebrand factor levels than VT patients with cancer who survived.

Strengths of our study are that we studied coagulation factor levels (procoagulant, anticoagulant and fibrinolytic) in four groups of participants: participants without venous thrombosis and without a cancer diagnosis, a cancer-only group, a venous thrombosis-only group and a group with both venous thrombosis and cancer. Furthermore, the plasma levels of a wide range of procoagulant and anticoagulant factors that are essential to the coagulation system were measured at the same time and with the same standardized assays for each factor. In addition, the levels were adjusted for age and sex.

Some limitations of this study have to be mentioned as well. First of all, blood was sampled at least three months after inclusion in the study. For this reason some of the MEGA-study participants who died after inclusion into the study but before the moment of blood collection are missing in our analyses. Also, participants with an advanced stage of disease and who were therefore unable to visit the hospital for blood sampling or participants who were not willing to visit the hospital for other reasons are not included in our analyses. Of 3227 cases in the MEGA study eligible for blood sampling, 851 (26%) did not provide a blood sample. For the partner controls this was 32% and for the RDD controls this was 51%. For abovementioned reasons, participants with cancer included in our analyses may have been less ill than those who did not participate, which can have diluted our results. Secondly, a drawback of this study is the relatively small sample size for some groups of participants, which did not allow us to study coagulation factor levels for different types of cancer. Furthermore, we missed some clinical details on the cancers diagnosed, such as stage of cancer and information on cancer treatment. However, a recent longitudinal study from Austria (n=112) in which hemostatic factors were measured in patients with various types of cancer at multiple time points showed that several coagulant factors were increased in patients with malignancy, at diagnosis, but also during the course of antineoplastic treatment with little difference in coagulation factor concentrations before and during antineoplastic treatment.[20]

Overall, we found increased levels of procoagulant coagulation factors in individuals with venous thrombosis without cancer and even higher levels of these factors in individuals with both venous thrombosis and cancer, suggesting a generalized role of procoagulant pathways in patients with cancer. These findings were most pronounced for levels of factor VIII and von Willebrand factor. Our finding of slightly increased levels of anticoagulant proteins, free protein S and TFPI in participants with cancer and venous thrombosis is suggestive of an additional role of anticoagulant pathways in cancer. For further studies it would be useful to study coagulation factor levels in relation to cancer and venous thrombosis for different types and stages of cancer and in patients with different cancer treatments in large enough numbers and sufficient follow-up.

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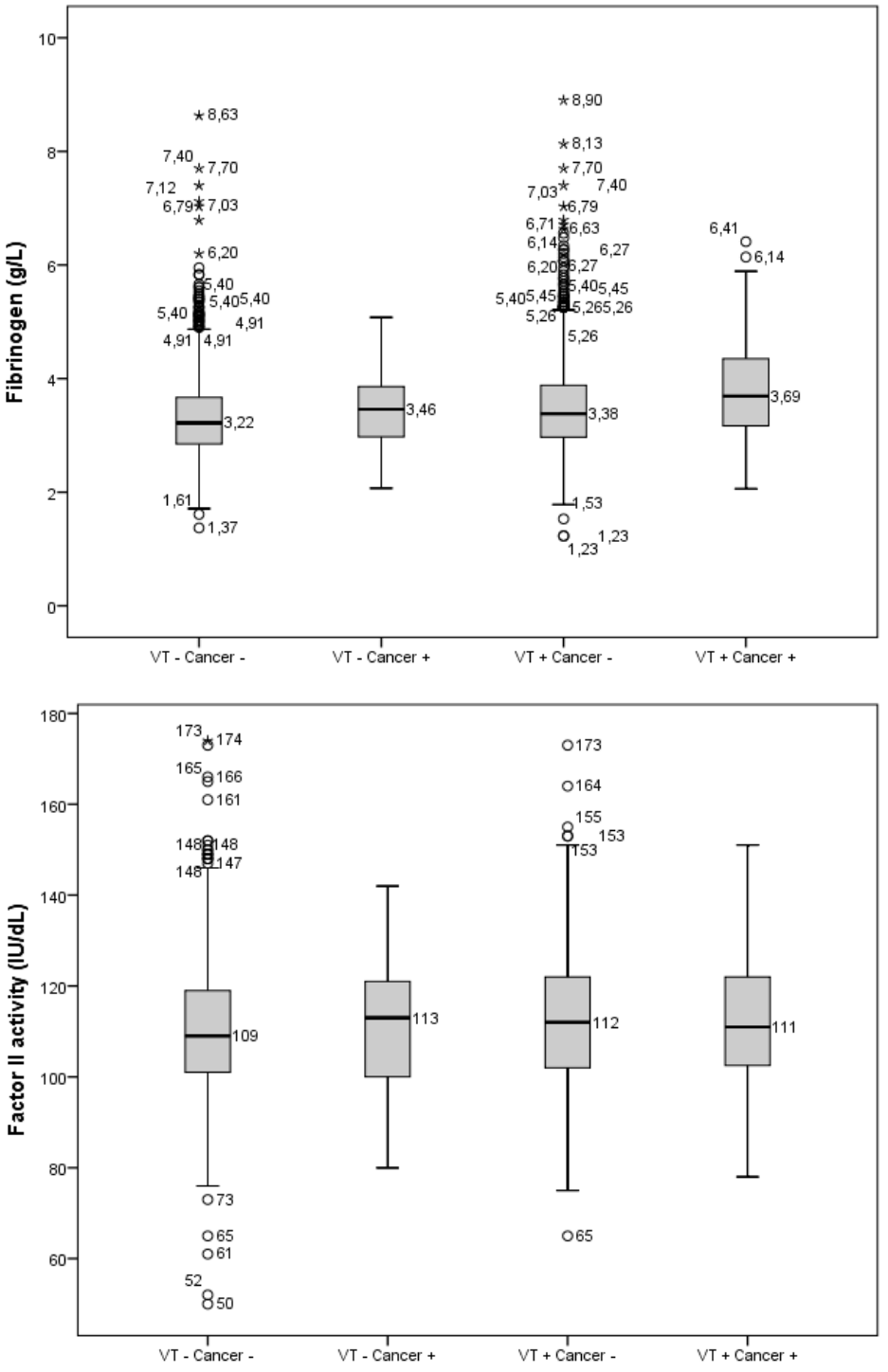
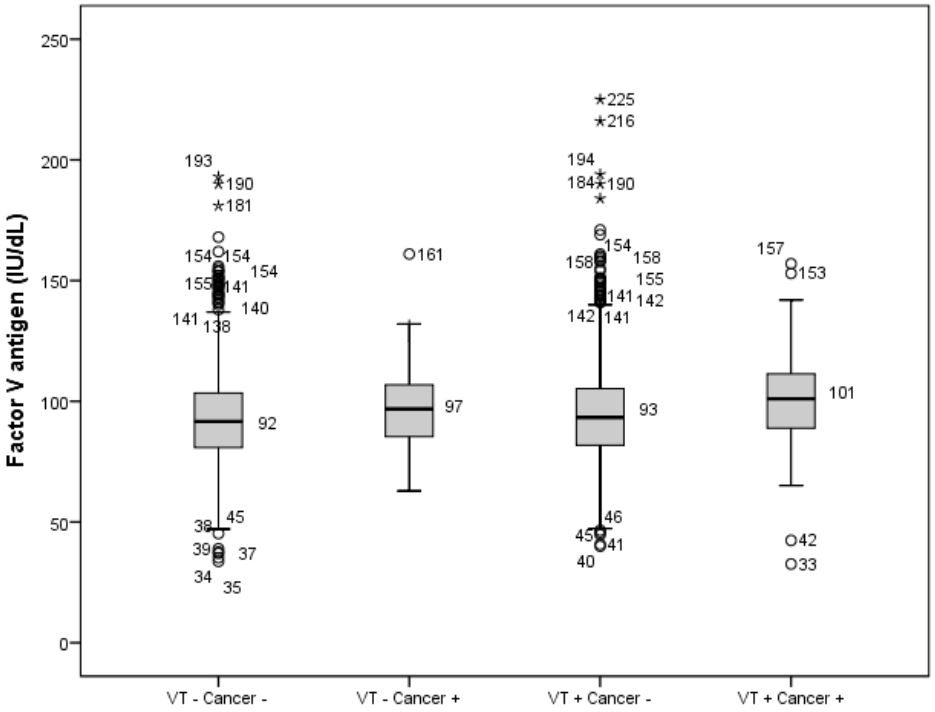
Supplemental Data

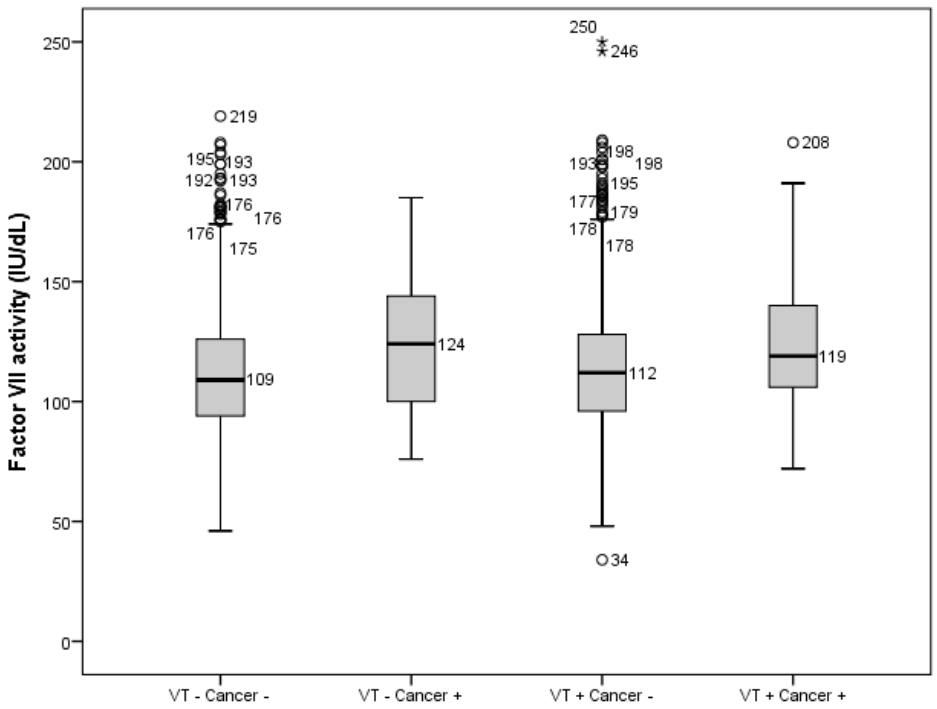
Supplemental Methods

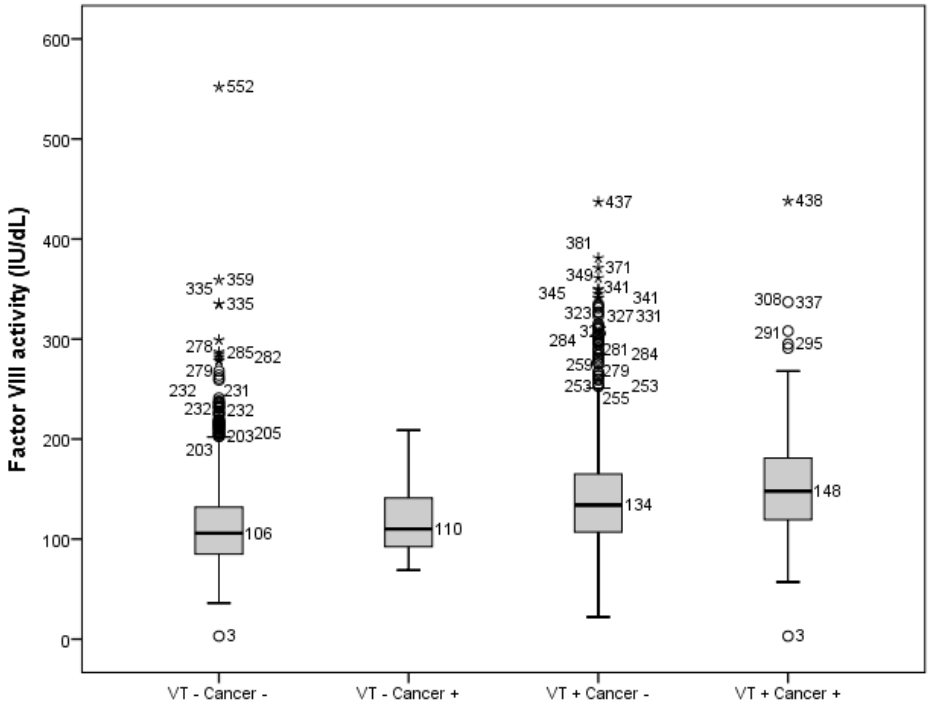
Prothrombin (factor II) activity, factor VII activity and factor VIII activity were measured with a mechanical clot detection method on a STA-R coagulation analyser following the instructions of the manufacturer (Diagnostica Stago, Asnieres, France). Levels of factor IX antigen, factor X antigen, factor VIII antigen, factor V antigen, factor XI antigen and total protein S levels were determined by enzyme-linked immunosorbent assay (ELISA). Fibrinogen activity was measured on the STA-R analyzer according to methods of Clauss. Von Willebrand factor (VWF) antigen was measured with the immunoturbidimetric method, using the STA Liatest kit (rabbit anti-hum VWF antibodies), following the instructions of the manufacturer. Measurement of antithrombin and protein C levels was performed with a chromogenic assay on the STA-R analyser. Free protein S was measured by an immune-turbidimetric method (Diagnostica Stago) accordingly to the manufacturer instructions. TFPI activity in plasma was measured by a chromogenic assay using the ACTICHROME TFPI activity assay (Sekisui Diagnostics, Stamford, Connecticut, USA) following the instructions of the manufacturer. TFPI activity was measured by inhibition of cleavage of a chromogenic substrate (spectrozyme Xa, Sekisui diagnostics) by factor Xa, after initiation of coagulation with an excess of factor X and Tissue Factor-Factor VIIa complex. D-dimer was assayed using the D-dimer HemosIL assay (Instrumentation Laboratory). The HemosIL D-Dimer HS is an automated latex enhanced immunoassay performed on the ACL TOP 700CTS (Instrumentation Laboratory, Warrington, UK).

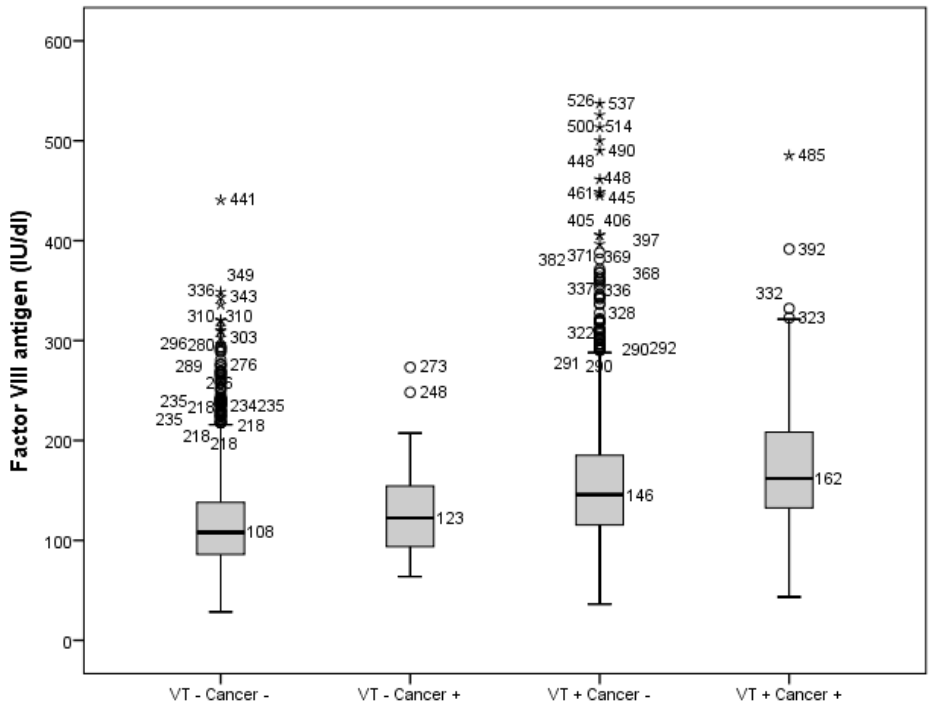
Supplementary Figure 1. Boxplots of factor levels for groups of participants.

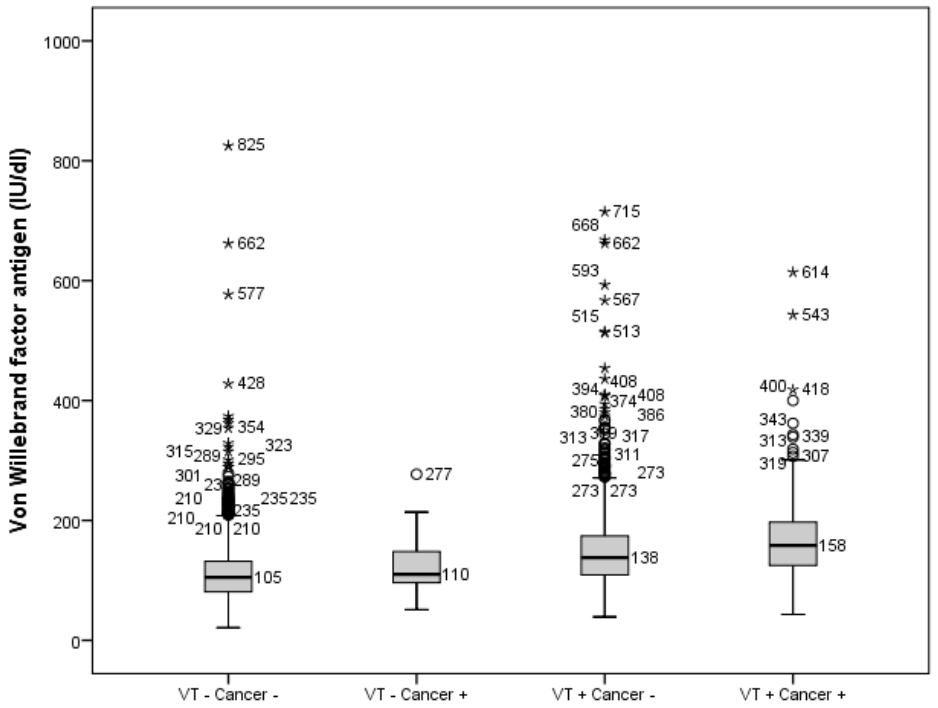


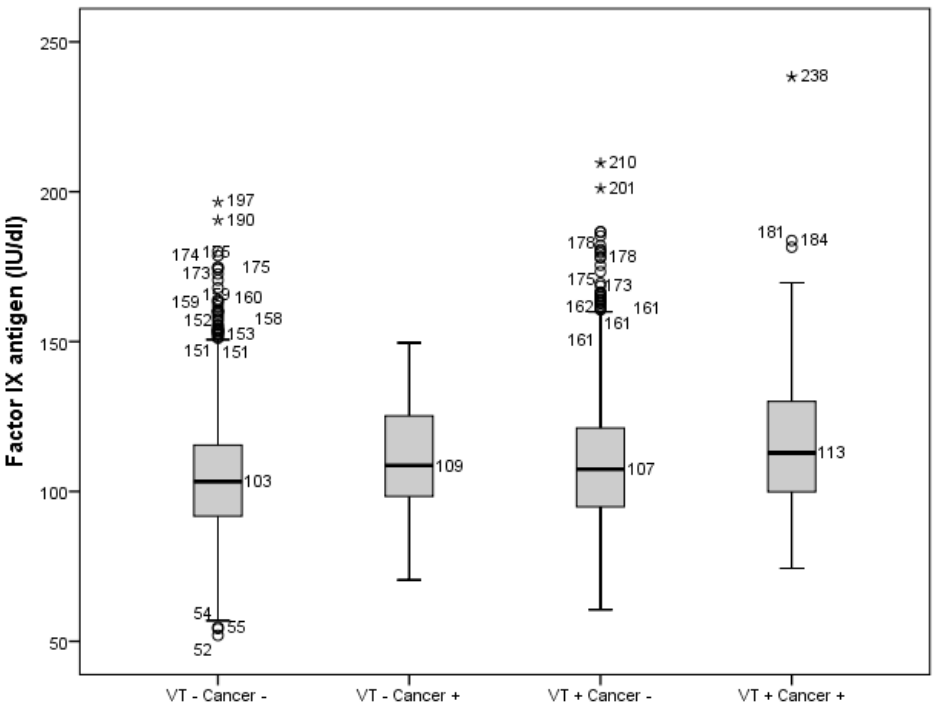

Factor V antigen (IU/dL)
250
200
150
100
50
0
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +

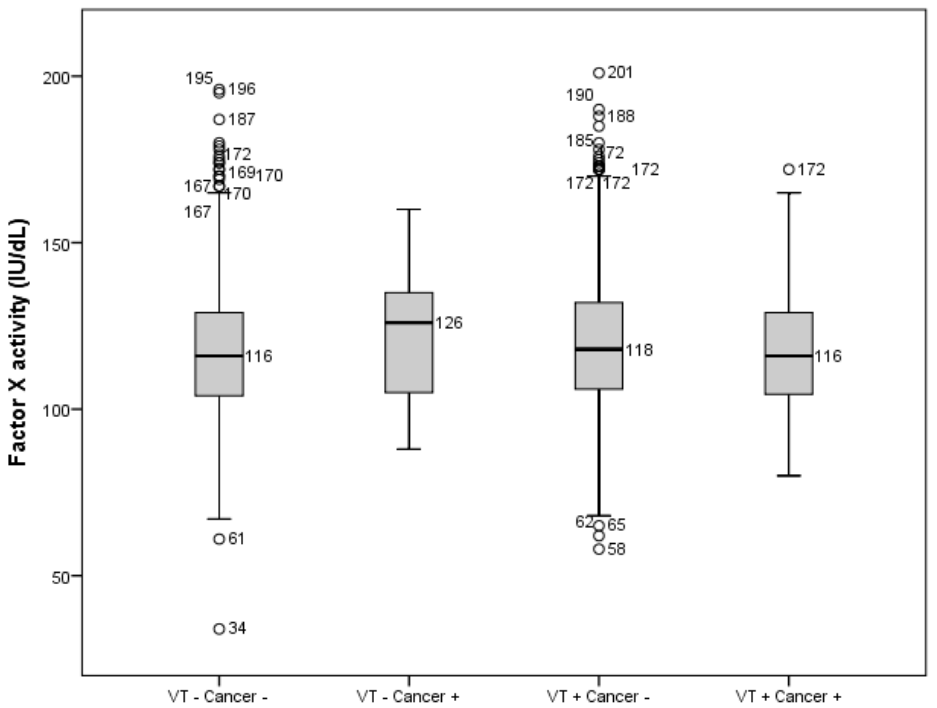

Factor VII activity (IU/dL)
250
200
150
100
50
0
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +

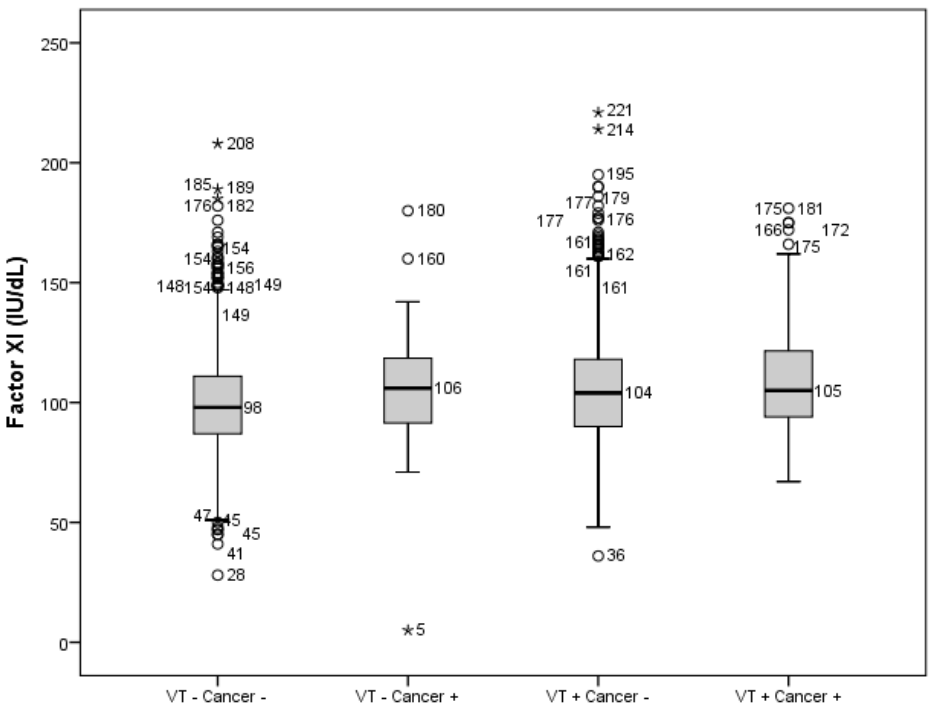

Factor VIII activity (IU/dL)
600
500
400
300
200
100
0
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +

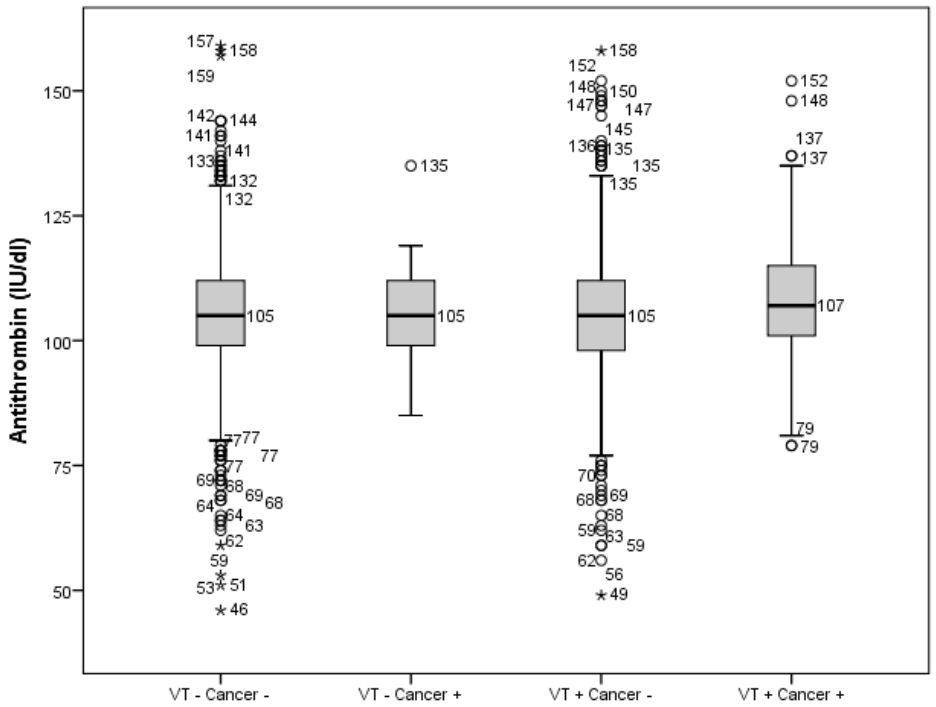

Factor VIII antigen (IU/dl)
600
500
400
300
200
100
0
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +

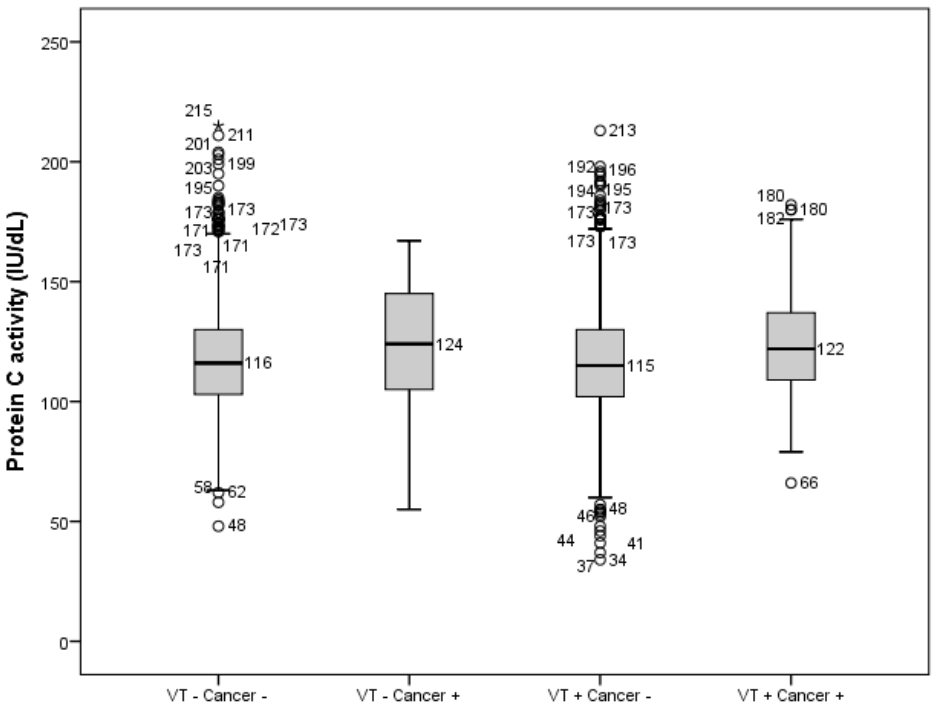

Von Willebrand factor antigen (IU/dl)
1000
800
600
400
200
0
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +
105
110
138
158

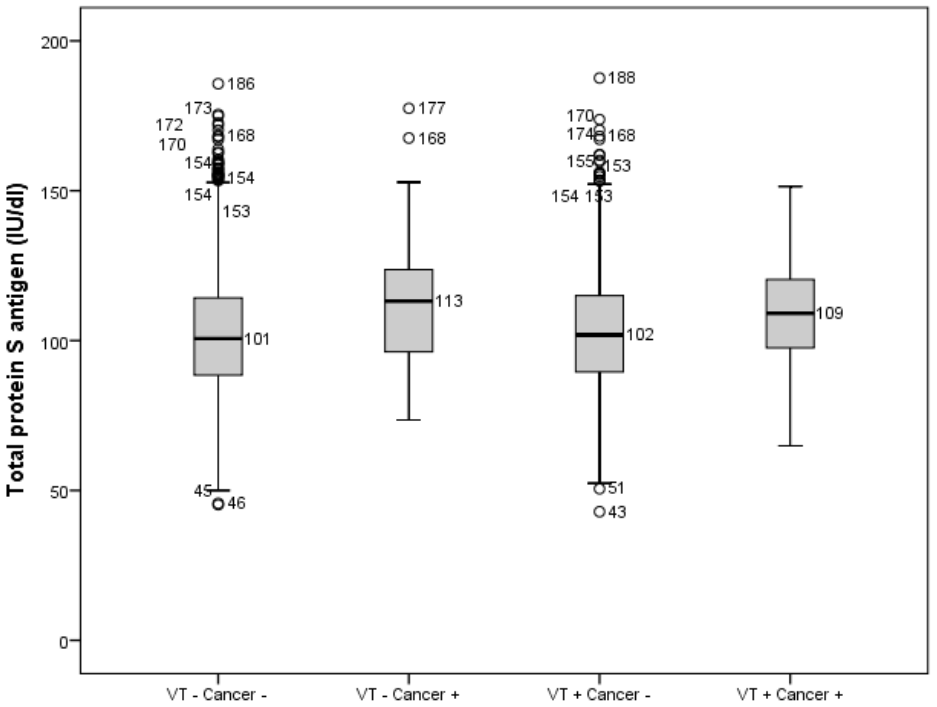

Factor IX antigen (IU/dl)
250
200
150
100
50
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +
103
109
107
113

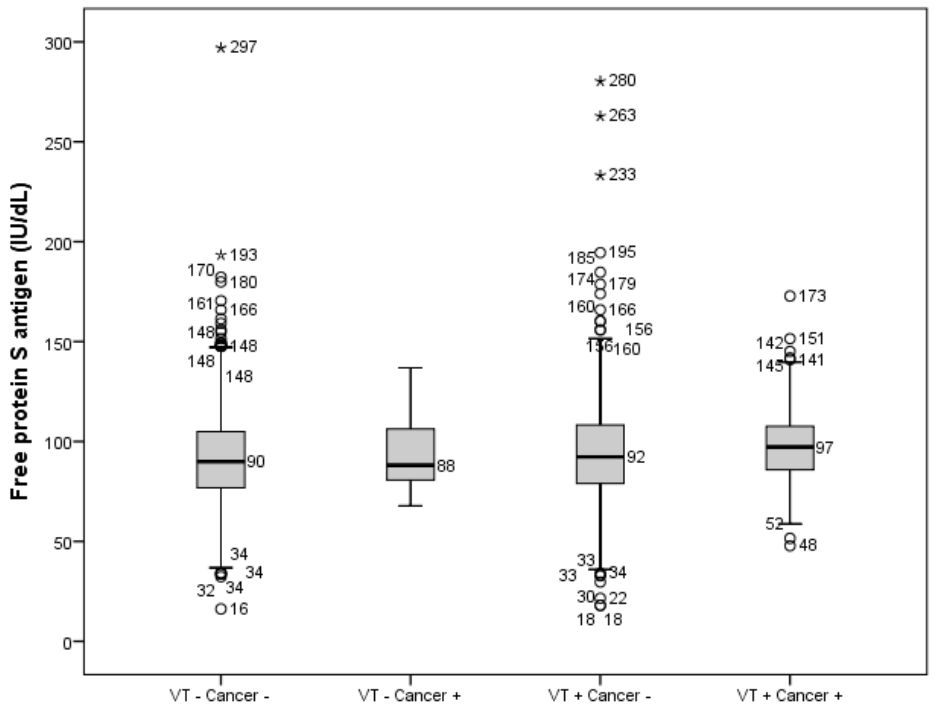

Factor X activity (IU/dL)
200
150
100
50
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +
116
126
118
116

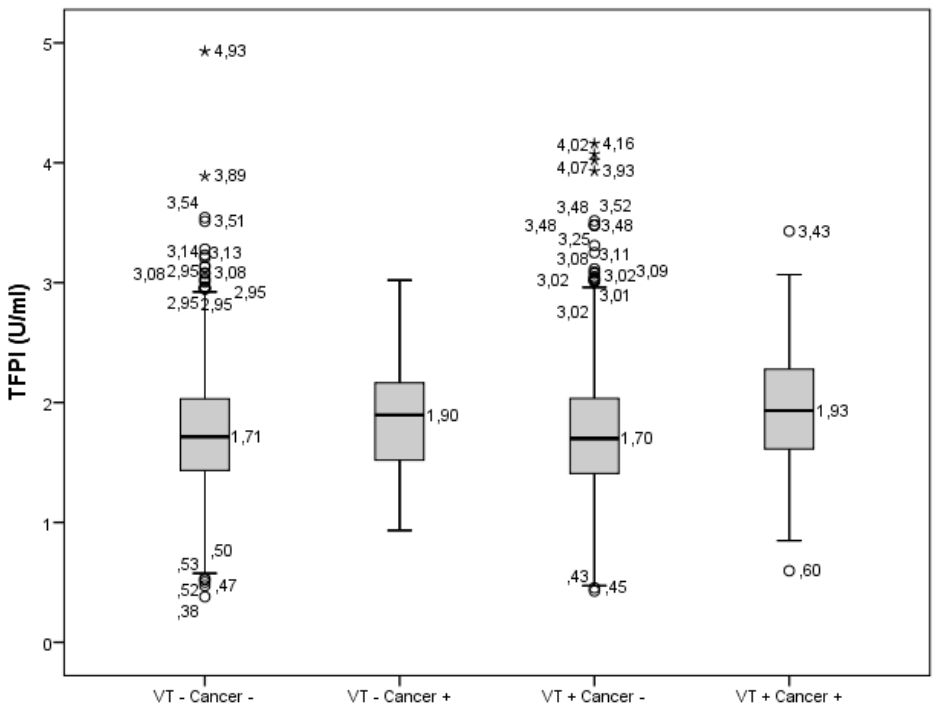

Factor XI (IU/dL)
250
200
150
100
50
0
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +
98
106
104
105

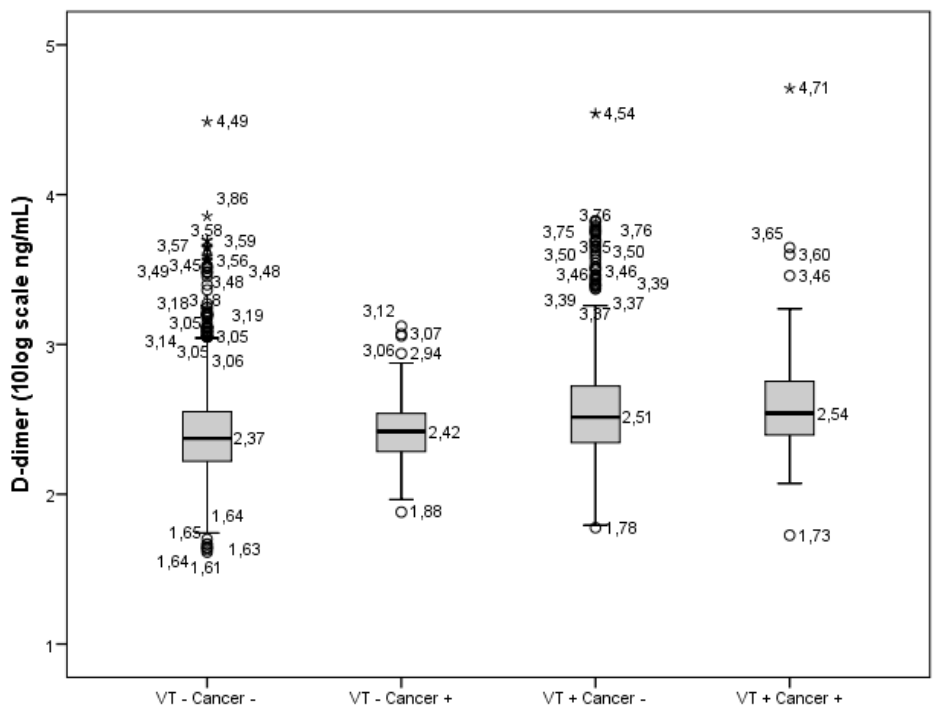

Antithrombin (IU/dl)
150
125
100
75
50
105
105
105
107
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +


Protein C activity (IU/dL)
250
200
150
100
50
0
116
124
115
122
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +


Total protein S antigen (IU/dl)
200
150
100
50
0
101
113
102
109
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +


Free protein S antigen (IU/dL)
300
250
200
150
100
50
0
90
88
92
97
VT - Cancer -
VT - Cancer +
VT + Cancer -
VT + Cancer +





Chapter 6

Predictive value of factor VIII levels for recurrent venous thrombosis: Results from the MEGA follow-up study

Jasmijn F. Timp, Willem M. Lijfering, Linda E. Flinterman, Astrid van Hylckama Vlieg, Saskia le Cessie, Frits R. Rosendaal, Suzanne C. Cannegieter

J Thromb Haemost 2015; 13(10):1823-1832

Abstract

Background
Prediction of recurrent venous thrombosis remains a challenge in the clinic.

Objective
To investigate the predictive value of coagulation factor VIII (FVIII) levels for recurrent venous thrombosis.

Patients/methods
Patients, aged 18-70 years with a first venous thrombosis were followed from discontinuation of anticoagulant treatment (1999-2010 MEGA follow-up study). Levels of FVIII activity, FVIII antigen and von Willebrand factor antigen (VWF) were measured at least 3 months after cessation of anticoagulant treatment.

Results
Out of 2242 patients followed for a median of 6.9 years, 343 developed recurrent thrombosis (incidence rate 2.7/100 patient-years (95%CI, 2.5-3.1)). Recurrence rates steadily increased with higher FVIII activity levels from 1.4 (95%CI, 1.0-1.9), 2.3 (95%CI, 1.8-2.9), 3.0 (95%CI, 2.4-3.7), 3.2 (95%CI, 2.5-4.1), 3.9 (95%CI, 2.8-5.3), to 5.1 (95%CI, 3.8-6.8) per 100 patient-years, for levels ranging from <100 IU/dL to >200 IU/dL. Patients in the highest category of FVIII (>200 IU/dL) had a three-fold higher recurrence rate than patients in the lowest category (≤100 IU/dL) (HR 3.4 (95%CI, 2.2-5.3)). Results were similar for FVIII antigen and VWF levels, in several sensitivity analyses and FVIII predicted recurrence rates over a long time period. Within subgroups of patients currently assumed to have low recurrence risks, a high level of FVIII was still predictive for recurrences. Adding FVIII to an existing prediction model (DASH-score) improved its predictive value, and after replacing D-dimer by FVIII, the model performed equally well if not better.

Conclusions
FVIII predicted recurrence in a dose-response fashion, overall and in several subgroups, and is a strong candidate component of recurrence prediction tools.

Introduction

The annual incidence of venous thrombosis (deep vein thrombosis (DVT) and pulmonary embolism (PE)) is 1-2 per 1000 inhabitants.[1] The five-year cumulative incidence of recurrent venous thrombosis, a condition with considerable comorbidity and mortality, is 12-25%.[2-4] Secondary prevention of recurrent venous thrombosis by prolonged anticoagulant treatment should, considering the risk of major haemorrhage (1-2% per year) [5,6], be targeted at high risk patients.[7] According to the latest ACCP guidelines, primary factors determining the risk of recurrence are the presence of a transient provoking risk factor (associated with a decreased incidence of recurrence) and the presence of active cancer at the time of the first event (associated with an increased incidence of recurrence).[7] Yet, only 50% of patients can be classified in either of these two categories. For the other 50% of patients prediction of recurrent venous thrombosis remains a challenge in the clinic and knowledge of good predictors is crucial.[8]

Factor VIII (FVIII) levels have been consistently reported as a potent predictor for first venous thrombosis.[9] These levels are fairly constant over time[10,11], and since FVIII levels are not affected by anticoagulant use, it is an interesting candidate for a prediction tool for recurrence. However, none of the existing guidelines or prediction models for recurrent thrombosis include levels of FVIII.[12-14] This is probably because research on this topic is scarce and results are not as consistent as in studies on first events.[10,15,16]

We aimed to investigate the predictive value of FVIII levels on recurrent venous thrombosis, both on its own as in combination with other variables in a prognostic model, in a large group of patients (n=2242) with a first venous thrombotic event. Since the aim of this study is to investigate the potential of FVIII as a predictor of recurrent venous thrombosis, and not to study the underlying mechanism, we will not take into account potential confounding factors.

Methods

MEGA follow-up study

Between March 1999 and August 2004, 4956 patients aged 18-70 with an objectively diagnosed first DVT of the leg or PE were included in a population-based case-control study (MEGA study). All patients filled in an extensive questionnaire on putative risk factors for venous thrombosis. Details of the MEGA study have been described previously.[17] Of the MEGA case-control study, only the cases were further followed for recurrence (MEGA follow-up study). For this, 225 of the 4956 patients did not consent, leaving 4731 patients (Supporting Fig1). Approximately three months after discontinuation of oral anticoagulant therapy, patients were invited for collection of a blood sample, unless they were still on anticoagulant therapy one year after their event, in which case blood was drawn during anticoagulant therapy.[18] Blood sampling was requested until June 2002, which included 3122 patients. 734 of these 3122 patients did not provide a blood sample, because of unwillingness or death, leaving 2388 patients for analyses on laboratory measurements.

Between 2007 and 2009 the vital status of all patients was acquired from the central Dutch population register.[19] For the patients who died, a cause of death (ICD-10-CM) was obtained from the national register of death certificates at the Central Bureau of Statistics. Questionnaires concerning recurrent venous thrombosis were sent by mail to all survivors and consenting individuals between June 2008 and July 2009, and supplemented by telephone interviews. Additional information was acquired from the regional anticoagulation clinics and from hospitals. Deaths due to recurrent venous thrombosis were counted as fatal recurrent events. Based on hospital discharge letters, the information from the anticoagulation clinic, questionnaires filled in by the patients and causes of death, possible recurrences were classified into certain and uncertain recurrences, following a decision rule (Supporting Methods).

This study was approved by the Medical Ethics Committee of the Leiden University Medical Center, and all participants gave written informed consent.

Current analyses

For the current analyses, follow-up was started at the moment of discontinuation of anticoagulant treatment because clinically it is more relevant to know recurrence rates and risks after discontinuation of treatment. Furthermore, because levels of FVIII were measured some time after the thrombotic event and some patients did not survive until blood collection, immortal time bias might play a role when follow-up is started before.[20] For these analyses 117 patients were excluded because their follow-up ended before discontinuation of anticoagulant treatment. An additional 29 patients were excluded because FVIII levels could not be accurately measured, leaving 2242 patients. MEGA follow-up patients included or excluded from the current analyses did not differ substantially on clinical characteristics, except for the proportion of patients with malignancy (Supporting Table1).

Laboratory measurements

Blood samples were drawn into vacuum tubes containing 0.1-volume 0.106- mol/L trisodium citrate and centrifuged for 10 minutes at 4ºC, after which plasma was aliquoted, frozen and stored at -80 ºC. FVIII activity (FVIII:C), FVIII antigen (FVIII:Ag) and von Willebrand factor antigen (VWF:Ag) levels were determined at a later point in time in series with the same method. The levels of these three measures are strongly correlated, since FVIII is stabilized when bound to VWF.[21] FVIII:C was measured with a mechanical clot detection method on a STA-R analyzer (Diagnostica Stago, Asnieres, France). FVIII:Ag was determined by enzyme-linked immunosorbent assay (ELISA). VWF:Ag was measured with a immunoturbidimetric method, using the STA Liatest kit (rabbit anti-human VWF antibodies). In this study, we focused on FVIII activity levels as this is the fastest test and the most commonly used in the clinic. 197 patients who were still on anticoagulant treatment at the time of blood collection were included for analyses, since FVIII is not vitamin K dependent and its levels are not affected by treatment with vitamin-K-antagonists.

ABO Blood group was determined by polymerase chain reactions using the TaqMan assay.[17] D-dimer was assayed using the D-dimer HemosIL assay (Instrumentation Laboratory). The HemosIL D-Dimer HS is an automated latex enhanced immunoassay for the quantitative determination of D-dimer performed on the ACL TOP 700 (Instrumentation Laboratory, Warrington, UK).

Statistical analysis

Duration of follow-up was counted from date of discontinuation of anticoagulant treatment to end of follow-up, which was defined as the date of a recurrence or, in its absence, the date of returning the follow-up questionnaire. The last questionnaire was returned on April 8, 2010. If patients did not complete the questionnaire, they were censored at the last date we knew them to be recurrence free (date of death (n=26), date of emigration (n=1), date last seen by the anticoagulation clinic or for research purposes (n=285)). Here we limit the analyses to certain recurrent events (n=343), and censored patients with uncertain recurrent events (n=84) at that time.

We used fixed cut-off levels of FVIII activity (100 IU/dL, 125 IU/dL, 150 IU/dL, 175 IU/dL and 200 IU/dL). Incidence rates of recurrent venous thrombosis were estimated as number of events over the accumulated follow-up time. Cox-proportional hazards models were used to evaluate rates between groups. Hazard ratios for recurrence were estimated for increasing levels of FVIII, using levels below 100 IU/dL as reference. The cumulative incidences of recurrence were estimated, treating death as competing risk using Stata's user-contributed *stcompet* suite. Survival curves were constructed to visualize the cumulative incidence of recurrent events over the years. By means of adding an interaction term between log(time) as a time-dependent variable and categories of FVIII in a Cox model the assumption of proportional hazards over time was tested.

To quantify potential misclassification of outcomes, several sensitivity analyses were performed (see Supporting Methods).

We stratified patients by those with first provoked and first unprovoked events, by sex (as unprovoked events and male sex are associated with higher recurrence risks)[22,23] and by blood group O and non-O (since VWF and FVIII levels are strongly determined by ABO blood group).[24,25] Stratified analyses with two age categories (cut-off 45 years) and two BMI categories (cut-off BMI 25 kg/m^2) were additionally performed. For a definition of both provoked and unprovoked venous thrombotic events see the Supporting Methods.

In a separate analysis we assessed both the predictive value of FVIII antigen levels and of VWF antigen levels on incidence of recurrent venous thrombosis.

Lastly, we studied how FVIII:C levels perform in an existing prediction model for recurrent venous thrombosis, i.e. the DASH-score.[14] For this, we first validated the DASH-score in our data. The DASH-score identifies patients with unprovoked first thrombosis at high or low recurrence risk by using the following risk variables: 1) abnormal D-dimer (cut-off 500 ng/mL; 2 points); 2) age (cut-off≤50 years at time of first event; 1 point); 3) sex (1 point for male); 4) hormone use at first event (-2 points if yes). We restricted our patient group according to the criteria that were used for derivation of the DASH-score, i.e. patients in whom the first event occurred in the absence of surgery, trauma, cancer, immobility or pregnancy/puerperium (n=1271; 57%) and in whom all items of the DASH-score were available. Furthermore, we excluded patients still taking anticoagulant therapy at time of blood sampling, finally leaving 1082 patients. Follow-up was started after discontinuation of anticoagulant treatment, similar as for the DASH-score. Next, we assessed the model accuracy for various versions of the model by means of the c-statistic.[27] This included models adding FVIII:C levels to the DASH-model and replacing D-Dimer by FVIII levels ('FASH'-model). We performed similar analyses for levels of VWF. Statistical analyses were performed using SPSS, version 20.0 (IBM Corp., Armonk, NY) and Stata, version 12 (Stata Corp., College Station, Texas).

Results

Clinical characteristics

2242 patients with a first episode of venous thrombosis were followed for recurrent events for a median of 6.9 years (interquartile range 2.7-8.0 years, total follow-up 12,499 years). Mean age at enrollment was 48 years and 1010 (45%) patients were men. Of the first events, 1578 (72%) were provoked, with trauma, surgery or immobilization accounting for most of these events (n=895; 57%) (Table 1). Most (n=1322; 59%) first events were DVTs. Median time between first event and blood collection was 10.0 months (interquartile range, 8.3-12.2 months).

Incidence of recurrent venous thrombosis related to levels of FVIII activity

343 patients developed recurrent thrombosis during follow-up, for an overall incidence rate of 2.7/100 person-years (95%CI, 2.5-3.1). Of these events 54 occurred before blood collection. Median FVIII activity level for all participants was 134 IU/dL (interquartile range 107-165 IU/dL). When FVIII was included as a continuous variable in the Cox model, the hazard ratio of recurrent venous thrombosis was 1.06 (95%CI, 1.04-1.08)

Table 1. Clinical characteristics

General characteristics	**MEGA follow-up cohort**		**Included for analyses**	
Total	4731	(100)	2242	100
Men	2164	(46)	1010	(45)
Age at enrollment, y	48	(18-70)	48	(18-70)
Classical venous thrombosis risk factors				
Provoked by*	3301	(72)	1578	(72)
Malignancy	426	(13)	127	(8)
Trauma/surgery/immobilization	1902	(58)	895	(57)
Plaster cast	219	(7)	109	(7)
Estrogen use (women)	1350	(41)	709	(45)
Pregnancy/puerperium (women)	173	(5)	92	(6)
Travel >4 hrs	717	(22)	374	(24)
Unprovoked	1299	(28)	621	(28)
Prothrombotic factor				
Blood group non-O	2913	(71)	1591	(71)
Type of index event				
Deep vein thrombosis only	2747	(58)	1322	(59)
Pulmonary embolism only	1549	(33)	702	(31)
Pulmonary embolism + deep vein thrombosis	435	(9)	218	(10)

Continuous variables denoted as mean (range), categorical variables as number (%).
Some data were missing for some variables.
* As concomitance of provoked risk factors occurred frequently, patients could be counted twice or more.

Table 2. Incidence rates (a) and cumulative incidences (b) of recurrent venous thrombosis for strata of factor VIII

2a. Incidence rates of recurrent venous thrombosis

Range	N	Observation years (n)	Recurrent Events	Incidence rate, per 100 py (95%CI)	Hazard ratio (95%CI)
Main analysis*					
FVIII:C (≤ 100 IU/dL)	438	2686	38	1.4 (1.0-1.9)	1 (reference)
FVIII:C (101-125 IU/dL)	516	3005	68	2.3 (1.8-2.9)	1.6 (1.1-2.4)
FVIII:C (126-150 IU/dL)	493	2775	83	3.0 (2.4-3.7)	2.1 (1.4-3.1)
FVIII:C (151-175 IU/dL)	382	2088	67	3.2 (2.5-4.1)	2.2 (1.5-3.3)
FVIII:C (176-200 IU/dL)	205	1028	40	3.9 (2.8-5.3)	2.7 (1.7-4.1)
FVIII:C (>200 IU/dL)	208	917	47	5.1 (3.8-6.8)	3.4 (2.2-5.3)
First DVT (n=1322)					
FVIII:C (≤ 100 IU/dL)	243	1522	22	1.4 (0.9-2.2)	1 (reference)
FVIII:C (101-125 IU/dL)	301	1744	44	2.5 (1.8-3.4)	1.7 (1.0-2.9)
FVIII:C (126-150 IU/dL)	303	1750	56	3.2 (2.4-4.2)	2.2 (1.3-3.6)
FVIII:C (151-175 IU/dL)	233	1312	42	3.2 (2.3-4.3)	2.2 (1.3-3.6)
FVIII:C (176-200 IU/dL)	117	622	20	3.2 (2.0-5.0)	2.2 (1.2-4.0)
FVIII:C (>200 IU/dL)	125	577	28	4.9 (3.2-7.0)	3.2 (1.8-5.5)
First PE (with or without DVT) (n=920)					
FVIII:C (≤ 100 IU/dL)	195	1164	16	1.4 (0.8-2.2)	1 (reference)
FVIII:C (101-125 IU/dL)	215	1260	24	1.9 (1.2-2.8)	1.4 (0.7-2.6)
FVIII:C (126-150 IU/dL)	190	1025	27	2.6 (1.7-3.8)	1.9 (1.0-3.5)
FVIII:C (151-175 IU/dL)	149	776	25	3.2 (2.1-4.8)	2.3 (1.2-4.3)
FVIII:C (176-200 IU/dL)	88	406	20	4.9 (3.0-7.6)	3.5 (1.8-6.7)
FVIII:C (>200 IU/dL)	83	340	19	5.6 (3.4-8.7)	3.9 (2.0-7.5)

CI denotes confidence interval; py, person-years; FVIII:C, factor VIII activity level
* Sensitivity analyses presented in the supplementary material (Supporting Table 2)

2b. Cumulative incidences of recurrent venous thrombosis

	Unprovoked first event (n=659)		**Provoked first event (n=1652)**	
Factor VIII category	*1-year (95%CI)*	*5-years (95%CI)*	*1-year (95%CI)*	*5-years (95%CI)*
FVIII:C (≤100 IU/dL)	4.9 (0.7-9.1)	16.3 (8.7-23.9)	2.0 (0.4-3.6)	5.9 (3.2-8.7)
FVIII:C (101-125 IU/dL)	5.8 (1.6-10.1)	17.7 (10.6-24.7)	4.2 (2.1-6.3)	8.8 (5.8-11.9)
FVIII:C (126-150 IU/dL)	9.2 (4.0-14.3)	21.9 (14.2-29.5)	3.6 (1.6-5.6)	11.1 (7.6-14.6)
FVIII:C (151-175 IU/dL)	8.2 (3.3-13.1)	20.9 (13.4-28.3)	6.1 (3.0-9.2)	13.4 (8.8-17.9)
FVIII:C (176-200 IU/dL)	15.0 (5.9-24.1)	32.5 (20.0-45.0)	3.1 (0.1-6.1)	12.6 (6.4-18.8)
FVIII:C (>200 IU/dL)	9.5 (2.2-16.8)	30.8 (18.9-42.7)	4.4 (0.6-8.3)	18.9 (11.1-26.7)
Total	8.2 (6.0-10.4)	21.9 (18.4-25.3)	3.8 (2.8-4.8)	10.5 (8.9-12.1)
	DVT (n=1322)		**PE (with or without DVT) (n=920)**	
Factor VIII category	*1-year (95%CI)*	*5-years (95%CI)*	*1-year (95%CI)*	*5-years (95%CI)*
FVIII:C (≤100 IU/dL)	3.9 (1.9-6.0)	8.6 (5.4-12.8)	1.1 (0.2-3.6)	7.8 (4.4-12.5)
FVIII:C (101-125 IU/dL)	3.9 (2.1-6.6)	11.0 (7.6-15.2)	5.5 (2.9-9.2)	10.9 (7.0-15.7)
FVIII:C (126-150 IU/dL)	6.3 (3.9-9.5)	15.6 (11.6-20.2)	2.9 (1.1-6.2)	10.7 (6.5-16.2)
FVIII:C (151-175 IU/dL)	6.2 (3.6-9.9)	14.7 (10.3-19.9)	7.4 (3.8-12.6)	17.3 (11.3-24.3)
FVIII:C (176-200 IU/dL)	5.3 (2.2-10.5)	18.6 (11.7-26.7)	8.9 (4.0-16.5)	19.9 (11.5-30.1)
FVIII:C (>200 IU/dL)	6.5 (2.9-12.2)	24.6 (16.5-33.6)	5.4 (1.7-12.2)	20.3 (11.4-31.0)
Total	5.2 (6.5-4.1)	14.1 (26.2-12.2)	4.6 (3.3-6.2)	12.8 (10.5-15.2)

CI denotes confidence interval; py, person-years; FVIII:C, factor VIII activity level

for each increase of 10 IU/dL. Incidence rates of recurrence clearly increased with categories of FVIII activity levels, from 1.4 (95%CI, 1.0-1.9) per 100 patient-years for levels below 100 IU/dL to 5.1 (95%CI, 3.8-6.8) above 200 IU/dL (Table 2a). Corresponding hazard ratios are additionally presented in Table 2a. The p-value for the interaction term between log(time) and categories of FVIII was non-significant (p=0.62), which means that hazards were found to be proportional over time.

All sensitivity analyses (Supporting Table2), in which we, amongst others excluded patients who donated blood during their anticoagulant treatment period, showed similar results, i.e., a graded increase in the incidence of recurrence for higher levels of FVIII activity. Results were similar for patients with a first DVT and patients with a first PE.

The cumulative incidence of recurrent venous thrombosis in patients with FVIII levels below 100 IU/dL was 5% after 2 years, 7% after 4 years and 10% after 8 years of follow-up (Fig1). For patients with FVIII levels above 200 IU/dL, these risks were 13%, 21%, and 31%, respectively. Cumulative incidences were higher for unprovoked than for provoked first events, and invariably peaked for high FVIII levels (Table 2b). Five-year cumulative incidences of recurrence for patients with a first unprovoked event were 16.3% (95%CI, 8.7-23.9) and 30.8% (95%CI,18.9-42.7) for levels ≤100 IU/dL and for levels >200 IU/dL, respectively. For patients with a provoked first event these cumulative incidences were 5.9% (95%CI, 3.2-8.7) and 18.9% (95%CI, 11.1-26.7), respectively.

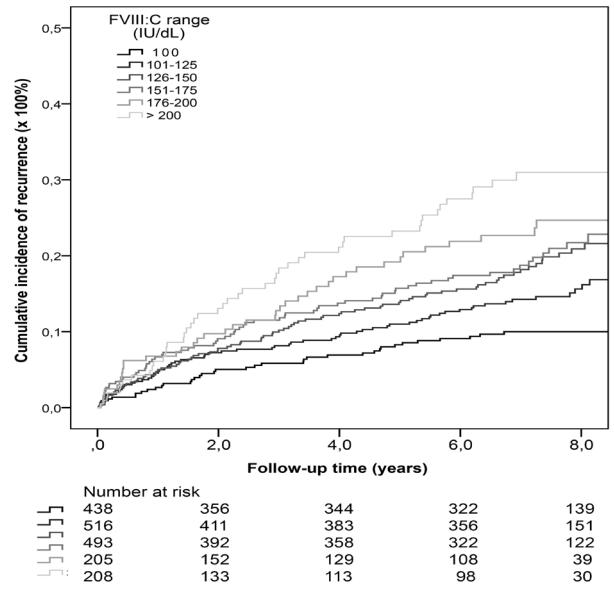


Figure 1. Survival curves of recurrent venous thrombosis for strata of factor VIII levels. FVIII:C denotes factor VIII activity.

Incidence of recurrent venous thrombosis related to FVIII antigen and VWF antigen levels

Median FVIII antigen level was 146 IU/dL (interquartile range 115-185 IU/dL) and median VWF antigen was 138 IU/dL (interquartile range 109-174 IU/dL). The recurrence rate was 1.1 per 100 person-years (95%CI, 0.7-1.6) for patients with factor VIII antigen levels ≤100 IU/dL, while it was 5.3 per 100 person-years (95%CI, 4.1-6.9) for patients with levels >225. For VWF these rates were 1.3 per 100 person-years (95%CI, 0.9-1.8) and 5.6 per 100 person-years (95%CI, 4.1-7.5), respectively. Incidence rates of recurrence increased gradually for increasing levels of FVIII antigen and VWF antigen, in a similar fashion as the FVIII activity levels (Table 3).

Table 3. Incidence rates of recurrent venous thrombosis for strata of factor VIII antigen and von Willebrand factor

Range	N	Observation years	Recurrent events	Incidence rate, per 100 py (95% CI)	Hazard ratio (95% CI)
FVIIIag (≤ 100 IU/dL)	347	2111	23	1.1 (0.7-1.6)	1 (reference)
FVIIIag (101-125 IU/dL)	383	2283	47	2.1 (1.5-2.7)	1.9 (1.1-3.1)
FVIIIag (126-150 IU/dL)	465	2673	64	2.4 (1.8-3.1)	2.2 (1.4-3.5)
FVIIIag (151-175 IU/dL)	366	1967	68	3.5 (2.7-4.4)	3.1 (1.9-5.0)
FVIIIag (176-200 IU/dL)	253	1422	47	3.3 (2.4-4.4)	3.0 (1.8-4.9)
FVIIIag (201-225 IU/dL)	182	943	36	3.8 (2.7-5.3)	3.4 (2.0-5.8)
FVIIIag (>225 IU/dL)	244	1087	58	5.3 (4.1-6.9)	4.6 (2.9-7.5)
VWFag (≤ 100 IU/dL)	424	2598	33	1.3 (0.9-1.8)	1 (reference)
VWFag (101-125 IU/dL)	446	2520	67	2.7 (2.1-3.4)	2.1 (1.4-3.1)
VWFag (126-150 IU/dL)	505	2957	62	2.4 (1.8-3.1)	1.6 (1.1-2.5)
VWFag (151-175 IU/dL)	320	1757	63	3.6 (2.8-4.6)	2.8 (1.8-4.2)
VWFag (176-200 IU/dL)	252	1320	47	3.6 (2.6-4.7)	2.7 (1.8-4.3)
VWFag (201-225 IU/dL)	109	563	27	4.8 (3.2-7.0)	3.7 (2.2-6.1)
VWFag (>225 IU/dL)	186	784	44	5.6 (4.1-7.5)	4.1 (2.6-6.5)

CI denotes confidence interval; py, person-years; FVIIIag, factor VIII antigen level; VWFAg, von Willebrand factor antigen

Subgroup analyses

We stratified patients into those with first provoked or unprovoked first events, by sex, by age and by BMI (Table 4). Mean FVIII levels were slightly higher in unprovoked than provoked thrombosis, and older and overweight patients (Table 4). Rates of recurrence were highest in men and after an unprovoked first event.[2-4,26] Within all subgroups there was a dose-dependent increase in recurrence incidence for increasing levels of FVIII. Excluding patients with a malignancy did not materially affect the results. Blood group non-O was associated with an increase in risk of recurrence of 30% (HR 1.3 (95%CI, 1.0-1.7) compared with blood group O. Both for blood group O and non-O a graded increase in recurrence risk was observed for increasing FVIII activity levels (Table 5).

Table 4. Incidence rates of recurrent venous thrombosis for strata of factor VIII, subgroup analyses

Range	Incidence rate, per 100 py (95%CI)	Hazard ratio (95%CI)	Incidence rate, per 100 py (95%CI)	Hazard ratio (95%CI)	Incidence rate, per 100 py (95%CI)	Hazard ratio (95%CI)
	Men (n=1010) Mean FVIII:C 141 (95%CI, 138-144)		*Women (n=1232)* Mean FVIII:C 139 (95%CI, 136-141)		*Unprovoked* (n=621)* Mean FVIII:C 146 (95%CI, 142-150)	
FVIII:C (≤ 100 IU/dL)	2.2 (1.4-3.2)	1 (reference)	0.9 (0.5-1.5)	1 (reference)	2.5 (1.4-4.2)	1 (reference)
FVIII:C (101-125 IU/dL)	3.1 (2.2-4.3)	1.4 (0.9-2.4)	1.6 (1.1-2.3)	1.8 (1.0-3.5)	3.3 (2.1-4.9)	1.3 (0.7-2.5)
FVIII:C (126-150 IU/dL)	4.8 (3.6-6.3)	2.1 (1.3-3.5)	1.9 (1.3-2.6)	2.1 (1.1-4.0)	4.9 (3.4-6.9)	1.9 (1.0-3.5)
FVIII:C (151-175 IU/dL)	4.8 (3.5-6.3)	2.1 (1.3-3.5)	1.7 (1.0-2.7)	1.9 (0.9-3.8)	4.9 (3.4-6.9)	1.9 (1.0-3.5|)
FVIII:C (176-200 IU/dL)	6.0 (3.9-9.0)	2.6 (1.5-4.6)	2.5 (1.4-4.1)	2.8 (1.4-5.8)	6.8 (4.2-10.5)	2.6 (1.3-5.0)
FVIII:C (>200 IU/dL)	5.8 (3.8-8.6)	2.5 (1.4-4.4)	4.5 (2.8-6.9)	4.9 (2.5-9.6)	7.1 (4.4-10.7)	2.6 (1.4-5.1)
	Provoked (n=1578) Mean FVIII:C 137 (95%CI, 135-140)		*Provoked minus malignancy (n=1451)* Mean FVIII:C 136 (95%CI, 134-138)		*Age <45 years (n=884)* Mean FVIII:C 130 (95%CI, 127-133)	
FVIII:C (≤ 100 IU/dL)	1.1 (0.7-1.6)	1 (reference)	1.1 (0.7-1.6)	1 (reference)	1.0 (0.6-1.7)	1 (reference)
FVIII:C (101-125 IU/dL)	2.0 (1.4-2.6)	1.8 (1.1-3.0)	1.9 (1.4-2.6)	1.8 (1.0-3.0)	2.8 (2.0-3.9)	2.7 (1.5-5.0)
FVIII:C (126-150 IU/dL)	2.4 (1.8-3.2)	2.2 (1.3-3.6)	2.3 (1.7-3.1)	2.1 (1.3-3.6)	2.3 (1.5-3.5)	2.2 (1.2-4.3)
FVIII:C (151-175 IU/dL)	2.4 (1.7-3.4)	2.2 (1.3-3.8)	2.5 (1.7-3.5)	2.2 (1.3-3.9)	2.4 (1.4-4.0)	2.3 (1.1-4.7)
FVIII:C (176-200 IU/dL)	2.7 (1.6-4.2)	2.5 (1.3-4.6)	2.9 (1.7-4.5)	2.6 (1.4-4.9)	2.0 (0.7-4.4)	1.9 (0.7-4.9)
FVIII:C (>200 IU/dL)	4.2 (2.7-6.3)	3.7 (2.1-6.6)	3.6 (2.2-5.6)	3.2 (1.7-5.9)	4.2 (2.0-7.7)	3.8 (1.7-8.6)
	Age>45 years (n=1358) Mean FVIII:C 146 (95%CI, 144-149)		*BMI<25 kg/m2 (n=805)* Mean FVIII:C 136 (95%CI, 132-139)		*BMI>25 kg/m2 (n=1335)* Mean FVIII:C 142 (95%CI, 139-144)	
FVIII:C (≤ 100 IU/dL)	1.9 (1.2-2.9)	1 (reference)	1.2 (0.6-1.9)	1 (reference)	1.7 (1.1-2.5)	1 (reference)
FVIII:C (101-125 IU/dL)	1.8 (1.2-2.6)	1.0 (0.6-1.7)	2.3 (1.4-3.3)	1.9 (1.0-3.7)	2.3 (1.6-3.1)	1.4 (0.8-2.3)
FVIII:C (126-150 IU/dL)	3.4 (2.6-4.4)	1.8 (1.1-2.8)	3.2 (2.1-4.5)	2.7 (1.4-5.0)	3.0 (2.3-4.0)	1.8 (1.1-2.9)
FVIII:C (151-175 IU/dL)	3.6 (2.6-4.7)	1.8 (1.1-3.0)	3.4 (2.2-5.0)	2.8 (1.5-5.4)	2.9 (2.1-4.0)	1.7 (1.0-2.9)
FVIII:C (176-200 IU/dL)	4.7 (3.2-6.5)	2.4 (1.4-4.0)	2.8 (1.3-5.3)	2.3 (1.0-5.3)	4.2 (2.8-6.2)	2.5 (1.4-4.3)
FVIII:C (>200 IU/dL)	5.4 (3.8-7.5)	2.7 (1.6-4.6)	5.1 (2.9-8.4)	4.1 (2.0-8.4)	5.5 (3.7-7.7)	3.1 (1.8-5.4)

CI denotes confidence interval; py, person years; FVIII:C, factor VIII activity level

* Numbers of unprovoked and provoked do not add up to a total of 2242 because some data were missing for some variables

Performance of FVIII in a prognostic model

We first validated the DASH-score in our study. The annual rates of recurrent venous thrombosis increased with every point increase of the DASH-score, although less pronounced than in the original article (Supporting Table3). The discriminative ability of different models, including FVIII, the DASH-score, the DASH-score + FVIII and the 'FASH'-score (replacing D-Dimer with FVIII) is shown in Table 6. The c-statistic was 0.64 (95%CI, 0.61-0.68) for the DASH-model, which became slightly higher when FVIII levels were added, i.e., 0.68 (difference 0.032, 95%CI, 0.004-0.061, p= 0.026). For the 'FASH'-score, the c-statistic became 0.67 (difference 0.022, 95%CI, -0.016; 0.059, p-value 0.264). When we added levels of VWF to the DASH-model the C-statistic was 0.67 (95%CI, 0.63-0.70), while it was 0.65 (95%CI, 0.61-0.69) in case D-dimer levels were replaced by levels of VWF.

Table 5. Recurrent venous thrombosis according to combinations of strata of factor VIII and blood group

Blood group	Range	N	Observation years (n)	Recurrent events	Incidence rate, per 100 py (95% CI)	Hazard ratio (95% CI)
O	FVIII:C (≤ 100 IU/dL)	236	1431	15	1.0 (0.6-1.7)	1 (reference)
O	FVIII:C (101-200 IU/dL)	378	2094	61	2.9 (2.2-3.7)	2.2 (1.4-3.6)
O	FVIII:C (>200 IU/dL)	31	129	7	5.4 (2.2-11.2)	4.5 (2.1-9.8)
Non-O	FVIII:C (≤ 100 IU/dL)	202	1255	23	1.8 (1.2-2.7)	1 (reference)
Non-O	FVIII:C (101-200 IU/dL)	1214	6784	197	2.9 (2.5-3.3)	1.8 (1.2-2.7)
Non-O	FVIII:C (>200 IU/dL)	175	783	40	5.1 (3.6-7.0)	3.3 (2.0-5.3)

CI denotes confidence interval; py, person-years

Table 6. Discriminative ability of different models within MEGA follow-up study

Model	C-statistic (95%CI)	
Factor VIII (continuous)	0.60	(0.56-0.64)
Factor VIII (categorical)	0.60	(0.56-0.64)
DASH	0.64	(0.61-0.68)
DASH + factor VIII (continuous)	0.67	(0.64-0.71)
DASH + factor VIII (categorical)	0.68	(0.64-0.71)
'FASH'	0.67	(0.63-0.70)

CI denotes confidence interval

Discussion

We followed 2242 patients with a first venous thrombosis for a median of 6.9 years and observed steadily increasing incidence rates of recurrent venous thrombosis with increasing FVIII levels, with a three-fold higher risk in those with FVIII levels over 200 IU/dL vs the lowest category (≤100 IU/dL). The assumption of proportional hazards over time held, which implies that levels of FVIII were able to predict recurrent thrombosis over long periods of time. We found higher incidence rates of recurrence for increasing levels of FVIII both in patients with unprovoked as in patients with provoked first events but the rate was maximum in patients with a high FVIII level who had a first unprovoked event. Lastly, we found that adding FVIII levels to an existing prediction model improved its performance.

Results from previous smaller studies have been contradictory.[10,15,26,27] In a study in our center (n=474), we did not find a relation between high FVIII activity levels and recurrent venous thrombosis (HR 1.1; 95%CI, 0.7-1.8),[26] possibly due to a low FVIII cut-off level (>166 IU/dL) to dichotomize patients.[28] Similar to Kyrle *et* al[15], we found the highest incidences of recurrence for patients with highest FVIII, although they reported an increased risk of recurrence only for patients with FVIII>234 IU/dL. We found recurrence rates to increase in a dose-response fashion with increasing levels of factor VIII. Cristina *et al.* found no relationship in patients with a provoked first event[27], which may be explained by a small group size (14 recurrences in 255 patients). We found an association between levels of factor VIII and recurrent venous thrombosis both in patients with a first unprovoked, as well as a first provoked event.

Within exposures that are currently thought to be associated with a low recurrence risk (provoked first event, women), high FVIII was still predictive for recurrent events. This implies that a more refined risk estimation is possible at an individual level. However, venous thrombosis is a multicausal disease and prediction of recurrence based on one factor is not sufficient to guide treatment duration.[29,30] Therefore we assessed the performance of FVIII in a prognostic model, in several combinations. When we added FVIII to the DASH-score, the model performed somewhat better. When we replaced D-dimer levels in the DASH-score by FVIII levels, the model performed equally well if not better. This may have considerable implications for the clinic since FVIII levels can be measured without error both during as well as after anticoagulant treatment with vitamin-K antagonists. Results of our sensitivity analysis in which we excluded patients who donated blood during their anticoagulant treatment period showed similar results as our main analysis. This would offer an important clinical advantage of FVIII over D-dimer measurement, which is affected by anticoagulant treatment.[31-33] Of note, measurement of factor VIII activity is also influenced by the new anticoagulant treatments (DOACs) that became recently available.[34,35]

In patients with a first venous thrombosis the average risk of recurrence is not high enough to outbalance the bleeding risk associated with continued anticoagulant treatment. Refinement of the size of the risk for subgroups is one way to aid in the decision to continue or not. For this, the results in Tables 2b and 4 can be of use. Furthermore, case-fatality rates of recurrent venous thrombosis and major bleeding events can be helpful in balancing the risks and benefits of different anticoagulant treatment strategies.[36] For example, the risk of recurrent venous thrombosis is 8.2% in the year after discontinuing anticoagulants in patients with an unprovoked first event and a FVIII level between 150 and 175 IU/dL (Table 2b). Should anticoagulant treatment be continued if this risk then drops to 0.5% at the cost of a 2.0% risk of a major bleeding event? In this example the additional mortality risk for bleeding would not outweigh the mortality benefit of reduced thrombosis (8.2% risk x 3.6% case-fatality rate of recurrent venous thrombosis[36] = 0.30% fatal events without continuation of anticoagulant treatment vs. 2.0% risk x 11.0% case-fatality rate of major bleeding[36] = 0.22% fatal bleeding events + 0.5% risk x 3.6% case-fatality rate of recurrent venous thrombosis = 0.24% fatal events with continued anticoagulant treatment).

Important strengths of our study are that this is the largest study till date on this issue in which patients were followed for a long period of time after their first event (median 7 years) and in which recurrent events were objectively confirmed and strictly classified as such. Furthermore, because of the large size several sensitivity analyses as well as subgroup analyses could be performed showing detailed and precise risk estimates. We are the first to externally validate the performance of the DASH-score and have shown the performance of the score with inclusion of factor VIII into the model.

Limitations of our study should also be mentioned. First, factor VIII can be increased in patients due to an acute phase reaction at the time of the first event. To avoid this problem, blood was drawn at least three months after the first event, in an outpatient setting. In a similar setting Tichelaar et al showed that factor VIII levels are fairly constant over time.[37] Second, FVIII levels were measured only once during follow-up. However, serial measurements of FVIII over time after venous thrombosis have shown that they remain reasonably constant and that high FVIII levels are a persistent phenomenon, which is in line with the predictive strength we found to be present over a prolonged period.[10,11,37] Third, FVIII levels were not measured in all patients, mainly because of logistic reasons. However, some may have been additionally missing in patients who were sickest. While this does not compromise the internal validity of our results, as we did not pose a causal question, it may imply that our results are generalizable only to those patients well enough to provide a blood sample. Fourth, since we used a strict definition of recurrent venous thrombosis and included only certain recurrences in our study, incidence rates and cumulative incidences may have been slightly underestimated. Fifth, our study consisted for 90% of Caucasians and hence our results may not be generalizable to other ethnic groups. Sixth, because our aim was to study whether levels of FVIII *predict* future recurrent events we did not

adjust for potential confounding factors. Therefore, this study provides no information on whether FVIII is causally related to recurrent thrombosis. Finally, although high FVIII levels predicted increased risks of recurrent venous thrombosis in both patients in whom the first event was either provoked or unprovoked, we cannot currently provide sufficient information whether the recurrent episodes were unprovoked or provoked in nature.

In summary, this study presents detailed evidence for a strong predictive value of FVIII levels for the risk of recurrent venous thrombosis in a large unselected group of patients with long follow-up. FVIII levels predicted recurrences in a dose-response fashion in various subgroups of patients over a long period of time. Addition of FVIII to an existing prognostic model, the DASH-score, improved the model's performance and D-Dimer could be replaced by FVIII levels without loss of predictive accuracy.

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SUPPLEMENT

Supporting Table1. Clinical characteristics of participants included and excluded from analyses

Supporting Methods

Supporting Table2. Incidence rates of recurrent venous thrombosis for strata of factor VIII; sensitivity analyses

Supporting Table3. Validation of the DASH score in the MEGA follow-up study

Supporting Figure1. Flowchart of patients included and excluded from analyses

Supporting Table 1. Clinical characteristics of participants included and excluded from analyses

General characteristics	Included in current analyses		Excluded from current analyses	
Total	2242	(100)	2489	(100)
Men	1010	(45)	1154	(46)
Age at enrollment, y	48	(18-70)	49	(18-70)
BMI	27	(16-58)	27	(14-63)
Chronic disease at baseline*	328	(15)	403	(16)
Classical venous thrombosis risk factors				
Provoked by†	1578	(72)	1723	(72)
Malignancy	127	(8)	299	(17)
Trauma/surgery/immobilization	895	(57)	1007	(58)
Plaster cast	109	(7)	110	(6)
Estrogen use (in women)	92	(45)	641	(37)
Pregnancy/puerperium (in women)	374	(6)	81	(5)
Travel >4 hrs	374	(24)	343	(20)
Unprovoked	621	(28)	678	(28)
Prothrombotic factor				
Blood group non-O	1591	(71)	1322	(71)
Type of index event				
Deep vein thrombosis only	1322	(59)	1425	(57)
Pulmonary embolism only	702	(31)	847	(34)
Pulmonary embolism + deep vein thrombosis	218	(10)	217	(9)

Continuous variables denoted as mean (range), categorical variables as number (%).
Some data were missing for some variables.
* Chronic disease defined as diabetes, liver disease, kidney disease, rheumatoid arthritis, multiple sclerosis, paralysis, hyperthyroidism, hypothyroidism, chronic bronchitis, eymphysema.
† As concomitance of provoked risk factors occurred frequently, patients could be counted twice or more.

Supporting Methods

To be classified as a certain recurrence, a reported recurrence should fulfil one of the following criteria.

1. A discharge letter was present concluding a diagnosis of recurrence, based on available clinical and radiological data. This recurrence should be in a different vein or in a different part of the body than the first event. The discharge letter had to contain information about instrumental diagnostic procedures. If location of either first or second thrombosis was not known or was similar to the first event, an event was still classified as certain if at least three months had passed since the first thrombosis.
2. A discharge letter was not available (e.g. when treating physician was unknown) but both the anticoagulation clinic and the patient reported a recurrence at a clearly different location than the first event (contralateral leg, DVT after PE or vice versa) or a time period of more than a year had passed between the two events.
3. A registered cause of death from PE or DVT at least six months after the first event.

Uncertain recurrences were defined by four criteria, one of which had to apply:

1. A diagnosis of a possible recurrence in the discharge letter, where clinical and radiological data could not distinguish between an extension of the first and a new thrombotic event.
2. A discharge letter was not available but both the patient and the anticoagulation clinic reported a recurrence within a year after the first event.
3. Information was only available from either the patient or the anticoagulation clinic.
4. A registered cause of death from PE or DVT within six months after the first event.

To quantify potential misclassification of outcomes, several sensitivity analyses were performed; one in which hazard ratios were corrected for time between the first thrombotic event and blood collection (n=2242), one in which all patients lost to follow-up were considered to have developed a recurrent event at the end of the study (for which the date of the recurrent event was set at the date on which the vital status was checked) (n=2242), one with start of follow-up from first thrombotic event (n=2357), one with patients with an active malignancy at time of the index date excluded from analysis (n=2115 included, n=127 excluded), one in which both certain and uncertain recurrent events are taken into account (n=2242, 427 recurrent events) and lastly, one with participants on anticoagulant treatment at the moment of blood collection excluded (n=2045 included, n=197 excluded).

Unprovoked first venous thrombosis was defined as venous thrombosis without surgery, trauma, plaster cast, pregnancy or immobilization in the first three months before the event, prolonged travel in the first two months before the event, active malignancies in the first five years before the event or hormone use (oral contraceptives or hormone

replacement therapy) at the time of the event. Patients who had one or more of these risk factors at time of their first event were classified as having a first provoked venous thrombosis.

Supporting Table 2. Incidence rates of recurrent venous thrombosis for strata of factor VIII; sensitivity analyses

Range	N	Observation years (n)	Recurrent events	Incidence rate, per 100 py (95% CI)	Hazard ratio (95% CI)
Sensitivity analysis 1*					
FVIII:C (≤ 100 IU/dL)	438	2686	38	1.4 (1.0-1.9)	1 (reference)
FVIII:C (101-125 IU/dL)	516	3005	68	2.3 (1.8-2.9)	1.6 (1.1-2.4)
FVIII:C (126-150 IU/dL)	493	2775	83	3.0 (2.4-3.7)	2.1 (1.4-3.0)
FVIII:C (151-175 IU/dL)	382	2088	67	3.2 (2.5-4.1)	2.3 (1.5-3.4)
FVIII:C (176-200 IU/dL)	205	1028	40	3.9 (2.8-5.3)	2.7 (1.7-4.1)
FVIII:C (>200 IU/dL)	208	917	47	5.1 (3.8-6.8)	3.5 (2.3-5.3)
Sensitivity analysis 2†					
FVIII:C (≤ 100 IU/dL)	438	2973	95	3.2 (2.6-3.9)	1 (reference)
FVIII:C (101-125 IU/dL)	516	3270	133	4.1 (3.4-4.8)	1.3 (1.0-1.7)
FVIII:C (126-150 IU/dL)	493	3063	154	5.0 (4.3-5.9)	1.6 (1.2-2.1)
FVIII:C (151-175 IU/dL)	382	2277	111	4.9 (4.0-5.9)	1.6 (1.2-2.0)
FVIII:C (176-200 IU/dL)	205	1172	72	6.1 (4.8-7.7)	2.0 (1.4-2.7)
FVIII:C (>200 IU/dL)	208	1066	90	8.4 (6.8-10.4)	2.7 (2.0-3.6)
Sensitivity analysis 3‡					
FVIII:C (≤ 100 IU/dL)	451	2978	39	1.3 (0.9-1.8)	1 (reference)
FVIII:C (101-125 IU/dL)	531	3359	72	2.1 (1.7-2.7)	1.6 (1.1-2.4)
FVIII:C (126-150 IU/dL)	522	3232	86	2.7 (2.1-3.3)	2.0 (1.4-2.9)
FVIII:C (151-175 IU/dL)	402	2439	72	3.0 (2.3-3.7)	2.2 (1.5-3.3)
FVIII:C (176-200 IU/dL)	219	1209	41	3.4 (2.4-4.6)	2.5 (1.6-3.9)
FVIII:C (>200 IU/dL)	232	1151	51	4.4 (3.3-5.8)	3.3 (2.2-5.0)
Sensitivity analysis 5§					
FVIII:C (≤ 100 IU/dL)	422	2591	37	1.4 (1.0-2.0)	1 (reference)
FVIII:C (101-125 IU/dL)	492	2898	65	2.2 (1.7-2.9)	1.6 (1.0-2.3)
FVIII:C (126-150 IU/dL)	464	2636	79	3.0 (2.4-3.7)	2.1 (1.4-3.1)
FVIII:C (151-175 IU/dL)	360	1992	65	3.3 (2.5-4.2)	2.2 (1.5-3.4)
FVIII:C (176-200 IU/dL)	191	955	39	4.0 (2.9-5.6)	2.8 (1.8-4.3)
FVIII:C (>200 IU/dL)	186	856	41	4.8 (3.4-6.5)	3.2 (2.0-5.0)
Sensitivity analysis 6¶					
FVIII:C (≤ 100 IU/dL)	438	2686	46	1.7 (1.3-2.3)	1 (reference)
FVIII:C (101-125 IU/dL)	516	3005	82	2.7 (2.2-3.4)	1.6 (1.1-2.3)
FVIII:C (126-150 IU/dL)	493	2775	95	3.4 (2.8-4.2)	2.0 (1.4-2.8)
FVIII:C (151-175 IU/dL)	382	2088	87	4.2 (3.3-5.1)	2.4 (1.7-3.4)
FVIII:C (176-200 IU/dL)	205	1028	53	5.2 (3.9-6.7)	2.9 (2.0-4.3)
FVIII:C (>200 IU/dL)	208	917	64	7.0 (5.4-8.9)	3.8 (2.6-5.6)

Sensitivity analysis 7**					
FVIII:C (≤ 100 IU/dL)	415	2605	28	1.1 (0.7-1.6)	1 (reference)
FVIII:C (101-125 IU/dL)	477	3118	53	1.7 (1.3-2.2)	1.7 (1.1-2.7)
FVIII:C (126-150 IU/dL)	454	2882	67	2.3 (1.8-3.0)	2.3 (1.5-3.6)
FVIII:C (151-175 IU/dL)	345	2156	52	2.4 (1.8-3.2)	2.4 (1.5-3.8)
FVIII:C (176-200 IU/dL)	182	1063	33	3.1 (2.1-4.4)	3.2 (1.9-5.2)
FVIII:C (>200 IU/dL)	172	886	35	3.9 (2.8-5.5)	4.0 (2.5-6.6)

Supporting Table 2. Incidence rates of recurrent venous thrombosis for strata of factor VIII; sensitivity analyses (continued)

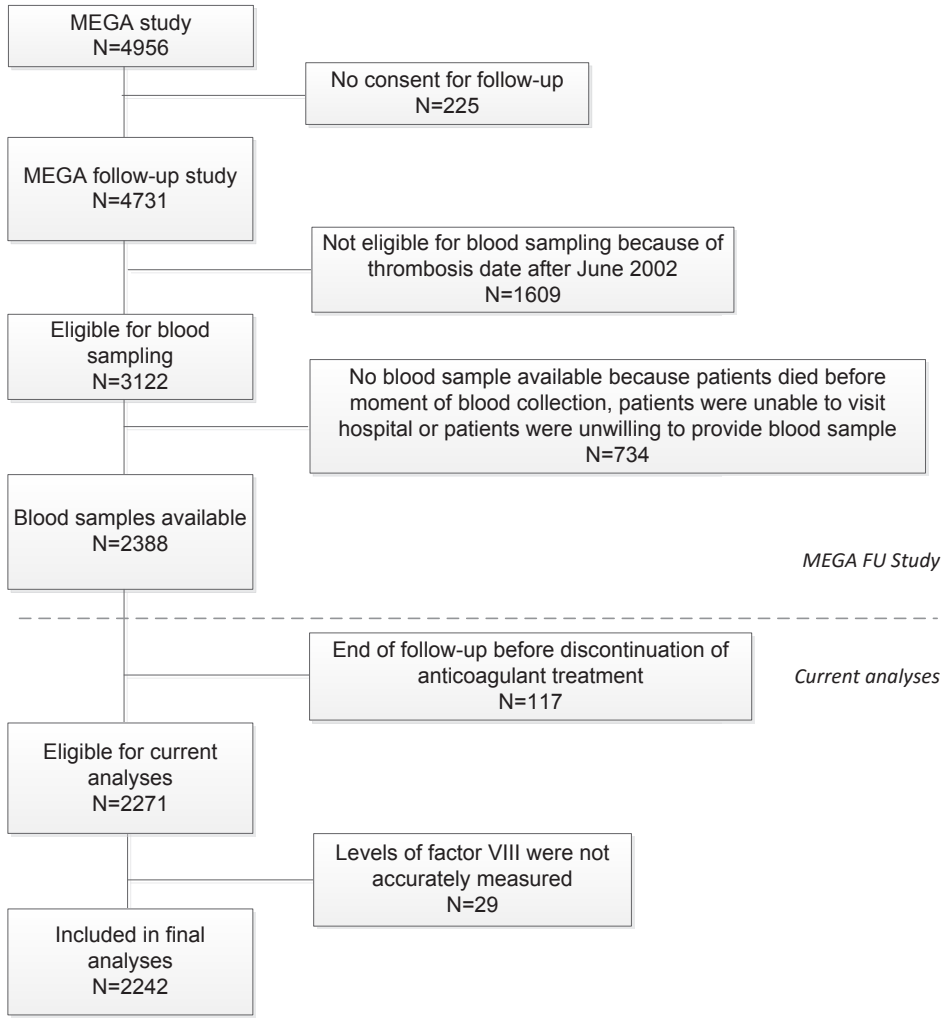
CI denotes confidence interval; py, person-years; FVIII:C, factor VIII activity level
* Hazard ratios corrected for time between first thrombotic event and blood collection
† Patients lost to follow-up all considered as having developed a recurrent event at end of the study
‡ Start of follow-up from first thrombotic event
§ Malignancy patients excluded
¶ Certain and uncertain recurrent events taken into account
** Participants on anticoagulant treatment at moment of blood collection excluded

Supporting Table 3. Validation of the DASH score in the MEGA follow-up study

DASH score	Recurrence/total	Incidence rate, per 100 py	(95% CI)
-2	1/69	0.23	(0.03-1.63)
-1	19/250	1.22	(0.78-1.92)
0	18/95	3.08	(1.94-4.90)
+1	50/285	2.96	(2.24-3.91)
+2	36/191	3.27	(2.36-4.53)
+3	44/162	4.95	(3.69-6.66)
+4	11/30	8.23	(4.56-14.85)

py denotes: person-years

Supporting Figure 1. Flowchart of patients included and excluded from analyses



Chapter 7

Recurrent venous thrombosis in premenopausal women: effect of continuing or starting hormonal contraceptive use

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Abstract

Background

There is a large body of literature available on hormonal contraceptive use and the risk of a first venous thrombotic event. Despite guideline recommendations to discontinue, a sizeable proportion of women continue or start using hormonal contraceptives after a venous thrombosis. The aim of this study was to evaluate the effect of this use on the risk of recurrence in premenopausal women.

Methods

Premenopausal female patients with a first venous thrombosis, included in the MEGA case-control study between 1999 and 2004, were followed for a recurrent venous thrombotic event up to 2010. Data on hormonal contraceptive use were available through a prescription database (from Dutch Foundation for Pharmaceutical Statistics). Time-dependent Cox-proportional hazards models were used to estimate hazard ratios (HR) with 95% confidence intervals (CI), adjusted for age and BMI at baseline and anticoagulation use.

Results

650 women were linked to the prescription database and followed for a total of 3537 person-years (median 6.1 years; range, 41 days to 9.7 years). 57 women had a recurrence, of which 14 were during hormonal contraceptive use. Irrespective of contraceptive use at the first event, any type of hormonal contraceptive use increased the risk of recurrence about two-fold (HR 1.8, 95%CI: 0.9 to 3.3). Using combined oral contraceptives after the first event increased the risk almost three-fold (HR 2.6, 95%CI: 1.3 to 5.2). The recurrence rate among IUD users was found to be similar to the rate among non-users (HR 0.9, 95%CI: 0.3-3.1).

Conclusion

Hormonal contraceptive use after a first venous thrombosis increases the risk of a recurrent venous thrombotic event. A levonorgestrel-releasing intra-uterine device may be a safe alternative.

Introduction

A large body of literature is available about combined oral contraceptive use and the increased risk of a first venous thrombosis.[1-4] National[5,6] and international[7] guidelines state to discontinue hormonal contraceptive use after a venous thrombotic event, in particular combined preparations (oral contraceptive, transdermal patch and vaginal ring) with the idea of preventing recurrences. Despite these guidelines, a large proportion of women either continues or starts hormonal contraceptive use after a first venous thrombosis. One study found that 39% of women using hormonal contraceptives at the first event either continued or restarted afterwards.[8] In the present study about three months after stopping anticoagulation therapy, 21% of combined oral contraceptive users continued their contraceptive use.[9]

Discontinuation of hormonal contraceptives could be a small intervention on a woman's lifestyle, with potentially large preventive effects with regard to the risk of recurrent venous thrombosis. Nevertheless, not much is known about the association between hormonal contraceptive use and recurrent venous thrombosis, except for one report by Christiansen et al.[10] In this study, the risk of recurrence was fourfold increased in users of hormonal contraceptives compared with non-users. The number of recurrences was too few to allow meaningful conclusions about the type of contraceptive.

The aim of the present study was to evaluate the effect of hormonal contraceptive use, administration route and type of combined oral contraceptive (dose of ethinylestradiol and type of progestagen) on the risk of recurrent venous thrombosis in premenopausal women with a first venous thrombosis.

Materials and methods

Participants

Participants were cases from a population-based case-control study; the Multiple Environmental and Genetic Assessment of venous thrombosis (MEGA) study. Details of the study have been described elsewhere.[11] In short, between 1 March 1999 and 31 August 2004, 4956 consecutive patients with an objectively diagnosed first deep vein thrombosis of the leg or pulmonary embolism were included. Patients were aged 18-70 years and were enrolled from six anticoagulation clinics in the Netherlands. Anticoagulation clinics monitor all patients taking vitamin K antagonists in a well-defined geographical area. All patients filled in a questionnaire on risk factors for venous thrombosis. About three months after discontinuation of the anticoagulation therapy, patients were invited to the anticoagulation clinic for a blood sample. During this visit participants were interviewed regarding the period from the venous thrombotic event until venepuncture. This interview included items on possible change of hormonal contraceptive methods since the diagnosis of venous thrombosis.

Of 4956 eligible patients, 4731 gave informed consent for follow-up. Short answer forms, regarding recurrent venous thrombosis, were sent by mail to patients between January 2008 and December 2009. Questions were asked by telephone interview when answer forms were not returned. During the same period information about recurrences was retrieved from the anticoagulation clinics where patients were initially included for their first event and, in case they moved house, at the clinic nearest to their new address. Deaths due to recurrent venous thrombosis were obtained at the Central Bureau of Statistics (CBS). To obtain information on diagnostic procedures, discharge letters were requested from the clinician who diagnosed the recurrence according to the patient or the anticoagulation clinic. A detailed questionnaire on risk factors for venous thrombosis during follow-up was sent to participants after they gave permission for this in the short answer form. Details of the follow-up study have been described elsewhere.[12] This study was approved by the Medial Ethics Committee of the Leiden University Medical Center.

For the current analyses, we focussed on premenopausal women with venous thrombosis before age 50 (N=1584). Women who had cancer in the five years before the first venous thrombosis or undergoing chemo- or radiotherapy were excluded (N=60). Women who were unlikely to use contraceptives due to various reasons were excluded, i.e., pregnant or postpartum women (N=35), current HRT users (N=53), self-reported peri- or postmenopausal women (N=52), underweight women (N=1) and 32 women who had undergone a hysterectomy or oophorectomy. The population of interest consisted of 1351 premenopausal women.

Hormonal contraceptives

Hormonal contraceptive use was defined as use of a contraceptive that contains steroid hormones, administered orally, transdermally or vaginally. Users of a copper-IUD were

considered non-users. Hormonal contraceptive use was categorised according to the route of administration into oral and non-oral preparations. Oral preparations were stratified into combined and progestagen-only preparations. Because many different preparations of combined oral contraceptives are available, these contraceptives were categorised according to the dose of ethinylestradiol and type of progestagen. Non-oral preparations were further stratified according to the specific application (vaginal ring, transdermal patch, implant, injectable, and levonorgestrel-releasing intrauterine device (IUD)).

Data on hormonal contraceptive use were available through two sources; a prescription database (the Dutch Foundation for Pharmaceutical Statistics (SFK) registry) [13] and the detailed questionnaire filled in at the end of follow-up. Participants in the MEGA follow-up study were linked to the prescription database via age, sex, 4 digits postal code and vitamin K antagonist use within the first month after the initial venous thrombosis. The national ID number was not available for linkage and abovementioned factors were not unique for every participant. Therefore, 650 (48%) of premenopausal women could be successfully linked to the prescription database. Linkage was a random process since being unique on the variables according to which linkage was performed is not associated with either recurrent venous thrombosis or use of contraceptives. The following information was available from SFK; date of prescription, name of the contraceptive and the amount and defined daily dosage (DDD) of the prescription. Periods of contraceptive use were defined as continuous use of contraceptive based on the normal duration of use. For instance, a prescription for 126 oral contraceptive pills was assumed to be taken for 24 weeks (three weeks of taking a contraceptive pill a day and a stopweek). An IUD was assumed to be used for five years. Women with a prescription for hormonal contraceptives just before the first venous thrombotic event, with enough contraceptive pills prescribed to continue áfter venous thrombosis, were considered exposed for these days after the event. This is because women are mostly advised to continue using contraceptives during the anticoagulant treatment period. [14] A prothrombotic effect of hormonal contraceptives is likely to be suppressed by anticoagulation, while the risk of menorrhagia associated with stopping hormonal therapy could be increased by anticoagulants.

The detailed questionnaire contained questions about hormonal contraceptive use after the first venous thrombosis; name of contraceptive used and starting date and date of discontinuation. Data on hormonal contraceptive use provided in the questionnaire was crosschecked with data retrieved at the time of the first venous thrombosis and at the time of venepuncture in the MEGA case-control study. 787 (58%) of premenopausal women filled in the detailed questionnaire and self-reported on their use of hormonal contraceptives after the first thrombotic event.

Recurrent venous thrombosis

A recurrent event was defined by information provided by patients through the questionnaire, anticoagulation clinics, discharge letters or causes of death. A decision

rule regarding certainty of the diagnosis was made according to the information collected per patient. Details of this decision rule have been described previously.[12] In short, reported recurrences were classified into certain recurrences when there was a discharge letter stating a diagnosis of a recurrent event based on clinical and radiological data, or when both the anticoagulation clinic and the patient reported a recurrent event at either a clearly different location than the first event or more than one year had passed since the first event, or when a registered death from a recurrent event at least six months after the first event was found. In the current analysis, certain recurrences were used as endpoint and patients with an uncertain recurrence were censored at time of their uncertain recurrence.

Statistical Analysis

Premenopausal women with information on hormonal contraceptive use after a first venous thrombosis were included. The start of follow-up was defined as the date of the first venous thrombosis. The end of follow-up was defined as the date of the recurrent event or when no recurrent event occurred, the date of returning the short answer form, or the last date until we knew patients to be recurrence free (last visit to the anticoagulation clinic, date of death, or emigration), whichever came first. Observation time was calculated as the time at risk from the first thrombotic event to the end of follow-up.

Hormonal contraceptive use was taken as a time-dependent exposure to allow women switching from use to non-use and vice versa during follow-up. Consequently, one woman could contribute follow-up time for hormonal contraceptive use as well as for non-use. Although anticoagulation use was not considered to be a confounder in the analysis, the risk of a recurrence is lower during a period of anticoagulation use. Therefore, analyses were adjusted for anticoagulation use (time-dependently) to obtain estimates of the incidence rate of a recurrence irrespective of anticoagulation use.

The relative risk of recurrent venous thrombosis was estimated separately for women using hormonal contraceptives at the first event and for women using hormonal contraceptives during follow-up. The effect of oral and non-oral preparations on the risk of recurrent venous thrombosis was assessed and compared with non-use. Data were analysed separately by data source (prescription database or questionnaire). Recurrence rates were calculated for combined oral contraceptives by dose of ethinylestradiol and progestagen.

All analyses were adjusted for the confounders age and BMI (at baseline) and for anticoagulation use. Time-dependent Cox-proportional hazards models were used to calculate hazard ratios (HR) with corresponding 95% confidence intervals. All statistical analyses were performed with STATA, version 13.0 (Statacorp LP, College Station, TX, USA).

Results

Of 1351 premenopausal women with a first venous thrombotic event, 650 were linked to the prescription database and followed for a total of 3537 person-years (median 6.1 years; range, 41 days to 9.7 years). 787 women filled in the detailed questionnaire and had a total follow-up of 5155 person-years (median 6.8 years; range 120 days to 9.9 years). Baseline characteristics of the study population by data source are given in Table 1. Characteristics were similar for the women linked to the prescription database and women who filled in the detailed questionnaire.

For 412 women data were available from both sources, and so could be checked for consistence. Based on the prescription data, 148 women (36%) did not use a contraceptive at any given time after the event and 109 women (26%) discontinued use some time after the event, for a total of 257 women (62%) who did not continue to use hormonal contraceptives after the first venous thrombosis. Data from the questionnaire are consistent with this, given that according to the questionnaire 241 of these 257 women (94%) did not continue to use hormonal contraceptives after the event. However, the rest of the periods and types of hormonal contraceptive use reported by the prescription database and the questionnaire are not consistent. Out of the 155 women who continued or started using hormonal contraceptives according to the prescription database, 111 women (72%) did not self-report on such use in the detailed questionnaire. Because of this discrepancy and because we assumed data to be more accurate from the prescription database (women may not precisely remember their contraceptive use over the past few years), we focussed our analyses on data from the prescription database. Results based on the questionnaire data can be found in Supplementary Table 1.

Prescription database

Among the 650 women linked to the prescription database, 57 recurrences occurred, of which 14 were during hormonal contraceptive use. The overall rate of recurrent venous thrombosis among premenopausal women was 16.1 (95%CI: 12.4 to 20.9) per

Table 1. Baseline characteristics of premenopausal women with venous thrombosis

	Prescription	Questionnaire
Variables	N=650	N=787
Age at 1st event, mean(range), yrs	37 (18-49)	36 (18-49)
BMI at 1st event		
<25 kg/m2	246 (42)	334 (44)
25-30 kg/m2	187 (32)	231 (31)
>30 kg/m2	156 (26)	190 (25)
HC use at 1st event	455 (70)	590 (75)

BMI denotes: body mass index, HC: hormonal contraceptive

Table 2. Recurrence rate in premenopausal women and the influence of hormonal contraceptive use

Questionnaire	N recurrence	Follow-up time (years)	IR (per 1000) (95%CI)	HR* (95%CI)	HR† (95%CI)	HR‡ (95%CI)
Non-use at first event	17	1057	16.1 (10.0-25.9)	1 (reference)	1 (reference)	1 (reference)
HC use at first event	40	2480	16.1 (11.8-22.0)	1.0 (0.6-1.7)	1.0 (0.6-1.7)	0.9 (0.5-1.5)
Non-use during follow-up	43	2910	14.8 (11.0-19.9)	1 (reference)	1 (reference)	1 (reference)
HC use during follow-up	14	627	22.3 (13.2-37.7)	1.7 (0.9-3.1)	1.7 (0.9-3.2)	1.8 (0.9-3.3)
Oral preparation						
COC	11	326	33.7 (18.7-60.9)	2.6 (1.3-5.2)	2.6 (1.3-5.2)	2.6 (1.3-5.2)
POP	0	2	0 (0-1844.4)	-	-	-
Non-oral preparation						
Vaginal ring	0	4	0 (0-922.2)	-	-	-
IUD	3	250	12.0 (3.9-37.1)	0.9 (0.3-2.9)	0.9 (0.3-2.9)	0.9 (0.3-3.1)
Injectable	0	43	0 (0-85.8)	-	-	-
HC use at first event and non-use during follow-up	27	1950	13.8 (9.5-20.2)	1 (reference)	1 (reference)	1 (reference)
HC use at first event and during follow-up	13	529	24.5 (14.3-42.3)	2.0 (1.0-3.9)	2.0 (1.0-4.0)	2.0 (1.0-4.0)

COC denotes: combined oral contraceptive, HC: hormonal contraceptive, HR: hazard ratio, IR: incidence rate, POP: progestagen-only pill
*Adjusted for anticoagulation
†Age and anticoagulation adjusted
‡Anticoagulation, age and BMI adjusted

1000 person-years. The recurrence rate in hormonal contraceptive users at the first event was similar (16.1, 95%CI: 11.8-22.0 per 1000 person-years) as in non-users at the first event (16.1, 95%CI: 10.0-25.9) (Table 2). This was also evident from the hazard ratio for users at the first event compared with non-users (HR 0.9, 95%CI: 0.5 to 1.5, adjusted for age, BMI and anticoagulation) (Table 2).

Among women using hormonal contraceptives during follow-up, we observed a recurrence rate of 22.3 (95%CI: 13.2-37.7) per 1000 person-years and among non-users during follow-up a rate of 14.8 (95%CI: 11.0-19.9) per 1000 person-years. This implied that hormonal contraceptive use after a first venous thrombosis increased the risk of recurrent thrombosis two-fold after adjustment for anticoagulation, age and BMI (HR 1.8, 95%CI: 0.9 to 3.3) (Table 2). Restriction to women who were using hormonal contraceptives at the first event, yielded a similar increased risk in women who continued to use hormonal contraceptives compared with those who stopped (HR 2.0, 95%CI: 1.0-4.0) (Table 2).

11 recurrences occurred during combined oral contraceptive use. The recurrence rate for combined oral contraceptive use during follow-up was 33.7 (95%CI: 18.7-60.9) per 1000 person-years, almost three-fold higher than for non-use during follow-up (HR 2.6, 95%CI: 1.3 to 5.2, adjusted for anticoagulation; HR 2.6 after adjustment for age, BMI and anticoagulation, 95%CI: 1.3 to 5.2). Notable was that out of 64 women using a levonorgestrel-releasing IUD (250 person-years of follow-up), only three had a recurrence (recurrence rate 12.0, 95%CI: 3.9-37.1 per 1000 person-years). The recurrence rate was similar for IUD users and non-users (HR 0.9, 95%CI: 0.3-3.1).

Recurrence rates were calculated by type of combined oral contraceptive. Numbers per type of combined oral contraceptive were, however, small. Incidence rates of recurrence were similar for the types of contraceptive mostly used in the Netherlands: IR 39.2 (95%CI, 17.6-87.1) for 30µg of ethinylestradiol and levonorgestrel, IR 33.4 (95%CI, 8.3-133.4) for 30µg of ethinylestradiol and desogestrel and IR 42.7 (95%CI, 6.0-303.5) for 30µg of ethinylestradiol and gestodene.

Questionnaire data

Among the 787 women who filled in a questionnaire during follow-up, 80 recurrences occurred resulting in a rate of recurrent venous thrombosis among premenopausal women of 15.5 (95%CI: 12.5 to 19.3) per 1000 person-years. All results for the analyses based on the questionnaire data were similar to results for analyses based on the prescription database (Supplementary Table 1).

In women who used hormonal contraceptives after the first venous thrombosis the risk of recurrence was almost three-fold increased (HR 2.8, 95%CI: 1.7 to 4.8, adjusted for anticoagulation, age and BMI) as compared with women who did not use hormonal contraceptives during follow-up. Restricting to women who were using hormonal contraceptives at the first event, a similarly increased risk was found with those who discontinued use as reference group (HR 2.9, 95%CI: 1.6-5.1). The risk of recurrent venous thrombosis was almost three-fold higher for combined oral contraceptive users than for non-users (HR 2.8, 95%CI: 1.6 to 5.0).

Discussion

Despite guideline recommendations, a large proportion of women either continues or starts hormonal contraceptive use after a first venous thrombotic event. This study assessed the association between the risk of recurrent venous thrombosis and hormonal contraceptive use after a first event. Information on hormonal contraceptive use was available from a large prescription database as well as from a detailed questionnaire filled in by premenopausal women from the MEGA follow-up study. Women using hormonal contraceptives, in particular combined oral contraceptives, after a first venous thrombosis had a two- to three-fold higher risk of recurrence than non-users. Use or non-use of hormonal contraceptives at the first event did not affect the risk of recurrent venous thrombosis. The use of a levonorgestrel-releasing intrauterine device appeared not associated with an increased risk of recurrent venous thrombosis.

To date, only one other study (LETS) evaluated the risk of recurrent venous thrombosis among women using hormonal contraceptives after their first event in a prospective follow-up study.[10] That analysis was restricted to women who used hormonal contraceptives at the first event. The authors observed a recurrence rate of 48.8 per 1000 person-years (95%CI: 24.3-87.2) among hormonal contraceptive users during follow-up and a recurrence rate of 10.5 per 1000 person-years (95%CI: 4.5-20.7) among those who had discontinued use. The recurrence rate among these non-users was similar as reported in the current study (14.8 per 1000 person-years); however, the recurrence rate in hormonal contraceptive users was higher (48.8 per 1000 person-years versus 22.3 per 1000 person-years). This difference may be due to differences in the distribution of types of contraceptives in the LETS and the MEGA study, between which a decade elapsed. The proportion of women using a second generation contraceptive had increased over time (MEGA study 55% vs LETS 25%), while the proportion of women using a third generation contraceptive and the proportion of women using triphasic preparations had decreased (35% vs LETS 49% and 5% vs LETS 11%). However, because of small numbers the difference in recurrence rates could be a chance finding as well.

Several limitations of this study should be mentioned. First, we aimed to combine data on hormonal contraceptive use from both the prescription database and the detailed questionnaires. 650 Women were linked to the prescription database and 787 women filled in the detailed questionnaire, with an overlap of 412 women. Combining both sources of information would have increased our power considerably. However, the lack of consistency between the two sources suggested that a substantial number of women had not correctly remembered periods of contraceptive use over the past years. Alternatively, as hormone use is actively advised against, women may have been reluctant to admit such use. We focussed our analyses on the objective data from the prescription database, where no misclassification is expected. Nevertheless, results for both data sources were similar.

A second limitation of our study is that only 48% of our population of interest could be uniquely linked to the prescription database. As a consequence, numbers were too small to assess reliably the risk of recurrence by type of contraceptive. The recurrence rate among women linked to the prescription database was similar (16.1, 95%CI: 12.4 to 20.9 per 1000 person-years) to the rate among women who could not be linked (17.9, 95%CI: 14.1-22.6), suggesting that bias due to the limited proportion that could be linked is unlikely.

A strength of our study is its size. Furthermore, as far as we know, we are the first to compare the risk of recurrent venous thrombosis between women using hormonal contraceptives versus those who did not throughout a long period of time after a first event, separately for those who used or not used hormones at the first event. Also we were the first to study the association for different administration routes of the contraceptive (oral vs non-oral). Furthermore, we used a decision rule to ascertain recurrence status by which we ensured that only certain recurrences were included in our analyses. Lastly, detailed information on participants hormonal contraceptive use during follow-up made it possible to perform a time-dependent survival analysis, allowing switches from exposed to non-exposed during follow-up and vice versa.

After a first venous thrombotic event recurrent venous thrombosis is common, with a five-year cumulative incidence of about 25%.[15,16,17] A large patient level meta-analysis has shown a one-year cumulative incidence of recurrence of 5% and a three-year cumulative incidence of 9% in women.[18] Recurrences are associated with considerable comorbidity (post-thrombotic syndrome, chronic pulmonary hypertension), mortality and health-care costs. Despite progress in identifying determinants of recurrence risk, its prediction and prevention in an individual patient remains a challenge. Prevention of recurrent venous thrombosis by extending anticoagulant treatment is dependent on a delicate balance between risk of thrombosis and bleeding. Discontinuation of hormonal contraceptives could be a small intervention on a woman's lifestyle, with potentially large preventive effects with regard to the risk of recurrent venous thrombosis.

Current guidelines[5,6,7] recommend women to discontinue hormonal contraceptive use after a first venous thrombotic event. These guidelines however, are based on the assumption that risk factors for a first event also increase the risk of a recurrence. Not much was known on the risk of recurrences in women who continued their contraceptive use. Our study supports current guidelines which advise women to refrain from the use of hormonal contraceptives after a venous thrombotic event. We found that the risk of recurrent venous thrombosis is two- to threefold increased during periods of use of, particularly combined, hormonal contraceptives. Women should be urged to discontinue the use of hormonal contraceptives, since there are alternatives, e.g. a copper-IUD, available. This study suggests that the use of a levonorgestrel-releasing IUD may also be a safe option after a venous thrombotic event.

Given that out of 53 women who continue or restart using a combined oral contraceptive after a first venous thrombotic event one woman develops recurrent

venous thrombosis (Number Needed to Harm: 1/ risk difference = 1/ (0.0337-0.0148)) and given that currently 20-40% of women continue or start using hormonal contraceptives after a first event[8,9], the overall burden of recurrent venous thrombosis in women could be significantly reduced by adherence to the guidelines.

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Supplementary Table 1. Recurrence rate in premenopausal women and the influence of hormonal contraceptive use

Questionnaire	N recurrence	Follow-up time (years)	IR (per 1000) (95%CI)	HR (95%CI)	HR† (95%CI)	HR‡ (95%CI)	HR§ (95%CI)
Non-use at first event	20	1284	15.6 (10.0-24.1)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
HC use at first event	60	3870	15.5 (12.0-20.0)	1.0 (0.6-1.7)	1.0 (0.6-1.7)	1.0 (0.6-1.7)	1.0 (0.6-1.7)
Non-use during follow-up	61	4615	13.2 (10.3-17.0)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
HC use during follow-up*	19	539	35.2 (22.5-55.2)	2.6 (1.5-4.3)	2.8 (1.6-4.7)	2.8 (1.6-4.7)	2.8 (1.7-4.8)
Oral preparation							
COC	15	399	37.5 (22.6-62.2)	2.7 (1.5-4.7)	2.8 (1.6-4.9)	2.8 (1.6-4.9)	2.8 (1.6-5.0)
POP	1	29	34.4 (4.9-244.5)	-	-	-	-
Non-oral preparation							
Vaginal ring	1	3	269.0 (37.9-1909.4)	-	-	-	-
IUD	0	77	0 (0-47.9)	-	-	-	-
Injectable	0	24	0 (0-153.7)	-	-	-	-
HC use at first event and non-use during follow-up	43	3378	12.7 (9.4-17.2)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
HC use at first event and during follow-up	17	491	34.6 (21.5-55.6)	2.6 (1.5-4.6)	2.9 (1.6-5.1)	2.9 (1.6-5.1)	2.9 (1.6-5.1)

COC denotes: combined oral contraceptive, HC: hormonal contraceptive, HR: hazard ratio, IR: incidence rate, POP: progestagen-only pill
*No data on the type of contraceptive used was available in seven women
†Adjusted for anticoagulation
‡Age and anticoagulation adjusted
§Anticoagulation, age and BMI adjusted

Chapter 8

Seated immobility, either through long-haul travel, seated work or confinement to a wheelchair, and the risk of recurrent venous thrombosis

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Abstract

Background
Several studies have shown an increased risk of venous thrombosis (VT) in otherwise healthy individuals during seated immobility. Temporary thromboprophylaxis during periods of immobility is not justified because of a low risk in absolute terms. This may be different for recurrent VT.

Objectives
To study whether seated immobility, either through exposure to long-haul travel or through prolonged immobility in daily life, increases the risk of recurrent VT.

Patients/ Methods
This study is a case-control study nested within a cohort of 4731 patients with a first VT who were followed for recurrence (MEGA follow-up study). Participants reported via a questionnaire on periods of seated immobility: 1) prolonged travel >4h or seated work >4h/day during a 3 month-period before the recurrence for the cases or a random period for the controls 2) confinement to a wheelchair anytime during the follow-up period. 2723 participants (58%) returned the questionnaire. For the first exposure, odds ratios (OR), adjusted for age, sex, comorbidity at baseline and anticoagulant treatment were estimated to compare risk of recurrence between groups with and without recent immobility. For the second, adjusted hazard ratios (HR) were estimated by means of time-dependent Cox regression analysis to compare risk of recurrence between groups with and without use of a wheelchair.

Results
No association was found between long-haul travel and recurrent VT (adjusted OR 0.8; 95%CI, 0.6-1.1) or daily seated work and recurrent VT (adjusted OR 0.8; 95%CI, 0.6-1.2). Within subgroups of different types and duration of travel or subgroups of days per week of work-related immobility results were similar. Five out of 47 patients who reported to use a wheelchair developed recurrent VT, but did not have an increased recurrence risk as compared with patients without a wheelchair (adjusted HR 1.1; 95%CI, 0.4-2.6).

Conclusions
For several seated immobility exposure categories, i.e. prolonged travel, seated work or confinement to a wheelchair, no association was found with recurrent VT.

Introduction

In 1856, Rudolf Virchow described three broad categories of factors contributing to venous thrombosis; endothelial injury, hypercoagulability and stasis. Stasis as a risk factor for thrombosis has been described predominantly in the context of plaster casts, prolonged bed rest and immobilisation after surgery. However, in these instances other factors like damaged tissue or comorbidities that contribute to hypercoagulability also play a role.

Immobilization per se as a risk factor for venous thrombosis, in otherwise healthy people, has been described for the first time in 1940, during the second World War, when an increase in number of deaths from pulmonary embolism was attributed to prolonged sitting in shelters during the bombardments of London.[1]

More recently, several studies have been published in which seated immobility, such as during work, travel or long haul flights, was found to be a risk factor for a first venous thrombotic event.[2-4] In a study by Healy and colleagues prolonged work- and computer-related seated immobility was associated with an almost three-fold increased risk of a first venous thrombotic event (OR 2.8; 95%CI, 1.2-6.1).[3] A meta-analysis by Chandra and colleagues reports on a three-fold increased risk (pooled RR 2.8; 95%CI, 2.2-3.7) for travellers as compared with non-travellers.[2] Prolonged immobility in a supine position however, has not been related to activation of coagulation.[5]

Although reported relative risks of a first venous thrombotic event during or after seated immobility are moderately high, the absolute risk of a first venous thrombotic event is low (1-2 per 1000 persons per year).[6] This is why clinical interventions such as temporary thromboprophylaxis (for example during long-haul flights) are not justified. This may be different for recurrent venous thrombosis, for which the absolute risk is high. The five-year cumulative incidence of recurrent venous thrombosis is reported to be around 12-25%.[7-9] However, the relation between seated immobility and recurrent venous thrombosis has not been studied before. Knowledge is needed to provide travellers, as well as individuals who are otherwise immobilised in the absence of morbidity, with solid advice regarding their actual risk and to evaluate the utility of prophylactic measures.

We aimed to study whether seated immobility, either through exposure to long-haul travel or through prolonged immobility in daily life, increases the risk of recurrent venous thrombosis.

Methods

Study design

Between March 1999 and August 2004, 4956 patients aged 18-70 with an objectively diagnosed first deep vein thrombosis (DVT) of the leg or pulmonary embolism (PE) were included in a population-based case-control study (MEGA study). All patients filled in an extensive questionnaire on putative risk factors for venous thrombosis. Details of the MEGA study have been described previously.[10] Of the MEGA case-control study, only the cases were further followed for recurrence until 2008-2009 (MEGA follow-up study). For this, 225 of the 4956 patients did not consent, leaving 4731 patients. Of these patients 134 participated in the MEGA follow-up pilot study for which follow-up ended in 2005. The MEGA follow-up study was approved by the Medical Ethics Committee of the Leiden University Medical Center, and all participants gave written informed consent.

Adjudication of immobility status

Data on periods of seated immobility of participants during follow-up came from a detailed questionnaire, filled in by the patients at the end of follow-up. We studied the following self-reported factors regarding seated immobility during follow-up: 1) Long-haul travel for >4 hours; 2) seated work for >4 hours per day, categorized as either seated work or work-related seated travel, and 3) confinement to a wheelchair.

Questions regarding long-haul travel or seated work were asked with regard to a 3 months period before the date of recurrence for the participants with a recurrent venous thrombotic event and a randomly picked three months period during follow-up for the participants without a recurrent venous thrombotic event (Figure 1).

The period of exposure to seated immobility is much longer for patients who are confined to a wheelchair than for patients who took e.g. a long-haul flight. Questions regarding the use of a wheelchair were therefore asked with regard to the complete duration of follow-up and focussed on the first date of using the wheelchair.

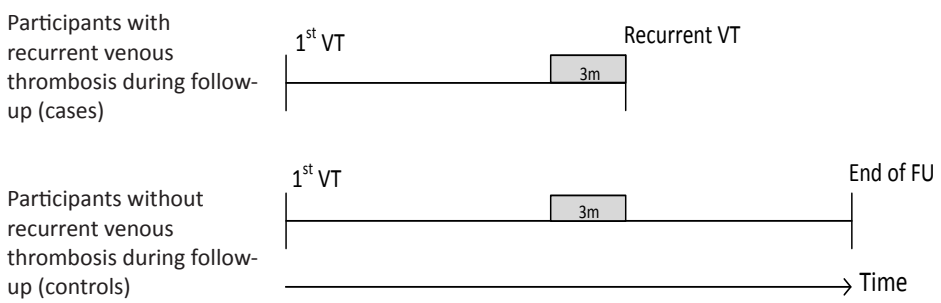

Figure 1. Three-months exposure period for cases and controls.

Adjudication of recurrent events

Between 2007 and 2009 the vital status of all patients was acquired from the central Dutch population register.[11] For the patients who died, a cause of death (ICD-10-CM) was obtained from the national register of death certificates at the Central Bureau of Statistics. Short answer forms concerning recurrent venous thrombosis were sent by mail to all survivors and consenting individuals between June 2008 and July 2009, and supplemented by telephone interviews. Additional information was acquired from the regional anticoagulation clinics and from hospitals. Deaths due to recurrent venous thrombosis were counted as fatal recurrent events. Based on hospital discharge letters, the information from the anticoagulation clinics, forms filled in by the patients and causes of death, possible recurrences were classified into certain and uncertain recurrences, following a decision rule published previously.[12] In short, reported recurrences were classified into certain recurrences when there was a discharge letter stating a diagnosis of a recurrent event based on clinical and radiological data, or when both the anticoagulation clinic and the patient reported a recurrent event at either a clearly different location than the first event or more than one year has passed since the first event, or when a registered death from a recurrent event at least six months after the first event was found.

Adjudication of use of prophylactic anticoagulant treatment

Information on the use of anticoagulant treatment during the three months exposure period, both for long-haul travel and seated work, was obtained from the SFK register. SFK stands for the Dutch Foundation for Pharmaceutical Statistics and is a register in which over 95% of the community pharmacies in the Netherlands are represented. [13] SFK data contain information about patient specific drugs dispensed; the generic name of a drug, the Anatomical Therapeutic Chemical (ATC) classification, the date of prescription, and the number of days for which a drug was prescribed. Information from this register was available for the years 1999 to 2009. Linkage was based on a combination of age, sex, 4-digit postal code and vitamin K antagonist use within the first month after the initial venous thrombosis. In total 2547 (54%) patients of the MEGA follow-up study could be individually linked with SFK. In analyses on the use of a wheelchair relative risks were time-dependently corrected for anticoagulant treatment during follow-up. Information on this use of anticoagulant treatment was derived from the anticoagulation clinics.

Statistical analyses

Patients reported on long-haul travel or seated work with regard to a three months exposure period. To study whether long-haul travel and daily seated work after a first thrombotic event were associated with recurrent venous thrombosis we used a nested case-control design, within the MEGA follow-up study. Cases were the participants with recurrent venous thrombosis and controls participants without recurrent venous thrombosis. Patients reported on the use of a wheelchair during follow-up and the

first date of use. To study whether confinement to a wheelchair was associated with recurrent venous thrombosis we used a traditional follow-up design.

1 Analyses on long-haul travel

For the analyses on long-haul travel participants of the MEGA follow-up pilot study (n=134), participants who did not return the follow-up questionnaire (n=1874), participants who did not fill in the question regarding long-haul travel (n=287) and participants with an uncertain recurrent event (n=50) were excluded. In total, 2386 participants were eligible for analyses. This group contained 402 cases, i.e. participants with a recurrent event and 1984 controls, i.e. participants without a recurrent event.

The cases were 1:1 matched on time since the first thrombotic event until the three months exposure period to take into account that the risk of recurrence decreases over time. After this matching procedure 804 participants were included for analyses, i.e. all 402 cases and 402 controls. Odds ratios (OR) with 95% confidence intervals were estimated with conditional logistic regression analysis to compare the risk of recurrence between groups with and without recent travel.[14] Odds ratios were estimated for long-haul travel for >4 hours yes or no, stratified by type of transport (airplane, bus, car or train), number of travels and duration of travel. Odds ratios were adjusted for age, sex and comorbidity at baseline. Comorbidity at baseline could be diagnoses of cancer, diabetes, liver failure, kidney failure, rheumatoid arthritis, multiple sclerosis, chronic bronchitis or emphysema. Additionally, adjustment for (prophylactic) anticoagulant use during the three months exposure period was performed.

2) Analyses on daily seated work

For the analyses on seated work participants of the MEGA follow-up pilot study (n=134), participants who did not return the follow-up questionnaire (n=1874), participants who did not fill in the question regarding seated work either because they were not employed at the time or because they did not want to answer the question (=1216) and participants with an uncertain recurrent event (n=31) were excluded, leaving 1476 participants eligible for analyses. This group contained 254 cases and 1222 controls. After the matching procedure 506 participants were included for analyses, of which 253 cases (out of 254) and 253 controls. Odds ratios were estimated for seated work for >4 hours per day, either through seated work or work-related seated travel, stratified by number of days per week. Odds ratios were adjusted for age, sex, comorbidities at baseline and anticoagulant treatment during the 3 months exposure period (as described above).

3) Analysis on use of a wheelchair

For the analyses on the use of a wheelchair participants of the MEGA follow-up pilot study (n=134), participants who did not return the follow-up questionnaire (n=1874), participants who responded with “Yes, I use a wheelchair’, but who did not fill in a starting date (n=7) and participants who did not answer the question regarding use

of a wheelchair (n=89), 2627 participants could be included for analyses. Duration of follow-up was counted from date of first thrombotic event to end of follow-up, defined as the date of a recurrence or, in its absence, the date of returning the short answer form. The last form in the current study population was returned on December 31, 2009. Here we limit the analyses to certain recurrent events (n=465) and participants with an uncertain recurrent event (n=86) were censored from the date of the uncertain recurrence onward. Incidence rates of recurrent venous thrombosis were estimated as the number of events over the accumulated follow-up time and with person time split and divided over participants with or without the need for a wheelchair during follow-up. The association between the use of a wheelchair and recurrent venous thrombosis was estimated by means of time-dependent Cox regression analysis with the use of a wheelchair as a time-dependent variable. Hazard ratios with corresponding 95% confidence intervals (CI) were estimated and corrected for age, sex and comorbidity at baseline. Additional adjustments for anticoagulant treatment during follow-up were performed with anticoagulant treatment as a time-dependent variable. Hazard ratios were estimated for confinement to a wheelchair yes or no and stratified for the duration of wheelchair use. As a sensitivity analysis we included patients who responded in the questionnaire “Yes, I use a wheelchair”, but who did not fill in a date (n=7) and considered them exposed over the full follow-up period.

Results

In the MEGA follow-up study 4731 patients with a first episode of venous thrombosis were followed for recurrent events for a median of 5.9 years (interquartile range 1.6-7.8 years, total follow-up 24 064 years). Mean age at enrolment was 48 years and 2164 (46%) patients were men. In 987 (21%) patients comorbidity at baseline was reported, with cancer (9%) accounting for most of the comorbidities. Baseline characteristics for patients who returned the follow-up questionnaire (n=2723) were similar to those of the total group of patients (mean age 48, 45% men) except for the proportion of patients with comorbidities at baseline (15% comorbidities) (Table 1).

Relation between long-haul travel and recurrent venous thrombosis

Of 402 cases with recurrent venous thrombosis, 127 (32%) reported long-haul travel of more than four hours, during the three months period prior to their recurrence. Of the matched 402 controls 148 (37%) reported long-haul travel during the 3 months exposure period. Long-haul travel appeared not related to recurrent venous thrombosis, shown by both the crude and adjusted odds ratios of 0.8 (95%CI, 0.6-1.1) and 0.8 (95%CI, 0.6-1.1) (Table 2). When results were split for either long-haul air travel or long-haul non-air travel, results did not change with adjusted odds ratios of 0.8 (95%CI, 0.5-1.2) and 0.8 (95%CI, 0.5-1.1). Results for number of travels and duration of travel were additionally similar and did not show an association between

travel and recurrent venous thrombosis. Only for patients with long-haul air travel for >12 hours we found a possibly increased risk of recurrence (OR 2.0; 95%CI 0.7-6.2), as compared with patients without long-haul air travel. When we stratified long-haul non-air travel for the type of transport we did not see elevated risks for any means of transport, with an adjusted odds ratio of 0.7 (95%CI; 0.5-1.1) for a trip by car, 0.4 (95%CI; 0.1-2.4) for a trip by train and of 0.9 (95%CI; 0.3-2.7) for travel by bus. In all of abovementioned analyses additional adjustment for (prophylactic) anticoagulant treatment did not change results (Table 2). We had data on other types of prophylactic measures, such as compression stockings or exercise, only for participants who took a long-haul flight. Of 56 cases who took a long-haul flight 17 (30%) reported not to have taken any prophylactic measures, while 21 (38%) reported to have exercised or moved during the flight and 28 (50%) reported to have worn compression stockings. For the 69 controls who took a long-haul flight, these numbers were 30 (43%), 27 (39%) and 24 (35%), respectively.

Relation between seated work and recurrent venous thrombosis

Of 253 cases with recurrent venous thrombosis, 147 (58%) reported to perform seated work of more than four hours, during the three months period prior to their recurrence. Of the matched 253 controls 155 (61%) reported to perform seated work during this period (Table 3). Work-related daily immobility was not associated with recurrent thrombosis, shown by an adjusted odds ratio of 0.8 (95%CI; 0.6-1.2). Also after

Table 1. Clinical Characteristics

	MEGA follow-up cohort with questionnaires (non-pilot)			MEGA follow-up cohort		
General characteristics	Cases	Controls	Total	Cases	Controls	Total
N	466*	2171	2723	673†	3839	4731
Age, mean (sd)	50 (12.7)	48 (12.4)	48 (12.5)	50 (12.9)	48 (13.1)	48 (13.1)
Sex, male (%)	300 (64%)	877 (40%)	1222 (45%)	427 (63%)	1618 (42%)	2164 (46%)
Baseline characteristics						
Comorbidity	64 (14%)	327 (15%)	411 (15%)	110 (16%)	791 (21%)	962 (20%)
Cancer	14 (3%)	94 (4%)	113 (4%)	41 (6%)	354 (9%)	421 (9%)
Diabetes	12 (3%)	67 (3%)	84 (3%)	21 (4%)	140 (4%)	171 (4%)
Liver failure	3 (1%)	5 (0%)	8 (0%)	3 (1%)	23 (1%)	27 (1%)
Kidney failure	3 (1%)	23 (1%)	26 (1%)	7 (1%)	43 (1%)	53 (1%)
Rheumatoid arthritis	20 (5%)	63 (3%)	87 (4%)	21 (4%)	104 (3%)	137 (3%)
Multiple sclerosis	0 (0%)	10 (1%)	12 (1%)	2 (0%)	22 (1%)	28 (1%)
Chronic bronchitis	18 (4%)	88 (4%)	113 (5%)	30 (5%)	191 (6%)	238 (6%)
Emphysema	5 (1%)	18 (1%)	24 (1%)	6 (1%)	49 (2%)	58 (1%)

*86 uncertain recurrent events not counted
†219 uncertain recurrent events not counted

Table 2. Odds ratios for risk of recurrent venous thrombosis according to long-haul travel

Exposure	Cases	Controls	OR (95%CI)	OR* (95%CI)	OR† (95%CI)
Long-haul travel >4 hours					
No	275	254	reference	reference	reference
Yes	127	148	0.8 (0.6-1.1)	0.8 (0.6-1.1)	0.8 (0.6-1.1)
Number of travels					
≤2	67	74	0.8 (0.5-1.2)	0.8 (0.5-1.2)	0.8 (0.5-1.2)
>2	60	74	0.8 (0.5-1.2)	0.8 (0.5-1.2)	0.8 (0.5-1.2)
Duration of travel					
≤12 hours	104	111	0.8 (0.6-1.2)	0.9 (0.6-1.2)	0.9 (0.6-1.3)
>12 hours	17	25	0.8 (0.4-1.6)	0.8 (0.4-1.6)	0.7 (0.3-1.5)
Long-haul air travel >4 hours					
No	345	331	reference	reference	reference
Yes	56	69	0.8 (0.5-1.1)	0.8 (0.5-1.2)	0.8 (0.5-1.3)
Number of flights					
≤2	41	43	0.9 (0.6-1.5)	1.0 (0.6-1.6)	1.0 (0.6-1.7)
>2	15	26	0.6 (0.3-1.1)	0.5 (0.3-1.1)	0.5 (0.2-1.0)
Duration of flight					
≤12 hours	42	60	0.7 (0.5-1.1)	0.7 (0.5-1.1)	0.7 (0.4-1.2)
>12 hours	9	5	1.8 (0.6-5.4)	2.0 (0.7-6.2)	2.0 (0.6-6.2)
Long-haul non-air travel >4 hours					
No	312	293	reference	reference	reference
Yes	86	106	0.7 (0.5-1.0)	0.8 (0.5-1.1)	0.8 (0.5-1.1)
Type of non-air travel					
Car	64	77	0.7 (0.5-1.1)	0.7 (0.5-1.1)	0.7 (0.5-1.1)
Train	2	7	0.3 (0.1-1.7)	0.4 (0.1-2.4)	0.5 (0.1-2.5)
Bus	12	10	1.0 (0.4-2.9)	0.9 (0.3-2.7)	0.8 (0.3-2.5)
Number of travels					
≤2	32	39	0.7 (0.4-1.2)	0.7 (0.4-1.3)	0.7 (0.4-1.3)
>2	54	67	0.8 (0.5-1.2)	0.8 (0.5-1.3)	0.8 (0.5-1.3)
Duration of travel					
≤12 hours	74	73	1.0 (0.7-1.4)	1.0 (0.7-1.6)	1.0 (0.7-1.6)
>12 hours	12	33	0.3 (0.1-0.7)	0.3 (0.1-0.7)	0.3 (0.1-0.7)

*Adjusted for age, sex, comorbidity
†Adjusted for age, sex, comorbidity and (prophylactic) anticoagulant treatment
Data on anticoagulant treatment was not available for all participants (linkage successful 54%)

stratification for daily seated work and daily work-related seated travel no association was found (adjusted OR 1.0 (95%CI; 0.7-1.5) and 0.7 (95%CI; 0.4-1.3), respectively). Within subgroups of days per week of work-related immobility results were similar and no association was found between seated immobility and recurrences. In all of abovementioned analyses additional adjustment for (prophylactic) anticoagulant treatment did not change results (Table 3).

Relation between confinement to wheelchair and recurrent venous thrombosis

In the follow-up questionnaire 47 patients reported to have a condition for which they were confined to a wheelchair. Five of these patients developed a recurrent venous thrombotic event for an incidence rate of 22.5 per 1000 person-years (95%CI; 9.5-54.0) while it was 26.9 per 1000 person-years (95%CI; 24.5-29.5) for patients without a wheelchair. Corresponding hazard ratio after adjustments for age, sex and comorbidity

Table 3. Odds ratios for risk of recurrent venous thrombosis according to immobility in daily life through work

Exposure	Cases	Controls	OR (95%CI)	OR* (95%CI)	OR† (95%CI)
Work-related daily immobility					
No	106	98	reference	reference	reference
Yes	147	155	0.9 (0.6-1.3)	0.8 (0.6-1.2)	0.8 (0.6-1.2)
Number of days per week					
1-2 days	14	20	0.5 (0.2-1.3)	0.5 (0.2-1.6)	0.5 (0.1-1.5)
>2 days	133	135	1.0 (0.7-1.4)	0.9 (0.6-1.3)	0.9 (0.6-1.3)
Daily seated work					
No	110	110	reference	reference	reference
Yes	141	142	1.0 (0.7-1.4)	1.0 (0.7-1.5)	1.0 (0.7-1.5)
Number of days per week					
1-2 days	12	16	0.4 (0.1-1.4)	0.7 (0.2-2.5)	0.7 (0.2-2.5)
>2 days	129	126	1.1 (0.7-1.6)	1.1 (0.7-1.6)	1.1 (0.7-1.6)
Daily work-related seated travel					
No	207	206	reference	reference	reference
Yes	35	32	1.1 (0.6-1.9)	0.7 (0.4-1.3)	0.7 (0.4-1.3)
Number of days per week					
1-2 days	9	13	0.5 (0.2-1.5)	0.3 (0.1-0.9)	0.2 (0.1-0.8)
>2 days	26	19	1.5 (0.8-3.1)	1.0 (0.5-2.2)	1.1 (0.5-2.3)

*Adjusted for age, sex, comorbidity
†Adjusted for age, sex, comorbidity and anticoagulant treatment
Data on anticoagulant treatment was not available for all participants (linkage successful 54%)

at baseline was 1.1 (95%CI; 0.4-2.6) (Table 4). In a sensitivity analysis in which we included seven participants who used a wheelchair but for whom we did not have a date of first use, we found similar results with an adjusted HR of 1.0 (95%CI, 0.4-2.2). 24 Out of 47 patients with a wheelchair started using their wheelchair before the first thrombotic event. They did not have an increased risk of recurrence (HR 1.0; 95%CI, 0.3-3.1) after adjustments for age, sex and comorbidity (Table 4). The other 23 patients who started using a wheelchair after the first thrombotic event developed 2 recurrences and did not have an increased recurrence risk (HR 1.2; 95%, 0.3-4.8 after adjustments). One of these recurrences was within one year after the start of using a wheelchair. Prophylactic anticoagulant treatment does not seem to explain our findings. Additional adjustments for anticoagulant treatment as a time-dependent variable did not change our results (Table 4). Of all 47 patients that reported to be bound to a wheelchair none started prophylactic anticoagulant treatment around the time of first use. Eight out of 24 patients who started using a wheelchair before their first thrombotic event received long-term anticoagulant treatment after the event. Three out of 24 patients received only therapeutic anticoagulant treatment shortly after the thrombotic event and thirteen out of 24 patients had one or several periods of prophylactic treatment during follow-up. Out of 23 patients who started using the wheelchair after the first event, 15 patients had no periods of anticoagulant treatment after the start of use, 5 patients had a period of long-term anticoagulant treatment after the start of use and 3 patients had one or several shorter periods of prophylactic treatment after the start of use.

Table 4. Odds ratios for risk of recurrent venous thrombosis according to confinement to a wheelchair

Exposure	Obs.yrs (n)	rec events	IR/1000	HR	HR*	HR†
Confinement to wheelchair						
No	16413 (2580)	441	26.9 (24.5-29.5)	1 (reference)	1 (reference)	1 (reference)
Yes	222 (47)	5	22.5 (9.4-54.0)	0.9 (0.4-2.1)	1.1 (0.4-2.1)	1.2 (0.5-2.9)
Duration of wheelchair use						
Start before the 1st thrombotic event	141 (24)	3	21.3 (6.9-66.1)	0.8 (0.2-2.4)	1.0 (0.3-3.1)	1.0 (0.3-3.3)
Start after the 1st thrombotic event	82 (23)	2	24.5 (6.1-97.9)	1.0 (0.3-4.1)	1.2 (0.3-4.8)	1.4 (0.4-5.8)

*Adjusted for age, sex, comorbidity
†Adjusted for age, sex, comorbidity and anticoagulant treatment
Obs.yrs denotes: observation years; rec. events: recurrent events; IR: incidence ratio; HR: hazard ratio

Discussion

In this study we found no association between seated immobility, either through long-haul travel, work-related seated immobility or through the use of a wheelchair and recurrent venous thrombosis. Odds ratios for long-haul air travel and long-haul non-air travel as compared with no travel were 0.8 (95%CI, 0.5-1.2) and 0.8 (95%CI, 0.5-1.1). Stratification for the duration of travel did not change results, although long-haul air travel >12 hours may be associated with an increased risk of recurrence. The risk of recurrent venous thrombosis was not increased during periods of daily seated work (OR 1.0; 95%CI, 0.7-1.5) or work-related seated travel (OR 0.7; 95%CI, 0.4-1.3) and results were similar in subgroups of days per week with seated work. For patients confined to a wheelchair the risk of recurrent venous thrombosis was not increased compared with patients who did not use a wheelchair (HR 1.2; 95%CI, 0.4-2.6).

Several studies that demonstrated a positive association between long-haul travel and *first* events of venous thrombosis have shown a dose-response relation with regard to increasing duration of travel and number of travels. In a large meta-analysis a dose-response relationship was found with a 20% higher risk for venous thrombosis for each 2-hour increase in travel duration.[2] Kuipers et al. have shown that the risk of a first event increases with exposure to more flights in a short time-frame and with increasing duration of flights.[15] A previous study has shown similarly increased risks of a first event after flying or traveling by car, bus or train.[16] For a first venous thrombotic event several studies have reported an association between both work- and computer-related seated immobility.[3,17-19] A severe case of a first venous thrombotic event after periods of prolonged sitting at a computer has even led to the proposal of the term 'eThrombosis; the 21st century variant of venous thromboembolism associated with immobility'.[18] The association between the use of a wheelchair, use of prophylactic anticoagulation and venous thrombosis has received little attention in the literature. However, a study by Arpaia and colleagues reported a frequency of asymptomatic DVT of over 40% in patients with advanced multiple sclerosis admitted to a neurology center, who were either wheelchair-bound or bedridden.[20] Currently, the ACCP guidelines recommend against the routine use of thromboprophylaxis in chronically immobilized patients who either reside at home or at a nursing home.[21]

Although previous studies on the relation between seated immobility, such as during travel or work, and first venous thrombosis have shown moderately strong associations, we did not find such an association between immobility and recurrences. A factor such as prolonged immobility, leading to stasis of blood in the venous system, which is strongly associated with first venous thrombotic events, could be expected to be a risk factor for recurrences as well. We cannot fully exclude the possibility of an increased recurrence risk during periods of seated immobility. An explanation for our null findings with regard to long-haul travel could be the large proportion of controls

who undertook long-haul travel. The frequency of long-haul air travel >4 hrs over three months was 17% in our control participants. Controls were asked to report on long-haul travel during a random three-month exposure period over the past few years. Perhaps they did not remember the exact date of travel, and responded 'yes' to the question while their actual date of travel was just outside the three months exposure period. This would result in an overestimation of number of travels in controls. However, in a study by Martinelli et al frequencies of long-haul flights of >8hrs and of flights of any duration were approximately 6% and 23%, respectively, over a three months period. [22] Our frequency of 17% for long-haul flights of >4hrs, lies in between these numbers. Another explanation may be that we may not have been able to correct for all potential confounding factors. Probably, patients who are able to travel have an overall better health status than patients who do not travel. We tried to correct for this by adjusting for comorbidities at baseline (at time of the first thrombotic event). However, we may not have had sufficient information on general health throughout the follow-up period. Such a 'healthy traveller effect' has been described before in a study on the association between air travel and first thrombotic events.[23] Another explanation for our null findings for both long-haul travel, seated work and confinement to a wheelchair is that we have not been able to correct for other prophylactic measures taken by patients themselves, such as compression stockings, or exercise.

In summary, in a large follow-up study we studied the association between seated immobility and recurrent venous thrombosis. For several exposure categories, like prolonged travel, seated work or confinement to a wheelchair, we did not find an association with recurrences. For participants with long-haul air travel >12 hours the risk of recurrent venous thrombosis may be increased. We cannot make a definite conclusion as to whether preventive measures, like prophylactic anticoagulant treatment, exercise or stockings during periods of seated immobility, would be beneficial in patients with a history of venous thrombosis. Randomized clinical trials are needed to answer this question.

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Chapter 9

Risk of first and recurrent venous thrombosis in individuals treated with antibiotics: Results from the MEGA study

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Abstract

Background

Previous studies have suggested a role for transient infections in the etiology of venous thrombosis (VT). We aimed to study whether individuals who receive antibiotic treatment (as a proxy for infections) have an increased risk of first or recurrent VT and what the joint effect is of antibiotics and genetic thrombophilia.

Methods and Results

4731 patients with a first VT from 1999-2004 were included in the MEGA-study and followed for a median of 5.9 years for recurrence (1999-2010 MEGA follow-up study). Information on antibiotic use was obtained via linkage to SFK-data (Dutch Foundation for Pharmaceutical Statistics). We used the self-controlled case-series method to study the risk of *first* VT during antibiotic prescriptions. VT, either PE or DVT, might at first sight be misdiagnosed as an infection. Therefore, patients for whom misclassification certainly played a role were excluded and we stratified for types of antibiotics for which misclassification is unlikely. 2547 VT patients could be individually linked to SFK-data, in whom 114 first events occurred during antibiotic use. After exclusion of patients who were misclassified we found a five-fold increased risk of *first* VT during antibiotic treatment: (incidence-rate-ratio [IRR] 5.0; 95%CI, 4.0-6.1). The IRR of DVT in patients receiving antibiotics for urinary tract infections (no misclassification) was 3.2 (95%CI, 1.9-5.6). By means of time-dependent Cox-regression, with correction for age and sex, antibiotic use was associated with a 2.0-fold (95%CI, 1.1-4.0) increased risk of *recurrent* VT, compared with no use. A joint risk of about 9 was found for antibiotic use and genetic thrombophilia (factor V Leiden or prothrombin G20210A mutation).

Conclusion

Individuals who receive antibiotics have a two to three-fold increased risk of a *first* VT or a *recurrent* VT during their antibiotic use, with highest risks during the first week of use.

Introduction

Venous thrombosis, defined as deep vein thrombosis (DVT) of the leg or pulmonary embolism (PE), is a major cause of mortality and morbidity. The incidence of venous thrombosis in the population is 1-2 per 1000 individuals per year[1], and the cumulative incidence of recurrent venous thrombosis within five years after a first event is 20-25%.[2-4] In about half of the patients with venous thrombosis no provoking risk factor can be identified. This is clinically important, as such patients are considered candidates for long term anticoagulant treatment.[5]

In 2006, Smeeth and colleagues observed an increased risk of venous thrombosis in patients who had a transient respiratory or urinary tract infection. The risk was highest in the first three months after diagnosis of an infection.[6] Also, as many as 36% of patients with acute venous thrombosis report, when asked, symptoms or signs of a transient infectious or inflammatory disease during the four weeks prior to presentation.[7] This adds credence to the suggestion that transient infectious diseases are associated with an increased risk of venous thrombosis. Nevertheless, infectious diseases are currently not considered as a provoking factor for venous thrombosis.[5] Moreover, the influence of infection on risk of recurrence has only received anecdotal attention in the literature and little formal study.[8] The mechanism that underlies the association has only been obtained in patients with sepsis[9], or in laboratory studies.[10]

We aimed to study whether individuals who receive antibiotic treatment (as a proxy for infection), have an increased risk of first and recurrent venous thrombosis. Additionally, we aimed to study whether the risk of venous thrombosis during antibiotic use is further increased in individuals with genetic thrombophilia. For this purpose we used three different study designs; a self-controlled case series design, a prospective follow-up design and a case-only analysis.

Methods

Patients

Consecutive patients aged 18 to 70 years with a first DVT or PE were included in the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study. Details of the MEGA study have been described previously.[11,12] In short, 4956 patients were recruited in the period between February 1999 to September 2004.

Of the patients included, 225 did not consent to participate in a follow-up study on recurrent venous thrombosis. Therefore, 4731 patients were followed from their first venous thrombotic event until 2008-2010 when they completed a questionnaire on recurrent venous thrombotic events.[13] This study has been approved by the Medical Ethics Committee of the Leiden University Medical Center, and all patients gave written informed consent.

Outcome classification first venous thrombosis

Patients with a first objectively identified DVT of the leg or a first PE were identified at six anticoagulation clinics in the Netherlands. The anticoagulation clinics monitor the anticoagulant therapy of all patients in a well-defined geographical area, which allowed the identification of consecutive and unselected patients with venous thrombosis. Unprovoked venous thrombosis was defined as venous thrombosis without surgery, trauma, plaster cast, pregnancy or immobilization in the first three months before the event, prolonged travel in the first two months before the event, active malignancies in the first five years before the event or hormone use (oral contraceptives or hormone replacement therapy) at the time of the event. Patients who had one or more of these risk factors at time of their thrombotic event were classified as having had a provoked venous thrombosis.

Outcome classification recurrent venous thrombosis

During the same period when patients were asked to self-report on any recurrent thrombotic events during follow-up, information about recurrences was additionally retrieved from the anticoagulation clinics and from hospital discharge letters. Furthermore, between 2007 and 2009 the vital status of all patients was acquired from the central Dutch population register.[14] For the patients who died, the cause of death (ICD-10-CM encoded) was obtained from the national register of death certificates at the Central Bureau of Statistics. Deaths due to recurrent venous thrombosis were counted as fatal recurrent events. Information from the anticoagulation clinics, hospital discharge letters, questionnaires filled in by the patients and death certificates was combined and based on this, recurrences were classified into certain and uncertain recurrences, following a decision rule as described previously.[13]

Antibiotic exposure definition

Information on antibiotic use was obtained by linkage to the SFK register (the Dutch Foundation for Pharmaceutical Statistics).[15] In the Netherlands, antibiotics are only available by prescription, and over 95% of the community pharmacies in the Netherlands are represented in this register. SFK contains information about patient specific drugs dispensed; the generic name of a drug, the Anatomical Therapeutic Chemical (ATC) classification, the date of prescription, and the number of days for which a drug was prescribed. Information from this register was available for the years 1999 to 2009. Linkage was based on a combination of age, sex, 4-digit postal code and vitamin K antagonist use within the first month after the initial venous thrombosis. In total 2547 (54%) patients of the MEGA study could be individually linked with SFK. After linkage to the SFK register, all MEGA patients with one or more prescriptions of antibiotics in the period 1999-2009 were identified.

Clinically, an early presentation of PE may at first be misdiagnosed as an infection. Early symptoms of PE are sometimes mistaken for a respiratory tract infection and antibiotics are prescribed. This misclassification would lead to spurious associations between antibiotic use and PE. For DVT and infections of the skin of the leg the same may be true. We reduced this possibility of misclassification step by step. First, we excluded patients in whom it was likely that such misclassification had taken place, from information of discharge letters. Second, we performed a subgroup analysis involving patients with DVT only, PE only or PE with or without DVT as the pathophysiology of DVT might be different from that of PE[16] and as misclassification (of for example an acute lung infection) is likely to be less for DVT than for PE. Furthermore, we stratified results for different types of antibiotics since misclassification will play a different role for different types of antibiotics. For example, we expect virtually no misclassification for antibiotics prescribed for urinary tract infections. We defined three main groups of antibiotics based on the condition for which these antibiotics are most often prescribed in the outpatient setting in the Netherlands: 1) penicillins, tetracyclines and macrolides (wide range of infections); 2) nitrofurane derivatives, sulphonamides and trimethoprim and quinolones (primarily urinary tract infections); 3) flucloxacillin (primarily skin infections).

Genetic thrombophilia testing

Venous blood was collected at least three months after discontinuation of anticoagulant therapy following the first event, or during anticoagulant treatment in patients who continued for more than one year. Blood was collected in trisodium citrate and processed within four hours. For logistic reasons this was done until June 2002. Patients who were unable or unwilling to provide blood samples or were recruited after June 1, 2002 were sent buccal swabs to collect DNA for genetic profiling and blood group determination. DNA from either buccal swab or blood samples was obtained by standard methods. Blood group was determined by a 5'nuclease assay (Taqman;Applied Biosystems, Foster City, California) using a standard PCR reaction mix

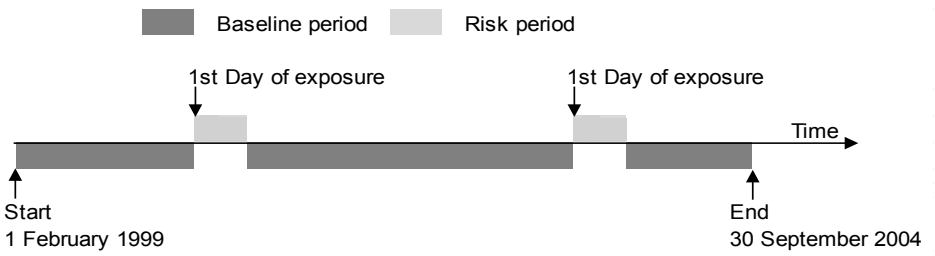
(Eurogentec, Seraing, Belgium) and an allele specific fluorescent probe equipped with a minor grove binding moiety (applied Biosystems). DNA analysis for the factor V Leiden (G1691A; rs6025) mutation and the prothrombin (G20210A; rs1799963) mutation was performed using a combined polymerase chain reaction method.[12]

Design and statistical analyses
Three designs were used to answer our research questions.

1) Antibiotic use and first venous thrombosis risk
We used the self-controlled case-series (SCCS) method to study whether patients who received antibiotic treatment have an increased risk of first venous thrombosis. The SCCS method relies on intra-person comparisons in a population of individuals who have had the outcome of interest, thereby eliminating fixed confounding.[17] Only those patients with a first venous thrombosis and at least one prescription of antibiotic use during the observation period from February 1999 to September 2004 (inclusion period for MEGA case-control study) were included in this analysis (n=1584).

We derived measures of the relative incidence of events during exposure to antibiotics as compared with all other observed time periods for each patient. The null hypothesis was that venous thrombotic event rates remain constant from day to day and are not affected by an acute exposure of antibiotic use. The period of exposure was defined as extending up to end of treatment with antibiotics. Additional analyses were performed in which only the first week after the prescription of an antibiotic was considered exposed person-time. All other observation time was taken as the baseline period (i.e., without exposure). This method and the time intervals used are illustrated in Figure 1. Conditional Poisson regression was used to estimate incidence rate ratios (with 95% confidence intervals [CIs]) for events occurring within the period of exposure as compared with the baseline period. Subgroup analyses were performed in patients

Figure 1. Risk periods in the self-controlled case series analysis



As shown in this example, the effect of each infectious stimulus was analyzed separately for the outcome of venous thrombosis. All individuals had at least one exposure to the stimulus (prescription of antibiotic), and had at least one venous thrombotic event. Risk periods were defined as total period of antibiotic drug use (not drawn to scale), which was further divided into the first week of use.

with either DVT only, PE only or PE with or without DVT and in patients with either a provoked or unprovoked first event. Additionally, incidence rate ratios were estimated for the three types of antibiotics.

2) Synergy between antibiotic use with genetic thrombophilia to venous thrombosis risk
The combination of genetic and environmental factors is often accountable for the development of venous thrombosis.[18] It is therefore likely that if infections increase the risk of venous thrombosis, the risk will be highest in combination with thrombophilic abnormalities such as factor V Leiden. We therefore assessed the extent of a joint effect on a multiplicative scale between antibiotic use and the presence of factor V Leiden, blood group non-O or prothrombin G20210A mutation to the risk for venous thrombosis in a case-only study.[19,20] In a case-only study one examines the association between an exposure and a genotype among case subjects only. The case-only study relies on the assumption that the two factors of interest are independently distributed in the general population which is a reasonable assumption for genetic risk factors and infectious diseases. Patients with first venous thrombosis were divided into those with a venous thrombotic event during a period of antibiotic use and patients who did not use antibiotics at the moment of the event. The odds ratios for genetic thrombophilia (i.e., factor V Leiden, the prothrombin mutation or blood group) then estimate the synergy index on the multiplicative scale.[20] This synergy index is the factor by which the odds ratios of genetic thrombophilia and antibiotic use have to be multiplied to obtain the joint odds ratio.

3) Antibiotic use and subsequent recurrent venous thrombosis risk
In a cohort study design we tested whether antibiotic use is associated with recurrent venous thrombosis. Duration of follow-up for recurrent venous thrombosis was estimated as the time at risk from the date of the index (first) thrombotic event to the end of follow-up. The end of follow-up was defined as the date of a recurrent event and in the absence of a recurrence, the date of filling in the follow-up questionnaire. If a patient did not fill in a questionnaire, they were censored at the last date we knew them to be recurrence free. This could be date of death (n=49), date of emigration (n=1), date of the last visit to the anticoagulation clinic (n=264) or the last moment known to be recurrence free from information of the MEGA case-control study (n=198). Details of assessment of end of follow-up have been described previously.[13] In the analyses we considered certain recurrent events only (n=367). Patients with uncertain recurrent events (n=120) were censored from this uncertain recurrent event onward.

Incidence rates of recurrent venous thrombosis were estimated as the number of events over the accumulated follow-up time and with person time split for periods with antibiotic treatment and periods without antibiotic treatment, without a wash-out period. This means that a patient with antibiotic use during follow-up contributes with one or several observation periods of exposed and non-exposed person-time. The association between antibiotic use and recurrent venous thrombosis was estimated

by means of Cox regression analysis with antibiotic use entered as a time-varying variable. Hazard ratios with corresponding 95% confidence intervals were estimated and corrected for age and sex. Exposure to antibiotics was first set at the total period of antibiotic use by the patient and additionally set at the first week of antibiotic use. The rest of the time was set at non-exposed person-time.

All statistical analyses were performed using SPSS, version 20.0 (IBM Corp., Armonk, NY) and Stata, version 12 (Stata Corp., College Station, Texas).

Results

2547 patients could be linked to the SFK data register and were included for analyses. Characteristics of these patients at the first venous thrombotic event are shown in Table 1. Median age of the patients was 51 years and 1197 (47%) patients were men. Most first venous thrombotic events were deep vein thrombosis (59%) and most first events were provoked by a provoking risk factor (68%). Baseline characteristics did not differ between those who could and could not be linked to SFK (Table 1).

1) Antibiotic use and first venous thrombosis risk

1584 patients with a first venous thrombotic event had at least one prescription of antibiotics in the period from February 1999 to September 2004. These patients were included in the SCCS analysis. During the aggregated period of antibiotic use the risk of a first venous thrombotic event was five-fold increased (Incidence rate ratio (IRR) 5.1; 95%CI, 4.1-6.3) as compared with periods without antibiotic use (Table 2). During the first week of antibiotic use the IRR was 5.3 (95%CI, 4.2-6.6)).

Clinically, a presentation of PE, and to a lesser extent DVT, may at first be misdiagnosed as an infection. We tried to reduce misclassification step by step. We excluded 13 individuals in whom such misclassification certainly played a role, based

Table 1. Clinical Characteristics*

	Patients linked to SFK	Patients not linked to SFK	Total
N (%)	2547 (54%)	2184 (46%)	4731 (100%)
Median age, years (range)	51 (18-70)	47 (18-70)	50 (18-70)
Male sex, n (%)	1197 (47%)	967 (44%)	2164 (46%)
DVT only, n (%)	1490 (59%)	1257 (58%)	2747 (58%)
PE +/- DVT, n (%)	1057 (41%)	927 (42%)	1984 (42%)
PE only, n (%)	826 (32%)	723 (33%)	1549 (33%)
Provoked†	1732 (68%)	1565 (72%)	3297 (70%)
Malignancy	247 (14%)	174 (11%)	421 (13%)
Trauma/surgery/immobilisation	1033 (60%)	869 (56%)	1902 (58%)
Plaster cast	107 (6%)	112 (7%)	219 (7%)
Estrogen use (women)	663 (61%)	687 (67%)	1350 (64%)
Pregnancy/puerperium (women)	86 (8%)	87 (8%)	173 (8%)
Travel >4 hours	367 (21%)	350 (22%)	717 (22%)
Unprovoked	742 (29%)	559 (26%)	1301 (28%)
Factor V Leiden, n (%)	344 (14%)	308 (14%)	652 (14%)
Prothrombin G20210A, n (%)	112 (4%)	106 (5%)	218 (5%)
Blood group non-O, n (%)	1590 (62%)	1323 (61%)	2913 (62%)

* At time of first venous thrombotic event
† Data were missing for some patients in some subgroups

Table 2. Incidence rate ratios of first venous thrombosis during exposure to all types of antibiotics

	Patients	Incidence rate ratio (95%CI)					
	N	All	DVT only	PE +/- DVT	PE only	Provoked	Unprovoked
All patients included							
Total duration of antibiotic use	1584	5.1 (4.1-6.3)	3.4 (2.5-4.8)	7.1 (5.4-9.3)	6.5 (4.8-8.9)	5.6 (4.4-7.1)	4.2 (2.7-6.4)
First week of antibiotic use	1584	5.3 (4.2-6.6)	3.5 (2.4-5.0)	7.5 (5.6-9.9)	6.9 (5.0-9.5)	5.5 (4.2-7.2)	5.2 (3.4-8.0)
Baseline period	1584	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
*Restriction analysis**							
Total duration of antibiotic use	1571	4.5 (3.6-5.6)	3.2 (2.3-4.5)	6.0 (4.5-8.1)	5.8 (4.2-8.0)	4.7 (3.7-6.2)	4.0 (2.6-6.3)
First week of antibiotic use	1571	4.6 (3.6-5.8)	3.2 (2.2-4.7)	6.2 (4.6-8.4)	6.0 (4.3-8.5)	4.6 (3.4-6.1)	5.0 (3.2-7.7)
Baseline period	1571	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)

*13 patients for whom misclassification was certain, based on information from discharge letters, were excluded from these analyses.

Table 3. Incidence rate ratios of first venous thrombosis during exposure to different types of antibiotics

	Patients	Incidence rate ratio (95%CI)					
	N	All	DVT only	PE +/- DVT	PE only	Provoked	Unprovoked
Penicillins, tetracyclines, macrolides (antibiotics prescribed for a wide range of infections)							
Total duration of antibiotic use	1357	4.7 (3.6-6.1)	2.7 (1.7-4.3)	6.9 (5.0-9.5)	6.7 (4.7-9.6)	4.7 (3.4-6.4)	5.1 (3.1-8.2)
First week of antibiotic use	1357	5.1 (3.9-6.7)	2.9 (1.8-4.7)	7.5 (5.4-10.5)	7.3 (5.0-10.5)	4.9 (3.5-6.9)	5.8 (3.5-9.3)
Baseline period	1571	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Flucloxacillin (antibiotic mainly prescribed for skin infection)							
Total duration of antibiotic use	218	4.1 (1.7-10.0)	3.8 (1.2-12.1)	5.1 (1.2-20.9)	3.2 (0.4-23.6)	5.4 (2.0-14.9)	2.1 (0.3-15.4)
First week of antibiotic use	218	4.9 (2.0-12.0)	4.7 (1.5-14.9)	5.8 (1.4-23.7)	3.7 (0.5-27.1)	6.7 (2.5-18.1)	2.5 (0.3-17.9)
Baseline period	1571	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Quinolones, nitrofurane derivatives, sulphonamides and trimethoprim (antibiotics mainly prescribed for urinary tract infections)							
Total duration of antibiotic use	622	3.4 (2.3-5.1)	3.2 (1.9-5.6)	3.6 (2.0-6.5)	3.3 (1.7-6.5)	4.0 (2.5-6.2)	1.7 (0.6-5.0)
First week of antibiotic use	622	3.0 (1.9-5.0)	3.4 (1.8-6.4)	2.7 (1.3-5.8)	3.0 (1.3-6.8)	3.2 (1.8-5.6)	2.6 (0.8-8.2)
Baseline period	1571	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)

on information from discharge letters. When we excluded these patients, the overall risk of venous thrombosis was 4.5-fold increased during antibiotic use (IRR 4.5; 95%CI, 3.6-5.6). For DVT only, the IRR remained 3.2-fold increased (95% CI, 2.2-4.7) during antibiotic use. Incidence rate ratios for a first provoked venous thrombotic event were somewhat higher than for a first unprovoked thrombosis.

Incidence rate ratios for the three types of antibiotics are shown in Table 3. There were 1357 patients who had at least one prescription of antibiotics for a wide range of infections, 218 patients who had at least one prescription of antibiotics mainly used for skin infections and 622 patients had at least one prescription of antibiotics mainly used for urinary tract infections. The risk of venous thrombosis was almost five-fold increased (IRR 4.7; 95%CI, 3.6-6.1) for the first group of antbiotics with a substantial difference between DVT (IRR~3) and PE (IRR~7). For antibiotics used mainly for infections of the skin the risk of venous thrombosis was about four-fold (IRR 4.1; 95%CI, 1.7-10.0) increased, with roughly similar risks for DVT and PE. For antibiotics used mainly for urinary tract infections the risk of both DVT and PE was three-fold increased, with IRR's of 3.2 (95%CI, 1.9-5.6) and 3.0 (95%CI, 1.3-6.8) for DVT and PE, respectively.

2) Synergy between antibiotic use with genetic thrombophilia

There were 114 patients with a first venous thrombotic event during a period of antibiotic use. Odds ratios, estimating the synergy indices for Factor V Leiden and prothrombin mutation, were both 1.0 (95%CI, 0.6-1.8) and 1.0 (95%CI, 0.4-2.5) respectively. This implies that the joint effect of both genetic factors and antibiotic use is equal to the product of the separate effects of both antibiotic use and the genetic factors (Table 4).[21] Given the effects of genetic variants and antibiotic use, this implies a high joint risk, which is about 9 for the joint presence of a genetic variant and antibiotics use. The synergy index for blood group non-O was somewhat lower (0.7; 95%CI, 0.5-1.1) and therefore the joint effect of both antibiotic use and blood group non-O does not become high.

3) Antibiotic use and subsequent recurrent venous thrombosis risk

Of 2547 patients included in this analysis 367 had a recurrent thrombosis, yielding an incidence rate of 29.1 /1000 person-years (95%CI, 26.3-32.3). During follow-up 1401 patients (55%) had at least one prescription of antibiotics. The incidence rate of recurrent venous thrombosis was 56.2/1000 person-years (95%CI, 29.2-108.0) during antibiotic use, while it was 28.8/1000 person-years (95%CI, 25.9-31.9) for periods without antibiotic use. The recurrence risk was two-fold increased during the use of antibiotics (HR 2.0; 95%CI, 1.1-4.0). During the first week of antibiotic use, the risk was 2.9-fold (95%CI, 1.4-6.1) increased.

Table 4. Multiplicative interaction between antibiotic use and genetic thrombophilia to venous thrombosis risk

Genetic thrombophilia	Venous thrombosis during antibiotic use		Odds ratio for joint effect
	+	-	(95%CI)
Factor V Leiden			
+	16	327	
-	84	1784	1.0 (0.6-1.8)
Prothrombin G20210A			
+	5	107	
-	95	2005	1.0 (0.4-2.5)
Blood group non-O			
+	65	1519	
-	35	586	0.7 (0.5-1.1)

Table 5. Age and sex adjusted risk of recurrent venous thrombosis in risk periods after antibiotic use

	Observation years (N)	Recurrent events	Incidence rate* (95%CI)	Hazard ratio† (95%CI)
Total duration of antibiotic use				
Baseline period	12439 (2547)	358	28.8 (25.9-31.9)	1 (reference)
Antibiotic use	160 (1401)	9	56.2 (29.2-108.0)	2.0 (1.1-4.0)
First week of antibiotic use				
Baseline period	12511 (2547)	360	28.8 (26.0-31.9)	1 (reference)
Antibiotic use	89 (1401)	7	78.7 (37.5-165.0)	2.9 (1.4-6.1)

*Per 1000 person-years
†Adjusted for age and sex

Discussion

Summary of findings

We found an increased risk for both first and recurrent venous thrombosis during periods of antibiotic use. Both for first and recurrent venous thrombosis relative risks were highest during the first week of use.

Incidence rate ratios of a first venous thrombotic event ranged from three to seven. Since symptoms of a venous thrombotic event might mimic an infection, we took several steps to reduce misclassification. After exclusion of patients for whom misclassification was likely and including patients with DVT only (for which we expect less misclassification than in patients with PE) we still found a three-fold increased risk of venous thrombosis, indicating that our results are robust. For antibiotics prescribed mainly for urinary tract infection, for which we expect no misclassification, we found an increased risk of DVT of 3.2 (95%CI, 1.9-5.6) and an increased risk of PE of 3.3 (95%CI, 1.7-6.5). In addition, we found a synergy index of around 1 between antibiotic use and both factor V Leiden and the prothrombin mutation, which leads to high joint relative risks. This appeared not to be the case for blood group non-O. We found a two-fold increased recurrence risk for patients with a history of venous thrombosis using antibiotics as compared with those patients not using antibiotics.

Previous studies

In the last decade several studies have been published that investigated the risk of a first venous thrombotic event after infections and inflammatory diseases.[6, 7, 22-24]

In a large register study from Denmark over 15 000 cases with venous thrombosis were matched to controls from the general population.[24] Within three months after a hospital diagnosed infection the risk of venous thrombosis was increased three-fold as compared with patients without infection (IRR 3.3; 95%CI, 2.9-3.8). The risk of venous thrombosis was almost three-fold increased after antibiotic treatment in the community (IRR 2.6; 95%CI, 2.5-2.8), with higher risks for antibiotics prescribed for both respiratory tract and skin or soft tissue infections than for antibiotics prescribed for urinary tract infections. The associations were strongest within the first two weeks and gradually declined thereafter. These results are quite similar to our findings. Ribeiro et al showed in the MEGA case-control study that self-reported pneumonia substantially increased the risk of venous thrombosis in the subsequent year (OR 4.8; 95%CI, 3.6-6.2) after adjustment for many confounding factors.[23] It was shown that the association could only partially be explained by a concurrent period of immobilization or lifestyle. In a large case-control study based on a general practice database from the UK 4.0% of DVT cases was reported to have a respiratory infection in the year before the index date as opposed to 2.3% in the controls.[22] An increased risk of DVT was found in the month following infection (OR 2.6; 95%CI, 1.6-4.3). In this study urinary tract infections were less strong risk factors for venous thrombosis than respiratory infections. There was only weak evidence for an association with subsequent DVT and no evidence of an

increased risk of PE following urinary tract infections. The authors suggest these latter findings might be explained by small numbers.

Misclassification of symptoms of either DVT or PE as an infection might have affected all of abovementioned studies. The study based on the UK general practice database[22] reduced possible misclassification by excluding patients with a respiratory infection in the month before PE. The other studies were not able to reduce misclassification. People with and without diagnosed infections probably are different in other aspects besides their infection, therefore comparison between individuals could be misleading and correction for potential confounders is crucial. In the large registry study from Denmark[24] correction for confounders affected results considerably and the covariate with the most influence was a measure of frailty or immobility. Although most of abovementioned studies corrected for many potential confounders, residual confounding remains possible. Smeeth and colleagues solved the part of the problem caused by intransient confounders by performing a self-controlled case study.[6] During the first week of a urinary tract infection the risk of both DVT and PE was increased two-fold. During the first week of a respiratory tract infection the risk of DVT was also increased two-fold (IRR 1.9; 95%CI, 1.5-2.4). Relative risks that we found are somewhat higher than the results from Smeeth (IRR ~3). This may be explained by the inclusion of objectively identified thrombotic events only, while events by Smeeth et al. came from an electronic database.

One previous study showed a moderately strong relation between inflammatory bowel disease and recurrent venous thrombosis[25] with a relative risk of 2.5 (95%CI, 1.4-4.2). This supports the hypothesis that infection/ chronic inflammation increases the risk of recurrent venous thrombosis as well.

Interpretation of our findings

Several explanations are possible for our findings of an increased risk of venous thrombosis during antibiotic use: 1) infections increase the risk of venous thrombosis through a systemic effect; 2) infections increase the risk of venous thrombosis through immobilisation/ bedrest; or 3) antibiotics have a direct effect on the risk of venous thrombosis. It has been described that oral application of some of the antibiotic drugs (i.e. macrolides, penicillins) can lead to overgrowth of Gram-negative bacteria in the gut.[26] This shift has been causally associated with entrance of Gram-negative bacteria into the blood stream and ultimately increased circulatory levels of lipopolysaccharides (LPS) inducing a pro-coagulant state.[27-29] This could lead to the hypothesis that some antibiotics might contribute to the development of clinical venous thrombosis by changing the gut microbiome. However, we have seen increased risks of venous thrombosis for all types of antibiotics and side-effects are rarely a class-effect. Since we have seen increased thrombosis risks for all types of antibiotics, amongst others antibiotics prescribed for urinary tract infection, immobilisation as the explanation for the increased risks is also improbable. This suggests the first explanation might be the right one.

Strong points

Strong points of this study are that for the association between antibiotic use and first venous thrombosis we used the self-controlled case series method. By using this method confounding by fixed factors, like the above mentioned frailty, is accounted for. Furthermore, since exposure to antibiotic treatment was recorded independent of the subsequent venous thrombotic event, biased ascertainment of exposure does not play a role. Requirements for the use of a self-controlled case series method are that the association concerns an acute event and a transient exposure, which was the case for our research question. Furthermore, the probability of exposure must not be altered by a previous event. We can safely assume that the probability of antibiotic use is not altered by a previous venous thrombotic event.

Another strong point of this study is that both first and recurrent venous thrombotic events were objectively confirmed. Other studies relied on thrombotic events reported in electronic databases often without diagnostic information. In our study possible recurrences were classified into certain recurrences and uncertain recurrences and only certain recurrences were taken into account. The main purpose of this was to distinguish extensions of a first event from truly new thrombosis.

Limitations

Some potential limitations should be mentioned as well. First, an early presentation of PE might be misdiagnosed as a respiratory tract infection. For DVT and infections of the skin of the leg the same may be true. To reduce this possibility of misclassification, we excluded patients for whom we were sure that such misclassification took place, from information of discharge letters. Furthermore, such misclassification will be unlikely for urinary tract infections and DVT and PE, and for skin infections and PE. During treatment for urinary tract infections the risk of both DVT only and PE only was still 3.2- and 3.3-fold increased in our study. In addition, the risk of PE during treatment for skin infections was 3.2-fold increased. Some misclassification of infectious diseases might have occurred since our definition of an infection was solely based on the antibiotic class that was prescribed. Second, our results may not be generalizable to all types of infection since we did not have data on antibiotic use during hospital stays and since we used antibiotic use as a proxy for infectious diseases, we do not have data on viral infections and the risk of venous thrombosis. Third, as cancer might increase the risk of both infections and venous thrombosis a diagnosis of cancer could account for some of the associations we observed. However, the results for patients with an unprovoked first venous thrombosis (in whom no patients with cancer were present) were in the same line as for total venous thrombosis; i.e. an increased risk of venous thrombosis at time of antibiotic use, especially in the first week after the start of antibiotic use. Therefore, cancer diagnoses do not explain our findings. Fourth, although we have used the self-controlled case series method to study the association between antibiotic use and first venous thrombotic events, in which intransient confounders do not play a role, residual transient confounders might account for some of the association we observed. Fifth,

in our analyses on recurrent venous thrombosis we were, because of small numbers, unable to correct for additional variables besides age and sex and residual confounding factors might play a role in the association we found there. Additionally, because of small numbers, we were not able to study different types of venous thrombosis and/or different types of antibiotics, so that we can not exclude misclassification of events. For the same reason we were not able to study the combined effect of antibiotic use and genetic thrombophilia on the risk of recurrences.

Conclusion

To conclude, individuals who receive antibiotics (which we used as a proxy for infection), have an approximately three-fold increased risk of a first venous thrombotic event and a two-fold increased risk of recurrent venous thrombosis. Our results should increase awareness of the risk of venous thrombosis in patients with infections, in treating physicians in and out of hospital. Furthermore, accuracy of treatment strategies might be improved by a revision of the current definition of 'unprovoked' events. Future clinical trials may be required to determine whether patients with prior venous thrombosis who use antibiotics should or should not receive thromboprophylaxis to decrease their risk of recurrence.

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Chapter 10

Summary and General discussion

Aim of this thesis

Although a long list of risk factors has been described for a *first* venous thrombotic event, the risk profile for *recurrent* venous thrombosis is not that well known. Also, data on factors able to predict the risk of recurrence are scarce. The aim of this thesis was to study several (modifiable) risk factors and predictors for their relation with recurrent venous thrombosis. In this chapter I provide an overview of the main findings. Furthermore, I will consider the clinical implications of this work and discuss directions for future research.

Overview of main findings

Incidence of recurrent venous thrombosis and known risk factors for recurrence

In **Chapter 2** the design and the first results from the MEGA follow-up study were described. Information on recurrent events from several sources of information was combined to obtain a valid estimate of the incidence of recurrent venous thrombosis. The overall incidence of recurrence in a group of patients with a first deep vein thrombosis of the leg or pulmonary embolism was 27.9 per 1000 person-years, with a 5-year cumulative incidence of 11%. These incidences were somewhat lower than reported in previous literature. This probably has to do with our use of a strict definition of a true recurrent event, to distinguish recurrence from an extension of the first. Men had about a two-fold increased risk of recurrent venous thrombosis as compared with women and a first idiopathic event was associated with a one and a half- to two-fold increased risk as compared with a first provoked event. Age did not affect recurrence risk. For deciding on the duration of anticoagulant treatment in patients with a first venous thrombotic event this information should be taken into account.

Cancer and (recurrent) venous thrombosis

A strong relation between cancer and venous thrombosis was identified already in the early 19th century. **Chapter 3** presented an overview of all knowledge gained on the incidence of and risk factors for cancer-associated venous thrombosis over the years. About 20-30 percent of all venous thrombotic events is cancer-associated and cancer increases the risk of thrombosis about four- to seven-fold. The risk of venous thrombosis in patients with cancer depends on several factors, e.g., cancer type and stage, treatment measures and patient-related factors. This information provides a basis for the identification of high-risk patients who could benefit from thromboprophylaxis and for further development and refinement of prediction models.

The risk of recurrent venous thrombosis in patients with cancer has not been studied extensively. In **Chapter 4** the risk of recurrent venous thrombosis in patients with cancer was evaluated, also in relation to time of diagnosis of the malignacy and in several types of cancer patients. Patients with cancer and thrombosis had an

increased risk of recurrent venous thrombosis compared with patients without cancer. Participants with cancer diagnosed before the first venous thrombotic event who died or had metastases had a two- to three-fold increased risk of recurrent thrombosis compared with patients without cancer, while patients with non-metastasized cancer or who did not die of cancer did not have an increased recurrence risk. Participants with cancer diagnosed *after* the first thrombosis had an increased recurrence risk, which was especially high in the first three months after cancer diagnosis (about five-fold compared with patients without cancer). Risk of recurrent venous thrombosis differed for different types of cancer, for different stages of cancer and for different time periods after cancer diagnosis. Currently, guidelines provide treatment recommendations for the group of patients with cancer and venous thrombosis as a whole.[1,2] Our study supports current thought that risk of recurrent venous thrombosis is not the same for all patients with cancer and that stratification of patients with cancer-associated venous thrombosis according to their recurrence risk is of relevance to offer these patients a better tailored treatment approach.

The pathophysiology underlying the association between cancer and venous thrombosis is largely unknown. Furthermore, it is not known in what way patients with cancer who develop thrombosis are different from those who do not. In **Chapter 5** several plasma coagulation factor levels (procoagulant, anticoagulant and fibrinolytic) were studied in four groups of individuals with and without cancer and with and without venous thrombosis. Increased levels of procoagulant coagulation factors in participants with thrombosis without cancer and even higher levels of these factors in participants with both venous thrombosis and cancer were found, suggesting generalized effects of procoagulant pathways in patients with cancer and emphasizing the importance of coagulation in cancer-associated venous thrombosis. Results were most pronounced for factor VIII and von Willebrand factor. Levels of factor VII were increased in participants with cancer and were unaffected by the presence or absence of thrombosis. The finding of slightly increased levels of anticoagulant proteins, free protein S and TFPI in participants with cancer and venous thrombosis is suggestive of an additional role of anticoagulant pathways in cancer. These data give more insight into the relation between venous thrombosis and cancer.

Risk factors and predictors for recurrent venous thrombosis

In **Chapter 6** the predictive value of coagulation factor VIII levels for recurrent venous thrombosis was studied. Recurrence rates steadily increased with higher factor VIII activity levels and patients in the highest category of FVIII (>200 IU/dL) had a three-fold higher recurrence rate than patients in the lowest category (≤100 IU/dL). Results were robust in several sensitivity analyses and factor VIII was able to predict recurrence rates over a long time period. Adding factor VIII to an existing prediction model (DASH-score) improved its predictive value, and after replacing D-dimer by factor VIII, the model performed equally well if not better. Factor VIII will be able to refine recurrence risk estimation at an individual level and factor VIII should be considered in recurrence

prediction tools. Whether measurement of factor VIII levels is to be preferred over D-dimer levels, which has as a major disadvantage that it can only be reliable measured after discontinuation of anticoagulant treatment, should be a topic for further research.

Despite guideline recommendations to discontinue hormonal contraceptive use after a first thrombotic event, still a sizeable proportion of women continue or start using hormonal contraceptives after a venous thrombosis. In **Chapter 7** the effect of this use on the recurrence risk was studied in premenopausal women. Hormonal contraceptive use during follow-up was associated with a two-fold increased risk of a recurrence. In particular, the use of combined oral contraceptives was associated with an almost three-fold increased risk. The data suggest that it would be wise for women with a history of venous thrombosis to adhere to the guidelines and refrain from this modifiable risk factor. Recurrence rate among hormonal IUD users was similar as in non-users, suggesting that a levonorgestrel-releasing IUD may be safely used after a first event.

The risk of thrombosis after long-haul travel in those with a history of venous thrombosis is not known, while thromboprophylaxis may be indicated in these patients. In **Chapter 8** the relation between long-haul travel (>4 hours) and recurrent venous thrombosis was studied. The risk of recurrent venous thrombosis was not increased in participants with recent long-haul travel, either after a flight or after other types of long-haul travel. This would suggest thromboprophylaxis is not needed in individuals with a history of venous thrombosis undertaking a long-haul trip. However, the lack of an effect may also be explained by a different health status in people who travel versus those who do not that could not be completely adjusted for. Also, for immobility in daily life due to seated work or confinement to a wheelchair no association with recurrent venous thrombosis was found. Again, results might be explained by residual confounding or perhaps use of anticoagulant treatment which we could not fully adjust for. Further studies are needed to give a definite answer as to whether refraining or intervening on this modifiable risk factor is beneficial.

The aim of **Chapter 9** was to study whether individuals who receive antibiotic treatment (as a proxy for infectious disease), have an increased risk of first or recurrent venous thrombosis. By means of a self-controlled case series study design the risks of both a first deep vein thrombosis and a first pulmonary embolism were found to be increased at least three-fold during antibiotic use. The major advantage of a self-controlled case series design is that fixed confounders, like frailty, do not play a role. For recurrent venous thrombosis similar results were found, with a two-fold increased risk of recurrent venous thrombosis during periods of antibiotic use as compared with periods with no use. These results should increase awareness in clinicians of the risk of venous thrombosis in in- and out-patients who are ill and get antibiotics. Furthermore, acute infectious disease should be added to the list of provoking factors for venous thrombosis.

Directions for future research

This thesis adds to the current knowledge on risk factors and predictors for recurrent venous thrombosis. This type of research is sometimes called prognostic factor research, in which prognostic factors are defined as factors able to distinguish between groups of people with a different average prognosis.[3] Such prognostic factors, however, do not yet provide enough distinctive power on their own to classify patients individually at high or low risk of recurrence. After identification of such prognostic factors the next step would be to create a prognostic model, the aim of which is to develop, validate and test the impact of statistical models that predict individual risks of a future outcome. For an individual with a given state of health, in our case patients with a first venous thrombotic event, a prognostic model converts the combination of predictor values to estimates of the risk of experiencing a specific endpoint within a specified time period. [4] Therefore, after this thesis, the next step should be to focus on taking all factors together and use them as *building blocks* for a prognostic model, which will be able to predict recurrences at a much more refined and individual level.

Current Prediction models

Currently three prediction models have been published for recurrent venous thrombosis; 1) the Men continue and HERDOO2 rule; 2) the Vienna prediction model and 3) the DASH score.

1. The 'Men continue and HERDOO2' rule (see Table 1) was published in 2008 by Rodger and colleagues.[5] In this multicentre prospective study, over 600 patients with a first unprovoked venous thrombosis were followed for a mean of 18 months. Clinical characteristics as well as blood samples were collected during anticoagulant treatment five to seven months after the start of treatment. The authors sought to determine the clinical predictors or combinations of predictors that identify patients with an annual recurrence risk of less than 3% after taking six months of anticoagulant treatment, which they considered sufficiently low to discontinue oral anticoagulants.

   The authors found no combination of clinical predictors for identifying a low-risk subgroup of men, which is why men were advised to continue anticoagulant treatment long-term. Additionally, women with ≥2 of the following risk factors: postthrombotic signs (hyperpigmentation, edema or redness in either leg), D-dimer level ≥250 µg/L, BMI ≥30 kg/m^2 and age ≥65 years were advised to continue treatment. Authors concluded that women with a score of ≤1 (52% of women) could safely discontinue anticoagulant treatment after six months following a first unprovoked event.

Table 1. 'Men continue and HERDOO2'	
Predictive factors	Advice
Men	Long-term anticoagulant treatment
None	
Women	Long-term anticoagulant treatment if score ≥2
	Score
• Postthrombotic signs (hyperpigmentation, edema or redness in either leg)	1
• D-dimer level ≥250 µg/L (during anticoagulant treatment)	1
• BMI ≥30 kg/m^2	1
• Age ≥65 years	1

2. Two years after the 'Men continue and HERDOO2 rule' the Vienna prediction model (see Table 2) was published by Eichinger and colleagues.[6] In this multicentre prospective cohort study over 900 patients with a first unprovoked venous thrombosis were followed for recurrence with the aim to develop a simple risk assessment model. Median follow-up of the patients was 43 months after discontinuation of anticoagulant treatment. Blood was drawn shortly after discontinuation of treatment. Eichinger et al. found male sex, proximal deep vein thrombosis, pulmonary embolism and elevated D-dimer levels to be associated with recurrence. Using these variables a nomogram (see Figure) was developed that can be used to calculate risk scores and to estimate cumulative probabilities of recurrence. C-statistics for the models at 12 and 60 months were 0.67 and 0.65, respectively. Additionally, a web-based risk calculator was developed (http://cemsiis.meduniwien.ac.at/en/kb/science-reserach/software/clinical-software/recurrent-vte/), to calculate risk scores and cumulative probabilities of recurrence in an individual patient. Based on these predicted risks the physician can decide whether to stop or continue anticoagulant treatment.

Table 2. Vienna prediction model	
Predictive values	*Points*
Sex	
Males	60
Females	0
Site of first venous thrombotic event	
Distal Deep Vein Thrombosis	0
Proximal Deep Vein Thrombosis	70
Pulmonary Embolism	90
D-dimer levels	
Continuous	0-100

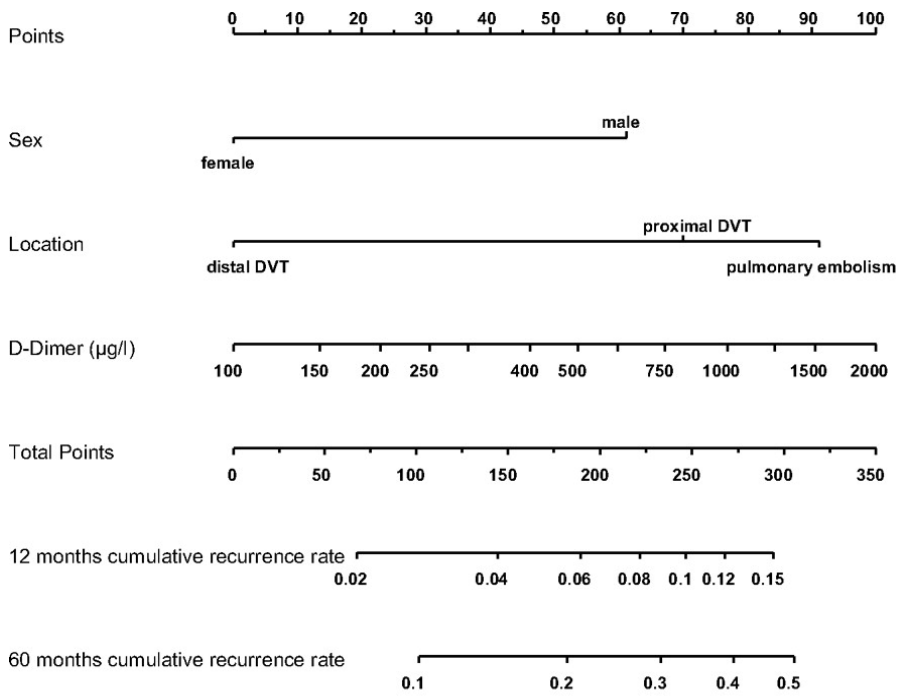

Figure from Eichinger S, Heinze G, Jandeck LM, Kyrle P. *Risk assessment of recurrence in patients with unprovoked deep vein thrombosis or pulmonary embolism.* Circulation 2010;121:1634. With permission from Wolters Kluwer Health, Inc.

An update of the Vienna prediction model was published in 2014.[7] Over 500 patients with an unprovoked venous thrombosis were seen at 3 weeks (baseline), 3, 9, 15 and 24 months after discontinuation of anticoagulant treatment. At every time point blood for measurement of D-dimer levels was collected. Separate nomograms for the prediction of recurrent venous thrombosis were developed for each time point including the same variables as the original Vienna prediction model. Another web-based calculator was developed for this dynamic Vienna prediction model which allows prediction of recurrence from any points between baseline and 15 months after anticoagulant treatment discontinuation (http://www.meduniwien.ac.at/user/georg.heinze/dvpm/).

3. The DASH score (see Table 3) was developed in 2012 by Tosetto and colleagues by pooling individual patient data from seven prospective cohort studies.[8] Over 1800 patients with an unprovoked venous thrombosis, treated for at least three months, were included for analyses and followed for a median of 22 months. Blood was sampled several weeks after discontinuation of anticoagulant treatment. Abnormal D-dimer, Age <50 years, male Sex and venous thrombosis not associated with

Hormonal therapy (in women) were the main predictors of recurrence and were used to derive the DASH score. C-statistic for the model was 0.71. Patients with a score ≤1 had an annualized recurrence risk of 3%, while the risk was over 12% for patients with score ≥3. With 52% of patients falling into the first group, the authors concluded that in about half of the patients with unprovoked thrombosis life-long anticoagulation might be avoided.

Table 3. DASH score

Predictive factors	Score
Elevated D-dimer levels one month after discontinuation of anticoagulant treatment	2
Age <50 years	1
Male sex	1
Women taking oral contraceptives	-2

Low risk of recurrence when the score ≤1

Before a prediction model can be adopted in practice it is necessary to show that predictions of the model are valid in another sample of patients than the specific context of the sample that was used for model development. This is important because the predictive performance of a model estimated on the development data is often optimistic, due to multiple testing with a limited sample size.[4] Such external validation of abovementioned models was not performed until recently which is probably why the models are not currently used in the clinic. The only model externally validated so far is the Vienna prediction model.[9,10] Pooled individual data from five prospective studies were used to test the prognostic value of the model.[9] The authors concluded that the ability to distinguish risk of recurrent venous thrombosis was at least as good in the validation cohort as in the derivation cohort, with a calibration slope of 1.17 (95%CI; 0.71-1.64) and a C-statistic of 0.63 (vs 0.65 in the derivation cohort). Performance of the model was less in an external cohort of elderly patients.[10] Lastly, studies would have to be performed in which the impact of a prediction model on decision making and patient outcomes is investigated.

Some disadvantages and differences between the three models should be outlined. In the 'Men continue and HERDOO2' model no combination of clinical predictors for identifying a low-risk subgroup of men was found and all men were advised to continue anticoagulant treatment on the long-term. The authors did not have an explanation for this finding, although it seems unlikely that risk prediction is not possible at all in men. In this thesis the predictive value of levels of factor VIII for recurrent venous thrombosis was described (**Chapter 6**). Recurrence rates increased steadily with increasing factor VIII also in men. This suggests that not all men have a similar risk of recurrent venous thrombosis. Furthermore, in the HERDOO2 model levels of D-dimer were measured while patients were on anticoagulant treatment. Although in the clinic

it would be a major advantage to assess recurrence risks while patients are still on anticoagulant treatment, several studies have shown that only 5-12% of patients have increased D-dimer levels during treatment with vitamin K antagonists.[11-13] One of abovementioned studies has actually suggested to omit the D-dimer measurement during anticoagulation.[13]

The Vienna prediction model has recently been updated for several time points after discontinuation of anticoagulant treatment and the model has both been internally and externally validated with reasonable outcomes. The model enables to predict recurrence rates both in men and women and D-dimer levels were measured after withdrawal of anticoagulant treatment. However, the Vienna model is considered complex for routine use. The model does not provide a simple scoring system and cut-off value for discontinuation or extension of anticoagulant treatment. This is probably the reason why this model is still not used much in the clinic.

The DASH-score provides a simple scoring system for both men and women and a cut-off value for when anticoagulant treatment may be safely discontinued. Interestingly, the DASH-model indicates age less than 50 as a risk factor for recurrence, while the Vienna model attributes a higher risk to age greater than 65. In the MEGA follow-up study age was not associated with recurrent venous thrombosis (Chapter 2). Additionally, hormone use at the first event (by women) is indicated to decrease the risk of recurrence, while in this thesis (Chapter 7) similar rates of recurrence for women who did or did not use hormones at time of the first event are reported.

Development of a prediction model in the MEGA follow-up study

The MEGA follow-up study is favourable for development of a prognostic model for recurrent venous thrombosis. In total, nearly 5000 patients with a first venous thrombotic event were followed over a long period of time for recurrences.

Currently existing prognostic models (described above) all focus on patients with a first unprovoked event. This is because the recurrence risk is higher in these patients as compared with patients who had an event related to surgery or trauma for example.[14] It is currently unknown for how long these patients should receive anticoagulant treatment. However, recurrent venous thrombosis in patients with a provoked first event is not uncommon.[15] In this thesis (Chapter 6) we have shown that although recurrence rates are low in patients with provoked first events, risk stratification is still possible in these patients. With a recurrence rate of 4% per year in patients with a provoked first event and factor VIII levels >200 IU/dL prolonged anticoagulation may still be warranted given the incidence rate of major bleeding of 1-2% per year.[16,17] Furthermore, the classification of an event as either unprovoked or provoked is artificial and controversial. In principle nearly all events are provoked by one or more factors. A prognostic model based on the MEGA follow-up study should take all patients into account, both patients with a provoked as well as patients with an unprovoked first event.

In the MEGA study blood was collected only in patients with their date of first thrombosis before June 1st 2002. This was for logistic reasons only. After multiple imputation of the factors measured in blood, all participants of the MEGA follow-up study can be included for the development of a prognostic model. Often recommendations are made for the maximum number of preselected predictors that should be estimated in a prognostic model. The reason for this is that including too many predictor variables would lead to the situation of 'overfitting' of a model, which causes optimism about a model's performance in new subjects out of the data under study. A common opinion is that the ratio of events to predictors (events per variable; EPV) should not be less than 10:1.[18] In 4731 patients included in the MEGA follow-up study, 673 recurrent venous thrombotic events were identified, meaning that 67 predictor variables may be preselected for the development of the prognostic model. This is more than the currently available prediction models could include, 9 (91/10), 17 (176/10) and 23 (239/10) for the HERDOO2, Vienna and DASH models, respectively.[5,6,8]

Since patients with a cancer-associated first venous thrombosis are so distinct from patients with a non-cancer associated event with regard to clinical characteristics and mortality risk, a separate prognostic model could be useful for this group of patients. Louzada et al. have published a prediction score for recurrent venous thrombotic events in patients with a cancer-associated first event, including four independent predictors (sex, primary tumor site, stage and prior venous thrombosis).[19] The performance of the score in an external cohort was reasonable.[20]

A prognostic model for the prediction of recurrent events on the moment of discontinuation, or the moment of deciding on whether to discontinue or extend anticoagulant treatment, will be most useful for the clinic. This time-point is where follow-up should start in the MEGA follow-up study when developing a prognostic model. Ideally, the model includes factors that can be measured or collected during the anticoagulant treatment period, so that a decision can be made before anticoagulant treatment is unrightfully stopped (or continued). Factors that should in any event be preselected for the model, and are available in the MEGA follow-up study, are: age, sex, type of first event (provoked vs unprovoked), first PE vs DVT, proximal vs distal DVT, BMI and levels of factor VIII (given the results from Chapter 6). Of note, genetic factors might additionally play a role in a prognostic model for recurrent venous thrombosis, and should therefore be included in the list of preselected variables. The development of a prognostic model in the MEGA follow-up study should result in an easy to determine risk score and cut-off value for decisions on the duration of anticoagulant treatment.

To be able to predict the risk of recurrence at different moments in time, e.g., directly after the first event, at the moment of intended discontinuation of anticoagulant treatment or several years after a first event, a prediction model should be time-dependent. For this, clinical characteristics as well as factors measured in blood should be collected at several time-points in a prospective follow-up study. Unfortunately, in the MEGA follow-up study we have data on factors measured in blood from one time-point only.

Conclusion

Secondary prevention of recurrent venous can be achieved in two ways, either by elimination of modifiable risk factors or by extending the anticoagulant treatment period in patients at high risk of recurrence. The aim of this thesis was to identify modifiable risk factors for as well as factors that might be able to predict recurrent venous thrombotic events. This thesis reports on an increased risk of recurrences in women who continue or start using hormonal contraceptives after a first venous thrombotic event, suggesting that refraining from this modifiable risk factor decreases the risk of recurrence. Furthermore, this thesis describes several factors, male sex, unprovoked first event, levels of coagulation factor VIII and antibiotic use to be associated with recurrent venous thrombosis. These factors should eventually be taken together and used to *build* a prognostic model, which will be able to predict recurrences at a refined and individual level.

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Appendices

Nederlandse samenvatting (Summary in Dutch)

Dankwoord (Acknowledgements in Dutch)

Curriculum Vitae

Nederlandse samenvatting

Inleiding

Veneuze trombose, voornamelijk optredend als een diep veneuze trombose in het been of een longembolie, komt voor bij 1 tot 2 per 1000 mensen per jaar. Het is qua frequentie de derde cardiovasculaire ziekte wereldwijd en heeft in de vorm van een longembolie ernstige, soms dodelijke, gevolgen. Na een eerste veneuze trombose is de kans op een recidief trombose groot. Ongeveer 25% van de mensen met een eerste trombose krijgt binnen vijf jaar een recidief. Een recidief trombose kan voorkomen worden door het wegnemen van risicofactoren óf, wanneer dit niet mogelijk is, door het verlengen van de behandeling met antistollingsmiddelen. Antistollingsmiddelen kunnen bij patiënten echter resulteren in bloedingen. Om deze reden zou alleen bij patiënten met een hoog risico op recidief trombose de periode van behandeling met antistolling verlengd moeten worden.

Hoewel een lange lijst met risicofactoren beschreven is voor een eerste veneuze trombose, is het risicoprofiel voor een recidief trombose nog niet goed bekend. Ook is niet veel bekend over voorspellers van een recidief.

Het doel van dit proefschrift was daarom om verschillende risicofactoren, met name die welke weggenomen kunnen worden, en voorspellers voor recidief trombose te bestuderen.

Overzicht van de belangrijkste resultaten van dit proefschrift

Incidentie van recidief veneuze trombose en bekende risicofactoren

In **Hoofdstuk 2** beschreef ik de opzet en eerste resultaten van de MEGA-vervolgstudie. Informatie over recidief trombose van verschillende bronnen werd gecombineerd om een precieze schatting te krijgen van de incidentie. De incidentie van recidief veneuze trombose was 27.9 per 1000 persoonsjaren in een groep patiënten met een eerste diep veneuze trombose van het been of een longembolie. De 5-jaars cumulatieve incidentie was 11%. Mannen hadden een tweemaal hoger risico op recidief veneuze trombose dan vrouwen en een eerste onverklaarde trombose was geassocieerd met een anderhalf tot twee keer verhoogd risico vergeleken met na een uitgelokte trombose. We vonden geen relatie tussen leeftijd en het recidiefrisico. Voor het bepalen van de juiste duur van behandeling met antistolling in patiënten met een eerste trombose dient deze informatie te worden meegenomen.

Kanker en veneuze trombose

De sterke relatie tussen kanker en veneuze trombose werd al in de 18^{e} eeuw beschreven. In **Hoofdstuk 3** gaf ik een overzicht van de kennis over de incidentie van en risicofactoren voor kanker-geassocieerde trombose die sindsdien bekend is geworden. Ongeveer 20-30 procent van alle veneuze tromboses is kanker-geassocieerd en kanker

blijkt het risico op trombose vier tot zeven keer te verhogen. Het risico op veneuze trombose in patiënten met kanker hangt af van verschillende factoren, onder andere het type kanker en het stadium, behandelingen, en patiënt-gerelateerde factoren. Deze informatie vormt de basis voor de identificatie van hoog-risico patiënten die baat zouden kunnen hebben bij tromboseprofylaxe én voor het ontwikkelen en verfijnen van predictiemodellen.

Het risico op recidief veneuze trombose in patiënten met kanker is nog niet goed bestudeerd. In **Hoofdstuk 4** heb ik dit risico in patiënten met kanker geëvalueerd, ook in relatie tot de tijd na de kankerdiagnose en voor verschillende types kanker. Patiënten met kanker en trombose hadden een verhoogd risico op recidief trombose, vergeleken met deelnemers zonder kanker. Patiënten met kanker die gediagnosticeered werd voor de eerste trombose hadden enkel een twee tot drie keer verhoogd risico op recidief trombose vergeleken met deelnemers zonder kanker, wanneer de kanker nog actief was na trombose (bij patiënten die uitzaaiingen hadden of de patiënten die later overleden aan kanker). Dit terwijl patiënten met kanker die waarschijnlijk in remissie was na trombose geen verhoogd risico hadden. Patiënten met kanker die gediagnosticeerd werd *na* de eerste trombose hadden ook een verhoogd recidiefrisico, dat met name hoog was in de eerste drie maanden na de kankerdiagnose (vijf keer verhoogd vergeleken met deelnemers zonder kanker). Het risico op recidief trombose verschilde voor verschillende types en stadia van kanker en voor verschillende tijdsperioden na de kankerdiagnose. Op dit moment geven de richtlijnen gelijke adviezen voor de hele groep patiënten met kanker en trombose tezamen. Hoofdstuk 4 laat zien dat het risico op recidief trombose niet voor iedereen gelijk is en dat het stratificeren van patiënten op basis van hun recidiefrisico belangrijk is om hen een beter passende behandeling te geven.

Het onderliggende mechanisme van de relatie tussen kanker en veneuze trombose is grotendeels onbekend. Bovendien is het niet bekend waarin patiënten met kanker die trombose ontwikkelen verschillen van patiënten die dat niet doen. In **Hoofdstuk 5** onderzocht ik concentraties van verschillende stollingsfactoren (procoagulant, anticoagulant en fibrinolytisch) in het bloedplasma van vier groepen deelnemers, deelnemers met en zonder kanker en met en zonder veneuze trombose. Wij vonden verhoogde spiegels van procoagulante stollingsfactoren in deelnemers mét trombose zonder kanker en nog hogere concentraties van deze factoren in deelnemers met zowel trombose als kanker. Dit suggereert dat procoagulante mechanismen een rol spelen in patiënten met kanker en benadrukt het belang van stolling in kanker-geassocieerde trombose. Resultaten waren het meest uitgesproken voor spiegels van stollingsfactor VIII en von Willebrand factor. Concentraties van stollingsfactor VII waren verhoogd in deelnemers met kanker en werden niet beïnvloed door de aan- of afwezigheid van trombose. Mijn bevinding dat de anticoagulante factoren, vrij proteïne S en TFPI in deelnemers met kanker en veneuze trombose verhoogd waren, suggereert een rol van de anticoagulante eiwitten in kanker. Deze data geven meer inzicht in de relatie tussen veneuze trombose en kanker.

Risicofactoren en voorspellers van recidief veneuze trombose

In **Hoofdstuk 6** onderzocht ik de voorspellende waarde van de concentratie van stollingsfactor VIII voor recidief veneuze trombose. De incidentie van recidieven nam gestaag toe met hogere factor VIII activiteit spiegels en deelnemers in de hoogste categorie van factor VIII (>200 IU/dL) hadden een drie keer verhoogd recidiefrisico vergeleken met deelnemers in de laagste categorie (≤100 IU/dL). De resultaten waren robuust in verschillende sensitiviteitsanalyses en deze informatie over factor VIII voorspelde de incidentie van recidieven over een lange tijdsperiode . Wanneer ik factor VIII toevoegde aan een bestaand predictiemodel, de DASH-score, verbeterde dit zijn voorspellende capaciteit, en na het vervangen van concentraties van D-dimer door factor VIII presteerde het model gelijk, zo niet beter. Factor VIII metingen kunnen de schatting van het recidiefrisico verfijnen en deze meting zou moeten worden betrokken in de ontwikkeling van predictiemodellen. Of het meten van factor VIII te prefereren is boven het meten van D-dimeer, dat als groot nadeel heeft dat het alleen betrouwbaar gemeten kan worden ná het stoppen met antistollingsmiddelen, zal in toekomstig onderzoek moeten worden uitgezocht.

Ondanks dat de huidige richtlijnen vrouwen na een eerste veneuze trombose aanraden te stoppen met het gebruik van hormonale anticonceptiva, gaat een aanzienlijk aantal vrouwen door of begint met het gebruik hiervan. In **Hoofdstuk 7** onderzochten wij het effect van dit gebruik op het recidiefrisico in premenopauzale vrouwen. Het gebruik van hormonale anticonceptiva na de eerste trombose was geassocieerd met een tweemaal verhoogd risico op een recidief. In het bijzonder was het gebruik van gecombineerde orale anticonceptiva geassocieerd met een drievoudig verhoogd risico. Het is van belang dat de richtlijnen gevolgd worden wat betreft het afzien van het gebruik van orale anticonceptiva na een veneuze trombose. De incidentie van recidief trombose onder vrouwen met een hormoonafgevend spiraaltje was gelijk aan die in vrouwen die geen anticonceptiva gebruikten, wat suggereert dat een spiraaltje met levonorgestrel veilig kan worden gebruikt na een eerste trombose.

Het risico op veneuze trombose na een lange reis in diegenen met een geschiedenis van trombose is niet bekend. Tromboseprofylaxe zou geïndiceerd kunnen zijn in deze patiënten. In **Hoofdstuk 8** onderzocht ik de relatie tussen lange reizen (>4 uur) en recidief veneuze trombose. Het risico was niet verhoogd in deelnemers na een recente lange reis, zowel na een lange vliegreis als na een lange reis met een ander transportmiddel. Dit suggereert dat tromboseprofylaxe niet nodig is in patiënten met een geschiedenis van veneuze trombose die een lange reis gaan maken. Ook voor immobiliteit in het dagelijkse leven, bijvoorbeeld door het uitvoeren van zittend werk of het gebruik van een rolstoel, werd geen relatie met recidief trombose gevonden. Het is echter mogelijk dat ik niet voor alle confounding factoren heb kunnen corrigeren. Daarom zijn meer studies nodig om een definitief antwoord te geven op de vraag of patiënten baat hebben bij tromboseprofylaxe tijdens periodes van immobiliteit of dat patiënten beter geen lange reizen kunnen maken.

Het doel van **Hoofdstuk 9** was om te bestuderen of tijdens het gebruik van antibiotica (als een maat voor infectieziekten) het risico op een eerste of recidief veneuze trombose verhoogd is. Door middel van een 'self-controlled case series' studieopzet vond ik een tenminste drievoudig verhoogd risico op zowel een eerste diep veneuze trombose als een longembolie tijdens antibioticagebruik. Het belangrijkste voordeel van een 'self-controlled case series' studie is dat niet-tijdsafhankelijke verstorende variabelen, zoals fitheid van de patiënt, geen rol spelen. Voor recidief trombose werden soortgelijke resultaten gevonden, met een verdubbeld risico op recidief trombose tijdens periodes van antibioticagebruik, vergeleken met periodes van geen gebruik. Deze resultaten laten zien dat artsen zich bewust moeten zijn van het risico op veneuze trombose bij zowel patiënten in als buiten het ziekenhuis die antibiotica krijgen. Verder zouden acute infecties toegevoegd moeten worden aan de lijst van uitlokkende factoren voor veneuze trombose.

Aanbevelingen voor toekomstig onderzoek

Dit proefschrift draagt bij aan de kennis van risicofactoren en voorspellende factoren voor recidief veneuze trombose. Dit type onderzoek wordt soms 'prognostic factor research' genoemd, waarin prognostische of voorspellende factoren gedefinieerd worden als factoren die groepen mensen kunnen onderscheiden met een verschillend risico op een bepaalde uitkomst. Zulke voorspellende factoren op zichzelf hebben echter nog niet voldoende onderscheidend vermogen om patiënten individueel te kunnen classificeren op een hoog of laag risico op recidieftrombose. Na identificatie van zulke voorspellende factoren is de volgende stap om een predictiemodel te maken. Voor een individu met een bepaalde gezondheidsstatus, in ons geval patiënten met een eerste veneuze trombose, zet een predictiemodel de combinatie van voorspellende factoren om tot schattingen van het risico op een specifiek eindpunt in een specifieke tijdsperiode. Daarom is de volgende stap alle factoren samen te voegen en deze te gebruiken als bouwstenen voor een model, dat recidief trombose op een meer verfijnd en individueel niveau zal kunnen voorspellen.

De MEGA-vervolg studie is zeer geschikt hiervoor. Met bijna 5000 deelnemers en 700 recidieven kunnen veel factoren geïncludeerd worden en getoetst op hun voorspellende waarde in het model. Anders dan de drie bestaande predictiemodellen voor recidief veneuze trombose zou dit model zich moeten richten op patiënten met zowel een uitgelokte als een idiopathische eerste trombose. Recidief veneuze trombose is niet ongewoon bij patiënten met een eerste uitgelokte trombose en stratificatie van het recidiefrisico is mogelijk en klinisch relevant in deze groep patiënten.

Conclusie

Preventie van recidief veneuze trombose kan worden bereikt op twee manieren, óf door het wegnemen van risicofactoren óf door de behandeling met antistollingsmiddelen te verlengen in patiënten met een hoog risico op recidieven. In dit proefschrift heb ik

risicofactoren voor recidief trombose onderzocht, alsmede factoren die een recidief kunnen voorspellen. Dit proefschrift laat een verhoogd recidiefrisico zien bij vrouwen die beginnen of doorgaan met hormonale anticonceptiva na een eerste trombose. Dit betekent dat het wegnemen van deze risicofactor het recidiefrisico zal verlagen. Verder laten we in dit proefschrift een associatie zien tussen verschillende factoren: man-zijn, een idiopathische eerste trombose, spiegels van stollingsfactor VIII en antibiotica gebruik, en recidief veneuze trombose. Deze factoren zullen uiteindelijk moeten worden samen genomen en gebruikt om een predictiemodel te *bouwen,* dat in staat zal zijn om recidief veneuze trombose te voorspellen op een meer verfijnd en individueel niveau.

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Jasmijn

Curriculum Vitae

Jasmijn Fleur Timp werd geboren op 14 januari 1988 in Zuidland. In 2006 behaalde zij haar VWO diploma cum laude aan het Penta College Angelus Merula in Spijkenisse, waarna zij aan de studie Biomedische Wetenschappen begon aan de Universiteit Leiden. Tijdens het tweede jaar van haar studie volgde zij zes maanden lang vakken aan het Karolinska Institutet in Stockholm. Tijdens haar master Biomedische Wetenschappen in Leiden, die zij in 2011 cum laude afsloot, volgde zij cursussen bij de afdeling Klinische Epidemiologie van het LUMC en deed daar haar afstudeerstage.

In 2011 werd Jasmijn aangesteld als promovenda op de afdeling Klinische Epidemiologie in Leiden onder leiding van Prof. dr Frits Rosendaal, Dr Suzanne Cannegieter en Dr Willem Lijfering. Tijdens het promotietraject volgde zij verschillende epidemiologische cursussen die zullen leiden tot haar registratie als Epidemioloog B. De resultaten van haar onderzoek naar risicofactoren voor recidief veneuze trombose zijn in dit proefschrift beschreven. Verder heeft zij haar resultaten gepresenteerd op nationale en internationale congressen en ontving hiervoor de Young Investigator Award van de International Society on Thrombosis and Hemostasis (2011, 2012 en 2013) en de Award for Scientific Excellence van de Nederlandse Vereniging voor Trombose en Hemostase (2012).

Jasmijn heeft haar promotie afgesloten met een rondreis van drie maanden door Australië en Nieuw-Zeeland. Hierna zal zij nog een jaar als postdoc werkzaam zijn op de afdeling Klinische Epidemiologie in Leiden om een prognostisch model voor recidief trombose te bouwen.