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Clinical and biochemical risk factors for first and recurrent episodes of venous thrombosis

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Clinical and biochemical risk factors for first and recurrent episodes of venous thrombosis

Sverre C. Christiansen



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Clinical and biochemical risk factors for first and recurrent episodes of venous thrombosis

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Part 1

General introduction

Venous thrombosis is a common disease with major effects on mortality and morbidity. In this chapter we will review the major features of venous thrombosis, and in particular the risk factors for first and second events.

First and recurrent events of venous thrombosis

Venous thrombosis (VT) is the formation of an obstructive blood clot within the venous blood flow, usually in the deep veins of the leg (deep vein thrombosis (DVT)). The clinical picture includes signs of impaired blood flow and classical local inflammation (erythema, pain, warmth, and swelling) (1). Pulmonary embolism (PE) occurs when the blood clot dislodges from the deep veins, passes via the right heart into the lungs and gets stuck in the pulmonary arteries. The clinical presentation will then often extend to involve chest pain at inspiration, dyspnoea, tachycardia, hypoxia, and sometimes haemoptoa (1). DVT and PE together are termed venous thrombosis, and PE occurs in approximately 1/3 of all cases (Table 1).

The annual incidence of a first episode of VT is approximately 1-3 per 1000 individuals per year (Table 1, 1-6), and increases with age (1-6).

Although not very common, VT is a potential lethal disorder, the cause of co-morbidity due to venous insufficiency or pulmonary hypertension, and lays a major financial burden on society in means of diagnostic procedures, hospitalisation, and clinical care. The treatment (thrombolysis, anticoagulation) is potentially harmful as it increases the risk of bleeding and causes delay if a patient is in need of immediate surgery.

The multicausal nature of VT makes it difficult to predict whether a healthy person is at risk, or whether a patient with the disease is at risk of a second event. It is widely thought that the combination of several predisposing conditions more readily leads to a crossing of the threshold to onset of the disease (7). Through the years clinical trials helped us to decide proper duration of anticoagulation on an individual basis. Still the duration and intensity of treatment is a matter of debate. The problem lies in the multiple ways a patient can develop VT, besides the heterogeneity of the clinical presentation. Most clinical trials and follow-up studies into recurrence risk are limited to cohorts of patients with so called “unprovoked” events. Authors have different opinions about the concept of the term “unprovoked”, but in general we can say that a venous thrombotic event is unprovoked in the absence of any known major prothrombotic condition at the time of diagnosis. Many assign this to be situations where clinical factors (surgery, immobilisation, vessel anomalies, iatrogenic vessel damage, and advanced cancer) are absent. Others expand the meaning of provoked to also include inherited or acquired coagulation disorders, pregnancy or the use of oral contraception.

During the last 2 decades, several follow-up studies have unraveled that the risk profile for recurrent thrombosis is quite different from that for a 1st VT. Table 2 shows the recurrence rates from some of the cohort studies. It appears that the risk of recurrence (8-11) remains increased for a long time after the index event, and that almost 1 out of 3 patients will have a 2nd event. One study found the risk on recurrence to increase even further after a 1st recurrence (9).

Table 1: Studies on the incidence of a 1st event of venous thrombosis

	Population	Type of follow-up	Period of follow-up	VT and PE IR/1000 py CI95	VT IR/1000 py CI95	PE IR/1000 py CI95	VT and PE men IR/1000 py CI95	VT and PE women IR/1000 py CI95
Silverstein ²	American	Retrospective	1966-1990	1.17 ¹ 1.12-1.22	0.48 ¹ 0.45-0.51	0.69 ¹ 0.65-0.73	1.30 ¹ 1.21-1.38	1.10 ¹ 1.04-1.16
Cushman ³	American ≥ 45 y	Prospective	1987-1989 1989-1990 1992-1993	1.61 ³ 1.43-1.81 1.92 ²	1.17 ³	0.45 ³		
Nordstrom ⁴	Swedish VT only	Prospective	1987		1.6		1.55	1.62
Hansson ⁵	Swedish Men 50-80 y	Prospective	1963-1993	3.87	1.82	Fatal: 1.07 Nonfatal: 0.98	3.87	
Andersson ¹	American VT and PE	Retrospective	1985-1986	0.71	0.56	0.23		
Oger ⁶	French	Prospective	1998-1999	1.83 1.69-1.98	1.24 1.12-1.36	0.60 0.52-0.69	1.52 1.34-1.72	2.03 1.83-2.26
Naess Chapter 2.1	Norwegian VT and PE > 20 y	Retrospective	1995-2001	1.43 ³ 1.33-1.54 ³	0.93 ³ 0.85-1.02 ³	0.50 ³ 0.44-0.56 ³	1.28 ³ 1.15-1.43 ³	1.58 ³ 1.44-1.74 ³

¹: Age and sex-adjusted

²: Age-adjusted

³: Unadjusted

Table 2: Studies on the incidence of recurrent venous thrombosis

	Population At baseline	Exclusion At baseline	Period of inclusion	2 year Cum.Inc % CI95	5 year Cum.Inc % CI95	8 year Cum.Inc % CI95	5 year Men Cum.Inc % CI95	5 year Women Cum.Inc % CI95
Hansson ⁹	Consecutive Swedish VT only N = 738	Tourists Emigrants Deceased <1 month after inclusion	1988-1993	12.1 9.3-14.9	21.5 17.7-25.4	-	-	-
Prandoni ¹⁰	Consecutive Italian VT only N = 355	No	1986-1991	17.5 13.6-22.2	24.6 19.6-29.7	30.3 23.6-37.0	-	-
Baglin ¹¹	Consecutive English VT and PE N = 570	Malignancy Antifosfolipid- syndrome Longterm- anticoagulation Cerebral VT Mesenteric VT	1997-2002	11 7.9-13.7	-	-	-	-
Kyrle ⁸	Consecutive Austrian VT and PE? N = 826	Malignancy Lupus anticoagulant Longterm- anticoagulation Surgery Trauma Pregnancy PC/PS/AT-def < 18 y	1992-2003	-	-	-	30.7 23.8-37.6	8.5 23.8-37.6
Christiansen Chapter 6	Consecutive Dutch VT only N = 474	Malignancy > 70 y	1988-1992	12.4 9.5-15.4	12.4 9.5-15.4	19.3 13.9-24.8	7.4 4.3-10.5	7.4 4.3-10.5

Genetic risk factors for venous thrombosis.

Genetic risk factors are either strong and rare, such as deficiencies of antithrombin, protein C or protein S, very frequent and weak, so as several recently described SNPs (12), or intermediately frequent and strong, such as factor V Leiden and prothrombin 20210A.

Factor V is a cofactor in the process where factor X activates thrombin (Figure 1). The mutation causes Arginine to be replaced by Glutamine at position 506 in factor V. This mutation makes activated protein C unable to inactivate factor V, which can be measured in plasma as resistance to activated protein C. The prevalence of FVL varies between 2-15 % in Caucasians, and 15-25 % in unselected patients with VT (13).

The evidence that carriership of factor V Leiden increases the risk of a first episode of VT or PE is convincing. A pooled analysis of 8 case control studies, with a total of 2310 cases and 3204 controls, found the odds ratio for a 1st VT in FVL-carriers to be five (14). The study also found the mutation to be less frequent among patients presenting with 1st PE, as compared to patients presenting with a 1st VT in a limb (OR: 0.7). This phenomenon is called the factor V Leiden paradox (15).

Several follow-up studies have addressed the prediction of recurrence in carriers of the mutation. In contrast to the risk of a first event, most of them found heterozygote carriers to not have a significantly increased risk (16-20). However, a few studies reported a mild effect on recurrence risk (21-23).

Prothrombin G20210A is associated with increased prothrombin-levels, the precursor of thrombin in the coagulation cascade (24) (Figure 1). Its prevalence is 1-3% in Caucasians and 6-16% in patients with VT (14). Carrying the mutation increases the risk on a 1st VT 3.8-fold (CI95: 3.0-4.9) according to a pooled analysis of 8 case control studies (including the Leiden Thrombophilia Study) (14). Heterozygous carriers probably have no or only a slightly elevated risk of recurrence (19, 25-26). A pooled analysis of 8 case-control studies, including 2310 cases and 3204 controls, found the pooled odds ratio on a 1st VT to be 20.0 (CI95: 11.1-36.1) in 51 cases co-carrying both FVL and Prothrombin G20210A (14).

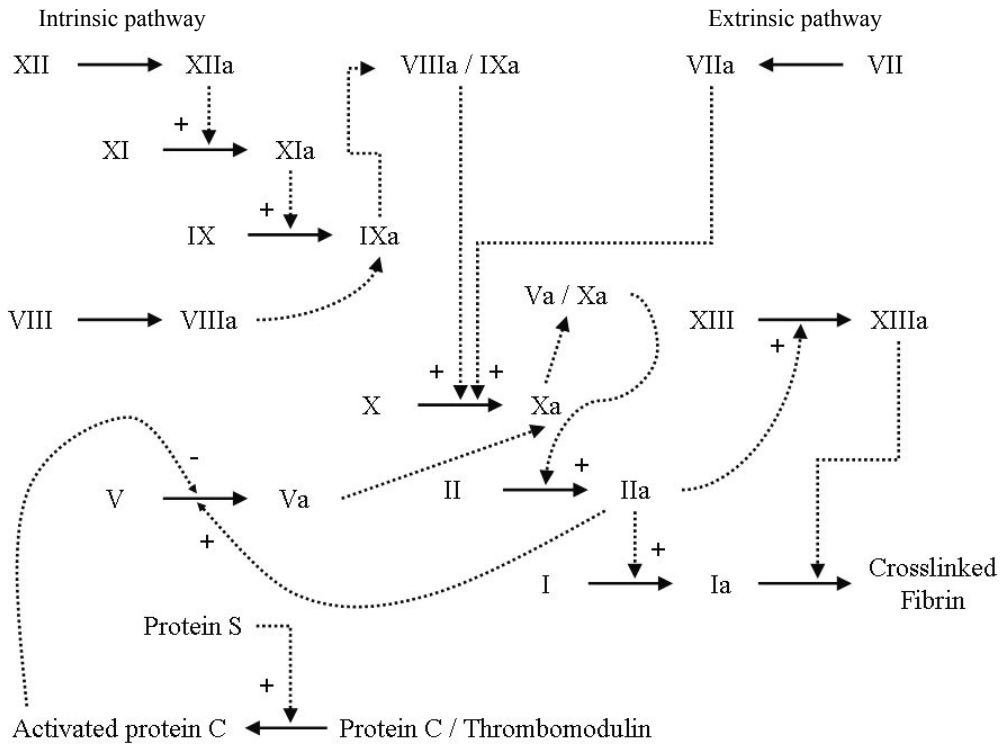


Figure 1. The intrinsic and extrinsic pathways of coagulation

Plasma abnormalities predisposing to thrombosis

Several plasma phenotypes which are not directly linked to a genetic defect increase the risk of thrombosis. These risks are usually, at the cut-off levels to denote abnormality that are commonly used, comparable to factor V leiden and prothrombin 20210A in strength and prevalence. They mainly refer to procoagulant proteins, but also to the aminoacid homocysteine and autoantibodies as anticardiolipins and lupus anticoagulants.

Elevated levels of clotting factors VIII and IX

Factor VIII is a co-factor in the intrinsic pathway of the coagulation cascade (Figure 1). It is an acute phase protein, and increases steeply at injury or stress. It is the only coagulation factor with a reservoir in the endothelial cells in the vessel walls. This reservoir can be released by hormonal factors, for instance in the pharmacological treatment (vasopressin-analogues) of patients with hemophilia type A. Patients with a non-O blood group have higher factor VIII levels than patients with an O blood group, due to increased levels of its carrier protein von Willebrand factor (27). Furthermore, the levels of factor VIII probably increase with age (28). High factor VIII levels increase the risk of venous thrombosis: In the LETS, factor VIII-levels above the 75th percentile were associated with a 5-fold increased risk of a first VT after correction for blood group and vWF-levels (27) and a more recent study found a 6.7-fold increased risk if factor VIII levels exceeded 270 IU/dl (29).

The AUREC-study found that factor VIII levels exceeding the 90th percentile in healthy controls (> 234 IU/dl) yielded a 6 -fold increased risk of recurrence as compared to those with factor VIII levels below the 25th percentile (30). An Italian study found factor VIII levels exceeding the 90th percentile (> 2.66-2.98 IU/ml) to increase the risk of recurrence; the risk was 2-fold increased if the 1st event was provoked, and up to 6-fold increased if it was unprovoked (28).

Factor IX is a vitamin K-dependent co-factor in the intrinsic pathway of the coagulation cascade (Figure 1). Few studies have assessed factor IX as a risk factor of VT. In the LETS, the risk of VT for factor IX levels above the 90 percentile of the healthy controls was 3-fold increased after correction for possible confounders as age, sex, and use of oral contraception (31).

The AUREC study followed 546 patients with a 1st idiopathic VT for a mean follow-up of 2.6 years. They found patients with factor IX-levels exceeding the 75th percentile ($\geq$ 138 IU/dl) in healthy controls to have a hazard ratio of 2-fold on recurrence as compared to those with lower levels (32).

Antiphospholipids

Phospholipids, amongst which cardiolipins, can be found in cell and mitochondrial membranes. Antibodies directed against phospholipids, such as cardiolipin, are found in patients with systemic lupus erythematosus, a disease involving arterial as well as venous thrombosis, but can also be found in a substantial number of individuals without apparent disease (estimates range up to 10 percent). The prevalence of antiphospholipid antibodies in patients with systemic lupus erythematosus is very high (30% to 50%) (33). In many individuals with antiphospholipid antibodies, antibodies against b2-glycoprotein I (b2-GPI) and prothrombin are found. When the interaction with clotting factors leads to a prolonged clotting time (APTT), it is called a lupus anticoagulant, which may lead to a severe prothrombotic tendency.

While a lupus anticoagulant clearly increases the risk of thrombosis, this is less clear for other types of antiphospholipid antibodies. A small case-control study found an increased risk of a 1st VT in carriers of isolated lupus anticoagulant, but not in patients carrying anticardiolipin antibodies (33). In the LETS, a case-control study including 474 cases and 474 controls, the presence of lupus anticoagulants clearly increased risk (3.6-fold), as did carriership of anti-b2-GPI antibodies (OR 2.4), while anti-prothrombin antibodies weakly affected risk (OR 1.4) (34).

Previously, a case-cohort study found the presence of IgM or IgG antibodies against b2-GPI not to predict venous thrombosis (35). In contrast, a case-cohort study including only men found high IgG anticardiolipin titers to increase the risk on a 1st VT 5-fold, but the assay failed to detect the subtype of antibody (36).

Addressing the risk of recurrence, a prospective cohort study found the risk of recurrence to be 2-fold increased if the patients had detectable IgG anticardiolipin antibodies (37). The drawing of blood samples for measurement of anticardiolipin antibodies was done 6 months after the index event, the lupus anticoagulant was not analyzed, and the subtype of anticardiolipin IgG was not reported.

So whether anticardiolipin antibodies increase the risk on a 1st VT is controversial and there is too little evidence to definitely conclude if their presence increases the risk on recurrent VT.

Hyperhomocysteinemia

Serum homocystein has been associated with venous as well as arterial disease. The most common cause of hyperhomocysteinemia is non-genetic as a result of deficiencies of folate or cobolamin. Other non-genetic causes are inflammatory diseases, renal insufficiency, endocrinological disorders as diabetes and hyperthyroidism, and partly life style factors (smoking, increased body mass index) (38). Homocystein is known to fluctuate in time, increase with age and to be higher in men than in women.

MTHFR677-TT is a genetic variant related to mildly elevated levels of homocystein, but carriership does not or only slightly increase the risk of a 1st VT (39-40).

Case-control-studies have shown hyperhomocysteinemia, exceeding the 90th or 95th percentile, to increase the risk on a first VT 2-fold (38, 41-42). Until now, a clear causal mechanism explaining how homocystein can activate coagulation is lacking.

Folate is known to decrease homocystein levels. Trials with supplementation of folate, vitamins B6 and B12, however, have failed to find a preventive effect on VT occurrence (43-44).

Several inflammatory conditions including inflammation in the vessel wall can increase levels of homocystein. It is therefore possible that the association between elevated homocystein and VT is a marker of post-hoc inflammation. Prospective designs may therefore be preferred to rule out the effect of consequences of thrombosis on homocystein levels. Only 2 studies have measured homocystein before the patients developed VT (45-46). One found a slightly increased risk of VT (46), and the other found a 3-fold increased risk of idiopathic VT (45). The recurrence rate in patients with hyperhomocysteinemia is poorly studied. One prospective study found the risk of recurrence to be 3-fold increased when homocysteine levels were above the 75th percentile (47).

Hyperfibrinogenemia

Fibrinogen is the final protein of the coagulation-cascade (Figure 1), and the substrate of fibrin. It is an acute phase reactant as well, and its levels measured in peripheral blood depend on various circumstances (48). For instance, fibrinogen increases in situations of somatic stress, with age, malignancy, menopause, pregnancy, unfavorable lipid profile, and fibrinogen could be a marker of arterial disease.

In the case-control part of the LETS high fibrinogen (fibrinogen > 4 g/l) increased the risk of a 1st VT 2-fold (49). A re-analysis of the same data found that this was mostly due to an increased risk in the older population (50). One prospective cohort study found that patients who suffered recurrence had higher plasma fibrinogen, higher plasma viscosity and higher red cell aggregation one year after the index event (51).

Several mechanisms through which high fibrinogen predisposes to venous thrombosis have been proposed. It could be that it increases blood viscosity which on its turn activates the coagulation cascade; it could be that it influences platelet aggregation, promotes tight fibrin network or impairs the fibrinolysis (49-50).

Elevated endogenous thrombin potential (ETP) and peak thrombin

Prothrombin is the precursor of thrombin (also known as activated factor II). Thrombin (Figure 1) is the central enzyme in the coagulation cascade as it is the outcome of the extrinsic as well as the intrinsic pathway, turns soluble fibrinogen into insoluble fibrin, and initiates anticoagulation which controls the process of clotting. The generation of thrombin starts when the tissue factor (TF) – activated factor VIIa complex is produced after damage to the vessel wall (52). Thrombin has a positive as well as a negative feedback on its own generation. The latter is mediated through thrombomodulin. When thrombin is binding this endothelial receptor, thrombin will lose its procoagulant potential and activate protein C which inhibits activated factor VIII and factor V (52).

The protein C anticoagulant system needs to be activated by thrombomodulin in order to exert its full anticoagulant activity, in which protein S is a cofactor (53). Thrombin is directly inhibited by antithrombin.

The generation of thrombin is increased in women who take oral contraceptives or hormonal replacement therapy (52), patients with natural inhibitor deficiencies (52) or high levels of clotting factors VIII, IX, and XI or who are carriers of Prothrombin G20210A or Factor V Leiden (52). Elevated d-dimer levels are an indicator of thrombin generation and fibrin formation.

The endogenous thrombin potential measures the capacity of an individual's plasma to produce thrombin. In the assay thrombin generation is initiated and continuously measured, which leads to a thrombin generation curve including parameters as peak thrombin and area under the curve; the latter is called the endogenous thrombin potential (54).

The AUREC cohort, including patients with unprovoked initial events revealed that peak thrombin generation may be a helpful tool in identifying risk groups: patients with ETP < 300 nmol/l had an almost 3-fold lower risk on recurrence as compared to those who had ETP > 400 nmol/l. A more recent report from the same authors, this time with a chromogenic assay measuring α 2-macroglobulin bound thrombin as a measure of thrombin formation, found that patients with an ETP level exceeding 100 % of normal had a 1.7-fold increased risk of recurrence as compared to those with lower values (54).

In the Prolong trial, a cohort of patients with a first unprovoked event, patients with ETP exceeding the upper tertile had a 2.5 fold higher risk of recurrent thrombosis than patients with ETP below the lowest tertile (53).

Environmental risk factors for thrombosis

Risk factors for thrombosis constitute a long list including cancer, immobilisation, surgery, bed rest, pregnancy, puerperium, long haul travel. Since the effects of these factors on the incidence of a first event of thrombosis are well-established, we will focus here on their effect on the risk of recurrence. As a group they certainly do, for most studies including consecutive patients indicate an increased rate of recurrent VT in those patients in whom there is no obvious risk factor present at the 1st event (9-11, 55-57). These events are assigned to be “unprovoked” (see earlier), but when related to VT the definition varies between authors. Studies that did not find an excess recurrence risk in patients with a 1st unprovoked event generally suffered from methodological weaknesses (55, 58).

Oral contraception

Oral contraceptives increase the risk of a first venous thrombosis about 4-fold. An early study found the recurrence rate to be lower in women with oral contraceptives at the 1st event as compared to women without (59). Since many women will discontinue oral contraceptive use after a venous thrombosis, this has been explained as an effect of the removal of a transient risk factor, analogously to the low recurrence risk observed in individuals with a short transient risk factor such as surgery (11, 60). This was subsequently confirmed in a study that found that women using oral contraceptives preceding the 1st VT or PE to have a low risk of recurrence (61). The AUREC-study, however, reported almost equal recurrence rates whether the women had or had not used oral contraceptives at the 1st VT (8).

Combined oral contraceptives have multiple effects on the pro- and anticoagulation system, which are reflected in an acquired APC-resistance (61). The effects of oral contraceptives include increased levels of prothrombin, factor VII, factor VIII, factor X, fibrinogen, and prothrombin fragments 1+2 and decreased levels of factor V during use (62). Several of these effects are pronounced for contraceptives containing third generation progestins, e.g. desogestrel, which also have a higher risk of first thrombosis than second generation containing oral contraceptives (63).

APC-resistance increases the risk of thrombosis, even in the absence of factor V Leiden, in a 'dose-dependent' way (64). Apart from factor V Leiden and oral contraceptive use, overweight and obesity also induce APC-resistance (65).

In the LETS, obesity itself was associated with a twofold increased risk for a first VT (66). The MEGA-study, a very large case-control study, confirmed this finding and demonstrated a synergistic effect of obesity and oral contraceptive use on the risk of first venous thrombosis (67).

There are few data on the recurrence risk in women continuing oral contraception after a first event.

Pregnancy

As dyspnea, tachypnea, and swelling of the calf are common features during pregnancy, it can be challenging to rule out VT in the pregnant patient. In addition to the alterations in the coagulation system, local factors are likely to play a role in the etiology of pregnancy-related thrombosis, as up to 80 % of VT during pregnancy occurs in the left leg (68).

Prothrombotic changes during pregnancy include elevation of the clotting factors VII, VIII, X, fibrinogen and von Willebrand factor, and lowering of anticoagulant protein S. Levels of prothrombin fragments, thrombin-antithrombin-complexes, and d-dimer increase during pregnancy, indicative of increased thrombin generation and a prothrombotic state. APC-resistance also increases during pregnancy, accompanied by a reduced fibrinolytic activity (69).

Overall 1 in 1500 pregnancies is complicated by VT (70), with the highest risk during the postpartum period (71). This risk is substantially increased for carriers of factor V Leiden or prothrombin 20210A (72). A few studies have investigated pregnancy as a determinant of recurrence, with one study estimating an incidence of 2.4% during the 2nd trimester (73).

The Trondheim-Leiden Study (TROL), a prospective study on predictors of a 1st event of venous thrombosis.

HUNT, data source of the TROL-study

The material used in the TROL-study was assembled from the HUNT-II study (Helseundersøkelsen i Nord-Trøndelag), a population-based survey carried out between August 1995 to June 1997. The study design has been extensively described in an article in the Norwegian Journal of Epidemiology (74).

All residents older than or reaching the age of 20 at the time of enrollment, were eligible. The participants filled in a questionnaire attached to the invitation, and a second one when arriving at the screening point. The response to the invitation and the study material is set out in the report by Holmen et al (74). The overall participation rate was 71% (N = 66140), with a good representation of all ages (Figure 2). In total, 98.7 % (N = 65291) of the participants donated serum, and 94.7 % (N = 62664) donated DNA-samples. In August 1996, the method of blood sampling was revised. Initially, fresh blood samples were assembled, and the clot separated from the serum. After August 1996 an additional blood sample of EDTA was added, as an alternative to clot extraction.

Participation meant a written consent for screening, contact for follow-up, and approval of the use of their data and blood sample for research purposes. The written consent was only valid as long as all information which could lead to the identification of the patients (names and personal ID numbers) was removed before publication. If the data were used for research purposes with external collaborators, all personal data were removed at the research centre in Verdal before the data were released to collaborators outside the centre. Some years after the enrolment new ideas came for DNA-tests, and demanded a new passive consent from the participants. Letters were sent out, and those who actively withdrew their consent (N = 1185), were excluded from further analyses.

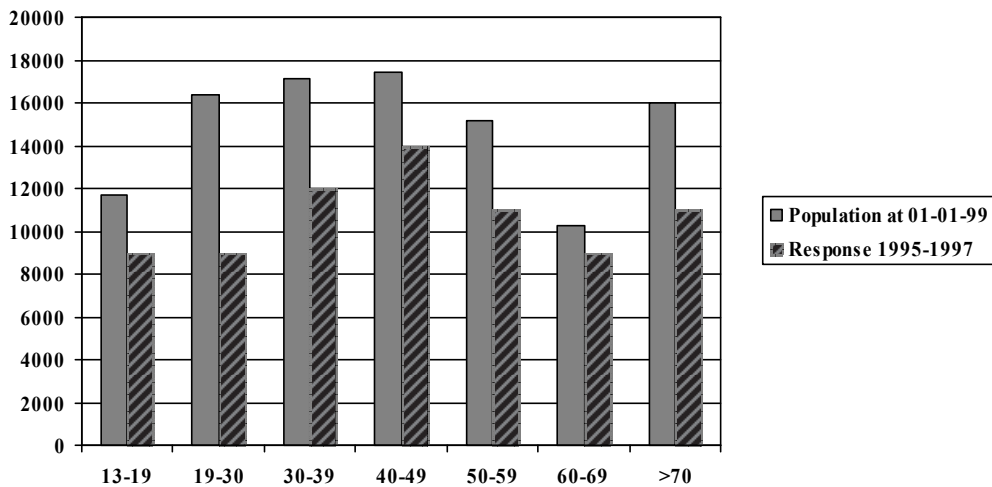


Figure 2. Number of participants in HUNT-II as compared to the number of inhabitants in Nord-Trøndelag county (source: Norsk Statistisk sentralbyrå) on 01.01.1999, in age-categories

TROL, a registry of venous thromboembolism during 1995-2002.

We set out to review all potential episodes of venous thrombosis in the cohort after inclusion. We assumed that all patients who had a VT or PE would be referred for diagnostic workup and treatment to the only two hospitals in the municipality (Levanger and Namsos). We selected all possible diagnostic codes assigning VT or PE in both ICD.9 and ICD.10, as ICD-10 replaced ICD-9 during the observation period (Appendix of this thesis). We also selected diagnostic codes assigning post-thrombotic syndrome or vascular complications in general, as these codes could have been used when a VT or PE occurred. Next, the lists of potential events were crosschecked with the codes for diagnostic procedures performed at the departments of radiology. In a few cases, patients were referred after diagnosis to a tertiary centre (St Olavs Hospital). If so we reviewed their records as well.

We found 2136 patients who according to the search procedure could have had a VT or PE in the period 01.01.1995 and 31.12.2001 (Figure 3). To adjudicate the diagnosis we used objective criteria as published earlier (75). These criteria for dividing an episode of VT or PE into low, medium and high probability are set out in the appendix of this thesis (2, 75-76). In addition to those with a non-definite diagnosis of venous thrombosis, we excluded 473 individuals who had not been included in HUNT-II at the time of their venous thrombosis, 210 individuals with a previous venous thrombosis and 73 patients with an isolated thrombosis in the veins of the eye. Of the remaining 515 cases, blood samples were missing in seven, so 508 cases with an objective diagnosis of 1st VT or PE were included in this analysis. In addition, we sampled 1505 individuals from the cohort as reference group of whom 1469 could be included as reference group (Figure 4).

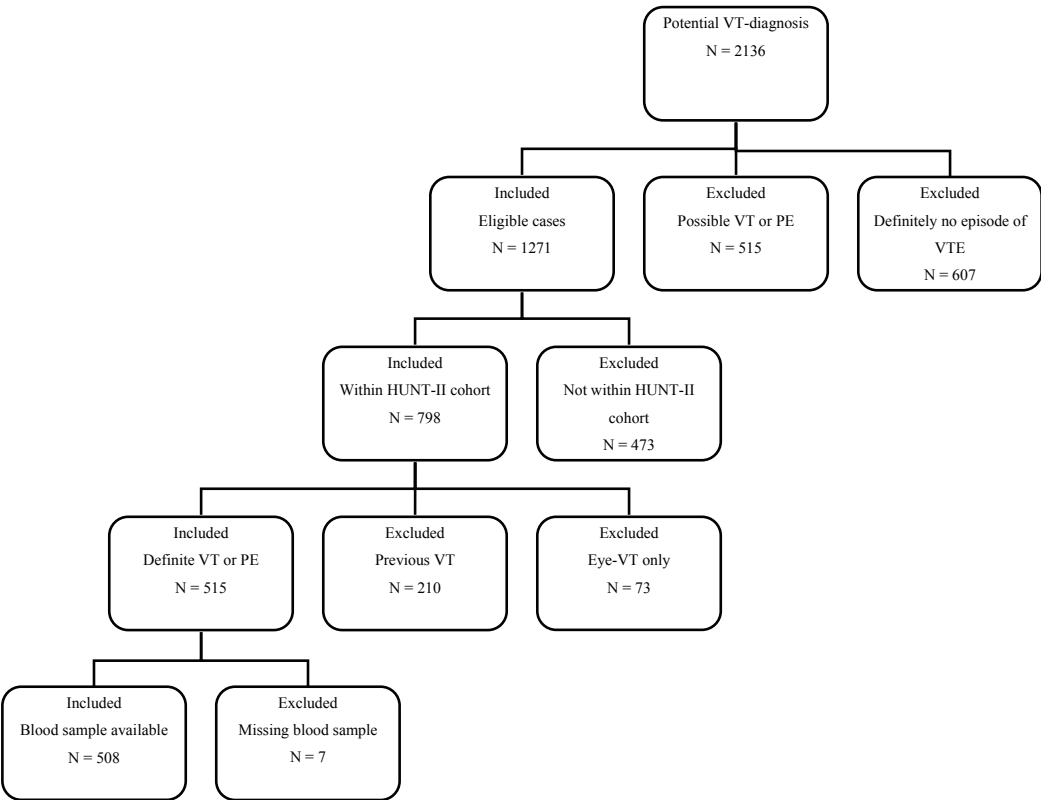


Figure 3. The selection of cases in the TROL-cohort

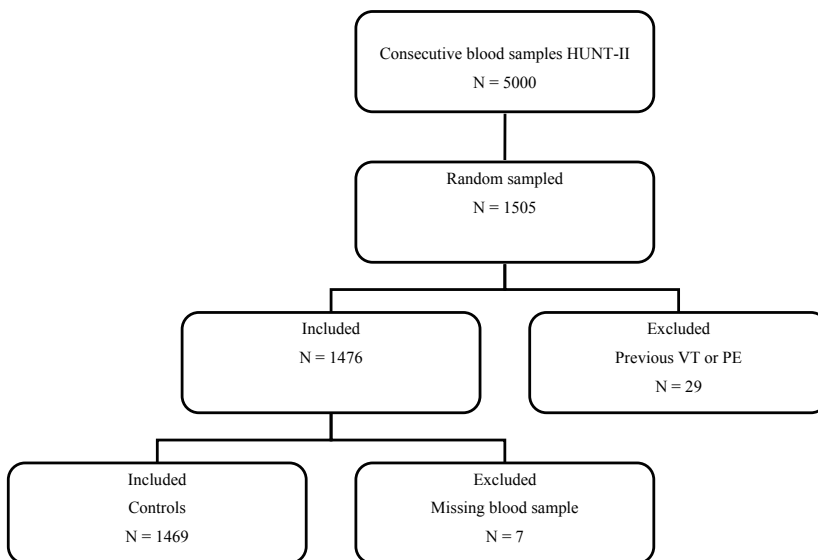


Figure 4. The selection of controls in the TROL-cohort

The Leiden Thrombophilia Study (LETS), a follow-up of the recurrence risk of venous thrombosis.

The Leiden Thrombophilia study was a case-control study, with the objective to find determinants of venous thrombosis, particularly genetic risk factors. It was the first population-based case-control study on venous thrombosis that adhered to strict criteria of objective diagnosis and inclusion of consecutive unselected patients. Four hundred and seventy four patients with a first time blood clot in veins of the leg (N=453) or the arm (N=21) were recruited. Patients with limited life expectancy, older than 70 years or with known cancer at enrollment, were excluded. On the basis of these criteria, about 10 % of the eligible patients were excluded.

Since its start in 1989, the study has contributed substantially to the knowledge of the etiology of venous thrombosis. It was instrumental in the identification of factor V Leiden and prothrombin G20210A as risk factors for venous thrombosis, and demonstrated for the first time that elevated levels of procoagulant factors (VIII, IX, XI) increased the risk of venous thrombosis. Its results were not limited to genetic abnormalities or plasma phenotypes, for the LETS was also one of the three studies that first showed an excess risk of venous thrombosis among users of third generation contraceptives as compared to users of second generation contraceptives, demonstrated that obesity leads to venous thrombosis and reported the synergistic effect of oral contraceptive use and factor V Leiden.

Patients with a first venous thrombosis are at risk of a second venous thrombosis. Anticoagulation will prevent most cases of recurrence. However, as anticoagulant therapy may be harmful in the sense of increasing bleeding tendency, it is of the greatest importance to limit the duration of treatment. Therefore, knowledge of risk factors for recurrence is of crucial value in prescribing personalized anticoagulant prophylaxis.

With the latter in mind, the 474 patients from the case-control part of the LETS were invited to take part in a long term follow-up. Follow-up was performed by repeated postal questionnaires to the patients on risk factor, and a telephone interview when a recurrence was suspected. These recurrent events were subsequently validated with the treating physician. We chose to exclude those “recurrent” events which occurred during the initial anticoagulation period, because we believe these events have another pathogenesis than recurrent VT later in the follow-up. Events during the initial anticoagulation period are more likely to be extensions of the initial event.

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Part 2

Risk factors for a first episode of venous thrombosis

Chapter 2.1

Incidence and mortality of venous thrombosis: a population-based study

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Summary

Background: Estimates of the incidence of venous thrombosis (VT) vary, and data on mortality are limited.

Objectives: We estimated the incidence and mortality of a first VT event in a general population.

Methods: From the residents of Nord-Trøndelag county in Norway aged 20 years and older (n=94,194), we identified all cases with an objectively verified diagnosis of VT that occurred between 1995 and 2001. Patients and diagnosis characteristics were retrieved from medical records.

Results: Seven hundred and forty patients were identified with a first diagnosis of VT during 516,405 person-years of follow-up. The incidence rate for all first VT events was 1.43 per 1000 person-years [95% confidence interval (CI): 1.33-1.54], that for deep-vein thrombosis (DVT) was 0.93 per 1000 person-years (95% CI: 0.85-1.02), and that for pulmonary embolism (PE) was 0.50 per 1000 person-years (95% CI: 0.44-0.56). The incidence rates increased exponentially with age, and were slightly higher in women than in men.

The 30-day case-fatality rate was higher in patients with PE than in those with DVT [9.7% vs. 4.6%, risk ratio 2.1 (95% CI: 1.2-3.7)]; it was also higher in patients with cancer than in patients without cancer [19.1% vs. 3.6%, risk ratio 3.8 (95% CI: 1.6-9.2)]. The risk of dying was highest in the first months subsequent to the VT, after which it gradually approached the mortality rate in the general population.

Conclusions: This study provides estimates of incidence and mortality of a first VT event in the general population.

Introduction

Venous thrombosis (VT) is the third most common cardiovascular disease after myocardial infarction [1,2] and stroke [3]. In the Western parts of the world, which have increasingly older populations, VT is a major health problem. The estimated incidence rates for VT vary between 1 and 2 per 1000 person-years [4-10]. In addition to real differences, variations in these estimates may also depend on the study design, case definition, and age distribution.

VT has genetic and acquired risk factors. Knowledge of the latter is important for prevention purposes. For example, a large proportion of cases is related to surgery (around 20%), which has a 6-fold increased risk of VT [5,11].

Reports on mortality after VT are scarce, and the estimates vary considerably. Reports of 30-day and 90-day case fatality rates have varied from less than 10% to 30% [5,12-20], and reports on 1-year case-fatality rates vary even more [4,15,21-23]. Many of these studies were randomized clinical studies [12,14,17,19,22], which are often based on selected groups of patients. Cohort studies [4,5,13,15,16,18] often included thromboses diagnosed by autopsy, and thus both the incidence rates and mortality rates were influenced by different autopsy rates.

Studies based on data from national registries suggest an increase in admission rates and mortality from VT after 1990 [24,25]

We estimated the incidence and mortality of a first VT event in the total population in the county of Nord-Trøndelag, in central Norway. We used validated VT diagnoses from hospital discharge registries linked to data from the HUNT2 study. These data provide a basis for both health care planning and future research on VT.

Materials and methods

The study population

The study population included all residents aged 20 years or more ($n = 94,194$) in Nord-Trøndelag county in central Norway in 1995-1997. During 1995-1997, all inhabitants of this county were invited to participate in a large-scale general health study (the HUNT2 study) [26]

We were provided with a database of the HUNT2 population. The population of Nord-Trøndelag is ethnically homogeneous (97% Caucasian), and the county is fairly representative of Norway with regard to geography, economy, industry, age distribution, morbidity, and mortality [<http://www.ssb.no>]. The population has low geographic mobility, and is served by a centralized health service. It is thus well suited for a population study with follow-up. The median age of the invited individuals was 46 years, ranging from 20 to 103 years.

Case identification

We identified all individuals registered with a diagnosis of VT, i.e. deep-vein thrombosis (DVT) or pulmonary embolism (PE), in the Nord-Trøndelag County from 1 January 1995 to 31 December 2001, by means of the electronic patient registry from the only two hospitals in the county, Levanger and Namsos Hospital. We identified inpatients and outpatients from all departments on the basis of International Classification of Disease, Ninth and Tenth Revision (ICD-9 and ICD-10) discharge diagnostic codes for DVT and PE (see Appendix).

We also identified positive diagnostic procedure codes for venography, duplex ultrasound and Doppler ultrasound from the two radiologic departments in the two hospitals. No radiology service was offered outside the hospitals. Finally, we performed a case-finding search at the tertiary-care center of the region, St Olav Hospital, Trondheim University Hospital, in the neighbouring county for residents of Nord-Trøndelag county discharged with a diagnostic code of VT.

Two physicians (I. A. Næss, S. C. Christiansen) reviewed the hospital records and validated each case. Adjudication differences were resolved by a consensus procedure. Each episode of VT or PE was categorized into three levels of diagnostic certainty (definite, probable and possible) on the basis of models used in other studies [5,9] and the 1995 revised PIOPED criteria [27,28]. Clinical diagnosis with no confirmatory test or with an indeterminate result was classified as possible VT and not included in the analyses. Definite DVT was defined by an intraluminal filling defect on ascending contrast venography in the leg or arm, a non-compressible venous segment in the popliteal, femoral or axillary veins on duplex ultrasound, or a positive computed tomography (CT) scan. Probable DVT required no venous filling on ascending contrast venography in the leg or arm or no venous flow in the popliteal, femoral or axillary veins on duplex ultrasound. Leg thrombosis was classified as proximal when the proximal extension was localized in the popliteal, femoral or iliac veins, and as distal when the thrombus was localized in the calf only (below the popliteal vein, diagnosed by venography). Upper-extremity thrombosis included thrombosis of arm veins, the superior vena cava, or the internal jugular, subclavian or axillary veins.

Definite PE was defined according to the PIOPED criteria [27,28] as a high-probability ventilation/perfusion (V/Q) scan, i.e. ≥ 2 segmental perfusion defects (V/Q mismatch), a perfusion scan with ≥ 2 segmental perfusion defects associated with normal chest X-ray (X/Q mismatch), or a positive CT scan. Probable PE was defined as an intermediate-probability V/Q scan (one moderate or large V/Q mismatch) or a positive echocardiogram/transesophageal echocardiogram.

Thrombosis events were classified as first or recurrent and secondary non-cancer, secondary cancer, or idiopathic. An event was regarded as secondary non-cancer VT when any of the following was registered in the medical record: trauma, surgery, marked immobility (specified as paresis, paralysis, prolonged bedrest because of an acute medical illness, or > 8 h travel) within the last 3 months, pregnancy or puerperium at the time of the event, or oral contraceptives used at the time of the event or up to 1 month before the event. An event was classified as secondary cancer VT when an active malignancy was registered at the event or within 6 months after the event. An event was considered idiopathic when none of the precipitating factors for a secondary VT was registered in the patient history.

Statistical analysis

The study population was followed from the date of entry until the event, emigration, death or end of follow-up, whichever occurred first. The end of follow-up was 31 December 2001 in the incidence analyses and 1 April 2004 in the case-fatality and mortality analyses. We used the unique person identification number assigned to every citizen in the country to link the study population to the death registry of Statistics Norway, which is a virtually complete and continuously updated register of all deaths and emigrations in Norway.

We used the observed number of cases of first VT as the numerator and the sum of individual person-time contributed by the total resident population of the area covered by the data (Nord-Trøndelag County) as the denominator to calculate incidence rates of first VT event. Five-year age-specific and sex-specific incidence rates were calculated using achieved age during follow-up. Thus, each person contributed person-time in different age categories while aging during follow-up.

Incidence rates for VT, DVT and PE were standardized by using the direct method, applying the age-specific rates in each 5-year age group to the world (Segi) standard population aged 20 years and above (<http://seer.cancer.gov>).

We used first events of VT as the denominator and deaths from any cause after the event as the numerator to calculate case fatality.

We used the Kaplan-Meier method to evaluate crude survival, and Cox regression with VT entered as a time-dependent covariate to study age-adjusted and sex-adjusted hazard ratios for mortality. VT cases were followed-up as non-VT cases until the event occurred, after which they were followed as VT cases. To assess the proportional hazard assumption, we used a log minus log plot.

Data were processed in SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL, USA) and STATA version 9.0 (StataCorp, College Station, TX, USA).

Ethics

The study was approved by the National Data Inspectorate and the Regional Ethical Committee. Informed consent was obtained from all participants.

Results

Cohort

Of the 94,194 inhabitants over 20 years of age living in Nord-Trøndelag between 1995 and 1997, we excluded four persons who were found to reside outside the county, and 421 persons who were registered with VT before the start of follow-up. Thus, we included 93,769 subjects, yielding 516,405 person-years at risk for a first VT event, and 705,558 person-years for the survival analysis.

Cases

Of the 2,136 patients for whom discharge records were identified, 421 were excluded from the study because they had had a VT before entry, and 189 because they did not belong to the cohort (i.e. not residing in the county or less than 20 years of age at date of entry). Patients not identified as VT cases in the validation procedure ($n = 497$), patients with possible VT ($n = 150$), patients with eye thrombosis ($n = 107$) and patients diagnosed post-mortem only (autopsy) ($n = 32$) were excluded as cases, but contributed person-time in the follow-up (Fig. 1).

The characteristics of the 740 included patients with a first VT event are given in Table 1. There were 411 women and 329 men, with a median age of 75 and 71 years, respectively. Two-thirds ($n = 482$) of the patients had DVT and one-third ($n = 211$) had PE alone. Six per cent of the patients ($n = 47$) had concurrent DVT and PE, and were classified as having PE in the analyses.

Fifty-seven percent of the VTs in the leg were localized in the left leg ($n = 260$), 42% were localized in the right leg ($n = 194$), and 1% ($n = 3$) were bilateral; three-quarters ($n = 328$) were proximal, and one-quarter ($n = 128$) were distal. Approximately half of the patients with PE (142 of 258) had a bilateral or central thrombus (Table 1). The acquired risk factors are given in Table 2.

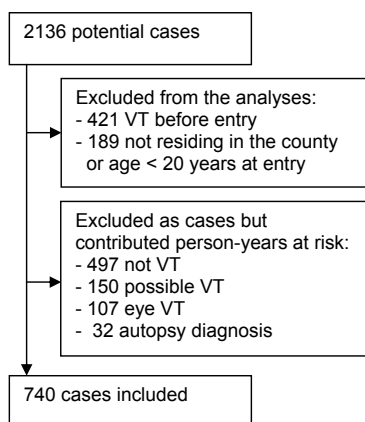


Figure 1. Case identification

Table 1: Characteristics of 740 patients with first VT events.

	Risk groups			
	Idiopathic, n (%)	Secondary non-cancer, n (%)	Secondary cancer, n (%)	All, n (%)
DVT	234 (63)	155 (66)	93 (71)	482 (65)
PE	139 (37)	81 (34)	38 (29)	258 (35)
Total VT	373 (100)	236 (100)	131 (100)	740 (100)
Women	210 (56)	137 (58)	64 (49)	411 (56)
Median age in years (range) ¹	74.3 (25-102)	69.1 (22-91)	75.6 (32-95)	73.2 (22-102)
Location				
PE unilateral	59 (16)	37 (16)	20 (15)	116 (16)
PE bilateral/central	80 (21)	44 (19)	18 (14)	142 (19)
DVT arm	3 (1)	7 (3)	3 (2)	13 (2)
DVT central ²	5 (1)	0	7 (5)	12 (2)
DVT leg	226 (61)	148 (63)	83 (63)	457 (62)
Distal leg	59 (26)	60 (40)	9 (11) ³	128 (28) ³
Proximal leg	167 (74)	88 (60)	73 (89) ³	328 (72) ³

DVT, deep vein thrombosis; PE, pulmonary embolism; VT, venous thrombosis.

¹ Age at event

² Vena lienalis ($n = 3$), vena mesenterica ($n = 3$), vena porta ($n = 3$), vena cava superior ($n = 3$), vena sagitalis superior ($n = 2$), vena cava inferior ($n = 1$), vena renalis ($n = 1$). Two of the cases had three locations.

³ In one DVT patient, leg distal or proximal location was not specified.

Table 2: Acquired risk factors for venous thrombosis in 740 cases.

Risk factor	n (%)
Surgery	148 (20)
Trauma	91 (12)
Marked immobility	77 (10)
Other*	18 (2)
Pregnancy/puerperium	13 (30) [†]
Oral contraceptives	7 (16) [†]
Active cancer	131 (18)
None (idiopathic)	373 (50)

Some of the cases had more than one risk factor.

*Tumor obstruction, central vein catheter, vessel anomaly.

[†]Among women < 45 years (n=43).

Incidence of first VT

The age-specific and sex-specific incidence rates are shown in Tables 3 and 4. The incidence rate of a first event of VT was 1.43 per 1000 person-years [95% confidence interval (CI): 1.33-1.54].

For DVT alone, the incidence rate was 0.93 per 1000 person-years (95% CI: 0.85-1.02), and for PE with or without DVT the estimated rate was 0.50 per 1000 person-years (95% CI: 0.44-0.56). The incidence rates increased exponentially with age. Incidence rates in subjects aged 70 years or above were more than three times higher than those in subjects aged 45-69 years, which again were three times higher than the rates in subjects aged 20-44 years. Women had an incidence rate of VT of 1.58 per 1000 person-years (95% CI: 1.44-1.74), as compared with 1.28 per 1000 person-years (95% CI: 1.15-1.43) in men. The incidence rate ratio was 1.2 (95% CI: 1.1-1.4) in women vs. men, but this difference disappeared in an age-adjusted analysis [incidence rate ratio 1.0 (95% CI: 0.9-1.2)]. During childbearing years, the incidence rate in women was twice the rate of incidence in men, but after 60 years, the rate was slightly higher in men.

The standardized incidence rate (world standard population) of a first event of VT was 0.94 per 1000 person-years (95% CI: 0.86-1.01), that for DVT alone was 0.61 per 1000 person-years (95% CI: 0.55-0.67), and that for PE with or without DVT was 0.33 per 1000 person-years (95% CI: 0.28-0.37).

Case fatality and mortality

Table 5 shows the 30-day and 1-year case-fatality rates after a first VT event. During these periods, 47 and 160 people died, respectively. The most frequent causes of the 47 deaths within 30 days after the event were cancer (43%), PE (28%), cardiac death (13%), infection (8%), central VT (2%), and sudden death (2%). The cause of death was unknown in 4%. Among non-cancer VT patients, 45% of the deaths within 30 days were because of PE. The 30-day case-fatality rate was twice as high in patients with PE as in patients with DVT [9.7% vs. 4.6%; risk ratio 2.1 (95% CI: 1.2-3.7)]. In patients without cancer, this difference was of similar magnitude [6.8% vs. 1.8%; risk ratio 3.8 (95% CI 1.6-9.2)]. Over time, the difference in case-fatality rate for PE and DVT gradually disappeared (Table 5).

Table 3: Incidence rates (IRs) among men per 1000 person-years and 95% confidence intervals (CIs) for first deep-vein thrombosis alone (DVT) and pulmonary embolism with or without DVT (PE $\pm$ DVT) in Nord-Trøndelag County (n = 93 857) in 1995 – 2001.

Age groups (years)	Person-years	DVT alone			PE $\pm$ DVT		
		n	IR	95% CI	n	IR	95% CI
20-24	15 197	0	0	-	2	0.13	0.03-0.53
25-29	27 981	1	0.04	0.01-0.25	0	0	-
30-34	26 045	4	0.15	0.06-0.41	1	0.04	0.01-0.27
35-39	25 065	4	0.16	0.06-0.43	3	0.12	0.04-0.37
40-44	25 446	5	0.20	0.08-0.47	6	0.24	0.11-0.52
45-49	26 082	13	0.50	0.29-0.86	4	0.15	0.06-0.41
50-54	25 077	18	0.72	0.45-1.11	4	0.16	0.06-0.42
55-59	19 195	17	0.89	0.55-1.42	7	0.36	0.17-0.76
60-64	14 893	17	1.14	0.71-1.84	11	0.74	0.41-1.33
65-69	14 181	23	1.62	1.08-2.44	12	0.85	0.48-1.49
70-74	14 045	26	1.85	1.26-2.72	22	1.57	1.03-2.38
75-79	11 620	41	3.53	2.60-4.79	17	1.46	0.91-2.35
80-84	7 243	27	3.73	2.56-5.44	18	2.49	1.57-3.94
≥ 85	4 686	19	4.05	2.59-6.36	7	1.49	0.71-3.13
Total	256 757	215	0.84	0.73-0.96	114	0.44	0.37-0.53

Table 4: Incidence rates (IRs) among women per 1000 person-years and 95% confidence intervals (CIs) for first deep-vein thrombosis alone (DVT) and pulmonary embolism with or without DVT (PE $\pm$ DVT) in Nord-Trøndelag County (n = 93 857) in 1995 – 2001.

Age groups (years)	Person-years	DVT alone			PE $\pm$ DVT		
		n	IR	95% CI	n	IR	95% CI
20-24	14 037	3	0.21	0.07-0.66	2	0.14	0.04-0.57
25-29	25 022	2	0.08	0.02-0.32	4	0.16	0.06-0.43
30-34	23 816	6	0.25	0.11-0.56	5	0.21	0.09-0.50
35-39	23 321	9	0.39	0.20-0.74	3	0.13	0.04-0.40
40-44	24 221	4	0.17	0.06-0.44	5	0.21	0.09-0.50
45-49	24 442	20	0.82	0.53-1.27	0	0	-
50-54	23 745	17	0.72	0.44-1.15	11	0.46	0.26-0.84
55-59	18 716	17	0.91	0.56-1.46	7	0.37	0.18-0.78
60-64	15 050	14	0.93	0.55-1.57	6	0.40	0.18-0.89
65-69	15 013	17	1.13	0.70-1.82	15	1.00	0.60-1.66
70-74	15 857	23	1.45	0.96-2.18	11	0.69	0.38-1.25
75-79	14 954	44	2.94	2.19-3.95	25	1.67	1.13-2.47
80-84	11 727	45	3.84	2.87-5.14	24	2.05	1.37-3.05
≥ 85	9 726	46	4.73	3.54-6.31	26	2.67	1.82-3.93
Total	259 648	267	1.03	0.91-1.16	144	0.55	0.47-0.65

Table 5: Thirty-day and 1-year case-fatality rate after first VT event

	Number at risk	Thirty days			One year		
		Number of deaths	Case-fatality rate (%)	95% CI	Number of deaths	Case-fatality rate (%)	95% CI
VT	740	47	6.4	4.6-8.1	160	21.6	18.7-24.8
DVT	482	22	4.6	2.9-6.8	101	21.0	17.4-24.9
PE	258	25	9.7	6.1-13.3	59	22.9	17.9-28.5
Idiopathic [§]	373	15	4.0	2.3-6.5	54	14.5	11.3-18.5
Secondary [¶]	236	7	3.0	1.2-6.0	23	9.7	6.6-14.3
Cancer [#]	131	25	19.1	12.3-25.9	83	63.4	55.6-71.9
Non-cancer							
VT	609	22	3.6	2.3-5.4	77	12.6	10.1-15.5
DVT	389	7	1.8	0.7-3.7	43	11.1	8.1-14.6
PE	220	15	6.8	3.9-11.0	34	15.5	10.9-20.9

VT, venous thrombosis; DVT, deep-vein thrombosis; PE, pulmonary embolism;.

[§]Idiopathic VT.

[¶]Secondary non-cancer VT.

[#]VT secondary to cancer.

The 1-year case-fatality rate was 21.6% (n = 160; 95% CI: 18.7-25.8) in all the cases, it was 12.6% (n = 77; 95% CI: 10.1-15.5) in patients with thrombosis without cancer, as compared to 63.4% (n = 83; 95% CI: 54.5-71.8) in thrombosis patients with cancer. The Kaplan-Meier overall survival plot showed poor survival in cases with cancer (Fig. 2). The survival for patients with secondary non-cancer VT and idiopathic VT was higher than for patients with VT secondary to cancer. Between patients with secondary non-cancer VT and idiopathic VT, the difference in observed survival was small.

In comparison to the general population and adjusted for sex and age, the hazard ratios for death for the whole follow-up period after VT were two times higher in patients with secondary non-cancer VT, 2.5 times higher for those with idiopathic VT and 13 times higher among VT patients with cancer (Table 6). We subdivided the follow-up after VT into three periods: 0-0.5 years after the event, 0.5-3 years after the event, and 3 years or more after the event. In patients with secondary non-cancer VT, there was no increased mortality beyond 6 months after the event in comparison to the general population. Among patients with idiopathic VT, the hazard ratio declined to 1.2 after 3 years as compared to the general population. In VT patients with cancer, the hazard ratio of death declined from more than thirtyfold during the first 6 months to less than 3-fold 3 years after the event as compared to the general population (Table 6). The mortality rate was slightly lower in women than in men [age-adjusted hazard ratio 0.8 (95% CI: 0.7-1.1)].

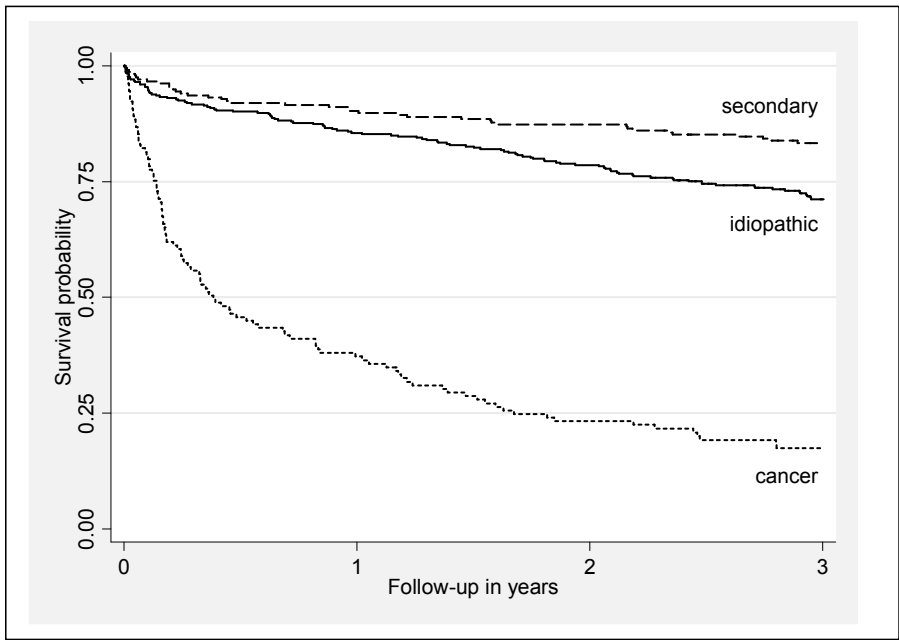


Figure 2. Kaplan-Meier survival probability after first venous thrombosis (VT) in the risk groups of secondary non-cancer VT (secondary), idiopathic VT (idiopathic) and VT secondary to cancer (cancer), respectively

Table 6: Hazard ratio of death after first venous thrombosis (VT) event in different risk groups compared to the general population according to different follow-up periods.

		Hazard ratio (95% CI)*			
		Periods of follow-up after VT			
	Number at risk	Total follow-up#	0-0.49 year	0.50-2.99 years	≥3 years
Total population	92 804	1	1	1	1
Idiopathic†	373	2.57 (2.17-3.04)	4.36 (3.12-6.08)	1.83 (1.44-2.32)	1.24 (0.87-1.75)
Secondary‡	236	1.97 (1.52-2.57)	4.85 (3.07-7.66)	1.05 (0.68-1.63)	1.09 (0.68-1.75)
Cancer§	131	12.73 (10.59-15.29)	33.78 (26.41-43.21)	7.29 (5.25-10.13)	2.48 (1.24-4.95)

*Adjusted for sex and age.
†Idiopathic VT.
‡Secondary non-cancer VT.
§VT secondary to cancer.
#From entry to end of follow-up. VT was entered as time dependent variable. Proportional hazard not met.

Discussion

We studied the complete population of Nord-Trøndelag County for 6.5 years, and found that the incidence rate of a first VT event in people aged 20 years or more was 1.43 per 1000 person-years. The risk of dying was highest shortly after the VT event. During the first year after the event, the risk of dying in the patients gradually approached that in the general population.

Our observed incidence rate of VT is similar to rates for first events in Brest, France [8], Göteborg, Sweden [7] and two studies in the USA: Olmsted County, Minnesota [9] and the LITE study [5].

The 30-day case-fatality rate of 6.4% and 1-year case-fatality rate of 21.6% in our study are similar to those in other cohort studies that excluded patients with autopsy-verified thrombosis [4,5,23]. In contrast, studies including autopsies reported a 30-day case-fatality rate of 28% [15] and a 1-year case-fatality rate close to 40% [15,21].

The high case-fatality rate in patients with a first VT event secondary to cancer is similar to that in other studies [4,5,15,23]. The increased mortality rate is most likely related mainly to the cancer itself. The follow-up study of Sørensen et al. [29] suggests that occurrence of thrombosis in cancer patients is associated with substantially increased mortality. It is unclear whether this is causally related to the thrombosis, or whether the thrombosis is a marker of an aggressive malignancy.

The strengths of our study are the high quality of the outcome data, the large population at risk, and the population-based design, with individual data from the HUNT2 study and the National death register on age, sex, death and emigration.

In comparison to existing studies, our advances are a complete population, a long follow-up time, exact person-time calculations, a complete case-finding procedure, and individually validated VT diagnoses.

However, we may have underestimated the incidence rate, for several reasons. We had data on emigration from Norway, but not on migration from the county to other counties within the country. Census data (<http://www.ssb.no/statistikkbanken/>) showed that the number of migrants to other parts of the country is about 2,500 each year from Nord-Trøndelag. Age-specific data showed that these persons are young people, perhaps students, who often move back within a few years. Nevertheless, this may have led to an overestimation of the person-time. We performed a sensitivity analysis to assess the potential impact on the incidence estimate. The amount of excess person-years contributed by migration within the country was estimated to be 41,000. After subtraction of the excess person-years in relevant 10-year age bands, the estimated overall incidence rate increased only slightly from 1.43 to 1.52 per 1000 person-years.

The presence of persons in the study population with an unknown previous thrombosis, and therefore not at risk for a first thrombosis, may also have led to underestimation of the incidence rate. However, this would lead to only a small change in the overall number of person-years at risk, and thus would have a negligible influence on the incidence rate estimate.

In addition to the 740 certain cases of VT identified during follow-up, we also identified 182 otherwise eligible cases among the 258 possible VT events (no objective diagnostic

procedures were performed, or the results were indeterminate) that were not included in the incidence estimate. If these cases had been definite cases and had been included, the estimated standardized incidence rate would have been 1.78 per 1000 person-years instead of 1.43 per 1000 person-years.

Cases diagnosed post mortem were not included in this study. The number of cases diagnosed post mortem will vary between studies, depending on differences in autopsy rates, and this makes it difficult to compare incidence rates between studies. The autopsy rate is low in Norway, and if they had been included, we would still have underestimated the incidence rate, because of an unknown number of undiagnosed events.

VT secondary to acquired risk factors was as common as idiopathic thrombosis in our study. Surgery and cancer were the most common risk factors, and improved prophylaxis in these patient groups might lower the incidence of VT.

In conclusion, this study provides an estimate of the incidence and mortality of first VT events in an unselected general population. The incidence increased nearly exponentially with age, and the proportion of VT events secondary to acquired risk factors was 50%. In comparison to the general population, the mortality rate was highest during the first months after the VT event, after which it gradually approached the rate in the general population.

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Appendix

Discharge codes used to identify potential cases of DVT and PE before the validation process were ICD-9 codes 415.x, 451.x, 452, 453.x, 325, 362.3, 433, 557.0, 634-638 (with decimals 6 and 7), 639.6, 639.8, 639.9, 671.x, 673.x, 674, and 997.2, and ICD-10 codes I26.x, I80.x, I81, I82.x, I63.6, I67.6, K55, H34.8, O08.x, O22.x, O87.x, and O88.x.

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Chapter 2.2

Inflammatory cytokines as risk factors for a first venous thrombosis: a prospective population-based study

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Summary

In case-control studies elevated levels of interleukins 6 and 8 have been found to be associated with an increased risk of venous thrombosis (VT). Because of the design of these studies, it remained uncertain whether these alterations were a cause or a result of the VT. In order to distinguish between the two, we set out to measure the levels of six inflammatory markers prior to thrombosis in a population-based cohort using a nested case-cohort design.

Between August 1995 and June 1997, blood was collected from 66 140 people in the second Norwegian Health Study of Nord-Trøndelag (HUNT2). We identified venous thrombotic events occurring between entry and 1 January 2002. By this date we had registered 506 cases with a first VT; an age- and sex-stratified random sample of 1464 controls without previous VT was drawn from the original cohort.

Levels of interleukins 1 β , 6, 8, 10, 12p70 and tumour necrosis factor - α were measured in the baseline sample that was taken 2 d to 75 mo before the event (median 33 mo). Cut-off points for levels were the 80th, 90th and 95th percentile in the control group. With odds ratios ranging from 0.9 (CI95: 0.6-1.5) to 1.1 (CI95: 0.7-1.8), we did not find evidence for a relationship between VT and an altered inflammatory profile.

In conclusion, the results from this population sample show that an altered inflammatory profile is more likely to be a result rather than a cause of VT, although short-term effects of transiently elevated levels cannot be ruled out.

Introduction

Venous thrombosis (VT) is a common and potentially lethal disorder with an estimated incidence of one to three per 1000 individuals per year¹⁻³. The occurrence of venous thrombotic events is influenced by acquired and inherited risk factors (e.g., oral contraception and Factor V Leiden)⁴⁻⁵. Combinations of these risk factors further increase the risk to potentially high levels⁶.

An important subgroup of risk factors comprises the levels of procoagulant proteins. Several studies have shown that elevated levels of coagulation factors VIII, IX and XI increase the thrombotic risk⁷⁻⁹. Genetic determinants of these high levels have not been found, with the exception of the well-known relation between ABO blood group and factor VIII levels. It is also possible that the increased procoagulant levels are acquired. Experimental studies in human volunteers injected with low dose endotoxin provide credence for this possibility, as they showed increases in procoagulant protein levels in parallel with an inflammatory response¹⁰⁻¹⁴. In other studies we have found increased levels of inflammatory markers in patients who had suffered from venous thrombotic disease¹⁵⁻¹⁷. A scenario that links all these data together is that an acquired inflammatory component is either wholly or in part responsible for the increased levels of at least some of the procoagulant proteins, and, either directly or through this mechanism causes VT.

The association between inflammation and VT leads to the question of whether inflammation is the cause or the result of VT. There are arguments for both. In favour of a causative role is the above-mentioned possibility that inflammation may increase procoagulant protein levels and thus increase the prothrombotic state of the blood. In addition, it is well known that inflammation may promote tissue factor expression on white blood cells and endothelial cells, thus providing a trigger that may lead to thrombotic disease¹⁸⁻²². Other arguments that could go with either role are: 1) the fact that levels of inflammation markers are high in the acute phase of VT and come down afterwards²³, and 2) the frequent occurrence of inflammatory post-thrombotic syndrome²⁴⁻²⁵.

Recent case-control-studies showed that raised levels of interleukins (IL) 6 and 8 are associated with a 2-fold risk for both first and recurrent thromboembolic events¹⁵⁻¹⁶. In the Leiden Thrombophilia Study (LETS), both cytokines, and tumour necrosis factor alpha (TNF- α) were associated with a 2- to 3-fold increased risk of a first VT, whereas the risk for IL-10 seemed to be decreased¹⁷. These studies don't answer the question of whether inflammation is a cause or a result of the event. No prospective studies have yet been undertaken to find out whether increased cytokine levels can be demonstrated in the blood before a venous thromboembolic event has occurred.

We tested the hypothesis that a chronic inflammatory state following a proinflammatory stimulus, regardless of origin, could precede future thrombotic events. In a population-based cohort, we tested levels of cytokines (TNF- α , IL-1 β , IL-6, IL-8, IL-12p70) in blood samples obtained at inclusion, and examined the association with the occurrence of subsequent thrombosis.

Patients and controls

Between August 1995 and June 1997, all inhabitants aged 20 y or older ($n=94,194$) in Nord-Trøndelag county (in Middle Norway) were invited to participate in a population-based health survey (HUNT2)²⁶. HUNT2 covers a wide range of topics such as chronic diseases, mental diseases, medication, education, employment, physical activity, and quality of life. The population in this county is considered representative of the rest of the Norwegian population regarding age and sex distribution, morbidity, mortality, and income. It has a low geographic mobility, which makes it suitable for a population survey with subsequent follow-up.

The overall participation rate was 71 % ($n=66,140$) at baseline (1995-1997), with a median age of 46 y (range 19-103 y)²⁶.

All consenting participants ($n = 66,140$) underwent a physical examination and filled out a questionnaire at inclusion. The questionnaire contained questions about risk factors for cardiovascular disease, life style, quality of life, and medication use. In addition, all participants were invited to donate 7.5 ml of blood, and acceptable serum samples were obtained from 65,291 participants (98.7 %)²⁶.

The follow-up for VT was performed as follows: the computerized diagnosis registries of all departments of the only two local hospitals (Levanger and Namsos hospitals) were checked for all in- and outpatient diagnoses containing ICD-9 and ICD-10 diagnostic codes for VT up until 1 January 2002. We completed case-finding by searching positive diagnostic procedure codes for venography, duplex ultrasound and Doppler ultrasound from the registries of the radiology departments in the two hospitals, and patients from Nord-Trøndelag County discharged from St.Olav University Hospital in the neighbouring county with diagnostic codes of VT. This search led to the identification of 2,136 cases with a diagnostic code of VT. Hospital records were obtained, and two physicians (I. Næss and S. Christiansen) validated each case. Cases were included only if they fulfilled the following criteria: deep venous thrombosis (DVT), an intraluminal filling defect or no venous filling on ascending contrast venography; or, no compressible venous segment or no venous flow in popliteal, femoral, or axillar veins on duplex ultrasound; or, a positive computed tomography scan or a positive autopsy; for pulmonary embolus (PE), a ventilation-perfusion scan with one or more segmental or subsegmental perfusion defects with normal ventilation; or, a contrast defect on pulmonary computed tomography scan; or, a positive autopsy. This way, 1,271 cases with a validated diagnosis of definite and probable VT were identified. These records were linked to the HUNT2 cohort, and 798 (63%) cases were identified within the cohort. We excluded all patients with previous VT, enrolment in the HUNT2 cohort after the event, or eye-vein thrombosis ($n=283$). As a consequence, 515 cases were included, out of which 506 had blood samples available in which reliable laboratory tests could be performed.

1,505 controls were randomly sampled from the baseline of the HUNT2 cohort by frequency matching to the cases by sex and 5-y age strata. 29 of them were excluded from the subsequent data-analysis because they had suffered one or more thrombotic events before the drawing of a blood sample. Another 12 had missing blood samples, leaving 1464 controls for the analyses.

In a case-cohort design, every person in the source population has the same chance of being included as a control. Since our control group was sampled at baseline, 29 controls later

became cases during the follow-up period. The odds ratio in a case-cohort study is to be interpreted as an estimate of the risk ratio, because all cohort participants from the baseline contribute to the denominator in absolute risks²⁷. As a consequence of this design, the 29 controls that later became cases were included in the case-group as well as in the control group.

Laboratory measurements

Whole-blood samples were collected from non-fasting participants at entry in the HUNT2 cohort, and within 2 h the serum was separated from blood cells (centrifugation at 1,010 g) and stored in a refrigerator at 4 °C. All samples were transported the same day in a cooler to the central laboratory at Levanger Hospital and stored in the HUNT biobank there the next morning at -70 °C. Samples taken on Friday were frozen the following Monday morning after being transported the same Friday evening²⁶. We have no indications that the time between phlebotomy and freezing affected the levels of the inflammatory mediators.

Serum levels of IL-1 β , IL-6, IL-8, IL-12p70 and TNF- α were measured using a commercial multiplex bead assay that allows accurate and reproducible measuring of multiple cytokines in a single small serum aliquot.²⁸⁻²⁹ To lower the detection limit of the kit, we used procedures that differed slightly from the recommendations of the manufacturer (BD Biosciences, Alphen aan den Rijn, Netherlands). In brief, 10 μ l of thawed serum was mixed with 10 μ l diluted capture bead suspension and 10 μ l of phycoerythrin-labelled detection reagent in a sample tube¹⁷. After 3 h of incubation in darkness, 200 μ l of wash buffer was added and the samples were centrifuged at 200 x g. The supernatant was aspirated and the bead pellet resuspended in 150 μ l of wash buffer, followed by analysis on a FACSCalibur flow cytometer. (BD Biosciences)¹⁷. In our hands, the detection limit of these ELISAs was 2.5 pg/ml for all cytokines. With the bead assays, the cytokine concentrations were often near the concentration of the lowest calibrator, and, depending on the analyte, the levels of cytokine in part of the samples remained undetectable. The technicians were not aware of the case or control status of the samples.

In some samples, outlier levels far above the standard curves (up to 4 % in cases and 3 % in controls) were seen. We believe that these measurements represent artefacts of the procedure, and the results from such samples were excluded prior to the analysis. In 12 controls (0.8 %) and 9 cases (1.7 %) the samples were missing, the volume of serum was too low to perform cytokine assays, or the cytokine measurements failed for technical reasons.

Statistical analysis

We used univariate logistic regression to calculate odds ratios and their confidence intervals. Cut-off points were detectable cytokine levels at the 80th, 90th, or 95th percentile calculated in control participants. We stratified for sex, three age categories (< 50, 50-69, and $\geq$ 70 y of age), time between blood sampling and the event, and whether the event was idiopathic or secondary. A secondary event was defined as an event that occurred after major surgery or trauma, during marked immobility (specified as paresis, paralysis, or > 8 h of travel), ante- or postpartum, during use of oral contraceptive pills, or in people with a history of malignancy. Linear regression was used to evaluate the relation between cytokine levels and time until the events in cases.

All analyses were performed with SPSS version 11.0 (SPSS, Chicago, Illinois, United States).

Consent

Participation was voluntary, and each participant signed a written consent. All surveys were approved by the Norwegian Data Inspectorate and by the Regional Committee for Ethics in Medical Research.

Results

General characteristics of the patients and controls are listed in table 1. The median age was 70 y in both cases and controls at baseline. 45.1 % of the cases were men, compared with 46 % in the control group (Table 1). Table 2 summarizes the cytokine and chemokine data. TNF- α , IL-6 and IL-10 were detectable in approximately two-fifths of the samples, whereas IL-1 β and IL-12p70 were detectable in three-fifths of the samples. IL-8 was unusual in that it was detectable in almost all cases (99 %), and in almost all the controls (98 %) as well (table 2).

Table 1: General characteristics of patients and controls.

		Patients n (%)	Controls n (%)
Total		506	1464
Sex	Men	228 (45.1)	671 (45.8)
	Women	278 (54.9)	793 (54.2)
Age groups	20-29	12 (2.3)	30 (2.1)
	30-39	17 (3.4)	46 (3.1)
	40-49	53 (10.5)	145 (9.9)
	50-59	72 (14.2)	195 (13.3)
	60-69	99 (19.6)	301 (20.6)
	70-79	169 (33.4)	499 (34.1)
	>80	84 (16.6)	248 (16.9)
Event	DVT	320 (63.3)	15 (51.7)*
	PE	153 (30.2)	12 (41.4)*
	Both	33 (6.5)	2 (6.9)*
Time from blood sample to event ** Median		33 months (2 days- 75 months)	

* The controls were collected at the entry of the HUNT2 study. During the follow-up, 29 controls got a first VT. They are included as both cases and as controls. Controls with previous events (i.e., events before the entry of the cohort) were excluded.

** Time in months from the date of blood sampling until the first objective event of VT in cases only.

Table 2: Levels of inflammatory markers in cases and controls

Analyte	Cases			Controls		
	Detectable n (%)	Range (pg/ml)	Median Detectable (pg/ml)	Detectable n (%)	Range (pg/ml)	Median detectable (pg/ml)
TNF- α	177 (35)	0-4507	15.2	499 (34)	0-3603	12.7
IL-1 β	289 (59)	0-4523	49.9	850 (60)	0-4967	46.6
IL-6	194 (39)	0-4370	35.0	547 (38)	0-4784	39.9
IL-8	498 (99)	0-4193	17.7	1437 (98)	0-4420	17.9
IL-10	201 (40)	0-3786	11.0	582 (40)	0-4831	11.8
IL-12P70	283 (58)	0-4024	36.1	849 (60)	0-4934	30.0

Whether markers were detectable or not was similar for cases and controls. Consequently, the odds ratios calculated based on detectable versus undetectable levels were about 1.0, with narrow confidence limits (Table 3). This indicates that a detectable level of any of the markers surveyed in this study was not related to a subsequent VT.

Table 3 lists the odds ratios that were calculated using P80, P90, and P95 as cut-offs. All interleukins demonstrated the same pattern, with no odds ratios exceeding 1.1 for the proinflammatory interleukins (TNF- α , IL-1 β , IL-6, IL-8, and IL-12P70) and an odds ratio of 0.9 for the anti-inflammatory IL-10.

We performed a sub-analysis on the samples that were taken within 1 y before the event to see if levels were higher shortly before a thrombosis occurred. They were not; if anything, they tended to be lower. In an analysis of only cases, IL-12-levels, for instance, showed a significant decrease with time closer to the event (figure 1). IL-1 showed a very similar pattern, while the other cytokines showed no clear trends in values nearer to the event.

Using the 90th percentile in the control participants as a cut-off point for all markers, again no effect was found when men and women were assessed separately, or when a distinction was made between an idiopathic and a secondary event. We found no effect within the three age categories either (table 4).

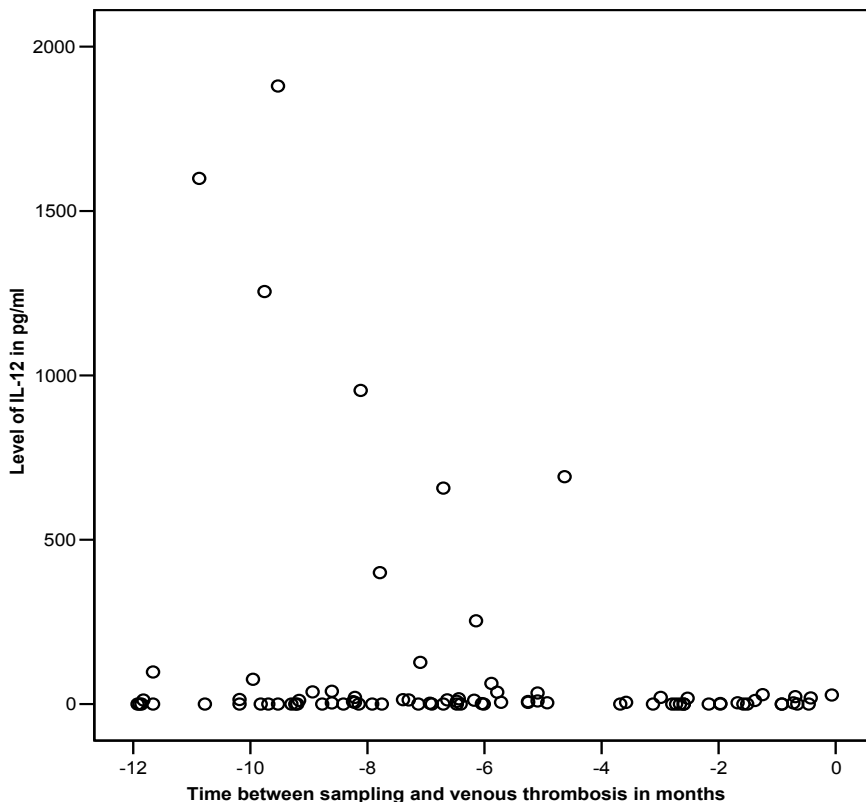


Figure 1. Regression Analysis of the Levels of IL-12 in the 12-mo Period before a Thrombotic Event ($p=0.04$). The y-axis shows levels of IL-12 from 85 individuals. Please note that IL-12 levels do not show a tendency to be increased when they are measured closer to the thrombotic event; in fact, the opposite appears to be the case.

Table 3: Odds ratios and 95% confidence intervals for detectability, and cut-offs at the 80th, 90th, and 95th percentile of inflammatory markers.

	TNF- α OR (CI95)	IL-1 β OR (CI95)	IL-6 OR (CI95)	IL-8 OR (CI95)	IL-10 OR (CI95)	IL-12p70 OR (CI95)
Detectable	1.0 (0.8-1.3)	1.0 (0.8-1.2)	1.1 (0.9-1.3)	1.5 (0.6-4.0)	1.0 (0.8-1.2)	0.9 (0.7-1.2)
P80	1.0 (0.8-1.3)	1.0 (0.8-1.3)	1.0 (0.8-1.3)	0.9 (0.7-1.2)	1.0 (0.7-1.2)	1.1 (0.8-1.4)
P90	1.1 (0.8-1.5)	1.1 (0.8-1.5)	1.0 (0.7-1.4)	1.1 (0.8-1.5)	0.9 (0.7-1.3)	1.0 (0.7-1.5)
P95	1.0 (0.6-1.5)	1.1 (0.7-1.8)	0.9 (0.5-1.4)	1.1 (0.7-1.8)	1.0 (0.6-1.6)	0.9 (0.6-1.5)

Table 4: Odds ratios and 95% confidence intervals for subgroups with inflammatory markers above the 90th percentile compared to those below.

	TNF- α OR (CI95)	IL-1 β OR (CI95)	IL-6 OR (CI95)	IL-8 OR (CI95)	IL-10 OR (CI95)	IL-12P70 OR (CI95)
Men	1.2 (0.7-1.9)	1.1 (0.6-1.8)	1.3 (0.8-2.1)	1.1 (0.7-1.8)	1.5 (1.0-2.3)	1.0 (0.6-1.7)
Women	1.0 (0.6-1.6)	1.0 (0.6-1.6)	0.8 (0.5-1.4)	0.9 (0.6-1.5)	0.7 (0.4-1.2)	1.1 (0.7-1.7)
Unprovoked events	1.0 (0.7-1.6)	1.3 (0.8-1.9)	1.1 (0.7-1.7)	0.9 (0.6-1.4)	0.9 (0.6-1.5)	1.3 (0.8-1.9)
Secondary events	1.1 (0.7-1.7)	0.9 (0.5-1.4)	0.9 (0.6-1.5)	1.2 (0.8-1.9)	0.9 (0.6-1.5)	0.8 (0.5-1.3)
Age < 50 years	0.7 (0.3-1.8)	1.1 (0.5-2.6)	0.7 (0.3-1.9)	1.0 (0.4-2.3)	0.5 (0.2-1.4)	0.9 (0.4-2.2)
Age 50-69 years	1.1 (0.7-2.0)	0.9 (0.5-1.6)	1.0 (0.6-1.8)	1.1 (0.7-2.0)	0.9 (0.5-1.7)	0.9 (0.5-1.7)
Age > 70 years	1.1 (0.7-1.7)	1.1 (0.7-1.8)	1.0 (0.6-1.6)	1.0 (0.7-1.7)	1.1 (0.7-1.8)	1.2 (0.8-2.0)

Discussion

We performed the current study to test the hypothesis that circulating levels of proinflammatory cytokines or chemokines are associated with a future event of VT. The data negate this hypothesis, as we found no evidence, neither in a primary analysis nor in post-hoc sub-analyses, that levels of inflammation markers were increased in individuals who later developed VT.

Due to the prospective design of this case-cohort study, there was considerable variation in the time between the blood sampling and the event (this varied between 75 mo and 2 d). Therefore, we could not fully rule out the possibility of a sudden increase of inflammatory markers in hours or days before a first thrombosis. However, this possibility seems less likely, considering the results of a sub-analysis of cases developing VT in the first year after blood sampling. These patients showed no increase whatsoever in cytokine levels shortly before the event.

We found a higher prevalence of detectable cytokines as compared with the prevalence recently found in the control group in the LETS, although the median levels were comparable¹⁷.

Both our cases and controls (with a mean and median age of respectively 66 y and 70 y, respectively) (Table 1) could be described as a substantially older population than the LETS control group (mean age 47 y)¹⁷. As some cytokines have been shown to increase with age while others remain constant, it is possible that the higher prevalence could be partly due to the high age of our cohort³⁰⁻³¹. This does not, however, explain the discrepancy of our study with the increased risks of VT recently found in the LETS.

The LETS found that the risk of VT increased parallel to several cytokines (TNF- α , IL-6 and IL-8). This was supported by a decreased risk with increasing levels of anti-inflammatory

IL-10. In the LETS, the association between cytokine levels and VT was found in samples that were collected after the event. Therefore, a model in which the thrombosis is the cause of subsequent cytokine release, and not the other way around, is the most likely explanation of the results. Such a model is also supported by the fact that C-reactive protein levels were not associated with VT in the LITE prospective study³².

A potential limitation of the study is that the inflammatory markers were evaluated with a method with lower sensitivity than high-sensitivity ELISAs. For example, a high-sensitivity ELISA of IL-6 may yield detectable levels in more than 90 % of the control samples. On the other hand, the claims that inflammatory markers are a risk factor for VT were based on studies of similar size with assays similar to ours.

In conclusion, we have investigated the role of cytokines before development of a first VT in the general population, and found no evidence of an association, not even shortly before the event. Therefore, it is unlikely that cytokine levels play an important role in determining the risk of VT.

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Chapter 2.3

A prospective study of anticardiolipin antibodies as a risk factor for venous thrombosis in a general population (the HUNT study)

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Summary

We prospectively examined whether there is an association between elevated anticardiolipin antibody levels and the risk for a future first venous thrombosis (VT) in a general population. We studied this in a large population-based nested case-cohort study of 508 VT cases and 1464 matched control subjects from a cohort of 66 140 participants in the Health Study of Nord-Trøndelag in Norway. Venous thrombosis was validated using standardized criteria for venous thrombosis and pulmonary embolism. Prethrombotic serum anticardiolipin antibodies were measured by an enzyme-linked immunoassay. There was no association between elevated anticardiolipin antibody levels and subsequent venous thrombosis, overall or after stratification by sex, different age groups or idiopathic vs. secondary thrombosis. The overall odds ratio was 1.11 (95% CI 0.71-1.74) for greater than vs. less than the 95th percentile of anticardiolipin antibody levels. In conclusion, in this general population sample elevated anticardiolipin antibody levels was not a risk factor for subsequent venous thrombosis.

Introduction

Antiphospholipid antibodies are a wide and heterogeneous group of antibodies, formerly believed to react to negatively charged phospholipids.¹ In recent years they have been shown to be directed against plasma proteins bound to anionic (not necessarily phospholipid) surfaces. Antibodies against β 2-glycoprotein I (β 2-GPI) and prothrombin are the two best known,²⁻⁴ and are detected in anticardiolipin antibody assays and in most lupus anticoagulant assays.^{2,5} The persistent presence of these antibodies, in two following tests at least 6 weeks apart, in combination with arterial and venous thrombosis, or recurrent fetal loss defines the antiphospholipid syndrome.^{6,7} The syndrome is termed primary antiphospholipid syndrome when there is no evidence of underlying disease, and secondary in the setting of autoimmune diseases, mainly systemic lupus erythematosus.⁸

Elevated anticardiolipin antibody levels have been associated with a twofold increased risk of venous thrombosis in presence of autoimmune disease (mainly systemic lupus erythematosus).^{9,10} In patients without autoimmune disease the association between anticardiolipin antibodies and risk of venous thrombosis has been inconsistent.¹¹⁻¹⁷

A meta-analysis of primarily case-control studies showed that the presence of anticardiolipin antibodies carried an odds ratio for venous thrombosis ranging from 0.3 to 2.5 regardless of site (arterial or venous), type (first event or recurrent) or the presence of systemic lupus erythematosus¹⁸. Higher levels of anticardiolipin antibodies were associated with higher risk for venous thrombosis.¹⁸

A major limitation of most of the studies published to date is that anticardiolipin antibodies were measured in blood collected after the thrombosis. Transiently elevated anticardiolipin antibody levels are found in many patients after a venous thrombosis, suggesting that the antibodies may be a result rather than a cause of thrombosis in these patients.¹⁹

Only two prospective studies, measuring anticardiolipin antibodies in blood collected before the venous thrombosis occurred in persons without autoimmune diseases, have been published.^{20,21} The first study, in male physicians, showed an association for a first venous thrombosis within the 5% highest immunoglobulin (Ig)G anticardiolipin antibody levels. The second study, which is the only population-based prospective study published, reported no association with different levels of anticardiolipin antibodies.

The aim of our study is to assess whether the presence of anticardiolipin antibodies is related to the risk of subsequent first venous thrombosis in a general population. Most studies published are concerned with the risk of recurrent thromboses in selected patient populations, and have measured anticardiolipin antibodies after the events. In contrast we have studied the risk for first events in an unselected population, and studied the relation prospectively by measuring anticardiolipin antibodies in blood samples collected prior to the events.

Methods

Study design

We included all cases with a validated diagnosis of a first venous thrombosis that occurred during a 7 year follow-up of the second Health Study of Nord-Trøndelag (HUNT 2) cohort, as well as controls selected at enrolment of the same cohort in a nested case cohort design.

The HUNT 2 cohort

The entire population (n=94 194) of the Nord-Trøndelag County in middle Norway, at the age of 20 years and older was invited to participate in the population-based HUNT 2 study in 1995.²² The population of Nord-Trøndelag County has a demographic composition similar to the general population of Norway and a low geographic mobility, which makes it well suited for a population survey. HUNT 2 is a comprehensive health study covering a wide range of topics, such as chronic diseases, mental diseases, medication, education, employment, physical activity and quality of life. Seventy-one percent of the whole population (n=66 140), with a median age of 46 years (range 19-103) were enrolled in the period 1995 -1997. Data were collected by questionnaires, clinical measurements and blood samples at inclusion.

Cases

We included all individuals registered with a first venous thrombosis, i.e. deep vein thrombosis or pulmonary embolism in the Nord-Trøndelag County from 1995 through 2001. All patients with venous thrombosis in the county were diagnosed and treated in Levanger hospital and Namsos hospital, the only two hospitals in the region. We collected the patients through the computerized diagnosis registry of the two hospitals by ICD-9 and ICD-10 diagnostic codes for venous thrombosis (see Appendix). Two-thousand-and thirty-six cases with a diagnostic code of venous thrombosis were thus identified. Hospital records were obtained and venous thrombosis diagnoses validated for each case by two physicians (IAN, SCC). Cases were only included for this analysis when they fulfilled the following criteria: for deep vein thrombosis having an intraluminal filling defect or no venous filling on ascending contrast venography; non-compressible venous segment or no venous flow in popliteal, femoral or axillar veins on duplex ultrasound; a positive CT scanning or a positive autopsy; for pulmonary embolism having ventilation-perfusion scans with one or multiple segmental or subsegmental perfusion defects with normal ventilation; a contrast defect on pulmonary CT scanning or a positive autopsy. Cases were also classified as first or recurrent events, and as idiopathic or secondary. An event was classified as idiopathic when no obvious cause was registered in the medical record within the last 3 months before the event. A secondary event was registered when a major trauma (specified with or without fracture to truncus, spine, pelvis, lower limb, upper limb, head, or other locations), major surgery (specified as orthopedic-, abdominal-, gynecological-, urological-, or other kind of surgery), marked immobility (specified as paresis, paralysis, or > 8 hour travel) within the last 3 months, obstetric cause (as pregnancy or delivery) within the last 2 weeks, oral contraceptive pills used at the time of or within 1 month before the venous thrombosis, or a malignancy was registered in the patient history. We identified 1226 cases with an objectively verified diagnosis of venous thrombosis.

The records were linked to the HUNT 2 cohort and 798 cases were identified within the cohort. Of these cases, 283 cases were excluded for the following reasons: previously diagnosed venous thrombosis, i.e. venous thrombosis before enrolment in the HUNT 2 study, or venous thrombosis located in the eye. Of the 515 cases included, blood samples were missing in 7 (1.4%). Thus the final study population consisted of 508 cases with a first venous thrombosis occurring after entry in the HUNT 2 study.

Controls

Control subjects were selected at random from the baseline of the HUNT 2 study. The controls were frequency matched to the cases by sex and 5 year age strata. We selected 1505 controls. The controls were excluded for the same reasons as the cases (previously diagnosed venous thrombosis, i.e. venous thrombosis before enrolment in the HUNT 2 study, or venous thrombosis located in the eye). Medical records were reviewed for both cases and controls after in- and out-patient diagnosis registries had been scanned for ICD-9 diagnostic codes for venous thrombosis (see Appendix) before entry of the HUNT 2 study. Thus 29 controls with a previous venous thrombosis (venous thrombosis before entry of the HUNT 2 study) were excluded, leaving 1476 individuals as control subjects. Blood samples were missing in 12 (0.8 %) control subjects, leaving 1464 controls for the analyses. Another 29 controls had a first venous thrombosis during the follow-up and they were included both in the 508 cases and the 1464 controls in the analyses.

Laboratory methods

Whole blood was drawn from non-fasting participants at HUNT 2 entry, centrifuged within 2 h, and the serum immediately placed in a refrigerator at 4 °C. The samples were sent in a cooler to the central laboratory in Levanger the same day and stored in the HUNT biobank at -70°C. After selection of cases and controls, stored samples from the HUNT biobank were retrieved.

Serum anticardiolipin antibodies were measured by a commercial sandwich enzyme linked immunosorbent assay (ELISA), the Varelisa Cardiolipin Screen test (Pharmacia Diagnostics, Uppsala, Sweden). The assay is adjusted to a set of established standard sera.²³ The test detects patient serum IgG, IgM and IgA antibodies to β 2-GPI bound to immobilized cardiolipin.

Plastic microtiter plates, coated with β 2-GPI from bovine heart in complex with bovine heart cardiolipin were incubated for 30 min. with 100 μ L of diluted [1:100 in phosphate-buffered saline (PBS)] patient samples, a negative control and a calibrator. After washing three times with a PBS buffer containing 0.1% sodium azide (NaN₃), the wells were incubated for 30 min with enzyme (horseradish peroxidase) labeled secondary antibodies to human IgG, IgM and IgA. After washing three times, the wells were incubated in the dark for 10 min with the substrate 3, 3', 5, 5' tetramethylbenzidine. Ten minutes after a stop solution (H₂SO₄) was added, we measured optical density (OD) at 450 nm in a spectrophotometer. The calibrator sample determined the OD cut-off value for each kit. The calculation of the cut-off, suggested by the manufacturer, was based on 432 apparently healthy blood donors. The results were expressed in screening ratios, calculated from OD sample / OD cut-off. The manufacturer's suggestions for interpretations of the results were anticardiolipin antibody screening ratio $\leq$ 1.0 as negative, 1.0 – 1.2 as low positive and $\geq$ 1.2 as high positive.

In a subsequent analysis we measured IgG anticardiolipin antibody (Varelisa Cardiolipin IgG) and IgM anticardiolipin antibody (Varelisa Cardiolipin IgM) separately in the 59 samples that had a positive anticardiolipin antibody screening ratio (ratio > 1.0). These specific tests use the same ELISA technique as the screening test, but express the result in anticardiolipin antibody concentrations calibrated to a standard curve for each kit. The technicians were blinded to whether the samples came from patients or control subjects.

Statistical analysis

In a univariate logistic regression model odds ratios and their 95% confidence intervals were calculated for the quintiles of anticardiolipin antibody levels and three cut-off levels, the 90th, the 95th, and the 98th percentile separately. The percentiles were calculated from the distribution in the control subjects. Subsequently, we stratified for sex, different age categories, type of thrombosis (idiopathic or secondary) and time between blood sampling and event, in order to evaluate a possible effect in some subgroups only.

Ethics

All participants gave their informed consent at enrolment in the HUNT 2 study. Each surviving adult HUNT 2 participant (n=61 426) received an information folder and a personal letter asking for a new consent to include genetic research in 2002. One thousand-one-hundredand-eighty-five persons (1.9%) withdrew from the cohort. The current case-cohort study was approved by the National Data Inspectorate and the Regional Ethical Committee.

Results

Participants

Table 1 shows the general characteristics of the 508 patients and 1464 control subjects. Most cases were elderly (50% > 70 years old) and few were younger than 50 years old (16%). The median age of both cases and controls at baseline was 70 years (range 20 – 98). Fifty-five percent of both patients and control subjects were women. Two-thirds of the patients had deep venous thrombosis and one-third pulmonary embolism. Among the 508 events, 245 were idiopathic venous thrombosis and 263 secondary according to the criteria described in the method section.

Table 1: General characteristics of the study population.

		Patients n (%)	Controls n (%)
Total		508	1464
Sex			
	Men	228 (44.9)	673 (46.0)
	Women	280 (55.1)	791 (54.0)
Age groups			
	20-29	12 (2.4)	30 (2.0)
	30-39	17 (3.3)	47 (3.2)
	40-49	53 (10.4)	145 (9.9)
	50-59	73 (14.4)	197 (13.5)
	60-69	99 (19.5)	301 (20.6)
	70-79	169 (33.3)	498 (34.0)
	>80	85 (16.7)	246 (16.8)
Event			
	DVT	322 (63.4)	15 (51.7) ^a
	PE	153 (30.1)	12 (41.4) ^a
	Both	33 (6.5)	2 (6.9) ^a
Time from blood sample to event			
	Median (range)	33 months (2 days – 75 months)	

^a The controls were collected at the entry of the HUNT 2 study. During the follow-up, i.e. after the blood sampling at the entry, 29 controls got a first VT. They are included both as cases and controls. Controls with previous events, i.e. events before the entry of the cohort, were excluded. DVT, deep vein thrombosis, PE, pulmonary embolism.

Anticardiolipin antibodies

The distribution of the anticardiolipin antibody levels was highly skewed with most of the observations at the very low levels (Fig.1). The median anticardiolipin antibody screening ratios for cases and controls were 0.386 and 0.376, respectively, and the distributions were very similar. The 90th, 95th and 98th percentiles calculated from the distribution in the control subjects were 0.675, 0.837 and 1.169, respectively.

Forty-six (78%) of the 59 subjects with positive anticardiolipin antibody screening levels (ratio ≥ 1.0) had elevated anticardiolipin IgG or IgM present in the specific tests, with an IgG/IgM ratio of 2:1. The IgG/IgM ratio was 5:1 in those with high anticardiolipin antibody screening levels (ratio ≥ 1.2).

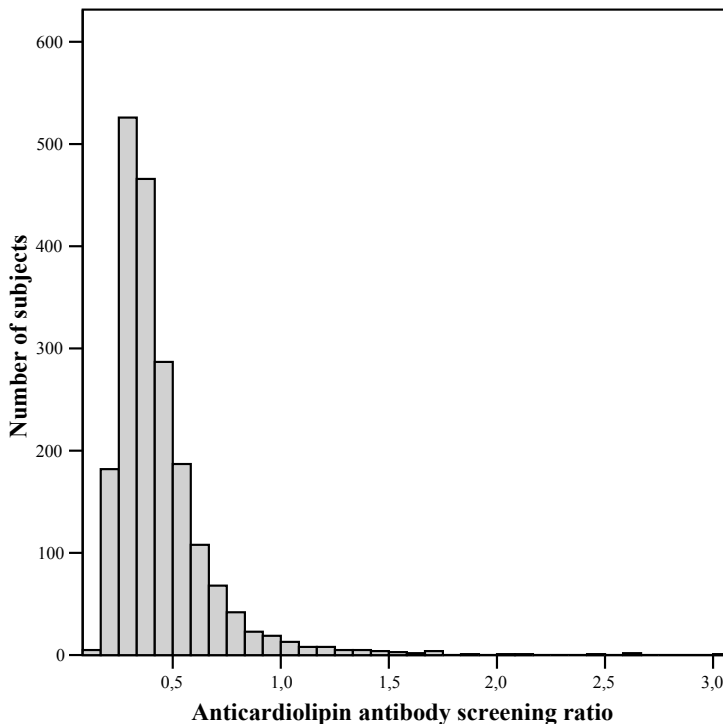


Figure 1. Distribution of anticardiolipin antibody levels in the study population (n = 1972).

Association with venous thrombosis

We observed no statistically significant associations between quintiles of anticardiolipin antibody levels and venous thrombosis (Table 2). Using cutoffs according to the 95th, 98th and 99th percentiles of anticardiolipin antibody levels, calculated from the distribution in the controls, no significant effect could be demonstrated, overall or in men and women separately (Table 3). However, the odds ratios tended to be higher in women than men. Further stratification showed no significant associations within subsets of patients, including idiopathic or secondary venous thrombosis, or different time between blood sampling and

the event, i.e. even in those with high anticardiolipin antibody levels the risk of venous thrombosis was not increased in the time immediately following the blood sampling (Table 4). The results did not change notably when we used the 98th percentile as a cutoff. However, high anticardiolipin antibody levels appeared to have some effect (albeit non-significant) on the risk of venous thrombosis in the youngest age group (< 50 years old).

Table 2: Odds ratios (OR) and 95% confidence intervals (CI) for venous thrombosis associated with quintiles of anticardiolipin antibody screening ratio (ACA).

Quintiles of ACA	No. of cases	No. of controls	Crude OR	Adjusted OR ^a	95% CI
<0.288	116	292	1.00	1.00	Reference
0.289-0.343	84	291	0.73	0.73	0.53-1.01
0.344-0.413	85	295	0.73	0.73	0.53-1.01
0.414-0.542	118	293	1.01	1.03	0.76-1.40
≥0.543	105	293	0.89	0.92	0.67-1.23

^aAdjusted for age and gender.

Table 3: Odds ratios (OR) and 95% confidence intervals (CI) for venous thrombosis in relation to the 95th, 98th and 99th percentiles (perc.) of anticardiolipin antibody levels.

Cutoff	cases (%) n=508	controls (%) n=1464	OR	95% CI
Overall (n=1972)				
≤ 95th perc.	480 (94.5)	1391 (95.0)	1*	
> 95th perc.	28 (5.5)	73 (5.0)	1.11	0.71 – 1.74
≤ 98th perc.	500 (98.4)	1435 (98.0)	1*	
> 98th perc.	8 (1.6)	29 (2.0)	0.79	0.36 – 1.74
≤ 99th perc.	501 (98.6)	1450 (99.0)	1*	
> 99th perc.	7 (1.4)	14 (1.0)	1.45	0.58 – 3.61
Men (n=901)				
≤ 95th perc.	217 (95.2)	632 (93.9)	1*	
> 95th perc.	11 (4.8)	14 (6.1)	0.78	0.40 – 1.55
≤ 98th perc.	225 (98.7)	658 (97.8)	1*	
> 98th perc.	3 (1.3)	15 (2.2)	0.56	0.17 – 2.04
≤ 99th perc.	226 (99.1)	665 (98.8)	1*	
> 99th perc.	2 (0.9)	8 (1.2)	0.74	0.16 – 3.49
Women (n=1071)				
≤ 95th perc.	263 (93.9)	759 (96.0)	1*	
> 95th perc.	17 (6.1)	32 (4.0)	1.53	0.84 – 2.81
≤ 98th perc.	275 (98.2)	777 (98.2)	1*	
> 98th perc.	5 (1.8)	14 (1.8)	1.01	0.36 – 2.83
≤ 99th perc.	275 (98.2)	786 (99.2)	1*	
> 99th perc.	5 (1.8)	6 (0.8)	2.38	0.72 – 7.86

The 95th, 98th, and 99th percentiles are ACA screening ratio 0.837, 1.169 and 1.369, respectively. The percentiles are calculated from the distribution in the control subjects. The same percentiles are used in men and women.

* Reference group.

Table 4: Odds ratios (OR) with 95% confidence interval (CI) for venous thrombosis (VT) in subgroups with anticardiolipin antibody levels above the 95th percentile compared with those below.

Subgroup (n cases)	Cases (%)	Controls (%)	OR	95 % CI
Overall (508) ^a	28 (5.5)	73 (5.0)	1.11	0.71 – 1.74
Idiopathic VT (245) ^a	17 (6.9)	73 (5.0)	1.42	0.82 – 2.45
Secondary VT (263) ^a	11 (4.2)	73 (5.0)	0.83	0.44 – 1.59
< 50 years (82)	4 (4.9)	4 (1.8)	2.80	0.68 – 11.45
50-69 years (172)	7 (4.1)	17 (3.4)	1.20	0.49 – 2.95
≥ 70 years (254)	17 (6.7)	52 (7.0)	0.96	0.54 – 1.69
Time between blood sampling and VT				
0 - 1 year (89) ^a	3 (3.4)	73 (5.0)	0.67	0.21 – 2.15
0 - 3 years (190) ^a	9 (4.7)	73 (5.0)	0.97	0.47 – 1.60
0 - 5 years (229) ^a	16 (7.0)	73 (5.0)	1.43	0.82 – 2.51

The 95th percentile is ACA screening ratio 0.837, calculated from the distribution in the controls (n=1464).

^a Number of controls =1464.

Discussion

This large prospective population-based study shows no evidence of an association between the presence of anticardiolipin antibodies and subsequent occurrence of first venous thrombosis in a general population. Neither did the study indicate any substantial effect in subgroups defined by age, sex, idiopathic vs. secondary thrombosis, or follow up time between the blood sample and the event.

Our results confirm those of the Longitudinal Investigation Thromboembolism Etiology (LITE) study.²¹ They found no association between anticardiolipin antibodies present at cohort entry and the risk of subsequent first venous thrombosis with an odds ratio of 0.66 (95% CI 0.34-1.28) for anticardiolipin antibody IgG levels above 95th percentile compared with those below. There was no effect in relation to different anticardiolipin antibody levels or in subgroups. The study design and anticardiolipin antibody assay used were similar to our study. Both studies were performed in a general population, with large sample sizes, anticardiolipin antibodies were measured in blood samples drawn before the event and both used standardized sandwich ELISA commercial kits that detect anticardiolipin antibodies that react to β 2-GPI bound to cardiolipin.

Our results contrast, however, to the Physicians' Health Study.²⁰ This study showed a significant association between anticardiolipin antibody IgG and risk of venous thrombosis in high anticardiolipin antibody levels only. The risk ratio was 5.3 (95% CI 1.55-18.3) for anticardiolipin antibody levels above the 95th percentile compared with those below the 90th percentile. The effect was not present for anticardiolipin antibody IgG levels in tertiles above the low positive cutoff (1.0 gamma-phospholipid [GPL] units), compared with those below the cutoff. Unlike our study this study was derived from a clinical trial, selected by sex, age, occupation and previous disease occurrence, and had a small sample size. They did not use a β 2-GPI -dependent assay to detect the anticardiolipin antibodies, possibly detecting

a different subset of antibodies. Recent reviews recommend for clinical practice assays detecting anticardiolipin antibodies binding to β 2-GPI immobilized on cardiolipin, as used in our study, as these are more reproducible and better correlated with venous thrombosis in patient populations.^{24,25}

Our study also contrasts to The Leiden Thrombophilia Study (LETS) that showed a 2.4-fold increased risk for a first venous thrombosis with positive anti- β 2-GPI-antibodies.²⁶ This study differs from ours by its retrospective design, with antibodies measured in blood collected after the thrombosis. The LETS study used a specific anti- β 2-GPI-assay where the antibodies bind to purified human β 2-GPI in absence of cardiolipin or other proteins, which differs from our assay. The conflicting results to our study could also be due to a different age distribution in the two studies, as the patients in the LETS study were younger than in our study (16-70 years, median age 45 years).

Possibly, high anticardiolipin levels have an effect in young people only. We observed a tendency in that direction in our study, but because of small numbers in the younger age groups, statistical power may have been too low to say much about subgroup effects here.

Anticardiolipin antibody assays are difficult to standardize and suffer from poor reproducibility.²⁵ We chose to use a commercial anticardiolipin antibody assay that is common to clinical practice, and which closely follows the “consensus” criteria of the European Antiphospholipid Forum.²⁵ The cutoff between a “positive” and “negative” anticardiolipin antibody test is arbitrary, and statistically determined in defined test populations. Calibration against Harris’ standard sera does not prevent large interlaboratory variations in results.²⁵ We chose to present the results of comparison of cases and controls at different anticardiolipin antibody levels, based on percentiles calculated from the distribution in the control subjects, which led to the same results as when the manufacturer’s cutoff was used.

Anticardiolipin antibody levels may be transient in healthy populations,²⁷ and an associated risk for venous thrombosis might be transient as well. A recent study showed that 79% of patients with idiopathic venous thrombosis that had elevated anticardiolipin antibodies within 1 month after the thrombosis reverted to normal after repeated testing beyond 1 month.¹⁹ This suggests that anticardiolipin antibodies may be a result of, rather than a cause of the thrombosis in many patients. This may explain the association between anticardiolipin antibodies and venous thrombosis in retrospective studies. Duplicate testing is included in the classification criteria for antiphospholipid syndrome to overcome this. We only measured anticardiolipin antibodies once and this is a potential limitation of our study.

The diagnosis of venous thrombosis is difficult, and clinical diagnosis is unreliable.²⁸ We validated carefully each individual case identified from the diagnosis registries, and included only cases with an objectively verified diagnosis. Thus a significant number of potential cases that had a clinical diagnosis with no or insufficient diagnostic tests performed were not included. A bias could theoretically result if these cases had anticardiolipin antibody levels different from the included cases, which is extremely implausible.

The negative results of this study can not be extrapolated to populations of patients with previous venous thrombosis or autoimmune disease, where the association between anticardiolipin antibodies and risk for venous thrombosis is well established.¹⁸

It is important to address the validity and generalizability of our study in the view of conflicting results from previous studies on presence of anticardiolipin antibodies and risk for venous

thrombosis. Our results were obtained by examining a large number of venous thrombosis events that occurred after blood samples were collected in an unselected, large population. We used a commercial test for β 2-GPI-reactive anticardiolipin antibodies chosen to closely resemble the situation in clinical practice. We thus feel that our results can be generalized to the primary health care setting, in a general population.

In conclusion, our prospective study shows no evidence of an association of elevated anticardiolipin antibody levels and risk for a subsequent first venous thrombosis. Our study does not support measuring anticardiolipin antibodies in primary risk evaluation of venous thrombosis nor primary anticoagulant prophylaxis for venous thrombosis in healthy individuals with elevated anticardiolipin antibody levels.

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Appendix

The ICD-9 codes for venous thrombosis diagnoses used were 415, 451, 452, 453, 997.2, 674, 673, 671, 634, 557, 437, 325 and 362.3 and the ICD-10 codes I26, I80, I81, I82, I67, I 63, K55, K75, O08, O22, O87, O88 and H34.8.

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Chapter 2.4

Prospective study of homocysteine and MTHFR 677TT genotype and risk for venous thrombosis in a general population - results from the HUNT 2 study

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Summary

This case-cohort designed study prospectively investigated whether elevated homocysteine levels measured in blood samples drawn before the event and methylenetetrahydrofolate reductase (MTHFR) gene polymorphism (MTHFR C677T) were associated with subsequent first venous thrombosis (VT) in a general population.

Between August 1995 and June 1997, blood was collected from 66 140 people in the second Norwegian Health Study of Nord-Trøndelag (HUNT2). During a seven-year follow-up, 505 VT cases were identified. 1458 age- and sex-matched controls were selected from the original cohort. Serum total homocysteine (tHcy) and MTHFR genotype were measured in stored samples that were drawn a median of 33 months before the events. The overall odds ratio (OR) was 1.50 [95% confidence interval (CI) 0.97-2.30] for homocysteine levels above *versus* below the 95th percentile.

There was no graded association with VT over quintiles of homocysteine. In men the OR was 2.17 (95% CI 1.20-3.91) for levels above *versus* below the 95th percentile, but no association was found in women (OR 1.00). Stratification by age, predisposing risk factors or time to event did not change these results. The MTHFR 677TT genotype was not related to risk for VT.

In conclusion, elevated homocysteine levels in the general population predicted subsequent first VT in men but not in women.

Introduction

Venous thrombosis (VT) is a common disease with an annual incidence of 1 to 3 in 1000 individuals and is caused by a combination of environmental and genetic risk factors (Rosendaal, 1999; Naess et al, 2007). Homocysteine has been considered as one of the risk factors for VT (den Heijer et al, 2005). However, most studies on the association between elevated homocysteine levels and VT have been case-control studies, for which blood samples used to measure homocysteine were collected after the event (den Heijer et al, 2005). Only two studies (on first VT) have been performed in which blood samples were collected before the event (Ridker et al, 1997, Tsai et al, 2003). The associations between elevated homocysteine levels and VT were weaker in the latter group than in case-control studies. The case-control studies could not determine whether the elevated homocysteine levels caused the thrombotic event, or whether they were a consequence of VT.

The discovery of a common mutation (677 C to T) in the gene coding for the enzyme methylenetetrahydrofolate reductase (MTHFR), which increases homocysteine levels (Frosst et al, 1995; Kluijtmans et al, 1997), introduced another means of testing the hypothesis of causality between homocysteine levels and VT.

Case-control studies and cohort studies have reported a weak or no association between MTHFR genotype and VT. Three meta-analyses assessing the association found an odds ratio (OR) of 1.60 [95% confidence interval (CI) 1.15-2.22] (Wald et al, 2002), OR 1.2 (95%CI 1.2-1.4) (Ray et al, 2002) and OR 1.20 (95%CI 1.08-1.32) (den Heijer et al, 2005).

The major disadvantage of meta-analysis is the possibility of publication bias, which has been shown to be common in the medical literature (Callahan et al, 1998). Recently, a large population-based case-control study, which was many times larger than previously published case-control studies, showed no association between MTHFR genotype and VT (Bezemer et al, 2007). This weakened the hypothesis of causality between elevated levels of homocysteine and VT.

The hypothesis that hyperhomocysteinaemia causes VT has been further attenuated by five secondary prevention trials that looked at the effect of lowering homocysteine levels with vitamin B supplementation on both arterial (Toole et al, 2004; Bonna et al, 2006; Lonn et al, 2006) and venous (Den Heijer et al, 2007; Ray et al, 2007) disease. Despite a decrease in homocysteine levels in the vitamin treatment group, none of these trials prevented recurrent VT or recurrent cardiovascular disease.

In this study, we prospectively examined the effect of elevated serum homocysteine on a first VT, and the association between the MTHFR 677TT genotype and VT, in a large population-based study where blood was collected at baseline before the disease occurred.

Methods

Study design

We conducted a case-cohort study embedded in the second Nord-Trøndelag Health Study (HUNT2). During a seven-year follow up after blood samples were banked at baseline, individuals with a subsequent first VT event were identified and matched to controls from the same cohort.

The HUNT 2 cohort

Between August 1995 and June 1997, all inhabitants aged 20 years and older ($n = 94\,194$) in Nord-Trøndelag County in central Norway, were invited to participate in HUNT 2. A total of 92 936 individuals were eligible to participate, and 66 140 (71%) attended. The study has been described in detail elsewhere (Holmen et al, 2003). Briefly, the participants were asked to complete a self-administered questionnaire, which included questions about risk factors for cardiovascular disease, life style, quality of life and medication use. At inclusion every consenting participant underwent a physical examination including measurements of blood pressure, height and weight, and a non-fasting serum sample and clot or EDTA-blood was obtained from 65 291 participants (98.7%). The population in this county is considered representative of the Norwegian population regarding age and sex distribution, morbidity, mortality and income. It has a low geographic mobility which makes it suitable for a population survey with a subsequent follow-up.

Cases

During a seven-year follow-up after HUNT 2, from 1 January 1995 to 31 December 2001, we identified diagnosed VT cases through all in-patient and out-patient clinics of the only two hospitals in the county (Levanger and Namsos hospitals). The International Classification of Diseases (ICD) codes ICD-9 and ICD-10 for VT was used to identify the patients from the electronic discharge register. We also used positive diagnostic procedure codes for venography, duplex ultrasound and Doppler ultrasound from the registries of the radiology departments in the two hospitals for potential VT identification. Finally, we identified cohort members among patients discharged with a diagnostic code of VT from the tertiary care centre of the region, the University Hospital, St Olav Hospital in the neighbouring county. The case-finding procedure led to the identification of 2136 cases with a diagnostic code of VT. To confirm the diagnosis, two physicians reviewed the hospital records and validated each case.

Deep vein thrombosis was defined by an intraluminal filling defect or no venous filling on ascending contrast venography in the leg or arm, non-compressible venous segment or no venous flow in popliteal, femoral or axillar veins on duplex ultrasound, a positive computed tomography (CT) scan or a positive autopsy. Pulmonary embolism was defined by a ventilation-perfusion scan with one or more segmental or subsegmental perfusion defects with normal ventilation, a contrast defect on pulmonary CT scan or a positive autopsy. A secondary non-cancer event was defined when (1) trauma, surgery or immobilization (specified as paresis, paralysis, prolonged bed rest because of an acute medical illness, or >8 h travel) within the last 3 months, (2) pregnancy or puerperium, (3) oral contraceptive use within the last 30 d was registered in the medical record. A cancer-associated event was defined when an active malignancy was registered at the event or within 6 months after. When none of the precipitating factors for secondary VT was registered in the patient history the event was defined as idiopathic.

Of the 1271 eligible patients with VT, 798 were identified within the HUNT 2 cohort. We excluded all cases with a history of VT before entry of the cohort and those with an eye vein thrombosis ($n = 283$). Thus, we included 515 cases with a first VT event, of whom 508 had blood samples available and 505 underwent successful analysis of serum homocysteine level.

Controls

We sampled 1505 control subjects from the HUNT 2 cohort by frequency matching to the cases by sex and age at baseline in 5-year bands. Twenty-nine of the controls were excluded because they had suffered one or more thrombotic event before inclusion in the HUNT2 cohort. Blood samples were missing in seven control subjects; the homocysteine analysis was not successful in 11 samples, leaving 1458 controls with analysis performed.

In a case-cohort design, every person of the source population has the same chance of being included as a control. Since our control group was sampled at baseline, 29 controls later became cases during the follow-up period. The OR in a case cohort study is to be interpreted as an estimate of the risk ratio, because all cohort participants from the baseline contribute to the denominator in absolute risks (Rothman, 2002). As a consequence of this design, the 29 controls that later became cases were included in the case-group as well as in the control-group.

Laboratory methods

Blood was drawn from non-fasting participants at entry in the HUNT 2 cohort and stored as serum, EDTA-anticoagulated whole blood or clots from whole blood at -70°C for research purposes. Before storage, serum was separated from blood cells within 2 h (centrifuged at 1010 g) and stored in a refrigerator at 4°C. All samples were transported the same day in a cooler to the central laboratory in Levanger and stored at -70°C in the HUNT biobank the next morning. Samples taken on Fridays were frozen the following Monday morning after being transported the same Friday evening. After selection of cases and controls, DNA and serum analyses were measured in stored samples.

The total homocysteine concentration (tHcy) in thawed serum was measured in one central laboratory (Laboratory of Paediatrics and Neurology in Nijmegen, the Netherlands) by an automated high-performance liquid chromatography method with reverse phase separation and fluorescent detection [Gilson 232-401 sample processor (Gilson Inc, Middleton, CT, USA), Spectra Physics 8800 solvent delivery system, and Spectra Physics LC 304 fluorometer (Spectra Physics, San Jose, CA, USA)], essentially according to the method by Fiskerstrand et al (1993), with modifications (de Bree et al, 2001).

The serum folate concentration was measured in the central laboratory in Levanger Hospital, Norway by an Architect Folic Acid assay (Abbott Diagnostics, IL, USA), using BioRAD Quantaphase II as reference standard.

The DNA for genotyping was extracted from peripheral blood leukocytes from EDTA whole blood or blood clots stored in the HUNT biobank, using the Puregene kit (Gentra Systems Inc., Minneapolis, MN, USA) manually or with an Autopure LS (Gentra System Inc.).

Assessment of MTHFR 677 C to T mutation in DNA was initially performed by restriction fragment length polymorphism analysis after conventional polymerase chain reaction (PCR). The presence of the MTHFR 677T allele was assessed by incubation with the restriction enzyme *Hinf*I. The polymorphism was determined by a 5' nuclease (Taq-man; Applied Biosystems, Foster City, CA, USA) assay using a standard PCR reaction mix (Eurogentec, Seraing, Belgium) and allele-specific fluorescent probes equipped with a minor groove binding moiety (Applied Biosystems). DNA analyses were performed in Leiden, at the Einthoven Laboratory for Experimental Vascular Medicine.

Statistical analysis

Frequencies and means were analyzed separately for VT cases and controls. The serum homocysteine measures were positively skewed, and we used the logarithmic transformations to normalize their distribution. Thus, all means presented here are geometric means. Logistic regression analysis was used to estimate relative risk in the form of OR with 95% CI. Odds ratios were calculated for total homocysteine concentration as a continuous variable and grouped into quintiles to examine whether there was a dose-response effect. In addition we used the homocysteine levels above *versus* below the 80th, the 90th, and the 95th percentile, based on the distribution of homocysteine among the controls, as cut-off points. In the final model we performed stratified analyses on sex and age, and subgroup analyses according to presence of predisposing risk factors for VT and according to time between entry and event comparing those above the 95th percentile of homocysteine levels with those below the 95th percentile. The sex-stratified model was adjusted for age as a categorical variable with 5-year age bands.

The age-stratified model was adjusted for sex as a categorical variable. The subgroup analyses according to predisposing risk factors for VT and according to time between entry and event were adjusted for both sex and age in 5-year bands.

To describe the relationship between the MTHFR genotype and serum homocysteine levels we calculated the geometric mean levels of homocysteine in individuals with normal (677CC), heterozygous (677CT), and homozygous (677TT) mutant genotypes. Odds ratios for MTHFR genotype and VT were analyzed for heterozygotes (677CT) and homozygotes (677TT), respectively, compared to the common genotype (677CC) as reference category. In addition, the OR for any T allele (677CT and 677TT) *versus* the reference category (677CC) was estimated.

Ethics

Participation was voluntary and each participant signed a written consent at enrolment in the HUNT2 cohort. The current case-cohort study was approved by the National Data Inspectorate and the Regional Committee for Medical Research Ethics of Central Norway.

Results

Participants

Table I shows the general characteristics of the 505 patients and 1458 control subjects. The median age of both cases and controls at baseline was 70 years (mean age 66, range 20–98). Fifty-five percent were women. Renal function and levels of folate, which may influence homocysteine levels, were similar. Two thirds of the patients had deep vein thrombosis and one third pulmonary embolism. Among the 505 events, 264 were idiopathic VT, 151 secondary non-cancer VT and 90 were cancer-associated VT. The mean homocysteine levels were 14.5 $\mu\text{mol/l}$ in the cases and 14.1 $\mu\text{mol/l}$ in the controls.

Table I: General characteristics of the study population.

	Patients [n (%)]	Controls [n (%)]
Total	505	1458
Sex		
Men	227 (45.0)	667 (45.7)
Women	278 (55.0)	791 (54.3)
Age groups		
20-29	12 (2.4)	30 (2.1)
30-39	17 (3.4)	47 (3.2)
40-49	53 (10.5)	144 (9.9)
50-59	72 (14.3)	194 (13.3)
60-69	99 (19.6)	300 (20.6)
70-79	167 (33.1)	496 (34.0)
>80	85 (16.8)	247 (16.9)
Event		
Deep vein thrombosis	321 (63.6)	14 (50.0)*
Pulmonary embolism	151 (29.9)	12 (42.9)*
Both	33 (6.5)	2 (7.1)*
Time from blood sample to event		
Median (range)	33 months (2d – 75months)	
Serum homocysteine (mean, nmol/l)	14.5	14.1
Serum folate (mean, nmol/l)	6.2	6.4
Serum creatinine (mean, µmol/l)	94	92
Body mass index (mean)	28	27
Smoking status (%)		
Never smoked	45	45
Past smoker	32	30
Current smoker	21	23

* Controls were collected at entry to the HUNT 2 study. During the follow up, i.e. after the blood sampling at the entry, 29 controls suffered a first VT in whom the blood sample was missing in one. These subjects were included both as cases and controls. Controls with previous events, i.e. events before the entry of the cohort, were excluded.

Association between homocysteine and VT

Table II shows the association between levels of serum homocysteine and VT. The sex- and age-adjusted OR for VT was 1.28 (95% CI 0.90-1.82) for the quintile with highest serum homocysteine levels compared to the lowest quintile, but no clear gradient of risk over homocysteine levels (quintiles) was observed. We found no trend over ordered categories of homocysteine levels (quintiles scored from 1 to 5) (P-value trend = 0.1). Using different cut-off points the adjusted OR was 1.32 (95% CI 1.02-1.70), 1.34 (95% CI 0.96-1.87) and 1.50 (95% CI 0.97-2.30) for homocysteine levels above *versus* below the 80th percentile, 90th percentile and 95th percentile, respectively. Several others parameters did not differ between the cases and control subjects (see Table I), thus we did not use them in the analyses.

Table III shows the odds ratios in subjects with homocysteine levels above *versus* below the 95th percentile after stratification according to age and sex. A 2-fold increased risk for VT

was found in men (OR 2.17, 95% CI 1.20-3.91), whereas no association was observed in women (OR 1.00, 95% CI 0.52-1.92). Stratifying according to age above or below 70 years at baseline did not suggest differences in effects for serum homocysteine levels using the 95th percentile as a cut-off point. Subgroup analyses according to presence of predisposing risk factors or time from blood sampling to the event did not suggest differences in effects between the groups. When we used the 80th and 90th percentile in the same analyses no essential difference was found, with the exception in men, in which the effect was slightly lower when the 80th and 90th percentiles were used [OR 1.55 (95% CI 1.08-2.22) and 1.60 (95% CI 1.03-2.50), respectively].

Table II. Odds ratios (OR) and 95% confidence intervals (CI) of first venous thrombosis for quintiles, 80th, 90th and 95th percentiles of serum homocysteine*.

Homocysteine levels	Cases n = 505	Controls n = 1458	OR†	95 % CI
quintile 1 (< 10.7 mmol/l)	100	282	1	ref
quintile 2 (10.8-12.6 mmol/l)	87	293	0.86	0.62-1.21
quintile 3 (12.7-14.8 mmol/l)	105	296	1.06	0.76-1.49
quintile 4 (14.9-17.9 mmol/l)	91	293	0.95	0.67-1.36
quintile 5 ($\geq$ 17.9 mmol/l)	122	294	1.28	0.90-1.82
< 80th percentile	383	1164	1	ref
$\geq$ 80th percentile	122	294	1.32	1.02-1.70
< 90th percentile	441	1311	1	ref
$\geq$ 90th percentile	64	147	1.34	0.96-1.87
< 95th percentile	469	1385	1	ref
$\geq$ 95th percentile	36	73	1.50	0.97-2.30

*The 80th, 90th and 95th percentiles are homocysteine concentrations of 17.9, 21.9 and 25.9 mmol/l, respectively. The quintiles and percentiles are calculated in the control subjects.

†Adjusted for age (in 5-years age bands) and sex.

Association between MTHFR genotype and VT

The MTHFR 677TT genotype was present in 40 (9%) of the cases and 122 (8%) of the controls (Table IV). Homocysteine concentration (geometric mean) was 20% higher in the MTHFR 677TT genotype than in the CC genotype. There was no increased risk for VT in the MTHFR heterozygotes (677CT) or homozygotes (677TT) relative to the 677CC genotype [OR 1.01 (95% CI 0.81-1.25) and OR 1.02 (95% CI 0.70-1.49), respectively]. When we studied the association between MTHFR genotype and VT in a subgroup of participants with low serum folate levels [<4.7 nmol/l (reference level)] no effect was found. The OR for any T allele (677CT and 677TT) compared to the 677CC genotype was 1.01 (95% CI 0.82-1.24).

Table III: Odds ratios (OR) with 95% confidence interval (CI) of venous thrombosis (VT) in subgroups with homocysteine levels above the 95th percentile* compared to those below the 95th percentile.

Subgroup	Cases	Controls	OR†	95 % CI
Men	22/205	33/634	2.17	1.20-3.91
Women	14/264	40/751	1.00	0.52-1.92
Age < 70 years	7/246	15/700	1.35	0.54-3.41
Age ≥ 70 years	29/223	58/685	1.54	0.95-2.51
Idiopathic events	17/247	73/1385	1.40	0.80-2.46
Secondary events#	10/141	73/1385	1.39	0.68-2.85
Cancer associated events§	9/81	73/1385	1.90	0.88-4.11
Time between blood sampling and VT				
< 1 year	8/80	73/1385	1.67	0.75-3.72
1 - 3 years	13/176	73/1385	1.44	0.76-2.71
≥ 3 years	15/213	73/1385	1.48	0.81-2.70

*The 95th percentile of homocysteine is 25.9 µmol/l, calculated in the control subjects (n = 1458).

†Adjusted for age (in 5-years age bands) and sex.

#Secondary non-cancer events.

§Events secondary to cancer.

Table IV: Odds ratios (OR) and 95% confidence intervals (CI) of first venous thrombosis for heterozygotes and homozygotes *versus* normal MTHFR genotype and geometric mean for serum homocysteine in the groups.

MTHFR genotype	Homocysteine geometric mean* (±SE) (mmol/l)	Cases n = 507	Controls n = 1430	OR†	95% CI
CC (wild type)	13.69 (±1.01)	255 (50)	726 (51)	1	ref
CT (heterozygote)	14.34 (±1.01)	208 (41)	582 (41)	1.01	0.81-1.25
TT (homozygote)	16.35 (±1.04)	44 (9)	122 (8)	1.02	0.70-1.49

*Calculated in the controls.

†Adjusted for age (5-years age bands) and sex.

Discussion

This population-based case-cohort found a weak association between homocysteine levels measured before the event and VT, which was only present in men (2-fold increased risk for levels over the 95th percentile, i.e. 25.9 µmol/l). There was no gradient of risk over lower homocysteine levels. The C677T polymorphism in MTHFR, the gene that codes for the enzyme MTHFR, which increases homocysteine levels, was not associated with the risk of VT.

Elevated tHcy was associated with about a 10-15% increased risk for coronary atherosclerosis and about 20% for cerebro-vascular disease (Wald et al, 2006). Studies on VT showed the

same level (den Heijer, 2003). Our results for VT were essentially in line with these findings. Most studies have reported positive associations between hyperhomocysteinaemia and VT (den Heijer et al, 2005). In most of these studies blood samples used to measure homocysteine were collected after the event (case-control studies). Only in two other studies (Ridker et al, 1997; Tsai et al, 2003) were blood samples collected before the event. The differences between the two types of studies are mainly quantitative, with weaker associations when blood was collected before the event. Having used a similar design we also found a weak association between hyperhomocysteinaemia and VT. This effect was only found in men, as reported by Ridker et al (1997), although this latter study did not include women. In contrast, the Longitudinal Investigation of Thromboembolism Etiology (LITE) study found the effect was higher in women (Tsai et al, 2003). Unlike these studies, we did not find a difference between idiopathic VT and secondary (non-cancer) VT.

Meta-analyses on homocysteine related to both VT (den Heijer et al, 2005) and cardiovascular disease (The Homocysteine Studies Collaboration, 2002) have shown lower relative risks in prospective studies than in retrospective studies. Reverse causality, with differences in homocysteine levels between cases and controls resulting from treatment or disease-related factors may explain results from retrospective studies. Our prospective design, where blood was collected before the onset of disease excluded such effects. However, an advantage of case-control studies is that they can measure levels at a point of time closely related to the event, while in prospective studies, such as ours, many years may have elapsed between the measurement and the event. When the thrombotic event does not affect homocysteine levels, which vary over time, the case-control results would be more pronounced and more accurate than those of follow-up studies.

If increased homocysteine levels cause VT, one would expect a relationship between the MTHFR 677TT genotype and risk of VT. This was not found in our study. This confirms the negative result from the MEGA (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis) study (Bezemer et al, 2007), where the association between MTHFR genotype and VT was studied in 4375 cases with first VT and 4856 control subjects. However, it is possible that the increase in homocysteine related to genotype is too small to produce an association. The finding of an association restricted to high levels of homocysteine supports this possibility.

Since we found an association with VT in high levels of homocysteine in men only and the confidence intervals were wide, we cannot rule out that the finding is due to chance. The higher risk for VT in women with hyperhomocysteinaemia found in the LITE study (Tsai et al, 2003) supports that our finding in men is a chance finding.

The serum levels of homocysteine were higher in our study than in other studies that used the same laboratory method (den Heijer et al, 1995, 1996). The 95th percentile calculated in the control subjects was 18.3 $\mu\text{mol/l}$ and 22.2 $\mu\text{mol/l}$, respectively in these studies, whereas it was 25.9 $\mu\text{mol/l}$ in our study. The mean age in the control groups in these two studies was 44 years and 51 years, respectively, whereas it was 66 years in our study. The differences may be due to different age distributions in the studies, reflecting increasing homocysteine levels by increasing age (Nygard et al, 1995). The serum samples were left at room temperature for up to 2 h before they were centrifuged, which could result in an increase in tHcy concentration of about 2 $\mu\text{mol/l}$ (Willems et al, 1998). This would not influence the difference between cases and controls, because it would happen to the same extent in both groups.

The diagnosis of VT is difficult, and clinical diagnosis is unreliable. We validated carefully each individual case identified from the diagnosis registries, and excluded all cases without an objectively verified diagnosis. We thus excluded a significant number of potential cases who had a clinical diagnosis with no or insufficient diagnostic tests performed. A bias could theoretically result if these cases had homocysteine levels different from the included cases, but this is unlikely.

A major strength of our study is the prospective design, with serum analyses performed in stored blood samples drawn before the event, which makes it possible to examine the effect of elevated homocysteine levels on the risk of a subsequent first VT. The population-based design in this study with a very high participation rate is an advantage, as it reduces the risk of selection bias and enables generalization of the results. It is the largest prospective study to date on homocysteine and VT risk, with high quality of the case ascertainment. It is one of few studies including both homocysteine and MTHFR 677TT genotype risk assessments in the same study. A drawback that may have diluted the risk estimates is the time that elapsed between venepuncture and the occurrence of thrombosis.

In conclusion, elevated levels of homocysteine were found to predict VT in men, but not in women. An association between MTHFR 677TT genotype and VT could not be demonstrated. The lack of association between MTHFR 677TT genotype and VT may suggest that the association between elevated homocysteine levels and VT is not casual.

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Appendix

The ICD-9 codes for VT diagnoses used were 415, 451, 452, 453, 997.2, 674, 673, 671, 634, 557, 437, 325 and 362.3 and the ICD-10 codes I26, I80, I81, I82, I67, I 63, K55, K75, O08, O22, O87, O88 and H34.8.

Chapter 2.5

**The risk of venous thrombosis related to increase
in body mass index is mediated by factor
VIII-induced APC-resistance**

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F.R. Rosendaal, S.C. Cannegieter

Abstract

High body mass index (BMI) is associated with an increased risk of venous thrombosis (VT). Clotting factor (F)VIII can be released by adipose tissue. High FVIII increases APC-resistance. This APC-resistance could be aggravated in FV Leiden carriers. Presence of high FVIII in non-O blood group individuals could worsen this further. We hypothesized that an association exists between BMI and APC-resistance, and that FV Leiden and/or blood group non-O modifies the risk of increasing BMI on VT occurrence. This was studied in a pooled analysis of 2 case-control studies (n=1801). APC-resistance increased linearly with increasing BMI, partly explained by increasing FVIII. Increasing BMI, independent of existing APC-resistance, was associated with 1.9-fold and 2.2-fold increased risk of VT for those with a BMI in the median or upper tertile, compared to the lowest tertile. Both risks decreased slightly after FVIII and APC-resistance adjustments. When BMI increased, APC-resistance, factor VIII, and risk of VT increased gradually in (non)-FV Leiden-carriers with (non)-O blood group, excepted for FV Leiden carriers with non-O blood group. These showed >20 fold increased risks of VT irrespective of BMI. We conclude that the increased risk of VT in individuals with high BMI is mediated by factor VIII induced APC-resistance.

Introduction

Venous thrombosis occurs in 1-3 per 1000 individuals per year,^{1,2} and is more frequent in individuals with overweight (body mass index [BMI] of 25-30 kg/m²),³ or obesity (BMI $\geq$ 30 kg/m²) than in lean individuals.³ Overweight or obesity lead to a 2-3 fold increased risk of venous thrombosis compared to normal weight.^{4,5} It is not understood how a high BMI predisposes to venous thrombosis. People with overweight or obesity tend to be more immobile which may lead to clot formation through stasis. It is also possible that these individuals acquire a prothrombotic state. Clinically at least, some studies have found a stronger effect of high BMI on the risk of first venous thrombosis in factor V Leiden carriers.^{5,6} Risk of venous thrombosis in factor V Leiden carriers is explained by APC-resistance.⁷ It was therefore speculated that a joint effect between factor V Leiden and a high BMI leads to increased APC-resistance which could explain the enhanced risk of venous thrombosis in heavyweight individuals with factor V Leiden.⁵ Data on whether a high BMI leads to APC-resistance is however limited to only one study that found an association between increasing BMI and increasing APC-resistance.⁸ High levels of factor VIII are also associated with an increased risk of venous thrombosis.⁹ This risk is partially genetically determined as individuals with blood group non-O have higher levels of factor VIII than individuals with blood group O.⁹ However, a high BMI is also associated with elevation in the level of factor VIII,^{4,10-12} which may be a result of release of factor VIII by adipose tissue through inflammatory cytokines.¹⁰⁻¹² As factor VIII can induce APC-resistance,⁸ the pathway of high BMI leading to venous thrombosis could run through factor VIII-induced APC-resistance. If so, a reinforcement in this risk in the presence of factor V Leiden, blood group non-O, or a combination of both genetic factors could be expected.

We used the data from two well-characterised case-control studies, in the Netherlands (LETS) and Norway (TROL), to study four hypotheses which we graphically illustrated in Figure 1. We set out to determine first of all whether an association exists between BMI and APC-resistance in LETS, and whether this association is mediated by factor VIII levels (black dashed arrows in Figure 1). Secondly, we studied whether the risk of venous thrombosis associated with high BMI was attenuated by APC-resistance or factor VIII levels in LETS (black arrow in Figure 1). Thirdly, in a pooled analysis of LETS and TROL, we verified if blood group non-O modified the risk of increasing BMI on the occurrence of venous thrombosis (grey dashed arrows in Figure 1) and whether these risks were further increased by the presence of factor V Leiden (grey arrows in Figure 1).

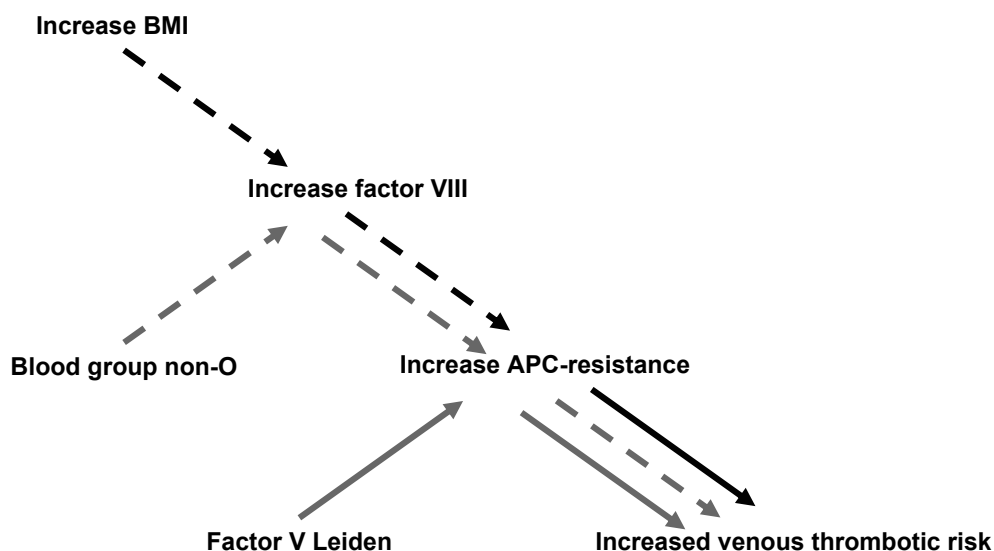


Figure 1. Hypotheses of the study

The black dashed arrows indicate the first study question, i.e. whether an association exists between BMI and APC-resistance, and whether this association is mediated by factor VIII levels. The black solid arrow indicates the second study question, i.e. whether the risk of venous thrombosis associated with high BMI is attenuated by APC-resistance or factor VIII levels. The grey dashed arrows indicate the third study question, i.e. if blood group non-O modifies the risk of increasing BMI on the occurrence of venous thrombosis. The grey arrows indicate the final study question, i.e. whether these risks were further increased by the presence of factor V Leiden.

Methods

LETS patients and controls.

The inclusion of patients and controls in the Leiden Trombophilia Study has been extensively described in the past.^{7,13,14} In short, cases were consecutive patients treated for deep vein thrombosis (DVT) at the Anticoagulation Clinics in Leiden, Amsterdam and Rotterdam during the period January 1, 1988 and December 30, 1992. Patients had to be without a diagnosis of active cancer, beneath 70 years of age at entry and to have a diagnosis of DVT established with objective diagnostic methods. Fulfilling these criteria, 90% participated resulting in a case-group with 474 consecutive DVT-patients. A control group was assembled where patients were to find their own healthy control subject of the same sex, same age (± 5 years), no biologic relative, and without a diagnosis of active cancer, a history of venous thrombosis, or having used coumarin therapy in the last three months before inclusion. This way 474 controls were found.^{7,13,14}

TROL patients and controls.

Between August 1995 and June 1997 all inhabitants older than 20 years of age ($n=94,194$) in Nord-Trøndelag County (in Middle Norway) were asked to be part of a population based health survey called "Helseundersøkelsen i Nord-Trøndelag" (HUNT2).¹⁵ All participants

(n=66,140, participation rate 71%) underwent a physical examination, donated 7.5 ml of blood and filled in a questionnaire. Participants had a median age of 46 years (range, 19-103).¹⁵ The follow-up of HUNT2 has been described before.¹⁶ In brief, cases with venous thrombosis were identified screening the computerized diagnosis registries of all departments in the only two local hospitals in the region (Levanger and Namsos hospitals) until 1 January 2002. The search was supplemented by crosslinking with all positive diagnostic procedures for venography, duplex and Doppler ultrasound, within the registries of the radiology departments at the two hospitals. Finally, patients from Nord-Trøndelag County discharged from St. Olav University Hospital with diagnostic codes of venous thrombosis were included. This search identified 2,136 cases with a diagnostic code of venous thrombosis, and their hospital records were reviewed to obtain information for validation of the diagnosis.

We used the following criteria as a confirmation of the diagnosis respectively for DVT; an intraluminal filling defect or no venous filling on ascending contrast venography; or, no compressible venous segment or no venous flow in popliteal, femoral, or axillary veins on duplex ultrasound; or, a positive computed tomography scan or a positive autopsy; for pulmonary embolus (PE); a ventilation-perfusion scan with one or more segmental or sub segmental perfusion defects with normal ventilation; or, a contrast defect on pulmonary computed tomography scan; or a positive autopsy.

We found 1,271 cases with a validated diagnosis of whom 798 (63%) were within the HUNT2 cohort. We excluded 283 patients with previous venous thrombosis, enrollment in the HUNT2 cohort after the event, leaving 515 cases with a definite diagnosis of venous thrombosis. Subsequently, we sampled 1,505 controls from the baseline of the HUNT2 cohort, frequency-matched to the cases by sex and 5-year age strata.

Laboratory measurements.

LETS.

Blood drawn from the participants was placed in 0.106 M of trisodium citrate, centrifugated at 2000 g for 10 minutes before storage at - 70 °C in a 1.5 mL container.^{7,12,13} A standard salting out method was used to extract DNA (3).

Activated Protein C Resistance and the Factor V 1691 (G→A) variant (factor V Leiden) were measured according to methods described earlier in the LETS.^{7,14}

The method of measuring plasma's APTT sensitivity to activated protein C was according to the technique used by Dahlbäck et al,¹⁷ and is expressed as normalized APC-ratio. APC-resistance measurements were missing or failing in 51 cases and 5 controls. DNA-samples were missing or measurement failed in 3 cases but in non of the controls. Factor VIII activity was measured by a 1-stage coagulation assay and blood group by polymerase chain reactions.⁹

TROL.

Available DNA descended from peripheral blood leukocytes (whole blood or blood clots) and was extracted using the Puregene kit (Gentra Systems Inc.) manually or with an Autopure LS (Gentra Systems Inc.). Factor V Leiden and blood group were measured using polymerase chain reaction according to the Taqman method.¹⁸ In 9 cases (1.7 %) and 50 controls (3.4 %), DNA-samples were either missing or the measurements failed. Technicians were not aware of the status of cases or controls since all samples were stored anonymously before the selection

of a control-group from the baseline cohort. In the TROL study plasma was not obtained, and therefore factor VIII levels and APC-resistance could not be determined in TROL.

Statistical analysis.

Linear regression was used to determine the relation between increasing APC-resistance and BMI, increasing factor VIII levels and BMI, increasing APC-resistance and factor VIII levels, and between increasing APC-resistance and BMI adjusted for factor VIII levels.

Cut-off points needed to create tertile categories of APC-resistance were derived from the control-group of the LETS population after the exclusion of factor V Leiden carriers and individuals who used oral contraceptives at time of blood draw. Cut-off points to create tertile categories for BMI were derived from the control groups of the LETS and the TROL population separately, irrespective of factor V Leiden or oral contraceptive use. Logistic regression was used to calculate odds ratios and their 95% confidence intervals (95% CIs), adjusted for age and sex.

The effect of factor V Leiden or blood group in combination with a high BMI was calculated in the LETS and the TROL population, both separately and combined. To have analyses directed at similar groups in the TROL and LETS populations, we restricted the analysis in the TROL subjects to those who were younger than 70 and to those who had a DVT only in the current study (n=183 cases and n=696 controls).

The statistical software used was SPSS version 16.0 (SPSS, Chicago, Ill, United States).

Results

LETS and TROL study populations.

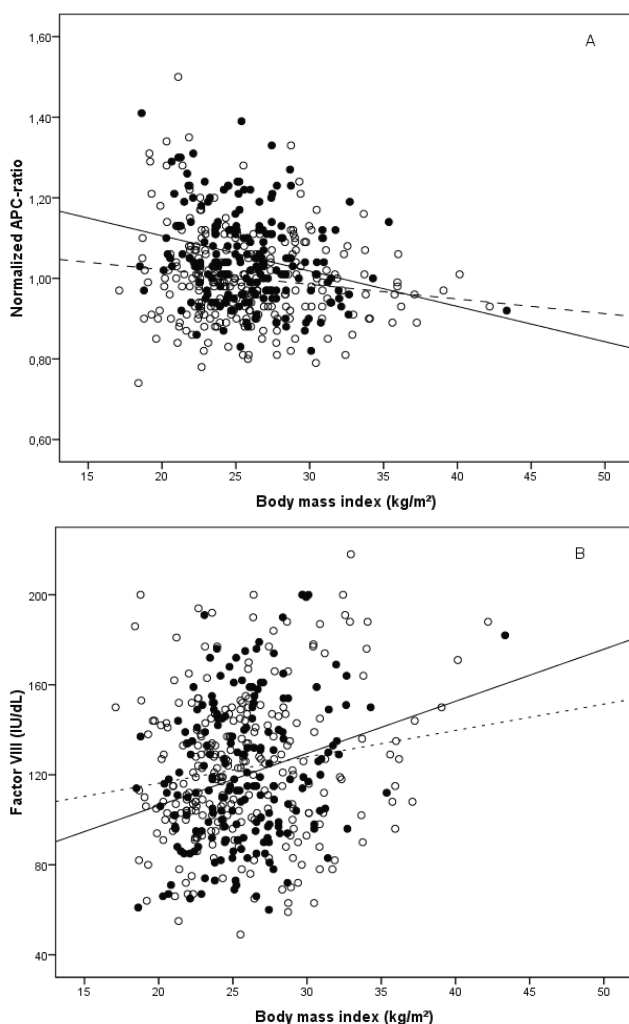
The descriptive characteristics of the patients and control individuals of both study populations are listed in Table 1. The TROL-population is an older population, since the upper limit of inclusion in LETS was 70 years whereas there was no age limit in the TROL study. Hence, LETS cases and controls had a median age of 45 years while this was 54 years in the TROL cases and 56 years in the controls. There were slightly more women than men in both studies.

Table 1: Baseline characteristics of the LETS and the TROL-study.

Category		LETS		TROL	
		Cases n (%)	Controls n (%)	Cases n (%)	Controls n (%)
Total		474	474	515	1476
Sex	Men	202 (43)	202 (43)	228 (44)	676 (46)
	Women	272 (57)	272 (57)	287 (56)	800 (54)
Age groups, years	10-19	10 (2)	16 (3)	-	-
	20-29	67 (14)	56 (12)	12 (2)	30 (2)
	30-39	75 (16)	79 (17)	17 (3)	47 (3)
	40-49	135 (29)	142 (30)	53 (10)	146 (10)
	50-59	92 (19)	93 (20)	74 (14)	199 (14)
	60-69	95 (20)	88 (19)	99 (19)	303 (21)
	70-79	-	-	172 (33)	501 (34)
	>80	-	-	88 (17)	250 (17)

BMI, APC-resistance, and FVIII.

Figure 2A shows that in the LETS control individuals, the normalised APC-ratio decreased linearly with increasing BMI, i.e. APC-resistance increased when BMI increased. This association was found in both men and women, but appeared to be slightly stronger in men (Beta -0.0088; 95% CI -0.0133 to -0.0042 for men and -0.0036; 95% CI, -0.0068 to -0.0004 for women). A same prothrombotic phenomenon was observed for factor VIII, i.e., an increase in BMI was associated with increased factor VIII levels both in men and women, which relation was again somewhat stronger in men than in women (Figure 2B). Increased APC-resistance was therefore also associated with an increase of factor VIII levels (Figure 2C), and factor VIII levels explained part of the relation between BMI and APC-resistance, as the slope of the regression line of APC-resistance on BMI levels decreased after adjustment for factor VIII both in men and in women (adjusted beta -0.0042; 95% CI, -0.0080 to -0.0003 for men and -0.0013; 95% CI, -0.0039 to 0.0013 for women).



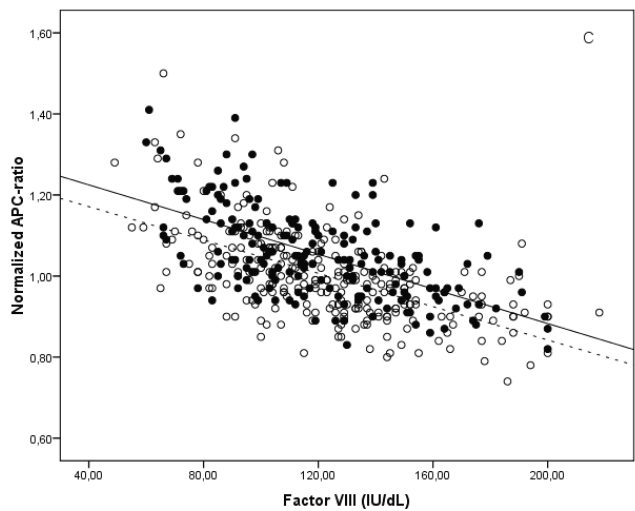


Figure 2. Scatter diagrams and linear regression models of normalized APC-ratio associated with body mass index and factor VIII*

Men without factor V Leiden (black dots and solid regression line; n=191)

Normalized APC-ratio = 1.18 - (0.0088 x BMI)	CI95 slope (-0.0133, -0.0042)	(Figure 1A)
Factor VIII (IU/dL) = 60.0 + (2.3159 x BMI)	CI95 slope (1.0547, 3.5771)	(Figure 1B)
Normalized APC-ratio = 1.31 - (0.0021 x FVIII)	CI95 slope (-0.0026, -0.0017)	(Figure 1C)
Normalized APC-ratio = 1.40 - (0.0042 x BMI)	CI95 slope (-0.0080, -0.0003), FVIII adjusted,	
data not shown		

Women without factor V Leiden (white dots and dashed regression line; n=257)

Normalized APC-ratio = 1.10 - (0.0036 x BMI)	CI95 slope (-0.0068, -0.0004)	(Figure 1A)
Factor VIII (IU/dL) = 92.8 + (1.1712 x BMI)	CI95 slope (0.2678, 2.0744)	(Figure 1B)
Normalized APC-ratio = 1.25 - (0.0021 x FVIII)	CI95 slope (-0.0024, -0.0018)	(Figure 1C)
Normalized APC-ratio = 1.28 - (0.0013 x BMI)	CI95 slope (-0.0039, 0.0013), FVIII adjusted,	
data not shown		

* Data subtracted from control individuals in LETS without factor V Leiden.

CI95 denotes 95% confidence interval ; FVIII, factor VIII

The risk of venous thrombosis with increasing BMI and APC-resistance.

To examine the effect of increasing BMI on the risk of venous thrombosis, separate from APC-resistance due to other factors, we restricted the analysis to individuals from the LETS without factor V Leiden or oral contraceptive use. In these individuals, the risk of venous thrombosis increased 1.9-fold for those with a BMI in the median tertile (odds ratio 1.9; 95% CI, 1.0-2.5) and 2.2-fold for those in the upper tertile (odds ratio 2.2; 95% CI, 1.4-3.4), as compared to individuals in the lowest tertile (Table 2). Adjustment for APC-resistance or factor VIII levels in a logistic regression model led to a slight decrease in these relative risk estimates, suggesting that the effect on risk is at least partly mediated through FVIII levels or APC-resistance.

Table 2: Risk of venous thrombosis according to BMI tertiles and APC-resistance *

BMI (kg/m ²)	Case	Control	Odds ratio (CI95)	Odds ratio (CI95)	Odds ratio (CI95)
	n (%)	n (%)		adjusted for APC	adjusted for FVIII
Lower tertile (< 23.0)	47 (20)	123 (33)	Reference	Reference	Reference
Median tertile (24.0-27.3)	87 (37)	123 (33)	1.9 (1.0-2.5)	1.6 (1.0-2.6)	1.7 (1.0-2.6)
Upper tertile (≥ 27.3)	103 (43)	123 (33)	2.2 (1.4-3.4)	1.7 (1.1-2.6)	1.9 (1.2-2.9)
Total	237	369			

BMI denotes body mass index (kg/m²); CI 95, 95% confidence interval; FVIII, factor VIII.

* Data subtracted from LETS, excluding individuals with factor V Leiden and women who used oral contraceptives.

The risk of venous thrombosis with increasing BMI, factor V Leiden and non-O blood group. Table 3 shows the combined effects of factor V Leiden and blood group within increasing BMI categories on the risk of venous thrombosis in the LETS and TROL-populations. When BMI increased, APC resistance and factor VIII levels also increased in a dose response way in non-factor V Leiden-carriers with blood group O. These individuals were at risk of venous thrombosis when they had a BMI in the upper tertile compared to the lowest tertile; adjusted odds ratio 1.9 (95% CI, 1.2-3.1). A similar but slightly stronger dose response pattern for increase in APC-resistance and increase of factor VIII levels when BMI level increased was observed in non-factor V Leiden carriers with blood group non-O. Venous thrombotic risk was modestly increased, in a dose-response way, when these individuals were compared with the reference group. Factor V Leiden carriers with blood group O also showed a dose-response increase of APC-resistance and factor VIII when BMI level increased. Risk of venous thrombosis was strongly increased, again in a dose response way, within the BMI tertiles compared to the reference group. Carriers of factor V Leiden with blood group non-O had highest factor VIII levels between the BMI tertiles. They showed the highest risk of venous thrombosis compared to the reference group, no longer in a dose-response way, with adjusted odds ratios of 40.6 (95% CI, 9.0-183), 23.3 (95% CI, 8.3-65.1) and 25.2 (95% CI, 10.4-61.2), respectively, within the BMI tertiles.

Table 3: LETS and TROL combined: Risk of venous thrombosis according to the combinations of factor V Leiden BMI tertiles and bloodgroup*

BMI	Factor V Leiden	Blood group	Mean normalized APC-ratio†	Mean factor VIII (IU/dL)‡	Case n (%)	Control n (%)	OR (CI95)	OR‡ (CI95)
Lowest tertile	-	O	1.06	111	35 (5)	137 (12)	Reference	Reference
Median tertile	-	O	1.02	115	40 (6)	163 (14)	1.0 (0.6-1.6)	1.0 (0.6-1.7)
Upper tertile	-	O	1.00	121	76(12)	160 (14)	1.9 (1.2-2.9)	1.9 (1.2-3.1)
Lowest tertile	-	non-O	0.98	132	87 (13)	230 (20)	1.5 (0.9-2.3)	1.5 (0.9-2.3)
Median tertile	-	non-O	0.98	140	121 (19)	216 (19)	2.2 (1.4-3.4)	2.4 (1.5-3.7)
Upper tertile	-	non-O	0.94	147	154 (24)	209 (18)	2.9 (1.9-4.4)	3.4 (2.2-5.4)
Lowest tertile	+	O	0.51	123	11 (2)	14 (1)	3.1 (1.3-7.4)	3.0 (1.2-7.2)
Median tertile	+	O	0.48	125	14 (2)	7 (0.5)	7.8 (2.9-20.9)	8.3 (3.1-22.5)
Upper tertile	+	O	0.47	132	14 (2)	6 (0.5)	9.1 (3.3-25.5)	9.7 (3.4-27.5)
Lowest tertile	+	non-O	0.53	154	20 (3)	2 (0.2)	39.1 (8.2-175)	40.6 (9.0-183)
Median tertile	+	non-O	0.50	136	29 (4)	5 (0.4)	22.7 (8.2-62.9)	23.3 (8.3-65.1)
Upper tertile	+	non-O	0.52	145	44 (7)	7 (0.5)	24.6 (10.2-59.3)	25.2 (10.4-61.2)
Total					645	1156		

BMI denotes body mass index (kg/m²); CI 95, 95% confidence interval; OR odds ratio.

* Tertiles derived from LETS and TROL control population, respectively.

* Cases and controls < 70 years of age and DVT cases only (PE excluded).

LETS: Upper tertile: BMI: ≥ 26.9. Median tertile: BMI: ≥ 23.7 < 26.9. Lowest tertile: BMI: < 23.7.

TROL: Upper tertile: BMI: ≥ 27.9. Median tertile: BMI: ≥ 24.9 < 27.9. Lowest tertile: BMI: < 24.9.

† Data subtracted from LETS.

‡ Odds ratio adjusted for age and sex.

Discussion

In this study we have shown that an increase in BMI is related to an increase in APC-resistance. Part of this relation could be explained by factor VIII level as the slope of the regression line of APC-resistance on BMI levels decreased after adjustment for factor VIII. There was a dose response relation between increase of BMI and increasing venous thrombotic risk (1.9-fold and 2.2-fold increased risk when median BMI tertile or upper BMI tertile, respectively, was compared with the lowest BMI tertile). This was partly a result of APC-resistance and factor VIII as odds ratios attenuated after we adjusted for APC-resistance or for factor VIII. These latter findings support evidence from other studies that also showed that increase of BMI is associated with APC-resistance,⁸ and elevation of factor VIII levels.^{4,10,11,19,20}

When individuals had other causes of APC-resistance, such as blood group non-O or factor V Leiden, we saw a gradual increase in APC-resistance and factor VIII levels with increasing BMI, and a joint effect on the venous thrombotic risk with increasing BMI. This confirmed our hypothesis that factor V Leiden and/or blood group non-O modifies the risk of increasing BMI on venous thrombosis occurrence through factor VIII induced APC-resistance. This conclusion is only excepted for individuals who had both blood group non-O and factor V Leiden, as these individuals had the highest risk of venous thrombosis (odds ratios >20) irrespective of their BMI tertile. Apparently, the combination of strong APC-resistance combined with high factor VIII levels was sufficient enough to generate very high thrombotic risks, irrespective of BMI, in these individuals. That factor V Leiden carriers with blood group non-O are at very high risk of venous thrombosis has been reported before,²¹⁻²³ and may deserve attention in the management of primary or secondary prevention for venous thrombosis in these individuals, although this implication should be handled with caution as numbers, particularly in the control group, were small.

Prevalence of overweight and obesity in the western world is 50-65%.^{5,24} It has been calculated that almost one third of all events of venous thrombosis could be prevented by weight loss, assuming that weight loss reduces venous thrombotic risk.^{5,25} For this reason, encouragement to manage weight control in individuals with overweight or obesity seems reasonable.

Some limitations of our data merit consideration. First, height and weight were self reported both in the LETS and in the TROL study. As in general individuals with underweight tend to overreport their body weight, while individuals with overweight tend to underreport their body weight,²⁶ actual risks should be somewhat higher if this phenomenon has occurred. Second, control individuals in LETS were introduced by the cases (friends, acquaintances). It is therefore possible that in LETS, cases and controls had similar life styles that may have resulted in similar BMIs. When this were the case, it also would have led to lower risk estimates in our study than actual risks.

We conclude that the increased risk of venous thrombosis in individuals with high BMI is mediated by factor VIII induced APC-resistance. Individuals with factor V Leiden or blood group non-O had a higher risk of venous thrombosis compared to non-carriers, which was enforced 2 to 10 fold by increase of BMI. Future studies are needed to show if these risks can be downgraded by weight loss.

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Part 3

*Risk factors for a recurrent episode of venous
thrombosis*

Chapter 3.1

Thrombophilia, clinical factors, and recurrent venous thrombotic events

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Summary

Context: Data on the recurrence rate of venous thrombotic events and the effect of several risk factors, including thrombophilia, remain controversial. The potential benefit of screening for thrombophilia with respect to prophylactic strategies and duration of anticoagulant treatment is not yet known.

Objective: To estimate the recurrence rate of thrombotic events in patients after a first thrombotic event and its determinants, including thrombophilic abnormalities.

Design, Setting, Patients: Prospective follow-up study of 474 consecutive patients aged 18-70 years without a known malignancy treated for a first objectively confirmed thrombotic event at anticoagulation clinics in the Netherlands. The Leiden Thrombophilia Study (LETS) was conducted from 1988 through 1992 and patients were followed up through 2000.

Main outcome measures: Recurrent thrombotic event based on thrombophilic risk factors, sex, type of initial thrombotic event (idiopathic/provoked), oral contraceptive use, elevated levels of factors VIII, IX, XI, fibrinogen, homocysteine, and anticoagulant deficiencies.

Results: A total of 474 patients were followed up for mean (SD) of 7.3 (2.7) years and complete follow-up was achieved in 447 (94%). Recurrence of thrombotic events occurred in 90 patients during a total of 3477 patient-years. The rate of thrombotic event recurrence was 25.9 per 1000 patient-years (95% confidence interval [CI], 20.8-31.8 per 1000 patient-years). The incidence rate of recurrence was highest during the first 2 years (31.9 per 1000 patient-years; 95% CI, 20.3-43.5 per 1000 patient-years). The risk of thrombotic event recurrence was 2.7 times (95% CI, 1.8-4.2 times) higher in men than in women. Patients whose initial thrombotic event was idiopathic had a higher risk of a thrombotic event recurrence than patients whose initial event was provoked (hazard ratio [HR], 1.9; 95% CI, 1.2-2.9). Women who used oral contraceptives during follow-up had a higher thrombotic event recurrence rate (28.0 per 1000 patient-years; 95% CI, 15.9-49.4 per 1000 patient-years) than those who did not (12.9 per 1000 patient-years; 95% CI, 7.9-21.2 per 1000 patient-years). Recurrence risks of a thrombotic event by laboratory abnormality ranged from an HR of 0.6 (95% CI, 0.3-1.1) in patients with elevated levels of factor XI to an HR of 1.8 (95% CI, 0.9-3.7) for patients with anticoagulant deficiencies.

Conclusion: Prothrombotic abnormalities do not appear to play an important role in the risk of a recurrent thrombotic event. Testing for prothrombotic defects has little consequence with respect to prophylactic strategies. Clinical factors are probably more important than laboratory abnormalities in determining the duration of anticoagulation therapy.

Introduction

The incidence rate of a first venous thrombosis is 1 to 2 events per 1000 patient-years.¹⁻³ Venous thrombosis manifests mainly as deep venous thrombosis (DVT) and pulmonary embolism. Environmental risk factors include immobilization, surgery, malignancies, pregnancy, puerperium, and exogenous female hormones.⁴ Genetic abnormalities increasing the risk of a thrombotic event have been known for several decades and include deficiencies of the natural anticoagulants antithrombin, protein C, and protein S. Additional biochemical risk factors for a thrombotic event are factor V Leiden, prothrombin G20210A, high levels of factor VIII, IX, or XI, homocysteine, and fibrinogen.⁵⁻¹¹ Knowledge of the risk of a thrombotic event recurrence and its determinants is relevant for clinical policy regarding screening for thrombophilia, duration of anticoagulant treatment, and prophylactic strategies in circumstances of increased risk.

Estimates of the 5-year cumulative incidence of recurrent thrombotic events are around 25%.^{12,13} One study reported a high recurrence rate of 20% (17 cases) in a total of 83 patients during a 10-months follow-up. While estimates of the overall risk of a thrombotic event recurrence vary, reports on contributing factors are contradictory. Several studies have been published linking factor V Leiden, the prothrombin G20210A mutation, and the recurrence of a thrombotic event.^{14,25} Most studies showed little effect of carriership of these mutations on the risk of recurrence.^{14-20,23} However, in some studies a 4- to 5-fold higher risk of a thrombotic event recurrence has been noted in carriers compared with non-carriers.^{21,22,24,25} A recent critical review²⁶ of 4 studies highlighted hazard ratios (HRs) ranging from 1.1 to 4.1 in carriers of factor V Leiden compared with noncarriers. In studies comparing carriers of prothrombin G20210A with noncarriers, HRs of a thrombotic event recurrence varied from 0.9 to 4.9.

A 6-fold increased risk of a thrombotic event recurrence was reported for patients with high plasma levels of factor VIII.²⁷ A slightly increased risk of a thrombotic event recurrence was reported for patients with high levels of factor IX.²⁸ No recurrence data are available for elevated levels of factor XI or fibrinogen, which have been shown to increase the risk for a first event.^{9,11} Hyperhomocysteinemia increases the risk of a thrombotic event.¹⁰ It was also found to be prevalent in patients with a recurrent thrombotic event.^{29,30}

Two reviews^{31,32} pointed out that the contradictory results on contributing factors of thrombotic event recurrence may have resulted from (1) differences in study design, (2) lack of proper inception cohorts, (3) incomparability of anticoagulation profiles, (4) differences in quality of documentation of events, or (5) differences in the interpretation of clinical outcomes and laboratory tests. A particular issue may be that selected subgroups of patients were studied, ie, those referred to specialized centers for thrombophilia work-up, who may well have harbored additional, yet unknown defects that could have affected risks. Most study cohorts were small and followed up for only a short period.

We set out to determine the risk of a recurrent thrombotic event in 474 patients who had participated in a large population-based case-control study of risk factors for a first DVT. Many risk factors for a thrombotic event were investigated and these patients were followed up for up to 12 years. In particular, we examined the effect of several thrombophilic risk factors on the risk of recurrence, as well as the effect of sex, oral contraceptive use, and whether the first event was idiopathic or provoked.

Methods

We included 474 consecutive patients with a first, objectively confirmed episode of DVT. Patients were diagnosed between January 1, 1988, and December 30, 1992, and were participants in the Leiden Thrombophilia Study (LETS),^{11,33} which was a case-control study of the etiology of DVT. In the Netherlands, patients with a thrombotic event are treated in anticoagulation clinics, which are regionally organized. Therefore, all patients living in a certain area are monitored by the same clinic, irrespective of the hospital they were admitted to or the physician who started the treatment. Patients participating in LETS were identified from the files at the anticoagulation clinics in Leiden, Amsterdam, and Rotterdam. Ninety percent of eligible patients were willing to participate in LETS. Patients older than 70 years and those with malignancies were excluded. There were no major differences at baseline in characteristics between the patient groups from the 3 clinics; 453 patients had a DVT and 21 had a thrombosis in the arm. LETS was approved by the medical ethics committee of the Leiden University Medical Center and has been described elsewhere.^{11,33}

Patients were initially seen at least 3 months after the discontinuation of oral anticoagulant treatment, except in cases when this treatment could not be stopped (n=48). Patients were seen in person by one of us (T.K.) between October 1990 and January 1994. The median time between a thrombotic event and venipuncture was 19 months (range, 6-68 months). At the examination, information on acquired risk factors was collected and a venous blood draw was performed. Information was also collected on surgery, trauma, immobilization, use of oral contraception shortly before the diagnosis of a thrombotic event, family history, and reproductive history.

Blood was collected from the antecubital vein and placed in 0.106 M of trisodium citrate. Plasma was prepared by centrifugation for 10 minutes at 2000g at room temperature and stored at -70°C in 1.5-mL container. DNA was extracted by standard salting-out methods. When a deficiency of protein C, protein S, or antithrombin was suspected, patients were asked to have blood redrawn to confirm the diagnosis and then were informed of their deficiency.³⁴ In subsequent years, we investigated resistance to activated protein C with factor V Leiden (1994) and prothrombin G20210A (1996) in all participants and informed the patients if they had an abnormal result.^{5,6,35} None of the other test results (on levels of factors VIII, IX, or XI, homocysteine, fibrinogen) were communicated to the patients.⁷⁻¹¹

Laboratory Measurements

Details regarding the methods of measuring levels of the coagulation factors were described in detail in LETS.¹¹ The factor VIII activity was measured by a 1-stage coagulation assay.⁷ Factor IX antigen levels were measured by sandwich enzyme-linked immunosorbent assays using commercial polyclonal antibodies (Dako A/S, Glostrup, Denmark).⁸ Factor XI antigen levels were measured by using a monoclonal anti-factor XI capture antibody and polyclonal anti-factor XI as tagging antibody.⁹ The fibrinogen concentration was measured according to the Clauss method using Dade reagent (Baxter, Miami, Fla). Protein C activity and antithrombin activity were measured with Coamate (Chromogenix, Mölndal, Sweden) on an ACL 200 (Instrumentation Laboratory, Milan, Italy).³⁴ Total protein S was measured by a polyclonal enzyme-linked immunosorbent assay.³⁴ All coagulation factors were expressed

as units per deciliter, in which 1 U is the amount of coagulation factor present in 1 mL of pooled plasma. Fibrinogen levels were expressed as grams per liter. Total homocysteine concentration was measured in a nonfasting state by a modified method of automated high-performance liquid chromatography with reverse phase separation and fluorescent detection with a 232-401 sample processor (Gilson Inc, Middleton, Wis), an 8800 solvent-delivery system (Spectra-Physics, Mountain View, Calif), and an LC 304 fluorometer (Spectra-Physics).¹⁰ Prothrombin G20210A and factor V Leiden (FV G1691A) genotypes were assessed by standard polymerase chain reaction.^{6,36}

Follow-up

All 474 patients gave informed consent for follow-up and for the collection of information from hospitals during a suspected thrombotic event recurrence. Follow-up started 90 days after the date of the initial thrombotic event that occurred in 1988-1992 (this 90-day period was defined as the period of initial anticoagulation) and ended on January 1, 2000. Information relevant to the follow-up after the thrombotic event was first gathered at the interview at baseline, and subsequently by repeated mailed questionnaires. The questionnaires were used as a screening tool for the occurrence of risk situations and recurrent thrombotic events, and also included items on relevant clinical circumstances such as anticoagulation (type, duration, indication), surgery, trauma, immobility, use of oral contraception, and pregnancies during the period covered by each questionnaire. Patients were further interviewed by telephone if they responded positively on any item from the questionnaire or if they did not respond to a questionnaire. Subsequently, confirmation of all relevant clinical information pertaining to recurrent thrombotic events or risk situations was obtained from the treating physicians. Recurrent thrombotic events were confirmed and reports on diagnostic methods were obtained by collecting the discharge letters from the treating hospitals. Recurrent thrombotic events were adjudicated when they were objectively confirmed with Doppler ultrasound, venography, or impedance plethysmography. Recurrences of pulmonary embolism required a positive perfusion lung scan (at least 1 segmental perfusion defect), a ventilation-perfusion lung scan (intermediate or high probability), or a computerised tomographic scan. Deep vein thromboses or pulmonary embolisms that occurred within the initial anticoagulation period (90 days) were not considered thrombotic event recurrences, but were considered a progression of the initial event (this occurred in 2 patients). Idiopathic was defined as an initial thrombotic event that occurred in the absence of (1) pregnancy, (2) puerperium, (3) oral contraceptive use within 30 days, (4) trauma, surgery, immobilization, or use of a plaster cast within 3 months before the event. All others were classified as provoked.

Statistical analysis

End of follow-up was at the first thrombotic event recurrence, date of death, date of emigration, or the end of the study, whichever occurred first. Observation time was calculated as the time at risk from the end of the anticoagulation treatment for the first thrombotic event to the end of follow-up. Incidence rates of recurrent thrombotic events were calculated as the number of events over the accumulated patient-time. Cumulative incidence was calculated by Kaplan-Meier survival analysis.

The Cox-proportional hazards model was used to evaluate risks between groups and was adjusted for age and sex. Anticoagulant therapy was entered in the model as a time-dependent covariate. Separate analyses were performed to assess the effect of prothrombotic abnormalities on the risk of recurrence (factor V Leiden, prothrombin G20210A, hyperhomocysteinemia, high levels of factor VIII, IX or XI or of fibrinogen, and deficiencies of protein C, protein S or antithrombin). We assessed the risk of thrombotic event recurrence by sex and by idiopathic or provoked classification of initial thrombotic event. The effect of oral contraceptive use was determined by calculating thrombotic event recurrence rates for women who used an oral contraceptive at any time (either continued use or restarted use) during the follow-up period, stratified by use at the time of the first event. In addition, we estimated the risk separately for a second contralateral compared with a second ipsilateral thrombotic event. During the follow-up period, some patients experienced periods with an increased risk of a thrombotic event (trauma, immobilization, operations, oral contraception, pregnancy) or a decreased risk (oral anticoagulation treatment). To determine the effect of blood abnormalities on risk of thrombotic event recurrence without the interference of these episodes, we repeated the analysis while excluding all such periods.

For continuous phenotypes, we used the following cut-off values: 166 IU/dL for factor VIII; 129 IU/dL for factor IX; 121 IU/dL for factor XI; 4.1 g/L for fibrinogen; and 16.7, 19.8 or 20.3 mmol/L for homocysteine (3 different cut-off levels were used as a consequence of different processing times in the 3 clinics). Patients were considered deficient for protein C or protein S when levels were below the lower limit of normal (67U/dL or 33U/dL when using oral anticoagulation at the blood draw). Patients were considered deficient of antithrombin when levels were repeatedly below 80 U/dL. All analyses were performed with SPSS 11.0 (SPSS Inc, Chicago, Ill).

Results

A total of 474 patients were followed up for a mean (SD) of 7.3 (2.7) years and complete follow-up was achieved for 447 patients (94%) for a total observation time of 3477 patient-years (FIGURE 1). Twenty-seven patients were lost from observation (22 untraceable, 4 refusals, 1 disabled) and were included until their last observation. Of the remaining 447 patients, 5 patients emigrated and 14 patients died during follow-up. Follow-up was complete until death for 6 patients. The others 8 patients died before the subsequent questionnaire was due and follow-up for a thrombotic event was considered complete up until the date of their last questionnaire. The response rates after each questionnaire varied between 96% and 99%. The general characteristics of the cohort are listed in TABLE 1. There were more women ($n = 272$) than men ($n = 202$) included in this follow-up study. The mean (SD) age of the cohort was 45 (13.7) years. The overall mean age was 6 years higher in men, which differed markedly according to the type of initial thrombotic event. Idiopathic first events were more common in men, who were on average 5 years younger than women with an idiopathic first event. Most provoked first events occurred in women. In the patients with provoked events, men were on average 9 years older than women, in whom oral contraceptive use was a common determinant. Apart from elevated levels of factor IX (more frequent in women) and hyperhomocysteinemia (more frequent in men), prothrombotic factors were equally distributed between the sexes (Table 1).

During follow-up, 90 patients had a recurrent thrombotic event. Of these patients, 73 had a DVT in the leg, 4 had a thrombosis in the arm, 12 had a pulmonary embolism, and 1 had Budd-Chiari syndrome with an extension into the vena cava. Two patients who initially had a DVT later had an arm thrombosis. One patient who initially had an arm thrombosis later had a DVT. Two other patients with an initial arm thrombosis later had a thrombosis in the opposite arm as their recurrent thrombotic event. Of the 72 patients who had a DVT as their recurrent event, 41 were ipsilateral and 31 were contralateral.

Table 1: Patient characteristics*

	Men (n = 202)	Women (n = 272)	All (n = 474)
Age, mean (SD), y†	49 (12.8)	43 (14.0)	45 (13.7)
First thrombotic event			
Idiopathic	163 (81)	96 (35)	259 (55)
Mean (SD) age, y	49 (13.1)	54 (10.7)	51 (12.5)
Provoked‡	39 (19)	176 (65)	215 (45)
Mean (SD) age y	46 (11.3)	37 (11.6)	39 (12.1)
Prothrombotic risk factors			
Factor V Leiden	40 (20)	52 (19)	92 (19)
Prothrombin G20210A	13 (6)	16 (6)	29 (6)
Anticoagulant deficiency§	13 (6)	12 (4)	25 (5)
High level of factor			
VIII (>166 IU/dL)	44 (22)	66 (24)	110 (23)
IX (>129 IU/dL)	29 (14)	57 (21)	86 (18)
XI (>121 IU/dL)	37 (18)	55 (20)	92 (19)
Hyperfibrinogenemia(>4.1 g/L)	39 (19)	48 (18)	87 (18)
Hyperhomocysteinemia‡	39 (19)	44 (16)	83 (18)

*Values are expressed as number (percentage) unless otherwise indicated. Cut-off points are in parentheses unless otherwise indicated.

†At start of follow-up.

‡Defined as pregnancy, puerperium or use of oral contraceptives within 30 days, or trauma, surgery, immobilization, or use of a plaster cast within 3 months before the event.

§Deficiency of protein C (<0.67 [0.33] IU/mL), protein S (<0.67 [0.33] IU/mL), or antithrombin (<0.80 U/mL).

yCut-off points: more than 16.7 μ mol/L (Leiden), 19.8 μ mol/L (Amsterdam), 20.3 μ mol/L (Rotterdam).

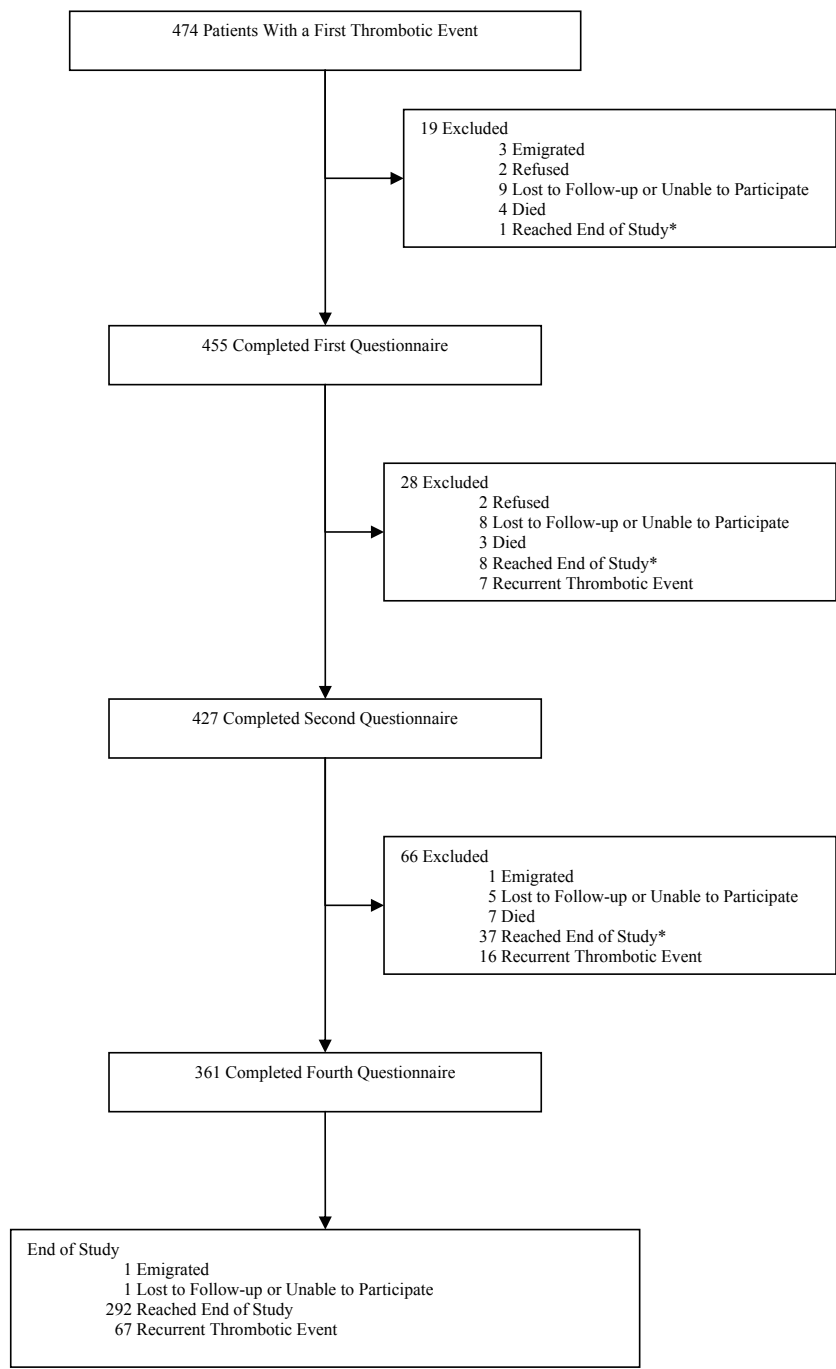


Figure 1. Flow of Patients During Follow-up

* A total of 9 individuals were lost during the study but later found. Thus, these individuals did not receive all 4 questionnaires, but information was complete during follow-up.

Anticoagulant Use During Follow-up

Follow-up started 90 days after the first event. At this time-point, 195 individuals had finished their initial anticoagulation treatment. Of the other 279 patients, 174 had a prolonged period of initial anticoagulation treatment, which was less than 3 months for the majority (n=106; TABLE 2). All others had additional periods of oral anticoagulant use during follow-up. For 116 patients (67%), the total duration of oral anticoagulant use was less than 12 months. The main reasons for anticoagulant use were prophylaxis of DVT during risk situations, such as surgery or pregnancy. Fifty-seven patients took an oral anticoagulant for more than 12 months in total, which was for cardiac reasons or arterial prophylaxis in 16 patients. The exact reasons for oral anticoagulant use were not known in 53 patients.

Of the 57 patients who took an oral anticoagulant for more than 12 months in total, 45 (79 %) had 1 or more prothrombotic abnormalities. The mean (SD) duration of oral anticoagulant use during follow-up of 45 patients was 4.7 (2.5) years per patient compared with 3.1 (2.3) years in the 12 patients without any abnormality. This difference was due mostly to patients with anticoagulant deficiencies (proteins C, protein S, and antithrombin) who received anticoagulation for a mean (SD) of 6.5 (0.9) years per patient. No major differences in prescription of anticoagulation were observed among carriers of the other prothrombotic risk factors, including factor V Leiden and prothrombin G20210A carriers compared with noncarriers.

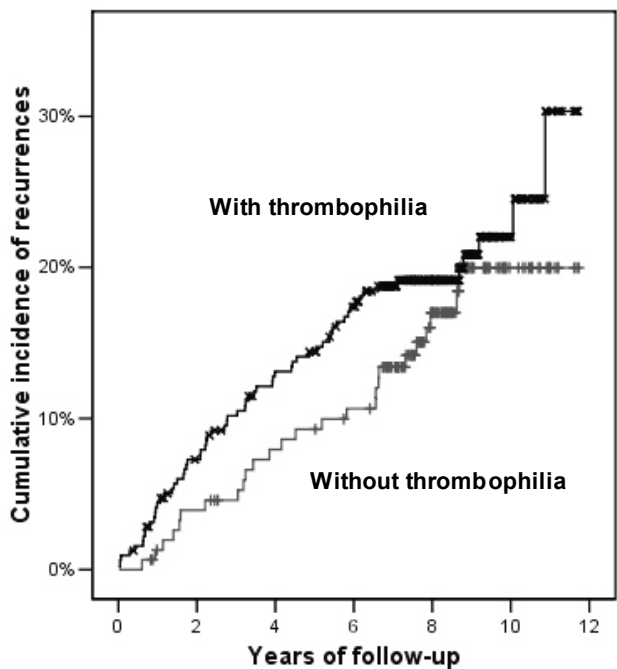
Table 2: Oral Anticoagulant Use During Follow-up

Oral Anticoagulant	Use During Follow-up*				Total
	None	<3 Months	3-12 Months	>12 Months	
Initial use					
<90 d	195	16	6	21	238
≥90 d	0	0	0	0	0
Continuous use after first event	0	106	51	17	174
Restart	0	13	30	19	62
Total	195	135	87	57	474

*Ninety days after first event.

Risk of recurrent venous thrombosis

The overall incidence rate of recurrent thrombotic event was 25.9 per 1000 patient-years (95% CI, 20.8-31.8 per 1000 patient-years), corresponding to an annual risk of 2.6%. FIGURE 2 shows the cumulative incidence of recurrence over the 12-year follow-up period. The risk of recurrence was 12.4% after 5 years of follow-up (95% CI, 9.5%-15.4%) and 16.5% (95% CI, 13.1%-19.8%) after 7 years of follow-up. During the first 2 years after the discontinuation of the initial anticoagulant treatment, the incidence rate was highest (31.9 per 1000 patient-years; 95% CI, 20.3 to 43.5 per 1000 patient-years). Subsequently, the incidence rate decreased slowly with time (FIGURE 3).

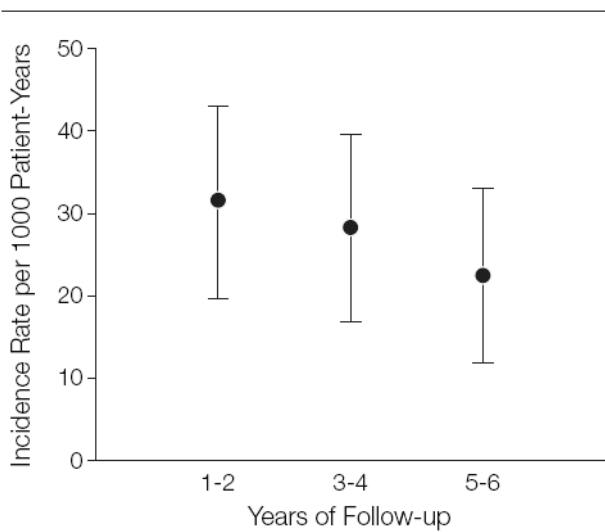


With Trombophilia	319	289	266	247	152	31
Without trombophilia	155	146	137	131	81	20
Total	474	435	403	378	233	51

Figure 2. Cumulative Incidence of Recurrent Thrombotic Events
Patients with and without thrombophilia during the period from the end of the initial anticoagulation period (90 days) until January 1, 2000. The crude hazard ratio of thrombophilia compared with no thrombophilia was 1.3 (95% confidence interval, 0.8-2.0); the hazard ratio adjusted for age, sex and oral anticoagulation as a time-dependent covariate was 1.4 (95 % confidence interval, 0.9-2.2).

Effect of sex

Men had a 5-year cumulative incidence of thrombotic event recurrence of 19.3% (95% CI, 13.9%-24.8%) compared with 7.4% (95% CI, 4.3%-10.5%) in women. The cumulative incidence after 7 years was 25.3% (95% CI, 19.3%-31.2%) in men compared with 9.9% (95% CI, 6.4%-13.5%) in women. The overall age-corrected HR for risk of thrombotic event recurrence in men compared with women was 2.7 (95% CI, 1.8-4.2).



Error bars indicate 95% confidence intervals.

Figure 3. Incidence Rate of Recurrent Thrombotic Event

Laboratory Abnormalities

Of all 474 patients, 319 (67%) had at least 1 laboratory abnormality at their first examination. After adjustment for age, sex and anticoagulation, no clear excess risk of recurrence was observed when we contrasted 319 patients with 1 or more prothrombotic abnormalities to those with none, (HR, 1.4; 95% CI, 0.9-2.2) (TABLE 3). We did not observe an increased risk of recurrence for any of the following prothrombotic risk factors (using those without the specific abnormality as reference group): factor V Leiden, prothrombin G20210A, elevated levels of factor VIII, elevated levels of factor IX, elevated levels of factor XI and hyperhomocysteinemia (TABLE 4). Adjustment for age, sex and periods of anticoagulation did not change these risk estimates (Table 4). In the patients with a deficiency of 1 of the natural anticoagulants protein C, protein S or antithrombin, a mildly increased risk of a recurrent thrombotic event was observed (HR, 1.8; 95% CI, 0.9-3.7). Fibrinogen levels exceeding 4.1 g/L were also found to be associated with a slightly increased risk of thrombotic event recurrence (HR, 1.6; 95% CI, 1.0-2.6; after adjustment for age, sex and anticoagulation the HR was 1.7 [95% CI, 1.1-2.8]). Only 1 of the 8 patients with homozygous for factor V Leiden experienced a recurrence during a mean (SD) follow-up of 8 (3.5) years. Their 5-year cumulative incidence of 12.5% did not differ from all patients, while none of the 8 received long-term anticoagulant treatment.

Table 3: Recurrence Rate for Number of Prothrombotic Laboratory Abnormalities in 474 Patients

	Abnormality			
	None	1	>1	Any
Incidence rate (95% CI)*	22 (14-32)	25 (17-37)	30 (21-42)	28 (22-36)
Hazard ratio (95% CI)†	Referrent	1.2 (0.7-2.0)	1.4 (0.8-2.3)	1.3 (0.8-2.0)
Hazard ratio (95% CI)‡	Referrent	1.2 (0.7-2.1)	1.6 (1.0-2.7)	1.4 (0.9-2.2)

Abbreviation: CI, confidence interval.

*Per 1000 patient-years.

†Relative to those without an abnormality (crude ratio).

‡Relative to those without an abnormality and adjusted for age, sex and anticoagulation as a time-dependent covariate.

Combinations of Thrombophilic Factors

Several patients had more than 1 of the abnormalities studied. Patients with 1 abnormality had a 1.2-fold increased risk of a thrombotic event recurrence compared with the 155 patients without an abnormality. In those with more than 1 abnormality, the recurrence rate increased 1.4 fold compared with those without an abnormality. After correction for age, sex and periods of anticoagulation (Table 3), the HR increased to 1.6 in those with more than 1 abnormality, while it stayed the same in those with only 1 abnormality.

Because factor V Leiden is the most common genetic abnormality, we studied its combinations with other prothrombotic defects in more detail. Sixty-three patients had factor V Leiden and 1 of the other prothrombotic risk factors (prothrombin G20210A, elevated levels of factors VIII, IX, or XI, fibrinogen, homocysteine, or deficiencies of protein C, protein S or antithrombin), while 29 patients carried factor V Leiden without any additional abnormality. The thrombotic event recurrence rate was 27.9 per 1000 patient-years (95% CI, 14.9-47.8 per 1000 patient-years) in those with factor V Leiden and any other abnormality and 33.9 per 1000 patient-years (95% CI, 13.6-69.9 per 1000 patient-years) in those with factor V Leiden without any other abnormality.

In patients with a combination of factor V Leiden and elevated levels of fibrinogen, a high recurrence rate of 69.8 per 1000 patient-years (95% CI, 25.6-152.1 per 1000 patient-years) was found. Other combinations of biochemical risk factors (prothrombin G20210A, elevated levels of factors VIII, IX, or XI, or homocysteine, deficiencies of protein C, protein S or antithrombin) with factor V Leiden did not show an additional increased risk.

Ipsilateral vs Contralateral Recurrent DVTs

The incidence rate of an ipsilateral second DVT ($n = 41$) was 12.4 per 1000 patient-years (95% CI, 8.9-16.9 per 1000 patient-years), while the incidence rate of a contralateral second DVT ($n = 31$) was only slightly lower at 9.5 per 1000 patient-years (95% CI, 6.5-13.5 per 1000 patient-years).

The incidence rate of an ipsilateral recurrent DVT in patients with a prothrombotic risk factor was 13.0 per 1000 patient-years (95% CI, 8.6-18.7 per 1000 patient-years), while the incidence rate of a contralateral recurrent DVT was 9.8 per 1000 patient-years (95% CI, 6.1-15.0 per 1000 patient-years). In patients without prothrombotic risk factors, the incidence rate of an ipsilateral recurrent DVT was 11.4 per 1000 patient-years (95% CI, 6.1-19.6 per

1000 patient-years), while the incidence of a contralateral recurrent DVT was 9.0 per 1000 patient-years (95% CI, 4.3-16.5 per 1000 patient-years).

Table 4: Recurrence Rates for Prothrombotic Laboratory Abnormalities in 474 Patients

Abnormality	No. of Recurrences	Incidence Rate (95% CI)*	Hazard Ratio (95% CI)†	Hazard Ratio (95% CI)‡
Factor V Leiden	20	30 (18-46)	1.2 (0.7-1.9)	1.3 (0.8 – 2.1)
Prothrombin G20210A	4	19 (5-48)	0.7 (0.3-2.0)	0.7 (0.3 – 2.0)
Anticoagulant deficiency§	8	45 (19-88)	1.8 (0.9-3.7)	1.8 (0.9 – 3.8)
High factor ¶				
VIII(>166 IU/dL)	23	29 (18-43)	1.1 (0.7-1.8)	1.3 (0.8 – 2.1)
IX(>129 U/dL)	13	21 (11-36)	0.9 (0.5-1.7)	1.2 (0.6 – 2.1)
XI(>121 U/dL)	11	16 (8-29)	0.6 (0.3-1.1)	0.6 (0.3 – 1.1)
Hyperfibrinogenemia‡	22	38 (24-58)	1.6 (1.0-2.6)	1.7 (1.1 – 2.8)
Hyperhomocysteinemia#	14	23 (13-39)	0.9 (0.5-1.6)	0.9 (0.5 – 1.6)

Abbreviation: CI, confidence interval.

*Per 1000 patient-years.

†Relative to those without the abnormality (crude ratio).

‡Relative to those without the abnormality and adjusted for age, sex and anticoagulation as a time-dependent covariate.

§Deficiency of protein C, protein S, or antithrombin.

¶Cut-off points: protein C: <0.67 (0.33) IU/mL; protein S: < 0.67 (0.33) IU/mL; antithrombin: < 0.80 U/mL; fibrinogen: >4.1 g/L.

#Cut-off points are in parentheses.

Cut-off points: homocysteine: >16.7 µmol/L (Leiden); 19.8 µmol/L (Amsterdam); 20.3 µmol/L (Rotterdam).

Initial Idiopathic and Provoked Thrombotic Events

The recurrence rate was highest in those with an idiopathic first thrombotic event at 33.2 per 1000 patient-years (95% CI, 25.4-42.6 per 1000 patient-years) compared with patients with a provoked first thrombotic event in whom the recurrence rate was 17.7 per 1000 patient-years (95% CI, 11.9-25.4 per 1000 patient-years) (HR, 1.9; 95% CI, 1.2-2.9). In both men and women, the risk of thrombotic event recurrence was higher in those who had had an idiopathic first thrombotic event, but the effect was slightly higher in women (TABLE 5). Likewise, the effect of sex on the risk of thrombotic event recurrence was the same irrespective of type (idiopathic or provoked) of thrombotic event, with a higher risk in men than in women (table 5).

In patients with an idiopathic first event, the recurrence rates were equal in those with prothrombotic abnormalities (33.6 per 1000 patient-years; 95% CI, 24.3-45.2 per 1000 patient-years) and without abnormality (32.4 per 1000 patient-years; 95% CI, 19.2-51.2 per 1000 patient-years) for a HR adjusted for sex, age and anticoagulation of 1.2 (95% CI, 0.7-2.2). In patients with a nonidiopathic first thrombotic event, the recurrence rate was somewhat higher among those with prothrombotic abnormalities (20.9 per 1000 patient-years; 95% CI, 12.9-31.9 per 1000 patient-years) compared with those without an abnormality (12.6 per 1000 patient-years; 95% CI, 5.4-24.8 per 1000 patient-years) for a HR adjusted for sex, age and anticoagulation of 1.7 (95% CI, 0.7-3.8).

Table 5: Recurrence Rates by Sex and Type of First Thrombotic Event

	Men		Women	
	Provoked*	Idiopathic	Provoked*	Idiopathic
No. of patients	39	163	176	96
No. of recurrences	8	49	21	12
Incidence rate (95% CI)†	29 (13-58)	44 (32-58)	15 (10-24)	17 (9-29)
Hazard ratio (95% CI)‡	Referrent	1.6 (0.8-3.4)	Referrent	1.9 (0.8-4.4)
Hazard ratio (95% CI)‡		2.7 (1.4-5.1)		Referrent
Hazard ratio (95% CI)‡	2.8 (1.2-6.6)		Referrent	

Abbreviation: CI, confidence interval

* Defined as pregnancy, puerperium or use of oral contraceptives within 30 days, or trauma, surgery, immobilization, or use of plaster cast within 3 months before the event.

†Per 1000 patient-years.

‡Corrected for 10-year age categories, duration of anticoagulation during the follow-up as a time-dependent covariate, and thrombophilia.

Oral Anticonceptive Use

A substantial number of women (n=128, 47%) used oral contraceptives at the time of the first thrombotic event, most of whom discontinued use after the event. However, 58 women continued or restarted use of oral contraceptive during follow-up. Eleven thrombotic event recurrences occurred during use of oral contraception or within 1 month after cessation. The recurrence rate in women who did not use oral contraceptives during follow-up was 12.9 per 1000 patient-years (95% CI, 7.9-21.2 per 1000 patient-years), while it was 28.0 per 1000 patient-years (95% CI, 15.9-49.4 per 1000 patient-years) in women who used oral contraceptives at some point during the follow-up period (either continuing or restarting).

Among women who did not use oral contraceptives during follow-up, recurrence rate was slightly higher in women who had never used an oral contraceptive (16.2 per 1000 patient-years; 95% CI, 8.7-30.2 per 1000 patient-years) compared with women who used oral contraceptives at the time of the first thrombotic event but discontinued use (9.7 per 1000 patient-years; 95% CI, 4.3-21.5 per 1000 patient-years) (TABLE 6). Among women who used oral contraceptives during follow-up, the risk of thrombotic recurrence was more or less equal in women who had also used an oral contraceptive at the time of the first thrombotic event (27.3 per 1000 patient-years; 95% CI, 14.7-50.7 per 1000 patient-years) compared with those who had not used oral contraceptives at the time of their first event (32.5 per 1000 patient-years; 95% CI, 8.1-130.0 per 1000 patient-years). These rates were higher when only the years that the oral contraceptives were actually used were taken into account (Table 6).

Two of the 11 thrombotic events that arose during oral contraceptive use occurred within 2 weeks after starting use. Two events occurred after 1 year of use, and the other events happened after a longer period of use, varying between 3 and 9 years. Of the 58 women who used oral contraceptives during follow-up, 15 had factor V Leiden. Only 1 thrombotic event recurrence occurred in this group (17.2 per 1000 patient-years; 95% CI, 2.4-122.0 per 1000 patient-years).

Table 6: Recurrence Rates by Oral Contraceptive Use in 215 Women Between 16 and 55 Years

	Taking Oral Contraceptive at First Event		Not Taking Oral Contraceptive at First Event	
	Used During Follow-up	Discontinued Use	Used During Follow-up	No Use
	(n = 50)	(n = 77)	(n = 8)	(n = 80)
No. of recurrences	10	6	2	10
No. of patient-years	366.6	621.0	61.5	616.4
Overall incidence (95% CI)*	27.3 (14.7-50.7)	9.7 (4.3-21.5)	32.5 (8.1-130.0)	16.2 (8.7-30.2)
No. of recurrences while taking oral contraceptive	10		1	
No. of oral contraceptive use patient-years	180.7		28.5	
Incidence (95% CI) while taking oral contraceptive*	55.3 (29.8-102.9)		35.1 (4.9-249.1)	

Abbreviation: CI, confidence interval.

*Per 1000 patient-years.

Rate of Spontaneous Recurrences

To determine the effect of prothrombotic abnormalities on recurrence risk without the interference of episodes with an increased or decreased risk of thrombosis, we repeated the analysis while excluding all postoperative periods (4 weeks following surgical interventions), pregnancy and puerperium (6 weeks following delivery), periods of oral contraceptive use, and all periods of anticoagulation treatment. This left 66 events over 2862 patient-years, for an incidence rate of 23.1 per 1000 patient-years (95% CI, 17.8-29.3 per 1000 patient-years). For those with prothrombotic abnormalities (factor V Leiden, prothrombin G20210A, hyperhomocysteinemia, deficiencies of the proteins C, S or antithrombin, elevated levels of the factor VIII, factor IX, factor XI or fibrinogen), the incidence rate was 24.8 per 1000 patient-years (95% CI, 18.2-33.1 per 1000 patient-years). For those patients without a prothrombotic abnormality, the incidence rate was 19.8 per 1000 patient-years (95% CI, 12.1-30.6 per 1000 patient-years) (HR, 1.2; 95% CI, 0.7-2.1). The effects of each of the prothrombotic defects separately on the risk of spontaneous recurrence were again equal to those found in the overall analysis (Table 4).

Discussion

In a large cohort of patients followed up for a prolonged time after a first venous thrombotic event, we found an annual risk of thrombotic event recurrence of 2.6%. The cumulative risk of recurrence was 12.4% after 5 years and 16.5% after 7 years of follow-up. Although the incidence was slightly higher in the first 2 years, at an annual rate of 3.2%, the risk of thrombotic event recurrence persisted at a high level of more than 2% during the following years. Others have reported higher recurrence rates of approximately 25% after 5 years of follow-up.^{12,13} An important difference of these studies was their inclusion of elderly and cancer patients—groups likely to have recurrent thrombotic events. Therefore, our results apply to patients who are younger than 70 years at their first thrombotic event, who do not have malignancy, but who are otherwise unselected.

We found a few clinical factors that affect the risk of recurrence (ie, male sex, an idiopathic first thrombotic event, and oral contraceptive use). Sex and the type of event were related: idiopathic first thrombotic events were more common in men, while provoked first thrombotic events were seen almost 5 times more frequently in women because of oral contraceptive use. Men had a 2.5-fold higher risk of thrombotic event recurrence than women. This effect of sex was the same in patients with a first idiopathic thrombotic event as in those with a first provoked thrombotic event. Similarly, the risk of thrombotic event recurrence in both men and women was higher in those who had had an idiopathic event. Although sex and type of first thrombotic event were strongly related, their effects on recurrence were not connected. The effect of prothrombotic abnormalities was small both in men and women and could therefore not explain the difference between the sexes. These findings confirm recent results from a British and an Austrian study.³⁷⁻³⁹ Use of oral contraception also increased the risk of thrombotic event recurrence. Advice to refrain from further oral contraceptive use would be a simple and effective way to reduce the risk of a second thrombotic event in women. We did not see a major effect of postthrombotic damage on the risk of recurrence. We found similar rates of thrombotic event recurrences in the ipsilateral and contralateral leg. This makes it tempting to hypothesize that a systemic effect is as likely to contribute to the overall recurrence risk as persisting remnants of the initial clot.⁴⁰

Sixty-seven percent of the patients had at least 1 prothrombotic abnormality. In these patients the recurrence risk was only slightly increased (1.4-fold) compared with those without such abnormalities. In patients with more than 1 abnormality, the recurrence rate was higher than in those with only 1 abnormality (1.6-fold vs 1.2-fold). The effect of the prothrombotic risk factors separately varied somewhat but on the whole, they seemed to be a weak determinant of recurrences. We found no evidence of an increased risk of recurrence for carriers of factor V Leiden or the prothrombin G20210A mutation. Similarly, we could not find an excess recurrence risk for individuals with high levels of factors VIII, IX, or XI. Hyperhomocysteinemia did not show any effect on recurrence risk either. It should be noted that in the Netherlands vitamin supplementation is not currently advised to patients with hyperhomocysteinemia. Therefore, these results represent the natural course of this condition. A mildly increased risk (1.8-fold) was observed in those with the strongest risk factors for first thrombotic events, deficiencies of protein C, protein S, and antithrombin. High fibrinogen levels also conferred a slightly increased risk of recurrence (1.7-fold). These results are at variance with some

other studies in which increased recurrence risks were found for protein C, protein S, and antithrombin,¹³ for hyperhomocysteinemia,^{29,30} for increased levels of factor VIII and factor IX,^{27,28} and for factor V Leiden and prothrombin G20210A.^{21,22,24,25} These studies differed considerably with respect to design and study population, methods, sample size, and duration of follow-up, which could explain the discrepancies.^{31,32} In a prospective cohort study of unselected patients with a similar design as our study, no effect of thrombophilia was found either.³⁷ In summary, we saw no major effect for any of these factors, which is internally consistent because it is difficult to understand why some prothrombotic abnormalities would increase the risk of recurrence and others would not.

Venous thrombosis is a multicausal disease.⁴ Individuals need a certain combination of risk factors, each adding to the thrombotic event potential, which exceeds the thrombosis threshold. When patients have similar thrombotic event potentials, recurrence risks may be similar, too. This explains the equal risks we found for all thrombophilic defects. In patients whose first thrombotic event was idiopathic, the recurrence rate was equal in those with and without a prothrombotic abnormality. This can be explained from the existence of a not yet identified prothrombotic abnormalities, which hold the same thrombotic event potential as the known prothrombotic abnormalities. The recurrence risk was actually only increased in those patients who had 2 or more abnormalities, or in other words, only those with a somewhat higher thrombotic event potential stood out.

Among patients with thrombophilia, those who had a provoked first thrombotic event had a recurrence risk that was still lower than patients who had an idiopathic first thrombotic event. This is remarkable because one would expect these rates to be equal after the environmental factor (surgery, puerperium) that contributed to the initial thrombotic event has been removed. This can only be explained when patients with thrombophilia and an idiopathic first thrombotic event have a higher thrombotic event potential than patients with thrombophilia and a provoked first thrombotic event—this could be an extra (a not yet identified) laboratory risk factor or a local factor such as an anatomical abnormality.

These findings have important implications for clinical strategies. Patients with an idiopathic first thrombotic event are often extensively tested for prothrombotic defects. However, a positive result of a defect does not predict the risk of thrombotic event recurrence and therefore has no clinical consequence.

Our study may be limited with respect to the generalizability of the findings because we excluded patients older than 70 years and cancer patients. Our main findings may therefore not be applicable to these groups. Also, in our study population the use of prophylactic anticoagulation for short periods was quite high. This may have affected the overall relatively low rate of recurrences. However, we do not expect that this has had an influence on the lack of effect we found for the separate prothrombotic abnormalities. In all analyses, the use of anticoagulation was adjusted for and even when we excluded all risk-enhancing (surgery, oral contraception use, pregnancy, puerperium) and risk-decreasing (oral anticoagulation) situations, we did not find an effect of thrombophilia. Another issue is the diagnosis of thrombotic event recurrence because a new event is often difficult to distinguish from postthrombotic syndrome.⁴¹ An incorrect classification of a thrombotic event recurrence could have affected the total rate of recurrent events. However, the incidence rate of a contralateral second DVT was only slightly lower than that of an ipsilateral DVT. It is therefore unlikely

that the low rate of recurrent events was overestimated. The distribution of the prothrombotic risk factors was also equal between ipsilateral and contralateral DVTs. Therefore, our conclusion with respect to the effect of thrombophilia would remain unchanged.

In conclusion, patients who had a first thrombotic event had a high risk of recurrence. This risk is higher in men, in patients whose first thrombotic event was idiopathic, in women who use oral contraceptives, and in patients with 2 or more prothrombotic risk factors. Solitary laboratory abnormalities appear not to predict the risk of recurrence. Therefore, extensive, if any, thrombophilic work-up after a first thrombotic event is not likely to confer a clinical benefit to the patient. Similarly, a differential treatment with regard to duration of oral anticoagulation in patients with prothrombotic abnormalities does not seem to be rational based on these data. Adequate prophylactic anticoagulation during risk situations for all patients with a history of thrombotic event may be the most important measure to reduce the risk of a recurrent event. Women using oral contraceptives should be advised to refrain from further use. The decision on optimal duration of anticoagulation therapy after a first thrombotic event will probably need to be based on clinical factors (male sex, oral contraceptives use, and idiopathic first thrombotic event) rather than laboratory abnormalities.

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3.2

Sex difference in risk of recurrent venous thrombosis and the risk profile for a second event

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Abstract

Background: The risk of recurrent venous thrombosis is higher in men than in women, which is so far unexplained. We set out to determine the influence of age, time between first and second event, type of first event, oral contraception, pregnancy and surgery.

Methods: We performed a prospective follow-up study of 474 patients with a first objective diagnosis of deep venous thrombosis, aged 18-70 (LETS-cohort).

Results: During 3477 person-years of follow-up, 90 recurrences occurred. The overall incidence rates of recurrence (IR) were 40.9/1000 person-years in men and 15.8/1000 person-years in women. Men with an unprovoked first event had the highest risk of recurrence, with almost one third experiencing a second unprovoked event within 8 years (IR 41.2/1000 person-years). This risk was 3-fold lower in women (IR 14.2/1000 person-years; hazard ratio 2.8 (95% CI 1.4-5.7)). Age at diagnosis had little effect on recurrence rate, nor had time elapsed since the first event. In women, almost half of the recurrences was provoked and mainly related to oral contraceptive use or pregnancy.

Conclusion: The higher recurrence rate in men than in women is not the result of differences in the environmental or transient risk factors we studied. The risk profile for a second thrombotic event is clearly different from that of a first.

Introduction

The occurrence of a first venous thrombosis depends on several factors which can be categorized into clotting disorders (both inherited and acquired) as well as environmental risk factors (e.g. surgery, pregnancy, and oral contraceptive use) [1]. A combination of several of these risk factors will enhance a patient's prothrombotic state, until a threshold is reached and a thrombus is formed [2].

After a first event, a patient has a 20-30% risk of a recurrence within the following decade [3-6]. Recent reviews pointed out the importance of clinical risk factors, which probably contribute more to the risk of recurrence than coagulation defects do [2,7,8]. Several studies found a higher recurrence rate after a first idiopathic than after a first provoked event, with relative risks of 2 to 3 [5,10-12]. Also, three large cohort studies and a cross-sectional study showed a sex difference in the risk of recurrence, with men having a 2-3-fold increased risk of recurrent venous thrombosis relative to women [6,13-15]. It has been proposed that the difference in recurrence risk between the sexes can partly be explained by exposure to hormonal factors in women, which contributed to their first event [2,16]. When these women are included in the comparison of women with men for recurrence risk, and their exposure to oral contraceptives or pregnancy has been discontinued, the comparison may not be fair as their risk will have been reduced. Age could also play a role, as these women were generally younger at the time of their first event than men [6,13,16]. Furthermore, the role of exposure to clinical risk factors (oral contraceptives, pregnancies and surgery) in the development of a recurrence is not well established [17-19]. Most reports advise to withhold the use of oral contraceptives in women with a personal history of venous thrombosis, but until now prospective data supporting this advice are lacking [20,21]. Some advise the use of progestin-only oral contraceptives, again without clear data supporting their safety.

We set out to analyse in detail the effect of sex, age and provoking factors on recurrence rate in a consecutive cohort of patients with a first objective deep vein thrombosis (LETS) who were followed for over a decade.

Methods

For the original Leiden Thrombophilia study (LETS), a case-control study into the etiology of venous thrombosis, 474 consecutive patients with a first episode of symptomatic proximal deep vein thrombosis in the leg (n=453) or arm (n=21) were enrolled between 1988 and 1992. Patients older than 70 years or with a recent malignancy were excluded. 90% of the invited patients agreed to participate. The study was approved by the medical ethics committee of the Leiden University Medical Center [22]. At least 3 months after cessation of the initial anticoagulation treatment, all patients were seen in the Leiden outpatient clinic for an extensive interview covering circumstances in the time window preceding the index event. At the same time a blood-draw was performed, with a median time between this venepuncture and the index event of 19 months (range 6-68 months).

All 474 patients gave informed consent for a follow-up study to establish the risk of a recurrent event. Follow-up was performed by sending questionnaires (four times during follow-up)

to the patients. These enquired after the occurrence of recurrent events, anticoagulation, surgery, immobilisation, trauma, pregnancies and the use of oral contraception or hormonal replacement therapy. Patients were further interviewed by telephone if they responded positively on any item from the questionnaire or if they did not respond to a questionnaire. Subsequently, confirmation of all relevant clinical information pertaining to recurrent thrombotic events or risk situations was obtained from the treating physicians. All patients gave informed consent to obtain discharge letters from all relevant in- and out-patient visits to confirm initial and subsequent events. The time span of follow-up was from the end of the initial anticoagulation period (90 days) until 01-01-2000. 195 individuals stopped using anticoagulant treatment after the initial period of 90 days. The others either continued for a prolonged period or restarted later during follow-up. For details on use of anticoagulant use during the follow-up we refer to our original publication [6]. Major outcomes of the follow-up part of LETS were published in 2005, [6] from which the present study forms a sub-analysis, with particular focus on risk factors for recurrence and the unexplained sex difference.

Initial venous thrombosis had to be confirmed by ultrasound, venography or impedance plethysmography. Recurrent thrombotic events were confirmed and reports on diagnostic methods were obtained by collecting the discharge letters from the treating hospitals. Recurrent thrombotic events were adjudicated when they were objectively confirmed with compression ultrasound, venography, or impedance plethysmography. Distal deep vein thrombosis was not classified as an initial or recurrent venous thrombotic event. When the recurrence was an episode of pulmonary embolism, this required positive perfusion lung scanning (≥ 1 segmental perfusion defect), ventilation-perfusion lung scanning ($\geq$ intermediate probability), or computerised tomography to be adjudicated as such. Recurrent thromboses in two patients were rejected as recurrent events because they occurred within the initial anticoagulation treatment for the first event.

A provoked event, both for first and recurrent events, was defined as occurring during pregnancy or puerperium (defined as a period of 6 weeks after delivery), during (or within 30 days after the cessation of) oral contraceptive use, during immobilization (i.e. a period of < 3 months being immobilized due to hospitalization or due to being bedridden at home) or within a period of 30 days after major trauma or surgery. All other events were assigned to be idiopathic.

Major surgery was defined as those procedures that required general or spinal anaesthesia. Brands of oral contraception were carefully recorded and subsequently arranged in groups according to their ability to elevate SHBG-levels [23], and increase APC-resistance [24]. Preparations containing triphasic levonorgestrel (LNG) were analysed as a separate group. Preparations containing monophasic 3rd generation progestagens (gestodene (GSD) or desogestrel (DSG)) were combined with preparations containing cyproterone acetate (CPA) into one group, known to have the highest ability to increase SHBG-levels and APC-resistance. Of all women who discontinued the use of oral contraception after their first event, three started using hormonal replacement therapy during the follow-up. One woman used a compound during the follow-up containing estradiol and norgestrel, mostly prescribed for patients with dysmenorrhoea. These 4 women were excluded from the analysis of the effect of oral contraceptives. Furthermore, we only included women between 16 and 48 for this analysis.

Statistical analysis

Observation time was calculated as the time from start of follow-up (set at 90 days after the first event even if anticoagulation lasted longer) until the end of the follow-up, i.e. date of the first recurrence, death, emigration or end of the study, whichever occurred first.

During the follow-up, time periods of increased risk were defined as the sum of exposure to (the different classes of) oral contraception, pregnancy (plus 6 weeks postpartum period), and postoperative periods (30 days after the date of surgery). Total follow-up time during which patients were not exposed to any clinical risk factor was calculated as the total follow-up time minus the sum of the periods of increased risk.

Incidence rates of recurrent thrombosis were calculated as the number of events over the accumulated person-time. We estimated cumulative incidences with Kaplan Meier survival analysis. To obtain hazard ratios of recurrence, we used Cox-proportional hazards model to correct for age differences. For the analysis of the effect of oral contraceptives and pregnancy we used a Poisson regression model to adjust for age. For this analysis, person-time was split for several women as during follow-up they could be both exposed to (several brands of) oral contraceptives, be pregnant, or not be exposed to hormonal risk factors. So some women could contribute to several exposure groups.

The effect of anticoagulation on recurrence risk has been published in our first paper on the LETS-cohort [6]. For the current paper we were not primarily interested in exact recurrence rates but in the effect of several clinical risk factors on recurrence with respect to the sex difference. As the use of anticoagulation did not differ for the sexes (there were 121 (60%) men and 158 (58%) women who used anticoagulation for more than 3 months; median duration of initial anticoagulant treatment time in both men and women was 4 months) and as the hazard ratio for recurrence in men compared to women remained the same after observation was restricted to time where patients did not use anticoagulants (2.6 (95% CI, 1.7-3.9) and 2.7 (95% CI, 1.8-4.1) respectively), we decided not to further include oral anticoagulant use in the subgroup analyses.

We used the Stata statistical software package (Stata Corp., College Station, TX) and SPSS version 14.0 (SPSS Inc, Chicago, ILL).

Results

Clinical characteristics of the study population are provided in Table 1. 474 patients were followed for a total follow-up of 3477 patient-years (py) (mean: 7.3 years, range 7 days to 11.7 years). Follow-up was complete for 94% of the patients, while 27 patients were lost to follow-up and therefore included until their last observation [6].

There were slightly more women (n=272) than men (n=202). Men were a little older at the start of follow-up, with a mean age of 48.5 years, which was 43.0 years in women.

When the follow-up ended at 01.01.2000, 90 recurrences had occurred. Of these patients, 57 were men and 33 were women. Seventy-three patients had a DVT in the leg (49 men, 24 women), twelve patients had a pulmonary embolism (7 men and 5 women), 4 had a thrombosis in the arm (1 man, 3 women), and one woman had Budd-Chiari syndrome with an extension into the vena cava inferior.

The overall rate of recurrence was 25.9 per 1000 py (CI95: 20.8 – 31.8 per 1000 py). In men, the overall rate of recurrence was 40.9 per 1000 py (CI95: 31.0 – 53.0/1000py) as compared to a rate in women of 15.8 per 1000 py (CI95: 10.9 – 22.1/1000py) (13).

In women, most first events were provoked (176/272, 65 %), mainly by oral contraceptive use (128 of these 176 women), while only 19% (39/202) of men had a provoked first event. The recurrence rate in women was similar following provoked (15.4 per 1000 py) or idiopathic (16.7 per 1000 py) first events, and in both cases it was clearly lower than in men (29.2 per 1000 py in men with a first provoked event and 43.8 per 1000 py in men with a first idiopathic event) (13). These results have been published in our earlier paper [6] but are repeated here as a basis for the following analyses into the effect of several clinical and transient risk factors on the recurrence rates.

Effect of age on recurrence risk

Table 2 shows the overall recurrence rate stratified by age and sex. The incidence rates were higher for men than for women in all age categories, with the exception of the patients who were younger than 30 at the time of their first event, where rates were about equal in both sexes. The recurrence rates in men varied somewhat over the age categories and did not clearly increase or decrease with age. In women, the recurrence rate went down with age, from an incidence rate of 27.3/1000 py for women aged under 30 at the time of the first event to 9.7/1000 py for women between 60 and 70 years of age. This pattern was the same when only the subgroup of subjects with a first idiopathic event was considered, i.e. no effect of age in men and a decreasing rate with age in women. When we further limited the analysis to the group with a first *and* a second idiopathic event, no effect of age was found in women any more either.

Risk of recurrence through time

Table 3 shows recurrence rates in men compared to women over time. Absolute recurrence rate in men and women was highest within the first 2 years of follow-up, and slightly decreased thereafter. The relative risk was invariably higher in men than in women within all subgroups.

Type of recurrence in relation to sex and type of first event

Table 4 shows the rates of idiopathic recurrences by sex and by type of the first event. Fifty-three men suffered from an idiopathic recurrence over a follow-up time of 1390 person-years (which excludes all person-time during which a subject was exposed to risk-enhancing situations) (IR 38.1/1000 py, 95%CI 28.6 – 49.9), as compared to 17 women with an idiopathic recurrence over a follow-up period of 1832 person-years (IR 9.3 /1000 py, 95% CI 5.4 – 14.9). This higher risk in men of an idiopathic recurrence was present both in those cases in whom the first event was provoked, as in those in whom the first event was idiopathic (table 3). The highest idiopathic recurrence rate was found in men with an idiopathic first event (IR 41.2/1000 py; 95% CI 30.2-54.9) and the lowest in women with a first provoked event (IR 6.2/1000 py; 95% CI 2.5-12.8).

Figure 1 summarises these differences as cumulative incidences of recurrence over time, stratified by sex and type of first event. Part 1a shows all recurrent events, and is therefore

equivalent to the incidence rates we published earlier (13). Part 1b shows only the idiopathic recurrences, i.e. the ones that could not have been prevented other than by prolonged anticoagulation therapy.

Overall, 20 provoked recurrent events happened, 16 of which in women. Of these, 11 occurred during or shortly after oral contraceptive use, 3 during or shortly after pregnancy, 1 after surgery, and one event occurred following a trauma. The total cumulative time that women were exposed to risk-enhancing situations (surgery, pregnancy and oral contraceptive use) was 243.3 person years, yielding an incidence rate of 65.8 per 1000 person-years (95% CI 37.6 – 106.5/1000py) during those time-windows. Of the 4 provoked events in men, 2 were related to surgery and 2 to trauma. Men were exposed to risk-enhancing situations for only 3.6 person-years in total, so this led to an incidence rate of 1123.6/1000 py (95% CI 305.6 – 2876.4/1000 py).

Of the 16 second provoked events in women, 14 occurred in women whose first event had been provoked as well. Of these 14, 10 happened in women who had been under 30, three who were between 30-40 and one between 40 and 50 at the time of their first event. Hence, no provoked second events happened in women who had been over 50 at the time of their first event.

Of the 4 provoked recurrences in men, one happened in a man whose first event had been provoked as well. No relation with age could be found here.

Table 1: Characteristics of men and women with a first episode of venous thrombosis

	Women, n=272	Men, n=202	All, n=474
First venous thrombosis			
Age at onset	43 (15-69)	48 (16-69)	45 (16-69)
10-30 years	59 (22)	22 (11)	81 (17)
30-40 years	52 (19)	26 (13)	78 (16)
40-50 years	77 (28)	61 (30)	138 (29)
50-60 years	41 (15)	45 (22)	86 (18)
60-70 years	43 (16)	48 (24)	91 (19)
Risk factors for first venous thrombosis			
Idiopathic	96 (35)	163 (81)	259 (55)
Provoked	176 (65)	39 (19)	215 (45)
Oral contraceptives	128 (47)	- -	128 (47)
Pregnancy/puerperium	21 (8)	- -	21 (8)
Surgery, trauma, immobilization	27 (10)	39 (19)	66 (14)
Duration of anticoagulation, months			
Total	4 (3-116)	4 (3-93)	4 (3-116)

Table 2: The absolute risk of recurrence according to sex and age and further stratified to first and second idiopathic events

Age	Men,All			Men,1 st idiopathic VT			Women,All			Women,1 st idiopathic VT				
	n ¹	n ² / FUy ¹	IR CI95	All recurrences		2 nd Idiopathic	n ¹	n ² / FUy ¹	IR CI95	All recurrences		2 nd Idiopathic		
				n ¹	n ² / FUy ¹					n ¹	n ² / FUy ¹		IR CI95	n ³ / FUy ²
10-30	22	4 / 173	23.1 6.3-59.1	19	3 / 153	19.6 4.0-57.2	59	12 / 440	27.3 14.1-47.7	2	0 / 18	0 0-207.2	0 / 17	0 0-212.7
30-40	26	11 / 168	65.6 32.7-117.4	18	8 / 113	71.0 30.6-139.8	52	7 / 413	16.9 6.8-34.9	10	2 / 80	25.0 3.0-90.1	1 / 80	12.5 0.3-69.8
40-50	61	20 / 425	47.1 28.8-72.5	48	19 / 321	59.2 35.7-92.4	77	7 / 603	11.6 4.7-23.9	24	4 / 184	21.8 5.9-55.7	3 / 175	17.1 3.5-50.1
50-60	45	9 / 303	29.8 13.6-56.5	36	7 / 248	28.2 11.3-58.2	41	4 / 317	12.6 3.4-32.3	23	3 / 171	17.6 3.6-51.3	3 / 170	17.6 3.6-51.5
60-70	48	13 / 326	39.9 21.2-68.3	42	12 / 285	42.0 21.7-73.6	43	3 / 310	9.7 2.0-28.3	37	3 / 265	11.3 2.3-33.1	3 / 264	11.4 2.3-33.2
Total	202	57 / 1394	40.9 31.0-53.0	163	49 / 1120	43.8 32.4-57.8	272	33 / 2083	15.8 10.9-22.2	96	12 / 718	16.7 8.6-29.3	10 / 706	14.2 6.8-26.0

n¹ Number of patients

n² Number of recurrences

CI95 95%confidence interval

FUy¹ Years of total follow-up

IR Incidence rate per 1000 patient-years

FUy² Years of idiopathic follow-up i.e. total follow-up after exclusion of periods of surgery, pregnancy, oral contraception

n³ Number of idiopathic recurrences

VT Venous thrombosis

Table 3: Recurrence rates of venous thrombosis in men and women according to time after first event

Time after 1st event, yrs*		n ¹	n ² /Fuy	IR	CI95	HR	CI95	HR**	CI95
0-2 yrs	Men	202	17 / 381	44.6	26.0-71.4	2.0	0.9-4.1	2.1	1.0-4.4
	Women	272	12 / 527	22.8	11.8-39.8	ref		ref	
2-5 yrs	Men	180	22 / 497	44.3	27.7-67.0	4.1	1.8-9.1	4.4	1.9-10.0
	Women	255	8 / 739	10.8	4.6-21.3	ref		ref	
5-8 yrs	Men	156	14 / 401	34.9	19.1-58.6	2.2	1.0-5.0	2.5	1.1-5.7
	Women	240	10 / 635	15.7	7.6-29.0	ref		ref	
> 8 yrs	Men	87	4 / 115	34.8	9.5-89.0	2.1	0.5-9.4	2.2	0.5-10.1
	Women	146	3 / 182	16.5	3.4-48.2	ref		ref	

* Follow-up until recurrent event, death, emigration or end of study, whichever occurred first

n¹ Number of patients

n² Number of recurrences

Fuy Years of total follow-up

IR Incidence rate per 1000 patient years

CI95 95% confidence interval

HR Crude Hazard Ratio

HR** Age-adjusted Hazard Ratio

Table 4: Hazard ratios of the risk of idiopathic recurrence in men compared to women, according to whether the 1st event was idiopathic or provoked.

	1st event	n ¹	2nd event	n ²	FUy	IR	CI95	HR	CI95	HR*	CI95
Men	Idiopathic	163	Idiopathic	46	1117	41.2	30.2 – 54.9	2.9	1.5 – 5.7	2.8	1.4 – 5.7
Women	Idiopathic	96	Idiopathic	10	706	14.2	6.8 – 26.1	ref		ref	
Men	Provoked	39	Idiopathic	7	273	25.6	10.3 – 52.8	3.9	1.4 – 11.0	4.0	1.4 – 11.8
Women	Provoked	176	Idiopathic	7	1103	6.3	2.6 – 13.1	ref		ref	

n¹ Number of patients

n² Number of idiopathic recurrences

FUy: Years of idiopathic follow-up i.e. total follow-up after exclusion of periods of surgery, pregnancy, oral contraception

IR Incidence rate per 1000 patient-years

CI95 95 % confidence interval

HR Crude Hazard Ratio

HR* Age-adjusted Hazard Ratio

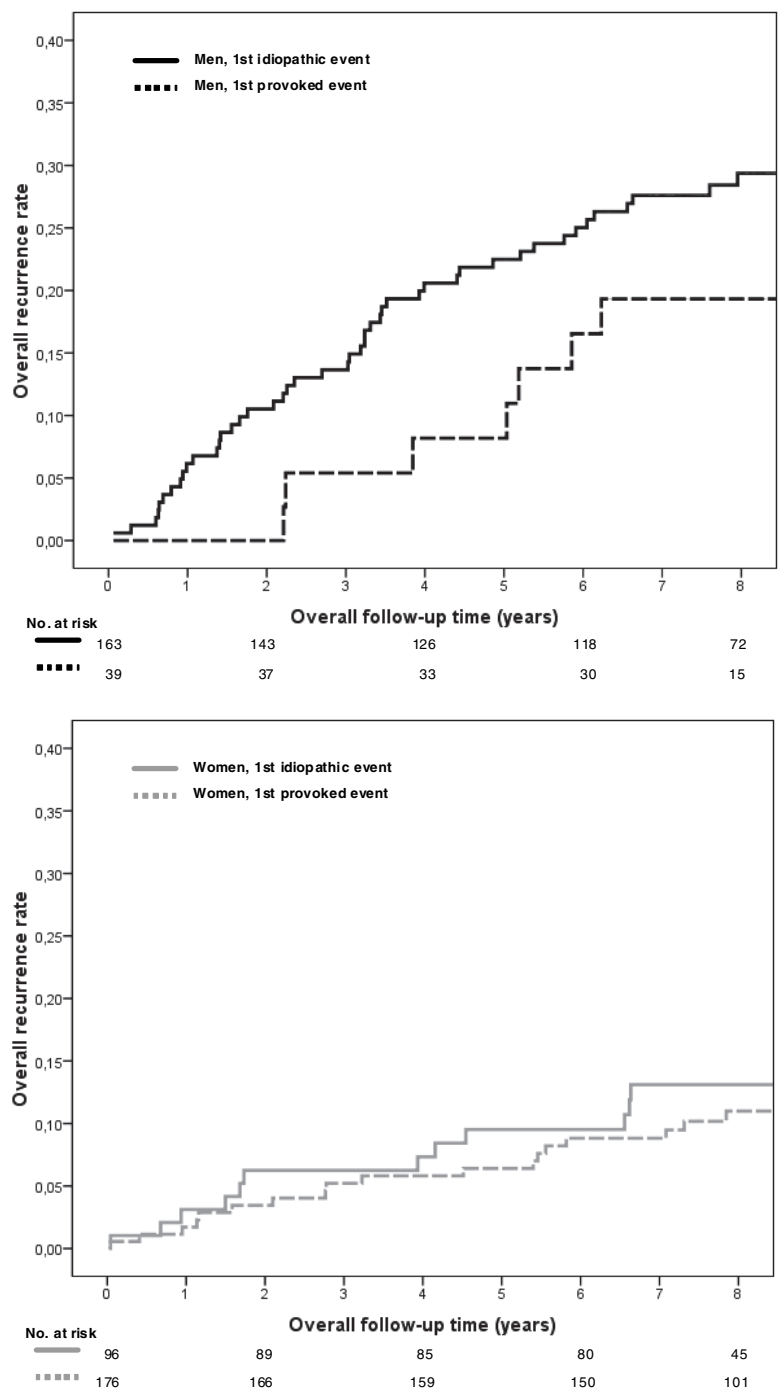


Figure 1a. Overall recurrence rate to whether the first event was idiopathic or provoked

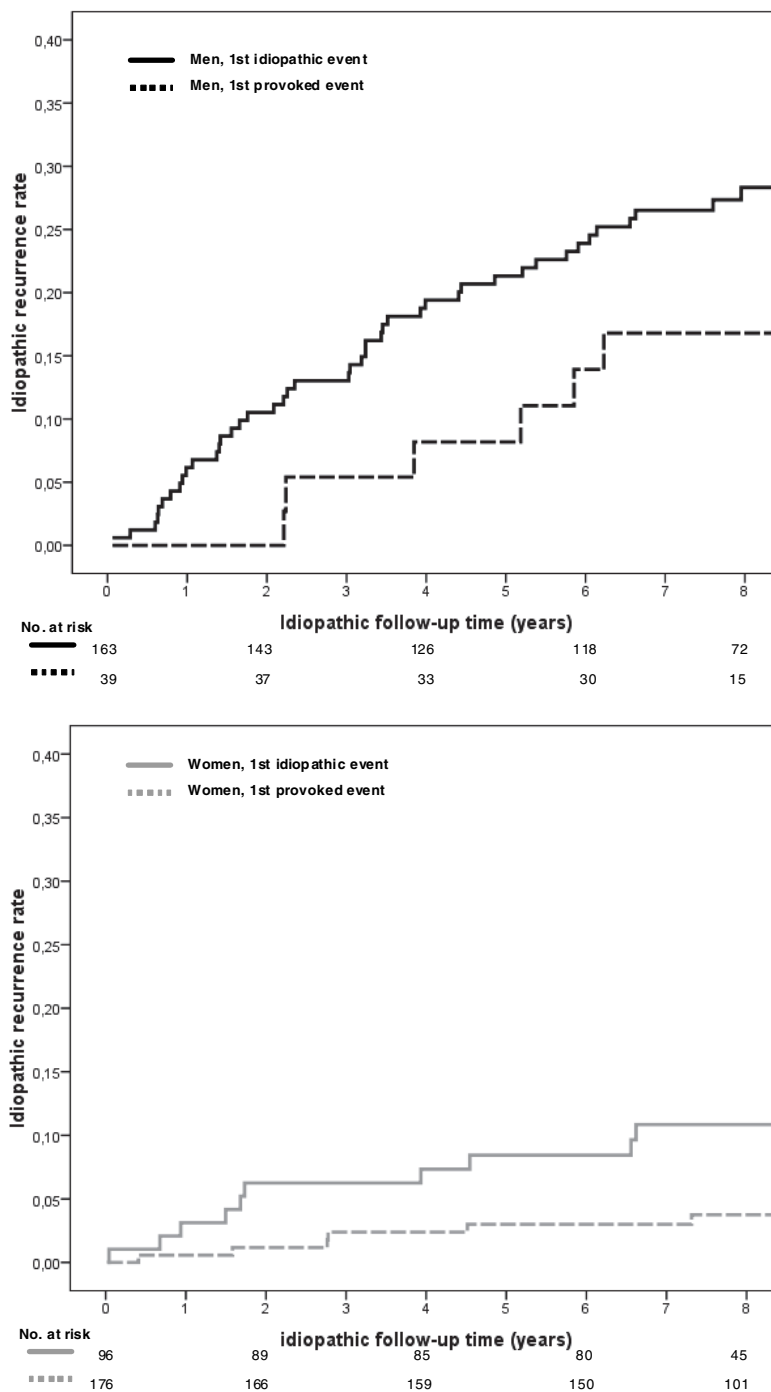


Figure 1b. Idiopathic recurrence rate according to whether the first event was idiopathic or provoked

Oral contraceptive use and pregnancy

A substantial number of women (n=128, 47%) had used oral contraception at the time of the initial thrombosis [13], 112 of whom were between 16 and 48 years old. In addition, the first thrombotic event was related to pregnancy in 21 women (8 ante and 13 postpartum). In these 133 women with a first event related to hormonal factors, we assessed the recurrence risk associated with oral contraceptive use and pregnancy during follow-up. Most of these women discontinued the use of oral contraception after the first venous thrombosis. Still, 53 women continued, started or restarted oral contraceptives during the follow-up. These women used 5 different types of oral contraceptives and eleven of them (20.8 %) suffered a recurrent event. With a total follow-up of 226 person-years of exposure to oral contraceptives, this led to a recurrence rate of 48.8/1000 py, (CI95: 24.3 – 87.2) for oral contraceptive use. This was a 4.6-fold (CI95: 1.9-11.5) higher recurrence rate than in women in the same age group (16-48) who stopped using oral contraceptives after their first event, and who were not pregnant during follow-up (adjusted for age) (Table 5).

The recurrence rate in users of monophasic oral contraceptives containing LNG was similar to the rate found in users of monophasic contraceptives containing GTD, DSG or CPA (44.4/1000 py (CI95: 5.4-160.5/1000 py) and 50.2/1000 py, (CI95: 16.3-117.1/1000 py)) (Table 5). Users of triphasic oral contraception with LNG had a higher risk with 2 events occurring in 6 women, leading to an incidence rate of 138.9/1000 py (CI95: 16.8-501.7/1000 py). Twelve women used oral or injectable progestin-only preparations and two recurred (who both used injectable medroxyprogesteron 150mg/ml), for a rate of 38.4/1000 py (CI95: 4.7-138.8/1000 py), which was only slightly lower than that of the 2nd and 3rd generation group. The age-adjusted hazard ratios for all subtypes were similar and roughly showed a 4-fold increase relative to non-use in all women aged between 16 and 48 (Table 5).

Of the 133 women who had been exposed to hormonal factors at the time of their first event, 31 had 1 or more pregnancies during follow-up (47 pregnancies all together) with a total exposure time of 34.3 patient-years (including a 6 week postpartum period). During this period, 3 pregnancy-related recurrences occurred. One of the 3 recurrences occurred in the 31st week of pregnancy in a woman who had used oral contraception when she had her first venous thrombosis. The second patient had her first event a few weeks post-partum and a recurrence at day 3 post-partum of a later pregnancy. The 3rd patient had both events during pregnancies. Overall, the recurrence rate during pregnancy/puerperium was 87.6 / 1000 py (CI95: 18.0 – 255.9), which corresponds to a 8.3 fold increased risk (CI95 2.2- 31.4) compared to women with a first thrombotic event related to hormonal risk factors who were not exposed during follow-up.

Surgery

One-hundred-and-four patients (32 men and 72 women) underwent 139 major surgical procedures during follow-up. Their mean age (45.7 years, range 20.0-68.8) was comparable to that of the overall cohort. Three recurrences related to surgery occurred in 2 men (age: 44.9 – 65.3) and 1 woman (age: 34.2) who all had had an unprovoked 1st event. The 3 recurrences occurred 5 days after a laparotomy, 6 days after a knee-arthroscopy, and 3 weeks after a diagnostic laparoscopy. We had only limited information regarding anticoagulant prophylaxis during these interventions.

The total exposure time to surgery added up to 11.2 person-years, which led to a recurrence rate of 267.1 / 1000 py (CI95: 55.0 – 780.1). Men's risk of surgery-related recurrence was

561.8 / 1000 py (CI95: 68.0 – 2028.1), and that of women was 130.4 / 1000 py (CI95: 3.3 – 726.2).

Table 5: Risk of recurrence among 133 women 16-48 years of age with hormonal risk factors at their first event (oral contraception use or pregnancy), according to exposure to hormonal risk factors during follow-up

Exposure during follow-up (n ¹)	n ²	FU ¹ (y)	IR	CI95	IRR ¹	CI95 ¹	IRR ²	CI95 ²
No hormonal contraception or pregnancy (80)	8*	760.3	10.5	4.5 – 20.7	ref	ref	ref	ref
Hormonal contraception (53)	11**	225.6	48.8	24.3 - 87.2	4.6	1.9 - 11.5	4.3	1.7 – 11.1
monophasic LNG (13)	2**	45.0	44.4	5.4 - 160.5	4.2	0.9 - 19.9	3.5	0.7 - 16.9
monophasic GTD, DSG, CPA (26)	5**	99.7	50.2	16.3 - 117.1	4.8	1.6 - 14.6	4.4	1.4 - 14.0
triphasic LNG (6)	2**	14.4	138.9	16.8 - 501.7	13.1	2.8 - 61.6	13.0	2.8 - 61.5
progestin only (12)	2**	52.1	38.4	4.7 - 138.8	3.7	0.8 - 17.2	3.6	0.7 - 17.3
monophasic LYN, NET (7)	0	14.4	0	0 - 256.4	-	-	-	-
Pregnancy (31)	3	34.3	87.6	18.0 – 255.9	8.3	2.2-31.4	NP	

LNG: levonorgestrel, GTD: gestodene, DSG: desogestrel, CPA: cyproterone acetate, LYN: lynestrenol, NET: norethindrone

n¹ Number of patients. The numbers do not add up as some women were exposed to several categories (e.g. both pregnancy and no hormonal exposure) during follow-up

n² Number of recurrences

FU¹ Years of total follow-up

IR Incidence rate per 1000 patient-years

CI95 95 % confidence interval

IRR¹ Crude incidence rate ratio

IRR² Age-adjusted incidence rate ratio

* Recurrence without oral contraception use or pregnancy within 1 month before event.

** Recurrence with oral contraception use within 1 month before event.

NP: Not possible to calculate as almost all pregnancies were in the same age category

Discussion

In our first paper on the LETS-cohort we reported, amongst several general aspects of recurrence risk of venous thrombosis, a higher recurrence rate for men as compared to women [6]. Some of the numbers and risks have been repeated in this second paper, as a necessary basis for the further analyses into the effect of several clinical and transient risk factors, which could possibly explain the sex difference in recurrence risk. In this study, we found that the clearly higher recurrence rate for men persisted through time. This higher risk could not be explained by age or by a sex difference in transient risk factors after the first event (such as oral contraceptives). Furthermore, the risk depended strongly on whether the 1st event was idiopathic or not, but men with a provoked 1st event still had a substantially higher overall recurrence rate than women. In men with an idiopathic 1st event almost one third was likely to suffer a 2nd idiopathic event within the next 8 years. This is therefore a group likely to benefit from long-term anticoagulant treatment. In women, the majority of the recurrences (48%) was provoked and occurred mainly during hormonal contraception use or pregnancy or post-partum.

In 2004, Kyrle et al first reported a difference in recurrence risk for men and women (HR 3.6) [13]. In retrospect, several previous papers contained information on this issue, some of which had found no or only a small difference [25-28]. However, most subsequent studies confirmed an increased recurrence risk for men, which was also the conclusion of a meta-analysis [29]. This meta-analysis included 11 cohort studies [11-14,25,27,30-34] with clearly defined inception cohorts that reached a > 90% complete follow-up. Five of these studies found an increased risk of recurrence in men (relative risk-range 1.7-6.3) [13,14,30,32,34], 5 studies found a weak or no effect (relative risk-range 1.2-1.5) [11,12,27,31,33], and one study found a decreased risk in men (relative risk 0.5) [25]. Of two more recent studies, one did not find a difference with respect to sex [9], while the other clearly did [35]. None of these studies could provide an explanation for the sex difference.

Surprisingly, even though the strongest risk factor for a first event is age [2,36], age appears to have no effect on the risk of a second event. None of the studies that looked into the effect of age on recurrence found a strong effect. Some described a slightly increased risk (HR 1.36 per decade increase in age [37], and others found no relation [9,13] or even a reduced risk with increasing age [38]. We found no effect of age in men, and a decreasing risk with age in women, which observation can be attributed to oral contraceptive use and pregnancy being a risk factor mainly in young women, whose risk reduces after removal of the risk factor. When we only considered idiopathic second events, no effect of age was seen in women either. Of note, these subgroups contained small numbers of patients, so the exact sizes of the rates should be interpreted with caution. They serve predominantly to demonstrate the generally absent effect of age. Still they are in line with recent reported findings from another prospective cohort study on recurrent venous thrombosis risk [39].

We described earlier, as did others [5,9], that the recurrence risk is highest when the first event was unprovoked. This difference was mainly observed in men, while men with a provoked first event still had a higher recurrence risk than women with an unprovoked first event. The recurrence risk in all women was low, and half of the recurrences in women were related to well known risk factors for thrombosis such as oral contraceptive use and pregnancy. Not many studies have been published on the effect of these factors on a second thrombosis, as generally patients are discouraged to use hormonal contraception after a first event (with the exception of progestin-only contraceptives), and because pregnancy is a relatively rare event in women with a history of thrombosis. We found a clear overall 4-fold increased risk for oral contraceptive use, without an indication of a difference per type of contraception. This means that women who stop using hormonal contraception after a first event can reduce their recurrence risk from 5% to 1% per year, which seems worthwhile, although the risk of pregnancy will have to be considered as well. We could not demonstrate a difference between the risk of 2nd and 3rd generation pills, which may be related to the small number of subjects. However, it could also be that no such difference exists in these patients, whose risk profile is different from that of a first event in many respects. Progestin-only compounds did not appear safer than the other types in our study. In 1998, the WHO found a weakly increased risk of a 1st venous thrombosis in users of progestin-only contraceptives [40] and one year later a study also initiated by the WHO found users of progestin-only preparations to have a 2-fold increased risk of a first venous thrombosis [41]. Studies into the effect of progestin only pills in women with a history of thrombosis are rare. A French series described

2 groups of 71 women with a previous venous thrombosis, one group using chlormadinone acetate (CMA) and the other without hormonal contraception. The recurrence risk was low in the CMA group in which 2 women developed a recurrence as compared to 6 in the non-treated group [42]. Our results are based on only a few users of progestin-only compounds, and the two recurrences that occurred in this group were both related to a specific type, i.e. injectable medroxyprogesteron. Therefore, our results suggest that the use of this preparation may have to be discouraged while we can not conclude about the safety of other types of progestin-only contraception. Interestingly, a recent study suggested that progesterone only oral contraceptives do not increase the risk of first venous thrombosis, [43] while two other studies, presented as an abstract, showed that injectable progesterone agents increased the risk of first venous thrombosis 2-3 fold [44,45], which is in accordance with our results on recurrent risk.

It has been suggested that the discrepancy in recurrence risk between the sexes could be explained by an age difference at the time of the first event, with women generally experiencing a first event at younger ages, due to oral contraceptive use or pregnancy. In this study, we observed a somewhat higher relative risk of recurrence in men compared to women in the first 5 years after the initial event, than when follow-up time was restricted to more than 5 years. A previous study found similar results and explained the sex difference by this lead time bias phenomenon [16]. However, our study showed that men were still at higher risk of recurrence compared to women when follow-up time was restricted to more than 5 years. Moreover, men still had higher recurrence rates than women when we restricted the analyses to idiopathic first events. Therefore, lead time bias may partially explain the sex difference but not totally.

This study has several limitations in addition to the small numbers in some subgroups. First of all, we did not have detailed information of the anticoagulant use around each surgical procedure or during or after pregnancy. We calculated the overall risk of exposure to pregnancy and surgery but it may well be that sufficient anticoagulant prophylaxis was lacking in some cases, and that the events occurred in this group. Furthermore, we started the follow-up of the study somewhat arbitrarily at 90 days after the first event, but the duration of anticoagulant treatment after the first event was often longer than 3 months. It may have been that these subjects were considered to be at high risk for a recurrent event by their physician. However, this cannot explain the strong sex differences as sex as a risk factor for recurrence was not expected at the time. Thirdly, our study results may not be generalizable to subjects who were 70 or older during their first event because such patients were not included in the original LETS-cohort. However, a large prospective study from Austria (AUREC) found similar results for age and sex on recurrent venous thrombosis risk that included patients up to an age of > 90 years [13,39]. Furthermore, for the results on contraceptives and pregnancy, this exclusion criterion is not relevant. Finally, an ipsilateral recurrent thrombotic event can be difficult to diagnose. Similarly, residual changes that were detected in some patients with recurrence may not have been detected initially (due to impedance plethysmography diagnosis) but may have been present at the end of the period of initial anticoagulation therapy. Hence, some misclassification may have occurred. However, one should expect similar misclassification in men and women, i.e. if misclassification occurred, it is expected to be non-differential. This type of misclassification would have led to an underestimation of the true relative risk estimates for men compared to women [46].

It is highly remarkable that apparently risk factors for a first thrombosis are different from those for a second thrombosis. Age, the strongest risk factor for a first event, does not appear to play a role in developing a recurrence. Male sex, not a risk factor for a first event, is a strong risk factor for recurrence. The known thrombophilic defects, which clearly affect the incidence of a first event, have little or no effect on the risk of a second event [2,5,6]. In contrast, clinical risk factors that increase the risk of a first event appear to have a similar, if not even more pronounced effect in causing a second event: oral contraceptive use, pregnancy and surgery. Explanations for these differences are hard to provide and should be the focus of further studies.

This study may have implications for patient care. First of all, women should be encouraged to avoid the use of sex steroids after a first venous thrombosis. Progestin-only preparations may still be an exception here although the 2 recurrences in users of medroxyprogesterone are not encouraging. Secondly, patients with a history of venous thrombosis need to receive strict anticoagulant prophylaxis around surgery or other procedures and possibly pregnancies. It may be helpful to educate patients and have them carry an emergency medical information card. Lastly, the risk of recurrence in men, especially in those whose first event occurred without any precipitating factors is sufficiently high to consider prolonged duration of anticoagulation in this group. Guidelines about the duration of anticoagulation should provide different policies for men and women [35,47,48].

In summary, this study determined the influence of sex, age and provoking factors on the risk of recurrent venous thrombosis. Neither age, nor time between first and second event, nor transient risk factors (oral contraceptives, pregnancy, and surgery) could explain the different recurrent risk for the sexes. The risk profile for a second event is clearly different from that of a first, which should be kept in mind for clinical care and further research.

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3.3

Elevated endogenous thrombin potential is associated with an increased risk of a first deep venous thrombosis but not with the risk of recurrence

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Summary

Measurement of the thrombin generating potential could provide a method for quantifying the composite effect of multiple risk factors. This study assessed the risk of a first as well as a recurrent venous thrombotic event associated with an increased endogenous thrombin potential (ETP). Analyses were performed in 360 patients and 404 control subjects of the Leiden Thrombophilia Study. The ETP was measured directly using a fluorogenic assay (Thrombinoscope™). Individuals with an increased ETP, i.e. above 90th percentile measured in control subjects ($>2109.0 \text{ nM} \times \text{min}$) had a 1.5-fold [95% confidence interval (CI): 0.9–2.3] increased risk of a first deep venous thrombosis. The risk was more pronounced after the analysis was restricted to idiopathic thromboses, i.e. 1.7-fold (95% CI: 1.0–2.8).

Overall, the hazard ratio of a recurrent thrombotic event associated with a high ETP, adjusted for age, sex, and oral anticoagulant use was 1.1 (95% CI: 0.5–2.2). Thus, a high ETP was not associated with an increased relative risk of recurrent venous thrombosis. At present, clinical relevance of the thrombin generation assay in predicting recurrent venous thrombosis remains uncertain.

Introduction

During the last decade, numerous risk factors for a first deep venous thrombotic event have been described, both environmental as well as genetic (Rosendaal, 1997). More recently, questions have been raised on whether these risk factors are also associated with an increased risk of recurrent thrombosis. It was shown that for individuals with a transient environmental risk factor, such as pregnancy, surgery, immobilization, or hormone use, the risk of recurrent thrombosis is low, whereas the risk of recurrence is high in patients with persistent risk factors, such as malignancies or lupus anticoagulant (Hansson *et al*, 2000). Thrombophilic conditions, such as protein C, protein S and antithrombin deficiency (De Stefano *et al*, 2006), as well as homozygous or double heterozygous carriership of the factor V Leiden (FVL) and prothrombin 20210A mutation have been only weakly associated with increased risk of recurrent venous thrombosis (Emmerich *et al*, 2001; Gonzalez-Porras *et al*, 2006).

Other risk factors for a first thrombotic event that have also been associated with the risk of recurrent venous thrombosis are heterozygous carriership of the FVL or prothrombin 20210A mutation (reviewed by (Ho *et al*, 2006), elevated TAFI levels (Eichinger *et al*, 2004), low vitamin B6 (Eichinger, 2006), low free TFPI (Hoke *et al*, 2005), hyperhomocysteinemia (Keijzer *et al*, 2002; Eichinger, 2003), and elevated levels of factor VIII and IX (Kyrle *et al*, 2000; Weltermann *et al*, 2003; Bobrow, 2005). However, only single studies have been performed or results were contradicted in other studies.

Overall, it can be concluded that most of the currently known risk factors do not appear to play an important role in the risk of recurrent thrombosis (Christiansen *et al*, 2005). Therefore, testing for these defects has little consequence with respect to prophylactic strategies. Clinical factors appear more important than laboratory abnormalities in determining the duration of anticoagulation therapy (Hansson *et al*, 2000; Baglin *et al*, 2003; Christiansen *et al*, 2005). It is still important to identify the group of patients that may benefit from prolonged treatment with oral anticoagulation therapy, i.e. those patients with an increased risk of recurrent venous thrombosis. In patients with a low risk of recurrence, prolonged treatment is associated with an increased risk of bleeding, which outweighs the benefits of the treatment (Prins & Marchiori, 2002). Testing for individual heritable thrombophilic defects reflects neither gene-environment interaction nor the connectivity of components of the coagulation network, i.e. it does not measure the composite phenotype. In this perspective, measurement of laboratory phenotypes for the stratification of thrombotic risk might be crucial. Palareti *et al* (2006) demonstrated that D-dimer testing could be used in the identification of individuals less prone to recurrent venous thrombosis.

Blood coagulability is determined by its capacity to generate thrombin. Measurement of the thrombin generating potential therefore could provide a method for quantifying the composite effect of multiple risk factors. This study assessed the risk of a first as well as a recurrent venous thrombotic event associated with an increased endogenous thrombin potential (ETP).

Material and Methods

Study design

Analyses were performed in the Leiden thrombophilia study (LETS). The design of this study has been described in detail previously (Koster *et al*, 1993; van der Meer *et al*, 1997). In brief, 474 consecutive patients younger than 70 years with a first, objectively confirmed episode of deep venous thrombosis (DVT) were included in this study. Four hundred and seventy-four control subjects were included who were friends or partners of patients with the same sex and approximately the same age (within 5 years). Individuals with known malignant disorders were excluded.

Patients and control subjects were initially seen at least 3 months after discontinuation of oral anticoagulation therapy, except in cases when this treatment could not be stopped ($n = 48$). Blood was collected from the antecubital vein into Sarstedt Monovette tubes, in 0.1 volume 0.106 mol/l trisodium citrate. Plasma was prepared by centrifugation for 10 min at 2000 *g* at room temperature and stored at -70°C until used. High molecular weight DNA was isolated from leucocytes and stored at 4°C .

Patients were subsequently followed-up to assess the risk of recurrent DVT. All 474 patients gave informed consent for follow-up. The LETS follow-up has also been described in detail (Christiansen *et al*, 2005). Follow-up started 90 d after the date of the initial thrombotic event, which occurred between 1988 and 1992 and ended on 1 January 2000. Recurrent thrombotic events were adjudicated when they were objectively confirmed with Doppler ultrasound, venography, or impedance plethysmography. Recurrent pulmonary embolus was confirmed by a positive perfusion lung scan, a ventilation-perfusion lung scan, or a computerized tomographic scan.

Measurement of the ETP becomes unreliable in plasma of patients using oral anticoagulant therapy; therefore, 48 patients and one control subject were excluded from analyses. Plasma was available for 360 patients not using oral anticoagulation therapy and 404 control subjects.

Measurement of the ETP, calibrated automated thrombography

The ETP was measured directly using a fluorogenic assay (Thrombinoscope™, Synapse BV, Maastricht, the Netherlands), which measures clot bound as well as free thrombin that is formed during coagulation.

A modified version of the original test as described by Hemker *et al* (1993) was used. In duplicate, 10 μL HEPES buffer [20 mmol/l HEPES, 140 mmol/l NaCl, 5 mg/ml bovine serum albumin (BSA), pH 7.35] with 15 pmol/l tissue factor (TF; American Diagnostica, Stamford, CT, USA) and 7 nmol/l thrombomodulin (TM; American Diagnostica) was added to 40 μL of one in four diluted plasma (10 μL plasma in 30 μL HEPES-buffered saline (20 mmol/l HEPES, 140 mmol/l NaCl, 5 mg/ml BSA, pH 7.35)], in a polypropylene round-bottomed microtitre plate (Greiner Bio-one Ltd, Stonehouse, UK). Phospholipids were added at a final concentration of 4 $\mu\text{mol/l}$ and were obtained from Avanti Polar Lipids (Alabaster, AL, USA) and consisted of 20 mol.% phosphatidylserine, 20 mol.% phosphatidylethanol-amine and 60 mol.% phosphatidylcholine prepared by extrusion. After addition of 10 μL of substrate reagent (2.5 nmol/l Gly-Gly-Arg-AMC, 0.1 mol/l CaCl_2 , 20 mmol/l HEPES, 60 mg/ml BSA, pH 7.35) the reaction was monitored in a Fluoroscan Ascent plate reader (Thermo Labsystems,

Helsinki, Finland) with an excitation filter at 390 nm and an emission filter at 460 nm. The fluorescent signal was converted to a thrombin concentration by continuous comparison with the signal generated by the thrombin calibrator added to a separate sample of the test plasma using Thrombinoscope™ software. A thrombin calibrator was added to a third plasma sample, to subsequently eliminate differences in the signal from the fluorophore due to the variability in the light absorption characteristics of different plasmas and to the inner effect which results in a non-linear relationship between the concentration of cleaved fluorophore and the emission signal. The ETP was calculated from the area under the thrombin generation curve, adjusted for α -macroglobulin-thrombin activity.

The intra- and inter-individual coefficient of variation (CV) was 4.2% and 8.0% ($n = 10$) respectively.

The determination of the FVL and the prothrombin 20210A mutation was performed by standard polymerase chain reaction, which has been described previously (Rosendaal *et al*, 1995; Poort *et al*, 1996).

Statistical analysis; Case-control study

Putative determinants of the ETP were studied in the healthy control group as reflecting the general population. Determinants were established mainly by comparing means.

The risk of a first deep venous thrombotic event (overall and an idiopathic event separately) associated with an increased ETP was assessed by calculating odds ratios with their 95% confidence intervals (95% CI). As a cut-off point we used the 90th percentile of ETP levels measured in the control subjects. Risks were calculated in subgroups defined by sex, age and hormonal status (i.e. pre- and postmenopausal women with and without oral contraceptive use). An idiopathic thrombotic event was defined as an initial event that occurred in the absence of pregnancy, puerperium, oral contraceptive use within 30 d, trauma, surgery, immobilization, or the use of a plaster cast within 3 months before the event (Christiansen *et al*, 2005).

Statistical analysis: follow-up study

The cumulative incidence of recurrent thrombosis was calculated by Kaplan-Meier survival analysis. The Cox-proportional hazards model was used to evaluate risks of the group with an elevated ETP (i.e. above the 90 percentile as measured in the healthy controls of the case-control study) compared with the group with a low ETP ($\leq$ 90th percentile). The risk of recurrent thrombosis was calculated for all patients and for patients who had an initial idiopathic thrombotic event. Hazard ratios were adjusted for sex, age and oral anticoagulation therapy during follow-up (added to the model as a time dependent variable).

Results

Table I describes the mean ETP (95% CI) in different subgroups. There was no difference in ETP level between men and women, ETP levels increased slightly with age, and ETP levels were higher in women using oral contraceptives compared with women not using oral contraceptives. Furthermore, ETP levels were associated with the FVL and the prothrombin 20210A mutation, where carriers had higher ETP levels than non-carriers.

Table I: Endogenous thrombin potential (ETP) levels in different subgroups.

	<i>n</i>	<i>Mean ETP (95% CI)</i>
<i>Control subjects</i>		
All	404	1641.5 (1607.1 – 1676.0)
<i>Subgroups</i>		
<i>Sex</i>		
Men	166	1642.1 (1587.6 – 1696.5)
Women	238	1641.2 (1596.4 – 1685.9)
<i>Age</i>		
≤35	103	1599.1 (1530.5 – 1667.8)
35–45	101	1693.2 (1623.0 – 1763.4)
45–55	93	1633.3 (1562.7 – 1704.0)
>55	107	1640.7 (1572.6 – 1708.9)
<i>OC*</i>		
Yes	48	1804.5 (1695.3 – 1913.6)
No	85	1574.3 (1504.3 – 1644.3)
<i>FVL</i>		
Yes	11 (heteroz)	1792.8 (1574.9 – 2010.7)
No	393	1637.3 (1602.4 – 1672.2)
<i>PT20210A</i>		
Yes	11 (heteroz)	1736.2 (1463.9 – 2008.5)
No	393	1638.9 (1604.1 – 1673.7)
<i>Patients</i>		
All	360	1673.1 (1634.4 – 1711.8)
<i>Subgroups</i>		
<i>First event</i>		
Idiopathic	187	1695.2 (1639.7 – 1750.6)
Provoked	173	1649.3 (1595.2 – 1703.4)

Idiopathic thrombotic event: absence of pregnancy, puerperium, oral contraceptive use within 30 d, trauma, surgery, immobilization, or the use of a plaster cast within 3 months before the event. ETP, endogenous thrombin potential; FVL, factor V Leiden.

* At the time of the venepuncture (women who were not pregnant, not within 30 d postpartum, did not have a recent miscarriage and did not use depot contraceptives).

Thrombotic risk of a first DVT associated with an increased ETP

Individuals with an increased ETP, i.e. above 90th percentile measured in control subjects (>2109.0 nM·min) had a 1.5-fold (95% CI: 0.9–2.3) increased risk of a first deep venous thrombotic event (Table II). The risk of a first deep venous thrombotic event was increased in all subgroups of men, women and menopausal status. The risk was more pronounced after the analysis was restricted to idiopathic thromboses, i.e. overall the risk of an idiopathic first event was 1.7-fold (95% CI: 1.0–2.8) increased in individuals with a high compared with a low ETP. The risk of a first DVT associated with a high ETP appeared highest in postmenopausal women who were not using oral contraceptives (all thromboses: OR = 2.3; 95% CI: 0.8–6.5; idiopathic thromboses: OR = 2.7; 95% CI: 0.9–7.8). Adjustment for age, sex, and carrier status of the FVL or the prothrombin 20210A mutation did not affect the results.

A clear dose-response relation could not be demonstrated when dividing the ETP levels into quartiles. Using individuals with an ETP level in the first (lowest) quartile (<1387.3 nM·min) as a reference category, we found no increase in the risk of venous thrombosis for individuals with an ETP level in the other quartiles ($OR_{2^{nd} \text{ quartile}}: 1.1$; 95% CI: 0.7–1.6, $OR_{3^{rd} \text{ quartile}}: 1.3$; 95% CI: 0.9–1.9, $OR_{4^{rd} \text{ quartile}}: 1.2$; 95% CI: 0.8–1.8). The increased risk of a first deep venous thrombotic event seemed present only in individuals with a pronounced ETP (i.e. above the 90th percentile).

The time between the venous thrombotic event and the venepuncture did not affect the ETP levels in the patients. After dividing the intervening time in four periods, the ETP levels remained approximately the same. The ETP levels ranged from 1626 nM·min in individuals with a venepuncture within 1 year after the thrombotic event, to 1695 nM·min in individuals with a venepuncture more than 3 years after the initial event.

Thrombotic risk of recurrent DVT associated with increased ETP

In the total group of 360 patients, 59 patients had a recurrent event (16.4%) over a mean of 7.6 years of follow-up. The overall incidence rate of recurrent thrombosis was 21.0 per 1000 patient-years in the group of patients with a high ETP and 21.6 per 1000 patient-years in the group of patients with a low ETP. The annual risk of recurrent thrombosis was 2.1% in patients with a high ETP compared with 2.2% in patients with a low ETP (Fig 1).

Overall, the hazard ratio of a recurrent thrombotic event associated with a high ETP, adjusted for age, sex, and oral anticoagulant use (added to the model as a time-dependent variable) was 1.1 (95% CI: 0.5–2.2). Using the same adjustments, the hazard ratio of a recurrent thrombotic event associated with a high ETP, after an initial idiopathic thrombotic event was 0.7 (95% CI: 0.3–2.1).

We also assessed the relative risk of recurrent thrombosis in the first year after venepuncture. Six patients had a recurrent thrombotic event within 1 year after venepuncture. There was no difference in the ETP level of the patients who had a recurrent event within 1 year after the initial event and those who had a recurrent event later (ETP levels 1459 and 1597 nM·min, respectively; mean difference: -138.2, 95% CI: -447.8 to 171.5).

Thrombotic risk associated with other test outcomes

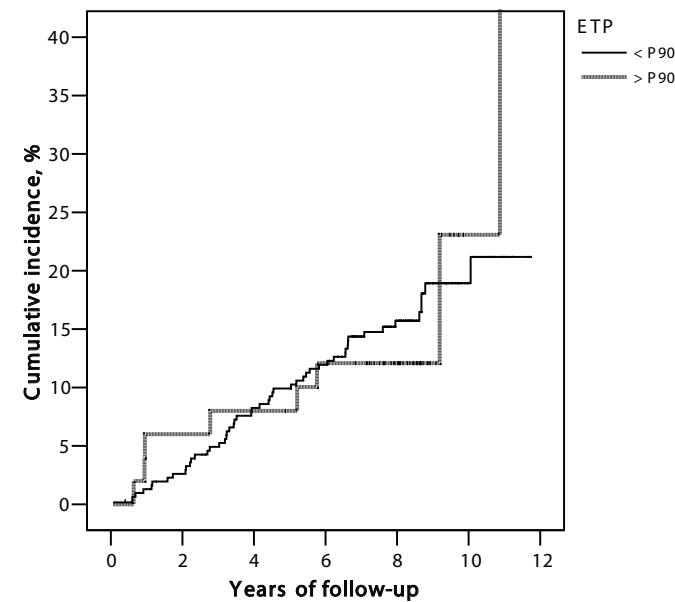
Other secondary components of thrombin generation test are the lag time (i.e. clotting time), the maximum amount of thrombin formed at a certain point in time (i.e. the peak), the time from initiation of coagulation to peak, and the maximum rate of thrombin formation. None of these components was associated with either a first or a recurrent venous thrombotic event (data not shown).

Table II: Risk of a first deep venous thrombosis associated with a high endogenous thrombin potential (ETP)*

Subjects	All	Idiopathic thrombosis
All	1.5 (0.9–2.3)	1.7 (1.0–2.8)
Sex		
Men	1.5 (0.7–3.2)	1.6 (0.7–3.5)
Women	1.4 (0.8–2.5)	2.0 (1.0–4.1)
Oral contraceptives†		
Premenopausal no OC	1.5 (0.3–6.8)	2.2 (0.5–10.2)
Premenopausal with OC	1.8 (0.5–6.0)	-
Postmenopausal no OC	2.3 (0.8–6.5)	2.7 (0.9–7.8)

* A High ETP is defined as an ETP level above 90th percentile as measured in the control subjects (P_{90} = 2109.0 nM·min).

† At the time of thrombosis (or similar index date for control subjects) as well as at time of the venepuncture, i.e. women who did not change their oral contraceptive use since the thrombotic event.



No. at risk

ETP>P ₉₀	50	47	46	43	29	2
ETP<P ₉₀	310	296	275	259	160	36

Figure 1. Cumulative Incidence of recurrent thrombosis
Patients with a high endogenous thrombin potential (ETP), i.e. above the 90th percentile as measured in control subjects and patients with a low ETP during the period from 90 d after the first thrombotic event until 1 January 2000.

Discussion

From a clinical perspective, it would be highly desirable to have a test that predicts the risk of developing a recurrent venous thrombotic event. Thrombin is the key enzyme in coagulation. Measurement of the thrombin generating potential therefore could provide a method for quantifying the composite effect of multiple risk factors. An elevated endogenous thrombin potential was associated with an increased risk of a first venous thrombotic event. As it was shown previously that the risk of a recurrent venous thrombotic event was low in individuals with a transient risk factor for venous thrombosis such as surgery or immobilization (Hansson *et al*, 2000), we investigated the association between an elevated ETP and the risk of an idiopathic venous thrombotic event (definition see Materials and methods). An elevated ETP was associated with a mildly increased risk of an idiopathic thrombotic event, with risk estimates being increased by 1.6- to 2.7-fold. There was no association between the overall risk of a recurrent thrombotic event and an elevated ETP. These results are applicable for patients aged less than 70 years at the time of their initial event.

The median time between the initial thrombosis and the venepuncture was 18 (6-56) months. Patient follow-up started 90 d after the initial thrombotic event. Although the levels of the ETP remained stable, as demonstrated by the lack of an effect of the time elapsed between the thrombotic event and the venepuncture, we also calculated the hazard ratio in the first year after the venepuncture. Consistent with the analysis where follow-up started 90 d after the initial event, no increase in the relative risk of a recurrent thrombosis in the first year after the venepuncture could be demonstrated.

Recently Hron *et al* (2006) reported on the relationship between recurrent venous thrombosis and peak thrombin generation. They concluded that patients with a peak thrombin generation less than 300 nmol/l had a lower risk of recurrence compared with patients with a peak thrombin generation above greater than 400 nmol/l (RR: 0.37) (Hron *et al*, 2006), a finding that could not be confirmed in the current study.

A major problem with thrombin generation assays is the interference from contact activation, especially with assays using a low TF trigger concentration (Luddington & Baglin, 2004). Contact activation can be avoided by sampling blood directly into anticoagulant containing corn trypsin inhibitor (CTI), an inhibitor of activated factor XII. However, addition of CTI after plasma separation was shown not to be sufficient to abolish contact activation (Luddington & Baglin, 2004), and as the plasma samples of the LETS have been stored for many years, contact activation could not be ruled out. When using a TF trigger concentration below 15 pmol/l, factor XIIa-driven thrombin generation can equal or exceed that due to TF. At a TF trigger concentration of 15 pmol/l there is little effect from contact factor activation (Luddington & Baglin, 2004) and so this concentration was used to trigger thrombin generation as samples had not been collected in CTI. However, we cannot exclude the possibility that different results would have been obtained if samples had been taken into CTI and the ETP triggered with a lower TF concentration. Thrombin generation after TF initiation can be divided into the propagation and initiation phase. Using a low TF trigger concentration in thrombin generation tests results in a more physiological situation. Therefore, preferably, to get a more global impression of the coagulation system including both the initiation as well as the propagation phase, a lower TF trigger concentration should

be used. The relatively high TF trigger concentration that was necessarily used in this study, might explain the absence of an increased risk in recurrent venous thrombosis associated with elevated ETP. Brummel-Ziedins *et al* (2005) calculated thrombin generation as well as several other determinants, e.g. rate of thrombin generation, maximum amount of thrombin formed also in the Leiden Thrombophilia case-control study. Consistent with the results presented in the current study, an increased risk of a first venous thrombosis associated with an elevated endogenous thrombin potential was demonstrated using a computer simulation of thrombin generation. However, for the management of patients with venous thrombosis, this model is less useful as all coagulation factors involved need to be measured in each patient. The risks reported in the study by Brummel-Ziedins *et al* (2005) are slightly higher than those reported here. In the described computer model, coagulation was activated with 5pmol/l TF, i.e. lower than the TF trigger concentration used in the present study. This again emphasizes that a thrombin generation assay that uses a low TF trigger concentration may be better in predicting the risk of a first and recurrent deep venous thrombotic event.

We added thrombomodulin to our assay as described in the Materials and methods. The concentration of thrombomodulin chosen was based on a previous study of selected patients in which this combination of TF/TM gave maximum discrimination between normal subjects and patients with a strong personal and family history of venous thrombosis (R. Luddington and T.P. Baglin, unpublished observations). The transmembrane glycoprotein thrombomodulin is the co-factor of thrombin and is necessary for effective activation of protein C by thrombin. Thrombomodulin amplifies the protein C activation more than a 1000-fold (Van de Wouwer *et al*, 2004). The addition of thrombomodulin therefore incorporates the protein C pathway in the assay thereby possibly providing a more global test of both the pro- as well as the anticoagulant system. The effect of this addition to the test needs to be studied in more detail. Interest is currently focussed on the use of a single laboratory test in the diagnosis of venous thrombosis. However, at present, the clinical relevance of the thrombin generation assay in predicting recurrent venous thrombosis remains uncertain. It is therefore not useful to perform these tests routinely in patients who experienced a first thrombotic event. No firm conclusions can be drawn from the outcome of this test until it has been studied in more detail and a positive predictive value has been confirmed by several studies.

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3.4

Contribution of high factor VIII, IX and XI to the risk of recurrent venous thrombosis in factor V Leiden carriers

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Introduction

Factor (F) V Leiden is a hereditary thrombophilic defect that is found in approximately 5% of Caucasians and in 20% of patients with a first venous thrombosis.^{1,2} Heterozygous FV Leiden carriers have a 5-7-fold increased risk of a first venous thrombosis compared with non-carriers,^{3,4} while its homozygous form is associated with an 80-fold increased risk.⁴ In a recent study of ours, FV Leiden was not associated with an increased risk of recurrent venous thrombosis, which is in agreement with others.⁵⁻⁷ A meta-analysis of these studies found a significant 1.4-fold increased risk of recurrent venous thrombosis in heterozygous FV Leiden carriers compared with non-carriers, but the estimated population-attributable risk from this type of patient was only 9.0 %.⁸ These results call into question the merit of extended duration of anticoagulation in these patients and cost-effectiveness of testing for FV Leiden in patients with first venous thrombosis.⁸ Studies on recurrent risk in heterozygous FV Leiden carriers did not account for co-segregation with high levels of FVIII, FIX, and FXI, which are common thrombophilic defects in patients with first venous thrombosis, and identified risk factors for first venous thrombosis.⁹⁻¹¹ We hypothesized that the risk of recurrent venous thrombosis might be increased in FV Leiden carriers who also have high levels of FVIII, FIX or FXI.

In the present follow-up study of 396 patients with first venous thrombosis, we assessed the contribution of high levels of FVIII, FIX or FXI to the risk of recurrent venous thrombosis in heterozygous FV Leiden carriers.

Materials and methods

Patients with a first, objectively confirmed episode of deep vein thrombosis, who were diagnosed between 1988 and 1992, were included in the study. They were participants of the Leiden Thrombophilia Study (LETS), a case-control study of the etiology of venous thrombosis, details of which have been described elsewhere.¹² Patients were prospectively followed-up through 2000. Major outcomes of this follow-up part of LETS were published in 2005,⁵ from which the present study forms a sub-analysis. Patients were tested for all currently known thrombophilic defects.⁵ The median time between a thrombotic event and venipuncture was 19 months (range, 6-68 months). Factor VIII activity was measured by a one-stage coagulation assay. Factor IX antigen levels were measured by sandwich enzyme-linked immunosorbent assays using commercial polyclonal antibodies (Dako A/S, Glostrup, Denmark). Factor XI antigen levels were measured by using a monoclonal antifactor XI capture antibody and polyclonal antifactor XI tagging antibody. The following cut-off values were used: 166 IU dL⁻¹ for factor VIII, 129 IU dL⁻¹ for factor IX, and 121 IU dL⁻¹ for factor XI, which correspond with the 90th percentile in controls. Factor V Leiden was demonstrated by polymerase chain reaction.⁴ For the current analysis, patients with antithrombin, protein C and protein S deficiency were excluded, as well as patients with homozygous FV Leiden or patients who were double heterozygous carriers of FV Leiden and prothrombin G20210A, as the risk of recurrence in these rare patients is uncertain and possibly high.^{7,13}

Observation time started 90 days after the date of the initial thrombotic event and ended on the date of recurrence or end of study. Patients who used vitamin K antagonists at time

of blood draw, were excluded from analysis as this treatment decreases FIX levels, and, obviously, the risk of recurrence.

Annual incidences of recurrent thrombotic events were calculated as the number of events over the accumulated patient time. A Cox-proportional hazards model was used to evaluate risks between groups after adjustment for age and sex. A separate analysis was performed to assess the effect of idiopathic or provoked classification of initial thrombotic event.

Results and discussion

Our study originally contained 474 consecutive patients with a first venous thrombosis who were followed-up for a median time of 8.0 years (range, 0.02-11.7). Median age at enrollment was 46 years (range, 15-69). Of these patients, 78 were excluded from further analysis, either because of antithrombin, protein C or protein S deficiency ($n = 25$), because of homozygous FV Leiden or double heterozygosity of FV Leiden and prothrombin G20210A ($n = 12$), because FV Leiden was not tested ($n = 3$), or because they used vitamin K antagonists at time of blood draw ($n = 38$). Thus, there were 396 patients in the final analysis. Their clinical characteristics are summarized in Table 1. Seventy patients (18%) were heterozygous for FV Leiden. High FVIII, FIX and FXI levels were noted in 23%, 21% and 19%, respectively in patients without FV Leiden and in 33%, 24% and 20%, respectively, in patients who were heterozygous for FV Leiden.

Table 1: Patient Characteristics

	Factor V Leiden	
	Wild type (n=326)	Heterozygous (n=70)
Women, n (%)	194 (60)	41 (59)
Median age at enrollment (range), years	46 (15-69)	43 (16-69)
Median follow-up time (range), years	8.1 (0.4-11.7)	7.9 (0.9-11.7)
First venous thrombosis		
Median age at onset (range), years	46 (15-69)	43 (16-69)
Idiopathic, n (%)	174 (53)	33 (47)
Recurrent venous thrombosis, n (%)	49 (15)	13 (19)
Median age at onset (range), years	54 (22-78)	51 (25-76)
Idiopathic, n (%)	36 (11)	9 (13)
High level of factor		
VIII (> 166 IU dL ⁻¹)	75 (23)	23 (33)
IX (> 129 IU dL ⁻¹)	68 (21)	17 (24)
XI (> 121 IU dL ⁻¹)	63 (19)	14 (20)

The annual incidence of recurrent venous thrombosis in patients without FV Leiden and normal FVIII, FIX and FXI levels was 2.0% (95% CI, 1.3-2.8) (Table 2). The crude hazard ratio for recurrent venous thrombosis in patients with heterozygous FV Leiden and normal FVIII, FIX and FXI levels was 1.1 (95% CI, 0.4-2.8) compared with patients without FV Leiden and normal FVIII, FIX and FXI levels. For patients without FV Leiden and high levels of FVIII, FIX or FXI, this crude hazard ratio was 1.0 (95% CI, 0.6-1.8), while it was 1.4 (95% CI, 0.6-3.0) in patients with heterozygous FV Leiden and high FVIII, FIX or FXI levels. Adjustment for age and sex did not change risk estimates in these groups, nor did an analysis in patients limited to those whose first event was idiopathic.

Our study suggests that patients with heterozygous FV Leiden and high levels of either FVIII, FIX or FXI are not at increased risk of recurrent venous thrombosis. This finding could be important for clinical practice. Both FV Leiden and high levels of FVIII, FIX and FXI are commonly found in patients with venous thrombosis,⁹⁻¹¹ as in our study, where 18% of patients had FV Leiden, and 20-30% had high levels of FVIII, FIX or FXI. Therefore, screening for these thrombophilic abnormalities in patients with venous thrombosis will probably reveal a high percentage of positive results, yet they do not give the clinician or the patient further information about the individual risk of recurrence. In our previous study we concluded that thrombophilic defects were not likely to play an important role in the risk of recurrence.⁵ One could argue, however, that specific combinations of thrombophilic defects were overlooked in that study that may well increase the risk of recurrence, as this has been shown in previous studies for first venous thrombosis, as well as for recurrence.^{7,13,14} The results of this sub-analysis indicate that this is not likely to be the case in patients with heterozygous FV Leiden and high levels of FVIII, FIX or FXI. Still, our study included a relatively small number of patients who had these thrombophilic defects, so these results should be interpreted with caution. Confirmation of our results in larger studies would therefore be advisable. In this respect, it is interesting to note a small study (n = 17) that suggests that the combination of FV Leiden with prothrombin G20210A increases the risk of recurrence substantially.⁷ The same holds for homozygous FV Leiden.¹⁵ However, small numbers may have played a role here as well. Our recurrence rate is low compared with other studies that reported on recurrence rates of venous thrombosis (for example, ¹⁶). Although a higher absolute risk of recurrence would have increased the number of cases, and hence the power of our estimates, it is unlikely that having more cases would have increased the point estimates of the relative risks on recurrence. These were low for each thrombophilic abnormality separately or in combination (point estimate 0.9-1.5, depending on the type of adjustment). We may have to make an exception for very high FVIII levels, as another study has shown that patients with FVIII levels ≥ 234 IU dl⁻¹ had a higher risk of recurrence.¹⁷ However, in our study only two patients had FVIII levels that were higher than 234 IU dl⁻¹. Therefore, patients with FVIII levels ≥ 234 IU dl⁻¹ may have an increased risk of recurrence, but according to our data these individuals are rare.

In summary, this study failed to show that patients with heterozygous FV Leiden and high levels of FVIII, FIX or FXI were at increased risk of recurrent venous thrombosis. Therefore, there seems to be no need to screen patients with venous thrombosis for FV Leiden and these coagulation proteins in order to determine recurrence risk.

Table 2: Risk of recurrent venous thrombosis in patients with factor V Leiden associated with co-segregation of clotting factors VIII, IX or XI

Factor V Leiden	Factors VIII, IX or XI	Observation years	Patients with event	Annual incidence, % (95% CI)	Crude hazard ratio (95% CI)	Adjusted* hazard ratio (95% CI)	Adjusted† hazard ratio (95% CI)
Wild type	Normal	1467 (n=189)	29	2.0 (1.3-2.8)	Reference	Reference	Reference
Heterozygous	Normal	232 (n=31)	5	2.2 (0.7-5.0)	1.1 (0.4-2.8)	1.1 (0.4-2.9)	0.9 (0.2-3.8)
Wild type	High	1016 (n=137)	20	2.0 (1.2-3.0)	1.0 (0.6-1.8)	1.1 (0.6-2.0)	1.1 (0.5-2.3)
Heterozygous	High	300 (n=39)	8	2.7 (1.2-5.3)	1.4 (0.6-3.0)	1.5 (0.7-3.3)	1.1 (0.4-3.2)

* Adjusted for age and sex.

† Limited to patients whose first event was idiopathic (n=207), adjusted for age and sex.

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General discussion

The content of this thesis and results from other research groups during the last decade have taught us that the nature of venous thrombosis undergoes a transformation when the disease occurs for the first time. Since the study of Prandoni (1), we have known that the overall risk of recurrence is increased for many years beyond the index event, and other cohort studies have also confirmed this.

The LETS study was designed to examine several baseline characteristics and to see if this knowledge could be helpful in the prediction of future recurrences.

This led to the important discovery that thrombophilia, clearly a risk factor for a first event, hardly affected the risk of a second event. Results on anticoagulant deficiencies were not entirely clear, due to the rarity of these defects, but seemed to indicate a mild effect on recurrence. The issue has since been much debated, and there exist several high quality studies with contradicting results (2-5), although most studies seem to confirm that FVL (6-9) and prothrombin G20210A (8, 10-12) at best play minor roles in the risk on recurrence. The discrepant results on anticoagulant deficiencies may stem from their low prevalence, and also from most cohort studies having included selected rather than consecutive patients. Regarding elevated levels of factors VIII and IX, the baseline risks on a 1st VT (13-14) were similar to that of prothrombin G20210A (15), and again follow-up failed to show an increased risk of recurrence in LETS. This was contradictory to results from the AUREC-study, where factor VIII and IX –elevation above the resp 90th and 75th percentile respectively yielded 6- and 2-fold increased risks on recurrence (16-17). Another study that included patients with first time VT as well as PE found factor VIII exceeding the 90th percentile to give higher risk on recurrence in patients with an unprovoked 1st VT (HR-range: 5.4-6.2) as compared to patients with a 1st provoked VT (HR-range: 1.7 - 2.6) (18).

A surprising discovery was the increased risk of recurrence in men (in the absence of a sex difference in the incidence of first events). Before the publication of the AUREC-study (19), other cohort studies had made minor notions of a sex difference (20) or found no or only a weakly increased risk on recurrence in men versus women (6, 10, 21-23). Following the AUREC, which found a 3.6-fold higher recurrence rate in men than in women, the Cambridge Venous Thromboembolism Study also reported a 2- to 3-fold higher risk in men than in women, also in an analysis restricted to consecutive men and women with an idiopathic 1st event. Also correction for age did not alter the results (19, 24). The authors of the AUREC-study recalculated the risk to be 3.6-fold increased (CI95: 2.2-6.0) when restricting the analysis to 633 patients with a proximally located VT, PE or both. They did so because distal VT more often occurs in women and has a lower recurrence rate, and lower rate of pulmonary embolism (25-26).

Contradictory to these findings, the largest cohort study, performed by Prandoni et al (27) found only a weak association of male sex with recurrent venous thrombosis (HR: 1.16, CI95: 0.94-1.43). When they restricted the analysis to patients with an idiopathic first event the relative risk of recurrence in men was 1.21 (CI95: 0.95 – 1.55) as compared to women.

A recent meta-analysis (28) included 11 prospective studies (6, 19, 22, 24-25, 29-34) with clearly defined inception cohorts and > 90 % complete follow-up. Eight of the 11 studies included consecutive patients (6, 19, 24-25, 30-31, 33-34) whereas 3 did not (22, 29, 32). Five of these studies found an increased risk of recurrence in men (relative risk-range 1.7-6.3) (19, 24, 29-30, 34), 5 studies showed little difference between the sexes (relative risk-range

1.2-1.5) (22, 25, 31-33), and one study found a decreased risk in men (relative risk 0.5) (6). The pooled risk estimate indicates a higher risk of recurrence in men than in women with a relative rate of 1.6 (CI95: 1.2-2.0). The increased risk in men could not be explained by status of anticoagulation, the distribution of initial distal VT, whether consecutive patients were enrolled, or whether the adjudication committee for the outcome (recurrent VT) was blinded for sex (28). An important finding of this meta-analysis was that the higher proportion of women than men with a transient risk factor (i.e. oral contraceptives) at the first event only accounted for a small part of the increased risk found in men. The difference in recurrence risk between men and women cannot be explained by a difference in the ratio of ipsi- versus contralateral recurrences of DVTs.

Prospective cohort studies are costly to perform, and rely on the long term cooperation of the participating patients. With 4 measuring moments over a 7.3 year follow-up, we had to rely on the memory of the patients. It became obvious that some data were better remembered than others. For instance, patient seldom had difficulty in recalling recent surgery, but would often fail to remember whether they received anticoagulation during the postoperative period. In the Leiden part of the population, the data on anticoagulation were cross-checked with the registries from the anticoagulation clinic (Trombosedienst). We had no possibility to do a similar crosscheck for the participants from Amsterdam and Rotterdam. It cannot be ruled out that some individuals with thrombophilia more often used anticoagulation during high risk periods than noncarriers. This raises the possibility that our estimate of the rate of recurrence in thrombophilia is an underestimate.

The TROL-study had a prospective design, which was excellent to study the effect of the presence of anticardiolipins, elevated levels of homocystein or of cytokines on the risk of thrombosis in the general population. In a retrospective case-control design, in which blood is sampled after the event, it cannot be ruled out whether observed abnormalities are the result rather than the cause of thrombosis. We did our best effort to secure the quality of the endpoints, and classified the endpoints according to the method used by the PIOPED investigators and Silverstein et al (36-38).

Elevated levels of cytokines did not increase the long term risk of venous thrombosis, but the possibility that a sudden increase in cytokine levels initiate the coagulation shortly before a thrombosis cannot fully be ruled out. Pre-existing elevated homocystein levels also did not predict future thrombosis, nor did the presence of cardiolipins. These results may indicate that these phenotypes do not causally affect the risk of thrombosis and reflect post-hoc phenomena, for instance due to a post-thrombotic syndrome and inflammation. However, in some cases blood was drawn long before the thrombotic event, and the power of the study may have been too low to detect whether transient increases in these factors could lead to a short-lasting increase in risk.

Male sex is a consistent risk factor of recurrence in populations of consecutively diagnosed patients with a first venous thrombosis, also if the analysis is restricted to those with an unprovoked 1st event. This may imply that men require prolonged anticoagulation after a first thrombotic event, and randomized studies should address this question. There is too little evidence to justify extended anticoagulation regimes in carriers of the most common thrombophilia factors.

The LETS follow-up has provided new evidence that women with hormonal risk factors at the first event have a reduced risk of recurrence if they avoid the exposition in the future. Advising women to quit oral contraception after a first event seems warranted. It is unlikely that in the near future we will again be able to collect estimates of the recurrence risk in oral contraceptive users as most women are now actively advised the discontinuation of oral contraception after a first event.

Although society undergoes major changes as for instance the increasing life expectancy, increasing body weight, increasing access to health care, and development of more accurate diagnostic tools, the TROL-study confirmed that the incidence of venous thrombosis has changed little over time. Therefore, studies aimed at identifying individuals at increased risk of first or recurrent venous thrombosis remain of the utmost importance and will hopefully in the future lead to the prevention of thrombosis, which remains a major disabling and often fatal disease.

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Summary

The objectives of this thesis were to study the magnitude of the risk of venous thrombosis in the general population, and to determine clinical and biochemical factors that influence this risk. We examined this separately for first and for recurrent thrombotic events and the content of the thesis is split accordingly in part 2 and part 3. We used data from two studies for both objectives: TROL, a Norwegian prospective follow-up study in the population, in which we counted patients with a 1st event of venous thrombosis and LETS, a follow-up of a Dutch cohort of 474 consecutive patients with a 1st objectively confirmed venous thrombosis. First we estimated the incidence and mortality of a first VT event in a general population, i.e. the residents of Nord-Trøndelag county in Norway, aged 20 years and older (n = 94 194). From this population we identified all cases with an objectively verified diagnosis of VT that occurred between 1995 and 2001. We found 740 patients with a first diagnosis of VT during 516,405 person-years of follow-up. The incidence rate for all first VT events was 1.43 per 1000 person-years (95% CI: 1.33-1.54). The incidence rates increased exponentially with age, and were slightly higher in women than in men. The 30-day case-fatality rate was higher in patients with PE than in those with DVT (9.7% vs. 4.6%, risk ratio 2.1 (95% CI: 1.2-3.7)); it was also higher in patients with cancer than in patients without cancer (19.1% vs. 3.6%, risk ratio 3.8 (95% CI 1.6-9.2)). (**chapter 2.1**).

As a first factor that could potentially influence the occurrence of thrombosis, we examined levels of cytokines. Former studies have associated increased levels of inflammatory parameters with an increased risk of venous thrombosis. As in most of these studies blood was sampled after the thrombotic event, the results were potentially biased if the occurrence of a thrombus itself initiates an inflammatory response. In our prospective design (TROL-study), we found no evidence of any pre-existing inflammatory state in those who developed a first event as compared to those who did not (**chapter 2.2**). Nevertheless, it can not be fully ruled out that high levels of cytokines predispose to VT, as the prospective study has as a drawback that there are for most individuals protracted time periods between blood sampling and thrombotic event.

In **chapter 2.3** we examined another risk factor for a first venous thrombosis, i.e. the presence of anticardiolipin antibodies, again in the TROL study. Patients with Systemic Lupus Erythematoses have an increased risk of both venous and arterial thrombosis. There has been a particular interest in the carriership of antibodies directed against phospholipids. The trigger inducing the production of these antibodies is not well understood, but it is believed to be a failure of the regulatory mechanisms of the autoimmune system. In the TROL we did not find evidence that anticardiolipin antibodies are risk factors for subsequent venous thrombotic disease, although this may be different for high titres (exceeding the 99th percentile in healthy controls) (**chapter 2.3**).

Next we examined in the TROL-study whether high levels of homocysteine are a risk factor for a 1st event of venous thrombosis in the general population. We found that high levels of homocysteine are at most a weak risk factor for venous thrombosis. The small increased risk that we found in men could have been the result of confounding by comorbidity that we were not able to fully adjust for (**chapter 2.4**). In addition to this negative finding for homocysteine levels, we also found no increased risk for recurrent VT in patients with hyperhomocysteinemia in the LETS (**chapter 3.1**).

In **chapter 2.5** we looked into detail whether obesity is related to APC-resistance and whether this could be mediated by increased levels of factor VIII. The results confirmed that this was the case, and furthermore that factor V Leiden carriership adds to the risk of venous thrombosis in obese as compared with non-obese patients.

In part 3 we focused on the incidence of recurrent VT and its risk factors in subjects with a first VT. 474 patients who participated in the LETS-study were followed for a mean of 7.3 years and complete follow-up was achieved in 447 patients (94%). Recurrence of thrombotic events occurred in 90 patients during a total of 3477 patient-years. The rate of thrombotic event recurrence was 25.9 per 1000 patient-years (95% CI: 20.8-31.8). The risk of recurrence was 2.7 times (95% CI, 1.8-4.2) higher in men than in women; patients whose initial thrombotic event was idiopathic had a higher risk of recurrence than patients whose initial event was provoked (HR 1.9; 95% CI, 1.2-2.9). Recurrence risk was also higher for women who used oral contraceptives during follow-up (28.0 per 1000 patient-years; 95% CI, 15.9-49.4) than for those who did not (12.9 per 1000 patient-years; 95% CI, 7.9-21.2). Surprisingly, there was no clear relation between recurrence risk and the presence of thrombophilia (**Chapter 3.1**).

In **chapter 3.2** we found that men with an unprovoked first event had the highest risk of recurrence, with almost one third experiencing a second unprovoked event within 8 years (IR 41.2/1000 person-years for men with an unprovoked first and second event). This risk was 3-fold lower in women (IR 14.2/1000 person-years; hazard ratio 2.8 (95% CI 1.4-5.7) for men vs women). Age had no effect on the rate of unprovoked recurrence, nor had time elapsed since the first event. In women, almost half of the recurrences were provoked and occurred mainly during oral contraceptive use, pregnancy or puerperium. Advising women to quit oral contraception after a first event seems warranted. (**Chapter 3.2**)

It has been proposed that the endogenous thrombin potential (ETP) could be a useful test to identify the group of patients who are at particular risk of developing recurrent venous thrombosis. Nevertheless, our study showed that the risk of recurrence in patients with increased ETP was only slightly increased, and it therefore seems unlikely that this test can identify patients at an increased risk of recurrence (**chapter 3.3**).

Lastly, as risk of recurrence could be increased in the joint presence of several risk factors, we studied whether FV Leiden carriers who also have high levels of FVIII, FIX or FXI have a higher risk of a second event. The results of our analysis in the LETS data failed to show such an association (**chapter 3.4**)

Studies aimed at identifying individuals at increased risk of first or recurrent venous thrombosis remain of the utmost importance and will hopefully in the future lead to lower risks of thrombosis than we found in our study, as venous thrombosis remains a major disabling and often fatal disease.

Samenvatting

Het doel van dit proefschrift was om de grootte van het risico op veneuze trombose in de algemene populatie te bepalen, en om vast te stellen welke klinische en biochemische factoren daaraan bijdragen. We deden dit separaat voor het optreden van een 1e trombose en voor het optreden van een recidief trombose. De inhoud van dit proefschrift is opgesplitst volgens deze indeling, waarbij het onderzoek rond een 1e trombose wordt behandeld in deel II, en het onderzoek rond een recidief wordt behandeld in deel III. Voor deze doeleinden maakten we gebruik van de gegevens van 2 verschillende studies: de TROL-studie, een prospectief vervolgonderzoek in een Noorse populatie, waarbinnen we keken naar het risico op een 1e trombose, en de LETS-studie, een vervolgonderzoek van een Nederlands cohort met 474 opeenvolgende patiënten die al een 1e veneuze trombose doormaakten.

Allereerst onderzochten we de incidentie en de mortaliteit van een 1e veneuze trombose in de algemene populatie, dat wil zeggen bij de inwoners van de provincie Nord-Trøndelag in Noorwegen die op het moment van het onderzoek 20 jaar of ouder waren ($n = 94\,194$). In deze populatie identificeerden we alle patiënten die een 1e veneuze trombose hadden in de periode 1995 tot en met 2001. We vonden 740 patiënten met een 1e veneuze trombose gedurende 516.405 persoonsjaren. Het incidentiecijfer voor een 1e veneuze trombose werd berekend op 1.43 per 1000 persoonsjaren (95% CI: 1.33-1.54). De incidentie nam exponentieel toe met de leeftijd, en was wat hoger in vrouwen dan in mannen. Het aantal overledenen 30 dagen na de diagnose was hoger in patiënten met een longembolie dan in patiënten met een diep veneuze trombose (9.7% versus 4.6%, relatief risico (RR) 2.1 (95% CI: 1.2-3.7)); de sterfte was ook hoger in patiënten met kanker dan in patiënten zonder kanker (19.1% versus 3.6%, RR 3.8 (95% CI 1.6-9.2)). (hoofdstuk 2.1).

Ten tweede wilden we uitzoeken welke factoren bijdragen aan een verhoogd risico op trombose. Hiervoor onderzochten we eerst of een verhoogd niveau van een aantal cytokines (ontstekingsfactoren) kon leiden tot het ontwikkelen van trombose. Eerdere studies hadden namelijk aangetoond dat verhoogde niveaus van cytokines een verhoogde kans op veneuze trombose met zich meebrachten. In de meeste van deze studies waren de bloedmonsters echter afgenomen nadat de trombose was vastgesteld, en het was niet uit te sluiten dat de gevonden associatie het resultaat was van een ontstekingsreactie die door de trombose zelf was teweeggebracht. Met onze prospectieve opzet (de TROL-studie) vonden we geen aanwijzingen dat verhoogde cytokinespiegels het risico op een veneuze trombose verhoogde. (hoofdstuk 2.2). Niettemin konden we niet helemaal uitsluiten dat een plotselinge verhoging van cytokines binnen korte tijd tot trombose zou kunnen leiden, aangezien er voor de meeste patiënten een aanzienlijke tijd zat tussen de bloedafname en de trombose.

In hoofdstuk 2.3 onderzochten we, eveneens in de TROL-studie, een andere risicofactor voor een 1e veneuze trombose, namelijk de aanwezigheid van anticardiopiline antistoffen. Patiënten met Systemische Lupus Erythematoses hebben een verhoogd risico op zowel veneuze als arteriële trombose, het geen mogelijk verklaard zou kunnen worden door dragerschap van antilichamen gericht tegen fosfolipiden. Het mechanisme van het ontstaan van deze antilichamen is nog niet goed bekend, maar het wordt beschouwd als het falen van regulatiemechanismen van het immuunsysteem. In de TROL-studie vonden we geen aanwijzingen dat de aanwezigheid van anticardiopiline-antilichamen in de algemene populatie het risico kon verhogen op veneuze trombose, hoewel het zou kunnen dat dit wel zo is voor patiënten met uitzonderlijk hoge titers (boven de 99^e percentiel in gezonde controles) (hoofdstuk 2.3).

Vervolgens keken we in de TROL-studie of hoge niveaus van homocysteïne het risico op een 1e veneuze trombose verhogen in de algemene populatie. We vonden dat hoge niveaus van homocysteïne ten hoogste een zwakke risicofactor waren voor een 1e veneuze trombose. Het iets verhoogde risico dat we vonden in mannen zou het resultaat kunnen zijn van confounding door comorbiditeit waarvoor we niet volledig konden corrigeren. (hoofdstuk 2.4). Naast deze negatieve bevinding voor homocysteïne voor een 1e veneuze trombose, vonden we in de LETS-studie geen verhoogd risico voor een recidief veneuze trombose in patiënten met een hoog homocysteïne gehalte in het bloed (hoofdstuk 3.1).

In hoofdstuk 2.5 keken we in detail of obesitas gerelateerd was aan APC-resistentie en of dit werd veroorzaakt door verhoogde niveaus van factor VIII. De resultaten bevestigden dat dit het geval was, en bovendien dat dragerschap van factor V Leiden meer bijdraagt aan het risico op veneuze trombose in zwaarlijvige vergeleken met niet-zwaarlijvige patiënten.

In het 3e gedeelte van dit proefschrift vermelden we de incidentie van recidief veneuze trombose, en de invloed van risicofactoren op recidief trombose in patiënten van de LETS-studie die al een 1e trombose hadden gehad. 474 patiënten die meededen aan de LETS-studie werden gemiddeld 7.3 jaar gevolgd, en een complete follow-up werd bereikt in 447 patiënten (94 %). 90 van hen kregen een recidief trombose gedurende 3477 patiëntjaren. Het incidentiecijfer voor een recidief trombose was 25.9 per 1000 patiëntjaren (95% CI: 20.8-31.8). Het risico op een recidief was 2.7 maal (95% CI, 1.8-4.2) hoger in mannen dan in vrouwen; patiënten bij wie voor de 1e episode geen duidelijke oorzaak werd gevonden hadden een hoger risico op recidief dan patiënten bij wie wel een oorzaak was gevonden (RR 1.9; 95% CI, 1.2-2.9). Het risico op een recidief trombose was ook hoger voor vrouwen die de anticonceptie pil gebruikten gedurende de vervolperiode (28.0 per 1000 patiëntjaren; 95% CI, 15.9-49.4) dan voor de vrouwen die geen pil gebruikten (12.9 per 1000 patiëntjaren; 95% CI, 7.9-21.2). Verrassend genoeg was er geen duidelijke samenhang tussen de kans op recidief trombose en de aanwezigheid van trombofilie (hoofdstuk 3.1).

In hoofdstuk 3.2 beschrijven we onze bevinding dat mannen die bij hun 1e trombose geen duidelijke verklaring hadden voor hun trombose het hoogste risico liepen op een recidief; , bijna 1/3e van hen kreeg een recidief trombose binnen een tijdsbestek van 8 jaar na de 1e trombose (incidentiecijfer 41.2/1000 persoonsjaren voor mannen wanneer er voor zowel de 1e als voor de 2e trombose geen duidelijke oorzaak was). Dit risico was 3-maal lager in vrouwen (incidentiecijfer 14.2/1000 persoonsjaren; RR 2.8 (95% CI 1.4-5.7) voor mannen versus vrouwen). Leeftijd had geen effect op het krijgen van een recidief, evenmin als de tijdsduur sinds de 1e trombose. Bij vrouwen kon voor bijna de helft van de recidieven een aanwijsbare oorzaak gevonden worden, nl. het gebruik van de anticonceptiepil, zwangerschap of kraambd. Het is daarom aan te bevelen dat vrouwen stoppen met de pil na een 1e veneuze trombose. (hoofdstuk 3.2)

Het meten van de endogene trombine potentiaal (ETP) zou mogelijk nuttig kunnen zijn voor het identificeren van patiënten die een bijzonder hoog risico hebben op een recidief trombose. Niettemin wees de LETS-studie uit dat het risico op een recidief in patiënten met verhoogd ETP beperkt was, en dat het daarom onwaarschijnlijk is dat deze test patiënten kan identificeren die een hoog risico op recidief trombose hebben (hoofdstuk 3.3).

Aangezien het risico op recidief trombose waarschijnlijk verhoogd wordt door het tegelijkertijd voorkomen van een aantal risicofactoren, hebben we in de LETS-studie onderzocht of dragers van FV Leiden met eveneens hoge niveaus van de factoren VIII, IX of XI een verhoogd risico op een 2e trombose hadden. De resultaten lieten echter zien dat dit niet het geval was (hoofdstuk 3.4).

Onderzoek dat zich richt op het vinden van individuen die een verhoogd risico hebben op een 1e of een 2e veneuze trombose blijft belangrijk, en hopelijk zullen toekomstige resultaten ertoe leiden dat het risico op trombose lager zal zijn dan wat wij in onze studies hebben gemeten, aangezien veneuze trombose een ziekte is die kan leiden tot invaliditeit, en in het ergste geval tot sterfte.

Dankwoord

Mijn carrière en leven was tot dan toe altijd gelijk verdeeld geweest tussen Nederland en Noorwegen, en na mijn artsexamen in 2000 stond ik voor de moeilijke keuze in welk land ik mijn werk wilde voortzetten. Na een zomer als huisartswaarnemer in Nord-Trøndelag in Noorwegen, besloot ik te solliciteren als promovendus op een project dat juist een samenwerkingsverband was tussen het bevolkingsonderzoek dat was uitgevoerd in Nord-Trøndelag, en de afdeling Klinische Epidemiologie in Leiden.

Daar kreeg ik ook de mogelijkheid om klinisch gericht werk te doen op het hemostaselaboratorium om de praktijk van de hemostase te leren, van de behandeling van patienten met hemofilie tot gecompliceerde stollingsafwijkingen op de Intensive Care.

Daar ik nogal ongeschoold was in de statistiek en de epidemiologie waren de aangeboden cursussen en het methodenuur welkom. Ik heb goede herinneringen aan de ietwat exotische cursus in 2001 op Calabrië, en in het bijzonder de colleges van Dimitri Trichopolous en Kenneth Rothman.

Het kunnen presenteren van het eigen onderzoek op Congressen zoals dat van de ISTH (International Society on Thrombosis and Haemostasis) in Birmingham in 2003, en de WEON-congressen (Vereniging voor Epidemiologie) in Rotterdam en Leiden, waren voor mij absolute hoogtepunten, naast de publicaties in de JAMA en de PLoS.

De samenwerking met Frits, Suzanne, Inger Anne en Jens is bijzonder vruchtbaar geweest en heeft goede publicaties opgeleverd. Om mijn voorgangers te citeren: Onderzoek doe je niet alleen.

Ik ben bijzondere dank verschuldigd aan mijn co-auteurs want zonder hun vertrouwen, geduld en inzet was het nooit zo ver gekomen met dit proefschrift.

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Mijn gedachten gaan deze dag vooral naar mijn vrouw Vanessa en mijn familie, die me al deze jaren zo onvoorwaardelijk hebben gesteund.

Curriculum Vitae

Sverre Christian Christiansen is op 12 oktober 1971 geboren te Leiden. In 1990 behaalde hij zijn gymnasium diploma aan het gymnasium in Ulsteinvik in Noorwegen. Aansluitend begon hij aan een studie wiskunde aan de Universiteit van Bergen in Noorwegen, voordat hij in 1991-1992 zijn dienstplicht vervulde bij de landmacht in Noorwegen.

Daaropvolgend voltooide hij in de jaren 1993-2000 de studie geneeskunde aan de Rijksuniversiteit Leiden, Nederland.

Na een korte tijd als waarnemend huisarts gewerkt te hebben in de gemeente Nærøy in Nord-Trøndelag in Noorwegen was hij in de jaren 2000-2004 werkzaam als promovendus op de afdeling Klinische Epidemiologie in het Leids Universitair Medisch Centrum op de projecten de Trondheim-Leiden studie (TROL) en het vervolgonderzoek van de Leiden Trombophilia Study (LETS).

In 2005 begon hij als arts-assistent op de afdeling Interne Geneeskunde in het ziekenhuis van Ålesund, Noorwegen. Sinds September 2009 werkt hij in het St Olavs Hospital in Trondheim, Noorwegen om de opleiding tot endocrinoloog te voltooien.

Appendix

ICD-9

- 325 Phlebitis and thrombophlebitis of intracranial venous sinuses
- 362.3 Retinal vascular occlusion
- 415.0 Acute cor pulmonale
- 415.1 Pulmonary embolism and infarction
- 433 Occlusion and stenosis of basilar artery
- 437 Cerebral atherosclerosis
- 437.6 Nonpyogenic thrombosis of intracranial venous sinus
- 451.0 Phlebitis and thrombophlebitis of superficial vessels of lower extremities
- 451.1 Phlebitis and thrombophlebitis of deep veins of lower extremities
- 451.2 Phlebitis and thrombophlebitis of lower extremities unspecified
- 451.8 Phlebitis and thrombophlebitis of other sites
- 451.9 Phlebitis and thrombophlebitis of unspecified site
- 452 Portal vein thrombosis
- 459.1 Postphlebotic syndrome
- 453.0 Budd-chiari syndrome
- 453.1 Thrombophlebitis migrans
- 453.2 Embolism and thrombosis of vena cava
- 453.3 Embolism and thrombosis of renal vein
- 453.8 Embolism and thrombosis of other specified veins
- 453.9 Embolism and thrombosis of unspecified site
- 557 Vascular insufficiency of intestine
- 557.0 Acute vascular insufficiency of intestine
- 572.1 Portal pyemia
- 634.6 Spontaneous abortion complicated by embolism
- 634.7 Spontaneous abortion with other specified complications
- 635.6 Legally induced abortion complicated by embolism
- 635.7 Legally induced abortion with other specified complications
- 636.6 Illegal abortion complicated by embolism
- 636.7 Illegal abortion with other specified complications
- 637.6 Legally unspecified abortion complicated by embolism
- 637.7 Legally unspecified type of abortion unspecified with other specified complications
- 638.6 Failed attempted abortion complicated by embolism
- 638.7 Failed attempted abortion with other specified complications
- 639.6 Embolism following abortion or ectopic and molar pregnancies
- 639.8 Other specified complications following abortion or ectopic and molar pregnancies
- 639.9 Unspecified complication following abortion or ectopic and molar pregnancies
- 671 Venous complications in pregnancy and the puerperium
- 671.2 Superficial thrombophlebitis in pregnancy and the puerperium
- 671.3 Deep phlebothrombosis antepartum unspecified as to episode of care
- 671.4 Deep phlebothrombosis postpartum
- 671.5 Other phlebitis and thrombosis in pregnancy and the puerperium
- 671.9 Unspecified venous complication in pregnancy and the puerperium
- 673 Obstetrical pulmonary embolism
- 673.0 Obstetrical air embolism
- 673.1 Amniotic fluid embolism
- 673.2 Obstetrical blood-clot embolism
- 673.3 Obstetrical pyemic and septic embolism
- 673.8 Other obstetrical pulmonary embolism
- 674 Other and unspecified complications of the puerperium not elsewhere classified
- 674.0 Cerebrovascular disorders in the puerperium
- 997.2 Peripheral vascular complications not elsewhere classified

ICD-10

- G08 Intracranial and intraspinal phlebitis and thrombophlebitis
- H34.8 Other retinal vascular occlusions
- I26.0 Pulmonary embolism with mention of acute cor pulmonale
- I26.9 Pulmonary embolism without mention of acute cor pulmonale
- I63.6 Cerebral infarction due to cerebral venous thrombosis, nonpyogenic
- I67.6 Nonpyogenic thrombosis of intracranial venous system
- I80 Phlebitis and thrombophlebitis
- I80.0 Phlebitis and thrombophlebitis of superficial vessels of lower extremities
- I80.1 Phlebitis and thrombophlebitis of femoral vein
- I80.2 Phlebitis and thrombophlebitis of other deep vessels of lower extremities
- I80.3 Phlebitis and thrombophlebitis of lower extremities, unspecified
- I80.8 Phlebitis and thrombophlebitis of other sites
- I80.9 Phlebitis and thrombophlebitis of unspecified site
- I81 Portal vein thrombosis
- I82 Other venous embolism and thrombosis
- I82.0 Budd-Chiari syndrome
- I82.1 Thrombophlebitis migrans
- I82.2 Embolism and thrombosis of vena cava
- I82.3 Embolism and thrombosis of renal vein
- I82.8 Embolism and thrombosis of other specified veins
- I82.9 Embolism and thrombosis of unspecified vein
- I87.0 Postphlebitic syndrome
- K55.0 Acute vascular disorders of intestine
- K75.1 Phlebitis of portal vein
- O08.2 Embolism following abortion and ectopic and molar pregnancy
- O08.7 Other venous complications following abortion and ectopic and molar pregnancy
- O08.8 Other complications following abortion and ectopic and molar pregnancy
- O08.9 Complication following abortion and ectopic and molar pregnancy, unspecified
- O22 Venous complications in pregnancy
- O22.0 Varicose veins of lower extremity in pregnancy
- O22.1 Genital varices in pregnancy
- O22.2 Superficial thrombophlebitis in pregnancy
- O22.3 Deep phlebothrombosis in pregnancy
- O22.4 Haemorrhoids in pregnancy
- O22.5 Cerebral venous thrombosis in pregnancy
- O22.8 Other venous complications in pregnancy
- O22.9 Venous complication in pregnancy, unspecified
- O87 Venous complications in the puerperium
- O87.0 Superficial thrombophlebitis in the puerperium
- O87.1 Deep phlebothrombosis in the puerperium
- O87.2 Haemorrhoids in the puerperium
- O87.3 Cerebral venous thrombosis in the puerperium
- O87.8 Other venous complications in the puerperium
- O87.9 Venous complication in the puerperium, unspecified
- O88 Obstetric embolism
- O88.0 Obstetric air embolism
- O88.1 Amniotic fluid embolism
- O88.2 Obstetric blood-clot embolism
- O88.3 Obstetric pyaemic and septic embolism
- O88.8 Other obstetric embolism

DEFINITIONS AND DIAGNOSTIC SCORE OF VT AND PE

Based on PIOPED (A72) and Silverstein (A73)

VT: (1) Definite: VT confirmed by one of the following methods:

- Venography (filling defect)
- Ultrasound of the proximal limb (popliteal-femoral- and inguinal veins) and the proximal arm (from v axillaris to fossa cubiti) (lack of full compression of the vein by compression duplex ultrasound eventually supported by Doppler ultrasound))
- spiral CT
- thrombus removed during surgery and autopsy.

(2) Probable: (a+b)

(a) Positive Doppler Ultrasonographic examination or compression duplex ultrasonography in other locations than in (1). (calf, pelvis)

(b) The patient underwent therapy with anticoagulant for VT

(3) Possible: confirmatory tests not done or results indeterminate and (a + b + c)

(a) the physician made a diagnosis of VT according to the medical report

(b) signs and symptoms consistent with VT (or possible VT)

(c) patient underwent therapy with anticoagulants (heparin, warfarin etc)

or a surgical procedure for VT

(4) not VT: not 1,2,3 or 5

(5) not specified:

No / to less information about the diagnosis in the medical record

PE:

(1) Definite: PE confirmed by

- Ventilation-perfusion lung scan (V/Q scan) interpreted as high probability of PE (≥ 2 segmental perfusion defects) (V / Q mismatch)
- Perfusion-scan with ≥ 2 segmental perfusion defects associated with normal chest x-ray (X / Q mismatch)
- Pulmonal angiography
- Spiral CT
- Thrombus removed during surgery and autopsy

(2) Probable: (a + b)

(a) - V/Q scan interpreted as intermediate probability of PE

(1 moderate to < 2 large mismatches) perfusion intermediate of PE – with signs and symptoms of PE

(hemoptysse, respiratory-dependent chest pain, tachycardia, tachypnoe, hypoxia, hypocapny, low A-a difference, positive D-dimer)

- perfusion scan with indeterminate result and normal CXR with signs and symptoms of PE
- ecco-cor / transoesophageal echo
- Intermediate results of other confirmatory tests with signs and symptoms of PE

(b) the patient underwent anticoagulant therapy for PE

(3) Patient underwent therapy with anticoagulants for PE

(3) Possible: (a + b)

(a) – confirmatory tests not done and signs and symptoms consistent with PE

- intermediate V / Q scan without symptoms and signs of PE

(b) patient underwent therapy with anticoagulant for PE

(4) not PE

- V / Q scan with low probability of PE (match) without signs and symptoms

(5) not specified:

No / to less information about the diagnosis in the medical record