

CHAPTER 2

A PROSPECTIVE STUDY OF ASYMPTOMATIC CARRIERS OF THE FACTOR V LEIDEN MUTATION TO DETERMINE THE INCIDENCE OF VENOUS THROMBOEMBOLISM

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Submitted for publication

SUMMARY

Background: The factor V Leiden mutation is a common genetic defect associated with an increased risk of venous thromboembolism. The clinical implications for asymptomatic carriers of this mutation and, consequently, the usefulness of screening families with a proband with the factor V Leiden mutation and venous thromboembolism, are unclear.

Methods: A prospective cohort study to assess the incidence of venous thromboembolism was performed in asymptomatic carriers of the factor V Leiden mutation, identified by means of screening first degree relatives above the age of 15 of probands with the mutation, who have had an episode of venous thromboembolism. The absolute annual incidence of venous thromboembolism was calculated, and the relationship with exposure to situations with an increased risk was evaluated.

Results: A total of 1564 observation years in 470 asymptomatic carriers (234 men, mean age 43 years [range 15-88], 12 homozygous) were recorded. Nine venous thromboembolic events were observed, all in heterozygous carriers, resulting in an annual incidence of 0.58% (95%CI 0.26-1.10). The incidence of spontaneous venous thromboembolism was 0.26% (0.07-0.65) per year, and 3.5% (0.1-17.8) per episode of surgery, trauma or immobilization, 0.0% (0.0-19.5) per pregnancy, 1.8% (0.4-5.2) per year of oral contraceptive use, and 2.9% (0.8-15.3) per year of use of hormone replacement therapy.

Conclusions: The absolute annual incidence of spontaneous venous thromboembolism in asymptomatic carriers of the factor V Leiden mutation is low and does not justify routine family screening of asymptomatic patients. The benefit of screening in order to adjust prophylactic strategies during high risk situations for venous thromboembolism, or to discourage estrogen use in all identified asymptomatic carriers of the factor V Leiden mutation, is unclear and should be addressed in future research that also includes potential side effects of such screening strategies.

2.1 INTRODUCTION

Until 1994, only isolated deficiencies of the physiological inhibitors of the coagulation system, antithrombin, protein C, and protein S, were recognized as inherited risk factors for venous thromboembolism. They could only be detected in a small minority of patients with the disease [1]. In 1994, resistance to activated protein C was reported as a new and prevalent risk factor for venous thrombosis [2] and soon thereafter was found to be caused by a single point mutation in the factor V gene at the major cleavage site of activated protein C (also called factor V Leiden mutation or FV:Q⁵⁰⁶ mutation) [3-5]. The factor V Leiden mutation has a prevalence as high as 20% to 50% in patients with documented venous thrombosis [2, 6], and an overall prevalence in western populations of approximately 5% [7]. Case-control studies have shown an increased risk of venous thromboembolism in heterozygous carriers of the factor V Leiden mutation compared with healthy controls of 3 to 7 [2, 6, 8, 9].

The clinical implications for asymptomatic carriers of the factor V Leiden mutation are a matter of debate [10, 11]. It is only worthwhile, or even necessary, to screen relatives of patients known to have the factor V Leiden mutation for the presence of the mutation if prophylactic strategies, either permanently or during high-risk situations, are indicated in asymptomatic carriers. As for any genetic disorder, the benefits of early detection and appropriate preventive measures need to be balanced against the disadvantages of screening asymptomatic persons, labeling them as having a disease, and the risks of complications of the preventive treatment. To rationally decide whether the benefits of screening for the factor V Leiden mutation outweigh the disadvantages, it is crucial to know the absolute risk of venous thromboembolism in carriers of the factor V Leiden mutation and its potentiating factors, such as surgery, trauma, immobilization, pregnancy, and use of oral contraceptives or hormone replacement therapy, and weigh the risk against the known risk of hemorrhage associated with anticoagulant prophylaxis. Earlier studies on the absolute risk of venous thromboembolism in carriers of the factor V Leiden mutation were carried out either retrospectively [12, 13], had only small numbers of patients [14], or consisted of selected populations [15]. Only one moderate-size prospective cohort was reported which observed a low absolute incidence (0.49% per year) [16]. We previously reported a retrospective study of first-degree relatives of consecutive patients with documented venous thromboembolism and the factor V Leiden mutation in which we found an overall incidence of venous thromboembolism of 0.45% per year after the age of 15, which varied between 0.25% for the group aged 15-30 years to 1.1% per year for persons over 60 years of age [12]. In the present study, we prospectively followed the extended cohort of 470 asymptomatic carriers of the factor V Leiden mutation for a mean period of 3.3 years (range 1.5-4.8), and determined the absolute incidence of venous thromboembolism and the relationship with established exogenous risk factors.

2.2 METHODS

2.2.1 Subjects and study design

In the participating centers, all patients with a proven episode of venous thromboembolism are tested for the presence or absence of an underlying coagulation disorder. First degree relatives (parents, siblings and children older than 15 years of age) of patients with the factor V Leiden mutation were interviewed for previous episodes of venous thromboembolism using a validated questionnaire [17], before the factor V Leiden mutation status (normal, heterozygous, and homozygous) was determined by a polymerase chain reaction-based assay, as described previously [5]. All asymptomatic carriers of the factor V Leiden mutation, who were identified before December 1, 1997, subsequently entered the prospective follow up study, whereas a few initially unresponsive relatives of already included families were included during 1998. The end date of the study was February 1, 2000.

Participating relatives were instructed to seek immediate medical attention when clinical signs or symptoms of deep vein thrombosis or pulmonary embolism occurred, so that objective diagnostic tests could be performed. Furthermore, there was a regular follow up contact every 6 months, using a standardized evaluation form to identify the occurrence of venous thromboembolism, the exposure to established risk factors for venous thromboembolism, and prophylactic or therapeutic use of anticoagulant drugs. For women, the use of oral contraceptives, hormone replacement therapy, and details about pregnancy and postpartum period were documented.

The institutional review boards of the participating centers approved the study, and all participants gave informed consent.

2.2.2 Definitions

Whenever clinical signs or symptoms of venous thromboembolism occurred, established objective tests were performed to exclude or confirm the diagnosis (compression ultrasound of the femoral or popliteal veins, if necessary followed by venography for suspected deep vein thrombosis and ventilation-perfusion lung scanning, and/or pulmonary angiography for suspected pulmonary embolism). At all times, the patients' charts were reviewed and if necessary the treating physicians were contacted.

Episodes of increased risk for venous thromboembolism included surgery, trauma, immobilization for more than 7 days, pregnancy, and use of oral contraceptives and hormone replacement therapy. Venous thromboembolism was considered to be related to such a situation if it occurred during or within 3 months of the last day of the particular situation, or

during use of oral contraceptives and hormone replacement therapy. The observation time was defined as the time since the day of inclusion in the study until February 1, 2000, or the date of the first venous thromboembolic event. The minimum follow up was 18 months.

2.2.3 Statistical analysis

The overall annual incidence of a first episode of venous thromboembolism was calculated. The 95% CIs were calculated according to normal approximation of the binomial distribution. In addition, the relationship of venous thromboembolism with the exposure to exogenous risk factors, was analyzed. We calculated a Kaplan-Meier estimate for visual assessment of the cumulative incidence of venous thromboembolism.

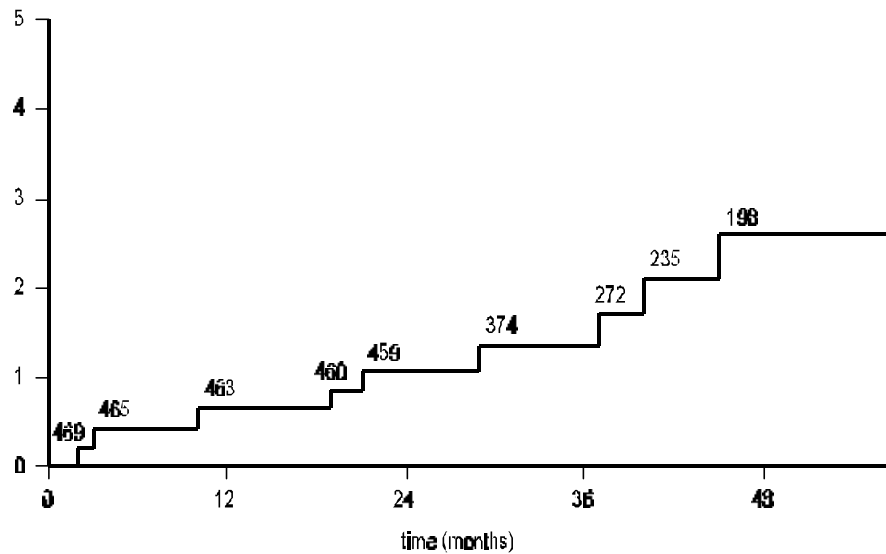
2.3 RESULTS

A total of 919 relatives (response rate of living relatives: 85%) were invited to participate in the study. Of these, 385 did not have the factor V Leiden mutation. Of the 534 carriers of the mutation, 61 were excluded because of a history of prior venous thromboembolism, and 3 were excluded because they used vitamin K antagonists permanently for other indications. Thus, a total of 470 asymptomatic carriers (234 men and 236 women) with a mean age of 43 years (range 15-88) at entry were included in the study. These carriers were first degree relatives of 247 symptomatic probands (234 heterozygous and 13 homozygous). Of the asymptomatic carriers, 12 (4 men and 8 women) turned out to be homozygous for the factor V Leiden mutation.

During follow up, 3 carriers died. One woman died at age 63 of pulmonary carcinoma, without evidence for symptomatic venous thromboembolism. Another woman, aged 57, died suddenly while asleep. Eight months prior to her death, she was diagnosed to have pulmonary cancer with mediastinal lymphnode metastases, for which she received radiotherapy. She also had a recent history of myocardial infarction and had undergone coronary artery bypass grafting. A postmortem examination was not performed. The third person who died during follow up, was a 55-year-old man who was known with sarcoidosis. He died of pulmonary bleeding in the presence of a bronchiolitis obliterans and organizing pneumonia. Pulmonary embolism was excluded by autopsy. Nine carriers withdrew consent (1 male aged 87 years because of old age; 1 female aged 77 years, because she was diagnosed to have esophageal cancer; 6 men, aged between 38 and 65 years, and 1 24-year-old woman for personal reasons;

none of these individuals experienced an episode of symptomatic venous thromboembolism, according to their participating relatives).

Figure 2.1 Cumulative incidence of venous thromboembolism in 470 asymptomatic carriers of the factor V Leiden mutation



There was a mean follow up of 3.3 year per carrier (range 1.5-4.8 years) with a total of 1564 observation years (including 44 for the homozygous carriers). Nine heterozygous carriers suffered from a venous thromboembolic event, so that the overall absolute annual incidence of venous thromboembolism was 0.58% (95% CI 0.26- 1.10). Four of these events occurred spontaneously (incidence 0.26%, 95% CI 0.07-0.65), and one occurred 3 weeks after surgery for a herniated disc, despite prophylaxis with low-molecular-weight heparin during the 7 days in-hospital stay. Another event occurred 5 months after start of hormone replacement therapy for climacterial symptoms, and 3 events occurred during oral contraceptive use. Two of these latter women had continuously been taking oral contraceptives for 4 and 10 years, respectively, whereas one woman had used a second generation preparation for 9 years, had an uneventful pregnancy, and restarted oral contraceptives use with a third generation preparation 1 year prior to the deep vein thrombosis. The clinical characteristics of these patients and details about the thromboembolic events are summarized in Table 2.1. Figure 2.1 shows the cumulative incidence of venous thromboembolism in the study cohort.

Table 2.1 Clinical characteristics of the individuals who experienced venous thromboembolism (VTE) during follow up

age at time of time VTE, yr	gender	kind of VTE	diagnostic method	eliciting risk factor
70	male	left popliteal vein	compression ultrasound	no
32	male	pulmonary embolism	ventilation-perfusion scan	surgery for a herniated disc*
55	male	right femoral vein and clinical signs of pulmonary embolism	compression ultrasound	no
25	female	left popliteal vein	compression ultrasound	use of oral contraceptives**
50	female	left popliteal vein	compression ultrasound	use hormone replacement therapy***
62	male	right popliteal vein	compression ultrasound	no
74	female	left popliteal vein	compression ultrasound	use of oral contraceptives****
53	female	left popliteal vein	compression ultrasound	no
23	female	left popliteal vein	compression ultrasound	use of oral contraceptives*****

* 3 weeks after surgery; prophylaxis with low-molecular-weight heparin during 7 days in-hospital

** during use of oral contraceptives for a period of 10 years; no change in used preparation

*** start of conjugated estrogens combined with hydroxyprogesteron 5 months prior to DVT

**** during use of oral contraceptives; change in used preparation (from 2nd to 3rd generation) 1 year prior to DVT

***** during use of oral contraceptives for a period of 4 years; no change in used preparation (second generation)

The number of high risk situations that were recorded during the study, are summarized in Table 2.2. Twenty-nine episodes of surgery, trauma, or immobilization for longer than 7 days were observed, for which the treating physicians initiated standard thrombosis prophylaxis in all. One of these episodes was complicated by pulmonary embolism 3 weeks postoperatively. The incidence of venous thromboembolism related to such conditions was therefore 3.5% (95% CI 0.1-17.8), despite the use of routine, in-hospital thrombosis prophylaxis.

Table 2.2 Relationship of venous thromboembolism with high risk situations

high risk situation	number	venous thromboembolism	
		n	incidence per event (95% CI)
major surgery, trauma, strict immobilization > 7 days*	29	1	3.5 (0.1-17.8)
pregnancy and postpartum period**	17	0	0.0 (0.0-19.5)
pregnancy, ongoing***	7	0	0.0 (0.0-41.0)
oral contraceptive use, yr	166 (66 women)	3	1.8 (0.4-5.2)
hormone replacement therapy, yr	34 (21 women)	1	2.9 (0.8-15.3)

* all episodes with standard low-molecular-weight heparin prophylaxis

** 2 caesarian section with 1 week prophylaxis postoperatively, 6 vaginal deliveries with 1 week low dose low-molecular-weight heparin prophylaxis

*** Including 1 with low dose low-molecular-weight heparin prophylaxis

There were 17 full term pregnancies, while 7 were still ongoing at the time of the end of the study. Of the 17 full term pregnancies, two were terminated by a caesarian section with standard prophylaxis with low-molecular-weight heparin during 1 week. Another 6 women used postpartum prophylaxis with low dose low-molecular-weight heparin for the first week. One woman used low dose low-molecular-weight heparin during the first and second trimester, and switched to higher doses during the third trimester and the 6 weeks postpartum, because of a strong family history and fear for venous thromboembolism. None of the pregnancies were complicated by venous thromboembolism. Four women had a total of 5 spontaneous abortions in the first trimester.

Sixty-six women, among whom 4 were starters, used oral contraceptives for a total period of 166 years. Three women experienced a deep vein thrombosis, so that the incidence of oral

contraceptive-related venous thromboembolism was 1.8% (95% CI 0.4-5.2) per year of use. Twenty-one women used hormone replacement therapy for a total period of 34 years. One woman had a deep vein thrombosis, so that the incidence of hormone replacement therapy-related thrombosis was 2.9% (95% CI 0.8-15.3) per year of use.

2.4 DISCUSSION

This prospective study of a large cohort of asymptomatic carriers of the factor V Leiden mutation demonstrated a low absolute overall incidence of venous thromboembolism of 0.58% (95% CI 0.26-1.10) per year, which is approximately five to six times higher than the annual incidence reported for the general population [18]. Almost half of the observed episodes occurred spontaneously. Our results are in agreement with the findings of two retrospective studies, that found an overall incidence of 0.45% (0.28-0.61) and 0.28% (0.15-4.10), respectively [12, 13].

How do these results affect the counseling of asymptomatic relatives of patients with venous thromboembolism in whom the factor V Leiden mutation has been detected? Screening all their family members in order to install prophylaxis with vitamin K antagonists in carriers of the mutation is not justified, since the risk of spontaneous venous thromboembolism (0.26% per year, 95% CI 0.07-0.65) is much lower than the yearly risk of major bleeding during therapeutic dose warfarin treatment which has been estimated to be between 2 and 10% [19]. Whether screening is indicated to adjust standard prophylactic strategies during high risk situations such as surgery, trauma, immobilization, pregnancy or the postpartum period, is unclear. In the present study, low-molecular-weight heparin prophylaxis was given routinely to all carriers undergoing surgery, trauma, or prolonged immobilization during hospitalization, as is common practice these days, irrespective of the factor V Leiden mutation. Out of 29 such episodes, one was complicated by pulmonary embolism three weeks postoperatively. Whether this absolute risk of 3.5% (95% CI 0-17.8%) justifies prolonged postoperative prophylaxis, i.e. for two to four weeks, is unknown and requires appropriate investigations. Hence, we believe that at present, family screening with the aim to adjust the current routine perioperative thrombosis prophylaxis is not indicated. The optimal approach of factor V Leiden carriers during pregnancy (including management of the postpartum period) is more complex and heavily debated. In the present cohort, only 17 pregnancies could be studied. One of these women received prophylaxis during pregnancy, and 6 women received low-molecular-weight heparin for the first week postpartum. No episodes of venous thromboembolism were observed either during or after pregnancy. This low rate is in agreement with previous retrospective findings in almost 400 pregnancies, which revealed a risk of approximately 2% without thrombosis prophylaxis [12, 13]. Thus, there is no convincing evidence to support a policy of screening all female

relatives who intend to become pregnant and prescribe anticoagulant prophylaxis during pregnancy, although in the cohort of the present study, 7 women chose to have anticoagulant prophylaxis postpartum or even during pregnancy. Since the majority of pregnancy-related venous thromboembolic complications occur postpartum [12, 13], it may be justified to screen pregnant relatives, in particular when there is a strong family history for venous thromboembolism, in order to consider postpartum prophylaxis. The remaining issue is whether screening young female relatives of indexpatients prior to oral contraceptive use should be advocated. In our study, 166 years of oral contraceptive use in 66 women resulted in 3 episodes of venous thrombosis in women who had used oral contraceptives for several years (1.8% per year, 95% CI 0.4-5.2%). Because there were only a few 'starters' in this cohort, some bias toward a low risk may have been introduced. However, this annual incidence is in between the findings in two retrospective studies that reported incidences of 0.48% (95% CI 0.10-1.40) and 2.0% (95% CI 0.3-7.2), respectively [12, 13]. It should be realized that 200 women need to be screened, to identify 100 carriers. If all these identified carriers would be advised not to use oral contraceptives, 2 venous thromboembolic events per year will be prevented. Whether this policy is justified, remains a matter of opinion and debate [10, 12, 20]. The same likely applies to hormone replacement therapy.

At least three methodological issues warrant comment. First, although this is the largest, prospectively followed cohort of asymptomatic carriers of the factor V Leiden mutation reported so far, a major limitation of this study is the moderate number of observed high risk situations. Therefore, firm conclusions from this study can only be drawn for the spontaneous absolute incidence of venous thromboembolism in these carriers. Since the prospectively observed overall incidence is in agreement with the results from the retrospective studies, the bias that might have been introduced by their retrospective design seem to be neglectable. Consequently, we believe that estimates for the risk of venous thromboembolism related to high risk situations observed in these studies, are likely valid. The second issue that needs consideration is a potential for bias toward a low incidence of venous thromboembolism that may have been introduced by the exclusion of symptomatic relatives. However, in the retrospective analysis of a large part of this study cohort, these excluded relatives were the outcome event, and the observed incidence was the same as found in the present prospective study [12]. Furthermore, there is a broad range of individuals (15 to 88 years of age) included in this prospective study with an average age of 43 years, while the average age of a first venous thromboembolic event in this study was 45 years. Hence, we believe that it is unlikely that the exclusion of symptomatic relatives has influenced our findings. Finally, we only studied the factor V Leiden mutation and are unable to investigate whether the combination with other hereditary thrombophilic defects influence the clinical expression of the mutation.

In conclusion, the absolute annual incidence of spontaneous venous thromboembolism is too low to justify family screening of symptomatic patients with the factor V Leiden

mutation. At present, screening in order to adjust prophylactic strategies during surgery, trauma, immobilization, pregnancy, or the postpartum period, or to discourage use of oral contraceptives and hormone replacement therapy, is unlikely to be beneficial. Future research should address whether a different approach is justified for highly thrombophilic families, but should also include the potential side effects of such screening strategies.

2.5 REFERENCES

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