Physical activity, immobilization and the risk of venous thrombosis

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Chapter 7

Mechanisms of the factor V Leiden paradox

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Abstract

Objective: Carriers of the factor V Leiden mutation (FVL-carriers) have a substantially increased risk of deep venous thrombosis (DVT) while the risk of pulmonary embolism (PE) is only mildly increased compared with non-carriers. So far few studies have investigated possible mechanisms for this so-called FVL paradox.

Methods and Results: Consecutive patients with a first DVT or PE were included in a large population-based case-control study (MEGA study). Patients, aged 18 to 70 years, provided a questionnaire, DNA (n=3313) or plasma (n=1474). Surgery, injury and travel were considered thrombosis-provocative. Out of 2063 patients with isolated DVT 20% was FVL-carrier, as was 8% of the 885 patients with isolated PE. Among DVT patients FVL-carriers had their thrombi more often proximal and a higher number of affected veins than non-carriers. No differences were observed between FVL-carriers and non-carriers in time between provocation and diagnosis, in vitro coagulation time and thrombus density. Compared with patients with both DVT and PE, isolated DVT patients more often had thrombi located distally and had a similar number of affected veins. Compared with isolated PE patients, isolated DVT patients had a shorter time between provocation and diagnosis, and similar in vitro coagulation time and thrombus density.

Conclusion: Although some effects were differential for FVL-carriers and non-carriers, and some were differential for PE and DVT patients, none of the potential mechanisms offered a clear explanation.

Introduction

The incidence of venous thrombosis is about 1 to 3 per 1000 individuals per year and is associated with life-threatening pulmonary embolism (PE)[1]. Both autopsy [2] and clinical [3;4] studies have shown that approximately 90% of the pulmonary emboli arise from thrombi in the deep veins of the lower limbs. Moreover, asymptomatic PE can be found in about half the patients presenting with deep venous thrombosis (DVT) [5]. For this reason many consider DVT and PE as a single disease which is referred to as venous thrombosis or venous thrombosis.

However, several studies have shown that the prevalence of some risk factors differs in patients with DVT compared with those with PE [6-9]. The factor V Leiden mutation, the most prevalent genetic factor known to increase the risk of venous thrombosis, has repeatedly been shown to be a strong risk factor for DVT, but at most a weak risk factor for PE. Shortly after the discovery of the Factor V Leiden mutation, it was hypothesized that the presence of Factor V Leiden would often lead to fatal PE, resulting in a lower number of Factor V Leiden positive subjects among those surviving PE. This would explain the weak effect of Factor V Leiden on the risk of PE found in studies of survivors of venous thrombosis, such as case-control studies. However, this hypothesis was rejected as autopsy studies have shown that among patients with fatal PE, the proportion of individuals with Factor V Leiden was no different from that in PE survivors or from that in the general population [10;11].

The differential effect of Factor V Leiden on DVT and PE is known as the "Factor V Leiden paradox" [13]. Although this paradox has been reported repeatedly [8;12-14], some still doubt whether it exists. We therefore studied the prevalence of Factor V Leiden among patients with an isolated DVT, isolated PE, or a combination of DVT and PE. Furthermore, we studied whether the effect was specific for Factor V Leiden, by assessing the effect of the prothrombin 20210A mutation, another well-known factor involved in the risk of venous thrombosis.

So far, few studies have investigated mechanisms that could lead to the Factor V Leiden paradox, except for a possible difference in thrombus location. In this study, we sought to investigate several potential explanations for the paradox. First we studied the difference in location. Second, we focused on differences in number of affected veins. A third possible mechanism was a difference in time interval between the provocation of thrombus formation and the actual diagnosis. The fourth possible mechanism was a difference in growth speed as expressed by *in vitro* coagulation time. A fifth, related, mechanism was a difference in clot structure with lower chances of thrombus breaking which might be expressed as a difference in *in vitro* clot density.

In this study we investigated these five possible mechanisms by determining a) whether Factor V Leiden affects thrombus location, number of affected veins, time until diagnosis, growth speed or clot density and b) whether these factors differ in prevalence between patients with isolated deep venous thrombosis of the leg compared to patients with isolated pulmonary embolism or combined deep venous thrombosis and pulmonary embolism.

Material and Methods

All analyses were done as part of the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA study), a large population-based case-control study. Between March 1999 and September 2004 all consecutive patients with a first episode of venous thrombosis were recruited from six anticoagulation clinics in the Netherlands. These clinics monitor the anticoagulant treatment of all out-patients within a well-defined geographical area. Eligible participants were between 18 and 70 years at time of their inclusion. Patients who died (n=280) and those who were at the end stage of disease (n=82) and were therefore unable to fill in a questionnaire were excluded. Of the 5969 eligible patients, 5051 (84.5%) were willing to participate.

Control subjects were recruited from two sources; first, by inviting partners of patients (82% of the partners participated), and second by using a random digit dialing method (69% of the eligible individuals participated). All participants provided informed consent in which they agreed to participate. This study was approved by the Medical Ethics Committee of the Leiden University Medical Center, Leiden, the Netherlands.

Data collection

Risk factors for venous thrombosis including surgery, injury and travel were reported in a standardized mailed questionnaire covering a period of one year prior to the venous thrombotic event. The questionnaire included a permission form to obtain information regarding the diagnostic procedure of the thrombotic event from existing medical records. Informed consent to obtain medical records was given by 4528 out of 5051 patients (90%). Diagnostic information regarding the thrombosis was obtained via hospital records or

general practitioners. Only those patients of whom information could be obtained and who had an objectively confirmed DVT or PE were included (n=3574), figure 1.

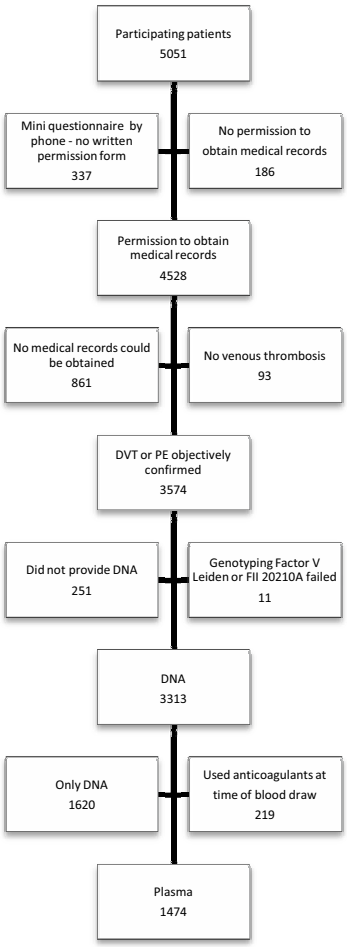

Figure 1. Flowchart of patients.

DVT was considered to be objectively diagnosed when a (Doppler) ultrasound showed the presence of a thrombus in the deep veins of the leg. PE was considered to be objectively confirmed when diagnosed with a high probability ventilation perfusion (VQ) scan, positive spiral computational topography (CT) or angiogram. A patient registered at the anticoagulation clinic with both PE and DVT, but with only one of these diagnoses

objectively confirmed according to the above mentioned criteria, was considered to have both PE and DVT.

We analyzed a subgroup of patients who had at least two diagnostic tests; at least one of the legs and one of the lungs. Patients who had a thrombus in the leg and tested negative for PE were considered to have isolated deep venous thrombosis. Patients with a positive lung scan but tested negative for DVT considered to have isolated PE.

Location of the thrombus and number of affected veins were abstracted from radiology reports and discharge letters without knowledge of the presence or absence of the Factor V Leiden mutation. Information regarding the location of the thrombus in the leg was available for 2083 patients with DVT, but obviously not for patients with an isolated PE. A thrombus in the calf veins only was defined as located distally, whereas a thrombus in any of the other veins was defined as proximal. For calculation of time between the onset of thrombus formation and diagnosis only patients who had surgery, an injury or had traveled in the 100 days prior to the diagnosis of venous thrombosis were included. In these patients we assumed that thrombus formation started shortly after provocation.

DNA collection and laboratory analyses

Patient included between March 1999 and May 2002 were asked to provide a blood sample 3 months after discontinuation of anticoagulant treatment, while those who were unable or unwilling to come to the anticoagulation clinic for a blood draw were sent a cotton swab for the collection of buccal cell DNA. From May 2002 onwards DNA was collected through buccal swab samples only. Assessment of the Factor V Leiden mutation was performed identically in DNA retrieved from whole blood and buccal swabs, as described previously [15]. Individuals who did not provide DNA (251 patients) and samples where genotyping of Factor V Leiden failed (11 patients) were excluded from the present analyses, resulting in a total of 3313 patients who were eligible for analysis, figure 1.

Blood samples were drawn into vacuum tubes containing 0.106 M trisodium citrate as anticoagulant. Fresh frozen plasma was obtained by centrifugation at 2000g for 10 minutes at room temperature and stored in aliquots at -80°C. Coagulation parameters were derived

from clot lysis experiments as described previously[16]. In short, a tissue factor-induced thrombus, which was lysed by exogenous t-PA, was studied by monitoring changes in turbidity during thrombus formation and subsequent lysis by measuring the optical density at 405 nm every 20 seconds. *In vitro* coagulation time was defined as time from adding the buffer till the midpoint of the clear to maximum turbid transition. Thrombus density was defined as the difference in light absorbance between the maximum turbidity minus the minimal turbidity, measured in optical densities (OD). For the calculation of *in vitro* coagulation time and thrombus density only those patients who donated plasma but did not receive anticoagulant treatment at time of blood draw were included in the analysis (n=1474).

Statistical Analyses

Percentages and 95 % confidence intervals (95% CI) were calculated using the exact method. Differences in time between provocation and diagnosis of venous thrombosis were determined using a log-rank test. All analyses were performed in SPSS for windows 14.0 (SPSS Inc, Chicago, Ill).

Results

A total of 3313 patients was included in the present analysis of whom 2063 were objectively diagnosed with DVT, 885 with PE and 365 with both. The characteristics of these three groups and the control subjects are shown in table 1.

Of the patients with DVT, 415 carried the Factor V Leiden mutation (20%), 60 patients with both DVT and PE carried the Factor V Leiden mutation (16%) and 75 patients with PE (8%) carried Factor V Leiden, compared with 256 control subjects (5%). Therefore the risk of DVT was 4.5 fold increased (OR 4.5 95% CI 3.8 to 5.3), while the risk of PE was only mildly increased (OR 1.7 95% CI 1.3 to 2.2) in carriers of Factor V Leiden, both compared with non-carriers.

Table 1. Characteristics of 3313 patients with isolated deep venous thrombosis of the leg (DVT), DVT combined with pulmonary embolism (PE), isolated PE and control subjects.

	DVT	DVT+PE	PE	Controls
N	2063	365	885	4857
Sex, women, N (%)	1093 (53%)	166 (46%)	502 (57%)	2589 (53%)
Age, mean (year)	48.2	50.3	49.1	48.1
Surgery, N (%)	440 (21%)	70 (19%)	210 (24%)	326 (7%)
FVL heterozygous, N(%)	393 (19%)	55 (15%)	71 (8%)	248 (5%)
FVL homozygous, N(%)	16 (1%)	5 (1%)	3 (0%)	8 (0%)
FII carrier, N(%)	121 (6%)	24 (7%)	38 (4%)	94 (2%)

DVT = deep venous thrombosis of the leg, PE = pulmonary embolism
FVL = Factor V Leiden; FII= factor II 20210A

When we studied the subgroup of patients who had had diagnostic tests performed of both lungs and legs, 30% of the patients with isolated DVT carried the Factor V Leiden mutation and only 7% of patients with isolated PE. When comparing these results with the control group, the risk difference was even more pronounced: the risk of isolated DVT for carriers of the Factor V Leiden mutation was almost 8-fold increased (OR 7.7 95% CI 3.9 to 15.3), while Factor V Leiden only mildly affected the risk of isolated PE (OR 1.4 95% CI 0.7 to 2.7), both compared with non-carriers.

This differential effect was specific for Factor V Leiden and not for prothrombin 20210A mutation, which was present in 121 out of 2063 patients with DVT (5.9%) and 38 out of 885 patients with PE (4.3%). Odds ratios for carriers of the prothrombin 20210A mutation were clearly elevated with overlapping confidence intervals for both DVT (OR 3.2 95% CI 2.4 to 4.2) and for PE (OR 2.3 95% CI 1.5 to 3.3).

Location

Differences in Factor V Leiden prevalence between patients with proximal and distal DVT were small. Among those with proximal DVT, 318 out of 1559 (20%) carried the Factor V Leiden mutation while this was 53 out of 329 (16%) patients with distal DVT; difference 4% (95% CI 0 to 9%), table 2. Patients with DVT more often had distally located thrombi (302 out of 1635 patients, 19%) compared with patients with both PE and DVT (27 out of 253 patients, 11%), difference 8% (95% CI 3 to 12%).

Table 2. Percentages of Factor V Leiden carriers in patients with different thrombus locations and number of affected veins.

	FVL	Total	Percentage FVL *(95% CI)
Location †			
Proximal thrombosis ‡	318	1559	20% (18 - 22)
Isolated inferior cava	*1*	*7*	*14% (-14 - 42)*
Isolated iliac vein	*6*	*49*	*12% (4 - 25)*
Iliofemoral vein	*12*	*61*	*20% (11 - 32)*
Isolated femoral vein	*40*	*170*	*24% (17 - 30)*
Popliteal-iliofemoral vein	*11*	*58*	*19% (9 – 29)*
Popliteal-femoral vein	*79*	*356*	*22% (18 - 27)*
Isolated popliteal vein	*169*	*858*	*20% (17 - 22)*
Distal thrombosis			
Isolated calf veins	53	329	16% (12 - 20)
Number of affected veins			
One vein	216	1208	18% (16 - 20)
≥ 2 veins	175	750	23% (20 - 26)

* FVL=Carrier of the Factor V Leiden mutation
† other locations for 70 patients
‡ On occasion combined with a thrombus in the calf veins

Number of affected veins

Of the 755 patients who had multiple veins affected, 175 carried the Factor V Leiden mutation (23%) while this was 216 out of 1208 (18%) patients who had only one vein affected, a difference of 5% (95% CI 2 to 9%), table 2. The number of affected veins was similar in patients who had an isolated DVT as in patients with a combination of DVT and

PE, 650 out 1690 (39%) had 2 or more veins affected while this was 100 out 268 patients who had combination of DVT and PE (38%), a difference of 1% (95% CI -7 to 5 %).

Time interval between provocation and diagnosis

We studied the time interval between Factor V Leiden carriers versus non-carriers in patients who were diagnosed with DVT or PE within the first 100 days after provocation of thrombus formation (n=1048). Within this time window, carriers of the Factor V Leiden mutation had a similar time interval between provocation and the diagnosis as non-carriers ($p>0.05$), figure 2a. Patients with PE were diagnosed slightly longer after provocation compared with patients with DVT ($p<0.05$), figure 2b.

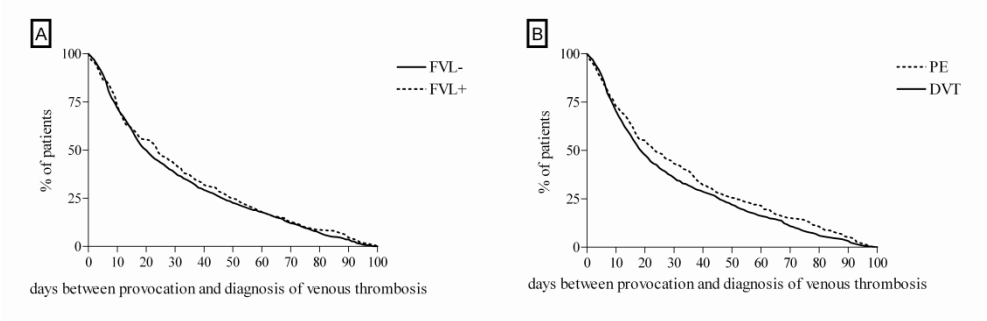

Figure 2. Time interval between provocation and venous thrombosis for Factor V Leiden carriers versus non-carriers (A) and in patients with deep venous thrombosis or pulmonary embolism (B).

In vitro coagulation time

In vitro coagulation time was similar in patients with Factor V Leiden (2.45 minutes) and non-carriers (2.46 minutes), a difference of 0.01 minutes (95% CI -0.07 to 0.08). Also no differences were observed in coagulation time between patients with DVT (2.45 minutes) and PE (2.47 minutes), difference (0.02 minutes 95% CI -0.04 to 0.09).

Thrombus density

Factor V Leiden carriers had a slightly lower thrombus density (mean OD 0.46) compared with non-carriers (mean OD 0.48), difference 0.02 (95% CI 0.01 to 0.04). However, thrombus density was similar in patients with isolated DVT (mean OD 0.47) and isolated PE (mean OD 0.47, difference 0.00 95% CI -0.01 to 0.01).

Discussion

The prevalence of Factor V Leiden is substantially higher in patients with DVT, in presence of absence of a concomitant PE, than in patients with isolated PE. In fact, Factor V Leiden is only a mild risk factor for isolated PE, whereas the risk of DVT is substantially increased by this mutation. We studied multiple explanatory mechanisms for the differential effect of Factor V Leiden on the risk of DVT and PE: thrombus location, number of affected veins, time between provocation and diagnosis, in vitro clot formation and in vitro clot density. Although some effects were different for Factor V Leiden carriers and non-carriers, and some were different for patients with PE and patients with DVT, none of the mechanisms offered a clear explanation.

Location

So far, studies have been inconsistent on whether the thrombus location is different in Factor V Leiden carriers compared with non-carriers. Some studies, including ours, showed that the presence of Factor V Leiden leads to increased risk of thrombosis in the proximal veins [19;20], while others have shown the opposite [17;18;22], or found no difference in location [21;23].

More distal located thrombi are less likely to be accompanied by PE, which is in agreement with other studies [4;24;25]. Therefore, if Factor V Leiden would lead to more distal located thrombi, and proximal located thrombi would lead to PE, one would expect that Factor V Leiden carriers were at lower risk for PE. However, as the results in the literature regarding the location of thrombi in Factor V Leiden carriers are inconsistent, and we even found an increased risk of a proximally located thrombus for Factor V Leiden carriers, it is unlikely that the location of the thrombus in the leg explains the risk difference of Factor V Leiden in DVT and PE risk.

Thrombus size

Murine models have shown that mice homozygous for the Factor V Leiden mutation had a larger thrombus volume compared with wild-type mice[26]. This is in line with our results as we showed that carriers of the Factor V Leiden mutation more often had multiple veins affected compared with non-carriers. It seems logical that when each thrombus has a certain probability of embolizing, the overall likelihood would increase with the number of veins

involved. From this finding it does not logically follow that DVT patients with Factor V Leiden have a decreased incidence of PE. Moreover, the number of affected veins was not different in patients with isolated DVT or both DVT and PE.

It should be noted that it is impossible to study the effect of the location and thrombus size in patients with isolated PE and that individuals with both DVT and PE have been used as a surrogate for the isolated PE population.

Growth speed

Factor V Leiden mice had faster growing thrombi compared with non-Factor V Leiden mice [26]. We studied growth speed in two ways, both epidemiologically and *in vitro*. First, we studied whether time between a clear thrombus provocation such as surgery, injury or travel, and diagnosis was similar in carriers versus non-carriers and found no difference. It took slightly more time to diagnose PE than to diagnose DVT. As a consequence it will be unlikely that the presence of Factor V Leiden will have resulted in earlier treatment and a reduction in risk of embolization. Secondly, we studied the growth speed by measuring clotting time *in vitro*. No differences were found between Factor V Leiden carriers and non-carriers in clotting time, nor was there a difference between PE and DVT patients. However, care should be taken in interpreting these results as the in vitro clotting was performed without the presence of activated protein C. Thus the effect of Factor V Leiden may not have become apparent by using this assay. Due to this limitation we cannot exclude a possible difference in growth speed of the thrombus as an explanation for the Factor V Leiden paradox. As mouse models have shown an increased speed of thrombus formation in Factor V Leiden mice and patients with PE had a longer time interval between provocation and diagnosis, there might be a relation. It should therefore be investigated more extensively whether the duration of thrombus formation could explain the Factor V Leiden paradox.

Thrombus density

Finally, we studied whether a difference in thrombus density could shed light on the Factor V Leiden paradox. We found that Factor V Leiden carriers had a slightly lower thrombus density than non-carriers. The results combined with the higher number of affected veins might suggest a different composition of the thrombus. Yet, no differences in thrombus density were found between patients with DVT or PE. Therefore, thrombus density does not seem to offer an explanation for the Factor V Leiden paradox.

Conclusion

These results confirm the existence of the Factor V Leiden paradox. However, none of the above mechanisms seems to be a solid explanation of the Factor V Leiden paradox. Future research might focus on a possible difference in growth speed and composition of the thrombus as these represent the most promising explanation.

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