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Rosendaal, F.R.

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D-Dimer as a Risk Factor for Deep Vein Thrombosis: The Leiden Thrombophilia Study

Astrid C. M. Andreescu¹, Mary Cushman^{1, 2}, Frits R. Rosendaal³

¹Department of Medicine, Division of Hematology, Oncology, and

²Department of Pathology, University of Vermont, Burlington, VT, USA;

³Department of Clinical Epidemiology and the Hemostasis and Thrombosis Research Center, Leiden University Medical Center, Leiden, The Netherlands

Keywords

Deep vein thrombosis, D-dimer, blood coagulation, risk factor

Summary

We studied the association of D-dimer with the risk of deep vein thrombosis (DVT). D-dimer was measured in 474 patients more than 6 months after diagnosis of a first DVT and in 474 age- and sex-matched controls. For D-dimer above the 70th percentile (130.5 ng/ml), the odds ratio (OR) for DVT was 2.2 (95% CI, 1.6-2.9). The association was unchanged with adjustment for other risk factors. Excluding participants with Factor V Leiden, prothrombin 20210A, or factors VIIIc or IX above the 90th percentile, the OR was 1.6 (95% CI, 1.1-2.3). The risks of DVT with the joint presence of high D-dimer and either factor V Leiden or prothrombin 20210A were increased 12.4-fold (95% CI 5.6-27.7) and 7.2-fold (95% CI 2.1-25.1), respectively. Higher D-dimer concentration was associated with the risk of DVT, and was supra-additive to the risks associated with factor V Leiden and the prothrombin 20210A variant. Persistence of this association in the absence of other hemostatic risk factors for DVT suggests that high D-dimer may be related to other, as yet unknown, risk factors for venous thrombosis. Confirmation of these findings is desirable.

Introduction

Deep vein thrombosis (DVT) has an incidence of 1 to 3 per 1000 per year. Factor V Leiden (1) and the prothrombin 20210A variant (2) are genetic risk factors for venous thrombosis, and are seen in 25% of unselected DVT patients (3). Recently, high levels of coagulation factors VIII and IX have also been associated with venous thrombosis risk (4,5). A particular area of controversy is the association of fibrinolytic activity with risk of DVT (6-9).

During fibrinolysis, fibrin fragment D-dimer is liberated upon degradation of fibrin by the major fibrinolytic enzyme plasmin. Thus, D-dimer concentration indicates both the recent production of and degradation of fibrin. D-dimer concentration increases with acute DVT (10) however, we are aware of only one study of this marker in relation to venous thrombosis risk. This recent case-control study showed an association between higher D-dimer and thrombosis in women aged 45-64 (9).

Others have studied the fibrinolysis markers plasminogen activator inhibitor-1 and tissue plasminogen activator in relation to venous

thrombosis. Most studies included patients during or a short time after thrombosis, and were not conclusive (6, 8, 11). The largest prospective study to date of baseline fibrinolytic state in healthy subjects and risk of future DVT failed to show a link between the two (7).

We measured concentrations of fibrin fragment D-dimer in a population-based case-control study. We hypothesized that a higher concentration of D-dimer, as an indicator of ongoing thrombin generation and fibrinolysis, would be associated with an increased risk of DVT.

Methods

Study Design and Subjects

The Leiden Thrombophilia Study (LETS) is a population-based case-control study of risk factors for first DVT (12). There were 474 patients recruited from three anticoagulation clinics in the Netherlands: Leiden, Amsterdam, and Rotterdam. In the Netherlands, anticoagulation clinics monitor all patients on coumarin treatment for venous thrombosis. Inclusion criteria were: (1) patients living in the geographical area of the anticoagulation clinics, (2) age under 70, (3) objective diagnosis of first DVT (positive compression ultrasound, impedance plethysmography, or venogram), (4) DVT occurring between January 1, 1988 and December 31, 1992, and (5) absence of malignancy. Evaluation for inclusion in the study was performed using medical records from the hospital and the anticoagulation clinics, and information from primary physicians. Patients received at least three months of anticoagulant therapy and were seen at least six months after their DVT diagnosis. Ninety percent of eligible individuals participated. Control subjects were 474 healthy persons with no prior history of venous thrombosis, the same sex and age within 5 years of the patients, and no biological relationship to the patients. They were recruited by asking each patient to find a control with the above characteristics, living in the same geographic area. Patients' partners were also invited to participate in the study. If a patient was unable to find a control, the first eligible person on the list of patients' partners was chosen. Two hundred and twenty-five (47%) of the controls were partners of other patients.

Participants provided informed consent allowing access to hospital records and use of stored specimens for research. The Medical Ethics Committee of the Leiden University Medical Center and the Institutional Review Board at the University of Vermont approved this analysis.

All subjects completed a questionnaire regarding acquired risk factors for venous thrombosis in a certain time period prior to the index DVT, or a similar date for the controls. The acquired risk factors considered were: surgery or hospitalizations in the year prior to the DVT, postpartum period within 30 days of the DVT, pregnancy at the time of DVT, and hormone use at the time of venipuncture and at the time of DVT.

Body mass index (BMI) was calculated (weight in kilograms/[height in meters]²). Obesity was defined as a BMI ≥ 30 kg/m². Elevated factors VIIIc and IX antigen were defined as values above the 90th percentile of the control population distribution. Considering factor V Leiden and the prothrombin 20210A allele, heterozygotes and homozygotes for each disorder were pooled for analysis.

Correspondence to: Mary Cushman, MD, MSc, Department of Medicine, 208 South Park Drive, Suite 2, Colchester, VT, 05446, USA – Tel.: +1 802 656-8968, Fax: +1 802 656-8965, E-mail: mcushman@zoo.uvm.edu

Table 1 Characteristics of the participants

Characteristic	Controls (n=474)	DVT Patients (n=474)
Mean age in years (SD)*	45 (13.5)	45 (13.7)
Male Sex (%)*	43%	43%
BMI \geq 30 (%)	14%	22%
Coumarin use at phlebotomy	0.2%	10%
OC use at DVT ††	39%	67%
OC use at phlebotomy ‡	33%	22%

* Matching Variable

† among controls, at the time the matched case had DVT

‡ women aged 16-45

DVT, deep vein thrombosis; BMI, body mass index; OC, oral contraceptive

Laboratory Methods

At the interview, blood was collected by venipuncture from the antecubital vein into tubes containing 106 mmol/L trisodium citrate. Plasma was prepared by centrifugation at room temperature for 10 min at $2,000 \times g$ and stored at $-70^\circ C$. D-dimer was measured by enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies against specific non-overlapping antigenic determinants (antibodies kindly provided by D. Collen and P. Declercq). This assay detects fragment D-dimer of cross-linked fibrin but not fragments of fibrinogen or non-cross-linked fibrin (10). The interassay coefficient of variation (CV) was 7.0%. The expected normal range (mean \pm 2 SD), established in 26 healthy volunteers is 6.8-189.9 ng/ml. As previously reported, factor VIIIc was measured using a 1-stage clotting assay (4), and factor IX was measured by ELISA (5).

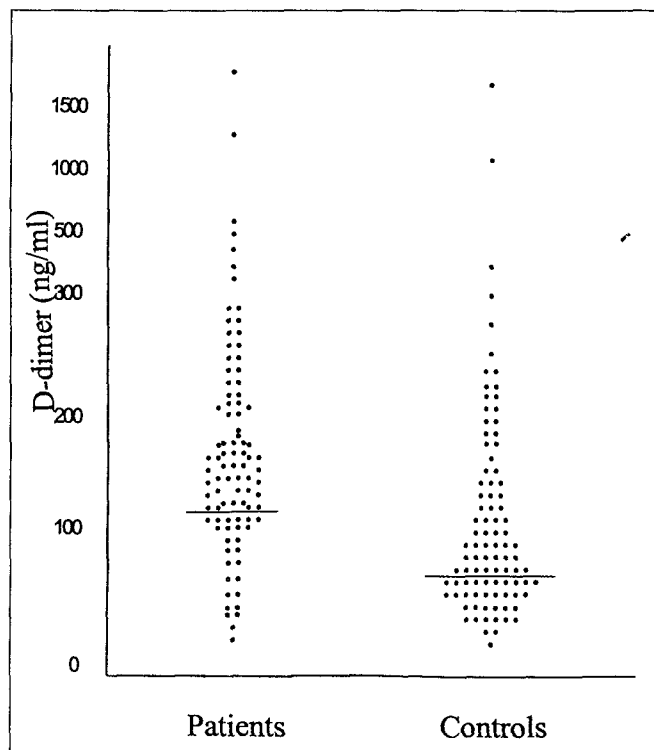


Fig. 1 Distribution of D-dimer in patients and controls. Each symbol represents the mean of five closest values. Horizontal lines represent median values

Statistical Analysis

Among control subjects, associations between D-dimer and other characteristics were analyzed using linear regression. Mean baseline D-dimer concentration was compared between cases and controls using t-tests. The measure of association between D-dimer and DVT was the odds ratio (OR), as determined by unconditional logistic regression, adjusting for the matching factors of age and sex. Assessment of confounding was done using multivariate logistic regression with D-dimer as the main independent variable. Analyses were repeated in subgroups defined by age, sex, oral contraceptive use, presence of elevated factor VIIIc or IX levels, and presence of factor V Leiden or prothrombin 20210A. To determine whether the association of D-dimer and DVT could be explained by the presence of other hemostatic risk factors, we repeated the analysis after excluding participants with either factor V Leiden, prothrombin 20210A, or elevated factor VIIIc or factor IX. We investigated the influence of time between thrombosis and phlebotomy on results using logistic regression analysis for different time intervals.

Results

Characteristics of patients and controls are shown in Table 1. The mean age was 45 years (16-70 for patients, 16-73 for controls), 43% of participants were men. Patients were more likely than controls to have used oral contraceptives at the onset of DVT and were more likely to be overweight. Among women aged 16-45, 67% of patients were using oral contraceptives at the time of DVT, compared to 39% of controls. In the same age group among women, at the time of venipuncture 22% of patients and 33% of controls were taking oral contraceptives. Twenty-two percent of patients had a BMI \geq 30 kg/m², compared to 14% of the controls. Only one control, compared to 48 patients (10%) was using coumarin derivatives.

Among controls, D-dimer was positively associated with age. For each 10-year increase in age, D-dimer was 19.8 ng/ml higher (95% CI, 11.0 to 28.6 ng/ml). Median D-dimer was 64.4 ng/ml in men, and 87.1 ng/ml in women (in controls). Among obese control subjects median D-dimer was 99.2 ng/mL, compared to 71.8 ng/mL in non-obese subjects. Among women aged 16-45, D-dimer was higher among 47 women using oral contraceptives; median 94.2 ng/ml compared to 72.8 ng/mL among non-users. When we take into account the biochemical thrombophilic defects, factor V Leiden, prothrombin 20210A, or elevated factors VIIIc or IX, median D-dimer was 104.7 ng/ml among 106 subjects with any one of these defects compared to 70.4 ng/ml among 361 subjects without any defect.

Figure 1 illustrates that the distribution of D-dimer differed in patients and controls, with higher values in patients. The median value among patients was 102.4 ng/ml (range 9.1-1946.3 ng/ml) and among controls it was 74.5 ng/ml (range 4.0-1608.9 ng/ml).

Table 2 shows the risk of DVT with each increasing decile of D-dimer, compared to the lowest decile. The relative risk of DVT was increased for values above the 70th percentile of the distribution of D-dimer. For values in the 8th, 9th and 10th deciles, relative to the first decile, the crude odds ratios of DVT were 1.6 (95% CI 0.9-2.9), 1.8 (95% CI 1.0-3.2), and 3.2 (95% CI 1.8-5.6), respectively. The association of D-dimer with DVT was not substantially altered with adjustment for age, oral contraceptive or coumarin use, and obesity (Table 3). With adjustment for these factors, the risk of DVT remained higher than 2.0 for values above the 70th percentile, and higher than 3.0 for values above the 90th percentile (compared to those with values below the 70th and 90th percentiles, respectively). Adjustment for C-reactive protein concentration also did not influence the odds ratios substantially

Table 2 Odds ratio of DVT by decile of the total distribution of D-dimer

Decile of D-dimer (ng/ml)	Number of Controls	Number of Patients	OR (95% CI)
1 (4 0-34 8)	46	33	1 (ref)
2 (34 8-48 6)	47	29	0.9 (0.5-1.6)
3 (48 6-59 1)	47	45	1.3 (0.7-2.4)
4 (59 1-70 2)	47	27	0.8 (0.4-1.5)
5 (70 2-88 1)	47	36	1.1 (0.6-2.0)
6 (88 1-107 9)	46	41	1.2 (0.7-2.3)
7 (107 9-130 5)	47	36	1.1 (0.6-2.0)
8 (130 5-165 7)	47	53	1.6 (0.9-2.9)
9 (165 7-223 6)	47	59	1.8 (1.0-3.2)
10 (223 6-1946 3)	46	105	3.2 (1.8-5.6)

OR, odds ratio, CI, confidence interval

Table 3 Odds ratio of DVT for D-dimer greater than the 70th or 90th percentiles of the total distribution

D-dimer*	Number of Controls	Number of Patients	OR (95% CI)†	OR (95% CI)‡
< P 70	327	247	1.0 (ref)	1.0 (ref)
≥ P 70	140	217	2.2 (1.6-2.9)	2.5 (1.8-3.5)
< P 90	421	359	1.0 (ref)	1.0 (ref)
≥ P 90	46	105	2.8 (1.9-4.0)	3.3 (2.2-4.9)

* D-dimer 70th percentile = 130.5 ng/ml, 90th percentile = 223.6 ng/ml
† OR adjusted for matching variables (age and sex)
‡ OR additionally adjusted for obesity, and coumarin or oral contraceptive use at the time of DVT and at the time of phlebotomy
P 70 = 70th percentile
P 90 = 90th percentile

(data not shown). Exclusion of subjects using coumarins at the time of phlebotomy did not alter these findings. Risks were slightly less after exclusion of subjects with hemostatic defects (factor V Leiden, prothrombin 20210A, and high Factor VIIIc or factor IX): OR 1.6 (95% CI 1.1-2.3) for D-dimer above the 70th percentile, and OR 1.9 (95% CI 1.1-3.2) for D-dimer above the 90th percentile.

There were some extreme values for D-dimer. While these values may be due to analytical variability of D-dimer, to assess whether very high values of D-dimer influenced the findings, we excluded 5 patients and 2 controls with D-dimer >1000 ng/ml from the analysis. The odds ratios remained the same (data not shown).

The risk of DVT for D-dimer above the 70th percentile was slightly higher in women than men, with odds ratios of 2.3 (95% CI 1.6-2.3) among women and 1.7 (95% CI 1.1-2.7) among men. For D-dimer above the 90th percentile these odds ratios were 2.4 (95% CI 1.4-4.1) and 3.0 (95% CI 1.8-4.9), respectively. D-dimer over the 70th percentile in combination with either oral contraceptive use or age ≥30 increased

the risk of DVT 12.7-fold (95% CI 4.0-41), and 2.0-fold (95% CI 1.2-3.6) compared to groups with lower D-dimer and absence of these characteristics.

The odds ratio of DVT in the joint presence of higher D-dimer and Factor V Leiden was 12.4 (95% CI 5.6-27.7). This value is larger than would be expected from the additive effect of the two factors (Table 4). A similar supra-additive joint risk of D-dimer in combination with prothrombin 20210A, but not elevated factor VIIIc or factor IX, was observed (Table 4).

The mean time from DVT to venipuncture among patients was 1.8 ± 0.9 years (range 0.5-5.6 years). There was no association of time between DVT and phlebotomy with D-dimer concentration ($r = 0.05$). We analyzed the association of D-dimer with DVT in categories of time between DVT and phlebotomy. The odds ratios for DVT with higher D-dimer in six time categories (0.5-1 years, 1-2 years, 2-3 years, 3-4 years, 4-5 years, and more than 5 years) are shown in Fig. 2, risks did not vary substantially by time.

D-dimer $\geq 70^{\text{th}}$ Percentile *	Hemostatic Defect *	OR (95% CI)**				
		Factor V Leiden	Prothrombin 20210A	High Factor VIIIc	High Factor IX	Any Defect
-	-	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
-	+	7.3 (3.2-16.8)	2.0 (0.8-5.1)	2.7 (1.5-4.8)	1.8 (1.0-3.2)	2.8 (1.9-4.2)
+	-	1.8 (1.3-2.4)	2.0 (1.5-2.6)	1.9 (1.4-2.5)	1.9 (1.4-2.5)	1.5 (1.0-2.1)
+	+	12.4 (5.6-27.7)	7.2 (2.1-25.1)	3.9 (2.4-6.3)	3.6 (2.2-6.0)	4.9 (3.4-7.2)

* presence of (+) or absence of (-) the listed risk factor
** unadjusted

Table 4 Odds ratio of DVT for D-dimer greater than the 70th percentile, with or without hemostatic defects

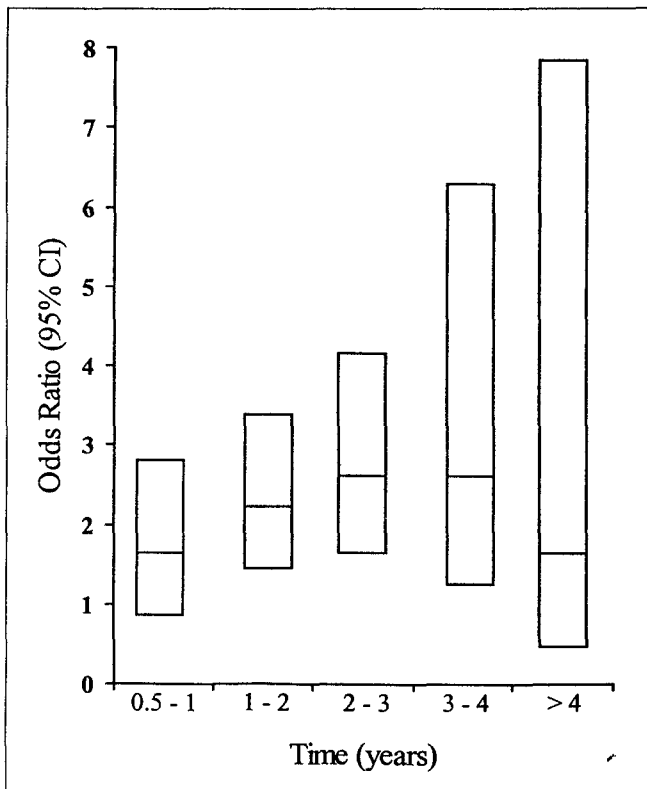


Fig. 2 Odds ratio of DVT for D-dimer above the 70th percentile by time between DVT and venipuncture. The time between DVT and venipuncture was divided into yearly increments. The odds ratio of DVT for D-dimer above the 70th percentile was calculated for patients and controls enrolled during each time interval

Discussion

The main finding of this study is an association of D-dimer with risk of DVT, with a 2.5-fold increased risk for D-dimer above the 70th percentile, and 3-fold increased risk for D-dimer above the 90th percentile. This association was not influenced by other risk factors such as obesity and oral contraceptive use, but risks were slightly lower with adjustment for known hemostatic risk factors for DVT. The joint effects of higher D-dimer and both the factor V Leiden and prothrombin 20210A variant on the risk of DVT were supra-additive.

Results here suggest a hypothesis that increased fibrin formation and fibrinolytic activity, assessed as D dimer, is associated with venous thrombosis, and can still be detected years after a DVT. Further, the persistent association of D-dimer with the risk of DVT after adjustment for known biochemical and genetic risk factors, suggests the presence of as yet undetermined risk factors, of which D-dimer may be a marker. There are two lines of genetic risk factors for DVT: first, a clear phenotype of heritable thrombophilia, approximately one-third do not have a definable hemostatic disorder (3). Second, in other family studies known defects do not necessarily explain the tendency to thrombosis (13).

Several findings here were compatible with other studies. These include the cross-sectional finding of increasing D-dimer with older age (14, 15). Also, as in a recent study of women aged 45-64 (9), we report an increased risk of DVT with higher levels of D-dimer, and extended these results to men. Considering the generally negative findings of studies that assessed the association of DVT with fibrinolytic factors, such as tissue plasminogen activator antigen and plasminogen activator inhibitor-1 activity or antigen, our findings suggest that the measurement of D-dimer may reflect fibrin formation rather than the fibrinolytic state, *per se*.

Although factor V Leiden is a common risk factor for DVT, the penetrance of thrombosis among heterozygotes is low, especially in the absence of a positive family history of thrombosis (16, 17). Our finding of an enhanced risk of DVT with the combined presence of higher D-dimer and factor V Leiden or prothrombin 20210A suggests that D-dimer might identify individuals with these defects who are at higher risk of DVT. If confirmed, this finding may have clinical implications for evaluating the role of testing for factor V Leiden. In this context, D-dimer may be a marker for the interaction of other, as yet unknown risk factors for DVT with factor V Leiden or prothrombin 20210A.

A point to discuss in the interpretation of our findings is that blood was drawn after the occurrence of thrombosis, and might not represent the levels prior to the thrombosis. Therefore higher D dimer may be a consequence rather than a marker of the causal pathophysiology of DVT. It is possible that venous damage from the DVT, resulting in venous stasis, might cause local thrombus formation and lysis, and result in a systemic increase in D dimer concentration on a chronic basis. The study design and analysis minimized this potential bias, as supported by the lack of differences in the association of D-dimer with DVT by time from DVT to phlebotomy. Also our finding is compatible with other observations (9) including those of a prospective association

between higher D-dimer and incidence of myocardial infarction, (18, 19). Regardless, prospective confirmation of these findings is desirable. A significant strength of the study is that it was based on all admissions to three large anticoagulation clinics, so was generalizable to a large population region.

In conclusion, we report that D-dimer appears to be a risk factor for DVT. Independence of this association from known hemostatic risk factors for DVT suggests that a high D-dimer concentration may be related to other, as yet unknown, risk factors for venous thrombosis. A clinical role for D-dimer assessment among patients with thrombosis requires further elucidation.

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