

Blood Coagulation, Fibrinolysis and Cellular Haemostasis

Influence of lipids and obesity on haemorheological parameters in patients with deep vein thrombosis

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Summary

It is not well established whether haemorheological alterations constitute independent risk factors for deep vein thrombosis (DVT). We have determined in 149 DVT patients and in 185 control subjects the body mass index (BMI), the haemorheological profile: blood viscosity (BV), plasma viscosity (PV), fibrinogen (Fg), erythrocyte aggregation (EA), erythrocyte deformability (ED) and plasma lipids. In the crude analysis BMI, Fg, PV, EA, triglycerides (TG) and ApoB were statistically higher and HDL cholesterol (HDL-Chol) statistically lower in DVT patients than in controls. No differences in BV and ED were observed. After BMI adjustment, Fg, PV and EA remained statistically higher in DVT cases than in controls ($P=0.013$; $P=0.012$; $P=0.013$; $P=0.028$, respectively). When the risk of DVT associated with these vari-

ables (using cut-offs that corresponded to the mean plus one SD of the control group) was estimated, $EA > 8.2$ and $PV > 1.28$ mPa·s were significantly associated with DVT even further adjustment for lipids and obesity ($OR=2.78$, $P=0.004$; $OR=1.91$, $P=0.024$, respectively). However, PV did not remain statistically significant after additional adjustment for Fg. When we consider together all the analyzed variables in order to control every variable for each other, $TG > 175$ mg/dl ($OR=3.2$, $P=0.004$) and $BMI > 30$ kg/m² ($OR=3.5$, $P=0.003$), were also independently associated with a greater risk of DVT. Our results suggest that increased EA constitute an independent risk factor for DVT. However, when associated to hyperlipidaemia and obesity it further increases thrombotic risk.

Keywords

Deep vein thrombosis, lipids, obesity, haemorheology

Thromb Haemost 2007; 98: 621–626

Introduction

Deep vein thrombosis (DVT) is a multifactorial disease in which inherited and acquired risk factors play an important pathogenic role (1). Since Virchow, it is assumed that blood flow alterations (haemorheological alterations) may favour the development of venous thrombus (2). The rheological hypothesis for venous thrombogenesis is supported by the association of many risk factors for DVT with systemic rheological alterations (3, 4) and the association of some rheological variables with DVT in case-control studies (5–10). However, there is not enough evidence to prove that haemorheological alterations constitute independent risk factors for DVT when patients are evaluated out of the acute phase, when the temporary fibrinogen increase (the protein

which modulates most of the haemorheological parameters) is strongly marked. Moreover, studies conducted so far consist mainly of a small sample size (6, 7, 9, 11, 12), do not determine all the rheological parameters (5–8, 10), do not exclude patients with malignant, inflammatory, autoimmune or infectious diseases (11, 12) that entail marked fibrinogen increases and, in addition, they do not always consider the presence of concomitant classical cardiovascular risk factors, i.e. obesity, hypertension, diabetes, dyslipidaemia and tobacco (6–9, 11, 12), which can not be overlooked as they affect rheological blood behaviour, acting as confounders. In particular, both obesity (13–18) and hyperlipidaemia (16, 19–26) appear to be associated with a two- to four-fold increased risk of DVT. The prothrombotic pathogenic mechanisms of obesity, as well as hyperlipidemia, are not clearly

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Financial support:
This study was partially supported by grants from Instituto de Salud Carlos III (CIBER CB06/03/0035 and Red RECAVA RD06/0014/0004), Spain.

Received February 7, 2007
Accepted after resubmission May 29, 2007

Prepublished online August 18, 2007
doi:10.1160/TH07-02-0095

established. One possible mechanism could be by modifying the blood flow characteristics, given that lipids and fibrinogen, which are increased in both conditions, may modulate most of the haemorheological parameters.

The aim of the present study was to assess the overall haemorheological profile six to 12 months after the acute episode in patients with DVT, i.e. blood viscosity (BV), plasma viscosity (PV) and erythrocyte aggregation (EA) and deformability (ED), to find out whether some haemorheological parameters constitute independent risk factors for DVT and to determine whether the lipid alterations themselves or those due to obesity could modify rheological parameters and thus promote DVT.

Materials and methods

Study group

One hundred and forty-nine patients with previously documented DVT (86 male, 63 female) aged 42 ± 12 years were referred to our Haemorheology and Thrombosis Unit between January 2003 and October 2006. They all had experienced a first DVT episode six to 12 months before sampling (mean, 9 ± 3 months). Exclusion criteria were: organic, malignant, infectious, autoimmune or inflammatory diseases, i.e. those pathologies that could influence rheological profile, as well as treatment with rheological drugs. Those patients with inherited (antithrombin, protein C or protein S deficiency, factor V Leiden, prothrombin G20210A mutation) or acquired (lupus anticoagulant or anticardiolipin antibodies) thrombophilic risk factors were also excluded as their thromboembolic episodes could be explained by haemostatic abnormalities.

The control group was made up of 185 subjects (aged 42 ± 13 years, 89 male, 96 female) undergoing a routine check up at our hospital, without a previous history of DVT confirmed with a validated questionnaire (27). Cardiovascular risk factors were recorded and considered for both groups. These included obesity (body mass index [BMI] >30 kg/m²), current tobacco use (>1 cigarette/day), hypertension (diastolic blood pressure >90 mmHg), hyperlipidaemia (total cholesterol >220 mg/dl and/or triglycerides >175 mg/dl), fasting glucose >126 mg/dl, or were receiving any pharmacological treatment for hypertension, hyperlipidaemia, or diabetes. Subjects gave their informed consent to participate in the study, which was approved by the Hospital Ethics Committee.

Circumstantial patient thrombotic risk factors, i.e. medical (oral contraceptives, varicose veins, pregnancy, bed rest >1 week, heart failure, chronic obstructive pulmonary disease, obesity, etc), surgery, immobilization, and trauma were recorded (secondary DVT), and those thrombotic events that occurred without any circumstantial risk factor were considered spontaneous DVT. From the 149 DVT patients, 111 showed some circumstantial risk factors (75%) and 38 did not (25%). In the 111 DVT patients, the percentages of circumstantial risk factors were as follows: medical 41.7%, surgical 17.5%, immobilisation 20.5% and trauma 20.3%. In addition, 16 of the 149 DVT patients had a pulmonary embolism (PE). DVT was documented with ultrasonography or venography, and PE with ventilation perfusion scanning, pulmonary angiography, or helicoidal computerised tomography.

Blood collection

Blood was collected at least six months after the acute event (range 6–12 months; mean: 9 ± 3 months). After a 12-hour overnight fast, blood was drawn between 8 and 10 a.m. by venipuncture into standard vacuum tubes containing EDTA K₃ for rheological and haematological measurements, 0.1 vol of 0.129 M trisodium citrate as an anticoagulant for fibrinogen measurement, or into plain tubes for glucose and lipids determination. Rheological parameters were examined within 2 hours of blood collection, to avoid deterioration of the rheological red blood cell properties (28).

Laboratory methods

Blood viscosity (BV) was determined in a Brookfield DVIII viscosimeter (Engineering, Stoughton MA, USA) at native and 45% corrected hematocrit with autologous plasma, at two shear rates of 230 s⁻¹ and 23 s⁻¹, at 37°C. Plasma viscosity (PV) was measured in a capillary plasma viscosimeter (Fresenius GmbH, Germany) at 37°C (29). Erythrocyte aggregation (EA) was determined in a Myrenne MA₁ aggregometer (Myrenne, GmbH, Roetgen, Germany) (30) after adjusting the hematocrit to 45% with autologous plasma, during completed stasis (EA₀) and while the sample was subjected to a low shear rate of 3 s⁻¹ (EA₁). The greater the tendency of red blood cell to aggregate, the higher the aggregability index. Erythrocyte deformability (ED) was determined in a Rheodyn shear stress diffractometer (Myrenne GmbH) at 12, 30 and 60 Pascals (31). The higher the erythrocyte elongation index (EEI), the more deformable the red blood cell is. Fibrinogen (Fg) was measured using coagulometric techniques on an ACL7000 autoanalyser (Instrumentation Laboratory, Milan, Italy). Haematocrit was measured by microcentrifugation at 15,000 x g for 10 minutes.

Total cholesterol (T-Chol), low-density lipoprotein-cholesterol (LDL-Chol), high-density lipoprotein-cholesterol (HDL-Chol), triglycerides, and glucose were measured by enzymatic techniques, using a DAX 72 autoanalyser (Bayer Diagnostics Division, Tarrytown, NY, USA). Apolipoproteins AI and B were quantified by immunonephelometry (Dade Behring, Marburg GmbH, Germany).

Basic haematological parameters and red cell indices, including mean cell volume (MCV), mean haemoglobin concentration (MHC), and mean corpuscular haemoglobin concentration (MCHC), were measured in a Sysmex ME 8.000 autoanalyser (TOA Medical Electronics, Kobe, Japan).

BV was determined in a random sample of 102 cases and 105 controls and ED in 40 cases and 59 controls. Cases and controls were well matched for age and sex. Patients and controls samples were processed simultaneously.

Statistical analysis

All continuous variables were checked for normal distribution. TG, glucose and Fg values were log-transformed for statistical testing. The data are expressed as mean \pm one standard deviation (SD). Student's t-test was used to assess the mean differences in continuous variables between patients and control subjects, and Chi²-tests were used to compare differences in percentages between patients and controls. Pearson correlation coefficients were calculated to describe the bivariate correlation among vari-

ables. Multiple lineal regression analysis (covariance method) was used to adjust for the influence of BMI or the other parameters on the unadjusted differences of means in lipids, fibrinogen, plasma viscosity and erythrocyte aggregation between DVT patients and controls. In these analyses, we used different models depending on the adjusted variable. First, we adjusted each variable that was statistically significant in the crude model for all the other variables that were statistically significant in the unadjusted analysis. Taking into account that after these adjusted BMI was the only variable that remained highly significant, we fitted additional multivariate models in which each lipid and haemorheological parameters were only adjusted for BMI. Logistic regression models (crude and adjusted for potential confounders) were fitted to estimate the odds ratio (OR) and 95% confidence interval (CI) of DVT associated with the presence of obesity and/or the other haemorheological parameters. In these analyses, dichotomised variables according to cut-off points that corresponded to the mean plus one SD of the control group were used. Standard regression diagnostic procedures were used to ensure the appropriateness of the models.

For statistical inference, a bilateral p-value <0.05 was considered statistically significant. All analyses were calculated using the Statistical Package for Social Sciences (SPSS, version 14) for Windows.

Results

Table 1 shows the percentage of DVT patients and controls with cardiovascular risk factors. DVT patients showed a higher percentage of hyperlipidaemia (46% vs. 35%, P=0.040) and obesity (23% vs. 6%, P=0.001) than controls. When hypercholesterolemia (T-Chol >220 mg/dl) and hypertriglyceridemia (TG >175 mg/dl) were considered separately, DVT patients showed a borderline increased percentage of hypercholesterolemia (P=0.040) and a higher percentage of hypertriglyceridemia (P=0.001). The percentage of hypertensives, diabetics and smokers was similar in DVT patients and control subjects.

Table 2 shows BMI, glucose, lipids and haemorheological parameters in the 149 DVT patients and 185 control subjects. Only BMI, TG, Apo B, B/AI ratio, Fg, PV, EA₀ and EA₁ were significantly higher and HDL-Chol lower in cases than in controls. After multivariate adjustment for the potential confounding factors, only BMI remained significantly different (P<0.001). After adjusting for BMI, only Fg, PV, EA₀ and EA₁ remained significantly different between patients and controls.

No statistical differences in rheological parameters were observed between patients with spontaneous and secondary DVT.

No differences in erythrocyte indices were observed between patients and controls (MCV: 91 ± 10 vs. 90 ± 5 fl, P= 0.782; MCH: 31 ± 2 vs. 30 ± 2 pg, P= 0.247; MCHC: 33 ± 1 vs. 33 ± 1%, P=0.875). The Pearson bivariate correlations between haemorheological and lipid parameters were statistically significant (P<0.01) between EA₁ and BMI (r=0.334), T-Chol (r=0.268), LDL-Chol (r=0.269), Apo B (r=0.344), TG (r=0.356), Fg (r=0.355), PV (r=0.412) and there was a negative correlation between EA₁ and HDL-Chol (r=-0.152) (P<0.05 in all cases). In addition, PV correlated with BMI (r=0.196), Apo B (r=0.186),

Table 1: Age, gender, BMI and incidence (%) of cardiovascular risk factor in DVT patients and controls.

	DVT (n= 149)	Controls (n= 185)	P
Age (mean ± SD)	42 ± 12	42 ± 13	0.982
Male/female	86/63	89/96	0.082
BMI (kg/m ²)	27.6 ± 4.23	24.5 ± 3.5	0.001
*Hyperlipidaemia (%)	46	35	0.040
**Hypercholesterolemia (%)	45	33	0.040
***Hypertriglyceridemia (%)	18.7	5.7	0.001
Obesity (%)	23	6	0.001
Hypertension (%)	13.5	8.2	0.114
Diabetes (%)	4.2	2.2	0.326
Tobacco (%)	31.1	38	0.183

* T-Chol>220 mg/dl and/or TG>175 ml/dl or were receiving any pharmacological treatment.
 ** T-Chol >220 mg/dl or were receiving any pharmacological treatment.
 *** TG>175 ml/dl or were receiving any pharmacological treatment.

TG (r=0.153) , and Fg (r=0.508) (P<0.01), and T-Chol (r=0.136) (P<0.05).

Given the multiple correlations between the rheological parameters, lipids and BMI, to estimate the risk of obesity associated with these parameters, a logistic regression analysis was carried out. Instead of continuous variables, dichotomized variables (according to cut-off points that corresponded to the mean plus one SD of the control group) were used for the analysed parameters to better estimate the risk associated with high values. Crude and multivariate models were estimated for each variable. Table 3 shows OR of DVT associated with haemorheological parameters (EA₁>8.2, PV>1.28 mPa·s, Fg>320 mg/dl) and obesity (BMI>30 kg/m²). In the crude Model I all parameters were significantly associated with a higher risk of DVT. This association remained statistically significant even further adjustment for lipids (Model II) and BMI (Model III). When PV was additionally adjusted for Fg (Model IV) this parameter did not remained statistically significant. However, EA₁, BMI and Fg remained statistically significant in Model IV.

Moreover, when we considered together all the analysed variables in order to control every variable for each other and to know whether they constitute independent risk factors for DVT, we obtained that TG >175 mg/dl (OR=3.2, 95% CI=1.4–7.3, P=0.004), Fg>320 mg/dl (OR=2.7, 95% CI=1.3–5.6, P=0.008), EA₁>8.2 (OR=2.8, 95% CI=1.3–5.9, P=0.007) and BMI >30 kg/m² (OR=3.5, 95% CI=1.5–8.1, P=0.003), but not PV >1.28 mPa·s nor T-Chol >220 mg/dl remained significantly associated with DVT. Therefore TG, Fg, EA₁ and BMI constitute independent risk factors for DVT when they reach high values (higher than mean plus one SD).

Discussion

The results obtained in the present study indicate that DVT patients do not show a higher BV compared to the control group,

Table 2: BMI, glucose, lipids and haemorheological parameters in DVT patients and controls.

	DVT (n= 149)	Controls (n= 185)	Unadjusted P-value	Adjusted	
				*P-value	**P-value
BMI (kg/m ²)	27.6 ± 4.23	24.5 ± 3.5	0.001	<0.001	-----
Glucose (mg/dl)	91 ± 14	90 ± 14.34	0.486		
Total cholesterol (mg/dl)	211 ± 47	203 ± 37	0.144		0.798
HDL-Cholesterol (mg/dl)	50 ± 15	55 ± 13	0.010	0.651	0.195
LDL-Cholesterol (mg/dl)	133 ± 38	130 ± 34	0.478		-
Triglycerides (mg/dl)	127 ± 91	94 ± 48	0.001	0.519	0.132
Apo AI (mg/dl)	144 ± 32	145 ± 25	0.716		-
Apo B (mg/dl)	103 ± 30	95 ± 23	0.016	0.300	0.883
B/AI	0.74 ± 0.26	0.67 ± 0.21	0.020		0.803
Fibrinogen (mg/dl)	282 ± 72	252 ± 46	0.001	0.514	0.013
Haematocrit (%)	43 ± 4	42 ± 4	0.184		
Plasma viscosity (mPas)	1.25 ± 0.07	1.22 ± 0.06	0.001	0.987	0.012
Erythrocyte aggregation stasis	3.85 ± 1.28	3.46 ± 1.02	0.002		0.013
Erythrocyte aggregation 3s ⁻¹	7.58 ± 1.36	6.99 ± 1.17	0.001	0.168	0.028
Native blood Viscosity 230s ⁻¹ (mPas)	4.34 ± 0.58	4.29 ± 0.52	0.493		
Native Blood viscosity 23s ⁻¹ (mPas)	6.61 ± 1.0	6.43 ± 1	0.225		
Corrected Blood Viscosity 230s ⁻¹ (mPas)	4.63 ± 0.39	4.61 ± 0.31	0.656		
Corrected blood viscosity 23s ⁻¹ (mPas)	7.09 ± 0.69	7.06 ± 0.61	0.756		
Erythrocyte elongation index 12 Pa (%)	47 ± 3	47 ± 4	0.648		
Erythrocyte elongation index 30 Pa (%)	53 ± 3	54 ± 4	0.462		
Erythrocyte elongation index 60 Pa (%)	56 ± 3	57 ± 3	0.220		

Values expressed as mean ± SD. *The corresponding P-value of each variable was adjusted for the other variables that were statistically significant in the un-adjusted analysis. **The corresponding P-value was adjusted only for BMI.

both at native and 45% corrected haematocrit. Little information is available on DVT case-control studies regarding BV evaluation. Our results agree with those previously reported (5, 6, 9, 11, 12) in not having found differences between both groups. It is well known that BV, when determined at high shear rate, is a global rheological parameter, depending on haematocrit, Fg concentration and ED. Given that DVT patients in the present study did not show a lower ED either, it is reasonable that, at the shear

rates used (230 s⁻¹ and 23 s⁻¹) where BV basically depends on ED, the former did not show any significant difference with respect to the control group. The significant increase in Fg observed in cases compared with controls does not seem to be high enough to produce significant increases in BV. Our results agree with several studies where DVT patients show significant Fg increases even three months or more after the acute event (5, 6, 8–12). In line with these results, in the population-based case control study

Table 3: Risk of DVT associated with haemorheological parameters and BMI. Crude and adjusted models.

	Model I unadjusted			Model II adjusted*			Model III adjusted**			Model IV adjusted***		
	OR	(95% CI)	P	OR	(95% CI)	P	OR	(95% CI)	P	OR	(95% CI)	P
EA ₁ >8.2	2.64	1.42–4.93	0.002	2.70	1.37–5.37	0.004	2.78	1.38–5.60	0.004	2.85	1.35–6.01	0.006
PV>1.28	2.29	1.40–3.86	0.002	2.09	1.21–3.59	0.008	1.91	1.09–3.37	0.024	1.47	0.81–2.71	0.205
Fg>320	4.40	2.29–8.48	<0.001	4.31	2.20–8.45	<0.001	3.32	1.66–6.67	0.001	+2.91	1.42–5.94	0.004
BMI>30	4.64	2.26–9.53	<0.001	5.12	2.32–11.3	<0.001	----	-----	----	+++4.19	1.84–9.52	0.001

Units: PV: mPas; Fg: mg/dl; BMI: kg/m². * Model II. Adjusted for lipids: T-Chol > 220 mg/dl and TG > 175 mg/dl. ** Model III. Adjusted for lipids and BMI > 30 kg/m². *** Model IV. Adjusted for lipids, BMI > 30 kg/m² and Fg > 320 mg/dl. +Adjusted for lipids and BMI > 30 kg/m². ++Adjusted for lipids and Fg > 320 mg/dl.

conducted by Koster (8), in which 199 patients with a first DVT episode were compared with 199 healthy subjects, those with plasma Fg higher than 500 mg/dl had an almost four-fold increased risk of DVT.

With respect to ED, as mentioned, we did not observe differences between cases and controls at any of the shear stresses tested. There have been no studies published, so far, where this rheological parameter has been evaluated using ektacytometric techniques, as in the present one. Previous studies (32, 33) that found controversial results regarding ED in DVT patients, used older filtration methods that may be influenced by extra-erythrocytic factors such as remnant leucocytes, red blood cell aggregates or plasma factors (34, 35), whereas ektacytometry does not.

As regards the other rheological parameters analysed in the present study, it is important to highlight that DVT patients have shown increased PV and EA when compared with controls. It is known that both rheological parameters are influenced by plasma lipids (10, 36–39) and Fg levels (40–42), which have also shown to be increased in our study. Additionally, obesity is also associated with increased Fg and TG levels (14, 16, 21, 43–46). In the present study, the risk associated with obesity (BMI ≥ 30 kg/m²) was 3.5 (95% CI 1.5–8.1), which is in line with previous studies carried out by our group where the risk of DVT associated with obesity was 2.5 (95% CI 1.2–5.1) (16). For this reason, the first question we should consider is: Is the increase in these rheological parameters, PV and EA, due to an independent increase in Fg and lipids or is their increase associated to obesity? In other words, if patients with DVT were not obese: Would they still have increased PV and EA? Our logistic regression models (Table 3) indicate that EA₁ > 8.2 and PV > 1.28 mPa·s were significantly associated with DVT even further adjustment for lipids (Model II) and obesity (Model III). These results suggest that PV and EA are increased in DVT patients independently of lipid levels and the obesity status. However, PV did not remain statistically significant after additional adjustment for Fg (Model IV), given the high influence of Fg on this rheological parameter. Therefore, in DVT patients EA₁ > 8.2 constitutes a risk factor for DVT, increasing the risk even though patients would not be obese. However, the fact of being obese would further increase the risk.

Several authors have also found EA to be increased in DVT patients 12 months after the acute event (6, 10–12). Some researchers have found higher EA only in those patients with persistent thrombotic risk factors but not in those with transient thrombotic risk factors (11). In this sense, we have not observed any differences in this rheological parameter in connection with the nature of the thrombotic episode, i.e. spontaneous or secondary DVT. It must be emphasised that some studies include patients with malignancy (11, 12) where increased Fg could have been responsible for erythrocyte hyperaggregability. Other authors (7) performed the study six weeks after the acute episode, where the acute phase could have influenced results and, in addition, most studies did not measure plasma lipids which may also account for erythrocyte hyperaggregability. In our study, the influence of acute phase or malignancy has been excluded, and the role played by lipids on EA has also been evaluated, allowing us to conclude that, according to our logistic regression analysis, EA₁ > 8.2 is an independent thrombotic risk factor, increasing the risk almost three times.

As regards PV in DVT patients, only some studies did determine this rheological parameter, finding it to be normal (9, 10) or increased (5, 6) in association with higher cholesterol and TG levels (5). Moreover, Balendra et al. (5) reported PV to be an independent risk factor for DVT. Although the present study has found PV to be increased in DVT patients and associated with lipids and mostly with Fg levels, it does not constitute an independent risk factor for DVT (Model IV). This is consistent with the pathophysiology of the different thrombotic locations, as increases in PV mostly promote thrombus development in the microcirculatory areas and small arteries, whereas increases in EA promote thrombus formation in low-shear areas such as pocket valves in the lower extremities.

In conclusion, the results obtained in the present study, performed in a large group of patients with a DVT episode in the previous 6–12 months, allow us to conclude that rheological parameters play a role in the pathogenesis of the thrombotic event. Increased EA constitute a risk factor for DVT independently of lipid levels and obesity status. However, when associated with hyperlipidaemia and obesity it further increases thrombotic risk. It is reasonable to advise losing weight and to decrease plasma lipid levels in order to reduce DVT risk.

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