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). 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

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 hyperlipidaemia, are not clearly
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 K$_2$ 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 MA$_1$ aggregometer (Myrenne, GmbH, Roetgen, Germany) (30) after adjusting the haematocrit to 45% with autologous plasma, during complete stasis (EA$_0$) and while the sample was subjected to a low shear rate of 3 s$^{-1}$ (EA$_1$). 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 autoanalyzer (Instrumentation Laboratory, Milan, Italy). Haematocrit was measured by microcentrifugation at 15,000 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 autoanalyzer (Bayer Diagnostics Division, Tarrytown, NY, USA). Apolipoproteins A1 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 autoanalyzer (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 ± one standard deviation (SD). Student's t-test was used to assess the mean differences in continuous variables between patients and control subjects, and Chi$^2$-tests were used to compare differences in percentages between patients and controls. Pearson correlation coefficients were calculated to describe the bivariate correlation among vari-
Table 1: Age, gender, BMI and incidence (%) of cardiovascular risk factor in DVT patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>DVT (n=149)</th>
<th>Controls (n=185)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>42 ± 12</td>
<td>42 ± 13</td>
<td>0.982</td>
</tr>
<tr>
<td>Male/female</td>
<td>86/63</td>
<td>89/96</td>
<td>0.082</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.6 ± 4.23</td>
<td>24.5 ± 3.5</td>
<td>0.001</td>
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<tr>
<td><strong>Hyperlipidaemia (%)</strong></td>
<td>46</td>
<td>35</td>
<td>0.040</td>
</tr>
<tr>
<td>*<strong>Hypercholesterolaemia (%)</strong></td>
<td>45</td>
<td>33</td>
<td>0.040</td>
</tr>
<tr>
<td>*<strong>Hypertriglyceridemia (%)</strong></td>
<td>18.7</td>
<td>5.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Obesity (%)</td>
<td>23</td>
<td>6</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>13.5</td>
<td>8.2</td>
<td>0.114</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>4.2</td>
<td>2.2</td>
<td>0.326</td>
</tr>
<tr>
<td>Tobacco (%)</td>
<td>31.1</td>
<td>38</td>
<td>0.183</td>
</tr>
</tbody>
</table>

* This indicates significance at a p-value <0.05.
** This indicates significance at a p-value <0.01.
*** This indicates significance at a p-value <0.001.

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

**Discussion**

The results obtained in the present study indicate that DVT patients do not show a higher BV compared to the control group.
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 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 2: BMI, glucose, lipids and haemorheological parameters in DVT patients and controls.](image)

![Table 3: Risk of DVT associated with haemorheological parameters and BMI. Crude and adjusted models.](image)
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 been 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 \(\geq 30\) kg/m\(^2\)) was 3.5 (95% CI 1.5–8.1), which is in line with previous studies carried out by using 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, EA\(_{1}>8.2\) 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.

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 is reasonable to advise losing weight and to decrease plasma lipid levels in order to reduce DVT risk.

References

venous thromboembolism in patients lacking
thrombophilic risk factors. Br J Haematol 2002; 118:
255–259.
17. Abdollahi M, Cushman M, Rosendaal FR. Obesity:
risk of venous thrombosis and the interaction with co-
gulation factor levels and oral contraceptive use.
passive motion in the prevention of deep-vein thrombo-
sis: a randomised comparison in trauma patients. J
factors for deep vein thrombosis in symptomatic out-
cholesterolemia as a risk factor for deep vein thromb-
(a), cholesterol and triglycerides in women with venous
thromboembolism. Blood Coagul Fibrinolysis 2000;
22. Griffin JH, Fernández JA, Deguchi H. Plasma lipo-
proteins, hemostasis and thrombosis. Thromb Haemost
23. Gómez-Ordóñez AJ, Fernández-Carreira JM, Fernán-
dez-Alvarez CR, et al. The concentrations of soluble
vascular cell adhesion molecule-1 and lipids are
independently associated with venous thromboembol-
lipid levels and the risk of venous thrombosis. Arte-
25. Deguchi H, Pechenik NM, Elias DJ, et al. High-
density lipoprotein deficiency and dyslipoproteinemia
associated with venous thrombosis in men. Circulation
2005; 112: 893–899.
26. Ageno W, Prandoni P, Romualdi E, et al. The meta-
bolic syndrome and the risk of venous thrombosis:
a case-control study. J Thromb Haemost 2006; 4:
1914–1918.
27. Frezzato M, Tosetto A, Rodeghiero F. Validated
questionnaire for the identification of previous person-
al or familial venous thromboembolism. Am J Epide-
miol 1996; 143: 1257–1265.
28. Guidelines for measurement of blood viscosity
and erythrocyte deformability. International Commit-
tee for standardization in Haematology, Expert panel on
Kapillarschlauch-Plasmaviskosimeter. Ein neues
Messgerät zur Quantifizierung der Blutplasmaviskosi-
hemorheological techniques for the routine laboratory.
31. Ruff P, Pöschl JMB, Linderkamp. The rheody
SSD for measuring erythrocyte deformability. Bior-
tionated sodium heparin at therapeutic doses on the
hemorheological parameters of patients with deep vein
33. Da Silva MA, Cardoso L, Gerales J, et al. Haemor-
heological and coagulation conditions in patients with
acute deep venous thrombosis compared with subjects
without vascular disease. Clin Hemorheol 1991; 11:
534 Abstract.
34. Kenny MW, Meakin M, Stuart J. Methods for re-
moval of leucocytes and platelets prior to study of ery-
throcyte deformability. Clin Hemorheol 1983; 3:
191–200.
35. Stuart J, Stone PCW, Bareford D, et al. Evaluation of
leucocyte removal methods for studies of erythro-
HDL subfractions on erythrocyte aggregation in hyper-
cholesterolemia men. Arterioscler Thromb 1994; 14:
361–366.
Highly significant negative correlations between ery-
throcyte aggregation value and serum value concen-
tration of high density lipoprotein cholesterol in a
sample from a normal population and in patients with
coronary heart disease. European Journal of Clinical
Investigation 1987; 17: 275–279.
cell aggregation and primary hyperlipoproteinemia.
39. Koenig W, Sund M, Ernst E, et al. Association be-
tween rheology and components of lipoproteins in
nogen levels on erythrocyte aggregation determined with
the Myrenne aggregometer and the Sefam ery-
33: 145–151.
41. Xong W, Roederer GO, Beaulieu R, et al. Contribu-
tion of acute-phase proteins and cardiovascular risk
factors to erythrocyte aggregation in normolipidaemic
and hyperlipidaemic individuals. Thromb Haemost
and erythrocyte factors on red blood cell aggrega-
gion in survivors of acute myocardial infarction.
43. Juhan-Vague I, Alessi MC, PAI-1, obesity, insulin
resistance and risk of cardiovascular events. Thromb
PAI-1 plasma levels in a general population without
clinical evidence of atherosclerosis: relation to envi-
ronmental and genetic determinants. Arterioscler
45. Woodward M, Lowe GDO, Rumley A, et al. Epi-
demiology of coagulation factors, inhibitors and activa-
tion markers: The Third Glasgow Monica Survey II.
Relationships to cardiovascular risk factors and preva-
lent cardiovascular disease. British Journal of Haema-
tology 1997; 97: 785-797.
analysis of clustered cardiovascular risks in adoles-
cence: obesity is the predominant correlate of risk