The concentrations of soluble vascular cell adhesion molecule-1 and lipids are independently associated with venous thromboembolism

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Background and Objectives. Venous thromboembolism (VTE) involves inflammation and a relation with dyslipidemia which remains controversial. The vascular cell adhesion molecule-1 (VCAM-1) is a ligand expressed by activated endothelium (and recruits leukocytes) whose soluble form (sVCAM-1) increases in atherosclerosis, severe hypertriglyceridemia or deep vein thrombosis (DVT) in acute phase. We analyzed the association between VTE (> 6 months after), sVCAM-1 and lipid concentrations.

Design and Methods. Case-control study involving 126 consecutive patients (aged 25-80 years, 49% males) and 125 controls of similar age and gender.

Results. The patients had a more unfavorable lipid profile than controls [higher triglycerides (p<0.001), LDLc/HDLc ratio (p<0.01) or total cholesterol (TC) (p=0.07)] and higher sVCAM-1 concentration (p<0.01) even adjusting for arterial diseases. VTE was associated with extreme values of TC, LDL-c, triglycerides (>P90) and HDL-c (<P10) (OR=2.1-3.6)(p<0.05) and mainly of sVCAM-1 (>P90) (OR=4.2)(p<0.0001). The sVCAM-1 values were age-related (r=0.26, p<0.001) but independent of lipid levels. Hazards ratios from five-fold to ten-fold appeared when combining the sVCAM-1 top quartile (>970 ng/mL) with TC >250 mg/dL or HDL-c <45 mg/dL (p<0.01) irrespective of thrombophilic status. Recurrent or severe VTE cases (pulmonary embolism or proximal DVT vs. distal DVT) showed higher sVCAM-1 values (p<0.05). All the associations weakened among females. In stepwise logistic regression, obesity (p<0.001), sVCAM-1 (p<0.001) and LDL-c (p=0.004) in men and sVCAM-1 (p=0.02) and triglycerides (p=0.04) in women retained their independent association.

Interpretation and Conclusions. Although the exact mechanisms linking abnormal lipid and sVCAM-1 concentrations to VTE await clarification, both seem to be independently associated.

Key words: VCAM-1, cholesterol, triglycerides, deep vein thrombosis, pulmonary embolism.

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The vascular cell adhesion molecule-1 (VCAM-1, CD106) is a ligand expressed by cytokine activated endothelium and monocytes that belongs to the immunoglobulin supergene family. Its receptor, the integrin VLA-4 (very late antigen-4) is also expressed on these cells and both react to produce cellular attachment. The function of VCAM-1 seems to be to recruit leukocytes (mainly mononuclear cells) to the sites of inflammation (e.g. atherosclerotic plaques). Upregulation of several endothelial adhesion molecules is accompanied by the release of their soluble fractions into the bloodstream. Therefore, higher concentrations of circulating VCAM-1 (sVCAM-1) have been reported in patients with an atherosclerotic aorta than in asymptomatic controls.[1] Furthermore, in patients with dyslipidemia, in whom advanced atherosclerosis could be expected, high serum concentrations of several adhesion molecules were demonstrated (sVCAM-1 mainly associated with hypertriglyceridemia).[2] Nevertheless, this relation between increased lipids and sVCAM-1 seems to be indirect and is attributed to the underlying arterial disease, since an aggressive lipid-lowering treatment does not cause the expected decrease of sVCAM-1.[2]

Probably, venous thrombosis (or the accompanying inflammatory process) could also involve some adhesive leukocytes-endothelium interactions. In fact, increased serum concentrations of sVCAM-1 were found during the acute phase of deep vein thrombosis (DVT) (when thrombus was present) in a relatively brief study.[3] Simultaneously, the same group of researchers demonstrated increased local concentrations of sVCAM-1 in blood taken from the affected veins in an experimental model of human venous thrombosis.[4] However, to our knowledge, the epidemiological association between venous thrombosis out of the acute phase, and sVCAM-1 concentrations has not yet been reported.

There are rational explanations to relate DVT with raised lipid concentrations, e.g. the positive correlation among cholesterol or triglycerides and prothrombin concentrations[5] or vitamin K-dependent procoagulant proteins[6] and the possible pathogenic role of lipoprotein(a).[7,8] Furthermore, hypertriglyceridemia is associated with higher plasma concentrations of plasminogen activator inhibitor type-1 (PAI-1).[9] The recently discovered *in vitro* anticoagulant properties of high density lipoprotein-cholesterol (downregulation of thrombin generation)[10] and the reported reduction in the risk of

DVT among older patients receiving treatment with statins also seem to highlight a possible relationship between lipids and thrombosis.[11] In spite of this, the epidemiological link between dyslipidemia and DVT remains a highly controversial topic. A significant association with high lipid concentrations was found among Asians, who had a 5-fold higher odds ratios when hypercholesterolemia and hypertriglyceridemia were both present,[12,13] and Europeans without risk factors.[14,15] Plasma triglycerides and body mass index (BMI) were increased in young women with DVT.[16] However, other studies did not confirm the association between DVT and increased lipids in children[7] or in the general population.[17,18]

Our study had three aims. The main aim was to establish the association between sVCAM-1 concentrations and venous thromboembolism (VTE) in a population-based study, delaying the sampling time until the conclusion of the therapeutic phase in order to avoid the inflammatory response from having a possible influence. The second aim was to explore the controversial relationship between VTE and serum lipids. Finally, we investigated the existence of a putative interaction between lipids and sVCAM-1 concentrations.

Design and Methods

Study design and population

We performed an observational analytic case-control study. We included all consecutive patients below 80 years diagnosed with VTE in our hospital, located in North Spain, between January 2000 and December 2001 who collaborated and survived until the sampling time. We required an objective confirmation of DVT by color Doppler investigations or venography and a compatible lung scan for the pulmonary embolism (PE). To avoid extrinsic influences (inflammatory status and coumarin therapy) all the patients had already completed the standard six-month period of oral anticoagulation at least three weeks before samples were taken. The overall interval between the index event and testing was seven months. Furthermore, at the sampling time we excluded patients and controls with obvious inflammatory conditions or a recent febrile episode.

The controls were randomly chosen from the same geographic area using census information. They had a similar age (±3 years) and sex distribution to the patients (block matching method). They were enrolled after an interview including a specific validated questionnaire to detect personal or family history of DVT or PE.[19] The study was approved by the local Ethics Committee and signed informed consent was obtained from all the participants.

Measurements

Height, weight (wearing light clothes) and waist and hip circumferences were measured by trained staff, using the same instruments for all the participants. Body mass index was calculated as weight (kg) divided by height (m^2) and we considered obesity as a BMI ≥30 kg/m^2, according to the guidelines of the National Institutes of Health.[20]

Blood samples were obtained from all the subjects by standard venipuncture at 8.00-9.00 a.m. after a 10-12 hours of fasting. Tubes of EDTAK3 were used to obtain DNA for genetic studies. Aliquots of serum and plasma (9:1 sodium citrate 0.129M) were either analyzed immediately (glucose and lipids) or frozen at –85°C until assayed (sVCAM-1 or thrombophilia studies).

Blood glucose was analyzed by the automated glucose oxidase method and total cholesterol (TC), triglycerides (TG), and HDL cholesterol (HDL-c) were measured using routine procedures with enzymatic assays from Roche Diagnostics on a Hitachi^R 917 analyzer. LDL cholesterol (LDL-c) was calculated by the Friedewald equation for triglycerides below 400 mg/dL. For two patients exceeding this limit we used the HDL-c and LDL-c data from a recent previous sample although the exclusion of both cases did not change the results significantly. We analyzed the standardized ratios of activated protein C resistance (APC-SR) with the Coatest^R APC Resistance kit (Chromogenix^R, Sweden) in a Sysmex^R CA-6000 coagulometer, using citrated plasma in a subgroup of 206 samples (82.1% of the overall, 109 patients and 97 matched controls). In order to increase the comparability of the obtained ratios we performed a normalization process (against a reference normal plasma); the normal values of these n-APC-SR, therefore, tend to 1.0.

Serum concentrations of the sVCAM-1 were measured in duplicate using the commercial enzyme-linked immunosorbent assay (ELISA) distributed by BioSource^R International. The assays were carried out according to the manufacturer's protocol. The sVCAM-1 concentrations of the samples were obtained by analysis of several dilutions of a commercial standard with a known concentration of this adhesion molecule and plotting the signal-concentration curve. The whole procedure was automated in a TRITURUS^R instrument (Diagnostic Grifols S.A.). The lower limit of detection (obtained from analysis of the standard curve) was 0.5 ng/mL of sVCAM-1 but according to the manufacturer's recommendation of a 1/50 dilution for the samples, the real limit was 25 ng/mL.

The patients were additionally screened, using conventional methods, for serum anticardiolipin antibodies and for lupus anticoagulant, antithrombin, proteins C and S in the plasma. Factor V R506Q and factor II G20210A DNA mutations were also searched for.

Statistical methods

ANOVA and χ^2 tests were performed to determine the differences between the two groups of participants. The continuous variables were evaluated for a normal distribution and their data are presented as mean (SD). Those variables with a non-Gaussian distribution (glucose, triglycerides and sVCAM-1) were presented as median and interquartile range (IQR) as well. To evaluate relationships involving the variables with a skewed distribution, the Spearman's correlation coefficients were calculated, while Pearson's correlation coefficients were used for the others. The differences between mean values were analyzed with an unpaired Student's t test or Mann-Whitney rank sum test.

Regarding the HDL-c and the n-APC-SR, we established different cut-off values to dichotomize their results by choosing the bottom quintile (20^{th} percentile) or decile (10^{th} percentile) instead of the top ones because of their protective pathogenic significance. In this way, it is possible to maintain the consistency of the results (prevalence ratios higher than 1.0). The significance of the differences in prevalence for each parameter was tested using the χ^2 test. The odds ratios (OR) and the 95% confidence intervals ($CI_{95\%}$) were calculated as a measure of relative risk. Stepwise logistic regression was used as a multivariate model to predict the presentation as VTE. Statistical significance was considered as a p value <0.05.

All calculations were performed with the SPSS for Windows Release 10.0 statistical package (SPSS, Chicago, IL, USA) and Epi Info, 6.0 version from the Centers for Disease Control and Prevention (CDC), Epidemiology Program Office, USA.

Results

Population and clinical features

During the period of the study we objectively confirmed 140 cases of VTE in patients under 80 years but 14 were not assayed (two denied consent, and by sampling time four had died, five did not attend the follow-up and three had deteriorated severely because of neoplastic disease or recurrent surgery). Overall, the study involved 251 participants (126 patients and 125 controls) with a similar age and gender distribution between groups. Table 1 shows the population's characteristics. At the time of sampling a definite diagnosis of arterial disease (cerebrovascular, coronary, aortic or peripheral) had been made in 21 patients and 11 controls (borderline difference). Active cancer had been diagnosed in eight patients and in no controls (p<0.01). The prevalence of obesity was clearly higher in patients than in controls reaching an OR of 3.3 for VTE among the obese subjects (CI 95%=1.9-5.6) (χ^2= 19.8, p<0.0001). A very similar situation was found for raised BMI, p<0.001). In contrast, waist to hip ratio (WHR) was similar in both groups (p=0.33) (Table 2).

Table 1. Population characteristics of the series.

	VTE patients	*Controls*	*p*
N	126	125	
Age, mean (SD) (range)	62.7 (13.7)(25-80)	62.3 (11.3)(26-83)	NS
Male gender (%)	49.2	48.8	NS
Ethnicity (% Caucasians)	99.2	100	NS
Obesity (%)	54.0	26.4	< 0.0001
Current smokers (%)	14.3	20.0	NS
Active cancer (%)	6.3*	0	0.01
Diabetes mellitus (%)	10.3	13.6	NS
Arterial disease (%)	16.7	8.8	0.06
Treatment with statins (%)	4.8	8.0	NS
Isolated venous thrombosis (location)	86 (22 distal and popliteal) (64 proximal)	–	
Pulmonary embolism	40 (31.7%)	–	
Recurrent VTE	35 (27.8%)	–	

**Excluding two cases of polycythemia vera. NS indicates not significant.*

Analytic characteristics of VTE patients and controls

The patients had clearly higher levels of triglycerides (p<0.001) and a weak trend towards higher TC and LDL-c (p=0.07 and 0.13, respectively) than the controls (Table 2). This difference is not attributable to the existence of a putative arterial disease or diabetes mellitus. The concentrations of HDL-c were distributed conversely to those of LDL-c between the two groups, thus the LDL/HDL ratio was also higher in patients (p<0.01) even excluding those patients with arterial disease (p<0.05) or receiving statin treatment (p<0.01). Unsurprisingly, the APC-SR was shorter among the patients (p<0.05) (Table 2) but showed no correlation with lipid or sVCAM-1 values either in patients or controls. The participants with VTE had higher sVCAM-1 levels [median= 882 (IQR= 701) ng/mL] than did healthy subjects [median= 765 (IQR= 338) ng/mL] (p=0.005, Mann Whitney test) (Table 2). This difference was particularly marked among males (Figure 1a). The difference remained after adjusting for arterial disease (p<0.05).

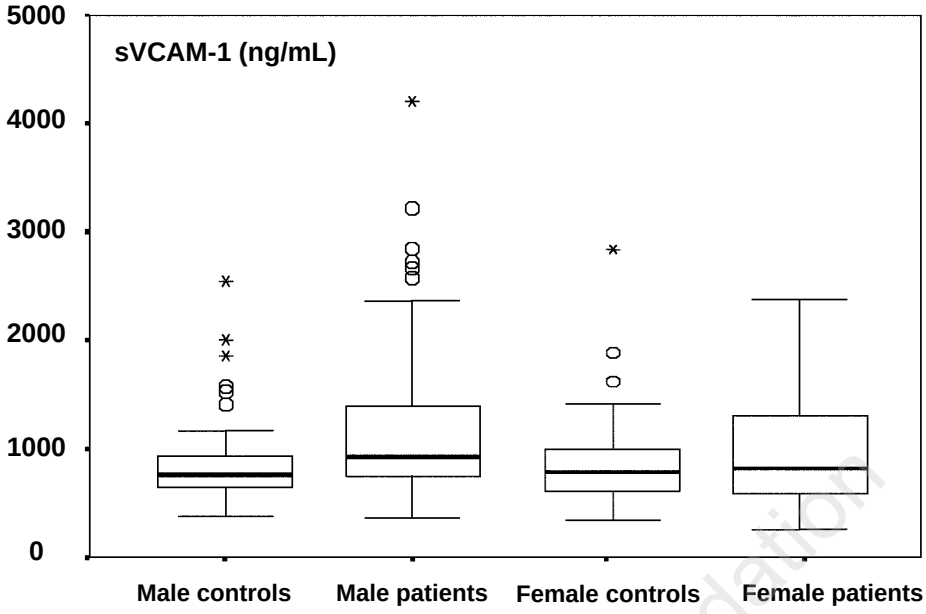

Figure 1a. The sVCAM-1 values and VTE considered the gender. Box plots.

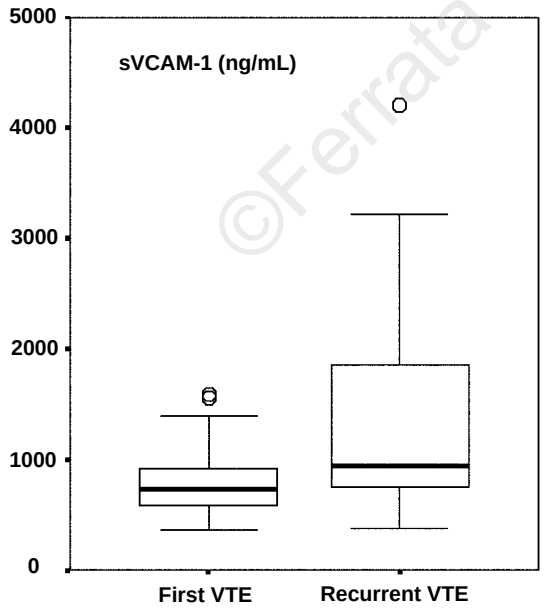

Figure 1b. The recurrent cases showed higher sVCAM-1 values than the cases with a first VTE episode. Box plots.

Table 2. Comparison of results in controls and VTE patients before and after exclusion of the thrombophilic patients.

	Controls (n=125)	*Patients (n=126)*	*Non-thrombophilic patients (n=71)*
BMI (kg*m^{-2})	**28.0 (4.3)**	**30.4 (4.7)$^{\#}$**	**30.7 (5.0)$^{\#}$**
WHR	0.90 (0.07)	0.91 (0.08)	0.91 (0.08)
Glucose (mg/dL)	107.0 (30.8) [98]	101.3 (24.9) [96]	103.7 (35.3) [94]
TC (mg/dL)	221.8 (35.6)	230.8 (42.6)¶	**233.4 (40.5)***
HDL-c (mg/dL)	58.6 (13.2)	55.5 (16.7)	56.6 (15.6)
LDL-c (mg/dL)	141.7 (32.9)	148.4 (37.5)	150.1 (33.5)
LDL/HDL	**2.55 (0.86)**	**2.88 (1.04)°**	**2.83 (0.91)***
TG (mg/dL)	**107.6 (54.7) [96]**	**141.0 (93.9) [118.5]$^{\#}$**	**144.9 (110) [127]°**
n-APC-SR (#)	**0.966 (0.146)**	**0.919 (0.189)***	**0.953 (0.154)**
sVCAM-1 (ng/mL)	**857.0 (380.8) [765]**	**1105.8 (659) [882]°**	**1054.1 (553.9) [885]***

*Mean (standard deviation) [median]; ¶p=0.07. Values written in bold type when statistical significance reached (*p <0.05, °p <0.01, $^{\#}$p<0.001); BMI: body mass index, WHR: waist/hip ratio, TC: total cholesterol, TG: triglycerides, n-APC-SR: (normalized) activated protein C-standardized ratio. (#) N= 206 participants (97 controls and 109 overall patients) or N= 154 participants (97 controls and 57 non-thrombophilic patients).*

Table 3. Associated odds ratios for VTE in the overall group of patients and in the subgroups (univariate analysis).

	Cut-off	*Patients (n=126) OR ($CI_{95\%}$)*	*Non-thrombophilic patients (n=71) OR ($CI_{95\%}$)*	*Males (n=62) OR ($CI_{95\%}$)*	*Females (n=64) OR ($CI_{95\%}$)*
BMI	P_{80}	**3.3 (1.9-5.7)@**	**3.7 (1.9-7.0)@**	**5.1 (2.3-11.5)@**	1.9 (0.9-4.4)
	P_{90}	**3.3 (1.6-6.7)#**	**4.5 (2.0-10.1)@**	**6.4 (2.4-17.0)@**	1.8 (0.6-5.2)
WHR	P_{80}	1.5 (0.8-2.7)	0.7 (0.4-1.4)	1.5 (0.7-3.6)	1.4 (0.6-3.3)
	P_{90}	1.7 (0.8-3.6)	2.0 (0.8-4.8)	1.4 (0.4-4.2)	2.5 (0.9-7.1)
Glucose	P_{80}	0.7 (0.4-1.4)	0.9 (0.4-1.9)	0.3 (0.1-1.0)¶	1.6 (0.7-3.6)
	P_{90}	0.6 (0.2-1.6)	0.9 (0.3-2.6)	0.2 (0.0-1.3)	2.3 (0.8-6.5)
TC	P_{80}	**2.1 (1.2-3.7)°**	**2.7 (1.4-5.2)°**	**2.8 (1.2-6.2)°**	1.4 (0.6-3.2)
	P_{90}	**2.1 (1.0-4.4)***	**2.2 (0.9-5.1)**	**2.7 (1.0-7.5)***	2.0 (0.7-5.8)
HDL-c	P_{20}	**2.0**	1.6	**2.8**	**1.7**
	‡	**(1.1-3.5)***	(0.8-3.2)	**(1.2-6.2)°**	**(0.7-3.7)**
	P_{10}	**3.6**	**3.6**	**2.9**	**3.0**
	‡	**(1.8-7.4)#**	**(1.6-8.1)#**	**(1.1-8.1)***	**(1.1-8.2)***
LDL-c	P_{80}	1.6 (0.9-2.9)	**2.0 (1.0-3.9)***	1.9 (0.8-4.4)	1.7 (0.7-3.7)
	P_{90}	**2.2 (1.1-4.7)***	2.0 (0.8-4.7)	**3.2 (1.2-8.8)***	1.4 (0.5 4.2)
LDL/HDL	P_{80}	1.7 (0.9-3.0)	1.5 (0.8-3.0)	**2.4 (1.1-5.4)***	1.7 (0.7-3.7)
	P_{90}	**2.8 (1.4-5.8)°**	2.2 (0.9-5.1)¶	**4.4 (1.6-11.8)°**	1.4 (0.5-4.2)
TG	P_{80}	**2.2 (1.3-3.9)°**	**2.4 (1.3-4.7)°**	**2.4 (1.1-5.4)***	2.0 (0.9-4.3)
	P_{90}	**2.2 (1.1-4.7)***	2.2 (0.9-5.1)¶	**3.2 (1.2-8.8)***	2.5 (0.9-7.0)
n-APC-SR (#)	P_{20}	**2.2**	1.3	2.4	2.1
	‡	**(1.2-4.2)***	(0.6-2.8)	(1.0-5.7)¶	(0.8-5.1)
	P_{10}	**3.2**	1.8	2.8	**3.5**
	‡	**(1.5-6.9)°**	(0.7-4.7)	(0.9-8.5)	**(1.2-10.3)***
sVCAM-1	P_{80}	**2.9 (1.7-5.1)#**	**2.9 (1.5-5.5)#**	**3.8 (1.7-8.5)#**	1.9 (0.9-4.3)
	P_{90}	**4.2 (2.1-8.5)@**	**3.8 (1.7-8.5)#**	**5.0 (1.9-13.6)#**	**3.2 (1.2-8.3)***
Effect of sVCAM-1 in the top quartile combined with abnormal lipid concentrations (mg/dL)					
TC > 250		**5.5 (1.6-19.5)°**	**7.7 (2.1-28.7)#**	**10.4 (1.3-84.5)***	3.8 (0.8-19.1)
HDL-c < 45		**9.6 (2.2-2.5)#**	**8.1 (1.7-39.1)°**	**6.5 (1.4-30.6)***	3.2 (0.6-16.5)
LDL-c > 160		2.1 (0.8-5.5)	2.5 (0.9-7.1)¶	2.9 (0.7-11.6)	1.6 (0.4-5.8)
TG > 150		**4.3 (1.2-15.5)***	3.9 (0.9-16.0)¶	3.8 (0.8-19.2)	2.6 (0.5-14.1)

*The specific cut off values were established for each distribution ¶ p=0.06-0.07. Values written in bold type when statistical significance reached (*p <0.05, °p <0.01, #p<0.001, @p<0.0001). BMI: body mass index, WHR: waist/hip ratio, TC: total cholesterol, TG: triglycerides, n-APC-SR: normalized activated protein C-standardized ratio. Observe that for HDL-c and n-APC-SR, P_{20} and P_{10} were used instead of P_{80} and P_{90}. (#) N= 206 participants (97 controls and 109 overall patients), N= 154 participants (97 controls and 57 non-thrombophilic patients) or N= 103 males and N= 103 females.*

After excluding 55 patients with thrombophilia (13 with factor V Leiden, 10 with prothrombin mutation, 27 with antiphospholipid antibodies, one with protein C deficiency, one with protein S deficiency, one with factor V Leiden plus antiphospholipid, one with prothrombin mutation plus antiphospholipid and one with factor V Leiden plus protein S deficiency) the main differences remained similar (Table 2). The observed differences only disappeared for the n-APC-SR because of the exclusion of the factor V Leiden carriers.

Association with VTE. Univariate analysis

The top quintile (P_{80}) of the TC, triglyceride and sVCAM-1 concentrations and the bottom one (P_{20}) of the HDL-c seem to be associated with an odds ratios between 2.0 and 2.9. The top decile (P_{90}) of the TC, LDL-c, and triglyceride concentrations and the bottom decile (P_{10}) of the HDL-c level associated with slightly higher odds ratios (2.1 to 3.6). Furthermore, the P_{90} value of the sVCAM-1 concentrations in the controls (1220 ng/mL) was found in 39 patients (31%) which indicates a stronger association (OR= 4.2) (χ^2= 17.7, p<0.0001). When combining the effect of the top quartile of the sVCAM-1 distribution (>970 ng/mL) with a low HDL-c (<45 mg/dL) the hazards ratios increased nearly ten-fold (p<0.001), while combining the top quartile of sVCAM-1 with a high TC (>250 mg/dL) or triglycerides (>150 mg/dL) the ORs rose about five-fold (p<0.01 and <0.05, respectively). After excluding the patients with thrombophilia these associations remained similar.

All these associations weakened (HDL-c and sVCAM-1) or disappeared (TC, LDL-c, LDL/ HDL ratio and triglycerides) among females. These cut-off points did not allow an association between BMI and VTE to be demonstrated among the women although the association with obesity (BMI ≥30 kg/m^2) was retained [OR= 2.7 (CI95%= 1.3-5.6) (χ^2= 7.6, p<0.01)] (Table 3).

Minor differences in sVCAM-1 concentrations were found between patients with DVT [median: 816 (IQR: 594) ng/mL, n=86] and those with PE [median: 1042 (IQR: 906) ng/mL, n=40] (p=0.09) although the strength of the association was related with the severity of the VTE. Thus the OR was 0.9 (0.3-2.6)(p=NS) for distal/ popliteal DVT, 2.4 (1.2-4.5) (p<0.01) for proximal DVT and 3.4 (1.6-7.0) (p=0.001) for PE, using top quartile level.

Patients with recurrent VTE showed significantly higher sVCAM-1 values than those with a single VTE episode [median: 1115 (IQR: 1190) ng/mL (n=36) vs. median: 830 (IQR: 590) ng/mL (n=90)] (p<0.01, Mann-Whitney test) (Figure 1b) but not higher lipid values. Therefore the association of the top quartile of sVCAM-1 was stronger for the patients with recurrent episodes [OR = 3.6 (CI 95% = 1.7-7.8) (χ^2 = 11.1, p<0.001) vs. OR= 1.8 (CI 95% = 1.0-3.3) (χ^2 = 4.2, p=0.04)].

Table 4. Spearman's correlation and regression coefficients for sVCAM-1 and main quantitative variables.

	Correlation coefficients			
	Overall series (n=251)	*Patients (n=126)*	*Controls (n=125)*	*Standardized β coefficients (n= 251)*
Age	**0.25#**	**0.24°**	**0.26°**	**0.27#**
BMI	–0.05	**–0.30°**	0.13	–0.04
WHR	0.12¶	0.13	0.09	0.02
Glucose	–0.04	–0.01	–0.04	–0.01
Total Cholesterol	**–0.16***	**–0.20***	–0.17¶	–0.35
HDL-c	–0.04	–0.02	–0.01	0.05
LDL-c	**–0.15***	**–0.18***	–0.16¶	0.19
LDL/ HDL	–0.07	–0.11	–0.11	–0.06
Triglycerides	–0.03	–0.11	–0.05	0.18
n-APC-SR (#)	–0.01	0.04	–0.01	0.06

*¶p=0.06-0.07. Values written in bold type when statistical significance reached (*p <0.05, °p <0.01, #p<0.001). BMI: body mass index; WHR: Waist/hip ratio, n-APC-SR: (normalized) activated protein C-standardized ratio. (#)N= 206 participants (109 patients and 97 controls).*

Table 5. Stepwise logistic regression analysis. Odds ratios (CI95%) associated with several major cut off points.

Cut off type	*Clinically valuable*		P_{80}		P_{90}	
	Cut off	*OR*	*Cut off*	*OR*	*Cut off*	*OR*
BMI (kg*m^{-2})	>30	**3.7 (2.0-7.0)#**	>31.2	**3.1 (1.7-5.6)#**	>33.5	**3.7 (1.7-8.2)#**
HDL-c (mg/dL)	<45	**2.5 (1.0-6.0)***	<46 ‡	—	<44 ‡	**4.3 (2.1-9.4)#**
LDL-c (mg/dL)	>160	—	>169	—	>180	2.9 (1.3-6.5)°
TG (mg/dL)	>150	**2.2 (1.0-4.9)***	>138	**2.2 (1.2-4.2)°**	>180	—
n-APC-SR (#)	<0.80	**3.6 (1.5-8.3)°**	<0.84 ‡	—	<0.79 ‡	**4.9 (1.3-17.9)°**
sVCAM-1 (ng/mL)	>970	**2.8 (1.4-5.5)°**	>1005	**3.3 (1.8-6.1)#**	>1220	**4.5 (2.1-9.8)#**

*Values written in bold type when statistical significance is reached (*p <0.05, °p <0.01, #p<0.001). ‡: For HDL-c and n-APC-SR, P_{20} and P_{10} were used instead of P_{80} and P_{90} were used. BMI: body mass index; TG: triglycerides. (#): N= 206 participants (109 patients and 97 controls).*

Among the patients, the sVCAM-1 concentration was independent of the carrier status for thrombophilic mutations (FV R506Q/ FII G20210A) [median: 800 vs. 884 ng/mL] (p=0.26), the presence of antiphospholipid antibodies [median: 869 vs. 892 ng/ml] (p=0.6) or an active cancer [median: 825 vs. 881 ng/ml] (p=0.9).

Correlations between variables

The sVCAM-1 concentrations were related to age in the overall series (r=0.25, p<0.001) and higher in males only among the patients [median= 924 (IQR: 690) vs. 820 (IQR: 739) (ng/mL) in female patients](p<0.05). Interestingly, sVCAM-1 concentration was inversely correlated with BMI among the patients (Table 4) and was weakly related to the lipids. sVCAM-1 levels showed an inverse correlation with serum concentrations of TC and LDLc (r close to –0.16, p<0.05) in the overall series, with a similar trend in both the patients and controls. sVCAM-1 levels showed no correlation with serum triglycerides (Table 4).

Association with VTE. Multivariate analysis

By multivariate logistic regression the BMI (p<0.001), HDL-c (p= 0.04), triglycerides (p=0.04), n-APC-SR (p=0.003) and s-VCAM-1 levels (p=0.003) retained their independent association with ORs for pathologic values close to 3 (Table 5). The main risk factors, ranked by their relevance, were BMI (p<0.001), sVCAM-1 (p<0.001) and LDL-c (p=0.004) in men and sVCAM-1 (p=0.02) and triglycerides (p=0.04) in women (*data not shown*). A n-APC-SR lower than 0.8 was weakly associated with disease in both genders (p=0.02).

Discussion

When the adhesion molecule VCAM-1 is overexpressed at the endothelium, mainly by the effect of TNF-α or interleukin-1, its soluble fraction (sVCAM-1) is released to the bloodstream. Thus, increased sVCAM-1 levels have been related with atherosclerosis in aortic and peripheral locations[1,21] and, more controversially, with coronary artery disease.[22,23]

Regarding venous pathology, some papers have described increased local expression of VCAM-1 in chronic venous insufficiency, venous hypertension and leg ulcers.[24,25] Searching for some distinctive markers useful for the early detection of post-operative venous thrombi, Smith and co-workers described high sVCAM-1 levels.[3] In this particular setting of total hip replacement the assay of sVCAM-1 was the most valuable because the concentration of this adhesion molecule remained in the normal range, despite the surgical aggression (28 controls), rising only when a DVT did appear (20 patients). Formerly, the same authors had reported that sVCAM-1 concentrations increased in blood taken from human veins with experimental throm-

bi, in contrast with several other molecules (sICAM-1, sE-selectin and sL-selectin) which remained unaffected.[4] To the best of our knowledge, this is the first report about the association between sVCAM-1 concentrations and DVT in an epidemiological study conducted in chronic phase, thus avoiding a potential influence of the inflammatory stimulus. In accordance with our hypothesis, VTE patients had higher sVCAM-1 values than did controls ($p<0.01$) even after excluding individuals with arterial disease ($p<0.05$). However, sVCAM-1 values seemed not to be affected when the VTE occurred in the most distal locations, such as tibial or popliteal DVT. In fact, the progressive tendency to a stronger association in parallel with the severity of the VTE (OR= 2.4 in proximal DVT, $p<0.01$ and 3.4 in PE, $p=0.001$) and its stronger association in recurrent cases (OR= 3.60, $p<0.001$) adds support to our hypothesis. No previous report has associated high sVCAM-1 concentrations with PE.

Technically, the sVCAM-1 values obtained in our control group are quite similar to those previously reported but superior to those stated by the manufacturer (550 ng/mL) probably in accordance with the older age of our patients.[21,26] The age-dependency of sVCAM-1 levels seems to be a function of the underlying atherosclerotic burden[2,21] and it was observed in both series of participants in our study.

By logistic regression analysis the level of sVCAM-1 seems to be a continuous and progressive risk factor. The observed association reached the significance for every sVCAM-1 value higher than the median (OR= 1.9)($p<0.05$), being stronger for the top quartile (OR= 2.8)($p<0.01$) and particularly significant for the top decile (OR= 4.5)($p<0.001$).

However, the role of sVCAM-1 in patients with either acute or late DVT remains unexplained. In acute DVT the increased sVCAM-1 may derive from local activation of endothelium at the site of thrombosis or marked leukocyte movement (mainly monocytes-macrophages) into the thrombus, as has been shown by the immunostaining for VCAM-1.[3] Nevertheless, in the chronic phase this process could be quite different. In fact, some epidemiological studies have demonstrated increased levels of several inflammation markers such as C-reactive protein (CRP), fibrinogen, and vWF/FVIIIc complex in the late stage of the DVT.[27,28] These could be enhancers of the coagulation process in some cases but may also be simple surrogate markers of endothelial dysfunction or hemostatic activation. Similarly, sVCAM-1 has been considered a sensitive marker of early endothelial dysfunction (even better than vWF or s-thrombomodulin in atherosclerosis),[29] although its expression on other cell types, such as leukocytes, implies that this interpretation must be viewed with caution. Anyway, sVCAM-1 seems not to react simply as an acute-phase protein because, unlike other acute phase proteins, it did not increase after a typical stimulus such as surgery.[3] Although very small, our sample of VTE patients with active cancer did not show further increased sVCAM-1 values ($p=0.9$).

Regarding new insights into this matter, we also examined the association of lipid concentrations and VTE. Increased plasma lipids are related to high levels of prothrombin[5] and other vitamin K-dependent factors[6] and can produce hypofibrinolysis through an Lp(a) effect[7,8] or through increased PAI-1 related to high TG concentrations.[9] A hypothetical clinical effect of the *in vitro* anticoagulant properties of HDL, described by Griffin *et al.*,[10] could be speculated. The relative risk reduction of DVT (22%) reported among older patients receiving statins also seems remarkable, even though the protection only marginally depends on the lipid-lowering effect and mainly on the drug itself.[11] Our data also support our second hypothesis but are less clear than those reported by Kawasaki *et al.*[12,13] Using a different cut-off value (250 instead of 220 mg/dL because of differences between Caucasians and Asians) we also concluded that hypercholesterolemia is associated with VTE, although to a lesser degree (OR= 2.1 instead of 2.6) or to a similar degree exclusively in males (OR= 2.7), ($p<0.01$). A cholesterol concentration higher than 220 mg/dL was also recently associated with DVT among Caucasians without thrombophilic risk factors in two reports by Vayá *et al.* The OR in these reports were 2.03[14] and 2.67;[15] according to our data (the exclusion of thrombophilic patients does not affect this association with OR= 2.7, $p<0.01$) their conclusions might be generalized.

In spite of the weak association found between cholesterol and VTE, the association was retained by the logistic regression analysis, even after including the BMI (data not shown). However, when considering HDL-c and LDL-c instead of TC (because of the co-linearity) the association was inconsistent until extreme values (HDL-c $<P_{10}$ and LDL-c $>P_{90}$)(Table 5).

Using less extreme cut-off points (HDL-c $<P_{20}$ and LDL-c $>P_{80}$) we would have agreed with McColl *et al.* who did not demonstrate an association with hypercholesterolemia but did with hypertriglyceridemia[16] (Table 5). The multivariate analysis of our data associated triglyceride levels with VTE among females as well. McColl *et al.* confined their report to women under 50 years old. The protective role of HDL-c is better established for arterial diseases and includes the inhibition of cytokine-dependent endothelial stimulus for the adhesion molecules.[30] Furthermore, HDL-c was related to a factor Va inactivation process acting *in vitro* as a protein C pathway cofactor[10] so a putative anticoagulant effect seems particularly applicable to VTE. Certainly, we found that extremely low HDL-c could be a risk factor for the VTE (mainly among males) but surprisingly, we did not observe any relation with APC-SR.

Other studies did not find any association between DVT and increased lipids in childood[7] or in middle

age.[17,18] Undersized samples in selected or particular populations, different criteria for choosing cut-offs and statistical methods could explain some of the quoted divergences. Our data in Tables 2, 3 and 5 suggest that by selecting different cut-off values we would reach alternative interpretations.

Concerning the third goal, the relationship between sVCAM-1 and lipids was different from that expected. sVCAM-1 showed a weak but inverse relation with serum concentrations of TC and LDL-c and no relation with triglycerides in either patients or controls. This could be explained because the relation between hyperlipidemia and sVCAM-1 seems to be indirect and conditioned by the atherosclerotic burden[2] in accordance with the observed stability of the sVCAM-1 values when lipid-lowering therapy operated successfully.[26] Furthermore the reported relationship between sVCAM-1 and triglycerides was mainly supported by studies including patients with severe hypertriglyceridemia (>475-500 mg/dL)[26] whereas no controls and only 1.6% of patients in our study had such high values. Our study confirms the strong association between obesity and VTE[15,16,18] in the general population and highlight large differences between the two genders.

The ORs for high sVCAM-1 or extreme lipid concentrations are lower than those established for some thrombophilia risk factors, such as heterozygous FV G1691A, but quite similar to others (FII 20210A allele).[31] They even exceed those described for prothrombin levels >1.15 U/mL (OR=2.1)[31] or fasting hyperhomocysteinemia (>16.6 mmol/L, OR= 1.9).[32] Considering interactions, numerous variables could modify the risk of VTE probably associated with increased sVCAM-1 levels, but dyslipidemia must be particularly prevalent and a high TC among males might be particularly serious.

As we were drafting this report, a paper stressing the association between atherosclerotic disease and spontaneous venous thrombosis was published. The authors concluded that either atherosclerosis can induce venous thrombosis or both processes may share common risk factors.[33] In any case, the matter is drawing great interest and we must consider that while fighting against atherosclerosis we are probably preventing numerous VTE episodes. Although both the observational character of our study and the small number of participants compel cautious interpretation of our data, assessing cholesterol fractions and triglycerides could be useful in the hazard evaluation of VTE in the general population. These analyses may be cost-effective for obvious public health reasons. Adding our data to recent evidence, we suggest a preliminary recommendation to correct any observed extreme lipid value, particularly among patients at a high risk of VTE.

Finally, sVCAM-1 might be a marker of a true VTE risk factor (VCAM-1). It fulfills a reasonable number of requirements: its precursor is localized in lesions and seems pathogenically related to the thrombotic process; we have demonstrated a progressive association between sVCAM-1 and VTE (strongest at higher levels) and an unusual rise in clinically severe or recurrent cases. However, the pathogenic mechanism of sVCAM-1 in VTE and its clinical relevance remain to be clarified, deserving experimental studies and prospective clinical investigations.

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Pre-publication Report & Outcomes of Peer Review

Contributions

AGO was the main investigator responsible for the study's conception and design, some laboratory assays, collection and interpretation of data and manuscript writing. JFC contributed to the design and statistical analysis. CFA, DMR and AGF obtained the clinical data of the participants. RV and AA performed several laboratory analyses. All the authors read and approved the manuscript after making their critical contribution. The criteria followed to assign the order of the authors are basically those related to qualification for authorship.

Disclosures

Conflict of interest: none.

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This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received May 19, 2003; accepted July 18, 2003.

In the following paragraphs, Professor Cazzola summarizes the peer-review process and its outcomes.

What is already known on this topic

Various observations suggest that circulating levels of VCAM-1 and lipds may represent risk factors for venous thromboembolism.

What this study adds

This study shows that patients with venous thromboembolism have higher concentrations of circulating VCAM-1 and a more abnormal lipid profile than controls.