Endothelial dysfunction in patients with spontaneous venous thromboembolism

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ABSTRACT

Background and Objectives

A high incidence of atherosclerotic lesions and cardiovascular events has been reported in patients with spontaneous venous thromboembolism. Endothelial dysfunction is an early marker of atherosclerosis and has predictive value for ischemic events. We have evaluated endothelial function in patients with a history of spontaneous venous thromboembolism.

Design and Methods

Patients with a history of symptomatic, objectively confirmed, spontaneous venous thromboembolism were included in a case-control study. Exclusion criteria were any known risk factors for cardiovascular diseases, other conditions associated with endothelial dysfunction, estro-progestinic therapy or pregnancy. Controls were age- (±5 years) and sex-matched subjects with the same exclusion criteria but without previous venous thromboembolism. Endothelial function was evaluated by the non-invasive measurement of flow-mediated vasodilation of the brachial artery and of plasma markers of endothelium activation; platelet activation parameters were also measured.

Results

Twenty-eight cases (8 females; mean age 59±15 years) and 28 controls (8 females; mean age 58±15) were studied. Flow-mediated vasodilation was 3.5±0.6% in cases (95% CIs: 2.2 to 4.8) and 5.7±0.6% (4.2 to 6.8) in controls (p=0.015). Brachial artery blood flow and hyperemic blood flow did not differ between the two groups. Plasma von Willebrand factor and soluble P-selectin levels were significantly higher in patients with venous thromboembolism, while plasma soluble CD40 ligand and urinary 11-dehydro-TxB_2 levels were similar in cases and controls.

Interpretation and Conclusions

Patients with spontaneous venous thromboembolism have endothelial dysfunction, unlike age- and sex- matched controls. This finding suggests that spontaneous venous thromboembolism may be a condition associated with an enhanced risk of atherosclerosis.

Key words: atherosclerosis, endothelium, thrombosis.

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Arterial and venous thromboses are major causes of morbidity and mortality in western countries. Due to their different pathogeneses and different clinical manifestations, arterial and venous thromboses have been considered as two separate disease entities. Indeed, arterial thrombi consist of platelet aggregates held together by small amounts of fibrin on the surface of atherosclerotic lesions, while venous thrombi are mainly composed of fibrin and red cells. On the other hand, both venous and arterial thrombi occur on the endothelial surface, which may thus profoundly influence the thrombotic process. The endothelium controls coagulant and anticoagulant interactions, mediates vascular vasodilatation, prevents platelet adhesion and activation and modulates smooth muscle cell proliferation and atherogenesis.[1,2] It is thus conceivable that a perturbation of the endothelium may represent a common pathogenetic background between venous thromboembolism (VTE) and arterial atherothrombosis.

Recently, in a prospective study on risk factors for pulmonary embolism (PE) in women, spontaneous venous thrombosis and atherosclerosis were found to share a number of risk factors, such as obesity, cigarette smoking and systemic arterial hypertension.[3] Another prospective study in men confirmed the association between cigarette smoking, obesity and PE.[4] A combined risk for both arterial and venous thrombosis has also been described in women on estro-progestinic therapy and in patients with lupus anticoagulant.[5] More recently, increased levels of lipoprotein(a), as well as hyperhomocysteinemia and the DD genotype of angiotensin converting enzyme (ACE), were shown to be independent risk factors for spontaneous VTE.[6-8] A higher prevalence of the metabolic syndrome has been detected in patients affected by spontaneous VTE than in patients with VTE associated with transient risk factors or in age and sex-matched healthy controls.[9]

In addition, the prevalence of asymptomatic carotid plaques has been reported to be significantly higher in patients with unprovoked VTE than in patients with secondary VTE or in age- and sex-matched controls.[10] Moreover, the incidence of myocardial infarction or stroke was significantly higher in patients with spontaneous, unprovoked PE than in patients with PE associated with transient risk factors in a recent study, PE being an independent predictor of cardiovascular events after adjusting for age.[11] Recently, a larger study confirmed that the incidence of ischemic cardiovascular events is significantly higher in patients with spontaneous VTE than in those with secondary VTE, as found at a median of 4 years follow-up after the diagnosis of VTE.[12] However, contrasting findings have been reported[13,14] and the issue of the association of spontaneous VTE and atherosclerosis is a subject of debate.[15]

Endothelial dysfunction is an early marker of atherosclerosis that derives from the impaired release of vasodilator and antithrombotic factors from the endothelium. Smoking, hypertension, high cholesterol levels, renal failure and obesity, as well as clinical conditions associated with increased levels of serum markers of chronic inflammation, such as C-reactive protein, are associated with endothelial dysfunction.[16] Several studies have shown a direct relation between the severity of endothelial dysfunction and the risk of initial or recurrent cardiovascular events.[16] The measurement of flow-mediated vasodilation (FMD) is a non-invasive method for the assessment of endothelial dysfunction, reflecting the ability of endothelial cells to induce nitric oxide (NO)-mediated vasodilation as a response to blood flow-provoked vessel wall shear stress. The evaluation of FMD of the brachial artery is a widely applicable method to screen, for endothelial dysfunction in large patient groups, and can be repeated over time. FMD is reduced in subjects with atherosclerosis and cardiovascular risk factors. More specifically, smoking,[17] high cholesterol levels,[18] arterial hypertension,[19] diabetes[20] and renal failure[21] are associated with NO-related endothelial dysfunction. In addition, FMD correlates with coronary vasodilator function and serological markers of endothelial perturbation.[22,23]

We evaluated endothelial function in patients treated for symptomatic, objectively confirmed spontaneous VTE and in age- and sex-matched healthy controls.

Design and Methods

Patients

Patients with a history of symptomatic, objectively documented, spontaneous deep vein thrombosis (DVT) of the proximal veins of the lower limbs, with or without PE, were included in a case-control study. Criteria for objectively documented VTE were a positive compression ultrasonography for DVT and a positive ventilation/perfusion lung scintigraphy and/or helical CT scan for PE. Spontaneous DVT was defined as that occurring in the absence of known cancer, known thrombophilia or of any transient risk factor for VTE.[24] The following criteria were necessary for inclusion into the study population: a history of spontaneous VTE, without abnormal findings concerning protein C, protein S, antithrombin activity, FV Leiden, prothrombin mutation, homocysteine levels, lupus anticoagulant or anticardiolipin antibodies. Patients were excluded from the study if they had risk factors for cardiovascular disease (smoking, arterial hypertension, hypercholesterolemia, diabetes, hyperhomocysteinemia), previous symptomatic manifestations of cerebro-, coronary or peripheral atherosclerosis or a family history of early atherosclerosis, other disease conditions known to be associated with endothelial dysfunction (heart failure, chronic renal failure, metabolic syndrome), and if they were taking estro-progestinic therapy or were pregnant. Smoking was defined as current or previous smoking; arterial hypertension as a blood pressure >140/90 mmHg; hypercholesteolemia as a total cholesterol concentration

>240 mg/dL; diabetes as a fasting plasma glucose level >126 mg/dL; and hyperhomocysteinemia, as a fasting homocysteine level >12 mmol/L. Total cholesterol, fasting plasma glucose and plasma homocysteine were measured in cases at the time of entry into the study by standard methods.

The control group was composed of age (±5 years) and sex-matched controls with the same exclusion criteria and without previous episodes of VTE. Exclusion of known risk factors for cardiovascular disease in controls was based on personal history and on previous analyses. Women in the fertile age were studied in the same phase of the menstrual cycle. For those patients with spontaneous VTE who were evaluated while on oral anticoagulant treatment, controls on anticoagulant treatment for atrial fibrillation or prosthetic heart valves, but with none of the exclusion criteria, were selected. Patients gave their informed, written consent to participate in the study. The study was approved by the Ethics Committees of the participating Institutions.

Flow-mediated vasodilation

All patients and controls underwent evaluation of endothelial function by the non-invasive assessment of FMD of the brachial artery, by B-mode ultrasonography, using a standardized procedure.[25] Subjects were examined in the morning (between 9 and 11 a.m.) in a quiet, temperature-controlled room, under fasting conditions; intake of caffeine in the morning of the evaluation was discouraged. Patients were checked for medical history, with particular attention to cardiovascular risk factors, medications and recent/current infections and the stage of menstrual cycle in women, before the examination was performed.

Ultrasonography of the brachial artery was carried out in the two centers by two expert operators, with a 10 MHz linear-array transducer and an Acuson 128 XP/10 System (Acuson, Mountain View, CA, USA) (n. 30 patients and controls) or an ESAOTE Caris 7230 (Esaote, Genova, Italy) (n. 26 patients and controls). The ultrasound evaluation was done on the right arm, with patients supine and kept at rest for 10 minutes before the evaluation. Blood pressure was measured by a sphygmomanometer before assessment of FMD.

The guidelines published by the International Brachial Arterial Reactivity Task Force were followed.[25] A blood pressure cuff was placed on the forearm, after which a baseline rest image was acquired. The brachial artery was imaged above the antecubital fossa in the longitudinal plane, a segment with clear anterior and posterior intima interfacies between the lumen and vessel wall was selected from continuous 2D gray scale imaging and recorded on super-VHS (basal diameter). Blood flow velocity was measured with a pulsed Doppler signal at a 70° angle to vessel, with the range gate (1.5 mm) in the center of the brachial artery. Basal blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by heart rate (continuously recorded by ECG) and cross-sectional vessel area. The cuff was then inflated to ≥50 mmHg above systolic blood pressure, to occlude arterial flow, for 5 minutes. The longitudinal image of the artery was recorded continuously from 30 sec before to 2 minutes after cuff deflation. The Doppler signal was obtained immediately after cuff release for a maximum of 15 seconds after cuff deflation to assess hyperemic flow. The increase in blood flow was calculated by dividing the maximum flow visualized in the first 15 seconds after cuff deflation by the flow at baseline.

Images recorded on super-VHS were digitized and the brachial artery diameter was measured offline, by an operator blinded as to the subjects and phase of the study, using M'ATH 2.0 software (Metris Argenteuil, France). Brachial artery diameter (basal and hyperemic) was measured at end diastole (onset of the R wave); four cardiac cycles were analyzed and averaged. FMD was measured as the change in post-stimulus diameter expressed as a percentage of the baseline diameter.[26]

Plasma and urinary markers of endothelial or platelet activation

Plasma, obtained by centrifugation of citrated venous blood at 3,000 g for 20 min, was immediately frozen and stored at -80°C for later assay. Levels of von Willebrand factor (vWF) activity were measured using an enzyme-linked immunosorbent assay kit (Axis-Shield, Scotland, UK). The wells of the microtiter plates are coated with a purified murine anti-vWf monoclonal antibody which recognizes a functional epitope of vWf. The results are expressed as the percentage of control.[27] The minimum detectable amount of vWf is typically 1.6%. Soluble P-selectin was also measured using an enzyme-linked immunosorbent assay kit (R&D System, Inc., Minneapolis, USA). This assay employs the quantitative sandwich immunoassay technique using a monoclonal antibody specific for soluble P-selectin precoated onto a microplate and a polyclonal antibody specific for soluble P-selectin conjugated to horseradish peroxidase.[27] The minimum detectable amount of soluble P-selectin is typically less than 0.5 ng/mL.

Soluble CD40L (sCD40L) was measured by an enzyme-linked immunosorbent assay (Bender MedSystem, Vienna, Austria). An anti-sCD40L monoclonal coating antibody is adsorbed onto microwells; sCD40L present in the sample binds to antibodies adsorbed to the microwells. A colored product is formed in proportion to the amount of sCD40L present in the sample.[28] The detection limit is 0.005 ng/mL.

The urinary excretion of 11-dehydro TxB_2 was evaluated by radioimmunoassay, as previously described.[29] Briefly, urine samples (40 mL) were taken from urine collected over a 24-hour period ending the morning of the FMD measurements, snap-frozen in liquid nitrogen and stored at -80°C until assay. Immunoreactive 11-dehydro-TxB_2 was assessed by a previously described gas chro-

matography/mass spectrometry–validated radioimmunoassay technique.[29]

Statistical analysis

Data are reported as mean±SEM together with 95% confidence intervals (CI). All data were analyzed with the D'Agostino-Pearson normality test and not normally distributed data were analyzed with the Mann-Whitney U-test; otherwise, data were analyzed with the unpaired Student's t-test. All tests were two tailed and a p value of <0.05 was considered statistically significant. For the correlation between different parameters, data were analyzed as separate observations by the Spearman's non-parametric test.

All analyses were performed using GraphPad Prism 4.00 for Windows software (GraphPad Software, San Diego California USA, *www.graphpad.com*). Stepwise general linear modeling with analysis of covariance (GLM-ANOVA) was performed to identify whether body mass index (BMI) is a predictor of FMD in our case series, using the NCSS 2001 software (Number Cruncher Statistical System, Kaysville, USA).

Results

Patients

Overall, 28 cases (8 females, mean age 59±2.8 years) and 28 matched controls (8 females, mean age 58±2.8 years) were included in the study. This case series was the result of the analysis of 312 consecutive patients with spontaneous VTE followed in the two centers. Most patients not enrolled had exclusion criteria, although one eligible patient did not give informed consent, and 42 were lost to follow-up. All the other patients meeting inclusion criteria were studied. Cases and controls were well matched for age, sex and BMI: all cases had fasting plasma glucose, total cholesterol and plasma homocysteine levels within normal limits (Table 1). Blood cell counts were within normal limits in cases (total red cell count: 4.4±0.5×10^9/L; white blood cell count: 6.7±0.5×10^9/L). Six subjects among cases and four among controls were on oral anticoagulants, of the latter two for atrial fibrillation and two for prosthetic heart valves (Table 1).

Flow mediated vasodilation

FMD was 3.5±0.6% in patients (95% CI: 2.2 to 4.8) and 5.7±0.6% (4.2 to 6.8) in controls (p=0.015) (Table 2). Brachial blood flow (88.0±9.3 vs 93.8±12.8 mL/min, respectively, p=0.72) and hyperemic blood flow (408.6±43.8 vs 448.2±52.9 mL/min, respectively, p=0.53) did not differ between the two groups. Basal brachial artery diameter was 4.3±0.1 mm in cases and 4.2±0.1 mm in controls (p=0.11), further confirming a good baseline comparability between the two groups (Table 2). Using the GLM ANOVA modeling, with FMD as the response variable, BMI ranking (low ≤23, medium≤23-28, high >28) as a factor variable, and BMI as a covariate, we found that FMD was not associated with BMI, and did not significantly differ between subjects with low, medium, or high BMI.

Table 1. Characteristics of the patients with spontaneous VTE (cases) and controls.

	Cases (n=28)	*Controls (n=28)*
Subjects, n	28	28
Age, years (mean ± SEM)	59±2.8	58±2.8
Gender, male	20	20
BMI (mean ± SEM)	26.9±0.9	24.5±0.6
OAC, n	6	4
Fasting plasma glucose (mg/dL)	98.7±2.6	93.6±1.9
Total cholesterol (mg/dL)	195.5±5.5	187.2±8.4
Red blood cell count (×10^{12}/L)	4.4±0.5	4.4±0.1
White blood cell count (×10^9/L)	6.7±0.5	7.5±0.4
Platelet count (×10^9/L)	199.6±10	212.1±12.4
Systolic BP mmHg (mean ± SEM)	135±1.8	131± 3.1
Diastolic BP mmHg (mean ± SEM)	82±1.2	80 ± 1.4
Type of event (DVT/DVT+PE)	19/9	0/0
Time from VTE, months (mean±SEM) (range)	25.8±5.2 (1-84)	

BMI: body mass index; OAC: on oral anticoagulants, BP: blood pressure; DVT: deep vein thrombosis, PE: pulmonary embolism; none of the parameters differed significantly between cases and controls.

Table 2. Brachial artery characteristics of VTE patients (cases) and controls.

	Cases (n=28)	*Controls (n=28)*	*p value*
Brachial artery basal diameter (mm)	4.3±0.1 (95%CI: 4.0 to 4.5)	4.2±0.1 (95%CI: 3.9 to 4.5)	0.11
Basal blood flow (mL/min)	88.0±9.3 (95%CI: 67.9 to 108)	93.8±12.8 (95%CI: 71.5 to 142.6)	0.72
Hyperemic blood flow (mL/min)	408.6±43.8 (95%CI: 314.8 to 502.5)	448.2±52.9 (95%CI: 360.3 to 596.2)	0.53
FMD absolute diameter increase (mm)	0.160±0.056 (95% CI: 0.044 to 0.276)	0.215±0.027 (95% CI: 0.160 to 0.270)	0.05
FMD (%)	3.5±0.6 (95% CI: 2.2 to 4.8)	5.7±0.6 (95% CI: 4.2 to 6.8)	0.015

FMD: flow-mediated dilatation.

Plasma and urinary markers of endothelial or platelet activation

Levels of vWF in plasma were significantly higher in cases than in controls (p=0.004) (Table 3). Similarly, soluble P-selectin plasma levels were significantly higher in cases than in controls (p=0.03). In contrast, plasma sCD40L did not differ between the two groups. Urinary 11-dehydro TxB_2 excretion was comparable in cases and controls, and the values were within the range of those found in healthy controls in our laboratory.[27,29]

Correlations

No correlation was found between FMD and soluble P-selectin, sCD40L or vWF levels; moreover neither sCD40L and vWF were correlated. In contrast, the levels of soluble P-selectin and vWF showed a statistically significant correlation (Spearman r =0.28, p=0.04).

Table 3. Plasma and urinary markers of endothelial or platelet activation.

	Cases (n=28)	*Controls (n=28)*	*p*
Plasma vWF (%)	150.1±9.2	117.7±9.7	0.004
Plasma soluble P-selectin (ng/mL)	41.4±7.2	24.1±2.9	0.03
Plasma sCD40L (ng/mL)	0.6±0.1	0.8±0.2	0.37
Urinary 11dehydro TxB_2 (pg/mg creatine)	463.3±48.4	549.7±62.6	0.28

Data are presented means±sem.

Discussion

Our study shows for the first time that patients with spontaneous VTE have impaired endothelial function, assessed by FMD, as compared to that in age and sex-matched controls. This finding was obtained in a population of VTE patients carefully selected to exclude any known risk factors or interfering conditions which might *per se* affect endothelial function, and thus shows that endothelial dysfunction is a peculiar, intrinsic characteristic of patients with unprovoked VTE. The magnitude of the impairment of FMD we observed in VTE patients is clinically relevant if compared with that in previous studies in other clinical conditions. In the Framingham heart study, for instance, compared with patients with a low Framingham score (lowest quintile), those with a high risk score (highest quintile) showed a difference of FMD of approximately the same magnitude we observed in our cases (-40%).[30] Moreover, in previous studies on the predictive value of FMD for the incidence of ischemic cardiovascular events in patients with peripheral arterial disease, the median FMD in patients having a vascular event during follow-up was approximately 25 to 40% lower than that of patients not experiencing events.[31,32] It is crucial to underline that the patients and controls we studied were carefully selected to exclude any confounding variable which might affect endothelial function. A subgroup of VTE patients were on oral anticoagulants, and this was taken into account by matching with controls on oral anticoagulants for atrial fibrillation or valve prosthesis. The small size of the sample does not allow subgroup analyses to be carried out. On the other hand, the presence of patients with cardiac diseases in the control group, if any, would have unbalanced the FMD values by reducing them in controls, thus further strengthening the evidence of endothelial dysfunction in patients with unprovoked VTE.

The impairment of endothelial function in patients with spontaneous VTE was associated with significantly higher levels of markers of endothelial activation in plasma, i.e. vWf and soluble P-selectin. Endothelial cells secrete Weibel Palade bodies in response to vascular injury, releasing vWf and externalizing P-selectin, which activates leukocyte trafficking. Endothelial exocytosis is one of the earliest responses to vascular damage and plays a pivotal role in thrombosis and inflammation. Several studies have reported that levels of soluble P-selectin and vWf antigen/activity in plasma are elevated in conditions associated with damage to the endothelium.[33,34] Our finding is consistent with a recent report showing elevation of endothelial microparticles and of other markers of endothelial activation in patients with VTE.[35] However, no alterations of platelet activation markers were observed in our patient population. In fact, sCD40L, a marker of *in vivo* platelet activation,[28] and the urinary excretion of 11 dehydroTxB_2, a sensitive indicator of *in vivo* platelet activation,[29] did not differ between patients with unprovoked VTE and healthy controls. The latter finding is in agreement with a recent study that failed to show, in patients with spontaneous VTE, any significant *in vivo* platelet activation.[36] The lack of *in vivo* platelet activation in patients with spontaneous VTE suggests that their defect is primarily endothelial; this is consistent with our previous observation that *in vivo* platelet hyperactivity is the consequence of associated risk factors, such as diabetes, hypercholesterolemia or smoking, and not of the vascular damage itself.[29]

P-selectin and vWF are also present in the storage granules of platelets and can be released in response to vascular injury. However, plasma vWF levels do not correlate with established platelet markers such as beta thromboglobulin, and aspirin, which is an inhibitor of platelet activation, has no effect on vWF levels. Therefore, abnormal plasma levels of vWF are thought to reflect mainly endothelial dysfunction and damage.[37] Similarly, platelets can also contribute to the increase in plasma soluble P-selectin. However, the fact that in our study the increases of soluble P-selectin and vWF were not associated with a

parallel increase of selective platelet activation markers (sCD40L and urinary 11-dehydro-TxB2) suggests that the plasma alterations we observed specifically reflect an endothelial perturbation. Indeed, soluble P-selectin and vWF levels correlated significantly in our study while no correlations were present between either soluble P-selectin or vWF and sCD40L. It remains to be established whether the endothelial dysfunction observed in patients with spontaneous VTE precedes the development of DVT or is simply a consequence of it.

While the latter hypothesis cannot be excluded, it seems unlikely considering that our patients were studied on average more than 2 years after the index event, a time by which the initial thrombus is completely recanalized in more than 70% of cases.[38] The impairment of endothelial function suggests the existence of a primary defect possibly acting as a common background to VTE and atherosclerosis. This finding confirms the biological plausibility of previous observations of a high incidence of atherosclerosis or arterial ischemic events in patients with spontaneous VTE.[11,12] Indeed, several observations establish a link between the role of the endothelium in arterial and venous thrombosis. Interestingly, endothelial microparticles of patients with myocardial infarction, similar to those detected in VTE patients,[35] impair endothelial NO-mediated relaxation of normal blood vessels.[39] Endothelial-derived NO participates in the regulation of venous tone in man,[40] and the impairment of arterial FMD in kidney failure[32] is associated with venous endothelial dysfunction which is corrected by L-arginine administration (the substrate for NO production) similarly to arterial endothelial dysfunction.[41]

We have previously shown that patients with unprovoked VTE present a high prevalence of atherosclerotic lesions, in a manner not related to the presence of risk factor for atherosclerosis,[10] and others have shown a significant association between VTE and coronary artery calcification.[42] The fact that impaired FMD has a predictive value for major cardiovascular events in different clinical settings[16] further strengthens the association between spontaneous VTE and atherosclerotic disease. Further clues to this association are the existence of common risk factors for VTE and atherosclerosis,[3,5] data suggesting a protective effect of statins on the incidence of VTE,[43] and recent findings that a dietary pattern reducing cardiovascular events is also associated with a lower risk of VTE.[44]

The present study has several limitations, including variability in the main end-point, which is known to be subject to possible methodologic artifacts.[25] However, in this study we minimized this problem by limiting FMD evaluation to two expert operators and by calculating FMD and blood flow centrally in a blind fashion. Moreover, it is known that FMD is influenced by basal arterial diameter:[25] in our study this was comparable in cases and controls, excluding that this variable may have affected the results. Another limitation is the lack of the assessment of nitroglycerin-induced (endothelial-independent) vasodilation; however, the aim of our study was to assess endothelium-dependent vasodilation, thus we decided not to submit patients and controls to unnecessary nitroglycerin administration. An additional limitation is that the absence of known risk factors for cardiovascular disease in controls was determined based on personal history and previous analyses thus it can not be excluded that some of the controls might indeed have had one or more unknown risk factors; however, this is unlikely because controls were mainly selected from among volunteer members of staff who were carefully instructed about the reasons of the study. Moreover, had some controls been indeed not completely devoid of risk factors, this would have reduced, and not enhanced, the difference from cases.

Conclusions

In conclusion, patients with spontaneous VTE have impaired endothelial function as compared to that in age-and sex-matched controls. This supports the suggestion that spontaneous VTE may be a condition at enhanced risk of atherosclerotic complications.

Authors' Contributions

MR and GP designed the research and analyzed the data; GP wrote the article, obtained funding; MR, BC, PR, DG, VMC, FE, CG and DVF contributed to data collection; GG performed the statistical analysis; MR, BC, PR, DG, PP and AG performed the critical revision of the article; all the authors approved the final version to be published.

Conflict of Interest

The authors reported no potential conflicts of interest.

References

1. Wiloughby S, Loscalzo J. Vascular control of platelet function. In: Platelets in thrombotic and non thrombotic disease: Pathophysiology, Pharmacology and Therapeutics. Gresele P, Page CP, Fuster V and Vermylen J, eds. Cambridge University Press, Cambridge, 2002. p. 432-55.
2. Reichenbach G, Momi S, Gresele P. Nitric oxide and its antithrombotic action in the cardiovascular system. Curr Drug Targets Cardiovasc Haematol Disord 2005;5:65-74.
3. Goldhaber SZ, Grodstein F, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, et al. A prospective study of risk factors for pulmonary embolism in women. JAMA 1997;277:642-5.
4. Tsai AW, Cushman M, Rosamond WD, Heckbert SR, Polak JF, Folsom AR. Cardiovascular risk factors and venous thromboembolism incidence. Arch Intern Med 2002;162:1182-9.
5. Rosendaal FR, Helmerhorst FM, Vandenbroucke JP. Female hormones and thrombosis. Arterioscler Thromb Vasc Biol 2002;22:201–10.
6. Marcucci R, Liotta AA, Cellai AP, Rogolino A, Gori AM, Giusti B, et al. Increased plasma levels of lipoprotein(a) and the risk of idiopathic and recurrent venous thromboembolism. Am J Med 2003;115:601-5.
7. Cattaneo M. Hyperomocysteinemia: a risk factor for arterial and venous thrombotic disease. Int J Clin Lab Res 1997;27:139-44.
8. Fatini C, Gensini F, Sticchi E, Batta-

glini B, Prisco D, Fedi S, et al. ACE DD genotype: an independent predisposition factor to venous thromboembolism. Eur J Clin Invest 2003; 33:642–7.
9. Ageno W, Prandoni P, Romualdi E, Ghirarduzzi A, Dentali F, Pesavento R, et al. The metabolic syndrome and the risk of venous thrombosis: a case-control study. J Thromb Haemost 2006;4:1914-8.
10. Prandoni P, Bilora F, Marchiori A, Bernardi E, Petrobelli F, Lensing AW, et al. An association between atherosclerosis and venous thrombosis. N Engl J Med 2003;348:1435-41.
11. Becattini C, Agnelli G, Prandoni P, Silingardi M, Salvi R, Taliani MR, et al. A prospective study on cardiovascular events after acute pulmonary embolism. Eur Heart J 2005;26:77-83.
12. Prandoni P, Ghirarduzzi A, Prins MH, Pengo V, Davidson BL, Sorensen H, et al. Venous thromboembolism and the risk of subsequent symptomatic atherosclerosis. J Thromb Haemost 2006;4:1891-6.
13. Reich LM, Folsom AR, Key NS, Boland LL, Heckbert SR, Rosamond WD, et al. Prospective study of subclinical atherosclerosis as a risk factor for venous thromboembolism. J Thromb Haemost 2006;4:1909-13.
14. van der Hagen PB, Folsom AR, Jenny NS, Heckbert SR, O'Meara ES, Reich LM, et al. Subclinical atherosclerosis and the risk of future venous thrombosis in the Cardiovascular Health Study. J Thromb Haemost 2006; 4:1903-8.
15. Agnelli G, Becattini C. Venous thromboembolism and atherosclerosis: common denominators or different diseases? J Thromb Haemost 2006;4:1886-90.
16. Lerman A, Zeiher AM. Endothelial function: cardiac events. Circulation 2005;111:363-8.
17. Migliacci R, Gresele P. Smoking and impaired endothelium-dependent dilatation. N Engl J Med 1996;334: 1674.
18. Felmeden DC, Spencer CGC, Blann AD, Beevers DG, Lip GYH. Low-density Lipoprotein Subfractions and cardiovascular risk in hypertension. relationship to endothelial dysfunction and effects of teatment. Hypertension 2003;41:528-33.
19. Perticone F, Sciacqua A, Maio R, Perticone M, Maas R, Boger RH, et al. Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. J Am Coll Cardiol 2005;46:518–23.
20. Rodriguez CJ, Miyake Y, Grahame-Clarke C, Di Tullio MR, Sciacca RR, Boden-Albala B, et al. Relation of plasma glucose and endothelial function in a population-based multiethnic sample of subjects without diabetes mellitus. Am J Cardiol 2005;96: 1273-7.
21. Migliacci R, Falcinelli F, Imperiali P, Floridi A, Nenci GG, Gresele P. Endothelial dysfunction in patients with kidney failure and vascular risk factors: acute effects of hemodialysis. Ital Heart J 2004;5:371-7.
22. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995; 26:1235–41.
23. Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, Halligan S, et al. Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: A statement by the Working Group on Endothelin and endothelial factors of the European Society of Hypertension. J Hypertens 2005; 23:7-17.
24. Agnelli G, Prandoni P, Santamaria MG, Bagatella P, Iorio A, Bazzan M, et al. Three months versus one year oral anticoagulant therapy for idiopathic deep venous thrombosis. N Engl J Med 2004;345:165-9.
25. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Internal brachial artery reactivity task force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. A report of the International brachial artery reactivity task force. J Am Coll Cardiol 2002;39:257-67.
26. Gresele P, Migliacci R, Procacci A, De Monte P, Bonizzoni E. Prevention by a nitric oxide donatine aspirin, but not by aspirin, of the acute endothelial dysfunction induced by exercise in patients with intermittent claudication. Thromb Haemost 2007;97: 444-50.
27. Gresele P, Guglielmini G, De Angelis M, Ciferri S, Ciofetta M, Falcinelli E, et al. Acute, short-term hyperglycemia enhances shear stress-induced platelet activation in patients with type II diabetes mellitus. J Am Coll Cardiol 2003; 41:1013-20.
28. Ahn ER, Lander G, Jy W, Bidot CJ, Jimenez JJ, Horstman LL, et al. Differences of soluble CD40L in sera and plasma: implications on CD40L assay as a marker of thrombotic risk. Thromb Res 2004; 114:143-8.
29. Davi G, Gresele P, Violi F, Basili S, Catalano M, Giammarresi C, et al. Diabetes mellitus, hypercolesterolemia and hypertension, but not vascular disease per se, are associated with persistent platelet activation in vivo: evidence derived from the study of peripheral arterial disease. Circulation 1997; 96:69-75.
30. Benjamin EJ, Larson MG, Keyes MJ, Mitchell GF, Vasan RS, Keaney JF Jr, et al. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. Circulation 2004; 109:613-9.
31. Brevetti G, Silvestro A, Schiano V, Chiariello M. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle-brachial pressure index. Circulation 2003; 108:2093-8.
32. Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, et al. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. J Am Coll Cardiol 2003; 41:1769-75.
33. Khare A, Shetty S, Ghosh K, Mohanty D, Chatterjee S. Evaluation of markers of endothelial damage in cases of young myocardial infarction. Atherosclerosis. 2005;180:375-80.
34. Blann AD. Plasma von Willebrand factor, thrombosis, and the endothelium: the first 30 years. Thromb Haemost 2006;95:49-55.
35. Chirinos JA, Heresi GA, Velasquez H, Jy W, Jimenez JJ, Ahn E, et al. Elevation of endothelial microparticles, platelets, and leukocyte activation in patients with venous thromboembolism. J Am Coll Cardiol 2005;45:1467-71.
36. Gerdsen K, Weber M, Langer F, Eifrig B, Lindhoff-Last E. Platelet activation markers in patients with venous thromboembolism without predisposing factors. Pathophysiol Haemost Thromb 2005;34:1-5.
37. Brunner H, Cockcrofr JR, Deanfield J, Donald A, Ferrannini E, Halcox J, et al. Endothelial function and dysfunction. Part I: association with cardiovascular risk factors and diseases. A statement by Working Group on Endothelial Factors of the Europena Society of Hypertension. J Hypertens 2005;23:233-46.
38. Prandoni P. Risk factors of recurrent venous thromboembolism: the role of residual vein thrombosis. Pathophysiol Haemost Thromb 2003/ 2004; 33:351-3.
39. Boulanger CM, Scoazec A, Ebrahimian T, Henry P, Mathieu E, Tedgui A, et al. Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. Circulation 2001;104:2649-52.
40. Blackman DJ, Morris-Thurgood JA, Atherton JJ, Ellis GR, Anderson RA, Cockcroft JR, et al. Endothelium-derived nitric oxide contributes to the regulation of venous tone in humans. Circulation 2000;101:165-70.
41. Hand MF, Haynes WG, Webb DJ. Hemodialysis and L-arginine, but not D-arginine, correct renal failure-associated endothelial dysfunction. Kidney Int 1998;53:1068-77.
42. Hong C, Zhu F, Du D, Pilgram TK, Sicard GA, Bae KT. Coronary artery calcification and risk factors for atherosclerosis in patients with venous thromboembolism. Atherosclerosis 2005;183:169-74.
43. Ray JG, Mamdani M, Tsuyuki RT, Anderson DR, Yeo EL, Laupacis A. Use of statins and the subsequent development of deep vein thrombosis. Arch Intern Med 2001; 161:1405-10.
44. Steffen LM, Folson AR, Cushman M, Jacobs DR, Rosamond WD. Greater fish, fruit and vegetable intakes are related to lower incidence of venous thromboembolism. Circulation 2007; 115:188-95.