CLINICAL RESEARCH

Factors influencing the level of circulating procoagulant microparticles in acute pulmonary embolism

Facteurs influençant le niveau des microparticules procoagulantes dans l’embolie pulmonaire

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KEYWORDS
Microparticles; Pulmonary embolism; Cardiovascular risk factors

Summary

\textit{Background.} — Flow cytometry has shown levels of platelet-derived microparticles (PMPs) and endothelial-derived microparticles (EMPs) to be elevated in deep-vein thrombosis. Cardiovascular risk factors can also contribute to hypercoagulability due to circulating procoagulant microparticles (CPMPs).

\textit{Aims.} — To investigate in a case-control study the respective contribution of pulmonary embolism and cardiovascular risk factors to the level of hypercoagulability due to CPMPs.

\textit{Methods.} — CPMP, PMP and EMP levels were measured in 45 consecutive patients (age 67.9 ± 11.6 years; 66.7% men) admitted to an intensive care unit for acute pulmonary embolism (APE), 45 healthy control subjects with no history of venous thromboembolism or vascular risk

\textit{Abbreviations:} APE, acute pulmonary embolism; Controls\textsubscript{CVRFs}, controls with cardiovascular risk factors; Controls\textsubscript{noCVRFs}, controls without cardiovascular risk factors; CPMP, circulating procoagulant microparticle; EMP, endothelial-derived microparticle; IQR, interquartile range; PMP, platelet-derived microparticle; VTE, venous thromboembolism.

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factors (Control\textsubscript{noCVRFs}), and 45 patients with cardiovascular risk factors (Control\textsubscript{CVRFs}). APE was diagnosed by spiral computed tomography or scintigraphy. CPMP levels were assessed using a prothrombinase assay on platelet-depleted plasma (results expressed as nmol/L equivalent).

**Results.** CPMP levels were higher in APE patients than in Control\textsubscript{noCVRFs} (medians 4.7 vs 3.2 nmol/L, interquartile ranges [IQRs] 2.9—11.1 vs 2.3—4.6 nmol/L; \(p = 0.02\)). Similar results were reported for PMPs (medians 2.2 vs 1.9 nmol/L, IQRs 1.7—5.8 vs 1.4—2.4 nmol/L; \(p = 0.02\)), whereas EMP levels were not significantly different. However, CPMP procoagulant activity was not significantly different in APE patients and Control\textsubscript{CVRFs}.

**Conclusions.** CPMPs and PMPs were significantly elevated in APE patients vs Control\textsubscript{noCVRFs}, but this correlation was not significant when APE patients were compared with Control\textsubscript{CVRFs}. Our observations highlight the importance of adjusting for the presence of cardiovascular risk factors in conditions in which microparticle levels are raised.

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**Introduction**

According to Virchow’s triad, the pathophysiology of VTE relies on the presence of blood hypercoagulability, stasis of blood flow and vessel-wall damage [1]. The factors involved in venous thrombogenesis could be defined as soluble coagulant factors, dysfunctional endothelium and circulating cells, especially platelets, lymphomonocytes and, potentially, CPMPs [2,3].

CPMPs are plasma membrane fragments that are released into the blood by stimulated cells during activation or apoptosis, and carry procoagulant phosphatidylserine and tissue factor on their surface [4—7]. CPMPs circulate in healthy humans and support low-grade generation of thrombin [8]. High levels of CPMPs have been reported in several scenarios, including, for example, in patients with an acute coronary syndrome [9—11] or atrial fibrillation [12]. Cardiovascular risk factors such as hypertension and diabetes [13—15] have been associated with elevated CPMPs and their
phenotypes. In interpreting the role of CPMPs in thrombogenesis, we need to take into account potential cofounders, the most prevalent of which appear to be cardiovascular risk factors.

Two previous clinical studies have reported elevated levels of CPMPs — mainly PMPs and EMPs — by flow cytometry, in patients hospitalized for deep-vein thrombosis. However, they had different phenotypic representations, and the authors did not take into account potential confounders, such as cardiovascular risk factors [16,17]. Experimental studies in vivo are supportive of the involvement of human cell-derived microparticles in venous thrombogenesis in a tissue factor-dependent manner, and have described a correlation of leukocyte- and PMPs with thrombus weight and tissue factor activity [18,19]. Recently, the crucial participation of circulating tissue factor-bearing microparticles released by tumour cells in cancer-associated hypercoagulability has been emphasized, depending on both the tumour cell origin and a critical threshold of microparticles [20—24]. In addition, we have shown in a case-control study that CPMP levels, defined by their procoagulant activity, were elevated in patients without cancer hospitalized with APE, and we analysed the influence of cardiovascular risk factors on this correlation [25].

Methods

Study subjects

Between November 2004 and December 2005, we included 45 consecutive patients admitted to our intensive care unit for APE associated with or without deep-vein thrombosis, and compared them with 45 healthy controls without (ControlsnoCVRFs) and 45 patients with (ControlsCVRFs) cardiovascular risk factors, matched for age and sex. ControlsCVRFs and patients with APE were also matched for the presence of hypertension. Demographic and clinical characteristics were recorded prospectively upon enrolment. The study was approved by the institutional review board and was performed in accordance with institutional guidelines. All patients gave written informed consent before participating in the study.

Acute pulmonary embolism cases

APE was confirmed by spiral computed tomography (n = 25), ventilation-perfusion scintigraphy (n = 20) or both (n = 12). Treatment on admission consisted of standard antithrombotic therapy with low-molecular-weight or unfractionated heparin. Exclusion criteria were conditions known or suspected to increase levels of CPMPs independently, such as acute coronary syndromes, acute heart failure, stroke, sepsis, chronic inflammatory disease, antiphospholipid syndrome, heparin-induced thrombocytopenia, thrombotic thrombocytopenic purpura and atrial fibrillation.

Transient VTE risk factors were defined as pregnancy, oestrogen therapy, surgery (< 60 days), trauma, confined to bed (> 5 days) and recent journey (> 10 hours). Cancer and thrombophilia were defined as chronic VTE risk factors. Haemodynamic status was considered over three levels: submassive APE (stable haemodynamics with signs of right heart failure on transthoracic echocardiography), massive APE (unstable haemodynamics with right heart failure on transthoracic echocardiography) and shock.

Controls with cardiovascular risk factors

ControlsCVRFs comprised patients with no history of VTE or atrial fibrillation who were undergoing routine screening physical examinations for cardiac symptoms at our outpatient cardiology clinic, with an electrocardiogram documenting sinus rhythm.

Controls without cardiovascular risk factors

ControlsnoCVRF included patients undergoing screening examination before orthopaedic surgery, with no known cardiovascular risk factors, history of atrial fibrillation, prior VTE, clinical evidence of disease or current cardiovascular treatment, and who had an electrocardiogram documenting sinus rhythm. These subjects were assessed by careful examination of their medical histories and by blood tests.

Circulating procoagulant microparticles

Blood was collected in the acute phase when the diagnosis of VTE was assessed and just before anticoagulation was started. Measurement of CPMPs was performed as described previously [26], with minor modifications.

Preparation of circulating procoagulant microparticle samples

All microparticle determinations were performed strictly according to Biro et al. [18]. Briefly, citrated blood was taken soon after admission and centrifuged at 1500 g for 15 min at room temperature within the hour after sampling. The supernatant was centrifuged again at 13,000 g for 2 min to avoid platelet contamination. Thrombin and factor Xa inhibitors (o-phenylalanyl-prolyl-arginy1 chloromethyl ketone and 1,5-dansyl-glutamyl-glycyl-arginy1 chloromethyl ketone, respectively) were added to plasma samples at a final concentration of 50 μM each, and CaCl₂ at a final concentration of 50 mM.

Quantitation of circulating procoagulant microparticles

After capture of microparticles onto annexin V-coated wells (for 30 min at 37°C), taking advantage of the strong affinity of annexin V for aminophospholipids present in microparticles at the calcium concentration used, four washing steps were performed with Tris buffer containing 1 mM CaCl₂ and 0.05% Tween 20, each for 5 min at 20°C, and the last one without Tween. The phosphatidylserine content of microparticles, directly responsible for their procoagulant activity, was then measured in a prothrombinase assay. Microparticles were incubated with factor Xa (50 pmol/L), factor Va (360 pmol/L), prothrombin (1.3 μmol/L) and 2.3 mmol/L CaCl₂ for 15 min at 37°C, and linear absorbance changes were recorded at 405 nm after the addition of chromozym TH (380 μmol/L).
Quantification of platelet-derived microparticles and endothelial-derived microparticles

After specific capture of PMPs onto anti-glycoprotein Ib antibody-coated wells and of EMPs onto anti-CD31 antibody-coated wells, quantitation was achieved after several washing steps using a prothrombinase assay as described above. Microparticle levels are expressed as nmol/L of phosphatidylserine equivalent.

Miscellaneous measurements

Quantification of C-reactive protein was determined by immunonephelometric tests and circulating brain natriuretic peptide levels by enzyme immunoassays.

Transthoracic echocardiography

To evaluate right ventricular dysfunction and haemodynamic status, transthoracic echocardiography was performed at the time of admission in all patients with APE. Systolic transtricuspid pressure gradient and left ventricular ejection fraction were also measured.

Statistical analysis

Based on previous studies[11,27,28], we hypothesized that patients with APE would have microparticle levels increased by approximately two standard deviations compared with healthy controls and by one standard deviation compared with subjects without APE but with cardiovascular risk factors. To achieve this with 90% power and \( p < 0.05 \) between the three groups, 35 subjects per group were required. To minimize the risk of a type II error and to account for possible confounders, we recruited in excess of this number of patients with APE and controls.

Categorical variables, expressed as percentages, were compared using the Chi² test or Fisher’s exact test. All analyses were performed using STATA 9 statistical software (STATA, College Station, TX, USA). A probability value of 0.05 was considered statistically significant.

Results

The baseline characteristics of the three groups are given in Table 1. The mean age of patients with APE was 67.9 ± 11.6 years and 66.7% were men. Deep-vein thrombosis was documented in 71.1% of patients, 26.7% (\( n = 12 \)) had haemodynamic instability and 4.4% (\( n = 2 \)) presented in cardiogenic shock. APE was submassive in 17 (37.8%) patients. Sixteen (35.5%) patients with APE had at least one transient VTE risk factor and eight (17.8%) had a permanent risk factor (cancer, \( n = 3 \); thrombophilia, \( n = 5 \)). APE was idiopathic in 21 (46.7%) patients.

There were no significant differences in terms of clinical cardiovascular risk factors between patients with APE and ControlsCVRFs, except for current smoking (Table 1). In contrast, by design, ControlsnoCVRFs were significantly different from the other two groups with regard to clinical cardiovascular risk factors. However, there was no significant difference regarding age and sex between ControlsCVRFs and ControlsnoCVRF, even if there was a slight predominance of men in the former.

Annexin-positive microparticles in acute pulmonary embolism patients and controls

Annexin V-positive microparticle levels were higher in patients with APE (median 4.7 nmol/L, IQR 2.9—11.1 nmol/L) than in ControlsnoCVRFs (median 3.2 nmol/L, IQR 2.3—4.6 nmol/L; \( p = 0.02 \)), but there was no significant difference compared with ControlsCVRFs (median 4.9 nmol/L, IQR 3.7—8.4 nmol/L; \( p = 0.99 \)) (Fig. 1). Annexin V-positive microparticle levels were significantly higher in ControlsCVRFs than in ControlsnoCVRFs (\( p = 0.01 \); Fig. 1).

Moreover, after adjustment for age, sex and hypertension, CPMPs were significantly correlated with C-reactive protein (\( p = 0.02 \)) and brain natriuretic peptide levels (\( p < 0.01 \), but not with the echographic haemodynamic status evaluated by systolic transtricuspid pressure gradient and left ventricular ejection fraction (Table 2).

Platelet-derived microparticles in acute pulmonary embolism patients and controls

PMP levels were not significantly different between patients with APE (median 2.2 nmol/L, IQR 1.7—5.8 nmol/L) and ControlsCVRFs (median 5.5 nmol/L, IQR 2.6—11.3 nmol/L; \( p = 0.23 \));
<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>APE (n = 45)</th>
<th>ControlsCVRFs (n = 45)</th>
<th>ControlsnoCVRFs (n = 45)</th>
<th>P value APE vs ControlsCVRFs</th>
<th>P value APE vs ControlsnoCVRFs</th>
<th>P value ControlsCVRFs vs ControlsnoCVRFs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.9 ± 11.6</td>
<td>67.1 ± 9.9</td>
<td>67.0 ± 9.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Men</td>
<td>30 (66.7)</td>
<td>30 (66.7)</td>
<td>26 (57.8)</td>
<td>—</td>
<td>0.38</td>
<td>0.38</td>
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<td>Hypertension</td>
<td>25 (55.6)</td>
<td>25 (55.6)</td>
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<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7 (15.6)</td>
<td>9 (20.0)</td>
<td>0</td>
<td>0.58</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>19 (42.2)</td>
<td>14 (31.1)</td>
<td>0</td>
<td>0.27</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Current smoker</td>
<td>4 (9.1)</td>
<td>16 (35.6)</td>
<td>0</td>
<td>&lt; 0.01</td>
<td>0.04</td>
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<tr>
<td>Coronary artery disease</td>
<td>3 (6.8)</td>
<td>9 (20.0)</td>
<td>0</td>
<td>0.07</td>
<td>0.08</td>
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<td>History of heart failure</td>
<td>1 (2.2)</td>
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<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>History of TIA or ischaemic stroke</td>
<td>2 (4.4)</td>
<td>1 (2.2)</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>History of atrial fibrillation</td>
<td>6 (13.3)</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Concomitant treatment</td>
<td></td>
<td></td>
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<tr>
<td>Aspirin</td>
<td>8 (17.8)</td>
<td>1 (2.2)</td>
<td>—</td>
<td>0.01</td>
<td>—</td>
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<tr>
<td>Beta-blocker</td>
<td>9 (20.5)</td>
<td>10 (22.2)</td>
<td>—</td>
<td>0.84</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>6 (13.3)</td>
<td>4 (8.9)</td>
<td>—</td>
<td>0.50</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Angiotensin-converting enzyme inhibitor</td>
<td>7 (15.9)</td>
<td>7 (15.6)</td>
<td>—</td>
<td>0.96</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diuretic</td>
<td>8 (17.8)</td>
<td>0</td>
<td>—</td>
<td>&lt; 0.01</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Nitrate</td>
<td>1 (2.2)</td>
<td>1 (2.2)</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Insulin</td>
<td>0</td>
<td>2 (4.4)</td>
<td>—</td>
<td>—</td>
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</tr>
<tr>
<td>Oral antidiabetic therapy</td>
<td>2 (4.4)</td>
<td>6 (13.3)</td>
<td>—</td>
<td>0.14</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation or number (%).
APE: acute pulmonary embolism; ControlsCVRFs: controls with cardiovascular risk factors; ControlsnoCVRFs: controls without cardiovascular risk factors; TIA: transient ischaemic attack.
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<table>
<thead>
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<th>Table 2</th>
<th>Baseline correlates of annexin V-positive microparticles and platelet and endothelial microparticle levels in 45 patients with an acute pulmonary embolism.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Annexin V-positive MPs</strong></td>
<td><strong>Clinical presentation</strong></td>
</tr>
<tr>
<td></td>
<td><strong>No. of patients (%)</strong></td>
</tr>
<tr>
<td><strong>Acute DVT</strong></td>
<td>32 (71.1)</td>
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<tr>
<td><strong>Clinical presentation</strong></td>
<td>25 (55.6)</td>
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<tr>
<td><strong>Baseline characteristics</strong></td>
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<td>19 (42.2)</td>
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<tr>
<td><strong>Current smoker</strong></td>
<td>4 (9.1)</td>
</tr>
<tr>
<td></td>
<td>3 (6.8)</td>
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<tr>
<td><strong>Coronary artery disease</strong></td>
<td>10 (22.2)</td>
</tr>
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<td>6 (13.3)</td>
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<td><strong>Biological criteria</strong></td>
<td><strong>C-reactive protein</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>Echographic criteria</strong></td>
<td><strong>L VEF &lt; 40%</strong></td>
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<td><strong>Platelet-derived MPs (anti-GP1b)</strong></td>
<td><strong>Clinical presentation</strong></td>
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<td><strong>No. of patients (%)</strong></td>
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<td><strong>Shock</strong></td>
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<td><strong>Acute DVT</strong></td>
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<tr>
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<td><strong>Echographic criteria</strong></td>
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<tr>
<td><strong>Endothelial-derived MPs (anti-CD31)</strong></td>
<td><strong>Clinical presentation</strong></td>
</tr>
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<td><strong>No. of patients (%)</strong></td>
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<td><strong>Shock</strong></td>
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<td><strong>Acute DVT</strong></td>
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Endothelial-derived microparticles in acute pulmonary embolism patients and controls

EMP levels were not significantly different between patients with APE (median 0.1 nmol/L, IQR 0.03–0.2 nmol/L) and

ControlsCVRFs (median 0.2 nmol/L, IQR 0.1–0.2 nmol/L; \( p = 0.44 \)) (Fig. 3). EMP levels were not higher in patients with APE compared with ControlsnoCVRFs (median 0.1 nmol/L, IQR 0.01–0.1 nmol/L; \( p = 0.54 \)). EMP levels in ControlsCVRFs were not significantly higher than in ControlsnoCVRFs (\( p = 0.06 \)).

After adjustment for age, sex and hypertension, EMPs were only correlated with brain natriuretic peptide levels (\( p = 0.026 \)) (Table 2).

Baseline characteristics and circulating procoagulant microparticles

Relations between other baseline characteristics and CPMPs (annexin V-positive, PMPs and EMPs) were investigated among patients with APE after adjustment for age, sex and hypertension (Table 2). Current smoking, significantly represented in ControlsCVRFs (Table 1; \( p = 0.003 \)), appeared to influence CPMP and PMP levels among patients with APE.
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Discussion

To our knowledge, this is the first study to compare CPMP levels and their phenotypes (using a functional assay based on prothrombinase) in APE patients. Comparing APE patients with two different control groups, our study design allowed us to analyse the relative effect of cardiovascular risk factors and APE on microparticle levels. In APE patients, CPMP procoagulant activity is significantly increased compared with the physiological status represented by a population with no prior thrombotic events or cardiovascular risk factors ($p = 0.02$). Nevertheless, procoagulant activity related to CPMPs in APE patients was not significantly different to that in ControlsCVRF.

Microparticles and venous thromboembolism

VTE results from an imbalance between procoagulant, anticoagulant and fibrinolytic activities. Platelet and endothelial microparticles are considered markers of ongoing or recent endothelial cell and platelet activation, or apoptosis. Microparticle procoagulant activity, mainly related to tissue factor activity in the presence of phosphatidylserine phospholipids, is dependent on the cellular origin, the initial stimulus and the secondary microparticle-induced cell activation [4,18,29]. Experimental animal studies with high-resolution online videomicroscopy have revealed that circulating microparticles mediate the accumulation of tissue factor on platelet-rich thrombi, and also in the venous thrombosis model [18,30]. Demonstrating the increased procoagulant activity related to CPMPs in APE patients vs ControlsCVRF, our results argue in favour of their functional participation in venous thrombogenesis in the interface of soluble coagulants factors, circulating cells and dysfunctional endothelium. As far as microparticle subtypes are concerned, only PMPs appeared to be significantly elevated in APE patients vs ControlsCVRF. We have to take into account the lower detection limits in the estimation of EMP involvement in APE to interpret this result, in terms of the variability in antibody affinity (CD31 vs CD62E or CD144), as in the availability of the antigen or in the level of endothelial markers borne by microparticles [31]. However, this feature has been used in studies reported by other groups to measure EMPs (CD31) and PMPs (glycoprotein 1b) distinctly [16]. In the future, more specific endothelial phenotypes, such as CD62E/CD144/CD146, should be targeted as a priority.

Data emerging from the literature are quite discordant concerning microparticles and phenotypes involved in APE, mainly because of the different techniques and methodological approaches used. To our knowledge, there are two previous clinical reports on this subject that differ from our study in terms of the design, the method of microparticle quantitation, the population studied and the comparator used [16,17]. In addition, increasing data correlated the risk of deep-vein thrombosis in patients with cancer to tissue factor-bearing tumour cell-derived microparticle levels measured by functional test or flow cytometry, with a pathophysiological role supported by P-selectin glycoprotein ligand 1 (or other mucin-like mucoproteins) and P-selectin interaction [21,23].

Relation between cardiovascular risk factors and microparticles in venous thromboembolism

Our observations highlight the importance of adjusting for the presence of cardiovascular risk factors in conditions in which microparticle levels are raised.

Diabetes [13,15,32] and hypertension [14] are associated with endothelial dysfunction and platelet activation, and increase CPMP levels. In a previous report, our group [15] found a higher level of CPMPs in patients with type 2 diabetes compared with healthy subjects. A strong positive correlation was also found between endothelial and platelet microparticle levels on the one hand, and the absolute level of both systolic and diastolic blood pressures on the other [14]. These findings and the present data emphasize that cardiovascular risk factors, especially hypertension, are confounders in the previously described relationship between circulating microparticles and VTE disease [16,17].

We suggest several hypotheses to account for the intriguing lack of difference in CPMP procoagulant activity between cases and ControlsCVRF. First, we could consider the predominant role of cardiovascular risk factors as vascular cell activators vs venous thrombosis risk factors such as hypoxaemia induced by blood stasis. A second hypothesis relies on an expected difference in microparticle consumption kinetics between both populations, which would be faster in cases of an acute thrombotic event, such as venous thrombosis [22,33—35]. Indeed, the production and consumption of microparticles are mainly local processes in the acute phase of VTE. This is in line with the recent experimental study by Ramacciotti et al., demonstrating not only variable kinetics for each subtype of microparticle, but also their evolutive thrombogenicity during the thrombotic process itself [19]. The sequestration of microparticles in the forming thrombus is also a diluting factor [23]. The last hypothesis is an underestimation of the CPMP effective procoagulant activity due to a lack of sensitivity with our test using a specific quantification of microparticle-linked phosphatidylserine in a functional assay, compared with tissue factor activity evaluation [36].

Microparticles: cause or consequence in venous thrombogenesis?

By evaluating microparticle-related procoagulant activity in only the acute phase of venous thrombosis, our study is unable to assess whether CPMPs have a causal role in venous thrombogenesis. Nevertheless, we found some interesting correlations.

The PMP subtype appeared to be an important source of procoagulant microparticles, and was correlated with CPMPs. This is in line with the hypothesis of platelet activation and participation in venous thrombogenesis as has been underlined recently in the literature [2,3], linking venous thrombosis to atherothrombosis. PMPs levels have been associated positively with thrombus weight and proteomics of microparticles after VTE revealed the upregulation of Gal
atherothrombosis. Moreover, Pomp et al. [41] showed that with current smoking, a major cardiovascular risk factor in CPMPs and PMPs appeared to be correlated in our cases with CPMP levels in pulmonary artery blood samples [31,40].

Finally, after adjustment for age, sex and hypertension, CPMPs and PMPs appeared to be correlated in our cases with current smoking, a major cardiovascular risk factor in atherothrombosis. Moreover, Pomp et al. [41] showed that current smoking remained a risk factor for venous thrombosis among young people after adjustment for age, sex and body mass index. In the literature, an increasing amount of evidence suggests the likelihood of a link between arterial and venous disease [42]. According to results from recent studies, atherosclerosis and VTE share common risk factors, including age, obesity and current smoking [43,44]. In a large population-based study, Sorensen et al. [45] provided strong evidence that patients with VTE are at increased risk of subsequent arterial cardiovascular events compared with population controls, which is most pronounced during the first year of follow-up. This is consistent with underlying common prothrombotic mechanisms such as thrombogenesis, endothelial damage and inflammation [46].

Study limitations

We did not evaluate the post-VTE prothrombotic state related to CPMPs using a second measure of CPMP procoagulant activity during the first year of follow-up. Moreover, taking into account the limitation of our test related to the choice of CD31 as single endothelial phenotypic marker [47,48], we should also consider the involvement of other microparticles subtypes, such as leukocyte microparticles. Several studies demonstrated that platelets, leukocytes and endothelial cells colocalize and interact in the milieu of a forming thrombus [49–52]. By binding the PSGL-1 counter receptor via P-selectin, platelets and PMPs could activate monocytes, enhancing tissue factor expression and procoagulant leukocyte microparticle release [53]. As suggested by experimental studies in vivo, leukocyte microparticle release might play a key role in thrombus initiation to vascular remodelling, and we are going to evaluate their procoagulant activity in APE [19,49]. Finally, we might have underestimated the procoagulant activity related to CPMP by using a prothrombinase assay, whereas the estimation of tissue factor procoagulant activity would be more relevant [36].

Conclusions

Our study confirms the correlation between APE and CPMPs through their own procoagulant functionality, relying mainly on platelet activation. This relation no longer holds true when patients with APE are compared with those with cardiovascular risk factors, stressing the fact that potential confounders should be taken into account when microparticle levels are analysed in VTE.

Further research is needed to demonstrate in which conditions (i.e., initial stimuli, cell origin, etc.) the circulating pool of microparticles could be sufficient to precipitate a venous thrombotic event and to evaluate the therapeutic implications.

Conflict of interest statement

None.

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References

Circulating procoagulant microparticles in pulmonary embolism


