Fibrinolytic inhibitors and fibrin characteristics determine a hypofibrinolytic state in patients with pulmonary embolism

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Dear Sirs,

Clot lysis time (CLT), measuring plasma fibrinolytic potential, provides a global overview of fibrinolysis. Hypofibrinolysis has been found to be a potential risk factor for arterial and venous thrombosis (1-3). The mechanisms determining pulmonary embolism (PE) and its resolution are not completely understood (4-6). Recent data (7) suggest that fibrin(ogen) characteristics may be relevant in determining the possible resistance to plasmin. It is reported that fibrin resistance to lysis occurs in patients with PE (8, 9). Aims of our study were: 1) to assess the function of the fibrinolytic system in patients with prior PE and no evidence of chronic thromboembolic pulmonary hypertension (CTEPH) by measuring CLT and a number of plasma fibrinolytic components, 2) to study in vitro plasmin-mediated lysis of fibrin clots and to evaluate if the characteristics of fibrin(ogen) could influence the CLT in these patients. Finally, we evaluated the role of fibrinolytic system and in vitro lysis of fibrin clots in determining PE.

The study sample included 100 (median age 57, range 46-69; females 55%) consecutive patients with prior PE and no clinical and echocardiographic evidence of CTEPH; 33 of these patients were included in a previous study (9). PE was associated with deep-vein thrombosis (DVT) in 63% patients. PE was associated with transient risk factors in 26 cases (recent trauma 6, recent surgery 20), and with permanent risk factors in 25 cases (deficiency of coagulation inhibitors 4, factor V Leiden 13, G20210A prothrombin polymorphism 8); in 46 patients it was unprovoked; 34.5% of females were on oral contraceptives at the
time of diagnosis. One hundred-six age- and sex-matched healthy subjects, with no history of venous thromboembolism and not on oral contraceptives, served as controls. The study was approved by the local Ethics Committee, and all participants gave their signed informed consent before entering the study.

The diagnosis of PE was established by computed tomographic angiography or by perfusion lung scan. Patients underwent a standard anticoagulant therapy with a first course of heparin, followed by vitamin K antagonists, at intended therapeutic range international normalised ratio (INR) 2-3. The duration of treatment was at least three months. Patients were studied not earlier than 12 months after PE, and at least one month after oral anticoagulant withdrawal. Blood was sampled after overnight fasting, plasma aliquots for plasmin-mediated lysis of fibrin, CLT, clot lysis ratio (CLR), thrombin activatable fibrinolysis inhibitor antigen (TAFI ag), plasminogen activator inhibitor-1 antigen (PAI-1 ag), tissue plasminogen activator antigen (t-PA ag) and plasminogen activator (t-PA ag), α2-antiplasmin activity (α2AP act), euglobulin clot lysis time (ECLT by Buckell [10]), fibrinogen activity according to Clauss (Fbg act) and factor XII activity (FXII act) assays were separated and stored at -80°C until determinations. We considered values above 75th percentile of control group as altered values. CLT was studied according to Lisman (11) with some changes regarding in particular t-PA concentrations (50 ng/ml final concentration) employed and CLT calculation model according to Cellai (12). To assess the contribution of TAFI activation to CLT, the assay was repeated in the presence of carboxypeptidase inhibitor (CPI) (25 (µl/ml), a specific inhibitor of activated TAFI. The contribution of TAFI to the CLT was quantified by calculating the CLR, which is defined as the ratio between CLT measured in the absence and in the presence of CPI (11). The procedures of fibrinogen purification, fibrin formation and degradation, and gel electrophoresis are described in detail elsewhere (8, 9).

CLT was more prolonged in PE patients than in controls (Mann-Whitney test, p = 0.003). PAI-1 ag and t-PA ag levels and the time course of fibrin β-chain degradation were significantly different in PE patients than in controls. No difference was found between the two groups as regards the other parameters (▷Table 1A). With the exception of PAI-1 ag and fibrinogen activity, that required log transformation, all the continuous laboratory data were normally distributed. Linear regression analysis was performed to assess the associ-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLT, min</td>
<td>63.6 (58.1–70.9)</td>
<td>60.4 (54.0–66.2)</td>
<td>0.003</td>
</tr>
<tr>
<td>CLR</td>
<td>1.46 (1.33–1.59)</td>
<td>1.41 (1.32–1.50)</td>
<td>ns</td>
</tr>
<tr>
<td>ECLT, min</td>
<td>282 (220–331)</td>
<td>315 (252–340)</td>
<td>ns</td>
</tr>
<tr>
<td>TAFI ag, g/ml</td>
<td>9.1 (8.1–10.3)</td>
<td>9.3 (8.1–10.7)</td>
<td>ns</td>
</tr>
<tr>
<td>PAI-1 ag, ng/ml</td>
<td>18 (10–30)</td>
<td>13 (9–21)</td>
<td>0.013</td>
</tr>
<tr>
<td>t-PA ag, ng/ml</td>
<td>8.9 (5.8–11.6)</td>
<td>5.9 (3.8–8.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plg act, %</td>
<td>106 (96–120)</td>
<td>105 (95–113)</td>
<td>ns</td>
</tr>
<tr>
<td>2-AP act, %</td>
<td>104 (99–112)</td>
<td>109 (103–113)</td>
<td>ns</td>
</tr>
<tr>
<td>FXII act, %</td>
<td>118 (101–134)</td>
<td>119 (100–134)</td>
<td>ns</td>
</tr>
<tr>
<td>Fbg act, mg/dl</td>
<td>318 (274–357)</td>
<td>313 (285–366)</td>
<td>ns</td>
</tr>
<tr>
<td>Fbg β 1h, %</td>
<td>83.7 (80.7–87.9)</td>
<td>78.3 (74.3–84.3)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fbg β 3h, %</td>
<td>58.6 (55.6–61.7)</td>
<td>48.7 (43.5–56.1)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fbg β 6h, %</td>
<td>32.60 (29.7–36.1)</td>
<td>23.60 (20.4–27.5)*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are presented as Median (Interquartile Range), * available only for 50 subjects. CLT, Clot Lysis Time; CLR, Clot Lysis Ratio; ECLT, Euglobulin Clot Lysis Time; TAFI ag, Thrombin Activatable Fibrinolysis Inhibitor antigen; PAI-1 ag, Plasminogen Activator Inhibitor antigen; t-PA ag, Tissue Plasminogen Activator antigen; Plg act, Plasminogen activity; 2-AP act, 2-antiplasmin activity; FXII act, Factor XII activity; Fbg act Fibrinogen functional; Fbg β 1h, 3h, 6h, Mean amount of intact fibrin α-chains at 1, 3, 6 hours of digestion.
ation of CLT with the other laboratory data (Table 1B). Further, a stepwise linear regression analysis to test the independent association between CLT and both fibrinolytic parameters and the amount of intact β-chain, after adjusting for all the variables significantly associated with CLT, was performed. We found that the fibrinolysis inhibitors (as PAI-1 levels and CLR) accounted for 41% of CLT variability. The addition of the model of fibrinogen plasma levels and the fibrin β-chain degradation at 3 hours allowed us to explain 48% of CLT variability. Adding PLG and α2AP produced a further change in the R² statistic, accounting for 53% of CLT variability. We also performed the statistical analysis for co-linearity and excluded confounding effects for the parameters of the multivariate model (VIF 1.0, tolerance 1.0).

To determine the association of laboratory parameters and clinical variables (hypertension, dyslipidaemia and current smoking) with PE a univariate logistic regression analysis was performed. All univariate associations were included in the multiple analysis model, setting PE as the dependent variable. Hypertension, CLT, t-PA and in vitro fibrin degradation were significantly associated with PE (odds ratio [OR]: 4.8; 2.2; 2.9; 23.9, respectively, p < 0.001 for all). The multivariate model showed that only CLT (OR: 6.6; confidence interval [CI]: 1.8-23.8) and in vitro fibrin degradation (OR: 22.4; CI: 6.4-78.3) were independent predictors of PE. No differences were observed between patients with and without (hereditary and/or acquired) thrombophilia and between patients with and without DVT associated with PE, and in patients with and without oral contraceptives regarding single fibrinolytic parameters, CLT and fibrin-β-chain degradation.

In this study we used different approaches, analysing for the first time in PE patients both endogenous fibrinolysis and fibrin susceptibility to lysis by added plasmin. Our data suggest that both fibrin resistance to lysis and fibrinolytic system are altered in about 90% and 42%, respectively, of PE patients, a feature that could influence their future clinical outcome. CLT was found to be explained not only by fibrinolytic inhibitors, but also by the impaired lysis of fibrin by added plasmin. This may suggest that the fibrinogen structure and/or the architecture of the fibrin clot have some bearing on the observed hypofibrinolytic pattern. With our statistical model including all factors associated with CLT, we could explain 53% of the variation in CLT.

We observed, in agreement with other authors (13), elevated levels of t-PA and PAI-1 in our PE patients suggesting that hypofibrinolysis caused by elevated PAI-1 levels increases thrombotic risk. In addition we found also an association between fibrin resistance to lysis and the risk of PE. Recently published results of our research group (9) showed that fibrin resistance to lysis occurs in patients with CTEPH, as it had been previously reported (8, 14). In the present study we increased the sample size of PE patients in which the endogenous fibrinolytic system was also explored in detail. Thus, for the first time, we observed that fibrin susceptibility to plasmin-induced lysis and endogenous fibrinolysis were independently associated with PE.

Interestingly, fibrinogen plasma levels were not different between patients and controls. Because fibrinogen is the substrate of plasmin, that is the main enzyme of fibrinolysis, the alterations observed with the two employed approaches probably indicate both a structural abnormality of fibrinogen, causing a modification of fibrin clot which makes it resistant to lysis, and an impaired endogenous fibrinolytic activity. This study has some limitations. Because the performance of fibrin degradation is time consuming, the evaluation was obtained only in half of controls and this explains the wide Confidence Intervals observed in logistic regression. Another limitation is the fact that CLT was measured after a thromboembolic event had occurred; however, these patients were clinically stable and the assay was performed after a standard antiagulation treatment, so suggesting that hypofibrinolysis is a feature of these patients that possibly explains in part their increased thromboembolic risk. Our findings indicate that an impaired fibrinolytic potential and an alteration of plasmin-mediated lysis characterise patients with a history of PE. These results need to be confirmed in larger studies. Probably these patients should be closely controlled and could benefit a prolonged anticoagulant treatment.

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Conflicts of interest
None declared.

References