Coagulation and fibrinolysis in patients with asthma

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Chapter 4

Loss of asthma control and activation of coagulation and fibrinolysis

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Abstract

Background: Epidemiologic studies have shown that patients with severe asthma have increased risk of pulmonary embolism, in particular patients with frequent asthma exacerbations. Therefore, we hypothesized that asthma exacerbations are associated with increased hemostatic activity.

Objective: To investigate if induced loss of asthma control is associated with changes in coagulation and fibrinolytic parameters in peripheral blood.

Methods: We performed a prospective, inhaled steroid-withdrawal study in 23 patients with moderate to moderately severe asthma, consisting of a baseline visit and a visit after loss of asthma control. During the visits we measured asthma control questionnaire (ACQ), atopy, lung function, inflammatory markers (eosinophils and neutrophils), and hemostatic parameters in plasma.

Results: Complete cessation of inhaled corticosteroids led to a loss of asthma control in 22 out of 23 patients. We found increased asthma symptoms (ACQ 0.9 vs. 2.9, p<0.01), significantly reduced lung function (forced expiratory volume in 1 second (FEV1) 3.51L vs. 3.13L, p<0.01) and increased levels of eosinophils in plasma (0.26x10^9/L vs. 0.16x10^9/L, p=0.03) in patients after loss of asthma control. However, we observed no significant changes in the coagulation and fibrinolysis parameters.

Conclusion: Loss of asthma control after cessation of inhaled corticosteroids does not lead to increased hemostatic activation in patients with moderate to moderately severe asthma. This suggests that more severe inflammation or additional risk factors are required for activation of coagulation or reduction of fibrinolysis in asthma.
Introduction

Asthma is a chronic inflammatory disease of the airways. Despite adequate treatment, a large proportion of patients with asthma are difficult to control and have frequent exacerbations, with increased eosinophilic airway inflammation\(^1\). Inflammatory processes can influence hemostasis\(^2,3\) and also in patients with asthma, hemostasis is activated\(^4\). Pro-coagulant factors such as tissue factor (TF), thrombin activity, thrombin-antithrombin complex (TATc)\(^5-7\), and fibrinolytic factors such as plasminogen activator inhibitor type-1 (PAI-1) have been shown to be increased\(^8-10\) in plasma or sputum of these patients. Some of these pro-coagulant factors have been shown to be associated with eosinophilic airway inflammation, airway hyperresponsiveness and asthma severity\(^5,7\).

Epidemiologic studies have shown that severe asthma is associated with increased risk of pulmonary embolism\(^11\), in particular in patients with frequent asthma exacerbations\(^12\). The mechanisms underlying the development of pulmonary embolisms in these patients are still unclear, but an important contribution of inflammation is likely. In the present study we hypothesized that an increase in airway inflammation due to loss of asthma control is associated with increased hemostatic activity. The aim of our study was therefore to investigate whether induced loss of asthma control in patients with moderate to moderately severe asthma is associated with changes in coagulation and fibrinolytic parameters in peripheral blood, and whether these changes are associated with increases in eosinophils or neutrophils in peripheral blood. Loss of asthma control was induced by complete withdrawal of inhaled corticosteroids.

Methods

Subjects

Twenty-three adult patients with asthma from the pulmonary outpatient clinic of the Academic Medical Center Amsterdam were included in the study. The diagnosis of asthma was confirmed by at least 12% reversibility in forced expiratory volume in 1 second (FEV\(_1\)) after 400\(\mu\)g salbutamol or airway hyperresponsiveness (PC\(_{20}\) methacholine <8mg/ml) during the past
5 years. All patients had moderate to moderately severe asthma according to the GINA criteria\textsuperscript{13} and used a daily dose of inhaled corticosteroids of at least 500µg fluticasone or equivalent. Patients were excluded if they were smokers or had a smoking history of more than 5 pack years, had a change in inhaled corticosteroids dose, had oral corticosteroids or had experienced a respiratory infection within 4 weeks prior to screening. Also, patients who were pregnant or had any concomitant disease or inherited coagulation disorder, which could interfere with the conduct of the study were excluded. The study was approved by the Medical Ethics Committee of the Academic Medical Center Amsterdam and all subjects gave their written informed consent. The study was registered at the Dutch trial registry (www.trialregister.nl): NTR3316.

**Design**

The study was part of a clinical trial investigating the effect of loss of asthma controls on biomarkers of airway inflammation. This study had a prospective follow-up design, consisting of a baseline visit, and a “loss of asthma control” visit. At the baseline visit spirometry, measurement of fractional exhaled nitric oxide (FeNO), skin prick test, nasal swabs, and blood collection were performed in eligible patients. Following this baseline visit patients discontinued the use of inhaled corticosteroids and were closely monitored on a daily base by email or text message for symptoms and electronic peak expiratory flow (PEF) measurements for a maximum period of eight weeks\textsuperscript{14}. As soon as the patients showed loss of asthma control according to predefined criteria outlined below, a second visit was scheduled at the lung function laboratory at which all measurements of the baseline visit were repeated, except for skin prick test. Loss of asthma control was defined by the subjects meeting two of the three following criteria: 1) a decrease in prebronchodilator morning PEF of more than 20% compared to baseline on at least two consecutive days; 2) wakening due to asthma on two consecutive nights; 3) increase in the need of short-acting inhaled β2-agonist to more than eight puffs per day on two consecutive days\textsuperscript{14}.

**Measurements**

Asthma control was assessed by the Juniper asthma control questionnaire (ACQ)\textsuperscript{15}. A trained lung function technician performed spirometry, according to the European Respiratory Society (ERS) recommendations\textsuperscript{16}. FeNO measurement was performed with a portable rapid-response chemiluminescent
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analyzer (flow rate 50mL/s; NIOX system, Aerocrine, Sweden) according to the guidelines of the American Thoracic Society. Allergy test was done by puncture skin testing for twelve common aeroallergens. Nasal swabs were assessed for the presence of common respiratory viruses by multiplex qPCR, as currently used for the diagnosis of respiratory infections in routine diagnostics at the Academic Medical Center Amsterdam. Venous blood was analyzed for C-reactive protein (CRP) and complete white blood cell count including differential cell count (to count the number of eosinophils and neutrophils). Measurements of TATc (Siemens healthcare diagnostics, Marburg, Germany), plasmin-antiplasmin complex (PAPc) (DRG, Marburg, Germany), and PAI-1 (Hyphen BioMed, Andrésy, France) were determined by ELISA in plasma. Von Willebrand factor (vWF) was determined in plasma by ELISA with a polyclonal rabbit anti-human vWF antibody as catching antibody and horse radish peroxidase-labelled rabbit anti-human vWF antibody as detecting antibody (both DAKO, Glostrup, Denmark). D-dimer levels were measured in plasma with a particle-enhanced immunoturbidimetric assay (Innovance D-dimer, Siemens healthcare diagnostics, Marburg, Germany).

Statistical analysis
Paired t-tests or Wilcoxon signed rank tests were used for comparison of the data between the visits. Fold changes (ratio of baseline and loss of control scores) were computed for each variable. Linear regression analysis was used to correlate fold changes of inflammatory parameters with coagulation and fibrinolytic parameters. P-value <0.05 was considered significant. SPSS Statistics (Version 20.0. Armonk, NY: IBM Corp) was used for the data analysis. Sample size estimation was based on measurement of PAI-1 levels in peripheral blood. The results of Tutluoglu et al. showed an difference of 8.3 ng/ml in PAI-1 levels in plasma of patients with asthma attacks and after 1 week of therapy (resp. 75.2 (27.2) ng/ml vs. 83.5 (29.6) ng/ml). Since this was an asthma attack instead of a loss of control we assumed a lower effect size (4.15 ng/ml for PAI-1). By including six patients we would detect this expected difference with a power of 80%. Furthermore, it was estimated that 50% of included patients would develop loss of control, therefore we had to include at least twelve patients to ensure power for the PAI-1 levels.
Results

Twenty-two out of 23 asthma patients reached the criteria for loss of asthma control (Figure 1) and were included in the analysis. Baseline characteristics of these patients are described in table 1. Gender was not equally distributed, 6 males (27%) and 16 females (73%). The median time until loss of control was 22 days (Interquartile range (IQR)=16.8–33.0). 4 out of 22 patients had a positive PCR for respiratory viruses at baseline or loss of control visit.

Figure 1 Flow diagram of the study

Table 1 Baseline characteristics of patients with moderate to moderately severe asthma (N=22)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>27% / 73%</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>28.3 (9.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)*</td>
<td>25.2 (4.4)</td>
</tr>
<tr>
<td>Duration of asthma (years) ‡</td>
<td>14.5 (6.8 – 23.0)</td>
</tr>
<tr>
<td>Inhaled corticosteroids average (500/1000 µg fluticasone)</td>
<td>73% / 27%</td>
</tr>
<tr>
<td>Long acting beta agonists (LABAs) (yes/no)</td>
<td>77% / 23%</td>
</tr>
<tr>
<td>Atopy (yes/no)</td>
<td>95% / 5%</td>
</tr>
<tr>
<td>Median days until loss of control (days)‡</td>
<td>22.0 (16.0 – 33.0)</td>
</tr>
<tr>
<td>Viral infection (yes/no)</td>
<td>18% / 82%</td>
</tr>
</tbody>
</table>

*Mean (standard deviation) ‡ median (interquartile range)
At the loss of control visit patients had increased ACQ scores (0.9 vs. 2.9, \(p<0.01\)), lower prebronchodilator FEV₁ (3.51 L vs. 3.13 L, \(p<0.01\)), increased FeNO levels (18.8 ppb vs. 33.3 ppb, \(p<0.01\)), and higher eosinophil counts in plasma (0.16x10⁹/L vs. 0.26x10⁹/L, \(p=0.03\)) as compared to baseline (Table 2). We observed no significant differences for neutrophils and CRP. Despite evidence of eosinophilic airway inflammation there were no significant changes in vWF, TATc, PAPc, PAI-1, D-dimer in plasma between baseline and loss of asthma control (Table 2).

Table 2 Clinical, inflammatory, coagulation and fibrinolytic parameters at baseline and loss of control in patients with moderate to moderately severe asthma (N=22)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Loss of control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
<td>Median</td>
</tr>
<tr>
<td>ACQ</td>
<td>0.9</td>
<td>0.4 – 1.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Pre FEV₁ (L)</td>
<td>3.51</td>
<td>3.09 – 4.04</td>
<td>3.13</td>
</tr>
<tr>
<td>FeNO (ppb)</td>
<td>18.8</td>
<td>9.5 – 40.5</td>
<td>33.3</td>
</tr>
<tr>
<td>Eosinophils plasma (10E9/L)</td>
<td>0.16</td>
<td>0.10 – 0.35</td>
<td>0.26</td>
</tr>
<tr>
<td>Neutrophils plasma (10E9/L)</td>
<td>3.66</td>
<td>2.83 – 5.31</td>
<td>3.53</td>
</tr>
<tr>
<td>CRP plasma (mg/L)</td>
<td>1.55</td>
<td>0.75 – 3.48</td>
<td>1.30</td>
</tr>
<tr>
<td>vWF plasma (%)</td>
<td>108</td>
<td>89 – 138</td>
<td>110</td>
</tr>
<tr>
<td>TATc plasma (µg/L)</td>
<td>2.8</td>
<td>2.3 – 3.4</td>
<td>2.9</td>
</tr>
<tr>
<td>PAPc plasma (µg/L)</td>
<td>514</td>
<td>416 – 630</td>
<td>459</td>
</tr>
<tr>
<td>PAI-1 plasma (ng/mL)</td>
<td>26.5</td>
<td>14.5 – 46.0</td>
<td>24.0</td>
</tr>
<tr>
<td>D-dimer plasma (mg/L)</td>
<td>0.19</td>
<td>0.10 – 0.25</td>
<td>0.21</td>
</tr>
</tbody>
</table>

IQR = interquartile range

In addition, there was no significant correlation between eosinophils or neutrophils and any of the coagulation or fibrinolytic parameters in plasma. However, we did observe a positive correlation between the fold changes (ratio of baseline and loss of control scores) of the two acute phase proteins CRP and vWF (R=0.81 \(p<0.01\)) in plasma (Figure 2).
Figure 2 Correlation of the fold changes (ratio of baseline and loss of control scores) of C-Reactive Protein (CRP) with von Willebrand Factor (vWF) in plasma of patients with moderate to moderately severe asthma (N=22)

Discussion

This study shows that loss of asthma control with increased eosinophilic airway inflammation after cessation of inhaled corticosteroids in patients with moderate to moderately severe asthma is not associated with activation of coagulation or fibrinolysis. No correlation between eosinophil or neutrophil counts in blood with any of the coagulation and fibrinolytic parameters was observed. However, there was an expected correlation between CRP and vWF. These results suggest that a flare-up of eosinophilic inflammation of the airways during an induced loss of control in asthma is not accompanied with hemostatic changes.

Our study is the first to investigate the effect of experimentally induced loss of asthma control on coagulation and fibrinolysis in patients with asthma. In a previous study hemostatic parameters were measured during a spontaneous asthma attack while patients were on inhaled corticosteroids treatment. That study showed increased plasma levels of PAI-1 in patients during an asthma attack as compared to healthy controls\textsuperscript{10}. We did not observe a change in PAI-1 during loss of control, which might be due to the difference in the cause of loss of asthma control. Other study showed a significant increase of sputum plasminogen but no change of thrombin activity, PAI-1 and D-dimer levels in
sputum before and after tapering of inhaled corticosteroids without leading to a loss of asthma control. Sputum may provide information about the local conditions in the lung, but systemic measurements provide better information about potential coagulation complications like thrombosis and therefore would be more clinically relevant.

How can we explain the lack of coagulation activation in patients with loss of asthma control? First, the extent and the severity of the airway inflammation during loss of asthma control might be of importance for the induction of hemostasis. In our study a relatively mild loss of asthma control was induced, which did not lead to increased hemostatic activity but when local airway inflammation is more severe, for example in patients with severe asthma, a severe loss of asthma control or exacerbation could potentially lead to increased hemostatic activity.

Second, the type of trigger causing loss of asthma control may to be important in inducing hemostatic activity. Studies have found that atopic sensitization and allergic rhinitis are more common among patients with venous thromboembolism as compared to healthy control subjects. Increased levels of PAI-1 and activated platelets have been found in patients suffering from allergic disorders suggesting that allergen exposure might be an important factor contributing to activation of coagulation and fibrinolysis.

In addition, previous studies showed that naturally occurring acute respiratory infections activate coagulation. Non-asthmatic, elderly subjects with influenza-like illness or flu-like symptoms showed increased levels of vWF, PAPc and D-dimer levels when compared to baseline. Another study showed that during severe influenza A virus infection in mice, platelet activation worsened the severity of lung injury. Furthermore, common cold infections increases PAI-1 levels in both the upper and lower airways of patients with asthma. Also, experimental rhinovirus infection in patients with mild asthma induced pro-coagulant changes, resulting in shortening of the fibrin generation test in bronchoalveolar lavage fluid and increased PAI-1 levels in plasma. Moreover, activation of coagulation correlated with viral load in both upper and lower airways. In our study, only 4 out of 22 patients had a positive viral PCR indicating that their loss of asthma control might have been caused by viral respiratory infection. Due to the small number of patients it was not possible to perform subgroup analysis although exclusion of these
patients did not change our results. So, patients with exposure to allergens and with viral respiratory infections who are non-compliant to therapy might be at the highest risk to develop venous thromboembolism due to increased coagulation activation.

The strength of our study is the prospective and experimental design, in which a loss of asthma control was induced under controlled conditions. However, we cannot exclude the influence of some potential confounding factors. First, one can argue that this study had not enough power to demonstrate differences in coagulation and fibrinolytic parameters. However, based on our own data, we performed a posteriori power calculations. Results indicated that with 80% power and a difference of 2.44 (0.63) µg/L for TATc levels, 17.05 (4.65) µg/L for PAPc levels, and 7.14 (1.83) ng/mL for PAI-1 levels only a minimum of 3 subjects would be needed.

Secondly, in this study we had an unequal male/female ratio, with more females. Since many women in our study (9 out of 16) used oral contraceptives, this might have influenced coagulation activity, towards a pro-thrombotic state. However, we did not find differences between women with or without oral contraceptive use, so we did not consider this potential confounder to be important.

Overall, the results of our study suggest that whilst steroid withdrawal induces a loss of asthma control, it does not lead to activation of coagulation and fibrinolysis despite evidence of a flare-up of eosinophilic airway inflammation. Whether activation of hemostasis occurs in other types of loss of asthma control, such as allergen or virus induced, or in patients with more severe disease cannot be excluded. Still, it is clinically reassuring that relatively mild losses of asthma control, which occur in the majority of patients with asthma, are not associated with increased hemostatic activity and risk of thromboembolic events.
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References


