Prothrombotic state in patients with severe and prednisolone-dependent asthma

Marlous M. S. Sneeboer, MSc,[a] Christof J. Majoor, MD,[a] Anne de Kievit, MSc,[a] Joost C. M. Meijers, PhD,[b,c] Tom van der Poll, MD, PhD,[d] Pieter W. Kamphuisen, MD, PhD,[e] and Elisabeth H. Bel, MD, PhD[a] *Amsterdam and Groningen, The Netherlands*

Background: Epidemiologic studies have shown that asthmatic patients, in particular those with severe disease, have increased risk of pulmonary embolism. It is unknown whether these patients have a prothrombotic state under stable conditions.
Objective: We sought to compare coagulation and fibrinolysis parameters between healthy subjects and patients with mild, severe, and prednisolone-dependent asthma under stable conditions and to investigate whether hemostatic markers correlate with airway inflammation.
Methods: In 126 adults (33 healthy control subjects, 31 patients with mild asthma, 32 patients with severe asthma, and 30 patients with prednisolone-dependent asthma) parameters of inflammation (peripheral blood eosinophils and neutrophils) and markers of hemostasis (endogenous thrombin potential [ETP], thrombin-antithrombin complex, plasmin-α_2-antiplasmin complex, plasminogen activator inhibitor type 1 [PAI-1], D-dimer, and von Willebrand factor [vWF]) were measured in plasma. One-way ANOVA with the *post hoc* Bonferroni test was used for group comparison, and linear regression analysis was used for correlations.
Results: We observed increased ETP (121% vs 99%, overall $P < .01$), plasmin-α_2-antiplasmin complex (520 vs 409 μg/L, overall $P = .04$), PAI-1 (10 vs 7 ng/mL, overall $P = .02$), and vWF (142% vs 87%, overall $P < .01$) levels in asthmatic patients compared with healthy control subjects. ETP, PAI-1, and vWF levels increased with increasing asthma severity. In addition, we found a correlation between ETP and vWF with neutrophil but not eosinophil counts.
Conclusion: Asthmatic patients have a prothrombotic state that increases with asthma severity. This might explain why patients with asthma, in particular those with severe disease, have an increased risk of venous thromboembolism. (J Allergy Clin Immunol 2016;137:1727-32.)

Key words: *Asthma, severity, airway inflammation, hemostasis, coagulation, fibrinolysis, comorbidity*

Asthmatic patients, in particular those with severe disease and frequent exacerbations, are at increased risk of venous thromboembolism.[1,2] Epidemiologic studies have shown that the risk of pulmonary embolism (PE) in patients with severe asthma is increased up to 9-fold compared with that in nonasthmatic subjects.[1] The reason why asthmatic patients are at increased risk of PE is unclear, but a procoagulant influence of the underlying inflammatory process has been suggested. This fits with the observation that also other chronic inflammatory diseases, such as inflammatory bowel disease and rheumatoid arthritis, are associated with increased risk of PE.[3,4] Another contributing factor could be the use of corticosteroids, the mainstay of asthma treatment, which has also been associated with altered hemostasis[5] and increased risk of PE.[6,7] Therefore it is conceivable that in asthmatic patients, in particular those with severe asthma, hemostasis is activated because patients with severe asthma have severe airway inflammation and require high doses of corticosteroids for control of their disease.

In the present study we hypothesized that asthmatic patients have a prothrombotic state, which increases with asthma severity and is related to the number of inflammatory cells (eosinophils and neutrophils) in peripheral blood. The aim of our study was to compare coagulation and fibrinolysis parameters between healthy subjects and patients with mild, severe, and prednisolone-dependent asthma under stable conditions. For this, we measured markers of hemostasis (thrombin-antithrombin complex [TATc], endogenous thrombin potential [ETP], plasmin-α_2-antiplasmin complex [PAPc], plasminogen activator inhibitor type 1 [PAI-1], D-dimer, and von Willebrand factor [vWF]) in peripheral blood.

METHODS

Subjects and design

One hundred twenty-six adults (33 healthy control subjects, 31 patients with mild asthma, 32 patients with severe asthma, and 30 patients with prednisolone-dependent asthma) were included in the study. Patients with asthma of different severities were recruited when visiting the outpatient pulmonary clinic of the Academic Medical Center Amsterdam, The Netherlands. Healthy subjects were recruited by advertisements in the local area outside the hospital. Assessment of asthma severity was based on the Global Initiative for Asthma 2002 guidelines[8] for mild asthma and Innovative Medicine Initiative criteria[9] for severe asthma. All asthmatic patients had stable asthma, and asthma was defined as a documented reversibility in FEV_1 of at least 12% after 400 μg of salbutamol or airway hyperresponsiveness

From the Departments of [a]Respiratory Medicine and [b]Experimental Vascular Medicine and [d]the Center of Experimental and Molecular Medicine, Academic Medical Center, University of Amsterdam; [c]the Department of Plasma Proteins, Sanquin Research, Amsterdam; and [e]the Department of Vascular Medicine, University Medical Center Groningen.

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Corresponding author: Marlous M. S. Sneeboer, MSc, Department of Respiratory Medicine, F5-260 Academic Medical Centre, University of Amsterdam, PO Box 22700, Amsterdam NL-1100, The Netherlands. E-mail: m.sneeboer@amc.uva.nl.
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Abbreviations used
CRP: C-reactive protein
ETP: Endogenous thrombin potential
PAI-1: Plasminogen activator inhibitor type 1
PAPc: Plasmin-α_2-antiplasmin complex
PE: Pulmonary embolism
TATc: Thrombin-antithrombin complex
vWF: von Willebrand factor

(concentration of methacholine <8 mg/mL causing a 20% decrease in FEV_1 from baseline [PC_{20} <8 mg/mL]) in the past 5 years. We divided patients into 3 categories based on the intensity of anti-inflammatory treatment: patients with mild persistent asthma (using 250-500 μg/d fluticasone or equivalent), patients with severe asthma (using ≥1000 μg/d fluticasone or equivalent and a second controller), and patients with severe prednisolone-dependent asthma (using ≥1000 μg/d fluticasone or equivalent and a second controller and >5 mg/d prednisolone). Healthy subjects had no history of airway disease, were nonatopic, and had no airway hyperresponsiveness.

None of the subjects were current cigarette smokers, and all had a smoking history of a maximum of 10 pack years. Subjects were excluded if they had signs of a respiratory tract infection or a change in inhaled or oral corticosteroid dose within 4 weeks before screening and if they used omalizumab. Also, patients using heparin, low-molecular-weight heparin, aspirin, nonsteroidal anti-inflammatory drugs, or vitamin K antagonists were excluded. Patients who were pregnant or had a history of venous thromboembolism and patients with concomitant disease or inherited coagulation disorders that could interfere with the study were also excluded.

This study was part of a research program aimed at investigating risk factors of venous thromboembolism in asthmatic patients. Study measurements included the Asthma Control Questionnaires, spirometry, venous blood collection, and sputum induction and were conducted at the Academic Medical Center Amsterdam. All samples were collected on the same day. The study was approved by the Medical Ethics Committee of the Academic Medical Center Amsterdam, and all subjects provided written informed consent. The study was registered at the Dutch trial registry (www.trialregister.nl) no. NTR3101.

Measurement of inflammation and hemostatic parameters in peripheral blood

Venous blood was obtained after 10 minutes of rest. Complete white blood cell counting, including automated differential cell counting, was performed to calculate the number of neutrophils and eosinophils in peripheral blood. C-reactive protein (CRP) levels were measured by using immunoturbidimetric determination. Citrated blood was used for measurement of the hemostatic markers vWF, TATc and D-dimer (*in vivo* coagulation), ETP (*in vitro* coagulation), and PAPc and PAI-1 (fibrinolysis). vWF levels were determined by using ELISA with a polyclonal rabbit anti-human vWF antibody as the catching antibody and horseradish peroxidase–labeled rabbit anti-human vWF antibody as the detecting antibody (both from DAKO, Glostrup, Denmark). TATc (Siemens Healthcare Diagnostics, Marburg, Germany), PAPc (DRG, Marburg, Germany), and PAI-1 (Hyphen BioMed, Andrésy, France) levels were determined by means of ELISA. D-dimer levels were measured with a particle-enhanced immunoturbidimetric assay (Innovance D-dimer, Siemens Healthcare Diagnostics). *In vitro* thrombin generation was measured by using the calibrated automated thrombogram. This assay determines the generation of thrombin in clotting plasma by using a microtiter plate reading fluorometer (Fluoroskan Ascent; ThermoLab systems, Helsinki, Finland) and Thrombinoscope software (Thrombinoscope BV, Maastricht, The Netherlands). The assay was carried out as described by Hemker et al[10] and in the Thrombinoscope manual. Coagulation was triggered with 5 pmol/L recombinant human tissue factor (Innovin, Siemens Healthcare Diagnostics), 4 μmol/L phospholipids, and 417 μmol/L fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem, Bubendorf, Switzerland). Fluorescence was monitored, and the different parameters (lag time [time to initiate coagulation], peak thrombin, and area under the curve or ETP) were calculated with Thrombinoscope software. Peak thrombin and ETP results were normalized to pooled normal plasma.

Statistical analysis

Nonnormally distributed data were log-transformed before analysis. Variables were summarized based on descriptive statistics. Continuous variables were expressed as means ± SDs or medians with interquartile ranges, depending on data distribution. Categorical variables were presented as percentages. Overall comparison between the groups was done with 1-way ANOVA, which resulted in an overall *P* value. In addition, *post hoc* Bonferroni comparisons were performed between groups, which resulted in separate *P* values. Multiple linear regression analysis corrected for age and sex was used to determine the association between coagulation and fibrinolysis parameters and inflammation parameters. A *P* value of less than .05 was considered significant. SPSS software (Version 20.0; IBM, Armonk, NY) and GraphPad Prism software (version 5.0; GraphPad Software, San Diego, Calif) were used for data analysis.

RESULTS

Characteristics of the patients with mild, severe, and prednisolone-dependent asthma, as well as healthy control subjects, are shown in Table I.[11,12] Asthmatic patients were older than healthy control subjects. Lung function (FEV_1 prebronchodilator) decreased with asthma severity, whereas Asthma Control Questionnaire scores increased with asthma severity (both overall $P < .01$, Table I). Compared with healthy control subjects, asthmatic patients had increased neutrophil (overall $P < .01$, Table I), eosinophil (overall $P < .01$, Table I), and CRP (overall $P < .01$, Table I) levels in peripheral blood.

Hemostatic parameters

Coagulation and fibrinolysis parameters of patients with mild, severe, and prednisolone-dependent asthma and healthy control subjects are shown in Table II. Compared with healthy control subjects, asthmatic patients showed increased thrombin generation, as demonstrated by a significantly higher ETP level in patients with asthma (overall $P < .01$, Fig 1). *Post hoc* analysis showed a significant difference in ETP levels between healthy control subjects (99%) and patients with severe asthma (114%, $P < .01$), between healthy control subjects (99%) and patients with prednisolone-dependent asthma (121%, $P < .01$), and between patients with mild asthma (105%) and patients with prednisolone-dependent asthma (121%, $P < .01$). A similar result was obtained with peak thrombin levels, which were higher in asthmatic patients compared with healthy control subjects (overall $P < .01$). There was again a trend with severity of disease and peak thrombin levels in patients with mild asthma of 109% ($P > .05$), in patients with severe asthma of 118% ($P < .01$), and in patients with prednisolone-dependent asthma of 132% ($P < .01$) when compared with 108% in healthy control subjects. There was also a longer lag time (overall $P < .01$) in asthmatic patients compared with healthy control subjects.

TATc levels showed a few outliers (values greater than the mean plus 5 times the SD) that were excluded from the analysis. Thus, for the analysis of TATc, we had data from 32 healthy control subjects, 28 patients with mild asthma, 30 patients with severe asthma, and 26 patients with prednisolone-dependent asthma. There was no significant difference for TATc levels