Asthma and coagulation: A clinical and pathophysiological evaluation

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Chapter 6

Effects of oral corticosteroids on coagulation in patients with asthma

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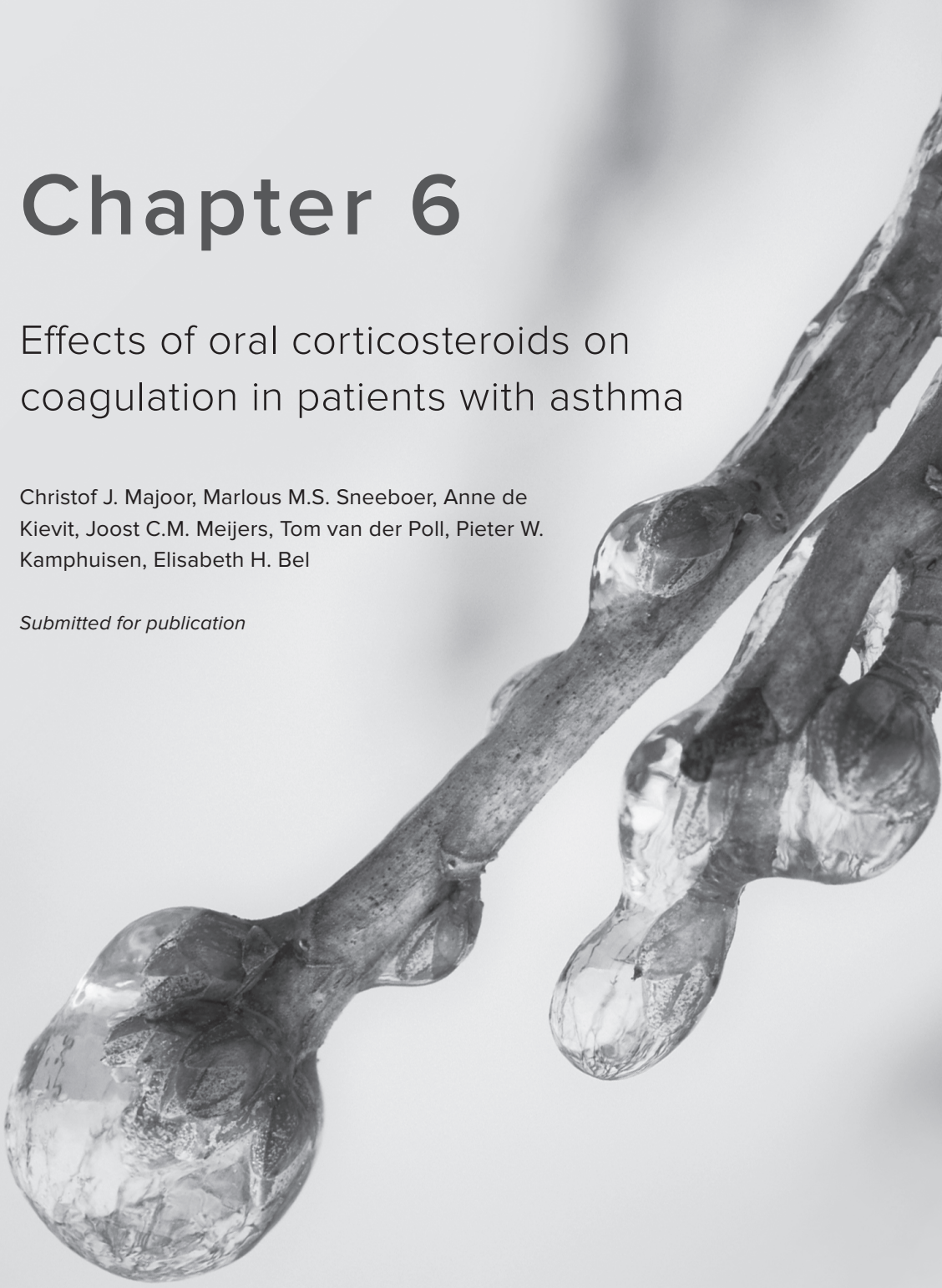

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Clinical Implications

Patients with severe asthma or asthma exacerbations are at increased risk of thromboembolic events because of the combined procoagulant effects of airway inflammation and oral corticosteroid use.

Capsule summary

This study shows that oral corticosteroids enhance coagulation and reduce fibrinolysis in patients with asthma, despite their anti-inflammatory effect.

Abbreviations

ACQ = asthma control questionnaire
BMI = body mass index
CRP = C-reactive protein
ETP = endogenous thrombin potential
FeNO = fractional exhaled nitric oxide
FEV1 = forced expiratory volume in 1 second
PAI-1 = plasminogen activator inhibitor type 1
PAPc = plasmin-α2-antiplasmin complex
PE = pulmonary embolism
TATc = thrombin-antithrombin complex
vWF = von Willebrand factor

Abstract

Background

Patients with asthma are at increased risk of pulmonary embolism, which is associated with disease severity and asthma exacerbations. Airway inflammation is known to activate coagulation. Whether oral corticosteroids also activate coagulation and thereby contribute to the increased thromboembolic risk is unknown.

Objective

To determine whether a 10-day oral corticosteroid course enhances coagulation in patients with asthma.

Methods

60 patients with stable asthma of varying severity were randomized to receive prednisolone 0.5mg/kg (n=30) or placebo (n=30) once daily for 10 consecutive days. Changes from baseline in plasma markers of coagulation (thrombin generation (peak thrombin and endogenous thrombin potential (ETP)), thrombin-antithrombin complexes (TATc)), fibrinolysis (D-dimer, plasminogen activator inhibitor type-1(PAI-1), plasmin-α2-antiplasmin complexes (PAPc)), and von Willebrand factor (vWF) were compared between the prednisolone and placebo groups. Also changes in peripheral blood eosinophils and neutrophils, C-reactive protein (CRP) and fractional exhaled nitric oxide (FeNO) were measured.

Results

Compared to placebo, prednisolone increased mean (SD) peak thrombin by 10.6 (14.8)% ($p=0.02$), vWF by 20.0 (15.3)% ($p<0.001$), and PAI-1 by 3.5 (IQR -1.0 – 9.0) ng/mL ($p=0.04$), and decreased PAPc by -53.6 (79.7) µg/L ($p=0.04$). TATc, ETP and D-dimer did not change. Peripheral blood eosinophils, CRP and FeNO decreased, while neutrophils increased (all $p<0.01$).

Conclusions

Oral corticosteroids induce a procoagulant state in plasma of patients with asthma by enhancing coagulation and reducing fibrinolysis. This suggests that corticosteroids, despite their anti-inflammatory effects, contribute to the increased risk of thromboembolic events in patients with asthma.

Introduction

There is accumulating evidence that patients with asthma are at increased risk to develop thromboembolic events including pulmonary embolism, acute coronary syndrome and stroke [1-5]. This increased risk is associated with asthma severity and severe exacerbations for which courses of oral corticosteroids are indicated[1-3, 5].

It is well established that inflammation enhances coagulation in animals and humans[6, 7]. This has not only been shown in sepsis and pneumonia, but also in chronic inflammatory diseases, such as inflammatory bowel disease, rheumatoid arthritis and asthma. Oral corticosteroids are known to reduce inflammation in these patients, and are thereby likely to mitigate the procoagulant state[8, 9] However, there is also evidence that these drugs may have a prothrombotic effect, as has been shown in healthy volunteers[10, 11] and in patients using maintenance oral corticosteroids for non-inflammatory conditions[12].

Since oral corticosteroids are frequently used in patients with asthma, in particular for those with severe disease or severe exacerbations, it is important to investigate whether these drugs are prothrombotic despite their anti-inflammatory effects. If so, this may have clinical implications. For example, in patients with additional risk factors for thromboembolic events, alternative treatment strategies might be considered.

In the present study we hypothesized that oral corticosteroids affect hemostasis in patients with asthma. Therefore, we investigated parameters of coagulation, fibrinolysis and endothelial activation in patients with stable asthma of varying severity after a 10-day oral prednisolone course. In addition, we assessed the effect of oral corticosteroids on markers of inflammation in peripheral blood. Some of the results of this study have been previously reported in the form of an abstract [13].

Methods

Study design

After a screening visit, eligible patients were randomly assigned in a 1:1 ratio by computer-generated randomization to receive either oral prednisolone (0.5mg/kg/day) or identical placebo tablets for 10 consecutive days. Patients were assessed at baseline, and after 1 and 10 days of treatment between 9 and 11 a.m. At all visits, venous blood was obtained after 10 minutes rest. Fractional exhaled nitric oxide measurement (FeNO) was measured at baseline and at day 10.

Patients

Adults with asthma were recruited from the pulmonary outpatient clinic of the Academic Medical Center in Amsterdam, The Netherlands. Patients had asthma diagnosed by a physician with at least 12% reversibility in forced expiratory volume in 1 second (FEV_1) after 400µg salbutamol and/or airway hyperresponsiveness (provocative concentration of methacholine causing 20% fall in FEV_1 (PC_{20}) <8mg/ml) in the past 5 years. All patients had stable asthma as measured by an Asthma Control Questionnaire (Juniper ACQ) score < 1.5 [14]. Thirty patients had mild-moderate asthma according to GINA guidelines (using 250-500µg/day fluticasone or equivalent) and 30 had severe asthma according to the IMI international consensus criteria (using ≥1000µg/day fluticasone or equivalent)[15, 16]. All patients were non-smoking or had stopped smoking for >1 year with a smoking history of <10 packyears in total. Participants were excluded if they had a change in inhaled corticosteroid dose within 4 weeks prior to screening or if they used NSAID, heparin, low-molecular-weight-heparin, vitamin K antagonists, oral contraceptives, or had any concomitant disease. 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 part of a research program aimed at investigating risk factors of venous thromboembolism in patients with asthma (Netherlands Trail Registry (NTR) 3101).

Study procedures

The study required a strict order in which all the procedures were performed. First, after a 10 minute rest, venous blood was obtained with a 19G needle through a Vacutainer system from the brachial vein after releasing the tourniquet. The venous blood was collected in citrate tubes to evaluate hemostatic parameters. All venous blood was sent to the laboratory to be processed within 15 minutes after collection of the blood. Venous blood was promptly centrifuged at 1500g for 10 minutes at room temperature. After transfer, plasma was re-centrifuged at 3000g for 15 minutes at room temperature to prepare cell-free plasma. Plasma was retransferred, mixed and transferred in storage tubes and immediately stored at -80°C until batch analysis.

After collection of the venous blood, FeNO measurement was performed with a portable rapid-response chemiluminescent analyzer (flow rate 50mL/s; NIOX system, Aerocrine, Sweden) according to the guidelines of the American Thoracic Society[17], followed by spirometry by a trained lung function technician according to the ERS recommendations[18].

Measurement of coagulation parameters

Measurements of TATc (Siemens Healthcare Diagnostics, Marburg, Germany), vWF antigen (antibodies from Dako, Glostrup, Denmark), PAPc (DRG, Marburg, Germany)

and PAI-1 (Hyphen BioMed, Andrésy, France) were performed by ELISA. D-dimer levels were determined with a particle-enhanced immunoturbidimetric assay (Innovance D-Dimer, Siemens Healthcare Diagnostics, Marburg, Germany). The Calibrated Automated Thrombogram® assays the generation of thrombin in clotting plasma 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.[19] and the Thrombinoscope® manual. Coagulation was triggered by recalcification in the presence of 5 pM recombinant human tissue factor (Innovin®, Siemens, Marburg, Germany), 4 µM phospholipids, and 417 µM fluorogenic substrate Z-Gly-Gly-Arg-AMC (Bachem, Bubendorf, Switzerland). Fluorescence was monitored using the Fluoroskan Ascent fluorometer, and peak thrombin level, velocity index and the area under the curve (endogenous thrombin potential or ETP) were calculated using the Thrombinoscope® software (Thrombinoscope BV). Peak thrombin, velocity index and ETP results were normalized to pooled normal plasma.

Measurement of inflammatory parameters in venous blood

In venous blood, eosinophils and neutrophils, and C-reactive protein (CRP) were performed by routine laboratory analysis by the clinical chemistry laboratory of the AMC. CRP was measured by immunoturbidimetric determination (Cobas 8000; Roche Diagnostics GmbH, Mannheim, Germany). Eosinophils and neutrophils were counted by Sysmex 5000 (Sysmex Europe GmbH, Norderstedt, Germany).

Sample size calculation

Sample size estimation was based on measurement of TATc in peripheral blood. Based on the results by Maeda (comparable inflammatory diseases) and de Kruijf (same laboratory) we expected the average TATc in controls to be about 2.56 ng/ml (SD 0.82) which we would expect to increase with 33% to 3.3 ng/ml (SD 0.85) after treatment with oral prednisolone for 10 days[11, 20]. A standard deviation of 30% was expected based on the same studies and also provided by the manufacturer of the commercial kit. With a significance level of 5% and a power of 80% we calculated that 15 subjects per group would be sufficient.

Statistical analysis

Continuous variables are expressed as mean +/ – SD. Not normal distributed variables were log-transformed before analysis and presented as mean +/ – SD. Log transformed continuous variables that were not normally distributed were expressed as median with interquartile range. Changes from baseline in TATc, PAPc, vWF, D-dimer, PAI-1, peak thrombin, velocity index, ETP, peripheral blood eosinophil and

neutrophil counts, levels of CRP and FeNO on days 1 and 10 were analyzed by paired or unpaired t-test, or Wilcoxon signed rank sum or Mann Whitney U tests, where appropriate. A two-sided p-value<0.05 was considered significant. Analyses were performed with SPSS Statistics, Version 21.0. Armonk, NY: IBM Corp.

Results

Sixty-five patients with asthma were enrolled of which 32 were assigned in the placebo group and 33 to the prednisolone group. Five patients did not complete the study, due to nephrolithiasis (n=1), infection (n=1), pyrosis (n=1) and withdrawal of consent (n=2). (Figure 1)

There was a trend toward higher age in the placebo group 50 (SD 15) yr. compared to the prednisolone group 43 (SD 14) yr. (p=0.08). More women were included in the placebo group as compared to the prednisolone group (11 (37%) vs 20 (67%), respectively; p=0.02). All other variables were similar between the two groups. Baseline patient characteristics are shown in Table 1.

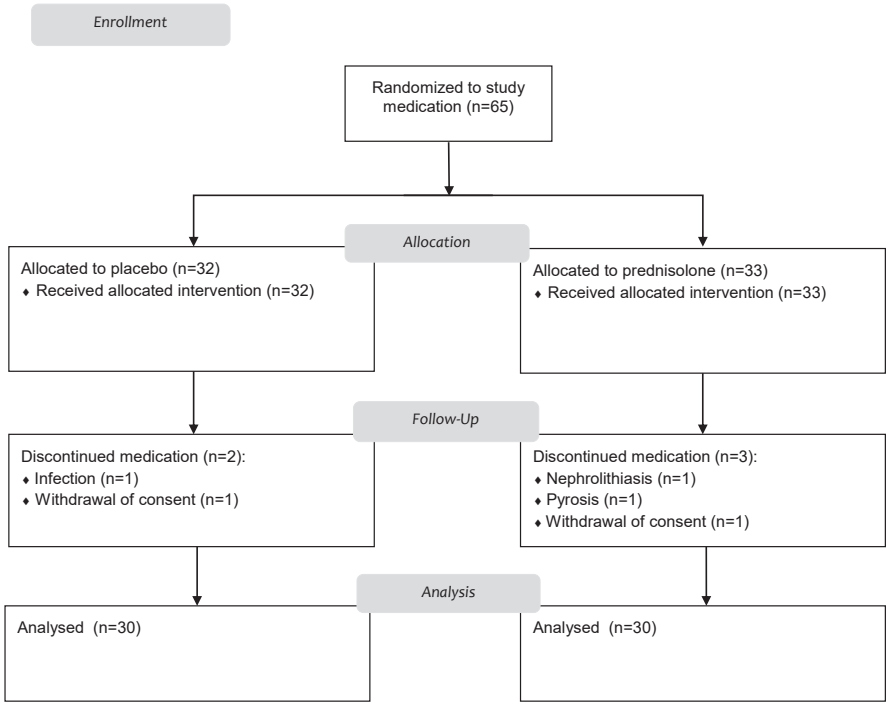

Figure 1.
Flow diagram of the study

Table 1. Baseline patient characteristics.

	Placebo	Prednisolone	
	N=30	N=30	p-value
Age (years) *	50 (15)	43 (14)	0.08
Gender (% males)	37%	67%	0.02
BMI (kg/m^2) *	26.0 (4.2)	25.8 (4.0)	0.89
Allergy (% yes)	80%	80%	1.00
Never smokers (% yes)	73%	73%	1.00
ACQ score ‡	1.3 (0.3 – 2.0)	0.8 (0.3 – 1.7)	0.25
FEV1 pre (%) *	95 (20)	91 (18)	0.40
FeNO (ppb) ‡	24.0 (16.0 – 37.5)	28.5 (15.5 – 40.0)	0.79

*mean ± standard deviation or ‡ median with interquartile range

Markers of coagulation and fibrinolysis

Measurements of markers of coagulation and fibrinolysis at baseline, day 1 and day 10 are shown in Table 2. Changes from baseline, expressed as the delta between baseline and day 1 or day 10, are presented in table 3. TATc did not change in the prednisolone or placebo group (p=0.24 between groups). However, there was a change in the peak thrombin and velocity index measured by the thrombin generation assay in the prednisolone group, but not in the placebo group. Peak

Table 2. Markers of coagulation and fibrinolysis.

	Placebo			Prednisolone		
	N=30			N=30		
	Baseline	Day 1	Day 10	Baseline	Day 1	Day 10
vWF, %	112 (47)	108 (48)	109 (46)	103 (35)	109 (30)	^123 (35)
TATc, µg/L*	2.5 (2.2-2.9)	2.5 (2.2-2.7)	2.7 (2.3-3.1)	2.4 (2.2-2.8)	2.6 (2.2-2.8)	2.4 (2.1-2.9)
PAPc, µg/L**	490 (302-955)	457 (257-851)	479 (257-832)	‡398 (257-676)	#347 (209-550)	˙363 (240-676)
PAI-1, ng/mL*	12 (5-15)	13 (5-15)	10 (6-18)	9 (6-13)	§12 (7-20)	§17 (6-22)
D-dimer, mg/L*	0.29 (0.19-0.41)	0.29 (0.10-0.39)	0.28 (0.18-0.40)	0.27 (0.10-0.36)	0.23 (0.10-0.32)	0.20 (0.10-0.37)
Peak thrombin, %	112 (21)	112 (21)	115 (17)	114 (23)	#120 (23)	^125 (19)
Velocity index, %	129 (45)	125 (42)	133 (36)	130 (51)	142 (47)	˙155 (49)
ETP, %	108 (15)	109 (16)	110 (14)	111 (20)	113 (19)	109 (14)

Mean with standard deviation, * Median with interquartile range or ** geometric mean with 95% confidence intervals

vWF von Willebrand factor; TATc Thrombin-antithrombin complexes; PAPc plasmin-α2-antiplasmin complexes; PAI-1 plasminogen activator inhibitor type 1; ETP endogenous thrombin potential. vWF, Peak thrombin, velocity index and ETP are presented as percentage of normalized pooled plasma.

‡ p<0.05 as compared to placebo; # p< 0.05 as compared to baseline (within group difference); § p<0.01 as compared to baseline (within group difference); ˙ p=0.001 as compared to baseline (within group difference); ^ p<0.0001 as compared to baseline (within group difference)

Table 3. Change in markers of coagulation and fibrinolysis.

	Change from baseline at day 1			Change from baseline at day 10		
	Placebo	Prednisolone		Placebo	Prednisolone	
	Mean (SD)	Mean (SD)	p-value	Mean (SD)	Mean (SD)	p-value
vWF, %	-3.3 (12.8)	5.8 (16.7)	0.02	-2.7 (16.4)	20.0 (15.3)	<0.001
TATc, µg/L	0.04 (0.74)	0.15 (0.71)	0.40	0.35 (1.10)	0.04 (0.82)	0.24
logPAPc, µg/L	-0.03 (0.08)	-0.04 (0.10)	0.74	-0.01 (0.09)	-0.06 (0.09)	0.04
PAI-1, ng/mL*	0.50 (0.0 – 4.0)	3.5 (-2.0 – 9.0)	0.38	0.0 (-2.0 – 2.0)	3.5 (-1.0 – 9.0)	0.01
Ddimer, mg/L*	0.00 (-0.05 – 0.02)	0.0 (-0.05 – 0.00)	0.53	0.00 (-0.04 – 0.02)	-0.00 (-0.13 – 0.00)	0.36
Peak thrombin, %	0.4 (11.7)	6.0 (13.0)	0.051	2.4 (12.3)	10.6 (14.8)	0.02
Velocity index, %	-3.7 (26.8)	11.9 (32.0)	0.045	4.1 (30.0)	25.6 (36.6)	0.02
ETP, %	1.5 (4.9)	1.9 (5.3)	0.54	1.4 (6.2)	-2.7 (10.6)	0.08

* Median, IQR

vWF von Willebrand factor; TATc Thrombin-antithrombin complexes; PAPc plasmin-α2-antiplasmin complexes; PAI-1 plasminogen activator inhibitor type 1; ETP endogenous thrombin potential. vWF, Peak thrombin, velocity index and ETP are presented as percentage of normalized pooled plasma.

thrombin increased in the prednisolone group by 10.6% (SD 14.8) (p=0.02 between groups), while velocity index increased by 25.6% (SD 36.6) (p=0.02 between groups) (Figure 2). D-dimer did not change in either group (p=0.36 between groups).

PAPc was lower in the prednisolone group as compared to the placebo group at baseline (Geometric mean: 398 µg/L (95% confidence interval (CI) 257-676) *vs.* 490 µg/L 95% CI (302-955), respectively; p=0.02), and decreased in the prednisolone group by 11.5% (geometric mean: -51.4 µg/L) as compared to no change in the placebo group (p=0.04 between groups) (Figure 2). PAI-1 slightly increased in the prednisolone group by 3.5 µg/mL (IQR -1.0 – 9.0), while no change occurred in the placebo group (p=0.01 between groups) (Figure 2).

Von Willebrand factor increased significantly in the prednisolone group 5.1% (SD 17.0), but not in the placebo group at day 1 (p=0.02 between groups) and increased further at day 10 (20.0% (SD 15.3) (p<0.001 between groups). (Figure 2) As gender was not divided equally between both groups, a correction for gender was performed by univariate general linear model for all significantly different coagulation markers. This model showed that gender did not influence the results.

Inflammatory markers

Changes in inflammatory markers are shown in Table 4. Blood eosinophils decreased and neutrophils increased after 1 and 10 days of oral prednisolone use (p<0.0001 between groups), while CRP and FeNO decreased (p=0.001 between groups).

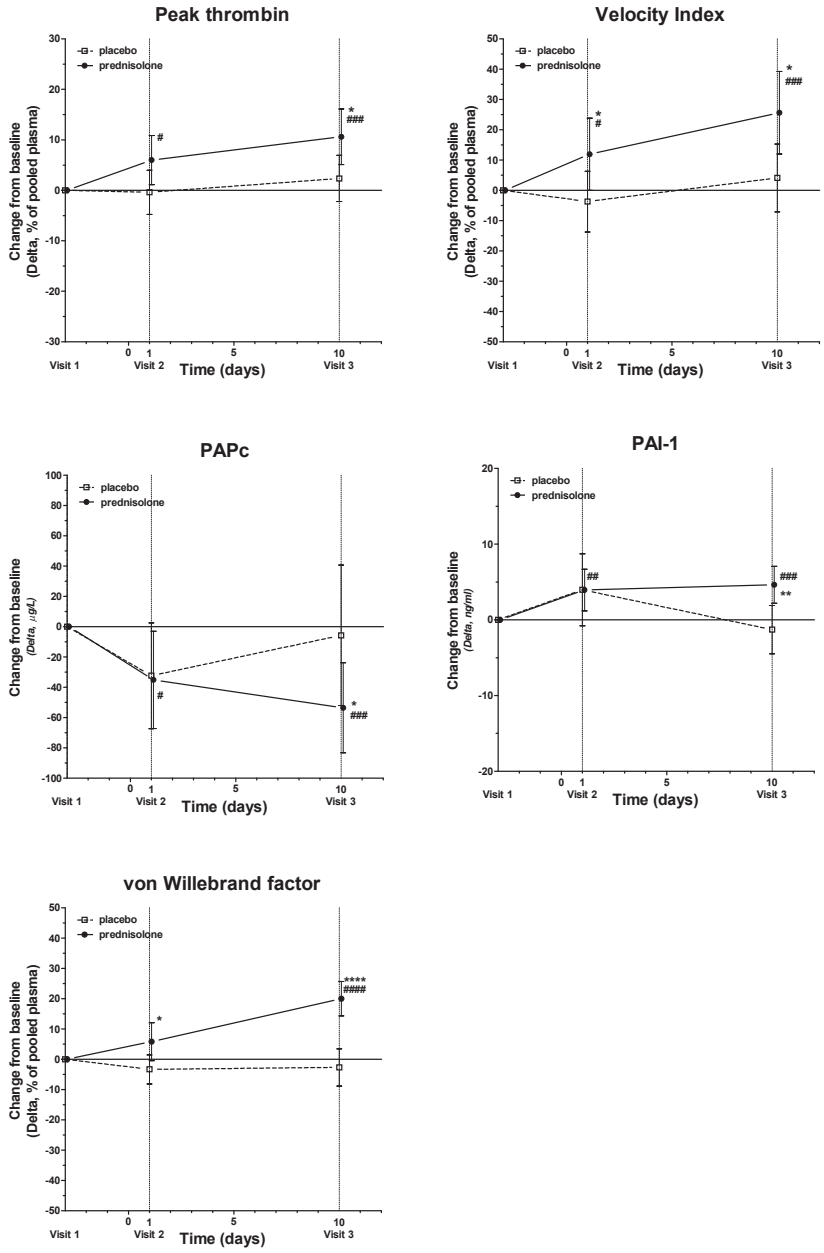

Figure 2.

Changes from baseline in hemostatic markers (peak thrombin and velocity index), fibrinolytic markers (PAPc and PAI-1) and WF.

(Baseline is set to zero and values are represented as mean and 95% CI; comparison between groups: * p< 0.05, ** p< 0.01, *** p< 0.001, **** p< 0.0001; comparison within group from baseline: # p<0.05, ## p< 0.01, ### p< 0.001, #### p< 0.0001)

Table 4. Inflammatory markers.

	Placebo			Prednisolone		
	Baseline	**Day 1**	**Day 10**	**Baseline**	**Day 1**	**Day 10**
Neutrophils, 10^E9/L	3.4 (1.1)	3.2 (1.1)	3.2 (1.1)	3.5 (1.0)	^{0}5.5 (2.0)	^{0}5.8 (2.0)
Eosinophils, 10^E9/L*	0.24 (0.18-0.47)	0.26 (0.16-0.48)	0.27 (0.16-0.49)	0.23 (0.13-0.49)	^{0}0.09(0.06-0.12)	^{0}0.11(0.06-0.16)
CRP, mg/L*	1.5 (0.4-3.5)	1.3 (0.4-3.1)	1.0 (0.4-2.7)	1.2 (0.6-3.9)	1.7 (0.7-2.6)	^{0}0.5 (0.3-1.1)
FeNO, ppb	34.1 (23.5)	ND	34.4 (21.2)	32.5 (25.5)	ND	*24.2 (13.9)

Mean with standard deviation or * Median with interquartile range
CRP C-reactive protein; FeNO fractional exhaled nitric oxygen; ND not determined
* p=0.001 as compared to baseline (within group difference)
^{0} p<0.0001 as compared to baseline (within group difference)

Discussion

This study shows that a 10-day course of oral prednisolone (0.5mg/kg/day) in patients with stable asthma caused a shift in the hemostatic balance to a procoagulant state by increasing *in vitro* coagulation and reducing fibrinolysis. Activation of coagulation occurred despite an anti-inflammatory effect of oral prednisolone, as shown by a decrease in peripheral blood eosinophils, CRP and exhaled nitric oxide. This suggests that the prothrombotic state in asthma patients and the increased risk of thromboembolic events not only depends on disease activity, but also on the adverse effects of oral corticosteroids.

Our study shows that corticosteroids enhance coagulation in patients with asthma. This is in contrast with the results of previous studies in patients with other inflammatory diseases such as SLE and connective tissue disease, showing that corticosteroids reduced coagulation[8, 9]. This might be explained by the fact that coagulation was studied during acute exacerbations of these diseases. Acute exacerbations are associated with intense inflammatory processes, known to have a strong procoagulant effect. We included patients with stable disease, to reduce the influence of acute inflammation as much as possible. Our results fit in with the findings in healthy subjects, showing an increase in coagulation factors after 5 days of oral dexamethasone[10]. Our results show a corticosteroid-induced increase in vWF, a factor known to be released by (activated) endothelial cells[21]. This is in conflict with the findings from diseases such as SLE and giant cell arteritis, showing decreases in vWF[9, 22, 23]. Most likely this is related to the anti-inflammatory effect of corticosteroids acting on the vascular wall, thereby counteracting the procoagulant effect of corticosteroids itself. Although isolated studies with corticosteroids in healthy subjects showed no change in vWF[10, 21, 24], a trend towards an increase in vWF was observed in a meta-analysis of these studies[25]. Finally, our observation of

a reduction in fibrinolysis by corticosteroids, shown by the decrease in PAPc and increase in PAI-1, is in line with studies in SLE and connective tissue diseases[8, 9, 23] and Cushing's disease[26, 27]. Together, these studies show that oral corticosteroids enhance coagulation and reduce fibrinolysis, as is evident from studies in healthy subjects and patients with stable disease.

Prednisolone induced an increase in thrombin generation (peak thrombin and velocity index) *in vitro*. The thrombin generation test assesses the whole coagulation system. It captures the end result of the whole extrinsic coagulation cascade and is a therefore a useful reflection of a prothrombotic state. Although we did not see an increase in TATc, which reflects actual activated coagulation, the increased thrombin generation suggests that activation of coagulation would occur after contact with a procoagulant trigger such as exposure to allergens or viruses.

Prednisolone also induced an increase in PAI-1 and a subsequent decrease in PAPc in our study, thereby reducing fibrinolysis. Our results fit in with previous studies showing an induction of PAI-1 gene expression by dexamethasone in several cell lines[28-30]. A reduction in fibrinolysis, in turn, has been shown to increase the venous thromboembolic risk[31].

Moreover, an increase in vWF by prednisolone was observed. Corticosteroids have been shown to activate endothelial cells and leukocytes in the absence of inflammatory stimuli leading to enhancement of vWF gene transcription[21, 32]. vWF binds to factor VIII and plays an important role in platelet adhesion[33]. Therefore, both induction of PAI-1 and enhanced vWF gene expression and secretion can explain the shift in hemostatic balance to a procoagulant state as shown in our study.

The procoagulant and anti-fibrinolytic state induced by high dose oral corticosteroid treatment has clinical implications. Not only does it provide better insight into the mechanisms as to why patients with severe asthma and frequent exacerbations are at increased risk for pulmonary embolism[1, 2], acute coronary syndrome[3, 4] and stroke[5], but it also suggests that any patient receiving frequent bursts or chronic administration of oral corticosteroids is at increased risk to develop thromboembolic events[34, 35]. In a post hoc analysis on our data we identified asthma patients at highest risk for prednisolone induced activation of coagulation. We found that asthma severity per sé did not influence this risk. However, the presence of eosinophilic inflammation, as measured by FeNO and peripheral blood eosinophils, did show an increased risk of coagulation activation (data not shown). These findings most likely reflect the additive effect of asthmatic inflammation on coagulation. Therefore, patients with severe asthma and persistent eosinophilic airway inflammation are probably better off with the new anti-inflammatory biologicals,

like anti-IL-5 or anti-IL-4/IL-13[36, 37]. Alternatively, one might consider treatment with prophylactic anticoagulants in asthma patients during exacerbations or in those receiving chronic oral corticosteroid treatment.

Our study may have some limitations. First the two groups were not well balanced as the male/female ratio was different between the two groups. However, by using a univariate general linear model the influence of sex did not alter the results. Second, we only studied patients with stable asthma, whereas it could be argued that the procoagulant effects of corticosteroids may be more important during an exacerbation. However, since most studies evaluating corticosteroid effects on coagulation and fibrinolysis have been performed during exacerbations of inflammatory diseases, we purposely focused on patients with stable disease to avoid the confounding effect of acute inflammation on coagulation[25].

In conclusion, this study shows that corticosteroids are procoagulant in patients with stable asthma by enhancing coagulation and reducing fibrinolysis. This finding has clinical implications, in particular for patients with severe eosinophilic asthma who require frequent or high doses of oral corticosteroids. These patients are at increased risk of thromboembolic events because of the combined procoagulant effects of severe airway inflammation and oral corticosteroids. Fortunately, in the near future, these patients will benefit from novel anti-inflammatory treatments with better safety profile than oral corticosteroids.

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Each author's contribution

conception and design: C.J. Majoor, P.W. Kamphuisen, T. v.d. Poll, E.H. Bel; *acquisition and analysis of data:* M.M.S. Sneeboer, C.J. Majoor, A. d. Kievit; *analysis*

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Conflict of interest

All authors declare no conflicts of interest

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