

BRIEF COMMUNICATION

Asthma is associated with reduced fibrinolytic activity, abnormal clot architecture, and decreased clot retraction rate

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Abstract

The aim of this study was to assess whether steroid-naïve asthma modulates hemostasis. We evaluated the clot retraction rate (CRR), fibrinolysis rate (FR), clot density (CD) (by confocal microscopy), plasma levels of plasminogen activator inhibitor (PAI-1), and factor XIII (FXIII), NO in exhaled breath (FE_{NO}), spirometry (FEV₁) and eosinophil count (EOS) in 36 patients with allergic, steroid-naïve asthma and in 34 healthy controls. We observed significantly ($P < 0.001$) reduced CRR, FR, and FEV₁ and increased FE_{NO}, EOS, PAI-1, FXIII, and CD in patients with asthma compared with controls. In patients with asthma, FR negatively correlated with CD, FXIII, PAI-1, FE_{NO}, and EOS and positively with FEV₁. FXIII positively correlated with CD. Clot retraction rate negatively correlated with FE_{NO} and positively with FEV₁ (all $P < 0.001$). These novel findings suggest that asthma itself is associated with decreased CRR and reduced fibrinolytic potential resulting from alterations in clot architecture and elevated levels of plasma FXIII and PAI-1.

Epidemiologic studies indicate that patients with asthma are at increased risk of pulmonary embolism (PE) (1, 2). The pathogenesis of PE is however not completely understood. Previous studies suggest that a prothrombotic state (3) and reduced fibrinolytic activity (4) might play a role in the development of PE. However, these studies were performed mainly on patients treated with corticosteroids which *per se* are known to affect hemostasis (5).

In this study, we studied the kinetics of clot formation, fibrinolysis, and retraction of the clot as well as factors known to affect the fibrinolysis rate (FR), *that is* clot architecture and plasma levels of PAI-1 and FXIII, in patients with steroid-naïve allergic asthma and compared these with healthy controls.

Methods

Subjects

Seventy adults (34 healthy control subjects and 36 patients with stable, mild steroid-naïve allergic asthma) were included in the study. The study protocol was approved by the Ethics of Research Committee of the Medical University of Białystok. Detailed information about diagnosis of patients with asthma and healthy subjects is provided in the Supporting information.

Abbreviations

BMI, body mass index; CD, clot density; CR, clot retraction; CRR, clot retraction rate; CT, clotting time; EOS, eosinophils; FE_{NO}, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in one second (1 s); FR, fibrinolysis rate; FXIII, coagulation factor XIII; MCF, maximum clot firmness; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; PPP, platelet-poor plasma; PRP, platelet-rich plasma; ROTEM, rotational thromboelastometry; TAFI, thrombin-activatable fibrinolysis inhibitor.

Table 1 Characteristics of healthy control subjects and patients with asthma

	Healthy subjects (<i>n</i> = 34)	Patients with asthma (<i>n</i> = 36)	<i>P</i>
Age (years)	43 (32–57)	45 (36–58)	0.546
Sex (M/F) (% male)	14/20 (41)	15/21 (42)	0.887
BMI	24.2 (22.5–27.2)	25.4 (22.45–28.4)	0.208
FEV ₁ (%)	102.4 (96.7–113.9)	88.1 (75.6–98.1)	<0.0001
FE _{NO} (ppb)	16.6 (2.0–36.0)	44.2 (29.3–80.0)	<0.0001
EOS (cells/ μ l)	125 (71–218)	334 (211–515)	0.0045
IgE (IU/ml)	32.3 (24–115)	112 (35.1–187)	0.0429
PLT ($10^3/\mu$ l)	237 (202–268)	257 (221–289)	0.132
Fibrinogen (mg/dl)	296 (272–341)	324 (287–412)	0.087
Thromboelastometry			
CT (s)	479 (356–516)	466 (319–598)	0.82
MCF (mm)	58 (54–62)	63 (60–68)	0.0003
Alpha angle ($^{\circ}$)	55 (51–62)	56 (51–64)	0.529
Clot density (fibers/10 μ m)	6.0 (5.0–7.1)	7.4 (6.4–8.5)	0.009
FXIII	22.6 (20.0–24.6)	32.6 (26.5–37.1)	0.0064
PAI-1 (ng/ml)	19.7 (12.4–25.6)	26.8 (19.2–39.6)	0.0069
TAFI (μ g/ml)	20.3 (13.5–27.5)	18.2 (14.2–29.7)	0.768
Fibrinolysis rate (% $\times$ min ⁻¹)	1.51 (1.21–1.59)	0.27 (0.16–0.38)	<0.0001
D-dimer	0.29 (0.21–0.39)	0.34 (0.12–0.55)	0.286
Clot retraction rate (% $\times$ min ⁻¹)	0.99 (0.87–1.14)	0.81 (0.74–0.87)	<0.0001
Degree of retraction at 120 min (%)	73.9 (68.05–77.25)	50.3 (46.55–54.43)	<0.0001

BMI, body mass index; CT, clotting time; EOS, eosinophils; MCF, maximum clot firmness; TAFI, thrombin-activatable fibrinolysis inhibitor. Results are present as median (1st–3rd quartile).

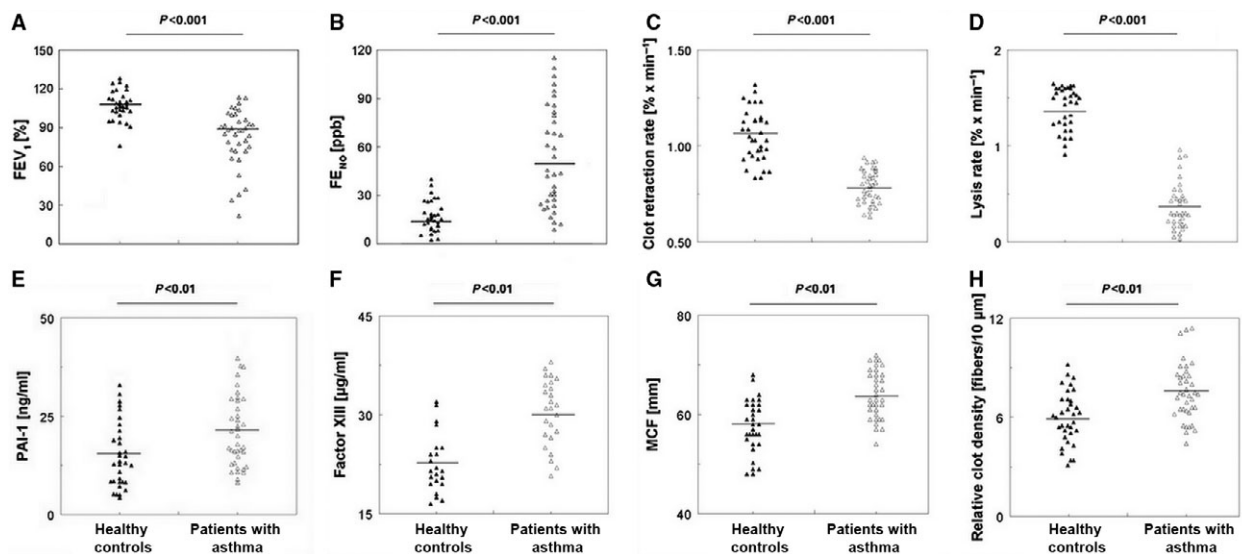


Figure 1 Comparison of FEV₁ (A), FE_{NO} (B), clot retraction (C) and fibrinolysis rates (D), plasma concentrations of PAI-1 (E) and factor XIII (F), maximum clot firmness (G), and relative clot density (H) in healthy controls and patients with asthma. Calculations of relative clot density were performed for clots made from platelet-poor plasma [PPP; more uniformly cross-linked compared with platelet-rich plasma (PRP)] and are expressed as a number of fibrin fibers

crossing a single, 10- μ m-long straight line. More details in Methods. Horizontal bars represent median values. The analyzed groups were compared by Mann–Whitney *U*-test. The number of individuals was 34 (healthy controls) and 36 (patients with asthma). FXIII levels were measured in groups consisting 22 healthy subjects and 25 patients with asthma.

Blood collection

Venous blood samples were collected into polypropylene tubes containing trisodium citrate (0.129 M). Platelet-rich plasma (PRP) was obtained by centrifugation of citrated blood at 200 *g* for 20 min. Platelet-poor plasma (PPP) was obtained by centrifugation of PRP at 1500 *g* for 15 min.

Measurements

Measurements of fractional exhaled nitric oxide (FE_{NO}), blood eosinophil count (EOS), and spirometric index (forced expiratory volume in 1 s, FEV₁) were performed as described previously (6). D-dimer levels were measured with an immunoturbidimetric assay (Innovance D-dimer; Siemens Healthcare Diagnostics Siemens Healthineers, Erlangen, Germany).

The kinetics of clot retraction (CR) was evaluated in whole blood by the optical method described previously (7). The kinetics of clot formation and fibrinolysis in whole blood were evaluated using rotational thromboelastometry (8), as described in the Supporting information.

Analyses of fibrin and platelet–fibrin clots labeled with AlexaFluor 488 were conducted using a confocal imaging system (Nikon ECLIPSE Ti/C1 Plus, Nikon, Tokyo, Japan), and the quantification of relative clot density (CD) was performed as described previously (9).

Plasma levels of PAI-1 and FXIII were measured by ELISA acquired from Abcam (Cambridge, UK). Plasma level of thrombin-activatable fibrinolysis inhibitor (TAFI) was measured using ELISA acquired from Flarebio Biotech (Wuhan, China).

Statistical analysis

The results are given as median and range. Differences between the two individual groups were analyzed using Mann–Whitney *U*-test. Correlations between parameters were assessed by a non-parametric test (Spearman's rank correlation coefficient, *r*). A *P*-value < 0.05 was considered to be of statistical significance.

Results

The characteristics of the patients with asthma and the healthy controls are shown in Table 1. Patients with asthma and control subjects were well matched for demographic variables and body mass index (BMI).

In comparison with healthy controls, the patients with asthma demonstrated decreased FEV₁ (86% of control; Fig. 1A), and higher FE_{NO} (266% of control; Fig. 1B), EOS (267% of control), and total IgE (347% of control) (Table 1).

There were no differences between patients with asthma and controls in clotting time (CT) and alpha angle, thromboelastometric variables characterizing the rate of initial thrombin formation, and contribution of activated platelets to the acceleration of thrombin generation, respectively (Table 1). By contrast, a distinct rise (109% of control) in maximum clot firmness (MCF; the parameter characterizing the mechanical stability of the clot) was noted (Table 1 and Fig. 1G).

Compared with the controls, patients with asthma had a significantly reduced FR (18% of control) which was accompanied by elevated plasma levels of PAI-1 (136% of control) and FXIII (144% of control) but unaltered D-dimers and TAFI levels (Table 1 and Fig. 1D–F). Fibrinolysis rate negatively correlated with CD, MCF, FXIII, PAI-1, FE_{NO}, and EOS and positively with FEV₁ (Table 2).

Impaired CR was observed in patients with asthma, as reflected by lower clot retraction rate (CRR) (81% of control) and reduced final clot volume (68% of control) (Table 1 and Fig. 1C). Table 2 shows that CRR correlated inversely with FE_{NO}, EOS, and FXIII and positively with FEV₁ and FR (this result (CRR vs FR) is not shown in Table 2).

Differences in the architecture of the clots formed in the blood of patients with asthma and healthy controls, visualized in a confocal microscope, are shown in Fig. 2A–D. As can be seen, in comparison with healthy controls, patients with asthma had distinctly more cross-linked clots and higher (123% of control) relative CD (Figs 1H and 2A–D; Table 1). The density of clots (formed in PPP) was positively associated with plasma FXIII level and with MCF and negatively associated with FR (Table 2).

The time course of CR in patients with asthma and in healthy controls is shown in Fig. 2E. As can be seen, in comparison with controls, patients with asthma demonstrate two-fold prolongation of the time required for obtaining 40% retraction of the clot.

Discussion

This study is the first designed to systematically compare and quantify fibrinolytic activity, CRR, and the architecture and

Table 2 Correlations between measured parameters in healthy controls and patients with asthma

	Clot density	MCF	FXIII	FEV ₁	FE _{NO}	EOS	PAI-1
CRR	−0.160	0.257*	−0.658***	0.505***	−0.554***	−0.393**	−0.289*
FR	−0.519***	−0.542***	−0.711***	0.558***	−0.590***	−0.419**	−0.472***
Clot density	–	0.860***	0.398*	−0.213	0.197	0.265	0.150
PAI-1	0.150	0.189	0.368*	−0.385**	0.457**	0.187	–

CD, clot density; CRR, clot retraction rate; EOS, eosinophils; FR, fibrinolysis rate; MCF, maximum clot firmness.

Statistical significance is shown as: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

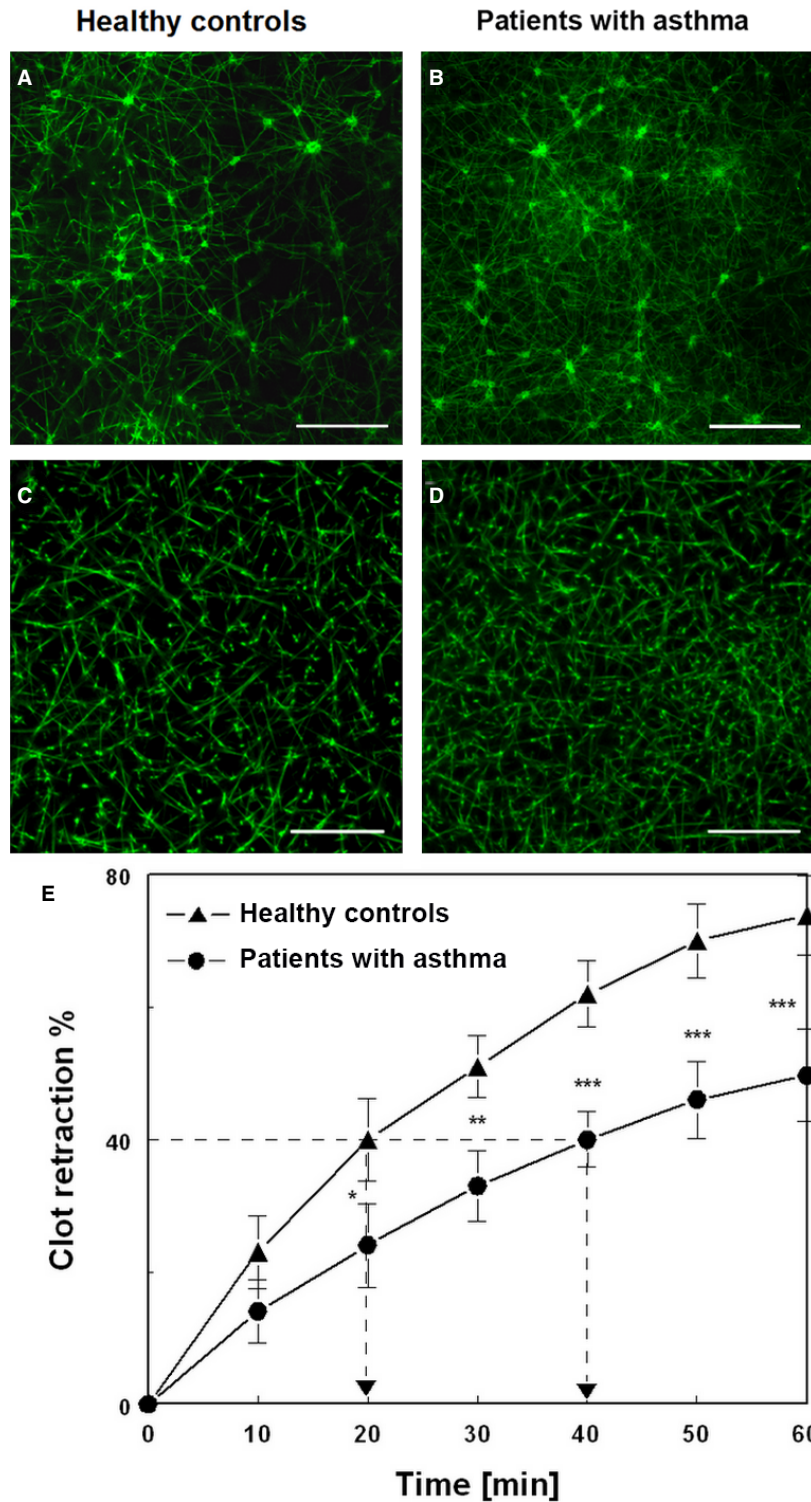


Figure 2 Comparison of architecture of platelet–fibrin and fibrin clots, and clot retraction (CR) kinetics in healthy controls and patients with asthma. Representative images from confocal microscope show the structure of clots formed in platelet-rich plasma (PRP) (A, B) and in platelet-poor plasma (PPP) (C, D). Magnification bar is 30 μm . Tracings

reflecting kinetics of CR in healthy controls and patients with asthma are shown in panel E. The dotted lines with arrowheads indicate the time required to obtain 40% of retraction. Experimental conditions as in Methods. The number of individuals was 34 (healthy controls) and 36 (patients with asthma). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

mechanical properties of clots formed *in vitro* in the blood of healthy subjects and patients with mild-to-moderate steroid-naïve asthma.

The results presented here show that not only steroid-treated (10) but also steroid-naïve asthma is associated with a significant reduction of fibrinolytic activity in peripheral blood (Table 1 and Fig. 1C).

In contrast to previous studies (3), which were performed on asthma patients treated with steroids, we did not observe marked activation of coagulation as measured in whole blood by thromboelastometry (unaltered CT and alpha angle; Table 1). This may exclude the contribution of platelet-related fibrinolysis resistance to reduction of fibrinolysis potential in steroid-naïve asthmatic patients (9, 11, 12).

This study shows that steroid-naïve asthmatic patients have elevated levels of PAI-1 in their plasma and that the PAI-1 level inversely correlates with the FR (Tables 1 and 2; Fig. 2D,E). This is consistent with previous studies performed on steroid-treated asthmatic patients (3, 4, 13, 14). Our results also show that the rise in plasma PAI-1 levels is better correlated with FEV₁ (reflecting structural changes in the lungs) than with FE_{NO} and EOS (reflecting an inflammatory state; Table 2). Such association may imply a pulmonary origin for this inhibitor.

The data presented here show for the first time that asthma is associated with a significant rise in plasma levels of FXIII (Table 1 and Fig. 1F). FXIII is known to reduce fibrinolytic activity directly (through the incorporation of α -2-antiplasmin into the clot structure) and indirectly, by supporting the formation of clots that are denser and thus more resistant to lysis (15).

It has been reported that FXIII can be produced in the bronchoalveolar compartment of subjects with chronic (14, 16) and allergen-evoked bronchoalveolar inflammation (17). It is therefore likely that in patients with asthma, FXIII can leak out from the bronchoalveolar compartment into systemic circulation where it can contribute to reduced fibrinolytic activity. Accordingly, we show that in patients with asthma, the level of FXIII is negatively correlated with FR and positively correlated with CD and MCF, indicating its influence on clot architecture and its contribution to reduced fibrinolytic activity.

The architecture of the clot has a great impact on the kinetics of its lysis. In general, clots consisting of thin and highly cross-linked strands (denser) dissolve at a lower rate (11, 15). The data presented here show for the first time that in comparison with healthy controls, clots from non-steroid-treated asthmatic patients are denser (more cross-linked) (Table 1; Figs 1H and 2A–D). The relative density of these clots (reflecting the clot architecture) strongly positively correlated with MCF (reflecting the strength of the fibrin clot; Table 2). In addition, both CD and MCF negatively correlated with FR indicating that reduction of fibrinolytic potential in patients with asthma may result from altered clot structure (Table 2).

To sum up, it is likely that in patients with asthma, PAI-1 and FXIII produced in the airways may diffuse into systemic circulation where they can reduce fibrinolytic activity directly (PAI-1 and FXIII) and indirectly (FXIII), by supporting the formation of clots that are more resistant to lysis.

Our results indicate that patients with asthma have impaired CR. This is an interesting finding, as studies performed on animal models have revealed that inhibition of CR may result in PE (18). Differences in the kinetics of CR between healthy controls and patients with asthma, reported in this study, strongly indicate that asthma is expected to be associated with a twofold prolongation of the time of anoxia of the neighboring tissues due to attenuation of the retraction of the formed clot (Fig. 2E). Accordingly, the final volume of the clots was increased (Table 1) and their strength (MCF) was reduced (Table 1; Fig. 1G). Impaired CR may thus be important for the development of PE in patients with asthma. The impact of impaired CR on the development of PE is even more likely when we realize that in patients with asthma, NO, produced in lungs, has recently been proposed to contribute to the reduction of CR rate (6).

Overall, our results are consistent with the hypothesis that steroid-naïve asthma is associated with the release into systemic circulation of factor(s) modulating clot architecture (FXIII), CRR (NO), and fibrinolytic activity (PAI-1, FXIII). The thus evoked reduction of fibrinolytic potential and impairment of CR might explain the increased risk of pulmonary thromboembolism in steroid-naïve asthma.

Author contributions

M.M.T.-L. and T.M. designed research, performed experiments, analyzed the data, and wrote the manuscript. T.R. and J.B.-J. performed experiments and analyzed the data. A.B.-L. contributed to the acquisition of data. M.T. designed research and wrote the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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Conflicts of interest

Authors declare no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Patient characteristics and Thromboelastometric analyses.

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