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Urinary fibrinopeptide-A as a predictive biomarker of exacerbation in asthma

Jonathan J. Owen [a], Suzanne L. Edgar [b], Scott Elliott [c], Sumita Kerley [c], Thomas L. Jones [c], Daniel Neville [c], Carole Fogg [c], Thomas P. Brown [c], Anoop J. Chauhan [c], Janis K. Shute [b,*]

[a] Department of Respiratory Medicine, Royal Hampshire County Hospital, Hampshire Hospitals NHS Foundation Trust, Winchester, SO22 5DG, UK
[b] Institute of Biomedical and Biomolecular Sciences, University of Portsmouth, PO1 2DT, UK
[c] Department of Respiratory Medicine, Queen Alexandra Hospital, Portsmouth Hospitals NHS Trust, Portsmouth, PO6 3LY, UK

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ABSTRACT

Background: Asthma is a prevalent and potentially life-threatening disease associated with exacerbation and costly hospital admissions. The coagulation cascade is up-regulated in severe asthma and increased fibrinogenesis in the airway may precede exacerbation in moderate asthma.
Objective: A longitudinal prospective study to test the hypothesis that levels of urinary fibrinopeptide A (FP-A), a marker of coagulation, increase prior to an exacerbation of asthma.
Methods: 24 non-smoking participants with moderate to severe asthma were recruited and followed to exacerbation and to recovery for up to 8 weeks afterwards. Baseline measurements included spirometry, full blood count, atopic status and plasma markers of coagulation. Participants provided daily Peak Expiratory Flow (PEF) readings and three urine samples per week for analysis of FP-A, a specific marker of activation of coagulation. A novel method to concentrate urinary FP-A for immunodetection and quantification was developed. Participants were followed up until exacerbation, when baseline measurements were repeated, and monthly thereafter for 2 months or to recovery.
Measurements and main findings: 17 participants exacerbated during the study. Significantly increased concentrations of plasma D-dimer (0.25(0.2–0.42) vs 0.21(0.12–0.29) μg FEU/ml, $p = 0.02$) were found at exacerbation. A peak in urinary FP-A concentration was detected on average 4.2 ± 2 days prior to exacerbation and was significantly ($p < 0.05$) higher than at exacerbation or 7 days later. Urinary FP-A concentrations correlated positively with time to recovery and negatively ($p < 0.01$) with IgE concentration.
Conclusion: FP-A is detectable in urine several days before the onset of an asthma exacerbation indicating disordered coagulation preceding asthma exacerbations.

1. Introduction

Asthma is a global health problem affecting 300 million people worldwide [1] and leading to 397,000 deaths per year [2]. Exacerbations account for the majority of mortality and costs attributable to asthma [3–5], and are one of the main reasons for hospitalisation. Viral respiratory tract infection and allergy are major risk factors for exacerbation of asthma [6] and uncontrolled Type 2 airway inflammation increases the risk of exacerbation in exacerbation prone patients [7].

Inflammation is closely integrated with innate immune defences and coagulation systems [8,9]. We have previously demonstrated that severe asthma requiring high dose inhaled corticosteroid therapy is associated with a pro-fibrinogenic, anti-fibrinolytic environment in the airways [10] and fibrin formation in the airways of a patient with fatal asthma has been described [11]. Both viral infection [12] and allergen exposure [13], major triggers of exacerbations, have been shown to activate the intrapulmonary coagulation cascade. The balance of the systemic coagulation cascade is affected in both non-allergic [14] and allergic asthma [15], with evidence of further increased systemic fibrin formation/decreased fibrinolysis on exacerbation in adults [15] and children [16].

Intrapulmonary fibrin formation is proposed to induce airway closure by neutralising surfactant and increasing the surface tension of airway surface liquids [11]. In addition, fibrinopeptides A and B, which

* Corresponding author.
E-mail address: jan.shute@port.ac.uk (J.K. Shute).

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are released during thrombin-mediated conversion of fibrinogen to fibrin, were reported to decrease lung compliance and ventilatory conductance and decrease pulmonary blood flow [17], to activate neutrophil elastase release and mast cell histamine release [18] and to have fibroblast mitogenic activity [19] important in airway remodelling.

However, little is known of the risk of exacerbations in relation to markers of coagulation in urine or plasma. Therefore, in this study we sought to prospectively assess changes from baseline in plasma markers of coagulation at exacerbation, and concentration of FP-A in urine before, during and after exacerbation of asthma.

2. Methods

2.1. Study population and assessments

Study participants aged 18–70 years were recruited from the severe asthma clinic at Portsmouth Hospitals NHS Trust (PHT), had a physician diagnosis of asthma for at least 12 months, at least one exacerbation in the previous 12 months, were exacerbation free for the preceding 4 weeks and were classified as British Thoracic Society (BTS) Steps 3–5, taking medium to high doses of inhaled steroids [20]. Participants were excluded if they were current or ex-smokers of greater than 20 pack years, had clinically significant cardio-pulmonary disease other than asthma, other significant co-morbidities uncontrolled with standard treatment, were on long-term anticoagulation, immune-modulators or interventional drug studies, had a bleeding diathesis, were alcohol or recreational drug abusers, were pregnant or had an abnormal chest X-ray or pulmonary imaging. The study was approved by the Berkshire Research Ethics Committee (reference 10/H0505/59).

Lung function was assessed by spirometry, static and dynamic lung volumes and fractional exhaled nitric oxide (FeNO). Emphysema was excluded by gas transfer measurements in all ex-smokers. Skin prick tests to common aeroallergens were performed. The panel of allergens tested consisted of positive and negative controls, tree pollen, grass pollen, house dust mite, cat epithelia, dog and Aspergillus fumigatus. Blood tests in samples taken at baseline and exacerbation were performed by the pathology services at PHT using commercial analysers and standard protocols and included full blood count, c-reactive protein (CRP), D-dimer (a marker of fibrin turnover), fibrinogen and IgE, INR and APTR. Plasma markers of platelet and endothelial activation (thrombin activatable fibrinolysis inhibitor (TAFI), plasminogen activator inhibitor-1 (PAI-1), platelet factor-4 (PF-4), transforming growth factor β1 (TGF-β1) and vascular endothelial growth factor (VEGF)) were analysed by enzyme-linked immunosorbent assay (ELISA). PAI-1, TGF-β1, VEGF and PF4 were analysed using ELISA kits from R&D Systems, and TAFI was measured using ELISA kits from Affinity Biologicals.

Participants were prospectively followed up for the study period of 11 months during which they recorded twice daily PEF measurements, kept a daily symptom diary (including SABA use) and collected thrice-weekly urine samples that were stored at home at 4 °C for collection every 5–7 days for subsequent storage at -80 °C prior to analysis.

Participants contacted the research team on exacerbation for review, with baseline assessments repeated. This was repeated at four week intervals until self-reported recovery to baseline state or up to 8 weeks. Exacerbations met the ATS/ERS 2009 criteria [21] for a severe exacerbation (use of/increase in systemic corticosteroids, 40 mg oral prednisolone once daily, for at least 3 days). Participants completed the study once recovered from exacerbation or at the end of the study period.

2.2. Laboratory methods

For FP-A analysis, protease inhibitors (Merck, UK) were added to 1 ml urine samples and centrifuged at 5000 g for 10 min at 4 °C. Cleared samples were passed through an Amicon Ultra-Concentrator centrifuge filter with 3 kDa cut off (Merck Millipore, UK) at 14,000 g for 60 min at 4 °C. The filtrate in the lower compartment (<3 kDa) was collected for FP-A (1500 Da) analysis. 1 ml of filtrate was added to a PD Miditrap G-10 (GE Healthcare) column equilibrated with deionised water and run into the bed. 0.7 ml water was run into the bed and any flow-through discarded. 1.5 ml water was added to the top of the column and the eluate collected and freeze-dried.

The lyophilised sample was reconstituted in 150 μl UHQ water and 1 μl of 3 mg/ml mouse anti-FPA [49D2] antibody (Abcam, UK). A standard curve was prepared using human FP-A (Sigma) in the concentration range 0–20 ng/ml in water and 1 μl of 3 mg/ml mouse anti-FPA [49D2] antibody added to 150 μl of each. Samples and standards were allowed to bind the antibody overnight at 4 °C, to facilitate subsequent binding of the small (1500 Da) peptide to nitrocellulose membranes.

Peptide-antibody complexes were bound to 0.2 μm nitrocellulose membrane (BioRad, UK) soaked in 20% methanol/PBS without Ca/Mg in a 96-well vacuum manifold (Jencons, UK). The samples, standards (150 μl) or blanks (20% methanol/PBS without Ca/Mg) were added and the fluid pulled through under vacuum. Proteins were fixed by baking the nitrocellulose membrane for 30 min at 100 °C in a pre-heated oven.

The membranes were stained for FP-A, with all steps at room temperature, as follows. Membranes were blocked for 1 h in 100 ml of 2% Tween-20 and 5% skimmed-milk powder in PBS without Ca/Mg, then washed 5 × 5 minutes in 100 ml of 0.1% Tween-20 in PBS without Ca/Mg.

Membranes were incubated for 1 h with 5 ml per blot of sheep anti-FPA 1-16-HRP (Affinity Biologicals, Canada) diluted 1:10,000 (200 ng/ml final concentration) in 2% Tween-20 and 5% skimmed milk in PBS without Ca/Mg.

Membranes were washed 5 × 5 minutes in 100 ml of 0.1% Tween-20 in PBS without Ca/Mg and HRP detected using West Pico Chemiluminescent (Thermo Fisher, UK) solutions applied for 5 min. Excess solution was removed and luminescence captured using the Syngene G: Box using a series of 10 × 3 minute captures.

2.3. Data analysis

Cohort characteristics were described using median and interquartile ranges, or mean and standard deviations for normally distributed data. Paired data were compared using the paired *t*-test and Wilcoxon matched-pairs test for parametric and non-parametric data respectively. Changes in symptom score and reliever use were analysed by one-way ANOVA with Tukey's multiple comparison test. Changes in urinary FP-A in relation to the time of exacerbation were analysed by one-way ANOVA with Dunn's multiple comparisons test. Data from sub-group analysis of urinary FP-A to time to recovery was analysed using the Mann-Whitney unpaired *t*-test. The correlation between plasma IgE and urinary FP-A was analysed by Spearman rank correlation for non-normally distributed data. Statistical analyses were conducted using GraphPad Prism 8 (GraphPad Software Inc., USA).

3. Results

24 participants were recruited, of which 17 suffered an exacerbation during the study period (Fig. 1).

Demographic data on participants is shown in Table 1.

Of the 17 exacerbating patients, one participant was withdrawn due to a diagnosis of pneumonia at the first recovery visit, one participant was withdrawn due to a second exacerbation prior to the second recovery visit, and one had not recovered by the second recovery visit. Participants exacerbated at a median of 52 days (IQR 28–70.5) post-study entry. There were no significant differences between participants who exacerbated and did not exacerbate in terms of age, gender or asthma severity, however participants who exacerbated were significantly more likely to have peripheral blood eosinophilia (≥400/μL) at baseline ($p < 0.01$).

PEF values were compared between baseline (average for the first two weeks of measurement) and the average value for the 7-day period

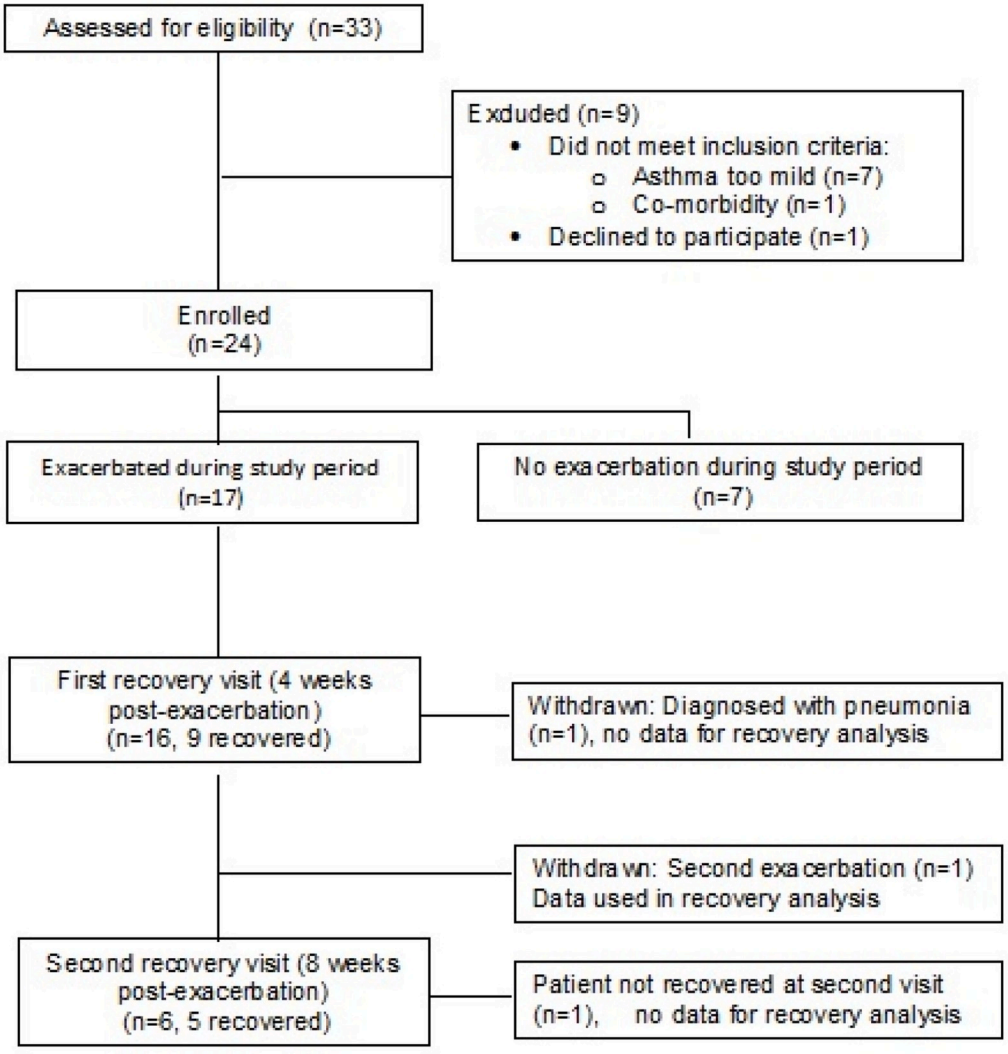

Fig. 1. STROBE diagram of study participation.

prior to exacerbation; the mean (±SD) baseline PEF was 304 (±106) litres per minute, and this dropped to 281 (±105) litres per minute in the week leading up to an exacerbation ($p = 0.01$, $n = 16$ (one patient had insufficient data recorded and was excluded from this analysis). PEFR variability did not increase significantly in the period prior to exacerbation.

A symptom score from 0-4 was derived from the presence or absence of wheeze, night wakening, chest tightness and breathlessness during a 24 h period. A significant ($p < 0.05$) increase in average symptom score was seen in the 7 days prior to exacerbation (2.33 ± 0.86) compared to the 7 day average at baseline (1.43 ± 1.01). A further significant ($p < 0.01$) increase in the symptom score was noted on the day of exacerbation (3.4 ± 0.83).

Reliever use, either number of puffs of SABA and/or number of SABA nebulisers in 24 h was also recorded. Reliever use was not significantly increased in the 7-day period prior to exacerbation (5.64 ± 4.6) compared to the baseline 7-day average (4.31 ± 4.9). Reliever use was increased significantly on the day of exacerbation (8.5 ± 6.93) compared to both the baseline period ($p < 0.01$) and the 7-day period prior to exacerbation ($p < 0.05$).

Plasma markers of fibrin turnover were assessed in samples collected at baseline and exacerbation, with the results shown in Table 2.

This demonstrates a small, but statistically significant, increase in plasma D-dimer concentration at exacerbation, and a 46% reduction in TGFβ1 at exacerbation (both $p = 0.02$). VEGF, PF4, fibrinogen, PAI-1 and TAFI did not change significantly from baseline to exacerbation. INR and APTR at baseline were in the normal reference range (0.8–1.2) of both parameters, and there was no significant change in the INR or APTR at exacerbation.

FP-A was analysed in urine samples collected in the 7 day periods before and after an exacerbation, and on the day of exacerbation (Fig. 2). The novel analytical approach to samples and standards is illustrated in Fig. 2A. Using this method, significantly higher peak FP-A concentration (0.395 (0.114–2.256) ng/ml) were detected 4.2 ± 2 days prior to exacerbation (0.054 (0.022–0.765) ng/ml, $p < 0.05$) and the average value for the 7-day post exacerbation period (0.038 (0.007–0.174 ng/ml, $p < 0.0001$) (Fig. 2B). Sub-group analysis (Fig. 2C) demonstrated significantly ($p < 0.05$) higher levels of urinary FP-A in the 5 patients that had not recovered within 4 weeks (2.866 (0.48–5.758) ng/ml) compared to those 9 patients that recovered within 4 weeks (0.189 (0.0725–1.508) ng/ml). The patient that was re-admitted with a second exacerbation prior to the 8 week follow-up had the highest level of FP-A measured pre-exacerbation (9.41 ng/ml).

Urinary FP-A concentrations (peak values or 7-day average values) did not correlate with PEF values, reliever use or symptom scores over the 7 day period prior to exacerbation. However, a significant ($p < 0.01$) negative correlation (Spearman rank correlation coefficient, $r = -0.635$) was noted between plasma IgE measured at baseline, and the peak

Table 1
Participant demographics (N = 24).

Characteristic		Mean (±SD)/Median (Q1-Q3)/n (%)
Age (years)		47.1 (±12.6)
BMI (kg/m^2)		28 (25–33)
Female gender		20 (83%)
FEV1% Predicted		71.6% (±19.7)
FEV1/FVC ratio		0.66 (±13.8)
FeNO (ppb)		19.5 (13.8–39.5)
Inhaled corticosteroid dose (BDP equivalents)		2000 (1150–2000)
Oral corticosteroids		7 (29%)
Montelukast (10 mg po, nocte)		12 (50%)
Theophylline		10 (42%)
Long acting β2-agonists		22 (92%)
Peripheral eosinophil count (10^9/L)		0.3 (0.1–0.4)
International normalised ratio (INR)		0.8 (0.8–0.9)
Activated partial thomboplastin ratio (APTR)		1.0 (0.9–1.1)
Exacerbations past 24 months		5.1 (±3.1)
BTS 2016 classification	Step 3	7 (29%)
	Step 4	9 (38%)
	Step 5	8 (33%)
Atopic	SPT positive	17 (71%)
	Raised IgE >81IU/ml	12 (50%)
Smoking status	Ex	83%
	Never	17%

Table 2
Plasma markers of fibrin turnover at baseline and exacerbation.

Variable	N	Baseline median (Q1-Q3) or mean (±SD)	Exacerbation median (Q1-Q3) or mean (±SD)	P-value
TAFI (ng/ml)	17	5406 (±2591)	4764 (±2030)	0.32
PAI-1 (pg/ml)	17	15000 (10336–30100)	16200 (11400–37850)	0.33
D-dimer (μgFEU/ml)	14	0.21 (0.12–0.29)	0.25 (0.20–0.42)	**0.02**
Fibrinogen (g/l)	13	3.08 (±0.57)	3.21 (±0.50)	0.40
Platelets (10^9/L)	13	305 (±61)	318 (±64)	0.28
PF4 (μg/ml)	17	3.9 (±2.9)	2.6 (±1.6)	0.12
TGFβ1 (pg/ml)	17	1801 (721–2570)	966 (682–2376)	**0.02**
VEGF (pg/ml)	17	23 (3–94)	20 (0–37)	0.22
Eosinophils (10^9/L)	13	0.30 (0.15–0.50)	0.30 (0.20–0.85)	0.28
C-reactive protein (mg/L)	15	<5 (<5–7)	8 (4.25–12.5)	0.07
INR	16	0.8 (0.8–0.8)	0.8 (0.8–0.9)	0.07
APTR	16	1.0 (0.9–1.1)	1.0 (0.9–1.075)	0.86

urinary FP-A concentration detected in the 7 days prior to exacerbation (Fig. 2D). A similar significant ($p < 0.05$) negative correlation (Spearman rank correlation coefficient, $r = -0.71$) was also noted with plasma IgE at exacerbation.

4. Discussion

These data demonstrate significant activation of the coagulation system in moderate to severe asthmatics prior to an exacerbation. An increase in coagulation is supported by evidence of higher concentrations of urinary FP-A, the first peptide cleaved from fibrinogen during thrombin-induced coagulation, in the 7 days leading up to an exacerbation relative to the week after the exacerbation. We propose this reflects coagulation in the airways, as indicated by our previous report of highly up-regulated fibrin formation in the airways of a patient with moderate asthma 5 days before a severe exacerbation requiring hospital admission [22]. FP-A is rapidly cleared by catabolism in the lung and the kidney, and urinary FP-A represents only a small fraction of that initially derived from cleavage of fibrinogen [23]. However, importantly, FP-A is the first urinary biomarker of coagulation to show promise in the prediction of an exacerbation of asthma. Maximum levels of FP-A in urine were detected, on average, 4.2 ± 2 days before exacerbation which offers a substantial period of time for intervention prior to an increase in symptoms that might normally warrant an escalation in treatment.

Exacerbations were defined per ATS/ERS 2009 criteria [21] as an increase in symptoms, sufficiently severe to warrant a course or an increase in oral corticosteroid. The exacerbations were confirmed by review with the study medical staff, and this is supported by the observed drop in PEF in the week leading up to exacerbation. However, the differences in PEF between pre-exacerbation and exacerbation measurements in our study were small, and are therefore useful objective markers of exacerbation but not clinically practicable to predict exacerbation in this study.

Others have reported that a combination of symptom score (increase of more than two standard deviations) and PEF (decrease to <70% personal best) optimally predicted an asthma exacerbation a mean of 4.1 days before occurrence [24]. It is interesting that we similarly detect a maximum increase in urinary FP-A at a mean of 4.2 days prior to exacerbation, which may therefore relate to increased pulmonary coagulation. In a large study of changes in PEF, symptom score and bronchodilator use over 14 days before and after an exacerbation, a relatively high level of false positive measures for PEF, symptom score and rescue treatment were reported outside the 28 day surrounding an exacerbation [25]. However, more work is needed to determine if changes in urinary FP-A will prove to be a more specific and sensitive test for predicting the onset of an asthma exacerbation.

Nine of twenty-four participants did not complete the study, primarily due to failure to exacerbate. This may be expected in an intensive study such as this due to improved medication compliance and the withdrawn participants were not significantly different to those who completed. Eosinophilia is a known marker for risk of exacerbation and that is demonstrated in this study. The relatively short time to exacerbation (median 52 days) in those who did exacerbate may reflect the predominantly female patient group [25] and their relative obesity which predisposes to early airway closure [26].

Changes in the balance of coagulation have previously been reported in peripheral blood and the airway in asthma. Previous work in our unit [10] has shown that moderate asthma is associated with a fibrinolytic intrabronchial environment which is corrected by inhaled steroid whereas severe asthma is associated with a pro-fibrinogenic, anti-fibrinolytic, airway environment, despite high dose inhaled steroids. Patients with asthma are at increased risk of pulmonary embolism [27,28] which is associated with increased risk of asthma exacerbation [28], and impaired systemic fibrinolysis was specifically associated with risk of severe exacerbation [29].

Sneeboer et al. [30] investigated the impact of an exacerbation on the coagulation system by withdrawing inhaled steroids of 23 participants with moderate to moderately severe asthma, but they did not find significant changes in von Willebrand Factor (vWF), D-dimer, thrombin-antithrombin complex (TATc), plasminogen activator inhibitor-1 (PAI-1) or plasmin-antiplasmin complex(PAPc) in peripheral blood. In our study, we did not induce exacerbations, but rather followed participants up until natural exacerbations occurred, and this may account for detection of significant changes in the plasma D-dimer marker of fibrin turnover. Manuyakorn et al. [16] followed a paediatric group from exacerbation to recovery and found increased vWF, PAI-1, and CRP during exacerbation and a positive correlation between D-dimer and exacerbation score, but not a significant difference between D-dimer at exacerbation and stable state. PAI-1 and CRP increased at exacerbation in our cohort but not significantly which may reflect differences between adult and paediatric populations.

Significantly lower levels of TGFβ in the circulation were previously reported in children with acute exacerbation of allergic asthma [31]. We also found significantly lower levels of plasma TGFβ1 at exacerbation.

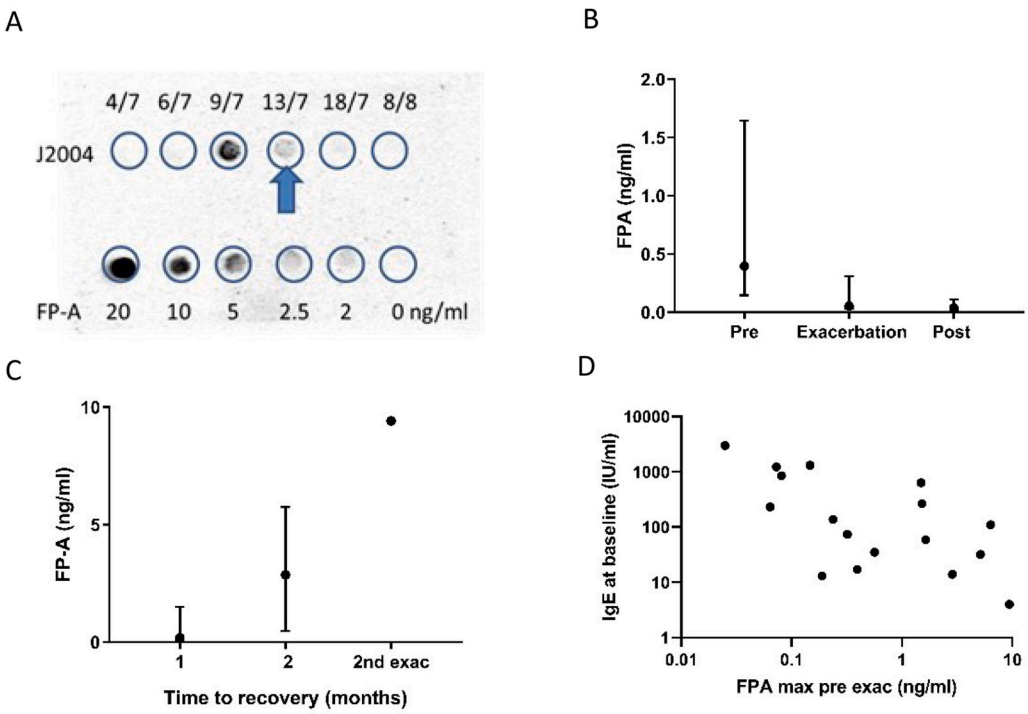

Fig. 2. Urinary FP-A analysis by immunoblot.
A; A representative immunoblot showing the FP-A standard curve and a set of urine samples collected from a patient before and after an exacerbation, indicated by the blue arrow. Blots were analysed by scanning densitometry using Quantiscan software.
B; FP-A concentration (median and interquartile range, n = 17) in urine in the 7-day period before and after an exacerbation of asthma. The data shows the highest value detected in urine before an exacerbation which on average was 4.2 ± 2 days prior to an exacerbation, and the average concentrations on the day of exacerbation and in the 7 days afterwards.
C; FP-A concentration (median and interquartile range) in urine in the 7-day period before an exacerbation in relation to time to recovery by one month (n = 9) and by 2 months (n = 5).
D; Plasma IgE at baseline in relation to the highest value of urinary FP-A detected in the 7-day period prior to exacerbation (n = 17).

Since TGFβ1 is a potent inducer of PAI-1 expression we might have predicted significantly lower levels of plasma PAI-1. However, D-dimers also stimulate the release of biologically active PAI-1 from monocytes [32] and may contribute to the sustained levels of PAI-1. D-dimers and other fibrin(ogen) fragments are reported to modulate the inflammatory response [33], and may therefore be both markers of coagulation and mediators of inflammation.

The urinary FP-A levels are significantly lower at exacerbation and afterwards than those detected prior to an exacerbation. This suggests that coagulation initiated in the airways prior to exacerbation is subsequently limited, potentially by serpins acting under the control of glycosaminoglycans, including mast cell-derived heparin [34]. However, anticoagulant protein C [35] and heparin [36] are relatively depleted in patients with asthma, supporting a pro-coagulant state. Factors that limit coagulation may therefore include tissue factor pathway inhibitor (TFPI) [37] or the endogenous thrombin inhibitor cartilage oligomeric matrix protein (COMP) [38], both major plasma and platelet derived inhibitors of thrombin.

The significant negative correlation of urinary FP-A prior to exacerbation with plasma IgE concentrations suggest an IgE-mediated anti-coagulation mechanism. This might reflect IgE-dependent mast cell activation and release of heparin or tryptase, which cleaves fibrinogen α and β-chains and renders it unclottable by thrombin [39]. Alternatively, IgE-dependent recruitment and activation of platelets in the airways contributes to many of the characteristic features of asthma, including bronchial hyperresponsiveness, bronchoconstriction, airway inflammation and airway remodelling [40]. However, the role of platelets in promoting pulmonary coagulation and/or IgE-dependent regulatory pathways to limit fibrin formation needs further investigation.

Bazan-Socha et al. [41] previously reported increased endogenous thrombin potential (ETP) and impaired fibrinolysis (prolonged clot lysis time) in asthma patients compared to healthy controls. They further reported no significant difference in ETP between individuals with allergic asthma and non-allergic asthma, although defective fibrinolysis, measured as a significantly longer clot lysis time, was reported in patients with non-allergic compared to allergic asthma [41]. However, a correlation with IgE was not reported. The same study also found that montelukast therapy, but not systemic corticosteroid therapy, in asthmatics was associated with significantly increased endogenous thrombin potential (ETP), an indicator of a pro-thrombotic/hypercoagulable state, as well as an increased clot lysis time. Half of our study participants were receiving montelukast as part of their standard therapy, but we found no significant difference ($p = 0.86$) in the subgroup analysis of urinary FP-A prior to exacerbation in those receiving montelukast therapy and those who were not.

Overall, our study explored the feasibility of detecting FP-A, a marker of coagulation, in urine before, during and after an exacerbation of asthma using a novel immunodetection method. A limitation of the study is that the results are from a small, but well characterised, mostly severe, predominantly female, Caucasian cohort with high BMI, predisposed to hypercoagulation [41], and need to be validated in other populations. Current smokers were excluded as cigarette smoking is known to affect the balance of the coagulation cascade [42–44], and so these results may not apply to people with asthma who smoke.

Triggers of the asthma attack were not identified in our study, but are likely to be viral infections and associated factors such as airway bacteria [45] and allergens [6]. It is estimated that up to 85% of asthma exacerbations in children and up to 80% in adults are linked to viral infections [45]. Viral infection [12] and allergen exposure [13] have been shown to promote pulmonary fibrin formation. Coagulation is believed to play a role in clearance and control of infectious agents [46] and, in a mouse model, fibrinopeptide B was recently identified as a potential serum marker of RSV infection [47].

This is the only study to date examining markers of coagulation in urine in acute exacerbation of asthma. A further prospective study is required to confirm the utility of the specific marker of coagulation, urinary FP-A, as a predictive biomarker of exacerbation, in the hope that, through escalation of treatment, it may change the natural course of the disease, and in particular the accelerated decline in lung function associated with exacerbation of asthma [48,49]. Others have reported that urinary bromotyrosine, measured by stable isotope dilution high-performance liquid chromatography with on-line electrospray ionisation tandem mass spectrometry, predicts asthma exacerbations in children [50]. However, detecting FP-A in urine presents the realistic prospect of translating these findings into a practical and acceptable point-of-care test, such as a lateral flow (dip-stick) test for patients with

asthma.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Jonathan J. Owen: Conceptualization, Investigation, Data curation, Writing - review & editing. **Suzanne L. Edgar:** Methodology, Data curation, Writing - review & editing. **Scott Elliott:** Investigation, Data curation, Writing - review & editing. **Sumita Kerley:** Investigation, Data curation. **Thomas L. Jones:** Data curation, Writing - original draft. **Daniel Neville:** Data curation, Writing - review & editing. **Carole Fogg:** Conceptualization, Data curation, Writing - review & editing. **Thomas P. Brown:** Conceptualization, Writing - review & editing. **Anoop J. Chauhan:** Conceptualization, Writing - review & editing. **Janis K. Shute:** Conceptualization, Data curation, Methodology, Writing - original draft.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yrmex.2020.100021.

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