



# Serum periostin in smokers and never smokers with asthma



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## KEYWORDS

Asthma;  
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## Summary

**Background:** Elevated serum periostin is associated with airway eosinophilia and may predict response to therapies targeting Th<sub>2</sub> inflammation. Smoking in asthma is generally associated with non-eosinophilic airway inflammation and corticosteroid insensitivity. We determined the effect of smoking status on serum periostin in asthma.

**Methods:** Serum periostin (ELISA; Aviscera Bioscience) was measured in 107 patients with stable asthma of different disease severity and 45 healthy controls. The effects on serum periostin of clinical indices including smoking status and of inflammatory biomarkers including sputum eosinophil count were analysed. Serum periostin was measured before and after two weeks of oral corticosteroids in a separate group of 33 non-smokers and smokers with stable asthma.

**Results:** Serum periostin (median [IQR], ng/mL) was reduced in smokers with asthma compared to never smokers with asthma; 9 (9, 307) versus 233 (34, 1108) respectively,  $p = 0.017$ . Periostin was not influenced by disease severity ( $p = 0.786$ ) or atopic status ( $p = 0.144$ ). There was a weak correlation between serum periostin and sputum eosinophil count in smokers with asthma ( $r = 0.315$ ,  $p = 0.020$ ). The proportion of patients with an elevated serum periostin concentration was greater in never smokers with asthma compared to smokers with asthma [65% versus 39% respectively,  $p = 0.003$ ]. Oral steroid treatment reduced serum periostin ( $p = 0.030$ ) in non-smokers with asthma.

**Conclusion:** Despite lower median serum periostin concentrations in smokers with asthma compared to never smokers with asthma, approximately forty percent of this group had a high

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level. The potential value of a raised serum periostin concentration in predicting a beneficial response to therapies targeting Th<sub>2</sub> inflammation in smokers with asthma requires to be investigated.

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## Introduction

Periostin is a matricellular protein expressed in mesenchymal cells [1] as well as in bronchial epithelial cells [2,3]. The Th<sub>2</sub> cytokine interleukin (IL)-13 induces bronchial epithelial cells to secrete periostin [2]. Periostin has been proposed as a biomarker of IL-13 activity in asthma [4] and elevated circulating levels of periostin may identify a Th<sub>2</sub>-high inflammatory phenotype of asthma [4,5]. In support of a possible role for serum periostin as a biomarker of airway inflammation in asthma, elevated serum periostin concentrations are associated with airway eosinophilia in non-smokers with moderate to severe asthma [6], with a greater decline in lung function [7] and with a favourable response to treatment of asthma with the IL-13 monoclonal antibody lebrikizumab [8,9] and the anti-IgE monoclonal antibody omalizumab [10].

Smoking in asthma is associated with poor symptom control, corticosteroid insensitivity and a non-eosinophilic airway inflammation compared to never smokers with asthma [11–16]. Atopy levels, as assessed as specific IgE levels to common environmental allergens, are lower in smokers with asthma compared to never smokers with asthma [15]. It is not known whether serum periostin concentrations are altered in smokers with asthma. Understanding the influence of cigarette smoking on serum periostin concentrations in asthma is important as it may help stratify these patients for therapies targeting these pathways. Our hypothesis was that circulating serum periostin concentration was reduced in smokers with asthma compared to never-smokers with asthma. We aimed to determine the effect of smoking status on serum periostin in patients with asthma of different severities and also the effects of oral corticosteroids on circulating periostin concentrations.

## Methods

### Subjects & study designs

A cross sectional study was performed in subjects with asthma and in healthy controls [17]. Clinical, physiological and induced sputum measurements were performed at baseline. Participants were recruited with mild, moderate and severe persistent asthma (GINA classification) [18], (both current smokers and never-smokers) as well as a group of healthy smokers and never smokers. *Asthma criteria:* Age range 18–75 years and duration of asthma  $\geq 6$  months; Symptoms of episodic wheezing, chest tightness and/or dyspnea; Objective confirmation by airway hyper-responsiveness determined by a  $\geq 20\%$  drop in FEV<sub>1</sub> at a methacholine dose of  $\leq 8$  mg/ml or when FEV<sub>1</sub>  $< 60\%$

predicted, by evidence of airflow variability with a  $\geq 12\%$  and 200 ml increase in FEV<sub>1</sub> following nebulised albuterol 2.5 mg. Smokers had at least 10 pack-year history and were currently smoking 5 or more cigarettes per day.

In a sub-study, thirty three patients with asthma received a two week open label, unblinded course of oral dexamethasone [19]. Airway corticosteroid sensitivity was assessed by change in pre-bronchodilator FEV<sub>1</sub> to a two week trial of oral dexamethasone (6 mg/1.74 m<sup>2</sup>; roughly equivalent to 40 mg prednisolone/day). On the day prior to initiation, and on completion of the dexamethasone course, all subjects performed spirometry, exhaled nitric oxide measurement at 50 ml/s (Niox Flex, Aerocrine AB, Sweden), had an induced sputum procedure conducted and completed asthma control questionnaires (ACQ) [20]. Serum cortisol was assessed on completion of the dexamethasone course and subjects were deemed to have been compliant if their blood cortisol was suppressed below 50 nmol/l. The West Glasgow Research Ethics Committee approved the study and all patients gave written informed consent.

## Measurements

### Questionnaire

Asthma control questionnaire (ACQ) score [20] was completed.

### Lung function tests

Spirometry was performed according to ATS guidelines [21]. In the asthma group, airway hyper-responsiveness to methacholine was measured [22]. Lung volumes and diffusing capacity for carbon monoxide (DLco) were performed using the body box technique (Zan500 Body Plethysmography, nSpire Health Limited, Hertford, UK). Exhaled nitric oxide levels were measured at 50 ml/s in concordance with standardised guidelines [23] (Niox Flex, Aerocrine, Sweden).

### Sputum induction

Sputum induction was performed using hypertonic saline and samples processed as previously described [17]. Samples with cell viability  $< 40\%$  or epithelial squamous cells  $> 80\%$  were discarded.

### Serum measurements

Serum periostin was measured by ELISA (Aviscera Bioscience). Serum periostin concentrations were reproducible when measured in samples from 58 subjects on two occasions ( $r = 0.927$ ;  $p < 0.001$ ). Definition of atopy: specific IgE concentration  $> 0.35$  AU/ml against one or more of three common allergens (house dust mite, cat, grass pollen). Serum cotinine was measured by enzyme immunoassay (Cozart Bioscience Ltd., Abingdon, UK).

**Table 1** Demographics and baseline characteristics.

|                                                     | Healthy             |                   | Asthma            |                    | p Value |
|-----------------------------------------------------|---------------------|-------------------|-------------------|--------------------|---------|
|                                                     | Never smokers       | Smokers           | Never smokers     | Smokers            |         |
|                                                     | n = 25              | n = 20            | n = 51            | n = 56             |         |
| Age (y)                                             | 49.0 (41.5, 55.5)   | 53.5 (45.0, 60.0) | 49.0 (38.0, 55.0) | 48.0 (40.5, 54.5)  | 0.287   |
| Gender (female: male)                               | 17:8                | 11:9              | 31:20             | 29:27              | 0.481   |
| Pack years                                          | —                   | 31.0 (23.5, 38.0) | —                 | 32.5 (21.2, 60.0)  | 0.841   |
| Serum cotinine (ng/mL)                              | 0.9 (0.3, 1.15)     | 29.2 (15.8, 44.7) | 0.4 (0.2, 0.67)   | 32.5 (23.5, 45.0)  | <0.001  |
| Duration of asthma (y)                              | —                   | —                 | 21.0 (9.0, 36.0)  | 18.5 (10.0, 33.7)  | 0.551   |
| GINA severity grade: mild; moderate; severe         | —                   | —                 | 18:16:17          | 19:17:20           | 0.967   |
| BMI, kg/m <sup>2</sup>                              | 26.0 (23.2, 29.4)   | 26.6 (22.2, 29.5) | 28.7 (25.9, 34.7) | 25.3 (22.7, 29.7)  | 0.010   |
| ACQ score                                           | —                   | —                 | 1.10 (0.85, 1.84) | 2.00 (1.50, 2.69)  | <0.001  |
| FEV <sub>1</sub> % predicted post-bronchodilator    | 109.5 (97.4, 120.0) | 96.8 (89.1, 98.5) | 91.0 (79.3, 98.4) | 84.0 (69.7, 101.8) | <0.001  |
| FEV <sub>1</sub> /FVC post-bronchodilator           | 0.99 (0.96, 1.02)   | 0.94 (0.90, 0.99) | 0.93 (0.86, 1.00) | 0.86 (0.79, 0.95)  | <0.001  |
| DL <sub>CO</sub> % predicted COHb                   | —                   | —                 | 86.3 (78.4, 95.0) | 75.4 (64.7, 87.0)  | <0.001  |
| Beclometasone equivalent dose of inhaled steroid µg | —                   | —                 | 750 (400, 800)    | 800 (400, 800)     | 0.397   |
| Blood eosinophils (10 <sup>9</sup> /L)              | 0.12 (0.08, 0.17)   | 0.21 (0.13, 0.30) | 0.26 (0.14, 0.41) | 0.19 (0.15, 0.23)  | <0.001  |
| Sputum eosinophils%                                 | 0.5 (0.3, 1.1)      | 0.5 (0.2, 0.8)    | 0.7 (0.2, 1.5)    | 0.9 (0.3, 2.1)     | 0.240   |
| Sputum neutrophils%                                 | 26 (19, 38)         | 35 (24, 47)       | 29 (21, 45)       | 38 (27, 45)        | 0.084   |
| FeNO <sub>50</sub> (ppb)                            | 16 (8, 22)          | 8 (5, 22)         | 27 (14, 44)       | 9 (7, 14)          | <0.001  |
| Total IgE (ng/mL)                                   | 8 (8, 35)           | 8 (8, 40)         | 60 (8, 387)       | 8 (8, 616)         | 0.060   |
| Atopic status*% positive                            | —                   | —                 | 80%               | 60%                | 0.031   |
| Serum periostin (ng/mL)                             | 113 (9, 2151)       | 15 (9, 178)       | 233 (34, 1108)    | 9 (9, 307)         | 0.004   |
| Th <sub>2</sub> -high number (%)**                  | 2 (12%)             | 3 (15%)           | 20 (39%)          | 20 (36%)           | 0.01    |

Data depicted as Median (IQR) unless otherwise specified. Boldface p values indicate  $p < 0.05$ .

Abbreviations: ACQ, asthma control questionnaire score; BMI, body mass index; DL<sub>CO</sub> % predicted, diffusing capacity for carbon monoxide, corrected for haemoglobin and carboxyhaemoglobin (COHb) as a percentage of the predicted value; FEV<sub>1</sub>, forced expired volume in one second; FeNO<sub>50</sub>, fractional exhaled nitric oxide, measured at a flow rate of 50 ml/s; FVC, forced vital capacity; GINA, global initiative for asthma; IgE, immunoglobulin E; IQR, inter quartile range.

Definition of: \*atopy: elevated in specific IgE concentration in one or more of three common allergens (house dust mite, cat, grass pollen), and \*\*Th<sub>2</sub>-high: IgE >100 IU/mL and blood eosinophil count  $\geq 0.14 \times 10^9/L$  [8].

## Statistical analysis

Continuous variables were summarised as median (inter-quartile range). Comparison between different patient groups was analysed by Mann–Whitney or Kruskal–Wallis tests, or chi-squared analysis for categorical data. Correlation analysis was performed using Pearson's rho after log transformation. Subgroups with high or low serum periostin levels were defined as above and below median value in all asthma and health control groups: <34 ng/mL;  $\geq 34$  ng/mL. A Th<sub>2</sub>-high sub-group was defined as those with a total IgE >100 IU/mL and a blood eosinophil count  $\geq 0.14 \times 10^9/L$  [8]. The change in the serum periostin concentration between pre- and post-corticosteroid treatment was established by paired-t-test.

## Results

### Demographics and baseline clinical characteristics

Results are summarised in Table 1. The asthma and healthy control groups were matched for age and smoking

histories and smoking status was confirmed independently by raised concentrations of serum cotinine ( $p < 0.001$ ). ACQ score was higher and post-bronchodilator FEV<sub>1</sub>% predicted, FEV<sub>1</sub>/FVC post-bronchodilator and DL<sub>CO</sub> were reduced in smokers with asthma compared to never smokers with asthma ( $p < 0.001$ ). The blood eosinophil count was elevated in never smokers with asthma compared to the other groups ( $p < 0.001$ ). Sputum eosinophil and neutrophil counts were similar between groups. FeNO<sub>50</sub> was reduced overall by smoking; 9 (6, 14) vs 21 (11, 33),  $p < 0.001$ . There was a trend for a raised total IgE concentration in the never smokers with asthma compared to the other groups ( $p = 0.060$ ). A higher proportion of never smokers with asthma were atopic compared to smokers with asthma (80% versus 60%,  $p = 0.031$ ). Of the 66 asthma patients, 40 had a Th<sub>2</sub>-high phenotype compared with 5 of the 45 healthy control subjects (chi-sq = 10.7,  $p = 0.001$ ), and this was unaffected by smoking category (Table 1).

The demographic and baseline characteristics of the participants in the oral corticosteroid sub-study are provided in Tables S1, supplementary file. Non-smokers with asthma had elevated FeNO<sub>50</sub> ( $p < 0.001$ ) compared to smokers with asthma.

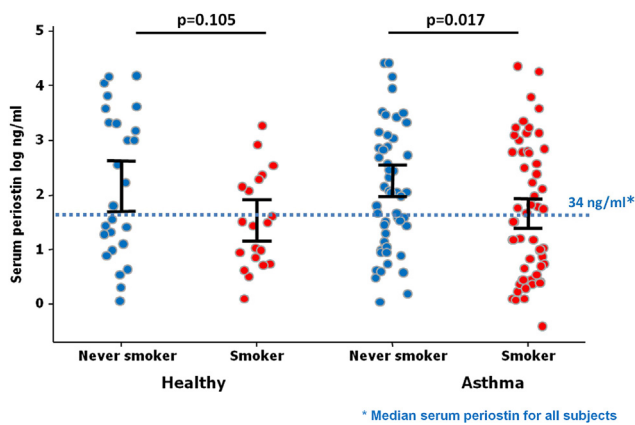


**Figure 1** Serum periostin concentration in healthy controls (never smokers and smokers) and in never smokers with asthma and smokers with asthma. Serum periostin (median [IQR], ng/mL) was reduced in smokers with asthma compared to never smokers with asthma; 9 (9, 307) versus 233 (34, 1108) respectively,  $p = 0.017$ . There was no difference in serum periostin concentration between the other sub-categories (healthy smokers and never smokers  $p = 0.105$ , never smokers with asthma and healthy never smokers  $p = 0.574$ , and smokers with asthma and healthy smokers  $p = 0.911$ ).

## Serum periostin concentration

### Smoking status

Serum periostin concentrations (ng/mL) were higher in never smokers with asthma compared with smokers with asthma [233 (34, 1108) versus 9 (9, 307) respectively,  $p = 0.017$ ] (Table 1 and Fig. 1). There was no difference in serum periostin concentration between the other sub-

categories (healthy smokers and never smokers  $p = 0.105$ , never smokers with asthma and healthy never smokers  $p = 0.574$ , and smokers with asthma and healthy smokers  $p = 0.911$ ) (Table 1 and Fig. 1).

### Asthma severity and atopic status

There was no significant difference in serum periostin (ng/mL) between severity grades in never smokers with asthma and smokers with asthma [never smokers with asthma, mild 131 (34, 464), moderate 288 (35, 2844), severe 233 (17, 2071),  $p = 0.444$ ; smokers with asthma, mild 19 (12, 829), moderate 9 (9, 169), severe 25 (9, 257),  $p = 0.251$ ] or between atopic and non-atopic subjects [104 (9, 750) versus 22 (9, 375) respectively,  $p = 0.144$ ] (Fig. 2).

### Inhaled corticosteroid use

The serum periostin concentration was not different comparing asthma patients who were taking or not taking inhaled corticosteroids; 78 (9, 750) vs 34 (9, 364),  $p = 0.647$ . There was no difference among the smokers ( $p = 0.676$ ) and never smokers ( $p = 0.466$ ). Among those taking inhaled corticosteroids there was no significant correlation between the corticosteroid dose and serum periostin concentrations in smokers ( $p = 0.730$ ) and in never smokers ( $p = 0.867$ ).

### Associations with clinical and inflammatory outcomes

There was no significant correlation between serum periostin and ACQ score, duration of asthma, pack years, FEV<sub>1</sub>% predicted post-bronchodilator, FVC% predicted post-bronchodilator or DLCO in the total asthma group or in either asthma subgroup. There was a weak correlation between serum periostin and sputum eosinophil count in all patients with asthma ( $r = 0.249$ ,  $p = 0.014$ ) and in smokers with asthma ( $0.315$ ,  $p = 0.020$ ). Serum periostin was not associated with FeNO<sub>50</sub>, blood eosinophil count or total IgE in either asthma group.

### Serum periostin high and low subgroups

The proportion of participants with an elevated serum periostin concentration ( $\geq 34$  ng/mL) was greater in never smokers with asthma compared to smokers with asthma [33 of 51 (65%) versus 22 of 56 (39%) respectively,  $p = 0.003$ ]; predominantly in the moderate plus severe categories of asthma [mild (10 of 18 (56%) versus 9 of 19 (47%) respectively  $p = 0.746$ ); moderate (12 of 16 (75%) versus 5 of 17 (29%) respectively  $p = 0.015$ ); severe (11 of 17 (65%) versus 8 of 20 (40%) respectively  $p = 0.120$ ); moderate plus severe  $p = 0.005$ ]. In smokers with asthma, the high serum periostin subgroup had a trend for a higher sputum eosinophil count compared with the low serum periostin subgroup (1.0% (0.8, 1.8) versus 0.5% (0.2, 1.8),  $p = 0.051$ ) (Table 2). In never smokers with asthma, the high serum periostin subgroup had a slightly longer duration of asthma compared with the low serum periostin subgroup (21.0 (13.5, 44.5) versus 14.0 (4.3, 27.0),  $p = 0.043$ ) (Table 2). There was no difference in all other variables including ACQ score, BMI, atopic status, total IgE, FeNO<sub>50</sub>, or blood eosinophil count between the smoker or never smoker subgroups (Table 2). Among the asthma patients there was no association between the proportions that had a Th<sub>2</sub>-high or low

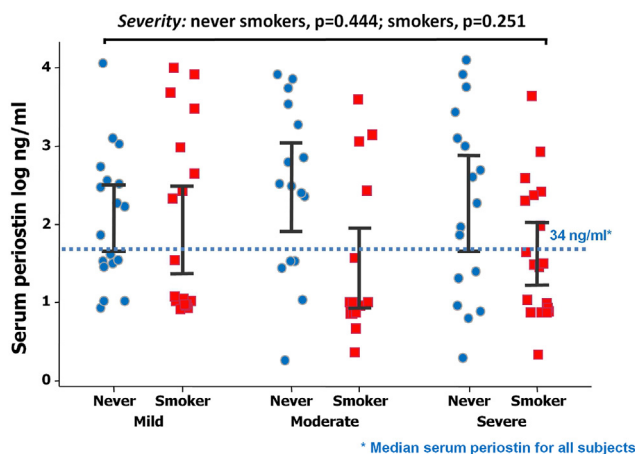


**Figure 2** Serum periostin in never smokers with asthma and smokers with asthma with mild moderate or severe severity grading. There was no significant difference in serum periostin (ng/mL) between severity grades in never smokers with asthma and smokers with asthma [never smokers with asthma, mild 131 (34, 464), moderate 288 (35, 2844), severe 233 (17, 2071),  $p = 0.444$ ; smokers with asthma, mild 19 (12, 829), moderate 9 (9, 169), severe 25 (9, 257),  $p = 0.251$ ].

**Table 2** Clinical and inflammatory outcomes in serum periostin high versus serum periostin low subgroups.

|                                                     | Never smokers with asthma        |                                   | p Value          | Smokers with asthma              |                                   | p Value          |
|-----------------------------------------------------|----------------------------------|-----------------------------------|------------------|----------------------------------|-----------------------------------|------------------|
|                                                     | Serum periostin low <sup>a</sup> | Serum periostin high <sup>a</sup> |                  | Serum periostin low <sup>a</sup> | Serum periostin high <sup>a</sup> |                  |
|                                                     | n = 18                           | n = 33                            |                  | n = 34                           | n = 22                            |                  |
| Age (y)                                             | 50.5 (36.8, 58.5)                | 48.0 (40.0, 54.0)                 | 0.643            | 49.0 (40.0, 53.5)                | 46.5 (42.8, 56.0)                 | 0.940            |
| Gender (female:male)                                | 11:7                             | 20:13                             | 0.972            | 19:15                            | 10:12                             | 0.446            |
| Pack years                                          | —                                | —                                 | —                | 34.0 (23.0, 53.3)                | 27.0 (19.5, 62.0)                 | 0.534            |
| Duration of asthma (y)                              | 14.0 (4.3, 27.0)                 | 21.0 (13.5, 44.5)                 | <b>0.043</b>     | 15.0 (6.5, 31.3)                 | 22.5 (11.5, 40.3)                 | 0.294            |
| GINA severity grade: mild; moderate; severe         | 8:4:6                            | 10:12:11                          | 0.496            | 10:12:12                         | 9:5:8                             | 0.543            |
| BMI, kg/m <sup>2</sup>                              | 30.4 (26.2, 36.6)                | 28.7 (24.6, 33.0)                 | 0.345            | 26.0 (23.3, 30.8)                | 24.6 (21.8, 27.9)                 | 0.191            |
| ACQ score                                           | 1.11 (0.84, 2.32)                | 1.07 (0.82, 1.75)                 | 0.686            | 2.00 (1.48, 3.00)                | 1.97 (1.50, 2.43)                 | 0.650            |
| FEV <sub>1</sub> % predicted post-bronchodilator    | 93.5 (81.4, 99.7)                | 87.2 (78.0, 97.9)                 | 0.454            | 84.3 (67.2, 101.9)               | 83.8 (70.2, 101.9)                | 0.854            |
| FEV <sub>1</sub> /FVC post-bronchodilator           | 0.94 (0.88, 1.02)                | 0.92 (0.80, 1.00)                 | 0.375            | 0.86 (0.78, 0.95)                | 0.87 (0.79, 0.95)                 | 0.603            |
| Inhaled corticosteroids: yes:no                     | 12:6                             | 25:8                              | 0.487            | 21:13                            | 13:9                              | 0.841            |
| Beclometasone equivalent dose of inhaled steroid µg | 400 (400, 1700)                  | 800 (400, 800)                    | 0.467            | 800 (400, 800)                   | 800 (500, 800)                    | 0.972            |
| Blood eosinophils (10 <sup>9</sup> /L)              | 0.28 (0.14, 0.40)                | 0.26 (0.13, 0.41)                 | 0.984            | 0.19 (0.15, 0.22)                | 0.20 (0.14, 0.30)                 | 0.420            |
| Sputum eosinophils%                                 | 0.7 (0.3, 1.1)                   | 0.9 (0.2, 2.5)                    | 0.802            | 0.5 (0.2, 1.8)                   | 1.0 (0.8, 1.8)                    | <b>0.051</b>     |
| Sputum neutrophils%                                 | 38 (23, 46)                      | 26 (21, 44)                       | 0.537            | 41 (30, 47)                      | 34 (26, 42)                       | 0.159            |
| FeNO <sub>50</sub> (ppb)                            | 26 (14, 32)                      | 27 (13, 45)                       | 0.716            | 10 (7, 17)                       | 9 (5, 13)                         | 0.460            |
| Total IgE (ng/mL)                                   | 24 (8, 410)                      | 74 (8, 393)                       | 0.766            | 8 (8, 514)                       | 8 (8, 683)                        | 0.768            |
| Atopic status**, yes:no                             | 13:3                             | 27:7                              | 0.841            | 18:14                            | 14:8                              | 0.653            |
| Serum periostin (ng/mL)                             | 17 (9, 34)                       | 550 (249, 3069)                   | <b>&lt;0.001</b> | 9 (9, 9)                         | 375 (254, 3079)                   | <b>&lt;0.001</b> |
| Th <sub>2</sub> -high number (%)***                 | 8 (44%)                          | 12 (36%)                          | 0.559            | 13 (38%)                         | 7 (32%)                           | 0.777            |

Data depicted as Median (IQR) unless otherwise specified. Boldface p values indicate  $p < 0.05$ .

Definition of: \*\*atopy: elevated in specific IgE concentration in one or more of three common allergens (house dust mite, cat, grass pollen), and \*\*\*Th<sub>2</sub>-high: IgE >100 IU/mL and blood eosinophil count  $\geq 0.14 \times 10^9/L$  [8].

<sup>a</sup> Subgroups with high or low serum periostin levels were defined as above and below median value in all asthma and health control groups: <34 ng/mL;  $\geq 34$  ng/mL.

inflammatory phenotype and the proportions that are serum periostin-high or low (chi-sq = 0.398,  $p = 0.533$ ), and the lack of association was maintained comparing the never smokers with asthma with smokers with asthma (Table 2). Furthermore, there was no difference in the proportion of Th<sub>2</sub>-high individuals in the serum periostin-high never smokers compared with serum periostin-high smokers (chi-sq = 0.121,  $p = 0.728$ ).

#### Effect of oral corticosteroids

As previously reported [19], the non-smokers with asthma group made a significant within-group improvement in lung function in response to oral dexamethasone (FEV<sub>1</sub> change; 202 ml (95% CI: 42, 362),  $p = 0.04$ ), in contrast to current smokers with asthma (smokers; 32 ml (−115, 178),  $p = 0.66$ ). After oral corticosteroid, serum periostin (ng/mL) was significantly reduced in non-smokers with asthma (pre-corticosteroid, 127 (15, 1159) versus post-corticosteroid, 15 (15, 1091)  $p = 0.030$ ), but not in the smokers with asthma group (pre-corticosteroid, 15 (15, 28) versus post-corticosteroid, 15 (15, 15)  $p = 0.277$ ).

## Discussion

An important goal of personalised medicine in asthma is to individualise treatment using biomarkers to predict a beneficial response and/or to identify individuals who are at risk of adverse effects. One method to stratify patients with asthma employs identifying those with a type 2 helper T-cell (Th<sub>2</sub>) subtype of asthma, where there is increased expression of cytokines such as IL-4, IL-5 and IL-13 [4,24], and who may benefit from therapies targeting Th<sub>2</sub> inflammation in addition to corticosteroids. Serum periostin has been proposed as a biomarker of a high-Th<sub>2</sub> eosinophilic phenotype of asthma [4,5]. Since smoking in asthma is associated with non-eosinophilic airway inflammation and corticosteroid insensitivity we postulated that smoking status might influence serum periostin concentrations in asthma. The main finding of our study was that serum periostin concentrations were lower in smokers with asthma compared to never smokers with asthma. However, despite the lower median serum periostin concentrations in

smokers with asthma, approximately forty percent of this group had levels above the group median, suggesting that a sub group within smokers with asthma could benefit from a targeted anti- $\text{Th}_2$  approach.

Serum periostin concentrations in asthma may not be comparable between studies due to the different assays that have been used [6,7]. We used a validated ELISA assay that was reproducible and gave serum values similar to previous reports. The source of serum periostin may include mesenchymal cells including bone, myocardium and skin [1] as well as from bronchial epithelial cells [2,3]. Serum periostin concentrations in asthma were not significantly different from healthy controls. It is assumed although not proven that a greater proportion of serum periostin comes from the airways in asthma compared to healthy controls.

Cigarette smoking has a prevalence of 20–30% in adults with asthma [16,25] and is associated with worse asthma control and an impaired therapeutic response to corticosteroids [11–16]. Serum periostin concentrations were reduced in smokers with asthma and levels were not associated with several clinical indices of asthma control including ACQ score and lung function, pack years or disease severity. Similar findings were found in the never smoker with asthma group. In a previous report, high serum periostin concentrations were associated with a greater decline in lung function in patients with asthma [7] and polymorphisms of periostin gene were related to higher serum periostin levels and a decline in  $\text{FEV}_1$  of 30 mL or greater per year [7]. Serum periostin levels have been reported to be increased in patients taking high dose inhaled corticosteroid treatment [7] although we found no association. Serum periostin concentration was weakly associated with sputum eosinophilia, which is in agreement with the findings of others where serum periostin level was the single best predictor of airway eosinophilia in patients with moderate to severe asthma [6]. In contrast, a recent study reported no significant association between serum periostin concentrations and sputum eosinophils in patients with mild to severe asthma [26]. Serum periostin was not associated with other potential biomarkers of  $\text{Th}_2$  high inflammation such as total IgE, exhaled NO or blood eosinophil count in either smoker or never smoker asthma subgroup.

We identified patients with elevated serum periostin concentrations defined in our study as above the median value in all asthma and healthy control groups,  $\geq 34$  ng/mL. Previous publications have used higher cut-off values to define elevated serum periostin, for example,  $\geq 50$  ng/mL [8] or  $\geq 95$  ng/mL [7], based on populations that did not include healthy controls or smokers with  $>10$  pack years. The proportion of patients with an elevated serum periostin concentration was greater in never smoker with asthma (65%) compared to smokers with asthma (39%) and a similar trend was found in each of the severity categories. A borderline higher sputum eosinophil count was the only clinical or inflammatory measurement that differed between periostin-high and periostin-low smokers with asthma. In asthma, an increased expression of epithelial periostin is associated with a beneficial response to inhaled corticosteroids in patients with mild asthma [4]. In a group of non-smokers with asthma in

whom there was an improvement in lung function in response to oral dexamethasone, serum periostin concentrations were reduced, whereas there were no changes in  $\text{FEV}_1$  or serum periostin in the smokers with asthma group. Elevated serum periostin concentration is associated with a favourable response to treatment with the IL-13 monoclonal antibody lebrikizumab in adults with asthma that was inadequately controlled despite inhaled corticosteroids [8] and with the monoclonal antibody omalizumab in patients with allergic asthma [10]. Despite the lower median serum periostin concentrations in smokers with asthma, approximately forty percent of this group had high levels suggesting that there may be a spectrum of  $\text{Th}_2$  high and  $\text{Th}_2$  low inflammation in smokers with asthma. The value of a raised serum periostin concentration in predicting a beneficial response to therapies targeting  $\text{Th}_2$  inflammation such as IL-13 blockers and omalizumab in smokers with asthma requires to be investigated.

The proportion of subjects with a  $\text{Th}_2$ -high inflammatory phenotype, as defined by serum IgE  $>100$  IU/mL plus a blood count of  $\geq 0.14 \times 10^9$  eosinophils/L, was higher in the asthma patients compared with healthy control subjects, but was unrelated to smoking status and to categories of high or low concentration of serum periostin.

We recognise that there are limitations in this study. The cross-sectional study design may have missed variations in circulatory periostin concentrations between patient groups over time and associations with clinical outcomes and inflammatory biomarkers. The value of serum periostin in predicting the therapeutic response to high dose inhaled corticosteroids or other treatments of  $\text{Th}_2$  inflammation in smokers with asthma was not investigated. Future studies need to examine the findings of this study prospectively as well to investigate the utility of serum periostin as a biomarker to predict responses to therapies, particularly biological agents, in different phenotypes of asthma including smokers with asthma and patients with the asthma-COPD overlap syndrome.

In conclusion, we have found that serum periostin concentrations were lower in smokers with asthma compared to never smokers with asthma. However, despite lower median serum periostin concentrations in smokers with asthma, approximately forty percent of this group still has a level above the group median, suggesting that these patients, despite their well recognised reduced clinical response to corticosteroids, may be responsive to targeted anti- $\text{Th}_2$  approaches. Future studies will need to determine the value of a raised serum periostin concentration in predicting a beneficial response to therapies targeting  $\text{Th}_2$  inflammation in smokers with asthma.

## Authors' contributions

Conception and design: NT, RC, MS, CMcS.

Data abstraction and statistics: NT, CMcS, RC, MS.

Drafting the manuscript for important intellectual content: NT, CMcS, RC, MS, JH.

Manuscript revision and final approval: all authors.

## Conflict of interest statement

Neil Thomson has participated in advisory boards and/or received consultancy fees from Chiesi, GlaxoSmithKline, Respivert, Roche and Takeda; received lecture fees from AstraZeneca, Boston Scientific, Chiesi, GlaxoSmithKline and Novartis; and industry-sponsored grant funding to the University of Glasgow from AstraZeneca, Boston Scientific, Glaxo SmithKline, Novartis, Respivert and Synairgen for participating in clinical trials.

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Mark Spears has received lecture fees from AstraZeneca, GlaxoSmithKline and Chiesi, support to attend conferences from GlaxoSmithKline and Boehringer Ingelheim and research funding from Genentech.

John Haughney has received reimbursements for attending symposia, fees for speaking, organising educational events, funds for research or fees for consulting from Almirall, AstraZeneca, Boehringer Ingelheim, Chiesi, Cipla, GlaxoSmithKline, Merck Sharp & Dohme, Mundipharma, Novartis, and Teva.

Charles McSharry has no financial interests to declare and has no competing interests associated with the scientific work contained in this manuscript.

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## Abbreviations

|                    |                                                                               |
|--------------------|-------------------------------------------------------------------------------|
| ACQ                | asthma control questionnaire                                                  |
| ATS                | American Thoracic Society                                                     |
| BMI                | body mass index                                                               |
| DLco               | diffusing capacity for carbon monoxide                                        |
| FeNO <sub>50</sub> | fractional exhaled nitric oxide, measured at a flow rate of 50 ml/s           |
| GINA               | global initiative for asthma                                                  |
| ICS                | inhaled corticosteroid                                                        |
| IgE                | immunoglobulin                                                                |
| IL                 | interleukin                                                                   |
| NO                 | nitric oxide                                                                  |
| PC <sub>20</sub>   | provocation concentration methacholine causing a 20% drop in FEV <sub>1</sub> |

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.rmed.2015.03.009>.

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## Supplementary file

**Table S1 Demographics and baseline characteristics of oral corticosteroid sub-study**

|                                                            | <b>Non-smokers* with asthma<br/>(n=20)</b> | <b>Smokers with asthma<br/>(n=13)</b> | <b>p value</b>   |
|------------------------------------------------------------|--------------------------------------------|---------------------------------------|------------------|
| <b>Age, y</b>                                              | 50 (38, 53)                                | 43(42, 51)                            | 0.897            |
| <b>Sex (M:F)</b>                                           | 10:10                                      | 8:5                                   | 0.515            |
| <b>Asthma Duration, y</b>                                  | 26 (15, 41)                                | 22 (18, 41)                           | 0.897            |
| <b>Daily ICS dose<br/>Beclomethasone equiv. «g</b>         | 800 (763, 1000)                            | 800 (700, 2000)                       | 0.663            |
| <b>Pack years</b>                                          |                                            | 25 (15, 35)                           |                  |
| <b>FEV<sub>1</sub> % predicted</b>                         | 77 (59, 84)                                | 80 (56, 96)                           | 1.000            |
| <b>FEV<sub>1</sub>/FVC ratio</b>                           | 66 (63, 73)                                | 70 (53, 78)                           | 0.472            |
| <b>ACQ score</b>                                           | 1.7 (0.9, 2.3)                             | 2.1 (1.2, 2.9)                        | 0.184            |
| <b>FeNO<sub>50</sub> (ppb)</b>                             | 30.6 (15.2, 83.3)                          | 11.4 (4.5, 13.3)                      | <b>&lt;0.001</b> |
| <b>Sputum eosinophils (%)</b>                              | 0.3 (0.0, 2.0)                             | 0.25 (0.0, 1.8)                       | 0.519            |
| <b>Sputum neutrophils (%)</b>                              | 24 (13, 52)                                | 30 (24, 60)                           | 0.215            |
| <b>Bronchodilator response<br/>(% FEV1 change)</b>         | 26 (14, 33)                                | 16 (15, 22)                           | 0.117            |
| <b>Oral corticosteroid response<br/>(post-pre/pre*100)</b> | 8.8 (-3.3, 22.0)                           | -1.9 (-14.0, 6.7)                     | <b>0.049</b>     |

**\* Current plus ex-smokers**

Data depicted as Median (IQR) unless otherwise specified. Boldface p values indicate  $p < 0.05$ .

Abbreviations: ACQ, asthma control questionnaire score; FEV<sub>1</sub>, forced expired volume in one second; FeNO<sub>50</sub>, fractional exhaled nitric oxide, measured at a flow rate of 50 ml/s; FVC, forced vital capacity.