

ORIGINAL ARTICLE

Asthma and Rhinitis

Pentraxin levels in non-eosinophilic versus eosinophilic asthma

Pengfei Gao¹  | Kun Tang¹ | Meijia Wang¹ | Qun Yang² | Yongjian Xu¹ | Jianmiao Wang¹ | Jianping Zhao¹ | Jungang Xie¹

¹Department of Respiratory and Critical Care Medicine, Key Laboratory of Pulmonary Diseases of Health Ministry, National Clinical Research Center of Respiratory Disease, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

²Department of Medical Ultrasound, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Correspondence

Jianping Zhao or Jungang Xie, Department of Respiratory and Critical Care Medicine, Key Laboratory of Pulmonary Diseases of Health Ministry, National Clinical Research Center of Respiratory Disease, Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China.
Emails: jpzhao@163.com or xiejgg@hotmail.com

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Summary

Background: Innate immunity has been thought to be involved in asthma pathogenesis. Pentraxins, acting as soluble pattern recognition molecules, play an important role in humoral innate immunity. Asthma is a heterogeneous inflammatory disease of airways and can be classified as eosinophilic or non-eosinophilic asthma.

Objective: To investigate whether pentraxin levels differ in subjects with eosinophilic versus non-eosinophilic asthma. Furthermore, to assess the predictive performance of pentraxin levels for discriminating asthma inflammatory phenotypes.

Methods: A total of 80 asthmatic patients and 24 healthy control subjects underwent sputum induction at study inclusion. Differential leucocyte counts were performed on selected sputum. Plasma C-reactive protein (CRP), serum amyloid P (SAP), pentraxin 3 (PTX3), and sputum SAP, PTX3, IL-8 levels were determined by enzyme-linked immunosorbent assay.

Results: Subjects with non-eosinophilic asthma had significantly increased pentraxin levels compared with those with eosinophilic asthma and healthy controls, with median (interquartile range) plasma CRP levels of 0.86 (0.28-2.07), 0.26 (0.14-0.85), and 0.15 (0.09-0.45)mg/L ($P < .001$), respectively, plasma SAP levels of 33.69 (19.79-58.39), 19.76 (16.11-30.58), and 20.06 (15.68-31.11)mg/L ($P = .003$), respectively, and sputum PTX3 levels of 4.9 (1.35-18.72), 0.87 (0.30-2.07), and 1.08 (0.31-4.32)ng/mL ($P < .001$), respectively. Conversely, sputum SAP concentrations of eosinophilic asthmatics (median, 21.49 ng/mL; IQR, 6.86-38.79 ng/mL) were significantly higher than those of non-eosinophilic patients (median, 8.15 ng/mL; IQR, 2.82-18.01 ng/mL) and healthy controls (median, 8.79 ng/mL; IQR, 2.00-16.18 ng/mL). Asthma patients with high plasma CRP ($P = .004$), SAP ($P = .005$) and sputum PTX3 levels ($P < 0.001$) also had significantly lower sputum eosinophil percentages. Sputum PTX3 levels had the best power (11.18-fold, $P < .001$) to predict non-eosinophilic airway inflammation in asthma patients.

Conclusion and Clinical Relevance: Pentraxin levels differed significantly between patients with non-eosinophilic asthma and those with eosinophilic asthma. Furthermore, elevated pentraxin expressions may predict non-eosinophilic airway inflammation in asthmatic patients.

KEYWORDS

asthma, clinical immunology, eosinophilic, innate immunity, non-eosinophilic, pentraxins

1 | INTRODUCTION

Asthma is a heterogeneous disease, with different underlying pathological processes, that is usually characterized by chronic airway inflammation.¹ Asthma can be classified as eosinophilic or non-eosinophilic based on the induced sputum.² Evidence from clinical trials suggests that approximately half of all asthmatic patients present with non-eosinophilic airway inflammation, which represents a stable phenotype associated with distinct lower airway pathology and a poor response to corticosteroid therapy.²⁻⁴ Although the mechanisms of eosinophilic asthma have been well characterized, those of non-eosinophilic inflammation are still poorly understood.

Both innate and adaptive immune responses play a critical role in the pathogenesis of asthma.⁵ It has been suggested that differences in T helper cell subsets between eosinophilic and non-eosinophilic asthma may participate in driving these phenotypes.⁶ In addition, increased expression of innate immune receptors, toll-like receptor (TLR) 2, TLR4, and cluster of differentiation 14, has been observed in subjects with neutrophilic asthma compared with other subtypes and controls, indicating more robust innate immune activation.⁷

Pentraxins are a superfamily of evolutionarily conserved, fluid phase pattern recognition molecules that play an important role in humoral innate immunity.⁸ C-reactive protein [CRP or pentraxin (PTX) 1] and serum amyloid P component (SAP or PTX2) constitute the short pentraxin arm of the superfamily, while PTX3 is the prototype of the long pentraxin arm. CRP and SAP are mainly produced in the liver in response to interleukin (IL)-6 systemically, while PTX3 is locally induced by primary proinflammatory factors and microbial components at the site of inflammation.⁹ Serum high sensitivity-CRP levels, which may be a useful predictor of airway inflammation, have been shown to be significantly higher in asthma patients compared to healthy controls.^{10,11} Elevated PTX3 expression in asthmatic airways has also been observed,¹² and sputum PTX3 levels, a candidate biomarker for evaluation of airway inflammation and remodelling, were also shown to be increased in children with asthma.¹³ However, currently, very little is known about expression of SAP in asthma. Although existing evidence has suggested that expression of CRP and PTX3 is increased in asthma patients and may participate its pathogenesis, whether the expression and/or roles of pentraxins differ in patients with eosinophilic versus non-eosinophilic asthma are still unknown. Therefore, the aim of the present study was to examine the innate immune response at local level and systemic levels, by measuring pentraxin concentrations, in asthma patients with eosinophilic and non-eosinophilic inflammatory airways compared to healthy control subjects. Furthermore, the diagnostic accuracy of each was evaluated to help better discriminate between inflammatory phenotypes of asthma.

2 | METHODS

2.1 | Subjects

Eighty subjects with asthma and 24 healthy control subjects were recruited from Tongji Hospital (Wuhan, China). None of the healthy control subjects had a history of respiratory disease or evidence of bronchial hyperreactivity to methacholine challenge. Diagnosis of asthma was established according to Global Initiative for Asthma guidelines. All asthma subjects were clinically diagnosed with symptomatic asthma and demonstrated evidence of a hyperresponsive airway (provacative dose of methacholine causing a 20% drop in FEV1 < 2.5 mg) and/or bronchodilator responsiveness (>12% improvement in FEV1% predicted following inhalation of 200 µg of salbutamol). Patients with any other respiratory, heart, renal, liver, or collagen disease were excluded. Patients were also excluded if they were current or ex-smokers with a history of more than 10 pack-years, underwent a course of oral corticosteroids, or had a respiratory tract infection in the 4 weeks prior to study entry. Blood and sputum samples were collected from all subjects upon study entry. All subjects provided written informed consent prior to study onset, and the ethics committee of Tongji Hospital, Huazhong University of Science and Technology (Wuhan, China), approved the study (IRB ID: 20150503).

2.2 | Sputum induction and analysis

Sputum induction with hypertonic saline (4.5%) was performed on all subjects, as previously described.¹⁴ For inflammatory cell counts, the sputum was isolated from saliva and dispersed using dithiothreitol, and total cell count and viability were assessed. Cytospin preparations were prepared and stained, and a differential cell count was obtained from 400 non-squamous cells. A cut-point of 3% eosinophils was used to distinguish eosinophilic from non-eosinophilic asthma.¹⁵

2.3 | Enzyme-linked immunosorbent assay

Plasma CRP, plasma and sputum PTX3, sputum IL-8 (R&D Systems, Minneapolis, MN, USA), and plasma and sputum SAP (Assay Pro, St. Charles, MO, USA) were assessed by enzyme-linked immunosorbent assay according to the manufacturers' instructions. Before measurement, plasma samples were diluted in 1:100 for CRP, 1:20000 for SAP, and sputum samples were diluted in 1:10 for SAP. The standard curves for these assays ranged from 0.8 to 50 ng/mL for CRP, 0.156 to 10 ng/mL for SAP, 0.3 to 20 ng/mL for PTX3, and 31.2 to 2000 pg/mL for IL-8. The lower detection limit of the assays was

0.22 ng/mL for CRP, 0.06 ng/mL for SAP, 0.116 ng/mL for PTX3, and 7.5 pg/mL for IL-8, respectively.

2.4 | Statistical analysis

Statistical comparisons were performed using unpaired *t* test for normally distributed data, with results reported as mean $\pm$ standard deviations. For non-normally distributed data, results were presented as median with interquartile range (IQR), and non-parametric tests (Kruskal–Wallis or Mann–Whitney test) were used to compare across groups. Fisher's exact test was used for analysis of categorical data. We analysed correlations using Spearman rank order correlation. Receiver-operating characteristic curves were generated to determine the cut-off value for the ability of plasma CRP, SAP, and sputum SAP, PTX3 levels to differentiate between non-eosinophilic and eosinophilic asthma based on curve points closest to (0, 1). Sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated for the optimal cut-points. Statistical analysis was performed with GraphPad Prism 5 (GraphPad, San Diego, CA, USA) and SPSS version 19 (SPSS Inc., Chicago, IL, USA). *P* < .05 was considered statistically significant.

3 | RESULTS

3.1 | Clinical features

Eighty asthma subjects and 24 healthy control subjects were enrolled in this study (Table 1). According to the percentage of sputum eosinophils in asthma patients, 44 (55%) had eosinophilic asthma and 36 (45%) had non-eosinophilic. There were no significant differences across subjects with eosinophilic, and non-eosinophilic asthma and healthy controls in terms of sex distributions and age. Body mass index of non-eosinophilic asthmatics was higher than those of eosinophilic patients and healthy controls, consistent with previous studies.¹⁶ Asthma patients had lower lung function than healthy controls. The serum concentrations of total immunoglobulin E were significantly lower in subjects with non-eosinophilic asthma compared to those with eosinophilic. As expected, subjects with eosinophilic asthma had an increased proportion of sputum eosinophils compared with non-eosinophilic asthma and healthy controls. The percentages of blood eosinophils were also significantly higher in subjects with eosinophilic asthma than in those with non-eosinophilic.

3.2 | Distinct pentraxins expression in eosinophilic and non-eosinophilic asthma

We measured the levels of plasma CRP, SAP, PTX3, and sputum SAP and PTX3 by enzyme-linked immunosorbent assay. Plasma CRP concentrations (Figure 1A) were higher in both asthma groups compared with healthy controls (median, 0.15 mg/L; IQR, 0.09–0.45 mg/L) and were highest in the non-eosinophilic asthma group (median, 0.86 mg/L; IQR, 0.28–2.07 mg/L) versus eosinophilic patients

(median, 0.26 mg/L; IQR, 0.14–0.85 mg/L). Plasma SAP concentrations (Figure 1B) of non-eosinophilic asthmatics (median, 33.69 mg/L; IQR, 19.79–58.39 mg/L) were higher than those of eosinophilic patients (median, 19.76 mg/L; IQR, 16.11–30.58 mg/L) and healthy controls (median, 20.06 mg/L; IQR, 15.68–31.11 mg/L). Conversely, patients with eosinophilic asthma (median, 21.49 ng/mL; IQR, 6.86–38.79 ng/mL) had significantly increased SAP concentrations in sputum (Figure 1D) compared with both patients with non-eosinophilic asthma (median, 8.15 ng/mL; IQR, 2.82–18.01 ng/mL) and healthy controls (median, 8.79 ng/mL; IQR, 2.00–16.18 ng/mL). No difference in plasma PTX3 levels (Figure 1C) was found between any of the three groups studied. However, PTX3 expressions in the sputum (Figure 1E) were significantly elevated in subjects with non-eosinophilic asthma (median, 4.9 ng/mL; IQR, 1.35–18.72 ng/mL) compared with eosinophilic patients (median, 0.87 ng/mL, IQR, 0.30–2.07 ng/mL) and healthy controls (median, 1.08 ng/mL; IQR, 0.31–4.32 ng/mL); no difference was observed between eosinophilic asthma patients and healthy controls. Furthermore, plasma CRP, SAP, and sputum PTX3 concentrations were significantly correlated when compared with each other (Figure 2).

3.3 | Comparison of sputum differential inflammatory cell counts in asthma patients with normal versus high pentraxin levels

The different pentraxin expression patterns in patients with eosinophilic versus non-eosinophilic asthma indicated that they may reflect the heterogeneity of asthmatic airway inflammation. We classified asthma patients into subgroups according to plasma CRP, SAP, and sputum SAP and PTX3 levels and selected the lower limit of the upper quartile for healthy control subjects as their cut-points; high levels were defined as a plasma CRP $\geq$ 0.45 mg/L, plasma SAP $\geq$ 31.11 mg/L, sputum SAP $\geq$ 16.18 ng/mL, and sputum PTX3 $\geq$ 4.32 ng/mL, respectively. Sputum eosinophil percentages were significantly lower in asthmatic patients with high plasma CRP and SAP, and sputum PTX3 levels (Figure 3A, B and D), while asthma patients with high sputum SAP levels have significantly higher sputum eosinophil percentages compared to asthmatics with normal sputum SAP levels (Figures 3C). Sputum neutrophil percentages were significantly higher in asthma patients with high plasma CRP versus normal CRP levels (Figure 3A). Furthermore, asthma patients with high sputum PTX3 levels also tended to have elevated sputum neutrophil percentages compared to asthmatics with normal PTX3 levels, although not significantly (Figure 3D). In contrast, there was no significant difference in sputum neutrophil percentages between asthmatic patients with high and normal plasma and sputum SAP levels (Figure 3B and C). Subanalysis of asthma patients according to the levels of all three pentraxins (ie CRP, SAP, and PTX3) revealed no difference in sputum macrophage or lymphocyte percentages (Figure 3).

Significant positive correlations were observed between sputum PTX3 levels and sputum neutrophil percentages and sputum IL-8 levels. In addition, the plasma CRP levels were also significantly

TABLE 1 Asthma (Eosinophilic and Non-eosinophilic) vs Healthy Control Subjects: Clinical Characteristics

Characteristics	Healthy control subjects	Eosinophilic asthma (EA)	Non-eosinophilic Asthma (NEA)	P value (EA vs NEA)
Number	24	44	36	
Sex (F/M)	12/12	21/23	21/15	.376
Age (y)	39.58 ± 13.01	42.27 ± 10.66	43.67 ± 12.27	.5936
BMI (kg/m ²)	21.29 ± 2.37	22.19 ± 2.88	23.97 ± 3.55***	.0175
Lung function				
FEV ₁ (L)	3.44 ± 0.83	2.33 ± 0.82*	2.47 ± 0.82*	.4441
FEV ₁ % predicted	98.75 (93.15-105.8)	87.45 (60.80-95.38)*	88.20 (70.78-98.43)*	.2194
FVC (L)	4.27 ± 0.92	3.57 ± 0.88*	3.64 ± 0.98*	.73
FVC % predicted	101.9 (96.78-111.3)	101.3 (88.33-112.7)	103.4 (94.1-115.6)	.3684
FEV ₁ /FVC (%)	81.17 (77.36-85.16)	67.24 (57.07-75.52)*	67.26 (61.83-75.02)*	.5233
Methacholine PD20 (mg)	NA	0.1449 (0.0278-0.6118)	0.2888 (0.0319-1.37)	.3258
IgE (IU/mL)	NA	168.1 (71.6-325.4)	68.68 (32.49-204.7)**	.0254
Sputum eosinophils (%)	0	14.56 (7.113-38.41)*	0.98 (0-1.803)***	<.0001
Sputum neutrophils (%)	43.17 (35.40-54.43)	51.8 (42.91-66.74)	66.88 (39.36-83.02)*	.0654
Blood eosinophils (%)	NA	4.8 (2.88-7.83)	1.6 (0.2-2.65)**	.0362
Blood neutrophils (%)	NA	56.09 ± 10.07	58.58 ± 7.7	.2263

Values are presented as mean ± SD or median (interquartile range). Eosinophilic asthma is defined as sputum eosinophils ≥ 3%, and non-eosinophilic asthma is defined as sputum eosinophils < 3%. NA, not applicable; PD20, provocation dose resulting in 20% fall in baseline FEV₁.

P* < .05 vs healthy control group. *P* < .05 vs eosinophilic asthma group. ****P* < .05 vs non-eosinophilic asthma group.

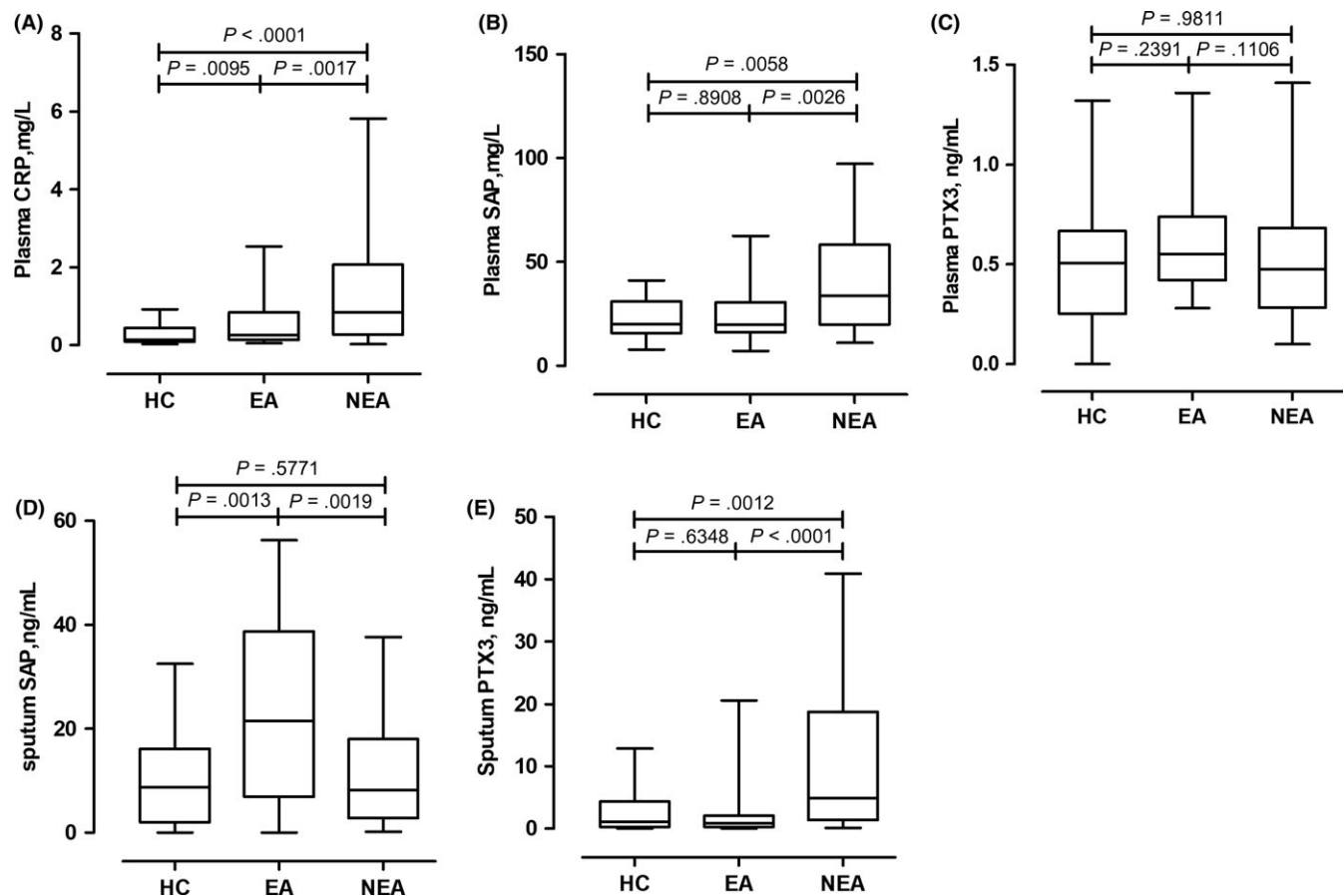


FIGURE 1 Pentraxin levels in subjects with eosinophilic, and non-eosinophilic asthma and healthy controls. Data are presented as median (interquartile range). Plasma CRP (A), SAP (B), and PTX3 (C) levels; Sputum SAP (D) and PTX3 (E) levels. HC, healthy control; EA, eosinophilic asthma; NEA, non-eosinophilic asthma

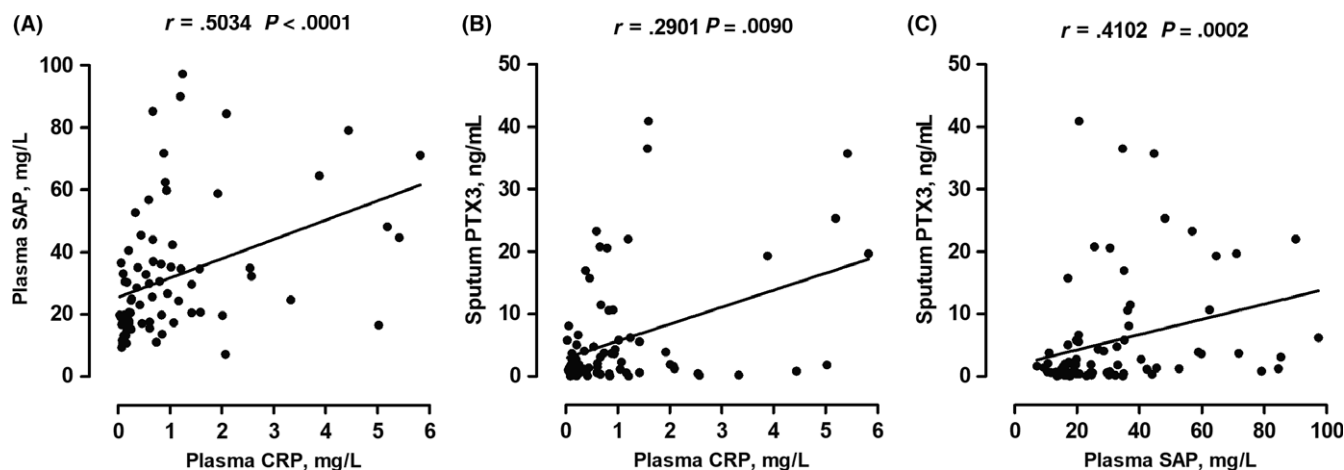


FIGURE 2 Correlation between plasma CRP and SAP (A), plasma CRP and sputum PTX3 (B), plasma SAP and sputum PTX3 (C) concentrations in subjects with asthma

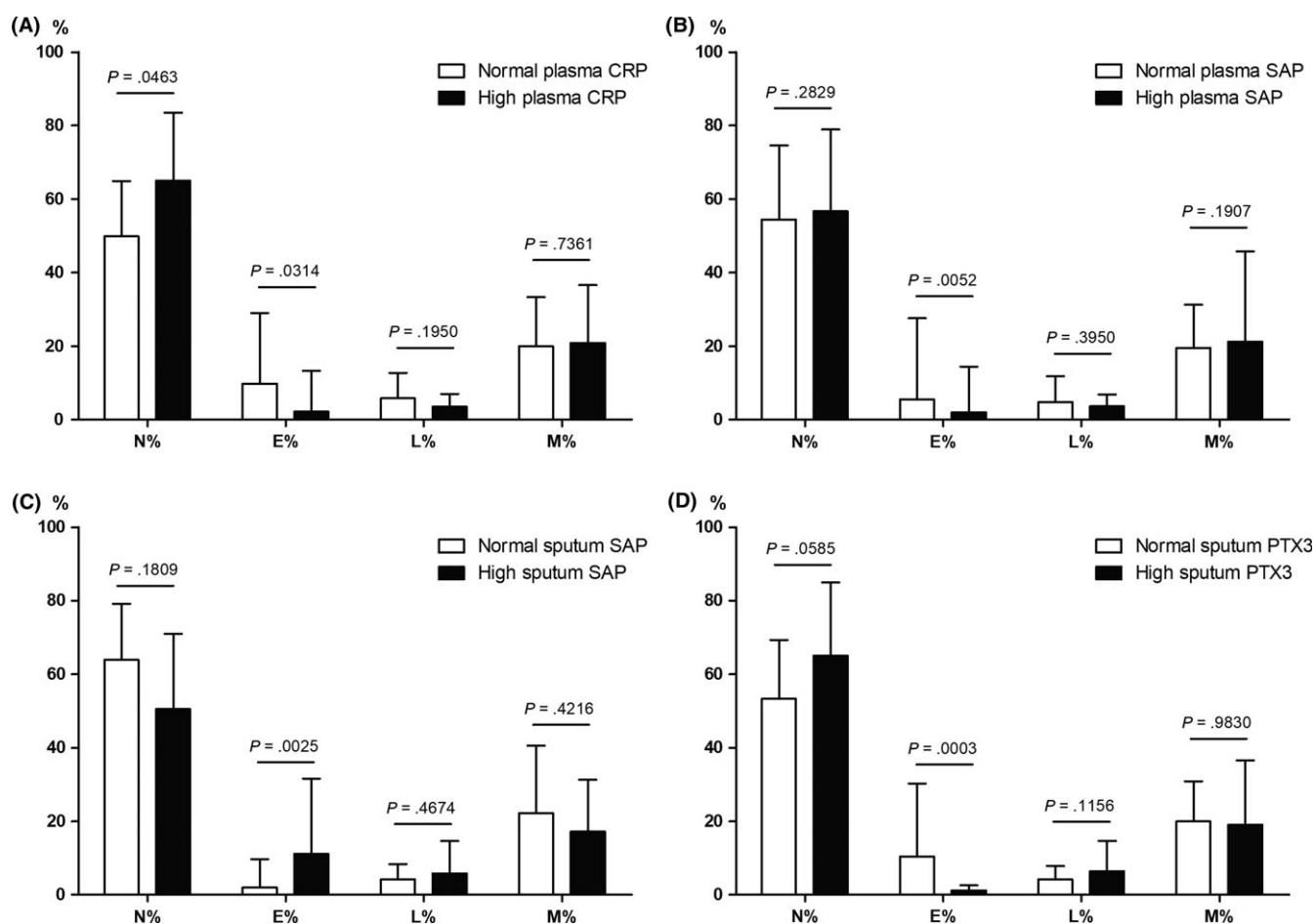


FIGURE 3 Comparison of sputum differential inflammatory cell counts in asthmatic subjects with normal versus high plasma CRP (A), SAP (B) and sputum SAP (C) and PTX3 (D) levels. High plasma CRP, SAP, and sputum SAP and PTX3 concentrations are defined as ≥ 0.45 mg/L, ≥ 31.11 mg/L, ≥ 16.18 ng/mL, and ≥ 4.32 ng/mL, respectively; (lower limit of upper quartile for healthy control subjects). Data are presented as median (interquartile range). N%, neutrophils (% total cells); E%, eosinophils (% total cells); M%, macrophages (% total cells); L%, lymphocytes (% total cells)

positively correlated with sputum neutrophils count (Table 2). The proportion of subjects with non-eosinophilic asthma was significantly higher in patients with high plasma CRP, plasma SAP, sputum PTX3,

and normal sputum SAP levels (Table 3). The likelihood of having non-eosinophilic airway inflammation was elevated in asthma patients with high plasma CRP, SAP, and sputum PTX3 levels, with

4.1 times, 3.75 times, and 11.18 times more likely to have non-eosinophilic airway inflammation, respectively. In contrast, asthma patients with high sputum SAP levels were less likely, with 0.31 times, to have non-eosinophilic airway inflammation (Table 3).

3.4 | Pentraxins as predictors of non-eosinophilic airway inflammation in asthma patients

The predictive performance of plasma CRP, SAP and sputum SAP and PTX3 for identification of patients with non-eosinophilic asthma is presented in Table 4, and the corresponding receiver-operating characteristic curves are shown in Figure 4. The predictive value of sputum PTX3 [area under the curve (AUC) = 0.78, 95% confidence interval (CI): 0.68-0.88] was superior to that of plasma CRP (AUC = 0.71, 95%CI: 0.59-0.82), SAP (AUC = 0.70, 95%CI: 0.58-0.81), and sputum SAP (AUC = 0.71, 95%CI: 0.60-0.83). The optimum cut-point for sputum PTX3 was 3.68 ng/mL, yielding a sensitivity of 61.11%, 86.36% specificity, 78.57% PPV, and 73.08% NPV. Plasma CRP with a cut-point of 0.45 mg/L showed 72.22% sensitivity, 61.36% specificity, 60.47% PPV, and 72.97% NPV for non-eosinophilic asthma. Plasma SAP with a cut-point of 30.39 mg/L had 58.33% sensitivity, 74.42% specificity, 73.68% PPV, and 63.64% NPV for non-eosinophilic asthma. Meanwhile, sputum SAP with a cut-point of 18.79 ng/mL had 78.79% sensitivity, 58.54% specificity, 60.47% PPV, and 72.97% NPV for non-eosinophilic asthma (Table 4).

4 | DISCUSSION

The present study demonstrates for the first time that pentraxin levels differ in patients with non-eosinophilic versus eosinophilic asthma. Furthermore, our data provided the first evidence regarding SAP expression in asthma patients. Significantly increased levels of plasma CRP and SAP and sputum PTX3, but not plasma PTX3, were observed in subjects with non-eosinophilic asthma compared to subjects with eosinophilic asthma and healthy controls. In contrast, sputum SAP levels in patients with eosinophilic asthma were significantly higher than those with non-eosinophilic asthma and healthy controls. Furthermore, the percentage of sputum eosinophils was significantly lower in patients with high plasma CRP, SAP and sputum PTX3 levels and normal sputum SAP levels, while the percentage of sputum neutrophils was significantly higher in patients

with high plasma CRP levels, indicating that pentraxin expression may reflect non-eosinophilic asthmatic inflammation. Meanwhile, asthma patients with high pentraxin levels, especially with high PTX3, were more likely to have non-eosinophilic airway inflammation. Moreover, within asthmatic patients, there were significant associations between plasma CRP and SAP and sputum PTX3 levels.

Unlike CRP and SAP, which are mainly synthesized in the liver, PTX3 is produced at the site of inflammation. It has been suggested that PTX3 plays a similar role in the periphery as CRP and SAP in the circulation.⁸ Therefore, sputum PTX3 is most likely a biomarker of local inflammation, while circulating CRP and SAP reflect a systemic inflammatory response, which might explain why there is no difference in plasma PTX3 levels between healthy controls and asthma subjects. It was noteworthy that SAP levels in sputum were far below that measured in plasma. As few studies reported other issues expressing SAP apart from liver, SAP detected in sputum was possible to mainly derive from circulation. The discordance between SAP levels in plasma and sputum may result from the heavier burden of aetiological factors, including endotoxin, viral, and bacterial infection,² seen in the airways of patients with non-eosinophilic asthma that SAP binds to,¹⁷ thus reducing the amount of SAP available for detection in the sputum enzyme-linked immunosorbent assay quantification. Thus, the lower SAP levels in sputum of non-eosinophilic asthma were supposedly not due to impaired expression, but rather to heavier burden of infection. In addition, the strong positive correlations between plasma CRP and SAP and sputum PTX3 levels indicated that systemic and local innate immune responses were highly concordant in asthma.

Several studies reported significantly higher circulating CRP concentrations in patients with asthma compared to healthy controls.^{10,18-21} Circulating CRP levels were also shown to be significantly elevated in steroid-naïve patients with asthma compared with healthy controls and steroid-treated asthma patients¹⁸ and increased in non-allergic, but not allergic, asthma.²⁰ Meanwhile, enhanced expressions of PTX3 were also observed in asthmatic biopsy samples¹² and in sputum of children with asthma.¹³ However, evidence to date has not explicitly linked pentraxin levels to airway inflammation phenotype in asthma.

In our current study, asthma patients were further subdivided according to airway eosinophilia. We found that plasma CRP, SAP, and sputum PTX3 levels were significantly higher in subjects with non-eosinophilic asthma. Furthermore, plasma SAP and sputum PTX3 levels were similar in eosinophilic asthma patients versus

TABLE 2 Associations between pentraxins and neutrophilic airway inflammatory markers of subjects with asthma

	Plasma CRP (mg/L)		Plasma SAP (mg/L)		Sputum SAP (ng/mL)		Sputum PTX3 (ng/mL)	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Sputum neutrophils (%)	.11	.3326	-.04	.7528	-.15	.1884	.26	.0223
Sputum neutrophils count (10 ⁶ /g)	.3	.0079	-.08	.4917	.09	.4464	.21	.0645
IL-8 (pg/mL)	.1	.4003	-.12	.3268	.05	.6706	.34	.0028

TABLE 3 EA and NEA frequencies in asthmatic patients with high or normal concentrations of plasma CRP, SAP and sputum SAP and PTX3

	EA, n (%)	NEA, n (%)	OR (95% CI)	P value
Plasma CRP				
Normal	27 (34)	10 (13)	4.1 (1.60-10.67)	.0035
High	17 (21)	26 (32)		
Plasma SAP				
Normal	33 (41)	16 (20)	3.75 (1.45-9.68)	.0064
High	11 (14)	20 (25)		
Sputum SAP				
Normal	17 (21)	24 (30)	0.31 (0.13- 0.79)	.0148
High	27 (34)	12 (15)		
Sputum PTX3				
Normal	40 (50)	17 (21)	11.18 (3.3-37.81)	<.0001
High	4 (5)	19 (24)		

ORs estimate the likelihood of high pentraxins levels for non-eosinophilic asthma as compared with normal pentraxins levels. CI, confidence interval; OR, odds ratio.

TABLE 4 Diagnostic performance characteristics of plasma CRP, SAP and sputum SAP and PTX3 for the identification of non-eosinophilic asthma

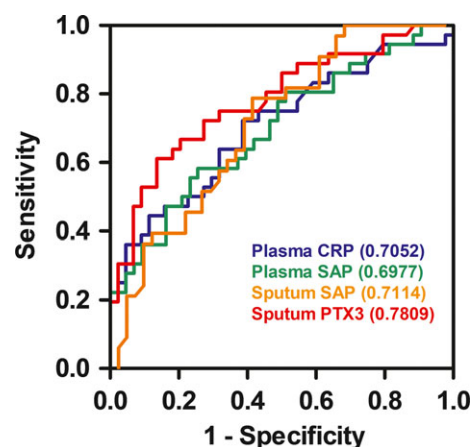
	Cut-point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (95% CI)	P value
Plasma							
CRP	>0.45 mg/L	72.22	61.36	60.47	72.97	0.71 (0.59-0.82)	.002
Plasma							
SAP	>30.39 mg/L	58.33	74.42	73.68	63.64	0.70 (0.58-0.81)	.003
Sputum							
SAP	<18.79 ng/mL	78.79	58.54	60.47	72.97	0.71 (0.60-0.83)	.002
Sputum							
PTX3	>3.68 ng/mL	61.11	86.36	78.57	73.08	0.78 (0.68-0.88)	<.001

Data for the optimum cut-points based on maximum AUCs from ROC analyses are given.

NPV, negative predictive value; PPV, positive predictive value; CI, confidence intervals.

healthy controls, while plasma CRP levels were only marginally higher, suggesting that increased expression of pentraxins mainly occurs in patients with non-eosinophilic airway inflammation.

Non-eosinophilic asthma is associated with upregulation of innate immune response, and current results demonstrate elevated expression of three markers of innate immunity (CRP, SAP, and PTX3) in these patients. This result is consistent with a previous study, which demonstrated that plasma CRP and IL-6, markers of systemic inflammation, were increased in neutrophilic asthma,²² and elevated IL-6 may be a major contributor to hepatic production of CRP and SAP in non-eosinophilic asthma. This finding also supports the results of Simpson et al⁷, who reported an enhanced innate immune response in neutrophilic asthma, with increased expression of innate immune receptors TLR2, TLR4, and cluster of differentiation 14 and cytokines IL-8, tumour necrosis factor α , and IL-1 β in sputum. Previously, tumour necrosis factor α and IL-1 β were found to significantly upregulate PTX3 expression in airway cells, particularly human airway smooth muscle cells,¹² which may be partly responsible for elevated sputum PTX3 expression in

**FIGURE 4** Receiver-operating characteristics curves for plasma CRP, SAP and sputum SAP and PTX3 concentrations for identification of subjects with non-eosinophilic asthma (see Table 4 for data)

non-eosinophilic asthma found in the current study. In addition, PTX3 is also constitutively stored in specific granules of neutrophils and released in response to TLR engagement or agonists,²³ which can rapidly induce PTX3 expression in several cell types,²⁴ while lymphoid cells (eg T, B, and natural killer cells) do not express PTX3.²⁵ Moreover, a previous study reported alteration of 317 genes in resting neutrophils from subjects with non-eosinophilic versus eosinophilic asthma, including genes related to cell motility and regulation of apoptosis.²⁶

CRP has been shown to exhibit anti-inflammatory effects in neutrophil-mediated inflammatory lung tissue injury, and high levels of plasma CRP predicted a favourable outcome in adult patients with acute lung injury/acute respiratory distress syndrome.²⁷ CRP transgenic animals were resistant to infections with *Streptococcus pneumoniae*,²⁸ and the Gram-negative pathogen *Salmonella enterica*.²⁹ Furthermore, CRP administration had a similar role in protection against *Haemophilus influenza*.³⁰ Besides protection against pathogens, CRP transgenic mice also played a protective role in a model of experimental allergic encephalomyelitis,³¹ and administration of human CRP offered protection against systemic lupus erythematosus (SLE) in SLE-prone mice.³² It was also reported that SAP can downregulate asthma phenotypes, through attenuating M2 activation of macrophages in an experimental model of *Aspergillus fumigatus*-induced allergic airway disease.³³ Moreover, PTX3 deletion has been observed to enhance airway inflammation, mucus production, and airway hyperresponsiveness in an ovalbumin-induced asthma model through a TH17-dominant phenotype.³⁴ PTX3 released from activated leucocytes can dampen neutrophil recruitment through P-selectin binding and regulate inflammation locally in acute lung injury,³⁵ which is consistent with results observed in an experimental model of post-ischemic acute and chronic kidney injury reported by Lech et al.³⁶ Similarly, PTX3 attenuated LPS-induced liver injury and inflammation in animal models.³⁷ Based on these data, it is possible that increased pentraxin may involve limiting excessive inflammation through a feedback mechanism in non-eosinophilic asthma. Additional studies are necessary to clarify the mechanisms and roles of increased pentraxins in non-eosinophilic asthma.

Furthermore, present results showed that plasma CRP, sputum SAP, and sputum PTX3 levels individually, but not plasma SAP, had AUCs >0.7, indicating clinically significant utility in prediction of non-eosinophilic airway inflammation in asthma patients. High sputum PTX3 levels were associated with the greatest likelihood of absence of sputum eosinophilia in asthmatic patients.

In conclusion, we have observed that pentraxin levels differed significantly between patients with non-eosinophilic asthma and those with eosinophilic. In addition, elevated pentraxin expressions may predict non-eosinophilic airway inflammation in asthmatic patients. Thus, the present study adds significantly to the current understanding of the heterogeneity of innate immunity in asthma.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

ORCID

Pengfei Gao  <http://orcid.org/0000-0002-0269-0127>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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