

ORIGINAL ARTICLE

Elevated serum levels of periostin in patients with allergic bronchopulmonary aspergillosis

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Summary

Background: Serum periostin levels have been reported to be an indicator of Th2 inflammation in asthmatic patients.

Objective: This study aimed to investigate serum levels of periostin in patients with allergic bronchopulmonary aspergillosis (ABPA) and to evaluate its diagnostic and monitoring value in the disease.

Methods: Patients with ABPA ($n = 19$) and asthma ($n = 24$), including severe asthma with fungal sensitisation (SAFS, $n = 11$) and severe asthma without fungal sensitization (SAwFS, $n = 13$), were enrolled. Serum levels of periostin were analysed by enzyme-linked immunosorbent assay. Serum total IgE and *Aspergillus fumigatus* specific IgE, IgG were measured by ImmunoCAP. Levels of cytokines (IFN- γ , IL-4, IL-5, IL-8, IL-10, IL-13 and IL-17A) were measured by Meso Scale Discovery (MSD).

Results: Serum levels of periostin in ABPA patients (85.55 ng/mL, [68.28-166] ng/mL) were higher than those in SAFS (50.99 ng/mL, [32.02-71.80] ng/mL; $P < 0.01$). Among the analysed cytokines, IL-5 levels in ABPA (1.55 pg/mL, [0.96-3.33] pg/mL) were higher than those in SAFS (0.31 pg/mL, [0.26-0.56] pg/mL; $P < 0.05$) or SAwFS (0.34 pg/mL, [0.21-0.56] pg/mL; $P < 0.01$). Serum periostin levels was positively associated with total IgE levels ($r = 0.319$, $P < 0.05$), serum IL-5 levels ($r = 0.484$, $P < 0.01$) and blood eosinophil counts ($r = 0.428$, $P < 0.05$). In ROC analysis, the clinical reference value of periostin was 68.8 ng/mL for differential diagnosis of ABPA and SAFS, with the area under the curve (AUC) of 0.81. Longitudinally, serum periostin levels did not change significantly after treatment in ABPA.

Conclusions: These findings suggested that serum levels of periostin were up-regulated in ABPA patients, which may be associated with eosinophilic inflammation.

KEYWORDS

allergic bronchopulmonary aspergillosis, asthma, biomarker, periostin

1 | INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disease caused by *Aspergillus fumigatus*. The pathogenesis is owing to allergic response against *A. fumigatus* colonising the airways rather than saprophytic or invasive of the fungi.¹ ABPA occurs in patients with asthma and cystic fibrosis (CF), and the prevalence of ABPA in asthma and CF is estimated to be 13% and 9%, respectively.²⁻⁴ The International Society of Human and Animal Mycology (ISHAM) has concluded a detailed diagnosis criteria for ABPA, and serum total IgE level >1000 kU/L is one of an important experimental diagnostic indicators.⁵ Nevertheless, it remains difficult in diagnosing ABPA because a certain number of patients with ABPA exhibit serum total IgE level <1000 kU/L. Hartl et al and their group have investigated indicators in ABPA patients with cystic fibrosis (CF) and concluded that thymus- and activation-regulated chemokine (TARC) can be a useful biomarker in the diagnosis of ABPA in CF patients.^{6,7} In asthma patients, however, there is still a lack of effective diagnostic markers.

Although the pathogenesis of ABPA remains controversial,⁸ it is generally considered that ABPA patients exhibit profound immune responses against *A. fumigatus* predominantly driven by Th2 CD4⁺ T cells.^{9,10} This leads to release of Th2 cytokines such as IL-4, IL5, IL-13 and others. In asthmatic patients, periostin is a matricellular protein produced by airway epithelial cells and lung fibroblasts under the induction of IL-13 or IL-4, and it is associated with lung fibrosis and airway remodelling.¹¹⁻¹³ Recent studies illustrate that periostin served as not only a predictive biomarker of response to treatment in asthma, but also a prognostic biomarker.¹⁴ Moreover, it is a potential therapeutic target of asthma.¹⁵

Periostin plays a critical role in allergic inflammation, especially in asthma, representing Th2 immune response which also occurs in patients with ABPA. By now, no study has been performed to investigate the role of periostin in ABPA. Thus, we analysed serum levels of periostin of patients with ABPA, patients with asthma sensitised to mould or allergens other than fungi, and examined the diagnostic value of it. Furthermore, we have observed a number of ABPA patients longitudinally in order to investigate whether serum periostin levels could be an indicator after treatment.

2 | METHODS

2.1 | Ethical statement

This study was approved by the ethics committee of First affiliated hospital of Guangzhou Medical University, with approval number: GYFYY-2016-73. All included patients or their legal guardian signed two copies of informed consent form before study commencement.

2.2 | Study subjects

This is a retrospective study. Patients' data were screen out from the database of the First Affiliated Hospital of Guangzhou Medical

University from January 2015 to December 2017. From the database, patients who were clinically diagnosed as APBA or severe allergic asthma with any sensitisation were screened out based on the clinical records. Patients complicated with other active, acute or chronic pulmonary diseases (eg tuberculosis, COPD), severe immune diseases, heart failure, coronary heart disease or malignant tumours were excluded. Finally, a total of 43 patients were screened out from the database of the hospital. Available information of each patient were collected from the database including age, gender, results blood routine, pulmonary function, fractional exhaled nitric oxide (FeNO), imaging, skin pick test (if any), IgE (total and specific), *A. fumigatus* specific IgG and clinical and medication records.

2.2.1 | Diagnosis criteria

The diagnosis of asthma was based on the Global Initiative for Asthma (GINA) guidelines, including asthma clinical features of the patient as well as presence of airflow limitation, and the definition of severe asthma was based on the description of American Thoracic Society.¹⁶ The diagnosis of ABPA was based on the criteria of ISHAM Working Group.⁵ Patients with bronchial asthma met the two obligatory criteria: (a) skin prick test positive to *A. fumigatus* or elevated specific IgE levels against *A. fumigatus*; (b) elevated total IgE levels (IgE level <1000 kU/L may be acceptable if other criteria was met); and at least two of the following criteria: (a) presence of precipitating or IgG antibodies against *A. fumigatus* in serum; (b) radiographic pulmonary opacities consistent with ABPA; (c) peripheral blood eosinophilia.

2.2.2 | Patient groups

According to the diagnosis of their clinical records, patients were assigned to ABPA (n = 20) and asthma (n = 24). Based on the results of ImmunoCAP or skin pick test, the asthma patients were further divided into two subgroups: severe asthma with fungal sensitisation (SAFS, n = 11) and severe asthma without fungal sensitisation (SAwFS, n = 13). Patients who had positive results of specific IgE or skin pick test to any fungal allergen but negative to other allergens were considered as SAFS, and patients who had positive results of specific IgE or skin pick test to any allergen (eg house dust mites or pollens) other than fungal allergens were considered as SAwFS. Table 1 demonstrates the details of the patient groups.

2.2.3 | Longitudinally observation of patients with ABPA

Serum levels of periostin and cytokines were also assessed during the ABPA episode.

According to the clinical records, patients who were initially diagnosed as ABPA at their first visit of our hospital were considered as baseline, and among these patients, those who took a visit after treatment for at least 3 months were considered as after treatment. A total of 12 ABPA patients with more complete information at the after-treatment-visit were selected for longitudinal observation. Goals of

TABLE 1 Baseline characteristics of the study population

	ABPA (n = 19)	Asthma (n = 24)		P value
		SAFS (n = 11)	SAwFS (n = 13)	
Demographic				
Age (y)	48 [27-57]	42 [31-53]	24 [14.5-30]**	<0.01
Gender (male/female)	11/8	6/5	6/7	0.81
BMI (kg/m ²)	21.51 [19.65-24.64]	24.67 [18.70-26.30]	21.03 [16.98-23.20]	0.24
Spirometry				
FVC (% pred)	93.50 [69.60-103.90]	91.10 [66.90-106.10]	97.70 [88.75-103.70]	0.45
FEV1 (% pred)	72.00 [54.34-85.30]	80.60 [62.55-93.80]	85.95 [74.55-95.12]	0.13
FEV1/FVC ratio (%)	69.91 [64.86-74.10]	78.13 [63.84-85.34]	77.34 [71.82-82.29]	0.41
FeNO (ppb)	51.00 [23.25-102.00]	27.00 [13.50-40.00]	—	0.26
Blood cell count				
Eosinophil (×10 ⁹ cells/L)	0.65 [0.27-1.18]	0.21 [0.15-0.33]	0.30 [0.24-0.90]	0.06
Neutrophil (×10 ⁹ cells/L)	3.9 [2.98-7.08]	3.45 [2.90-4.73]	5.80 [4.20-7.70]	0.52
Immunological characteristic				
Total IgE level (kUA/L)	2007 [1346-2967]	504 [102-1947]**	464 [147-732.5]**	<0.01
A f sIgE positivity (%)	100%	81.8%	0%	—
A f sIgE levels (kUA/L)	8.19 [0.89-13.60]	1.25 [0.71-2.02]*	—	0.03
A f sIgG levels (mg _A /L)	53.25 [23.74-101.50]	5.56 [3.35-7.85]*	—	0.02
Glucocorticoids				
Use of oral glucocorticoids (%)	21.05%	36.36%	23.07%	0.63
Use of inhaled glucocorticoids (%)	42.11%	18.18%	30.77%	0.28

^aData were given as medians with interquartile range (IQR).

*P < 0.05 compared with ABPA.

**P < 0.01 compared with ABPA.

treatments of ABPA included control symptoms of asthma; prevent exacerbations; reduce pulmonary inflammation; and mitigate progression of fibrotic or cavitary disease.¹⁷ Treatment details and outcomes are shown in Table 2. A decreased level of total serum IgE by at least 25% of the baseline level after 3 months' treatment or longer, along with clinical improvements, were considered as responding stage of ABPA.^{5,18}

2.3 | Measurements of lung function and FeNO

Baseline levels of lung function and FeNO tests were done before the final diagnosis was made or before treatments. Lung function tests were performed on a computerised spirometer (MasterScreen). The examination parameters included forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), percentage predictors' parameters (%FVC, %FEV1) and the FEV1/FVC ratio. FeNO measurement (Niox Mino; Aerocrine) was performed prior to bronchial provocation at a flow rate of 50 mL/s using signals fed back for the control.

2.4 | Laboratory tests

Serum samples were obtained from venous blood collecting with separation gel containing vacutainer tubes by centrifuged at 1500g for 10 minutes. Aliquots of serum were stored in -80°C freezer to

avoid repeated freezing and thawing. Serum total IgE, specific IgE and *A fumigatus* specific IgG were analysed by ImmunoCAP 1000 system (Thermo Fisher Scientific Inc) according to the procedures offered by the manufacture.

2.5 | Detection of periostin and cytokines

Serum level of periostin was analysed by ELISA according to the manufacturer's instruction (R&D Systems Inc). Briefly, diluted samples were pipetted into wells pre-coated with antibody and detected by biotinylated mouse anti-human periostin antibody. The values of optical density were read by microplate reader (Varioskan Flash; Thermo Fisher Scientific) at 450/540 nm. The diluted concentrations were calculated from standard curves, and the original concentrations were figured out by multiplied the dilution factor. Levels of cytokines (IFN-γ, IL-4, IL-5, IL-8, IL-10, IL-13 and IL-17A) were measured by Meso Scale Diagnostics (MESO QuickPlex SQ 120, Model 1300) according to the manufacturer's instruction. The level of periostin was shown in ng/mL while the levels of cytokines were shown in pg/mL.

2.6 | Statistical analysis

Non-parametric quantitative data were expressed as median (interquartile range). Differences of the three groups were analysed using

TABLE 2 Longitudinally observation of 12 patients with ABPA

Patient number	Oral glucocorticoids (mg/d)				Inhaled glucocorticoids (µg/d)	β2-agonists (µg/d)	Anti-fungal (mg/d)	Baseline total IgE level (kUA/L)	Total IgE level after treatment (kUA/L)
	1st week	2nd week	3rd week	From 4th week					
1	20	20	20	20	500	100	200	2095	227
2	30	30	30	30	500	100	200	2858	398
3	25	25	25	25	—	—	200	1350	95
4	20	20	20	20	—	100	200	5000	207
5	20	20	20	20	128	100	250	5000	2411
6	20	20	15	15	—	—	200	2504	640
7	30	20	20	20	320	20	200	2412	836
8	25	20	15	10	—	—	200	3294	1375
9	30	25	20	15	—	—	200	5000	5000
10	20	15	15	15	—	—	200	1663	612
11	20	20	20	20	320	20	200	2679	2059
12	20	20	20	20	—	—	200	863	2086

the non-parametric Kruskal-Wallis test, while differences between two groups were analysed by Mann-Whitney test. Chi-square test was applied to analyse the difference in the ratio data between groups, and Spearman's ρ test was applied for correlation analysis. Optimal scaling was carried out with categorical principle component analysis. Briefly, optimal scaling quantifies categorical data by assigning numerical values to the categories, resulting in an optimal linear regression equation for the transformed variables. When the calculated data are put down on two dimensional graphic, the distance between two points represents the strength or correlation between two factors (ie the shorter the distance between the two points, the higher the correlation). The diagnostic value of periostin and total IgE levels between ABPA and SAFS was calculated using receiver operator characteristic (ROC) curves with area under the curve (AUC). Cut-off value was determined according to Youden's index. Statistical analysis was performed with SPSS 22.0 (SPSS) and GRAPHPAD PRISM 6.0 software (GraphPad Software Inc). A value of $P < 0.05$ was considered to be statistically significant.

3 | RESULTS

As shown in Table 1, among 24 asthma patients, 11 were sensitised to fungal allergens and 9 of them (81.8%) were sensitised to *A. fumigatus*. The lung functions, FeNO, peripheral blood eosinophil and neutrophil counts did not differ in the three groups. The median serum total IgE levels in ABPA were significantly higher than those in other groups. Moreover, the median serum levels of *A. fumigatus* specific IgE and IgG were significantly increased comparing with those in SAFS. Notably, patients with SAwFS were younger than those with ABPA ($P < 0.01$). Regarding the use of glucocorticoid (either oral or inhaled), no statistical difference was found among the three groups.

3.1 | Serum levels of periostin were elevated in ABPA

Serum level of periostin in patients with ABPA (85.55 ng/mL, [68.28-166] ng/mL) was significantly higher than patients with asthma (58.17 ng/mL, [36.29-82.39] ng/mL; $P < 0.01$; Figure 1A). When compared with the subgroups, statistical significance was found between ABPA (85.55 ng/mL, [68.28-166] ng/mL) and SAFS (50.99 ng/mL, [32.02-71.80] ng/mL; $P < 0.01$), rather than SAwFS (63.05 ng/mL, [41.76-90.51] ng/mL; Figure 1B). Among the detected cytokines, we found no significant difference between patient groups except serum levels of IL-5. Similarly, ABPA patients (1.55 pg/mL, [0.96-3.33] pg/mL) had significant higher levels of IL-5 than asthma patients (0.34 pg/mL, [0.24-0.56] pg/mL; $P < 0.001$; Figure 1C). In the comparison of the subgroups, serum levels of IL-5 in ABPA was significantly higher than those in both SAFS (0.31 pg/mL, [0.26-0.56] pg/mL) and SAwFS (0.34 pg/mL, [0.21-0.56] pg/mL; $P < 0.05$; Figure 1D).

3.2 | Serum periostin levels was positively associated with serum total IgE levels, IL-5 levels and peripheral blood eosinophil counts

Among the studied subjects, serum levels of periostin were positively correlated to serum total IgE levels ($r = 0.32$, $P < 0.05$), IL-5 levels ($r = 0.46$, $P < 0.01$) and peripheral blood eosinophil counts ($r = 0.49$, $P < 0.01$; Figure 2A-C). In addition, serum IL-5 levels were positively correlated to serum total IgE levels ($r = 0.37$, $P < 0.05$), *A. fumigatus* specific IgE levels ($r = 0.50$, $P < 0.01$) and peripheral blood eosinophil counts ($r = 0.54$, $P < 0.01$; Figure 2D-F), but negatively correlated to lung function (%FVC [$r = -0.39$, $P < 0.05$]; %FEV₁ [$r = -0.41$, $P < 0.05$]; Figure 2G,H). No association was found between serum levels of periostin and IL-13 in our study ($r = 0.14$, $P = 0.54$). Besides,

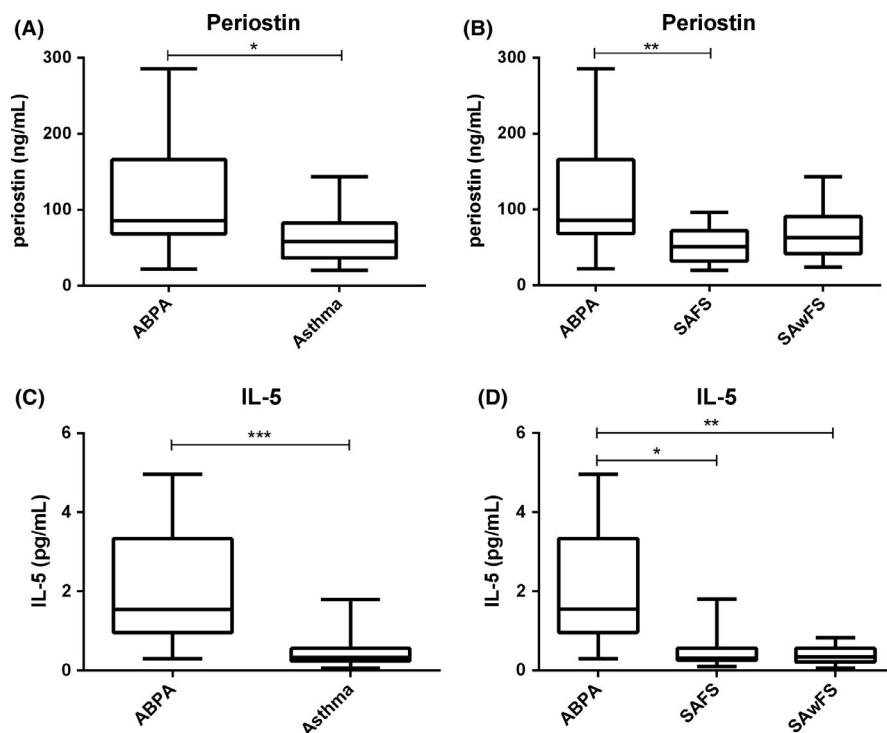


FIGURE 1 Serum levels of periostin and IL-5 in patients with ABPA and asthma. Serum levels of (A) periostin and (C) IL-5 in ABPA and asthma. Further comparison of serum levels of (B) periostin and (D) IL-5 was performed as asthma was classified into two subgroups: severe asthma with fungal sensitization (SAFS) and severe asthma without fungal sensitization (SAwFS). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

there was no correlation between results of lung functions and serum levels of periostin, total IgE, *A fumigatus* specific IgE and any cytokines other than IL-5 (Table S1).

Results from optimal scaling demonstrated that a close association was found between IL-5 levels and peripheral eosinophil counts as well as total IgE levels and *A fumigatus* specific IgE levels. Furthermore, levels of periostin and IgE (either total or *A fumigatus* specific) were associated with levels of IL-5 and peripheral eosinophil counts, but in different pathways. Interestingly, levels of periostin and IL-13 were at the opposite site of the scale, indicating that the synergistic effect of IL-13 and periostin was not found in these patients (Cronbach's $\alpha = 92.0\%$; Figure 3).

3.3 | The clinical value of serum periostin levels in the diagnosis of ABPA

To evaluate the diagnostic value of serum periostin level, ROC curves of baseline levels of periostin and total IgE in patients with ABPA and SAFS were analysed (Figure 4A,B). The optimal cut-off value for total IgE and periostin was 1036 kUA/L and 68.81 ng/mL, respectively. At the cut-off values, the sensitivity and specificity was 88.9% and 72.7% for total IgE and 73.7% and 81.8% for periostin, respectively. The area under the curve (AUC) was 0.79 for serum total IgE levels and 0.81 for serum periostin levels ($P < 0.01$).

3.4 | Serum levels of periostin did not alter in ABPA patients responding to the treatment

We observed 12 patients with ABPA longitudinally. As shown in Table 2, there were nine patients who had decreased levels of total

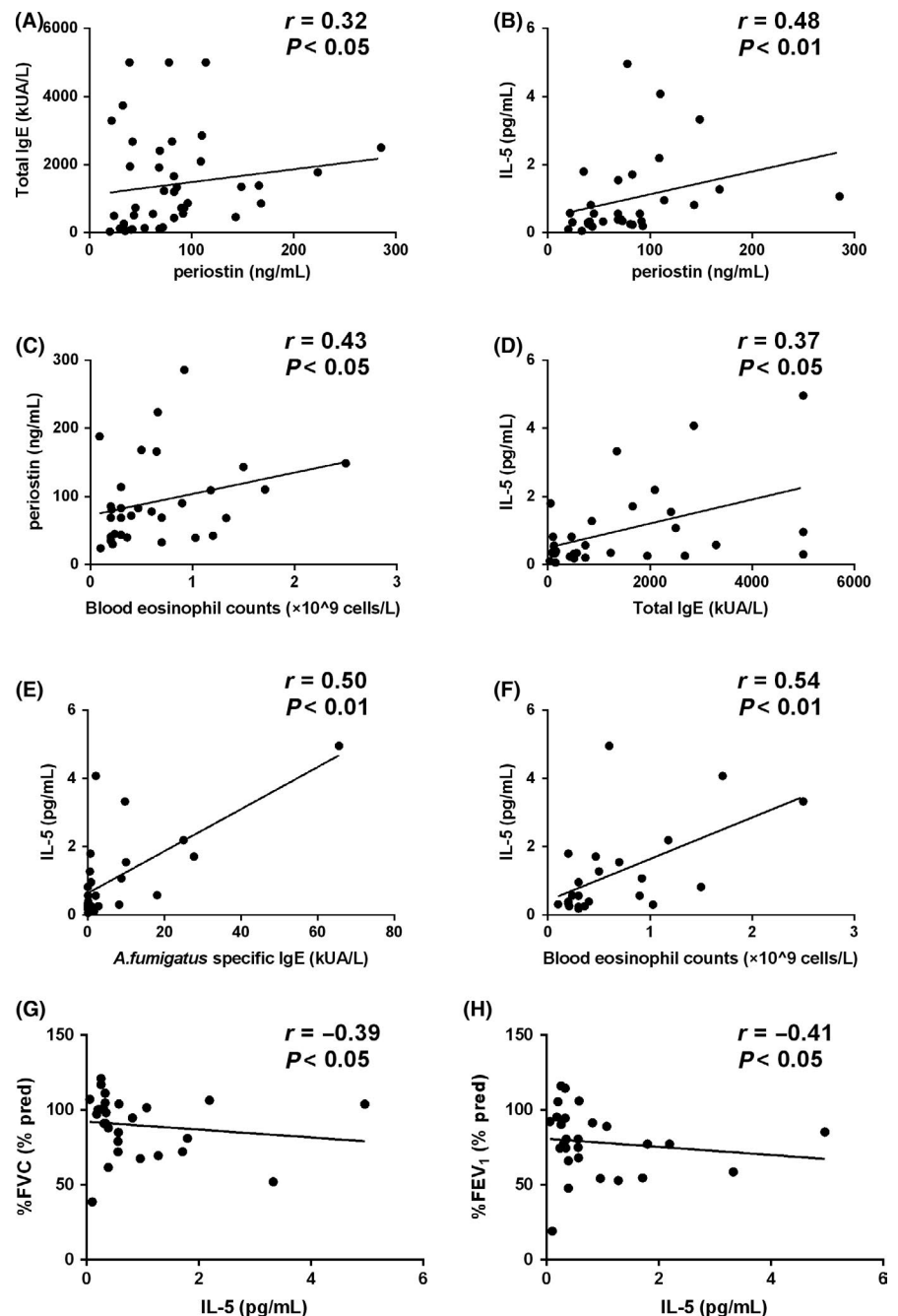
serum IgE by at least 25% of the baseline levels after treatment for 3 months or longer (Figure 5A), along with clinical improvements according to their clinical records. These patients were responding to the treatment based on the staging in ABPA.^{5,18} Within these patients, the blood eosinophil counts were also significantly decreased after treatments (Figure 5B), but there was no significant difference of serum periostin levels (Figure 5C, $P = 0.20$). Among the detected cytokines, decreased levels of IL-4 ($P < 0.05$), IL-5 ($P < 0.01$), IL-13 ($P < 0.05$) and IL-8 ($P < 0.05$) were found after 3 months treatment (Figure 5D-F,G).

4 | DISCUSSION

This study was the first to investigate the clinical value of periostin in ABPA and asthma patients. We demonstrated increased serum levels of periostin in ABPA patients compared with asthma patients, particularly with those sensitised to fungi. Correlation and optimal scaling analysis showed that serum levels of periostin were positively associated with serum levels of IL-5 and blood eosinophil counts. Besides, serum periostin levels might be a potential marker for the differential diagnosis of ABPA in asthma patients with fungal sensitisation.

Allergic bronchopulmonary aspergillosis is a chronic disease, and the long-lasting period may result in pulmonary fibrosis, bronchiectasis and persistent asthma with loss of lung function.^{17,19} Lung infection of *A fumigatus* can cause various clinical syndromes due to different immunity of the hosts: in addition to lung damage, immune dysfunction may end up with invasive aspergillosis (IA), while immune hyperactivity may lead to severe asthma with fungal

FIGURE 2 Correlation analysis of serum levels of periostin, IL-5 and clinical data. Serum periostin levels were positively correlated to (A) serum total IgE levels, (B) IL-5 levels and (C) peripheral blood eosinophil counts, while serum IL-5 levels were positively correlated to (D) serum total IgE levels, (E) *Aspergillus fumigatus* specific IgE levels and (F) peripheral blood eosinophil counts. Additionally, serum IL-5 levels were negatively correlated to results of lung functions: (G) FVC (% pred) and (H) FEV1 (% pred)



sensitisation (SAFS) or ABPA.²⁰ SAFS is a newly described entity of worse controlled asthma, and Agarwal has hypothesised that as the Th2 immune responses increase, patients with asthma associated with fungal sensitisation can develop ABPA.¹ It has been reported that periostin could be a biomarker of Th2 inflammation, since up-regulated gene expression of *POSTN* was detected in the airway epithelial cells of asthma patients.²¹ Consistently, our result demonstrated that significant elevated levels of periostin was found in ABPA patients, suggesting that ABPA patients exhibited more profound Th2 inflammation and/or higher level of fibrosis than asthma patients, especially than those with fungal sensitisation. We noticed that periostin levels in ABPA were higher than those in SAwFS, but without statistical significance. This might mainly due to small

sample size. Another possibility should not be neglected is younger age of SAwFS, because periostin was suggested to be involved in development of bone, tooth, heart and myoseptum in the youths, and study has found that teenagers had higher levels of periostin than adults.²² Generally, the Th2 immune response leads to secretion of cytokines such as IL-4, IL-5 and IL-13. Previous study showed that serum levels of these Th2 cytokines were significantly higher in CF-ABPA patients and ABPA patients with asthma compared with patients with CF colonised with *A. fumigatus*, but did not differ significantly from patients with CF sensitised to *A. fumigatus*, atopic CF patients and atopic control subjects.⁷ In our study, significant increased serum levels of IL-5 were observed in ABPA. Since IL-5 plays a critical role in the eosinophilic conditions,²³ this result implied that

patients with ABPA may have higher level of eosinophilia than atopic asthma patients.

Indeed, correlation analysis showed that serum levels of periostin were positively associated with serum levels of IL-5, peripheral blood eosinophil counts and serum total IgE levels, while levels of IL-5 were negatively correlated to lung functions. This means that periostin may involve in the pathophysiology of Th2 immune response, especially in the process of eosinophilia. The release of Th2 cytokines leads to a cascade of response in the airway, including recruitment of eosinophil and production of IgE,²⁴ which may lead to

reduce of lung functions. Similarly, the optimal scaling analysis also showed that levels of periostin and IgE (either total or *A fumigatus* specific) were associated with levels of IL-5 and peripheral eosinophil counts, but in different pathways. IgE bound to receptors of human mast cell and basophils and the activation of these cells causes secretion of cytokines such as IL-5, resulting in eosinophilia.²⁵ The mechanism of the involvement of periostin in eosinophilia is still no clear. Blanchard et al²⁶ reported that periostin facilitated eosinophil accumulation in Th2-associated mucosal inflammation in allergic lung in both mice and humans. Although previous study illustrated that periostin was induced by IL-13 in airway epithelial cells,²⁷ our data showed no association between the serum levels of periostin and IL-13. Moreover, the result of optimal scaling showed that levels of periostin and IL-13 were at the opposite site of the scale, suggesting that high levels of periostin in ABPA patients may not be dominant by the effect of IL-13. In addition to eosinophilia, periostin also mediates collagen synthesis and fibrillogenesis, resulting in airway fibrosis.²⁸ In patients with ABPA, the immune response against the colonised *A fumigatus* persisted in the airway, and subsequently, higher level of inflammation and fibrosis can also be found.^{1,5,29} This may partly explain the increase serum levels of periostin in ABPA patients.

It is easy to misdiagnose ABPA as SAFS because of the similarity of clinical features of these diseases.¹ Currently, serum total IgE level has been considered to be a crucial indicator in the diagnosis of ABPA. One of the major difficulties in the diagnosis is in patients with ABPA who exhibited serum total IgE levels <1000 kUA/L. Thus, more effective biomarkers are required. Agarwal et al reported the cut-off value of total blood eosinophil counts, total serum IgE, *A fumigatus* specific IgE and *A fumigatus* specific IgG,^{30,31} while Zheng et al¹⁹ introduced specific IgE against mixed fungal cultures (sIgE-M.x.) for the differential diagnosis of ABPA from asthma. We here examined the diagnostic value of periostin. Our results demonstrated that serum levels of periostin showed similar diagnostic value as total IgE levels, as the AUC were both around 0.8. To date, the utility of periostin for differential diagnosis of ABPA and asthma has not been reported before. Although the sample size is limited in our study, serum levels of periostin seem to be a promising biomarker in the diagnosis of ABPA, which requires further investigation to verify.

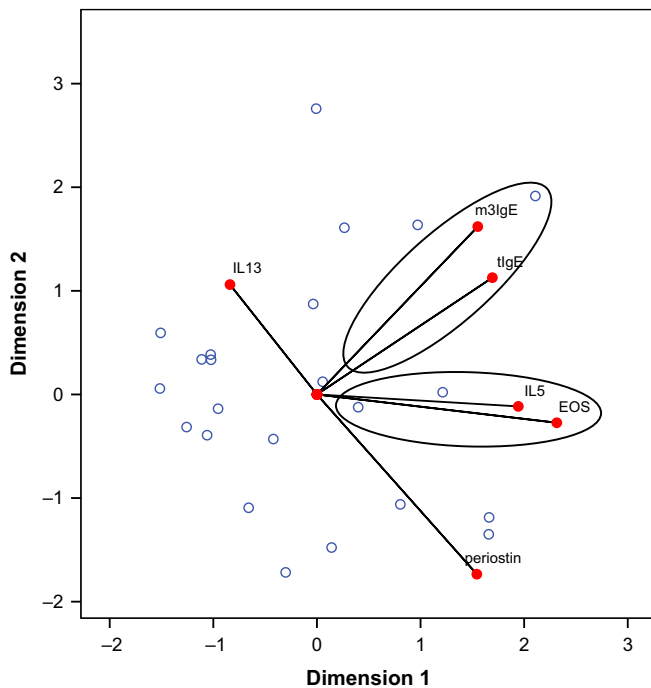


FIGURE 3 Optimal scaling analysis of serum levels of periostin, total IgE, *Aspergillus fumigatus* specific IgE, IL-5, IL-13 and peripheral blood eosinophil counts in patients with ABPA and asthma. A closer distance between the dots represented a stronger correlation of the corresponding data (Cronbach's $\alpha = 92.0\%$). tIgE: total IgE, m3IgE: *A fumigatus* specific IgE, EOS: peripheral blood eosinophil counts

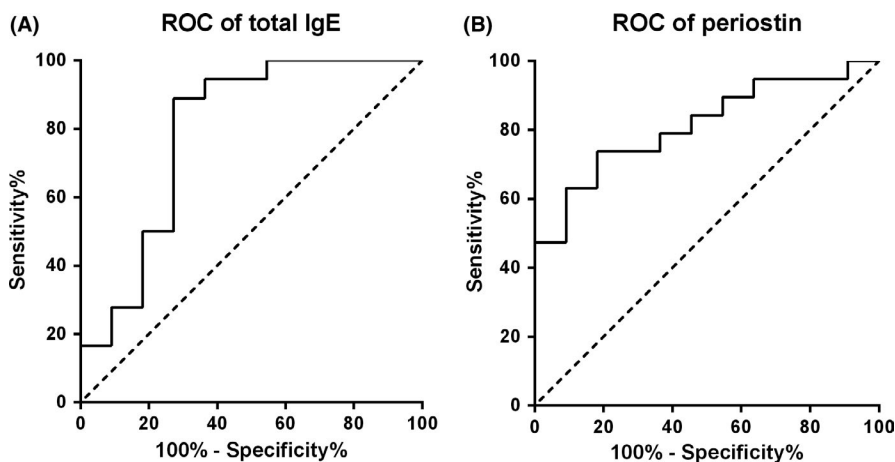
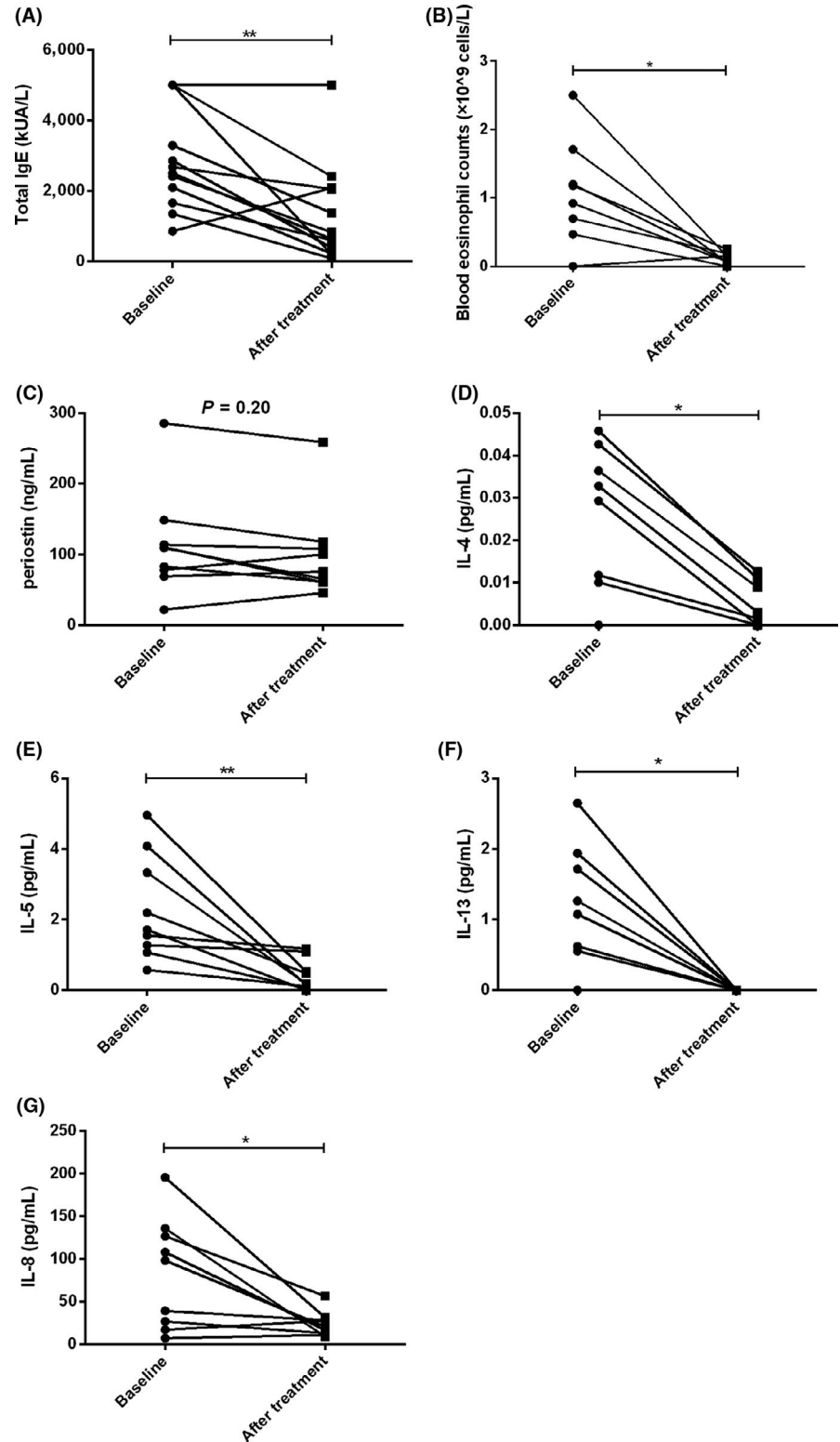


FIGURE 4 ROC curve analysis. ROC curve analysis was performed to evaluate the diagnostic effect of serum levels of (A) total IgE and (B) periostin in the differential diagnosis of ABPA from severe asthma with fungal sensitization (SAFS)

FIGURE 5 Longitudinally observation of serum levels of total IgE, periostin, IL-4, IL-5, IL-13, IL-8 and peripheral blood eosinophil counts in patients with ABPA. In 12 patients with ABPA we observed, 9 of them showed decreased levels of total serum IgE by at least 25% of the baseline level after treatment (A). Among these nine patients, we further observed peripheral blood (B) eosinophil counts, (C) serum levels of periostin, (D) IL-4, (E) IL-5, (F) IL-13 and (G) IL-8 after their treatments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$



In the longitudinal observation during ABPA episode, patients who were responding to the treatments exhibited significant decreased levels of total IgE, total eosinophil counts and various cytokines levels (IL-4, IL-5, IL-13 and IL-8). The principles of ABPA treatments were anti-inflammatory agent and anti-fungal agent,³² and thereby, the Th2 driven inflammation reduced remarkably in the responding patients. The decline of IL-8 partially reflected the

attenuation of fungal infection because proteases released by *A. fumigatus* induced the production of IL-8.³³ However, serum levels of periostin in these patients did not alter significantly after treatment. One possible explanation was that the repair of the damaged airway was still in progress though inflammation has been controlled, and periostin served as a vital molecule in the process of fibrosis and remodelling.^{12,34} Besides, studies have proposed a periostin-high

asthma phenotype with low response to ICS and low pulmonary function.³⁵⁻³⁷ This should also be noticed in patients with ABPA, and further investigation is needed to examine the responsiveness to glucocorticoids in ABPA patients with high serum levels of periostin.

There are several limitations in the study. Firstly, the enrolled subjects were asthma patients with/without ABPA. Hence, our results are not suitable for patients with CF and ABPA. Secondly, since it was a retrospective analysis, some of the clinical data were not available. Furthermore, patients aged under 16 years were not excluded in the study, as periostin levels were known to be higher in children than in adults.^{28,38} However, this might not be an impact to our outcomes. Since most of young patients were in the SAwFS group, the median level of periostin in ABPA would not be affected by such issue. Last but not least, the sample size was limited in this study. Future studies should aim to verify the diagnostic value of serum periostin levels in ABPA, as well as to explore the role of periostin in the disease.

In conclusion, serum levels of periostin were up-regulated in ABPA patients, which may be associated with eosinophilic inflammation. In addition, it could be a promising biomarker in the diagnosis of ABPA.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Baoqing Sun conceived and designed the experiments; Ge Wu and Lu Li performed the experiments; Ge Wu, Xun Meng, Lu Li, Peiyan Zheng and Haisheng Hu collected and analysed the data; Ge Wu, Xun Meng, Peiyan Zheng, Xiaohua Douglas Zhang and Baoqing Sun led the writing; Ge Wu, Xun Meng, Xiaohua Douglas Zhang and Baoqing Sun revised the manuscript.

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REFERENCES

- Agarwal R. Severe asthma with fungal sensitization. *Curr Allergy Asthma Rep.* 2011;11:403-413.
- Agarwal R, Aggarwal AN, Gupta D, Jindal SK. Aspergillus hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma: systematic review and meta-analysis. *Int J Tuberc Lung Dis.* 2009;13:936-944.
- Denning DW, Pleuvry A, Cole DC. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. *Med Mycol.* 2013;51:361-370.
- Maturu VN, Agarwal R. Prevalence of Aspergillus sensitization and allergic bronchopulmonary aspergillosis in cystic fibrosis: systematic review and meta-analysis. *Clin Exp Allergy.* 2015;45:1765-1778.
- Shah A, Panjabi C. Allergic bronchopulmonary aspergillosis: a perplexing clinical entity. *Allergy Asthma Immunol Res.* 2016;8:282-297.
- Latzin P, Hartl D, Regamey N, Frey U, Schoeni MH, Casaulta C. Comparison of serum markers for allergic bronchopulmonary aspergillosis in cystic fibrosis. *Eur Respir J.* 2008;31:36-42.
- Hartl D, Latzin P, Zissel G, Krane M, Krauss-Etschmann S, Griesse M. Chemokines indicate allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Am J Respir Crit Care Med.* 2006;173:1370-1376.
- Tracy MC, Okorie C, Foley EA, Moss RB. Allergic bronchopulmonary aspergillosis. *J Fungi.* 2016;2:17.
- Kauffman HF. Immunopathogenesis of allergic bronchopulmonary aspergillosis and airway remodeling. *Front Biosci.* 2003;8:e190-e196.
- Schuyler M. The Th1/Th2 paradigm in allergic bronchopulmonary aspergillosis. *J Lab Clin Med.* 1998;131:194-196.
- Ito Y, Al Mubarak R, Roberts N, et al. IL-13 induces periostin and eotaxin expression in human primary alveolar epithelial cells: Comparison with paired airway epithelial cells. *PLoS ONE.* 2018;13:e0196256.
- O'Dwyer DN, Moore BB. The role of periostin in lung fibrosis and airway remodeling. *Cell Mol Life Sci.* 2017;74:4305-4314.
- Takahashi K, Meguro K, Kawashima H, et al. Serum periostin levels serve as a biomarker for both eosinophilic airway inflammation and fixed airflow limitation in well-controlled asthmatics. *J Asthma.* 2018;56:236-243.
- Izuhara K, Conway SJ, Moore BB, et al. Roles of periostin in respiratory disorders. *Am J Respir Crit Care Med.* 2016;193:949-956.
- Li W, Gao P, Zhi Y, et al. Periostin: its role in asthma and its potential as a diagnostic or therapeutic target. *Respir Res.* 2015;16:57.
- Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. *Am J Respir Crit Care Med.* 2000;162:2341-2351.
- Greenberger PA, Bush RK, Demain JG, Luong A, Slavin RG, Knutsen AP. Allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol Pract.* 2014;2:703-708.
- Agarwal R, Chakrabarti A, Shah A, et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy.* 2013;43:850-873.
- Zheng PY, Wu SQ, Xie JX, et al. Clinical and immunological characteristics of severe asthma with fungal sensitization and allergic bronchopulmonary aspergillosis: potential utility for differential diagnosis. *Int J Clin Exp Med.* 2017;10:16540-16547.
- Kosmidis C, Denning DW. The clinical spectrum of pulmonary aspergillosis. *Thorax.* 2015;70:270-277.
- Woodruff PG, Boushey HA, Dolganov GM, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci U S A.* 2007;104:15858-15863.
- Walsh JS, Gossel F, Scott JR, Paggiosi MA, Eastell R. Effect of age and gender on serum periostin: relationship to cortical measures, bone turnover and hormones. *Bone.* 2017;99:8-13.
- Roufosse F. Targeting the interleukin-5 pathway for treatment of eosinophilic conditions other than asthma. *Front Med.* 2018;5:49.

24. Venkayya R, Lam M, Willkom M, Grunig G, Corry DB, Erle DJ. The Th2 lymphocyte products IL-4 and IL-13 rapidly induce airway hyperresponsiveness through direct effects on resident airway cells. *Am J Respir Cell Mol Biol*. 2002;26:202-208.
25. Bradding P, Roberts JA, Britten KM, et al. Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol*. 1994;10:471-480.
26. Blanchard C, Mingler MK, McBride M, et al. Periostin facilitates eosinophil tissue infiltration in allergic lung and esophageal responses. *Mucosal Immunol*. 2008;1:289-296.
27. Zhao J, Minami Y, Etling E, et al. Preferential generation of 15-HETE-PE induced by IL-13 regulates goblet cell differentiation in human airway epithelial cells. *Am J Respir Cell Mol Biol*. 2017;57:692-701.
28. Takayama GO, Arima K, Kanaji T, et al. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J Allergy Clin Immunol*. 2006;118:98-104.
29. Kennedy JL, Heymann PW, Platts-Mills TA. The role of allergy in severe asthma. *Clin Exp Allergy*. 2012;42:659-669.
30. Agarwal R, Aggarwal AN, Garg M, Saikia B, Chakrabarti A. Cut-off values of serum IgE (total and *A fumigatus* -specific) and eosinophil count in differentiating allergic bronchopulmonary aspergillosis from asthma. *Mycoses*. 2014;57:659-663.
31. Agarwal R, Maskey D, Aggarwal AN, et al. Diagnostic performance of various tests and criteria employed in allergic bronchopulmonary aspergillosis: a latent class analysis. *PLoS ONE*. 2013;8:e61105.
32. Agarwal R, Sehgal IS, Dhooria S, Aggarwal AN. Developments in the diagnosis and treatment of allergic bronchopulmonary aspergillosis. *Expert Rev Respir Med*. 2016;10:1317-1334.
33. Wark PA, Gibson PG. Allergic bronchopulmonary aspergillosis: new concepts of pathogenesis and treatment. *Respirology*. 2001;6:1-7.
34. Izuhara K, Nunomura S, Nanri Y, et al. Periostin in inflammation and allergy. *Cell Mol Life Sci*. 2017;74:4293-4303.
35. Kato G, Takahashi K, Izuhara K, Komiya K, Kimura S, Hayashi S. Markers that can reflect asthmatic activity before and after reduction of inhaled corticosteroids: a pilot study. *Biomarker Insights*. 2013;8:97-105.
36. Kanemitsu Y, Matsumoto H, Izuhara K, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J Allergy Clin Immunol*. 2013;132:305-312.e3.
37. Matsusaka M, Kabata H, Fukunaga K, et al. Phenotype of asthma related with high serum periostin levels. *Allergol Int*. 2015;64:175-180.
38. Sanchez-Garcia S, Habernau Mena A, Quirce S. Biomarkers in inflammation pediatric asthma: utility in daily clinical practice. *Eur Clin Respir J*. 2017;4:1356160.

SUPPORTING INFORMATION

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

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