

Decreased expression of indolamine 2,3-dioxygenase in childhood allergic asthma and its inverse correlation with fractional concentration of exhaled nitric oxide

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ABSTRACT

Background: The tryptophan metabolic pathway mediated by indolamine 2,3-dioxygenase (IDO), a tryptophan-degrading enzyme, plays an important role in controlling the development of allergic inflammation. The fractional concentration of exhaled nitric oxide (FeNO) is closely associated with the allergic state and is extensively used for the clinical evaluation of airway allergic inflammation. Clinical trials have rarely assessed the expression of IDO in childhood allergic asthma and its correlation with FeNO.

Objective: To evaluate the IDO level in children with childhood allergic asthma and the relation between IDO levels and FeNO.

Methods: Thirty children older than 5 years who were diagnosed the first time with allergic asthma were selected from the pediatric outpatient department. Another 30 healthy children were selected as controls. The subjects were evaluated by complete medical history, pulmonary function test results, skin prick test reaction, FeNO concentration test result, eosinophil count, and a disease severity score. Peripheral venous blood and induced sputum were obtained to measure the concentrations of IDO metabolites (ie, tryptophan and kynurenine).

Results: The IDO levels in the peripheral blood and induced sputum were significantly lower in patients with childhood allergic asthma than in children in the control group. The IDO level was negatively correlated with FeNO but was not significantly correlated with age, sex, blood eosinophil count, or disease severity scale.

Conclusion: The expression of IDO was significantly lower in childhood allergic asthma, particularly in children with high FeNO levels. There was no significant relation between IDO levels and asthma severity.

Trial Registration: Chinese Clinical Trial Register (www.chictr.org.cn) Identifier: ChiCTR-COC-15006080.

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Introduction

Allergic asthma is an allergic inflammatory disease characterized by airway hyperresponsiveness. An imbalance in the ratio of T-helper cell type 1 to type 2 (T_H1/T_H2) is a key step in the pathogenesis of asthma, and T_H2 cells in particular play an important role. However, in recent years, T_H17 and regulatory T cells also have been found to have important roles in the pathogenesis of asthma.

Tryptophan is an essential amino acid that functions as an important amino acid substrate used by many human cells. There is growing evidence that the tryptophan metabolic process plays a major role in the development of allergic diseases.¹ Ciprandi et al²

found that concentrations of plasma tryptophan and its metabolites in patients with pollen allergy were significantly higher than those in healthy blood donor controls. Kositz et al³ noted that a high basal tryptophan concentration in patients with pollen allergy was associated with hypo-responsiveness to specific immunotherapy with pollen extracts. The key mechanism of specific immunotherapy is immune tolerance. Thus, tryptophan catabolism is believed by some researchers to be an important mechanism in the development of immune tolerance.⁴

Indolamine 2,3-dioxygenase (IDO) is the rate-limiting enzyme of the tryptophan catabolic pathway, with high expression in immune-privileged organs. IDO plays an important role in peripheral immune tolerance. The role of IDO in pregnancy, organ transplantation, and tumor immune tolerance has led to research hotspots in recent years. A clinical trial found that the plasma IDO activity in patients with allergy was significantly lower than that in healthy individuals.⁵ Another clinical trial found that in patients with asthma and airborne allergen exposure, the plasma

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IDO activity was significantly higher in patients with asymptomatic allergy than in those with symptomatic allergy and healthy individuals.⁶ Maneechotesuwan et al⁷ observed lower IDO activity in induced sputum but a significantly higher activity of inhaled corticosteroid treatment in patients with mild to intermittent and mild to moderate persistent asthma compared with healthy subjects, which suggests that glucocorticoids, at least in part, play their anti-inflammatory role by upregulating IDO activity. Thus, IDO-mediated tryptophan catabolism could play an important role in the development and progression of allergic diseases.⁸

Notably, the fractional concentration of exhaled nitric oxide (FeNO), a marker commonly used for noninvasive clinical monitoring of airway allergic inflammation, as recommended by the American Thoracic Society, is closely related to IDO. It has long been confirmed that NO catabolism also plays an important role in allergic diseases.¹ Significantly increased inducible nitric oxide synthase (iNOS) was found in the airway epithelium and inflammatory cells of patients with asthma. The exhaled breath of patients with allergic rhinitis or asthma was shown to contain higher levels of nitric oxide (NO) than that of healthy controls during the non-pollen season. The FeNO of patients with allergic rhinitis was significantly increased during the non-pollen season, and a further increase of FeNO occurred in the pollen season.⁹ Therefore, IDO and FeNO are indicators of airway allergic inflammation. The specific interaction between NO and IDO has been reported to be important in asthma and allergic rhinitis.¹ NO production can inhibit IDO expression and activation in symptomatic patients with allergy.^{10,11}

These findings indicate that IDO-mediated tryptophan catabolism could play an important role in the development of allergic diseases. In allergic diseases, NO can inhibit IDO expression, leading to high tryptophan levels. The relation among IDO, NO, and allergic diseases has become a research hotspot. Asthma mainly develops in children, whose basic characteristics are growth and development. Allergy and immune tolerance *in vivo* can change with advancing age and with environmental changes. No researchers have been involved in clinical trials on IDO, FeNO, and childhood allergic asthma. Therefore, we investigated IDO levels in childhood allergic asthma and compared the results with those in normal children. Furthermore, we explored the possible relation of the IDO level with the FeNO level and disease severity in children with childhood allergic asthma.

Methods

The trial was registered at and approved by the Chinese Clinical Trial Register (registration number ChiCTR-COC-15006080). This trial also was reviewed and approved at a meeting of the ethics committee of Southwest Hospital, Chongqing, China. Family members of all participating children signed an informed consent form.

Subjects

The subjects were recruited at the pediatric outpatient department of Southwest Hospital. Thirty children (sample size calculated from the expected significant difference) older than 5 years who had allergic asthma (retrospective grading based on disease severity) were included. Another 30 age- and sex-matched children who had a physical examination during the same period were selected as normal controls. The representativeness and universality of age and sex were taken into account during the selection of patients and controls to decrease the offset and make the distribution as normal as possible. The clinical data of the 2 groups were comparable.

Determination of sample size

The sample size was calculated using the superiority test formula for a 2-sample mean comparison based on a 2-group, parallel, controlled, randomized blocks design:

$$N = \frac{(Z_{\alpha} + Z_{\beta})^2 \times (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2 - \delta)^2}$$

The observation indicator was IDO activity. According to relevant studies in China and other countries, the characteristic value of IDO activity, which is the ratio of kynurenine to tryptophan (percentage), was 12.8 in the experimental group and 7 ± 1 in the control group ($\alpha = .025$ [unilateral], $1 - \beta = .90$, $\mu_1 = 12.8$, $\mu_2 = 7$, $\sigma_1^2 = 2^2$, $\sigma_2^2 = 1^2$, and $\delta = 3$). The sample size of each group was calculated to be 29. Considering interference factors such as the dropout of subjects during the clinical trial, we determined that each group should have 30 subjects.

Selection criteria for observation group

1. Selection criteria for patients in the observation group
 - a. Patients older than 5 years
 - b. Patients diagnosed with asthma for the first time or previously diagnosed with asthma but without standard treatment for asthma
 - c. Patients did not receive inhaled glucocorticoid or leukotriene receptor antagonist treatment within 2 weeks
 - d. Patients were classified by disease severity according to the Global Initiative for Asthma 2016 guidelines for the diagnosis and treatment of childhood bronchial asthma after 6 months of follow-up as outpatient treatment
2. Diagnostic criteria for asthma
 - a. Recurrent wheezing, cough, shortness of breath, and chest tightness, mostly related to contact allergens, cold air, physical and chemical stimuli, respiratory infections, and sports and often with onset or exacerbation at night and (or) early morning
 - b. Scattered or diffuse, mainly expiratory, wheezing in the 2 lungs at onset, with an extended expiratory phase
 - c. Effective or spontaneous remission of these symptoms and signs after antiasthma treatment
 - d. Exclusion of wheezing, coughing, shortness of breath, and chest tightness caused by other diseases
 - e. Atypical clinical manifestations (eg, no obvious wheeze), with at least 1 of the following (those who met criteria a–d or d and e were diagnosed with asthma):
 - i. Positive results from the bronchial challenge test or exercise challenge test
 - ii. Confirmed reversible airflow limitation from a positive result on the bronchial dilation test: greater than 12% increase in forced expiratory volume in the first second (FEV₁) at 15 minutes after inhalation of the short-acting β_2 -receptor agonist salbutamol sulfate
 - iii. 20% daily variability of peak expiratory flow (continuous monitoring for 1–2 weeks)
3. The classification of childhood asthma severity was performed as follows.¹² The severity of the asthma condition was subjected to retrospective assessment and classification based on the level of treatment required to achieve asthma control. The assessment was usually performed a few months after the standard treatment with control drugs. Mild persistent asthma was defined as asthma that was well controlled by treatment with a level 1 or 2 escalation therapy regimen (1 point); moderate persistent asthma was defined as asthma that was well controlled by treatment with the level 3 escalation therapy regimen (2 points); and severe

persistent asthma was defined as asthma that was well controlled with the level 4 or 5 escalation therapy regimen (3 points).

Selection criteria for control group

Thirty healthy children who underwent physical examination in the pediatric outpatient department during the same period were recruited (no history of asthma; no recent infectious diseases, immune diseases, or cardiopulmonary disease; and no use of glucocorticoids or immunomodulators within 2 weeks). Age and sex showed a good distribution and representativeness in the control group.

Exclusion criteria

1. Patients with oral or intravenous administration of glucocorticoids or immunomodulators within 2 weeks
2. Patients with other immune diseases, such as tuberculosis, systemic lupus erythematosus, or juvenile idiopathic arthritis
3. Patients with infectious diseases, such as upper respiratory tract infection and pneumonia, within 4 weeks after onset
4. Patients with cardiopulmonary failure

Trial Design

In part I, subjects were examined by a complete medical history, a physical examination, pulmonary function test results, a skin prick test reaction, and an eosinophil count (EC).

In part II, a peripheral venous blood test was performed. Blood was collected once from children in the observation and control groups immediately after enrollment. The following procedure was used.

1. Specimen collection: Four milliliters of venous blood (in heparin anticoagulant) was taken from each subject. After whole blood centrifugation, plasma was collected and frozen at -80°C . Peripheral blood mononuclear cells were separated using Ficoll density gradient centrifugation.
2. Indicator measurement: Tryptophan and kynurenine concentrations (micromoles per liter) were measured by high-performance liquid chromatography. The level of IDO activity was reflected as the ratio of kynurenine to tryptophan.

In part III, an induced sputum test was performed.

1. Specimen collection: Induced sputum was collected once from children in the observation and control groups immediately after enrollment. Aspiration of sputum was conducted after aerosolization with 3% hypertonic saline (1.5 mL of 10% NaCl + 2.5 mL of 0.9% NaCl). The supernatant of the induced sputum was stored at -70°C .
2. Indicator measurement: Tryptophan and kynurenine concentrations were measured by high-performance liquid chromatography. The level of IDO activity was reflected by the ratio of kynurenine to tryptophan.

In part IV: the FeNO test was performed. FeNO was measured according to the American Thoracic Society and European Respiratory Society guidelines for exhaled NO measurement in children in the observation and control groups immediately after enrollment. The subject was in the sitting position. After a calm breath, the subject inhaled NO-free gas ($\text{NO} < 5$ ppb) through the mouth with a disposable filter to nearly total lung volume and then immediately exhaled evenly at a flow rate of 50 mL/s. The exhalation time was 6 seconds for children younger than 12 years and 10 seconds for those older than 12 years. The test was considered a failure if it was not completed within 9 repeated

tests after detailed explanation of the method and the watching of a demonstration video. FeNO measurements were conducted in all children before the pulmonary function test.

Statistical Methods

All data were subjected to statistical analysis using SPSS 18.0 (SPSS, Inc, Chicago, Illinois). Multiple comparisons were conducted only after the normal distribution test (Kolmogorov-Smirnov and Shapiro-Wilk tests) and analysis of variance (Levene test). For data satisfying a normal distribution and homogeneity of variance, a group comparison was conducted using the independent-sample *t* test, and the results were expressed as the average (95% confidence interval). For data not satisfying a normal distribution, nonparametric statistical analysis was performed using the Mann-Whitney U test, and the experimental results were expressed as the median (interquartile range). The correlation between multiple groups was analyzed by multiple linear regression analysis. Differences were considered statistically significant at a *P* value less than .05.

Results

Clinical and Laboratory Test Characteristics of Allergic Asthma and Control Groups

The study enrolled 30 patients in the observation group (5–13 years old; 17 boys and 13 girls) and 30 age- and sex-matched healthy children in the control group. The clinical characteristics of the subjects are presented in Table 1. Allergen positivity was defined as a skin test reaction to more than 1 of the 12 common aeroallergens, including 2 types of dust mites, pollen, cat and dog epithelium, *Alternaria* species, *Aspergillus* species, birch, oak, mugwort, and so on. As expected, the children with allergic asthma showed significantly increased positive reactions for the skin prick test ($P < .001$), EC in blood ($P < .001$), and FeNO ($P < .001$) compared with those of controls.

IDO Activity in Induced Sputum and Blood of Allergic Asthma and Control Groups

In the allergic children, the level of IDO activity in the sputum was significantly lower than that of the controls ($P < .001$; Fig 1A). The level of IDO activity in peripheral blood also was significantly lower than that of the controls ($P < .001$; Fig 1B).

Table 1

Clinical and Laboratory Characteristics of Subjects Without Allergy (Controls) and Patients With Allergic Asthma

Variable	Patients (n = 30)	Controls (n = 30)	P value
Age (y), median (IQR)	7.5 (5–9.25)	7 (5.75–11)	.77
Boys/girls	17/13	14/16	.606
Allergen (positive/negative)	21/9	6/24	<.001
Eosinophils in blood (%), average (95% CI)	2.97 (2.662–3.285)	2.207 (2.032–2.381)	<.001
FEV ₁ /FVC (%), average (95% CI)	75.3 ± 12.03	91.2 ± 8.76	<.001
Severity score, median (IQR)	1 (1–1.25)	—	—
FeNO (ppb), average (95% CI)	37.833 (34.626–41.041)	24.7 (23.027–26.37)	<.001
IDO in sputum (%), average (95% CI)	5.442 (4.950–5.935)	8.912 (.398–9.426)	<.001
IDO in blood (%), average (95% CI)	3.136 (2.787–3.485)	4.12 (3.638–4.603)	.001

Abbreviations: CI, confidence interval; FeNO, fractional concentration of exhaled nitric oxide; FEV₁, maximum forced expiratory rate in the first second; FVC, forced vital capacity; IDO, indolamine 2,3-dioxygenase; IQR, interquartile range.

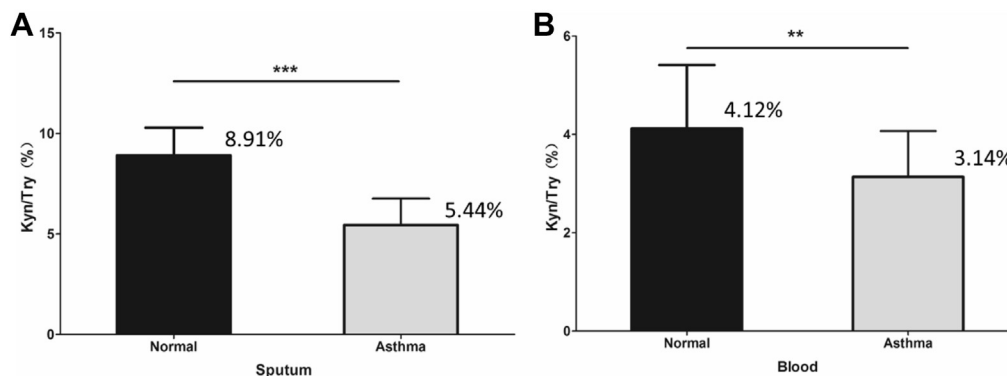


Figure 1. (A) Bar plot showing the level of indolamine 2,3-dioxygenase activity in induced sputum in subjects with or without allergic asthma. (B) Bar plot showing the level of indolamine 2,3-dioxygenase activity in blood in subjects with or without allergic asthma. * $P < .05$; ** $P < .01$; *** $P < .001$. Kyn, kynurenine; Try, tryptophan.

Relation of IDO Levels in Induced Sputum and Blood With FeNO Level and Other Factors

Taking into account the level of the effect of multiple factors on the level of IDO activity, we found that IDO levels in induced sputum and blood had no significant correlations with age, sex, or EC in blood. We also did not find any significant correlation between IDO levels and asthma severity (Tables 2 and 3). IDO levels showed significant negative correlations only with the FeNO level ($P = .009$, $P = .001$; Fig 2A, B).

Predictive Value of IDO Activity in Induced Sputum and FeNO Alone or in Combination for Childhood Allergic Asthma

Based on receiver operating characteristics curve analysis (Table 4, Fig 3), in the predictive value analysis of FeNO alone for childhood allergic asthma, the maximum value of the Youden index at 0.667 was taken as the best cutoff value for diagnosis. This value corresponded to the specific cutoff of the NO concentration at 33, with a sensitivity of 70% and a specificity of 96.7%.

In the predictive value analysis of IDO activity level in induced sputum alone for childhood allergic asthma, the maximum value of the Youden index at 0.833 was taken as the best cutoff value for diagnosis. This value corresponded to the specific cutoff of the IDO ratio at 7.28%, with a sensitivity of 93.3% and a specificity of 90%.

In the predictive value analysis of IDO in induced sputum combined with FeNO for childhood allergic asthma, the maximum value of the Youden index at 0.9 was taken as the best cutoff value for diagnosis. This value corresponded to the specific cutoff of the combined indicators at 7.59% to 28.5%, with a sensitivity of 96.7% and a specificity of 93.3%.

Table 2

Results of Multivariable Linear Regression Analysis For the Relation Between the Level of Indolamine 2,3-Dioxygenase Activity in Induced Sputum and Other Variables

Variable	B	SE	Partial r	t	P value
Age (y)	-1.565	.815	-0.319	-1.922	.067
Sex	-0.086	4.347	-0.003	-0.020	.984
Severity	-3.819	4.274	-0.151	-0.894	.380
Eosinophils (%)	-3.790	2.618	-0.240	-1.448	.161
FeNO (ppb)	-0.702	0.247	-0.466	-2.839	.009
Analysis of variance					
F ratio		3.696			
P value		.013			
R^2	0.435				
Adjusted R^2	0.317				
Multiple correlation coefficient	0.660				

Abbreviation: FeNO, fractional concentration of exhaled nitric oxide.

Discussion

Tryptophan is an essential amino acid that is used by many human cells as an important amino acid substrate. The tryptophan metabolic pathway has strong relevance to immunoregulation.¹³ IDO, a tryptophan-degrading enzyme, is considered an important regulator of the immune system; however, whether IDO activity is beneficial or harmful to inflammatory tissue has not been universally established. Some studies have shown that IDO inhibits T-cell activity, downregulates T_H2 expression, and improves the long-term tolerance of T cells to antigen stimulation^{14,15}; however, other studies have found that in lung dendritic cells IDO upregulates T_H2 -mediated allergic airway inflammation but cannot inhibit the T_H1 response to achieve immune tolerance.^{16,17} Pavaglio et al¹⁸ reported that IDO overexpression in the lungs of a transgenic mouse model might induce an antiasthma effect by decreasing regeneration and the production of $CD4^+$ T-cell-associated cytokines. It is currently believed that IDO-mediated peripheral immune tolerance could have the following associations: (1) IDO catabolizes tryptophan in the local microenvironment to inhibit the activation of T-cell differentiation and proliferation, whereas the products of tryptophan catabolism by IDO induce T-cell apoptosis; and (2) IDO mediates the binding of ligands (toll-like receptor-9, cytotoxic T-lymphocyte-associated protein-4, CD200, and glucocorticoid-induced tumor necrosis factor) on plasmacytoid dendritic cells to the corresponding receptors on T cells, thereby inducing the production of antigen-specific regulatory T cells. Moreover, these regulatory T cells induce IDO expression in plasmacytoid dendritic cells through reverse signaling (the inhibitor of κB kinase- α -mediated nonclassical nuclear factor- κB signaling pathway), which inhibits T-cell activation.¹⁹

Table 3

Results of Multivariable Linear Regression Analysis for the Relation Between the Level of Indolamine 2,3-Dioxygenase Activity in Blood and Other Variables

Variable	B	SE	Partial r	t	P value
Age (y)	0.0430	0.060	0.124	0.713	.483
Sex	0.135	0.322	0.073	0.420	.678
Severity	0.085	0.317	0.047	0.267	.792
Eosinophils (%)	0.278	0.194	0.248	1.433	.165
FeNO (ppb)	-0.068	0.018	-0.639	-3.729	.001
Analysis of variance					
F ratio		2.990			
P value		.031			
R^2	0.384				
Adjusted R^2	0.255				
Multiple correlation coefficient	0.620				

Abbreviations: FeNO, fractional concentration of exhaled nitric oxide; IDO, indolamine 2,3-dioxygenase.

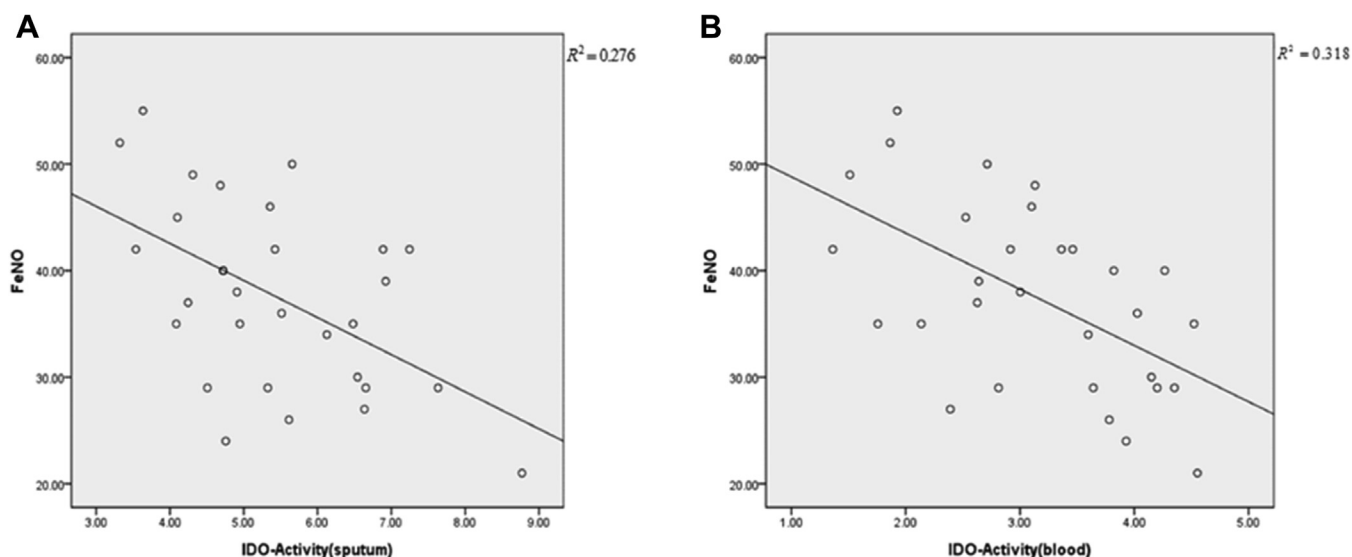


Figure 2. (A) Scatterplot showing the correlation between the level of indolamine 2,3-dioxygenase (IDO) activity in induced sputum and fractional concentration of exhaled nitric oxide (FeNO) in patients with allergic asthma. (B) Scatterplot showing the correlation between the level of IDO activity in blood and the FeNO in patients with allergic asthma.

The role of IDO in immune regulation and immune induction has been extensively recognized in infections, pregnancy, autoimmune issues, and tumor-like lesions. IDO also plays an important role in the control of allergic inflammation. In vivo clinical trials have shown that IDO is significantly associated with the pathogenesis of pollen allergy, allergic rhinitis, and allergic asthma.^{5–7} Allergic diseases, such as asthma, rhinitis, and even early allergic dermatitis, are immune environments mainly expressing T_H2 . T_H2 -type cytokines are upregulated in allergic inflammation, whereas the T_H1 -type immune response and associated cytokines, such as interferon- γ , are downregulated. Interferon- γ is a strong inducer of IDO.¹ IDO can degrade the essential amino acid tryptophan and, as part of the antiproliferative phase of immunoreactive cells, prevent infection and the growth of malignant cells; this response also is an immunomodulatory intervention of T cells, which can prevent over-activation of the immune system.²⁰ One study found that in patients with allergic asthma, experimental exacerbations of rhinovirus asthma were associated with systemic tryptophan concentrations; in addition, the IDO activity in lung tissue was low, whereas the tryptophan concentrations in plasma were increased.²¹ Thus, von Bubnoff and Bieber⁸ recognized that IDO-mediated tryptophan degradation is a central regulatory pathway in the allergic process. However, no study has reported on the activity of IDO in the airway-induced sputum of patients with childhood allergic asthma in China or overseas. In the present study, we found that the IDO levels in peripheral blood and induced

sputum were significantly lower in patients with childhood allergic asthma than in age- and sex-matched healthy children. This finding suggests that IDO activity can inhibit the airway allergic reaction and could play a protective role in the pathogenesis of childhood allergic asthma.

It has been widely reported that NO metabolism plays an important role in allergic diseases. NO might be associated with allergic airway obstruction and inflammation. Increased iNOS expression has been reported in airway epithelial cells and inflammatory cells in patients with allergic asthma. The expression of FeNO, the biomarker most widely used for airway inflammation, is significantly higher in patients with asthma than in the healthy controls.⁹ The results of the present study also showed that the FeNO levels were significantly higher in patients with childhood allergic asthma than in the controls. One study found no reason why high tryptophan concentrations occur in patients with pollen allergy during the non-pollen season; nonetheless, this phenomenon can be explained to a certain extent by the specific interaction between NO and IDO.²² NO can reversibly inhibit IDO activity by binding to activated heme sites.¹⁰ Thus, when NO synthesis increases, NO consequently inhibits IDO, eventually leading to an increase in absolute tryptophan concentration.²² The production of IDO and iNOS is induced by interferon- γ . By decreasing the production of NO, iNOS inhibitors limit NO production and thus improve IDO activity.²³ Our results showed that the IDO level was not significantly correlated with age, sex, EC in blood, or asthma severity, and the IDO level exhibited a significant negative correlation only with the FeNO level. A higher FeNO level corresponded to lower IDO activity in children. This finding also demonstrated that an interaction exists between NO and IDO in the airway of patients with childhood allergic asthma and that NO production inhibits IDO activity, thus aggravating airway allergic inflammation.

Furthermore, the present study shows that the level of IDO activity in sputum might be a biomarker with diagnostic value for children older than 5 years with allergic asthma. The use of IDO in sputum combined with FeNO could improve the diagnostic value for childhood allergic asthma. Therefore, this study has provided a strong supplement to the diagnosis of childhood allergic asthma. There were a few limitations to this study. For instance, the number of subjects enrolled in the study was relatively small owing to the

Table 4

Receiver Operating Characteristics Curve Analysis for the Value of IDO Alone, FeNO Alone, and IDO and FeNO for Classifying Children as Those With or Without Allergic Asthma

	FeNO	IDO in sputum	FeNO and IDO
Area under the curve (95% CI)	0.899 (0.822–0.976)	0.969 (0.932–1)	0.977 (0.937–1)
Youden index	0.667	0.833	0.9
Best cutoff value	33	7.28	7.59–28.5
True positive rate (sensitivity)	0.7	0.933	0.967
True negative rate (specificity)	0.967	0.9	0.933

Abbreviations: CI, confidence interval; FeNO, fractional concentration of exhaled nitric oxide; IDO, indolamine 2,3-dioxygenase.

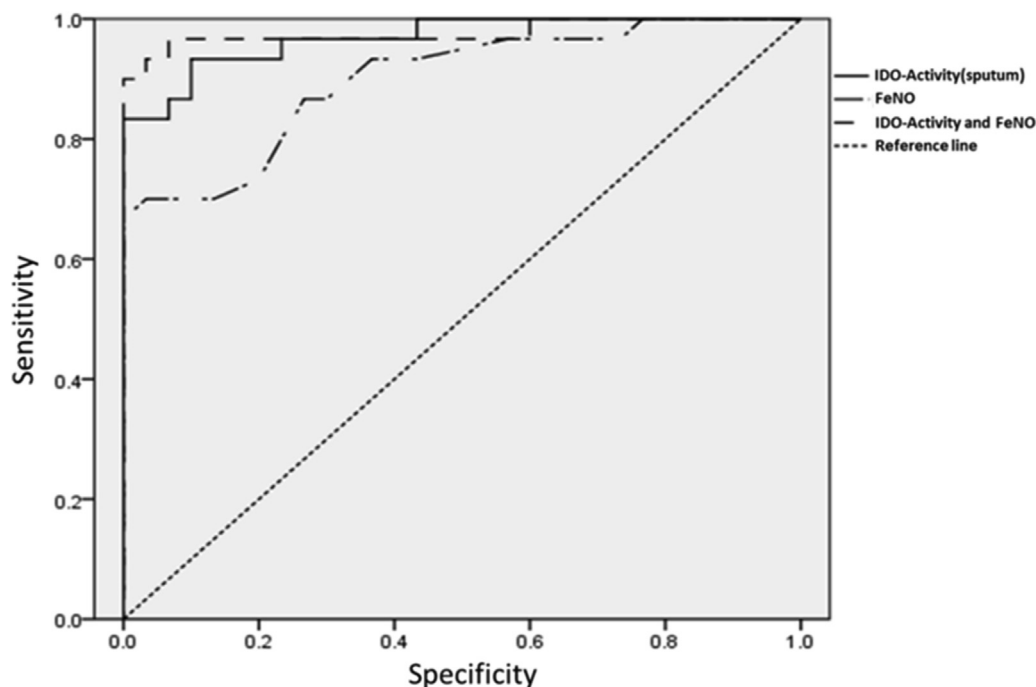


Figure 3. Receiver operating characteristics curve for the value of indoleamine 2,3-dioxygenase (IDO) alone, fractional concentration of exhaled nitric oxide (FeNO) alone, and IDO and FeNO for classifying children as those with or without allergic asthma.

difficulties in clinical specimen collection. In addition, the study focused on children older than 5 years with asthma, and research on the characteristics of preschool children with wheezing is lacking. Moreover, the investigation on the interaction mechanism between IDO and NO was not thorough in this clinical trial, and further in vitro trials are needed as a powerful supplement.

The levels of IDO activity in airway and blood were significantly lower in patients with childhood allergic asthma than in healthy children. The IDO level was not significantly correlated with age, sex, EC in blood, or asthma severity scale but had a significant negative correlation with the FeNO level. This finding suggests that IDO could play a protective role in the pathogenesis of childhood allergic asthma and have a close interaction with NO. Combined testing of IDO and FeNO can be used as a biomarker for an auxiliary diagnosis of childhood allergic asthma in clinics.

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