

# Fraction exhaled nitric oxide and IOS as potential biomarkers for HDM immunotherapy in patients with allergic rhinitis and asthma

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## Research

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# Abstract

**Background** Allergen specific immunotherapy (AIT) is the only potential disease-modifying treatment of house dust mite (HDM) allergic asthma and rhinitis. The conventional treatment procedure lasted for 3 years, but biomarkers indicating early efficacy from this long-term treatment has not been well illustrated. In this single arm, observation study, we evaluated non-invasive airway inflammation parameters such as Exhaled nitric oxide (FeNO), nasal NO levels (FnNO), and small airway function parameter-impulse oscillometry system (IOS) as well as serum parameters Dermatophagoides pteronyssinus (Derp) specific IgG4 and sIgG4/IgE ratio as potential biomarkers for AIT.

**Methods** Patients with mild allergic asthma concomitant with allergic rhinitis were treated with subcutaneous immunotherapy of Der p extract. Clinical evaluations including symptom score and medication score, small airway function of spirometer and non-invasive airway inflammation parameters from the nose (FnNO) and lung (FeNO) were collected before and after half year and one year of AIT. Serum Derp specific IgE and IgG4 measurement data were also collected.

**Results** Symptoms and medication score of patients were alleviated both after half and one-year HDM immunotherapy according to VAS (visual analogue score) and medication score. Improved small airway function (evaluated through IOS) and reduced non-invasive airway allergic inflammation parameters both from the nose (FnNO) and lung (FeNO) were achieved after half and one year AIT. As to serum biomarkers, Der p specific IgG4 increased and Der P specific IgE/IgG4 decreased significantly after one year treatment.

**Conclusions:** One year HDM subcutaneous allergen immunotherapy improved allergic symptoms and reduced medication score in patients with allergic asthma concomitant with rhinitis. Non-invasive airway inflammation parameters such as FeNO and FnNO and small airway function parameter IOS are potential biomarkers for evaluating early efficacy of AIT. Derp specific IgG4 as well as IgE/IgG4 ratio maybe good therapeutic predictors to reflect the efficacy of AIT in the first year of treatment.

## Introduction

House dust mite (HDM) allergy is a major cause of respiratory allergic diseases and often implicated as a trigger for asthma and perennial allergic rhinitis, especially in East and South Asia [1]. Allergen specific immunotherapy (AIT) provides an alternative option for treating HDM allergy that target to the underlying cause and modify the development of the disease. A treatment course of 3 years is commonly considered to achieve long-term efficacy [2–4]. The World Allergy Organization (WAO) recommends that the primary outcomes used in AIT include measurement of symptoms and the use of concomitant medications and/or a combination of the both of the measures. Currently there are no validated or generally accepted candidate biomarkers predictive of the clinical response to AIT. Most of the candidate biomarkers used in clinical trials of allergic rhinitis patients with/without asthma in the literature could not be used with routine clinical testing procedures [5]. Conventional AIT treatment lasts for 3–5 years. Feasible and

practical biomarkers reflecting the efficacy of the treatment at a relative early phase, e.g. one year, are important for both physician and patients.

Recently some simple, rapid, non-invasive parameters have been used in evaluating the severity of airway allergic inflammation, such as exhaled nitric oxide (FeNO) and nasal NO levels (FnNO) [6–9]. As to mild allergic asthma patients, the classical lung function parameters, e.g. FEV1, FEV1/FVC, are normal and difficult to change after treatment because of ceiling effect. However, small airway function evaluated by impulse oscillometry system (IOS) may showed the treatment effects. The mechanisms for AIT are still not fully elucidated, especially for the lung parameters. In this study, we investigated two non-invasive airway inflammatory parameters, i.e. FeNO and FnNO, as well as IOS as the biomarkers for evaluating the efficacy with allergic asthma patients during one year of AIT.

## Methods

### Subjects

All subjects were non-smoking adults with mild allergic asthma concomitant with allergic rhinitis. Patient were considered eligible for HDM AIT according to the following factors: diagnosed with asthma under control with standard pharmacotherapy (without exacerbation or systemic corticosteroid use within the last 6 months), HDM allergy and perennial asthma (medical history consistent with HDM allergy). Positive skin prick tests (wheal diameter  $\geq 6$  mm greater than a negative control) to the house dust mite *Dermatophagoides pteronyssinus* extract (ALK, Hørsholm, Denmark), FEV1  $\geq 80\%$  predicted value.

The study was performed with the approval by the Ruijin hospital Research Ethics Board (#2017-117). All subjects gave written informed consent.

### AIT protocol

The patients were treated with subcutaneous injection of aluminium-formulated *Dermatophagoides pteronyssinus* Alutard® SQ (; ALK, Hørsholm, Denmark). The treatment protocol followed the recommended up-dosing schedule of 5 months with 1 injection/week and 2.5 years treatment with a maintenance dose of 100,000 SQ with injection interval of 4 $\pm$ 2weeks.

### Clinical evaluations and serum allergen specific IgE and IgG4 measurement

Allergy symptoms were recorded using the visual analog scale (VAS) of 100 mm range and the patients marked on a line the point they felt represented their perceptions of their current state. Medication score was scored on a scale of 0-5 (0, not at all; 1, occasionally seldom; 2, occasionally often; 3, almost daily; 4, continuously; 5, continuously with the maximal dose) that included oral or nasal anti-histamine, leukotriene antagonist(LTRA),nasal corticosteroid spray as well as ICS or ICS/LABA treatment for asthma.

Serum samples were collected before and after one year AIT. Der p-specific IgE was determined by the Unicap 100 fluorescence enzyme immunoassay system (Phadia, Uppsala, Sweden) according to the

manufacturer's instructions. Serum Der p-specific IgE and IgG4 levels were reported as kAU/L with a cut off value of 0.35 kAU/L and upper limit of 100 kAU/L.

### **Measurement of FeNO, FnNO, IOS and pulmonary function**

FeNO levels were measured according to the guidelines of American Thoracic Society (ATS) by the single-breath method (on-line measurement) using a fast response (0.02 s) chemiluminescence analyzer (NOA 280; Sievers Instruments Inc., Boulder, CO)[10]. Before FnNO measurements, the patient should blow their nose and assure free airflow in both nostrils. Aspiration is done through one nostril with the use of a tightly fitting nasal olive with the other nostril open. The subject performs a deep inhalation and holds their breath to obtain velum closure, while air circulates from one naris to the other around the posterior nasal septum. Velum closure may also be obtained by oral expiration against a resistance of 10 cmH<sub>2</sub>O, by pursed-lip breathing via the mouth, or by voluntary elevation of the soft palate. Velum closure can be monitored by measuring nasal CO<sub>2</sub>. Measurement is done after a deep inhalation with NO-free gas, followed by slow exhalation through the nose (with the mouth closed) into a tightly fitted mask covering the nose. [9,7]

IOS measurements were obtained using a commercially available IO device (Master Screen IOS, Jaeger, Germany) according to the manufacturer's recommendations.[11] After the IOS measurements, the MasterScreen IOS-Jaeger(Germany) device was used to perform spirometry. To avoid any negative effects of forced expiration on the airway, spirometry was never performed before the IOS measurements, The percent predicted forced vital capacity(%FVC), the percent predicted forced expiratory volume in 1 s(%FEV<sub>1</sub>), the FEV<sub>1</sub>/FVC ratio, the percent predicted the maximal mid-expiratory flow(%MMEF), and the percent predicted peak expiratory flow(%PEF) were also obtained.

### **Statistical analysis**

Statistical analysis was conducted via SPSS version 20 and R version 3.6.0. Numeric variables were expressed as means±SEM. The differences between different time point were tested by repeated measurement analysis of variance. Differences between different groups were tested via one-way analysis of variance. P <0.05 was considered statistically significant.

## **Results**

Totally 22 subjects (female 59.1%) with mean age of 34.41 ± 2.13y were included in this study. The subjects suffered from well controlled or partially controlled mild allergic asthma with rhinitis (ACT score, 23.32 ± 0.22; FEV<sub>1</sub>% of predicted value, 82.10 ± 0.45%) But their airway eosinophilic inflammation were significant high according to FeNO evaluation (FeNO, 56.45 ± 4.47 ppb). Almost every subject has been treated with medication regularly (medication score, 3.23 ± 0.11; only one subject less than 3). Detailed baseline symptom, lung function and airway or serum inflammation indicators were listed in **Table 1**.

<b>Table 1. Baseline Characteristics of subjects.</b>	
Baseline characteristics	Values
Number of subjects	22
Gender	
Male	9 (40.9%)
Female	13 (59.1%)
Age (mean $\pm$ SEM, y)	34.41 $\pm$ 2.13
ACT score	23.32 $\pm$ 0.22
VAS score	62.27 $\pm$ 2.05
Medication score	3.23 $\pm$ 0.11
FEV1% of predicted value	82.10 $\pm$ 0.45
FEV1/FVC (%)	81.70 $\pm$ 0.55
PEF% of predicted value	81.81 $\pm$ 0.53
R20(Kpa*L <sup>-1</sup> *s <sup>-1</sup> )	103.05 $\pm$ 3.52
R5(Kpa*L <sup>-1</sup> *s <sup>-1</sup> )	124.82 $\pm$ 5.57
FeNO (ppb)	56.45 $\pm$ 4.47
FnNO (ppb)	688.05 $\pm$ 41.25
Der p IgE concentration (kU/L)	81.17 $\pm$ 18.93
Der p IgG4 concentration (AU/ml)	489.27 $\pm$ 183.81
IgE/IgG4	0.19 $\pm$ 0.04

Symptoms and medication score significantly declined after half year and one-year AIT treatment. The changes of asthma control test (ACT), VAS and medication score indicated a trend of symptom alleviation. VAS and medication score decreased statistical significance compared with baseline values (VAS score, 62.27  $\pm$  2.05 vs. 43.41  $\pm$  1.75 vs. 37.27  $\pm$  1.57, baseline, 6 months and 1 year respectively, both 6 months and 1 year  $P < 0.001$ ; medication score, 3.23  $\pm$  0.11 vs. 1.91  $\pm$  0.14 vs. 1.68  $\pm$  0.10, both 6 months and 1 year  $P < 0.001$ , Fig. 1).

For spirometry analysis, there were no significant change of %FEV1, %PEF and FEV1/FVC during one year AIT, showing in Fig. 2C-E. But the airway resistance significantly reduced after treatment (R5, 124.82  $\pm$  5.57 vs. 106.32  $\pm$  3.52 vs. 102.09  $\pm$  1.91 Kpa\*L<sup>-1</sup>\*s<sup>-1</sup>, both 6 months and 1 year  $P < 0.001$  compared with baseline value, and R20 with a reduction trend) (Fig. 2A-B). More importantly, as the indicator of airway allergic inflammation, FeNO and FnNO level significantly reduced after AIT (FeNO, 56.45  $\pm$  4.47 vs. 34.91

$\pm 2.49$  vs.  $21.82 \pm 1.01$  ppb; FnNO  $688.02 \pm 41.27$  vs.  $580.35 \pm 28.65$  vs.  $489.84 \pm 20.15$  ppb, both 6 months and 1 year  $P < 0.001$  compared with baseline value, Fig. 3).

Der p Specific IgG4 level significantly increased during the treatment ( $489.27 \pm 183.81$  vs.  $2330.78 \pm 1097.30$ , pre- and post-treatment,  $P < 0.01$ , Fig. 4B), while specific IgE concentration did not change after one year (Fig. 4A). The ratio of sIgE/IgG4 was reduced significantly ( $0.19 \pm 0.04$  vs.  $0.05 \pm 0.01$ , pre- and post-treatment,  $P < 0.05$ , Fig. 4C).

## Discussion

The clinical data of the current study show that HDM AIT in patients with allergic asthma concomitant with rhinitis results in a significant improvement in symptom and reduction in medication. We also found that non-invasive airway inflammation parameters FeNO and FnNO (which reflect total airway and upper airway allergic inflammation) and small airway function parameter (which represented by IOS R5) decreased after both half and one-year AIT in these mild and almost well controlled asthma patients. We saw a substantial increase of IgG4, while IgE remained unchanged, but ratio of IgE/IgG4 decreased along with the first year of immunotherapy process. The weakness of our study is lack of control group. As AIT has been a routine treatment for mild allergic asthma for more than a decade in our clinic, it is difficult to find enough patients willing to receive placebo or delayed AIT for controlled observation. We've only found 6 patients as the control group (data not shown), but the number of cases was too small for statistical analysis.

The standard AIT efficacy is the evaluation of clinical symptoms and rescue medications during natural allergen exposure, as defined by the EAACI task force following regulatory guidelines [5, 12]. For traditional clinical efficacy evaluation, the visual analog scale (VAS) is generally recommended for subjective symptom evaluation by allergic patients. The VAS can be a good means for assessing allergic patient's feelings about the severity of their disease, especially during long-term follow-up of AR [2, 3]. The traditional clinical efficacy of AIT in allergic asthma is measured using medication scores as well as lung function test. Although the medication scores were used as primary endpoints in AIT trials done both worldwide and China [12–13], the score system has not covered mild to moderate asthma routine medication, for example ICS/LABA combination or LTRA. Thus, in this study, we use the medication score system including all the medication for allergic rhinitis and asthma in order to evaluate overall treatment response for both AR and asthma. ACT (asthma control test) is widely used in evaluating asthma control status according to GINA guideline and numerous publications. But in patients with different disease severity, the improvement of ACT was more predominant in moderate asthma group when receiving AIT [14, 15]. In our study, most patients are well and partially controlled their asthma before treatment, so the subjects had a trend of symptom alleviation reflected by decreased ACT. With one year of HDM specific immunotherapy, both VAS and medication score were significantly improved, consistent with the results of most of the previous studies [13–15]. In addition, for subjects with continuous improvement (there was improvement during both the first and the second half year), most of this effect was gained

during the first half year which means early effect of AIT in these mild asthma concomitant with rhinitis patients .

Measurement of FeNO is a simple and non-invasive assessment of airway inflammation severity in asthmatic patients [16]. It has been used in epidemiological studies of allergic asthma as well as rhinitis [17], and recently it had been used as a supplemental tool for monitoring AIT treatment efficacy in asthmatic children [18]. Within the nasal region, altered nasal NO levels (FnNO) have also been described in a number of conditions, including allergic rhinitis, sinusitis, and nasal polyps [7–9]. Few studies have yet investigated the effect of AIT on exhaled nitric oxide concentration [18], although AIT with *D. pteronyssinus* and *D. farinae* extracts has been found to induce exhaled NO in asthmatic children with mite allergy [19]. However, the results from the previous studies are controversial and a clear demonstration of a reduction in exhaled NO in asthmatic patients taking subcutaneous immunotherapy (SCIT) is lacking [18, 20]. According to our data, exhaled NO levels decreased after SCIT with HDM, no matter from the airway reflected by FeNO or from the nose reflected by FnNO. It indicates that AIT is effective in reducing airway allergic inflammation. Again, most of this effect was gained during the first half year which means early effect of AIT.

Peripheral airway disease as indicated by increased frequency dependence of resistance and reactance measured by Impulse oscillometry system (IOS) which is a novel device for respiratory functional assessment especially in evaluation of lung mechanics [21]. Peripheral airway impairment detected by IOS or spirometry (i.e., forced expiratory flow between 25% and 75%) commonly occurs, and each measurement may be complementary in predicting loss of control even with normal forced expiratory volume in 1 second [22]. In recent years, IOS has been used in assessing airway resistance after bronchoprovocation [23, 24]. In small-airway obstruction situation like mild asthma, the value of both R5 (low frequencies which represents resistance from large and small airways) and R5-R20 (resistance from more peripheral airways) would elevate. In our study, R5 decreased significantly after half and one-year AIT. This may indicate R5 is a sensitive biomarker for pulmonary physiologic changes during AIT process. Besides, IOS reflects changes in the caliber of the small airways occur earlier in asthma, before the abnormalities in the larger airways characterized by spirometry [25, 26].

According to the indication of AIT, patients receiving HDM immunotherapy in our study were mild asthma patients with almost normal value of FEV1 and FEV1/FVC. It was difficult to improve these parameters after treatment because of lung function ceiling effect. As we know, for children, lung parameters like IOS were superior in identifying asthmatics than the conventional spirometry [27]. But until now as we know, no related study has been done in adult patients with allergic asthma and rhinitis. Our results indicate IOS evaluation may be another lung mechanics biomarker during adult AIT process.

Elevated serum allergen specific IgE and related allergic symptom on allergen exposure to sensitized allergy like HDM are currently the sole standard for allergy diagnosis and inclusion criteria for starting AIT [28–30]. Serum biomarkers are important indicators reflecting AIT efficacy. Levels of specific IgE (sIgE) transiently increase during treatment and followed by gradually decreasing. Specific IgG subclasses

especially antigen specific IgG4 has been fully studied during AIT [31]. A correlation between allergen sIgG4 and clinical outcomes has been reported in some studies which had used immunoblotting and Pharmacia CAP system as we did in this work [32, 33]. Longitudinal data on serum specific sIgE and sIgG4 to Der p allergen during AIT are limited in Chinese populations. In the previous study, Chinese doctors found sIgE/sIgG4 ratios for Der p 1 and Der p 2 decreased continuously from 6 through 24 months of AIT in asthma and rhinitis children [34]. Allergen specific IgE/total IgE ratio is one of the biomarkers for monitoring clinical efficacy of AIT for allergic rhinoconjunctivitis and allergic asthma[5]. But it is still controversial about the usefulness of serum t-IgE levels in the clinical diagnosis of allergy to common aeroallergens [35]. As total IgE level in Chinese adult population may influenced by more factors such as parasite exposure during younger age [36]. Thus, we combined allergen specific immunological parameter IgE and IgG4 level and use HDM IgE/IgG4 ratio to clinical outcome. It showed that HDM specific IgG4 increase as well as IgE/IgG4 decreased in the first year of AIT which may be a better biomarker to evaluate the early treatment efficacy compare to the traditional allergen specific IgE/total IgE ratio[37].

In conclusion, one-year HDM AIT improved allergic symptoms and reduced medication score in patients with allergic asthma and rhinitis. Non-invasive airway inflammation parameters such as FeNO and FnNO and small airway function parameter IOS were good parameters for evaluating early efficacy of AIT. HDM specific IgG4 and IgE/IgG4 may be good therapeutic indicator to reflect the efficacy of AIT during the first year of treatment.

## Abbreviations

AIT

allergen immunotherapy

HDM

house dust mite

FeNO

Exhaled nitric oxide

FnNO

nasal NO exhaled nitric oxide

IOS

impulse oscillometry system

Der P

Dermatophagoides pteronyssinus

FEV<sub>1</sub>

forced expired volume in 1 second

FEV<sub>1</sub>/FVC

forced expired volume in 1 second versus forced vital capacity ratio

IgE

immunoglobulin E  
IgG4  
immunoglobulin G subtype 4  
VAS  
visual analogue score  
ATS  
American Thoracic Society  
FVC  
forced vital capacity  
MMEF  
maximal mid-expiratory flow  
PEF  
peak expiratory flow  
ACT  
asthma control test  
SCIT  
subcutaneous immunotherapy  
ICS  
inhaled corticosteroid  
LABA  
long-acting  $\beta$ 2 agonist  
LTRA  
leukotriene antagonist

## **Declarations**

### **Acknowledgements**

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### **Competing Interest**

The authors declare that they have no competing interests.

### **Author's Contribution**

Wei Tang and Ranran Dai participated in the design of the study and coordinated the asthma patients.. Jun Zhou collected the clinical data. Wei Du analyses and performed the statistical analysis. Guofang Xu and Yi Tao performed the HDM immunotherapy. Ping Wang performed lung function testing. Bo Peng and Lin Sun helped to collect clinical data and draft the manuscript. All authors read and approved the final manuscript. Jun Zhou and Wei Du contributed equally to this study.

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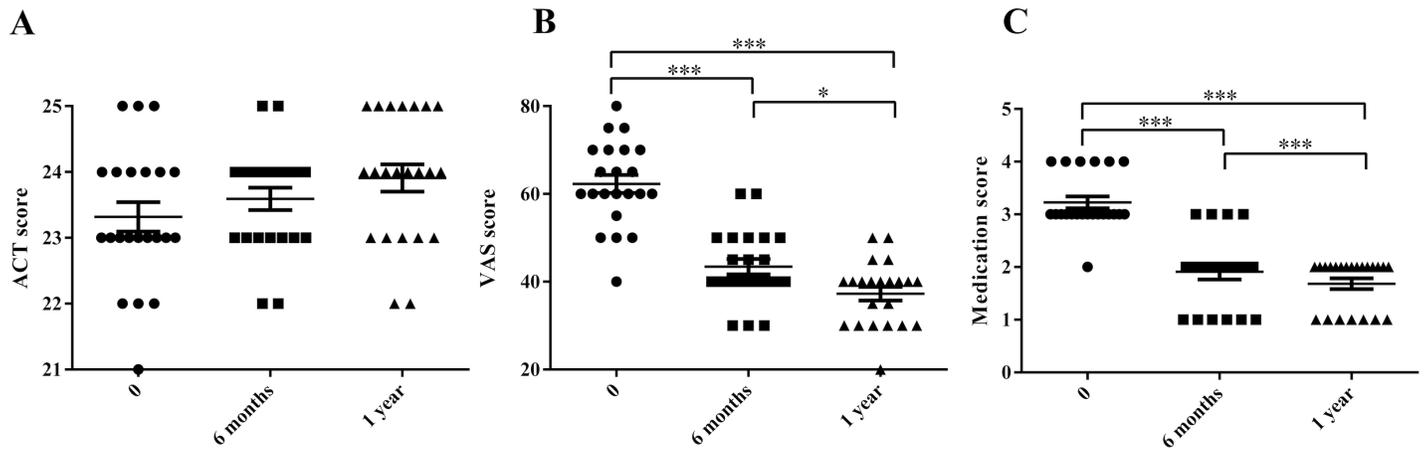
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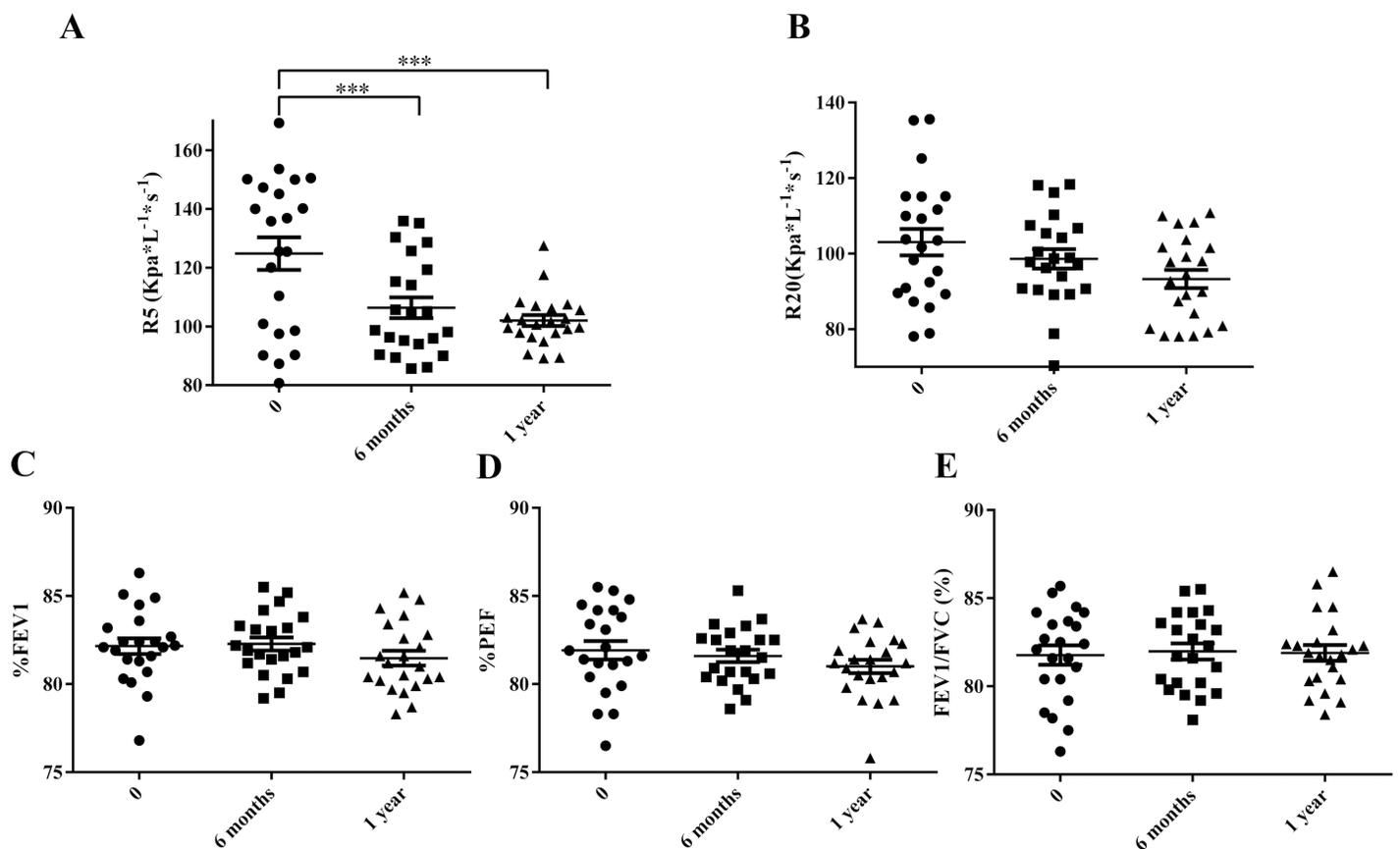
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## Figures



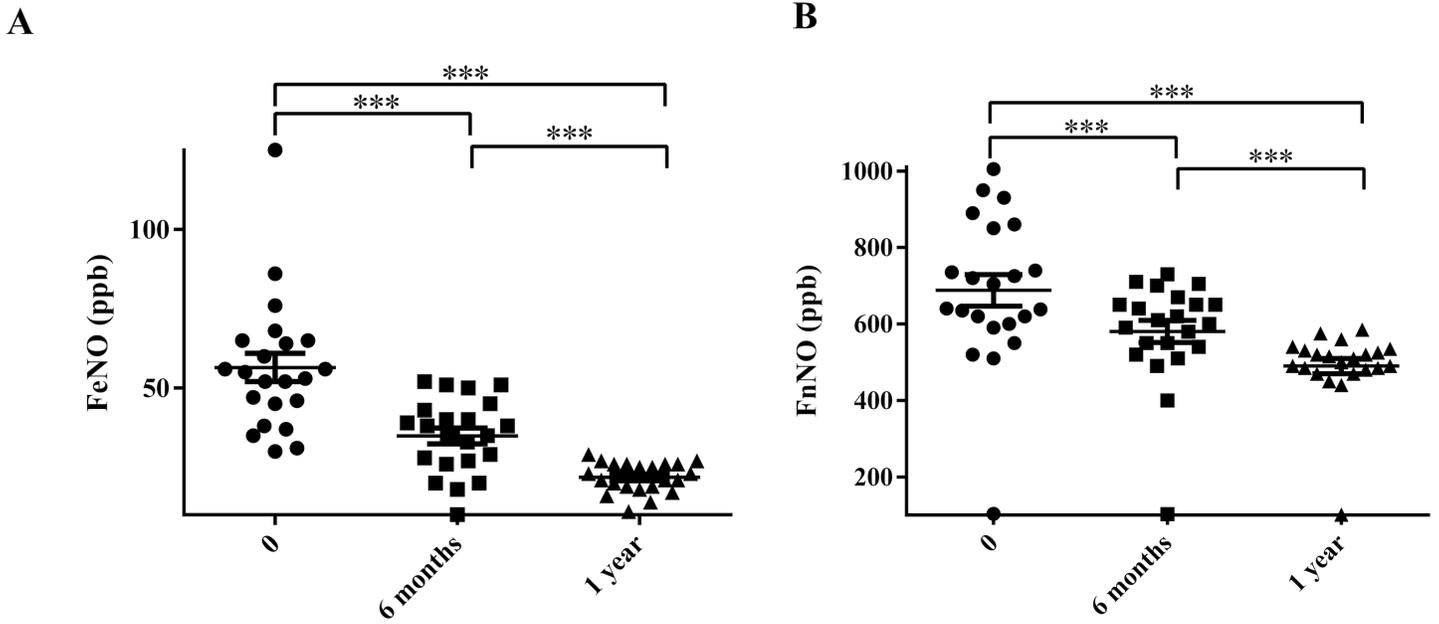
**Figure 1**

Asthma symptoms and medication use were decreased after immunotherapy. A) ACT score, B) VAS score and C) Medication score during treatment period were as shown. \*  $P < 0.05$  and \*\*\* $P < 0.001$ .



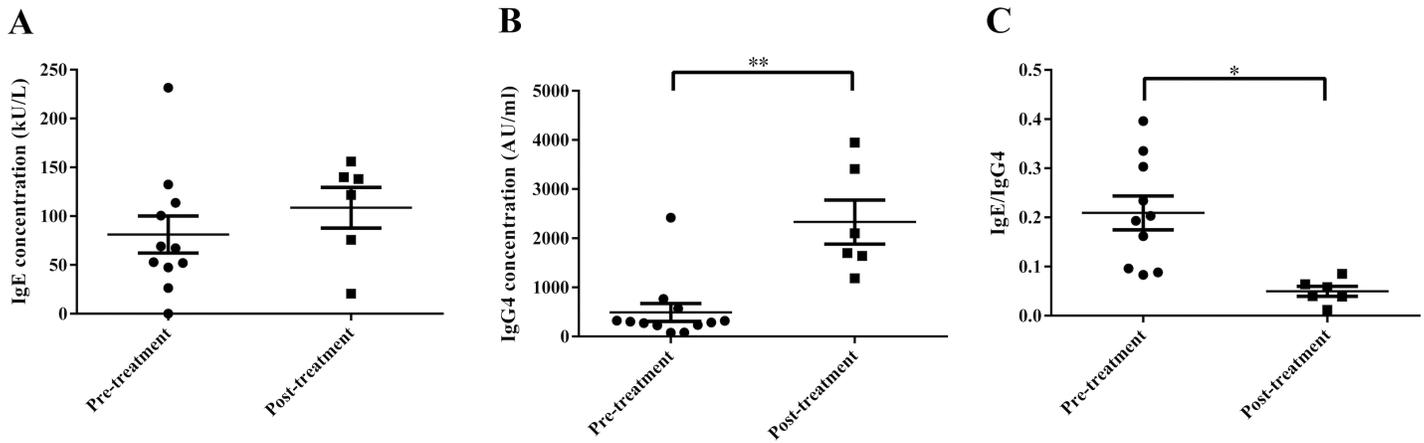
**Figure 2**

Airway resistance was reduced after immunotherapy. A) R5, B) R20, C) %FEV1, D) %PEF and E) FEV1/FVC were as shown. \*\*\* $P < 0.001$ .



**Figure 3**

A) FeNO and B) FnNO were significantly reduced after immunotherapy. \*\*\*P < 0.001.



**Figure 4**

Change of Serum biomarkers B) IgG4 and their ratio C) IgE/IgG4 were significant pre- and post-treatment while there was no significant difference for IgE. \*P < 0.05, \*\*P < 0.01.