

# Clinical Significance and Levels of NLRP3, MUC5AC, and MUC5B in Asthma

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**Abstract:** *Objective:* To explore the role of NLRP3 in mucus hypersecretion in asthmatic patients. *Methods:* From January 2020 to June 2022, 90 patients with asthma and 60 healthy patients under the Department of Pulmonary and Critical Care Medicine of the First Affiliated Hospital of Xi'an Medical University were selected. Immunohistochemistry and enzyme-linked immunosorbent assay were performed. NLRP3 inflammasome and mucins MUC5AC and MUC5B levels in lung tissue and sputum were detected. *Results:* Compared to the healthy control group, the asthma group had significantly higher sputum MUC5A ( $20.12 \pm 5.07$  versus  $36.21 \pm 6.13$ ) and NLRP3 ( $72.31 \pm 15.13$  versus  $119.21 \pm 31.21$ ) levels ( $P < 0.05$ ) but lower MUC5B levels ( $1.35 \pm 0.12$  versus  $0.53 \pm 0.11$ ,  $P < 0.05$ ). Immunohistochemistry showed that NLRP3, MUC5AC, and MUC5B expressions were consistent with the sputum results. *Conclusion:* NLRP3 and MUC5AC levels are significantly increased in asthmatic patients, whereas MUC5B levels are reduced in these patients. They can be used as targets for the diagnosis and treatment of asthma.

**Keywords:** Asthma; NLRP3; MUC5AC; MUC5B; Airway hypersecretion

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## 1. Introduction

Bronchial asthma, or asthma for short, is a common chronic airway inflammatory disease. The main pathophysiological features of asthma include chronic airway inflammation, airway hyperresponsiveness, and airway mucus hypersecretion<sup>[1,2]</sup>. The morbidity rate of asthma is on the rise globally, which brings a serious burden to both patients and health resources<sup>[3,4]</sup>. As an important part of the innate immunity of the respiratory system, airway mucus can adhere to inhaled particles and protect the body. However, excessive mucus secretion in asthmatic patients not only blocks the airway and causes airflow limitation, but also leads to poor control of asthma and increased morbidity and mortality. Current studies have revealed that the release of inflammatory mediators will aggravate the damage to the airway in asthma, leading to increased mucin secretion and the hypersecretion of mucus will cause the weakening of airway anti-inflammatory function<sup>[5,6]</sup>. The interaction between airway inflammation and airway mucus hypersecretion is the main cause of mortality in patients with severe asthma<sup>[7]</sup>.

NLRP3 inflammasome has been shown to be involved in the occurrence of asthma, highly expressed in asthmatic patients, and closely related to disease progression<sup>[8,9]</sup>. However, the effect and specific mechanism of NLRP3 on mucus hypersecretion are still unclear.

Therefore, we intended to compare the changes of NLRP3 and mucins MUC5AC and MUC5B in the sputum of asthmatic patients and normal healthy individuals to explore the relationship between the changes and the occurrence of asthma and provide a theoretical basis for targeting NLRP3 as a new treatment

strategy for asthma.

## **2. Materials and methods**

### **2.1. Clinical information**

A total of 90 asthmatic patients admitted to the Department of Pulmonary and Critical Care Medicine of the First Affiliated Hospital of Xi'an Medical University from January 2020 to June 2022 were selected. Their age ranged from 18 to 70 years, with an average age of  $45.32 \pm 3.62$  years; all of them met the diagnostic criteria in the 2016 Bronchial Asthma Guidelines for Prevention and Treatment of Asthma. In addition, 60 healthy individuals who underwent physical examination during the same period were selected as the control group, with an average age of  $41.22 \pm 4.12$  years. The gender and age of both groups were consistent with no statistical significance ( $P > 0.05$ ). This study was approved by the Hospital Ethics Committee (No. XYYFY2022LSKY-014).

Exclusion criteria: (i) patients who were uncooperative during sputum collection; (ii) patients with allergies to salbutamol and other drugs; (iii) patients with severe hypertension, diabetes, autoimmune diseases, and other diseases; (iv) patients with a history of lung surgery; (v) patients with a recent history of alcoholism.

### **2.2. Methods of collecting induced sputum**

The subjects inhaled 200  $\mu\text{g}$  of nebulized albuterol, with a flow rate of 4L/min, followed by 3% hypertonic saline for 20–30 min. Sputum was collected in sterile containers, and sputum plugs were selected for microscopic observation; samples were considered qualified if less than 5% of squamous cells and pulmonary macrophages were observed. Then, 0.1% dithiothreitol was mixed with 4 times the amount of sputum, and the mixture was placed in a constant-temperature water bath at 37°C for 15 min; thereafter, phosphate-buffered saline (PBS) was added 4 times, and the solution was placed in the water bath for another 5 min. Then, 48  $\mu\text{m}$  of diluted sputum was filtered with a nylon membrane, centrifuged at 2000 r/min for 10 min, and the supernatant was collected and frozen at -80°C until testing. An enzyme-linked immunosorbent assay (ELISA) kit was used to detect the concentration of MUC5AC, MUC5B, and NLRP3 in the sputum. According to the instructions on the kit, the absorbance at 450 nm was measured with a microplate reader (SynergyMx M5, Molecular Devices), and a standard curve was drawn based on the standard measurement results provided by the kit to calculate the content of each factor.

### **2.3. Immunohistochemistry staining**

The normal segmental bronchial stump distal to the surgical lesion was selected to avoid suspicious mass tissue. Samples were fixed in formalin, routinely dehydrated, and embedded in paraffin. The samples were cut into slices with a thickness of 5  $\mu\text{m}$ , dewaxed into water, inactivated with endogenous enzymes in 3% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) at room temperature for 5–10 min, washed with distilled water 3 times, underwent heat-induced antigen retrieval, and washed 1–2 times with PBS after cooling. 5% bovine serum albumin (BSA) blocking solution was added dropwise, and it was kept at room temperature for 20 min. The primary antibody (MUC5AC 1:100; MUC5B: 1:300; NLRP3 1:500) was added dropwise, and it was kept overnight at 4°C. Subsequently, it was washed with PBS 3 times over 2 min; the secondary antibody was added dropwise, and it was incubated at room temperature for 30 min. It was then washed again with PBS 4 times over 5 min. It was stained with DAB, and the reaction time under the microscope was controlled; distilled water was then used for washing. It was lightly counterstained with hematoxylin, dehydrated, mounted with neutral gum, observed under a microscope, and photographed. A medical image analysis system was used for image analysis, 200 $\times$  and 400 $\times$  images were selected, and 5 high-power fields of view were randomly selected for each slice to measure the average optical density.

## 2.4. Statistical analysis

SPSS 18.0 was used for statistical analysis. Measurement data were expressed as mean  $\pm$  standard deviation; t test was used for comparison between two groups, while analysis of variance was used for comparison between multiple groups; chi-square test was used to analyze the correlation between indicators by Person correlation;  $P < 0.05$  was considered statistically significant.

## 3. Result

### 3.1. Sputum NLRP3, MUC5AC, and MUC5B levels in the asthma group and the control group

The asthma group had higher sputum NLRP3 and MUC5AC levels but lower MUC5B level than the healthy control group ( $P < 0.01$ ), as shown in **Table 1**.

**Table 1.** Comparison of NLRP3, MUC5AC, and MUC5B levels between the asthma group and the healthy control group

Group	NLRP3 ( $\mu\text{g/mL}$ )	MUC5AC (g/mL)	MUC5B (g/mL)
Control group	72.31 $\pm$ 15.13	20.12 $\pm$ 5.07	1.35 $\pm$ 0.12
Asthma group	119.21 $\pm$ 31.21**	36.21 $\pm$ 6.13**	0.53 $\pm$ 0.11*
F value	56.62	20.12	12.21

\* $P < 0.05$  and \*\* $P < 0.01$ , compared with the control group. Data are given as mean  $\pm$  standard deviation.

### 3.2. Correlation analysis

The correlation analysis between NLRP3, MUC5AC, and MUC5B showed that NLRP3 was positively correlated with MUC5AC ( $r = 0.4732$ ,  $P < 0.05$ ) but negatively correlated with MUC5B ( $r = -0.3273$ ,  $P < 0.05$ ).

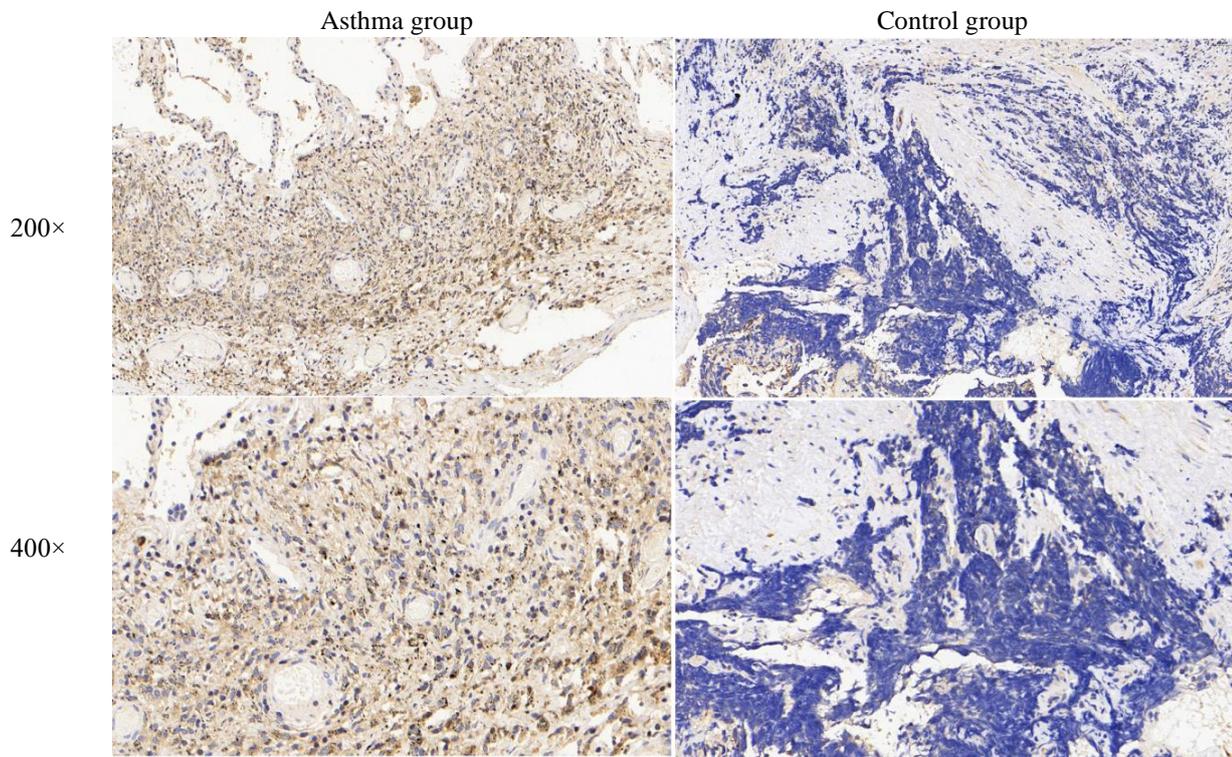
### 3.3. Immunohistochemistry results

In the control group, only a few goblet cells were observed in the ciliated columnar epithelium of the bronchial mucosa, and no hyperemia and inflammatory cell infiltration were observed in the tube wall, airway lumen, and surrounding areas. In the asthma group, goblet cells in the airway increased, with part of the lungs fused into the alveoli, and the number and size of alveoli also increased to varying degrees. Immunohistochemistry results showed that MUC5AC was mainly distributed in the cytoplasm and widely expressed in the cytoplasm of goblet cells (**Figure 1**); its expression was observed to be significantly higher in the asthma group than in the control group. The expression of MUC5B, on the other hand, was lower in the asthma group than in the control group (**Figure 2**). Compared with control group, the asthma group showed higher NLRP3 expression (**Figure 3**).

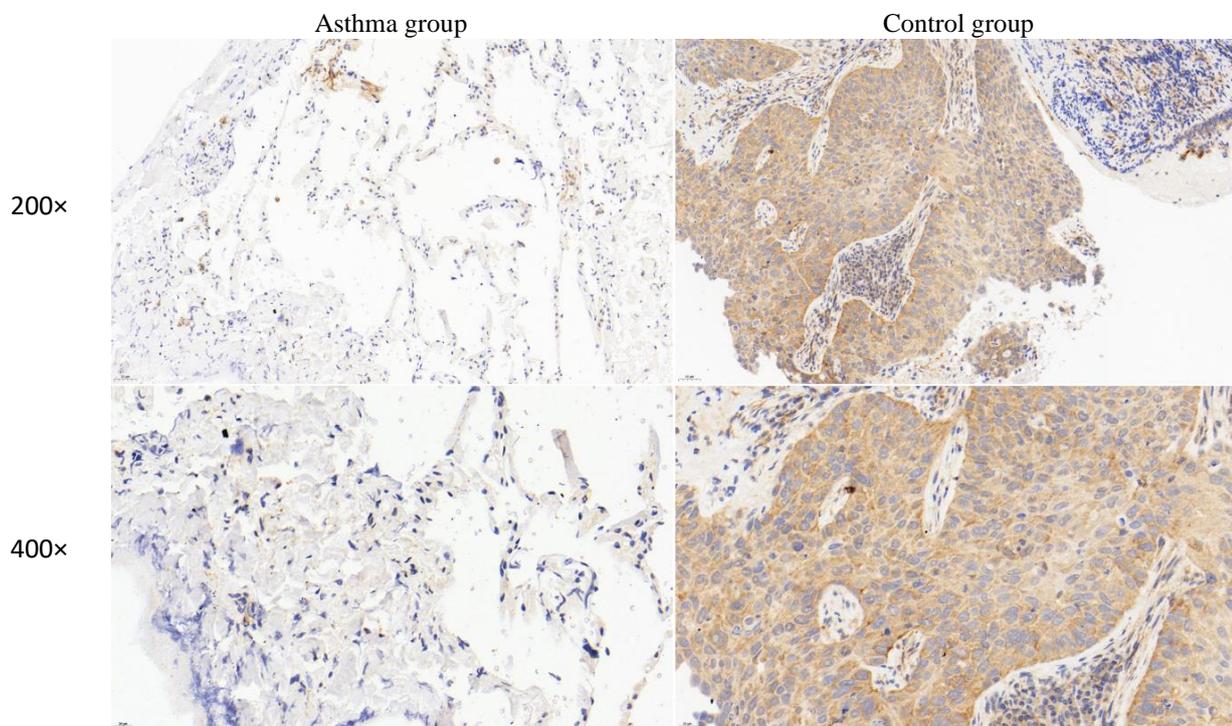
## 4. Discussion

Asthma is an airway disease characterized by airway hyperresponsiveness, inflammation, and mucin protein hypersecretion. The secretion of mucus in the airway is for cell protection, airway surface lubrication, and capture of invading microorganisms [10,11]. To date, 21 human mucin genes have been identified, 14 of which have demonstrated expression in the airway. Among these airway-associated mucins, MUC5AC and MUC5B glycoproteins are associated with gel formation. The abnormal expression of mucin genes can lead to airway obstruction [12,13]. The former is a major gel-forming protein from surface mucus or goblet cells, while the latter is a major polymeric protein secreted from submucosal glands that is also expressed in bronchiole goblet cells. MUC5AC and MUC5B have been found to play significant roles in asthma, chronic obstructive pulmonary disease, and pulmonary cystic fibrosis. In a healthy state, MUC5B

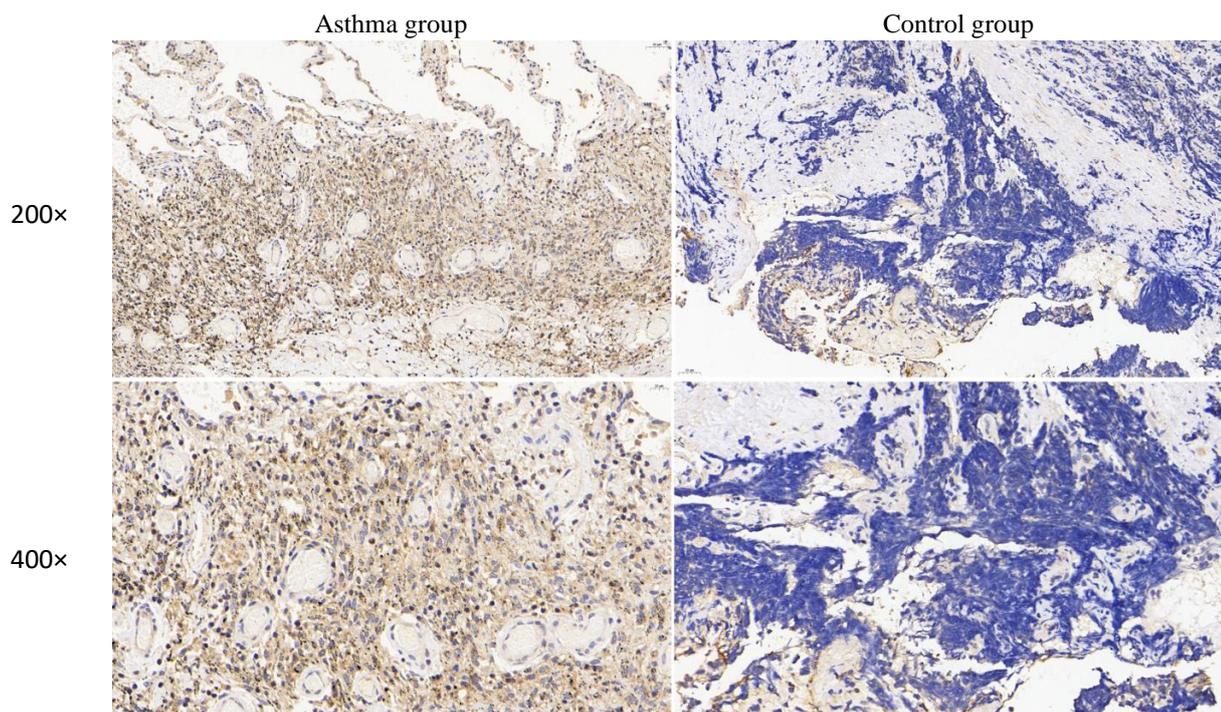
is the major gelatinous mucin in the lung and is required for mucociliary clearance, while MUC5AC is a minor mucin in healthy lungs <sup>[14,15]</sup>.



**Figure 1.** Expression of MUC5AC in the asthma and control groups



**Figure 2.** Expression of MUC5 B in the asthma and control groups



**Figure 3.** Expression of NLRP3 in the asthma and control groups

The results showed that MUC5AC in induced sputum and tissues of asthmatic patients was significantly higher than that of the control group, while MUC5B was significantly lower in asthmatic patients than in the control group. It is suggested that the abnormal expressions of MUC5AC and MUC5B play an important role in the abnormal secretion of mucus in asthma, which may be related to the role of MUC5B as a defense mechanism in the peripheral airway rather than the central airway.

In recent years, NLRP3 has been found to play an important role in the pathogenesis of asthma, participating in the process of airway inflammation and remodeling. In this study, NLRP3 in induced sputum and tissues of the asthma group was significantly higher than that of the control group. This result is consistent with other studies [16,17]. Sputum NLRP3 was found to be positively correlated with MUC5AC but negatively correlated with MUC5B, indicating that there is a correlation between inflammatory response and mucus hypersecretion.

By observing the levels of NLRP3 inflammasome and specific mucins in patients with asthma, we found that the asthmatic patients in our study showed increased NLRP3 and MUC5AC levels but decreased MUC5B levels. These components can be detected as indicators for the clinical diagnosis of asthma and the evaluation of asthmatic patients, thus providing a basis for treatment.

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### Disclosure statement

The authors declare no conflict of interest.

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