

# The Expression and Significance of $\rm CD_4{}^+T$ Lymphocyte in the Peripheral Blood of Patients with Asthma

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## Abstract

**Research Article** 

**Objective:** To detect the  $CD_4^*T$  lymphocyte proportion in the peripheral blood of the patients with bronchial asthma at different stages by flow cytometry.

**Methods:** 53 patients with asthma were recruited and divided into three groups: the acute exacerbation group of 28 cases, the mild remission group of 20 cases and the moderate to severe remission group of 5 cases. At the same time, the normal controlled group of 16 cases was set up as contrast. The proportion of Th1, Th2, Th17 and Treg in the peripheral blood of the patients with asthma and normal controlled cases were detected by flow cytometry respectively. Lung function tests were simultaneously performed in the patients with asthma groups and normal controlled group. High-resolution CT was taken in the remission and the normal group, then the ratio of 2 airway wall thickness to outer diameter (2T/D), the ratio of wall area to total airway area (WA%), lung densities in both the inspiratory and expiratory phases and the two phase difference were measured in everyone who took the HRCT.

**Results:** The acute group had a much lower Th1 and Treg proportion than the mild remission group and the normal group (P<0.05), and the remission group also had a lower proportion than the normal controlled group (P<0.05), but the Th1 and Treg proportion difference was not statistically significant between the two remission groups; The proportion of Th2 and Th17 in peripheral blood in acute group was higher than that in the mild remission group and the normal group (P<0.05), the proportion of Th2 and Th17 in peripheral blood in acute group was also higher than that in the mild remission group and the normal group (P<0.05), and that in the two remission group was also higher than that in the normal group (P<0.05), the proportion of Th2 in the acute group was higher than that in the moderate-severe remission group, but Th17 was not statistically significant between the two groups, the proportion of Th1, Th2 and the ratio of Th17, Treg was obviously significant between the acute group, the mild remission group and the normal group (P<0.05), whereas the ratio of Th17 and Treg between any two groups was statistically significant (all P<0.05), whereas the ratio of Th1 and Th2 was not significant between the mild remission group and the moderate to severe remission group, but it was also significant among the other two groups; 2T/D, WA%, expiratory phase CT values and the different CT values between inspiratory phase and expiratory phase Wer statistically significant among the two remission and the normal groups (P<0.05), but inspiratory phase CT value in the two remission and the normal groups (P<0.05), but inspiratory phase CT value in the two remission and the normal groups (P<0.05), but inspiratory phase CT value in the two remission and the normal groups (P<0.05), but inspiratory phase CT value in the two remission and the normal groups (P<0.05), but inspiratory phase CT value in the two remission and the normal groups (P<0.05), but inspiratory phase CT value in the two remission and the normal groups (P<0.05),

**Conclusions:** There were  $CD_4^{+}$  T lymphocyte immunological function disorders in peripheral blood of patients with acute exacerbation and remission of asthma, of which Th2 and Th17 had an enhanced immune response phenomenon, with an obviously enhanced expression for Th17 cells in moderate-severe remission asthma patients, However, Th1 and Treg cells with a protective effect had a lower functioning. Therefore there existed not only Th1/ Th2 imbalance but also Th17/Treg imbalance in peripheral blood of asthma; airway wall thickness pathological changing phenomenon and gas retention phenomenon existed in asthmatic patients too; the diffusion capacity of patients with bronchial asthma and the normal controlled group did not be obviously different.

**Keywords:** Bronchial asthma; CD<sub>4</sub><sup>+</sup>T lymphocyte; Flow cytometry; HRCT; Lung function

### Introduction

Bronchial asthma is a chronic inflammatory disorder of the airways which are involved with eosinophils cells (EOS), neutrophils, mast cells, T lymphocytes, airway epithelial cells, other cells and cytokines. Airway inflammation of asthma is an inflammatory response with EOS and Th2 cytokine predominantly high expressed. Experimental and clinical studies have confirmed that Th1/Th2 imbalance is involved in airway inflammation response. However, for some refractory asthma patients and early asthma patients who have the significant airway structural changes, Th1/Th2 imbalance cannot fully explain the clinical characteristics and phenotype. Especially in some non-EOS based refractory asthma patients, the phenotype of airway inflammation is neutrophils or smooth muscle cell proliferation based less inflammatory cells of which Th17 cells play an important role. Th17 cells are a new type of  $CD_4^+T$  lymphocytes discovered recently, both experimental and clinical studies found that Th17 cells and airway neutrophilic

inflammation mediated by Th17 cells are closely related to asthma severity [1]. In recent years, some researchers have suggested that Th17/ Treg imbalance plays a key role in the occurrence and development of asthma, particularly in patients with refractory or special inflammation phenotype [2-6]. This study detected the expression of Th1, Th2, Th17, Treg of asthma patients in the acute, paracmastic phase and the normal group respectively, and moreover, explored the changes of lung function

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and airway wall structure by high-resolution CT. This study aims to clarify that how Th1/Th2 imbalance and Th17/Treg imbalance play a role in the pathogenesis of asthma patients, especially in the patients with airway structural changes, and also aims to tell the phenotypic characteristics of Th1, Th2, Th17 and Treg in the peripheral blood of different types of asthma patients.

### Patients and Methods

## Patients

The asthma group: The asthma patients were selected from respiratory Department of the First Hospital of Shanxi Medical University, in line with "bronchial asthma prevention and treatment guidelines" [7] developed by the Chinese Medical Association in 2008. These patients were older than 18 years old, they did not smoke and did not have other chronic respiratory disorders and other diseases which affected the airway structure. Patients with chronic inflammatory diseases should also be ruled out. Selected patients were divided into acute stage (n=28) and remission stage, the remission group was divided into mild remission (n=20) and moderate to severe remission (n=5) groups by symptom assessment and pulmonary function test results.

**The normal contrast group:** The patients of this group were healthy by physical examination. All of them didn't have a history of allergic disease and family history, and they didn't take drugs such as hormones and immunomodulatory drugs in the past three months (n=16).

The four groups above have no significant differences in age distribution, gender ratio and disease history.

**Laboratory equipment and reagents:** 1), Equipment Flow cytometry (U.S. BD Biosciences), 64-slice spiral CT (GE's), ME511L PACS system (Japan TOTOKU Company), Vmax229 pulmonary function analyzer (U.S. Sen Disi company), auto trim levels centrifuge, -70° refrigerator (Japan SANYO).

### Reagents

U.S.BD Biosciences LEUKO ACTIVE CKTL CD4 monoclonal antibody (FITC-labeled), CD25 mAb (PerCP-Cy5.5 mark) CYTOFIX/ CYTOPERM BUF KIT, HUMAN FOXP3 BUF SET IL-4 antibody (PE marker), IFN-r antibody (PerCP-Cy5.5 marker), IL-17 antibody (Alexa Fluor 647 labeled), Foxp3 antibodies (PE labeled) corresponding to the various antibody isotype control; lymphocyte separation medium, RPMI-1640 complete medium, PBS solution.

### Methods

# Analysis of the ${\rm CD}_4^{\ +}{\rm T}$ lymphocyte subpopulations by flow cytometry

• Cell preparation and activation: Fasting peripheral blood of 5 mL was collected in sterile heparinized blood collection tube. PBS solution was diluted with an equal proportion, then the blood and PBS solution were mixed; 3 mL lymphocyte separation medium was added to a new 15 mL centrifuge tube, the diluted whole blood was added slowly to the liquid surface of the lymphocyte separation medium, centrifuged 2000 rpm for 20 minutes; The white lymphocyte layer was suctioned to another centrifuge tube, then 5 mL PBS liquid was added, 1500 rpm centrifuged for 5 minutes, the supernatant was discarded; Then 5 mL RPMI-1640 complete medium was added to resuspend the cells, 1500 rpm for 5 minutes, the supernatant was throwed away. Then the cells were resuspended with RPMI complete medium and the cell concentration was adjusted to  $1 \times 10^7/mL$ , the cell suspension was

transferred to a sterile 12-well plates, a cell stimulating agent (including PMA and ionomycin, monensin) 2 uL/mL was added into it, mixed. 12-well plates were trained at 5%  $\rm CO_2$ , 37°C incubator for 4-6 hours, to the time after the cultured cells were harvested , centrifuged at 1000 rpm for 5 minutes, the supernatant was removed, then the cells were suspended with 2 mL PBS, centrifuged 1500 rpm for 5 minutes , and the supernatant was discarded .

• Cell surface staining:  $2 \times 10^6$  cells were put into per tube, then 20 uL CD4 labeled antibody was added into each tube, then 5 uL CD25 labeled antibody was added into the Treg tube, reacting in the dark at room temperature for 20 minutes; then 2 mL PBS liquid were added into each tube, 1000 rpm centrifuged for 5 minutes, the supernatant was throwed away.

· Cells fixed and rupture

1. Th1, Th2, Th17, 500 uL 4°C for precooling fixative (Fixation) was added to the corresponding test tube, mixing, reacting in the dark at room temperature for 20 minutes, after adding 2 mL1×Perm/Wash lotion, 1500 rpm centrifuged for 5 minutes, the supernatant was added broken film formers 2 mL 1×(Perm/Wash solution) to resuspend cells, incubated in the dark place at room temperature after 15 minutes, 1500 rpm centrifuged for 5 minutes, the supernatant was throwed away.

2. Treg 2 mL 1×Human Foxp3 Buffer A was added to the test tube, mixed, incubated in the dark at room temperature for 10 minutes, centrifuged at 1000 rpm for 5 minutes, the supernatant was throwed away, and then 2 mL PBS lotion was added, mixed, 1000 rpm centrifuged for 5 minutes, the supernatant was throwed away, and then 0.5 mL 1×Human of Foxp3 Buffer C was added to each tube, mixed uniformly, incubated in the dark at room temperature for 20 minutes, 2 ml PBS washings was added to each tube, 1000 rpm centrifuged for 5 minutes, the supernatant was discarded.

3. Intracellular staining: 5 uL IFN-r antibody, 5 uL IL-4 antibody, 20 uL IL-17 antibody and 20 uL Foxp3 antibody were added to each tube of Th1,Th2, Th17 and Treg respectively. The corresponding isotype control antibody were also added to isotype control tubes, mixed in the dark at room temperature for 30 minutes, added 2 ml PBS washings was added, centrifuged at 1000 rpm for 5 minutes, 150 uL Buffer was added to the supernatant to resuspend cells.

4. Acquisition and analysis by flow cytometry

High-resolution CT scan: The remission asthma patients and the healthy group were took the chest HRCT inspection. The imagines were viewed at the end of expiratory breath holding and inspiration breath holding respectively, thickness 0.625 mm, layer interval of 1 mm continuous scanning. Scan condition was 200 mA, 140 kV, matrix 512×512 bone algorithm reconstruction. Measurements were conducted by two experienced thoracic radiologists who didn't know the patients' disease working independently in PACS system on a window width of 1500 Hu, window level of-450 Hu. Five sections were obtained: top of the aortic arch, main carina, 1 cm below the main carina, level of the pulmonary veins, and 2 cm above the right hemidiaphragm. The bronchus of more than 1 mm in diameter clearly seen in cross section was measured at the above level. The imagines were viewed on a work station using a magnification of an uniform ratio, and measurements of overall (D) and internal (L) diameter of the bronchi were made using electronic calipers, with wall thickness (T) being derived from these measurements(T=(D-L)/2). Airway wall thickness ratio of the outer diameter of the airway 2 times 2T/D, accounting for the percentage of the total cross-sectional area of the airway wall area WA% {WA%=[ $\pi$ 

(D/2)  $2-\pi$  (L/2)  $2]/\pi$  (D/2)  $2\times100$ }. And select the aortic arch, 1cm above the main carina, 2cm above the right hemidiaphragm 3 levels respectively as representatives of the upper, middle and lower of the lung, select 50 mm<sup>2</sup> region of interest (ROI) at each image before and after the subpleural 5 mm, measured CT values (as much as possible to make the ROI avoid blood vessels and bronchial). Left and right lungs 6 CT values of the ROI, then obtained the average CT value at the end of the whole lung inspiratory, and expiratory.

**Pulmonary function tests:** Acute exacerbation of asthma patients in stable condition and the other inductees routine were took pulmonary function tests, relaxation experiments when necessary, measured comprehensive to reflect pulmonary flow volume indicators FEV<sub>1</sub>, FEV<sub>1</sub>% predicted, FVC, FVC% predicted, FEV<sub>1</sub>/FVC% FEF25%, FEF50%, FEF75%, FEF25-75%, etc. and reflect pulmonary diffusing capacity indicators DLCO.

### Statistical analysis

All data were analyzed by SPSS17.0 software, the data were tested by normality and homogeneity of variance. The data fitting both normal distribution and homogeneity of variance adopted single-factor analysis of variance (F test). The comparations among Groups were adopted the LSD-T test ( $\alpha$ <0.05 level). The description of the experimental results were  $\overline{x} \pm s$ ; While the Kruskal-Wallis (H test) was adopted for unequal variances, and the pairwise comparisons used the Wilcoxon test ( $\alpha$ <0.05 level of inspection) results with a median (interquartile range).

### **Results and Discussion**

### Results

### Flow cytometry results:

1. The expression of Th1 cells of Each group in peripheral blood : the proportion of CD<sub>4</sub><sup>+</sup> IFN- $\gamma^+$  (Th1 cells) of patients with acute exacerbation of asthma was 3.10 ± 0.34%, lower than the mild remission group (5.74 ± 0.37%), the moderate-severe remission group (4.91 ± 0.72%) and the healthy group (8.33 ± 0.09%). Furthermore, the proportion of CD<sub>4</sub><sup>+</sup> IFN- $\gamma^+$  (Th1 cells) in the mild group and the moderate-severe remission group were lower than that of the healthy group. There were significant differences between these groups (P<0.05); but the expression of Th1 cells in mild group and that of moderate-severe remission group was not statistically significant.

2. The expression of Th2 cells of Each group in peripheral blood: the proportion of  $CD_4^+$  IL-4<sup>+</sup> (Th2 cells) of patients with acute exacerbation of asthma in peripheral blood was 44.81 ± 0.35 %, higher than the mild remission (33.74 ± 1.08%), the moderate-severe remission group(32.90 ± 1.27%) and the healthy group (21.74 ± 1.61%). Meanwhile, the proportion of Th2 cells in the mild group and in moderate-severe remission group was higher than that of the healthy group. There were significant differences among these groups (P<0.05); but the expression group was not statistically significant.

3. The expression of Th17 cells of Each group in peripheral blood: the proportion of  $CD_4^{+}IL-17^+$  (Th17 cells) in peripheral blood of patients with acute exacerbation of asthma was  $3.20 \pm 0.14\%$ , higher than that in the mild remission period ( $1.81 \pm 0.14\%$ ) and the healthy group ( $1.18 \pm 0.12\%$ ), the proportion of Th17 cells of the moderatesevere mitigation phase group was  $3.09 \pm 0.09\%$ , also higher than that in the mild group and the healthy group, and that in the mild remission phase was also higher than the healthy group, the comparisons among these groups were significant (all P<0.05); but the expression of Th17 cells in the acute stage and in moderate-severe remission asthma group was not statistically significant .

4. The expression of Treg cells of Each group in peripheral blood: the proportion of  $CD_4^+ CD_{25}^+$  Foxp3<sup>+</sup> (Treg cells) in peripheral blood of the acute exacerbation of asthma was  $2.57 \pm 0.48\%$ , lower than that in the mild remain group ( $6.80 \pm 0.39\%$ ), the moderate-severe remission group ( $5.95 \pm 0.80\%$ ) and the healthy group ( $8.70 \pm 0.61\%$ ), while that in the mild group and the moderate-severe remission group was lower than that in the healthy group, any of the above comparison between the two groups were P<0.05; but the expression of Treg cells in mild group and that in moderate-severe remission group was not statistically significant.

5. The ratio of Th1/Th2, Th17/Treg of each group: the ratio of Th1/Th2, Th17/Treg of the acute exacerbation, the mild and the moderatesevere remission was statistically significant with the healthy group (P<0.05), and the ratio of Th17/Treg between any two groups was statistically significant (all P<0.05), while the ratio of Th1/Th2 in mild group and the moderate-severe remission group was no statistically significant. All of the remaining two groups were also statistically significant (all P<0.05).

The above results can be seen in tables 1 and 2; Flow cytometry results shown in figures 1-4.

The results of HRCT: Table 3 is the HRCT results, as shown in this table, the airway wall thickness and 2 times of airway lumen diameter ratio (2T/D) of the moderate-severe remission period group (0.414  $\pm$  0.03) and the mild remission group (0.403  $\pm$  0.01) were higher than that of the healthy group (0.352  $\pm$  0.01) (P<0.05), there was no statistical significance between the moderate-severe remission period group and the mild remission group; airway wall area accounts for a percentage of the total cross-sectional area of the airway (WA%) of the moderate-severe remission period group (0.648  $\pm$  0.01) were higher than that of the healthy group (0.593  $\pm$  0.02)

Group	n	Th1 (%)	Th2 (%)	Th1/Th2*
The acute	28	$3.10 \pm 0.27^{abc}$	44.81 ± 1.49 <sup>abc</sup>	0.071 (0.087-0.051) <sup>abc</sup>
The mild remission	20	5.74 ± 0.37 <sup>e</sup>	33.74 ± 1.08 <sup>e</sup>	0.167 (0.218-0.145) <sup>e</sup>
The moderate-severe	5	4.91 ± 0.72 <sup>f</sup>	32.90 ± 1.27 <sup>f</sup>	0.119 (0.217-0.104) <sup>f</sup>
The healthy	16	8.33 ± 0.41	21.75 ± 1.61	0.399 (0.483-0.270)
F/H		39.081	43.090	51.933
Р		0.000	0.000	0.000

**Note:** Compared with the mild remission group, <sup>a</sup>P<0.05; compared with the moderate-severe remission group, <sup>b</sup>P<0.05; compared with the healthy group, <sup>c</sup>P<0.05; compared with the moderate-severe remission group, <sup>d</sup>P<0.05; compared with the healthy group <sup>c</sup>P<0.05; compared with the healthy group; <sup>f</sup>P<0.05; \*This set of data used non-parametric tests

**Table 1:** The expression of Th1, Th2 ( $\overline{x} \pm s$ ) and the ratio of Th1/Th2 (median).

Group	n	Th17 (%)	Treg (%)	Th17/Treg*
The acute	28	$3.20 \pm 0.14^{ac}$	$2.57 \pm 0.31^{abc}$	1.372 (2.728-0.793) <sup>abc</sup>
The mild remission	20	1.81 ± 0.14 <sup>de</sup>	$6.80 \pm 0.39^{e}$	0.282 (0.355-0.181) <sup>de</sup>
The moderate-severe	5	$3.09 \pm 0.09^{f}$	5.95 ± 0.80 <sup>f</sup>	0.487 (0.765-0.403) <sup>f</sup>
The healthy	16	1.18 ± 0.12	8.70 ± 0.61	0.116 (0.199-0.081)
F/H		41.493	40.682	53.968
Р		0.000	0.000	0.000

**Note:** Compared with the mild remission group, <sup>a</sup>P<0.05; compared with the moderate-severe remission group, <sup>b</sup>P<0.05; compared with the healthy group, <sup>c</sup>P<0.05; compared with the moderate-severe remission group, <sup>d</sup>P<0.05; compared with the healthy group, <sup>e</sup>P<0.05; compared with the healthy group, <sup>f</sup>P<0.05; \*This set of data used non-parametric tests

**Table 2:** The expression of Th17, Treg ( $\overline{x} \pm s$ ) and the ratio of Th17/Treg (median).

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(all P<0.05), there was no statistical significance between the moderatesevere remission period group and the mild remission group; the expiratory CT value of the mild remission group (818.3 ± 11.0 HU) and the moderate-severe remission group (870.8 ± 16.3 HU) was higher than that of the healthy group (777.9 ± 12.1 HU) (P<0.05), while there was no statistical significance between the moderate-severe remission period group and the mild remission group; the differences of the CT values between expiratory and inspiratory in the mild group (32.0 ± 3.6 HU) and the moderate-severe remission group (18.9 ± 3.5 HU) were higher than that in the healthy group (105.8 ± 11.1 HU) (P<0.05), while there was no statistical significance between the moderate-severe remission period group and the mild remission group; the inspiratory CT value of the three groups was not statistically significant. The results of pulmonary function: Table 4 is the results of pulmonary function, as shown in this table, the FVC/predicted value (%) and FEV<sub>1</sub>/predicted value (%) of the healthy group and the mild remission group were higher than that of the acute exacerbation group, while the two values in the mild remission group were also higher than that in the moderate-severe remission group and the healthy group, FEV<sub>1</sub>/predicted value (%) in the healthy group was higher than that in the moderate-severe remission group, all P<0.05; FEV<sub>1</sub>/FVC (%) was statistically significant among groups other than the acute attack phase group with the mild remission group (P<0.05); the DLCO was not statistically significant among the groups (P=0.061).

### Discussion

The pathogenesis of bronchial asthma is complex, while immune and inflammatory mechanism of CD<sub>4</sub><sup>+</sup>T lymphocyte has reached a consensus. The theory of Th1/Th2 imbalance in the past few decades has been considered to be the core of the pathogenesis of asthma; allergens in patients with asthma can selectively promote Th1 cell proliferation predominant expression of Th2 cells, as a consequence, the Th1/Th2 balance to Th2 conversion [8], which means that Th2 cells have a polarization response. The results of this study confirmed that there existed Th1/Th2 imbalance and the high expression of Th2 cells which have a role of pro-inflammatory in the peripheral blood of asthma patients, and its expression of the acute group was higher in a serious condition, while Th2 expression in acute asthma was also higher than that in moderate-severe remission group, but that in mild group and the moderate-severe remission group was not statistically significant. The expression of Th1 cells which have a role of cell-mediated immunity in the peripheral blood of asthma patients was low, while its expression in the acute phase group was also lower than that in the remission group, but that in the mild group and the moderate-severe remission group was not statistically significant. This result suggested that the expression of Th1, Th2 and Th1/Th2 imbalance in the peripheral blood might be related to the disease severity in patients with bronchial asthma, but the expression of the two values in the mild remission group and the moderate-severe remission group was not statistically significant. However, the treatment effect of the targeted therapy by other animals and clinical studies against the Th1/Th2 imbalance cytokines is not ideal. In recent years, some studies have found that Th1 cells are proinflammatory, and its role of proinflammatory outstrips the anti-inflammatory. In other animal studies, adoptive Th1 cell transfusion has not only failed to reduce the Th2 cell-induced airway hyper responsiveness and inflammation, it can

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cause serious airway inflammation [9] as well. Therefore, the Th1/Th2 imbalance theory does not fully explain the pathogenesis of asthma.

Meanwhile, the experiment also suggested there was another imbalance of  $CD_4^{+T}$  lymphocytes in the peripheral blood of bronchial asthma, the Th17/Treg imbalance, which means that the expression of Th17 cells which have a role of pro-inflammatory in the peripheral blood of asthma patients was high, and its expression in the acute group was higher than that in the mild remission group, and its expression in moderate-severe remission group was also higher than that in the mild remission group and the moderate-severe remission group was not statistically significant. Whereas, the expression of Treg cells in the peripheral blood of patients with asthma which have a role of immunosuppression and immune tolerance was

Group	n	2T/D	WA%	Inspiratory phase CT value (HU)	Exspiratory phase CT value (HU)	Different CT values (HU)
The mild remission	20	0.403 ± 0.01 <sup>b</sup>	0.648 ± 0.01 <sup>b</sup>	850.3 ± 11.0	818.3 ± 11.0 <sup>b</sup>	32.0 ± 3.6 <sup>b</sup>
The moderate- severe	5	0.414 ± 0.03°	0.665 ± 0.03°	889.6 ± 17.0	870.8 ± 16.3°	18.9 ± 3.5°
The healthy	9	0.352 ± 0.01	$0.593 \pm 0.02$	$883.7 \pm 4.7$	777.9 ± 12.1	105.8 ± 11.1
F		5.196	4.654	3.045	7.033	43.920
Р		0.011	0.017	0.062	0.003	0.000

**Note:** Compared with the moderate-severe remission group, <sup>a</sup>P<0.05; compared with the healthy group, <sup>b</sup>P<0.05; compared with the healthy group <sup>c</sup>P<0.05

**Table 3:** The results of HRCT ( $\overline{x} \pm s$ ).

Group	n	FVC/predicted (%)	FEV1/predicted (%)	FEV1/FVC (%)	DLCO (mL.mmHg <sup>-1</sup> . min <sup>-1</sup> )
The acute	14	87.1 ± 5.8 <sup>ac</sup>	$69.3 \pm 6.2^{ac}$	66.1 ± 3.5 <sup>bc</sup>	
The mild remission	20	113 ± 4.3 <sup>d</sup>	98.5 ± 3.7 <sup>d</sup>	73.0 ± 2.3 <sup>de</sup>	97.4 ± 4.8
The moderate- severe	5	92.6 ± 7.6	59.0 ± 5.5 <sup>f</sup>	51.8 ± 3.5 <sup>f</sup>	96.4 ± 12.4
The healthy	9	109.1 ± 3.6	109.6 ± 3.3	86.4 ± 1.8	118.0 ± 6.0
F		6.118	16.700	13.988	3.062
Р		0.001	0.000	0.000	0.061

**Note:** Compared with the mild remission group, <sup>a</sup>P<0.05; compared with the moderate-severe remission group, <sup>b</sup>P<0.05; compared with the healthy group, <sup>c</sup>P<0.05; compared with the moderate-severe remission group, <sup>d</sup>P<0.05; compared with the healthy group, <sup>e</sup>P<0.05; compared with the healthy group, <sup>f</sup>P<0.05; compared with

**Table 4:** The results of pulmonary function ( $\overline{x} \pm s$ ).

low, its expression in the acute group was lower than that in the two remission groups, but that in mild group and the moderate-severe remission group was not statistically significant. This phenomenon also speculated that the expression of Th17, Treg and Th17/Treg imbalance in the peripheral blood was related to the disease severity and symptom control. The findings of the topics confirmed that there existed  $CD_4$  'T lymphocyte immune dysfunction in the peripheral blood of patients with asthma. Not only did the Th1/Th2 imbalance exist, there was Th17/Treg imbalance as well. The severity of asthma is closely related to them. At the same time, the study found that the expression of Th17 cells was not statistically significant between the acute attack phase group and the moderate-severe remission group, while its expression in the moderate-severe remission group was higher than that in the

mild remission group. This finding indicated that Th17 cells in asthma patients was more closely related to the severity of the disease and symptom control.

Airway eosinophil and neutrophil infiltration is one of the characteristics of asthma, their presence or absence and their extent and strength may be associated with the patients' clinical manifestations and treatment outcome. It is generally believed that patients with asthma which is characterized with eosinophil infiltration respond well to hormone therapy, treatment with inhaled corticosteroids can inhibit eosinophil inflammation, improving both lung function and symptom scores. However, neutrophilic asthma patients have poor response to steroid therapy. A number of studies have shown that Th17 cells are closely related to respiratory neutrophils inflammation [10-11]. Neutrophilic inflammation and severe asthma is positively correlated. Wang et al. [12] and other researchers have shown that IL-17 involved in airway remodeling in bronchial asthma. Therefore, clinical treatment targeting Th17 cells could be more meaningful.

HRCT is currently the most objective and accurate measurement of airway wall thickness by means of CT imaging, because of its spatial resolution and noninvasive, it is widely used in assessment of airway remodeling in bronchial asthma. Wu et al. [13] proposed that the severity of asthma is related to airway wall thickness and lung density difference between inspiration and expiration. The results of this study indicated that 2T/D, WA% and expiratory CT values in the two remission group were higher than the healthy group, and the difference of CT values in the two remission group was lower than the healthy group, but the above four indicators in the mild remission group and in the moderate-severe remission period were not statistically significant. The inspiratory CT value was no significant difference among the three groups. These results suggest that there are different degrees of airway wall thickening and gas retention in the asthma patients. Asthma is a disease characterized by expiratory flow limitation, which may be the reason that inspiratory phase CT value among the three groups was not significant difference. Airway wall thickening is related to airway remodeling, so by HRCT measuring airway wall thickness can provide valuable information for the evaluation of airway remodeling in patients with asthma, while it can also provide the basis for early clinical interventions. In this study, the affection factors of acute exacerbation of bronchial asthma patients such as acute airway wall edema, bronchospasm, mucus embolism, airway secretions and other reversible factors affecting the airway wall thickness can lead to overestimate their airway wall thickness disease, so that HRCT was not performed. In addition, the some enrollers had poor compliance, therefore, the experimental results failed to do correlation analysis with the other related measuring.

Pulmonary function test is an important function testing means to evaluate respiratory physiology function of patients with asthma. The results of this study showed that FVC% expected value and FEV<sub>1</sub>% predicted values in the healthy group and the mild remission group were both higher than those in the acute exacerbation group, and those in the mild remission group were also higher than those in the moderate-severe group. FEV<sub>1</sub>% predicted value in the healthy group was higher than that in the moderate-severe remission group; FEV<sub>1</sub>/ FVC in each two groups other than the acute attack group and the mild remission group was statistically significant; DLCO had no significant difference among the three groups. Acute patients were hospitalized, they could only do bedside lung function, but the bedside lung function machine in our hospital cannot detect DLCO. So the acute patients in the study didn't do DLCO. The results indicated asthma patients in terms of acute exacerbation or remission period had varying degrees of airflow obstruction, and disease aggravated as the degree of airflow obstruction. This study also found that the dispersion function of asthma patients and the healthy group had no significant difference. In the past, it was considered that lung function of asthma patients in remission was relatively normal, but the recent studies found that asthma could lead to continued damage to lung function, some of which could develop to reversible airflow obstruction or even worse to irreversibility. Therefore, early assessment of lung function of asthma patients is particularly important, especially to clear whether there is airflow obstruction and its reversible possibility.

 ${\rm CD_4}^+{\rm T}$  lymphocytes is a group of plasticity, the relationship among their subsets is intricate. The ratio imbalance among them is an important cause to asthma, and the release of inflammatory cytokines may be the key to break the balance. The studies found that Th17 cells in asthma patients are more closely related to both the severity of the disease and symptom control. They may be the important reasons of refractory asthma, severe asthma and airway remodeling in asthma, they provide the biological targeting for the treatment of refractory and severe asthma. At the same time, they provide a new research direction for asthma airway remodeling. "Th17/Treg imbalance" makes up the flaws of "Th1/Th2 imbalance" theory, which provides a more comprehensive understanding of the pathogenesis of asthma, further to clarify its mechanism of action. The development of new anti-inflammatory factor and immunotherapy is expected to be a new avenue for the treatment of asthma.

Bronchial asthma is characterized by chronic airway inflammation and airway remodeling. The results of this study showed that there existed Th1/Th2 and Th17/Treg immunoregulatory imbalance in the peripheral blood of patients with asthma; the study confirmed that the expression of proinflammatory Th2 cells and Th17 cells was high in acute exacerbation, while the expression of Th17 cells in the moderatesevere remission group was also higher than the mild remission group. This study also confirmed there were changes of airway wall thickness, gas retention, changes of lung function (airflow obstruction) in asthma patients. The study further clarifies the differences of  $CD_4^+$ T lymphocyte subsets expression in the peripheral blood in asthma patients, which has important implications for the clinical judgment of asthma staging and clinical classification, it can guide clinical treatment, and especially it will bring the research direction for the biological targeted therapy for refractory asthma and special type of asthma patients.

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