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# The clinical differences between cough variant asthma cells and humoral immunology indicators

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**ABSTRACT**

The Purpose of this study was to study and analyze the clinical differences between cough variant asthma (CVA) cells and humoral immunology indicators. For this aim, 73 sick children with CVA were enrolled in this study and were admitted to the Pediatric Inpatient Department of Weifang Maternal and Child Health Hospital for treatment from April 2019 to May 2021. They were divided into the attack stage group (n=45) and the remission stage group (n=28). Meanwhile, 30 children with normal physical examination results were selected as normal controls. Differences in serum levels of TNF-, hs-CRP, IL-4, IL-5, IL-6 and IL-13 were compared among the three groups, as well as the differences in humoral immunology indicators such as T lymphocyte subsets $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+$/ $CD8^+$ and IgA, IgG, IgG, IgM, IgE, IgE and IgG subtypes. Results showed that serum levels of TNF-α and hs-CRP were significantly higher in the attack stage group than those in the remission stage group and normal control group, and the difference was statistically significant ($P<0.05$). The serum levels of TNF-α and hs-CRP were higher in the remission stage group than those in the normal control group, and the difference was not statistically significant ($P>0.05$). Serum levels of IL-4, IL-5, IL-6 and IL-13 were significantly higher in the attack stage group than those in the remission stage group and normal control group, and the difference was statistically significant ($P<0.05$). $CD4^+$ and $CD4^+/CD8^+$ were significantly higher in the attack stage group than those in the remission stage group and normal control group, and the difference was statistically significant ($P<0.05$). The difference in serum levels of $IgG_1$, $IgG_2$ and $IgG_3$ was not statistically significant ($P>0.05$) among the three groups. While the level of $IgG_4$ subsets in the attack stage group was significantly higher than that in the remission stage group and normal control group, and the difference was statistically significant ($P<0.05$). Then the cytokines, cells and humoral immunology indicators of CVA patients are not in their normal range. They are involved in the pathogenesis of CVA. The combined detection is of great clinical significance in the diagnosis of early CVA to avoid misdiagnosis and missed diagnosis.

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## Introduction

Cough variant asthma (CVA), also known as allergic cough, refers to a special type of asthma with chronic cough (> 8 weeks) as the main or only clinical manifestation and not accompanied by wheezing, choking sensation in the chest, tightness in breathing and so on typical asthma symptoms. It is a chronic disease in nature (1). The new study has found that the global prevalence of chronic cough is as high as more than 9%. Many studies in China show that CVA accounts for more than 30% of chronic cough in the Chinese population (2). The etiology and pathogenesis of CVA are still unclear. It is believed to be similar to typical asthma, mainly influenced by genetic and environmental factors (3). The principle of CVA treatment is the same as in typical asthma. It is mainly inhaling low-dose glucocorticoids and bronchodilators, which can effectively improve asthma symptoms. The disease is a chronic disease and requires long-term treatment and monitoring (4). CVA is a type of asthma with more hidden clinical conditions that can occur at any age. Its main clinical symptoms include chronic cough with no obvious positive signs. It is easy to misdiagnose CVA as bronchitis and other diseases. If CVA is misdiagnosed and not treated in time, the condition will gradually aggravate and even appear with typical asthma symptoms such as wheezing, choking sensation in the chest and tightness in breathing, etc. About 30-40% of CVA will develop into typical asthma (5). Therefore, we mainly study the pathogenesis of CVA and investigate the important significance of the level of

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cough variant asthma cytokines in CVA diagnosis and treatment. Some studies have shown that most CVA patients have a personal history of allergies. That is, the patients have an allergic constitution, resulting in a variety of different allergic reactions and allergic diseases (6). Some studies have found a close link between the patient's allergic constitution and antibody IgE and so on the class switch. However, there are few studies on the changes in CVA cells and humoral immunity-related indicators (7). This study is aimed to analyze the changes in their clinical differences by selecting CVA patients as the study subjects and testing the levels of their CVA cytokines and humoral immunology-related indicators.

## Materials and methods

### General materials

A total of 73 children with CVA were enrolled in this study and were admitted to the Pediatric Inpatient Department of Weifang Maternal and Child Health Hospital for treatment from April 2019 to May 2021. Inclusion criteria: ① All of them met the clinical diagnostic criteria of CVA; ② Those agreed to participate in the study and had signed the informed consent form. Exclusion criteria: ① Those were combined with other respiratory infections. ② Those were combined with serious functional diseases in the important organs such as the heart, liver and kidneys. ③ Those were combined with sinusitis and gastroesophageal reflux, etc. ④. The CVA attack stage refers to the period that the patient suffers from different degrees of dyspnea and wheezing, and the remission stage refers to the period that the patient has their symptoms obviously relieved spontaneously or after treatment. Among them, there were 45 patients included in the attack stage group, including 20 boys and 25 girls, aged 1-11 years and a mean age of (6.15±4.21) years. There were 28 patients included in the remission stage group, including 16 boys and 12 girls, aged 2-12 years and a mean age of (6.42±4.37) years. During the same period, 32 healthy children were selected as normal controls via physical examination. There were 15 boys and 17 girls aged 1-10 years and a mean age of (5.98±3.87) years. They had no history of asthma or personal history. The difference in sex and age was not statistically significant ($P<0.05$) among the three groups but comparable. This study was approved by the Medical Ethics Committee of Weifang Maternal and Child Health Hospital.

### Methods to observe indicators

(i) Detection of tumor necrosis factor -α(TNF-α), interleukin-4 (IL-4), interleukin-5 (IL-5), hypersensitive C-reactive protein (hs-CRP), IL-6 and IL-13: Fasting venous blood (3ml) was extracted from the patients in the morning and their serum levels of TNF-α, IL-4, IL-5, IL-6 and IL-13 were measured by enzyme-linked immunosorbent assay (ELISA), as well as hs-CRP levels by immunoturbidimetry (ITM).

(ii) T Lymphocyte subpopulation: The extracted venous blood was treated and stained with anti-$CD3^+$, $CD4^+$ and $CD8^+$ monoclonal antibodies and then tested by flow cytometry (FCM).

(iii) Detection of immunoglobulin A (IgA), IgG, IgM, IgE and IgG subgroups: The levels of IgA, IgG, and IgM were detected by scattering turbidimetry, while IgE and IgG subgroups were tested by ELISA.

### Statistical method

The data of this study were analyzed by the SPSS20.0 software package. The measurement data met the normal distribution, expressed by ($\bar{x}\pm s$). An Independent sample t-test was used for comparison among groups and paired sample t-test for comparison before and after treatment. $P<0.05$ was considered that the difference was statistically significant.

## Results and discussion

### Comparison of TNF-α and hs-CRP levels among the three groups of children

Serum levels of TNF-α and hs-CRP were significantly higher in the attack stage group than those in the remission stage group and normal control group, and the difference was statistically significant ($P<0.05$). Whereas the serum levels of TNF-α and hs-CRP were higher in the remission stage group than those in the normal control group, and the difference was not statistically significant ($P>0.05$). See Table 1.

### Comparison of serum levels of IL-4, IL-5, IL-6, and IL-13 among the three groups

Serum levels of IL-4, IL-5, IL-6 and IL-13 were significantly higher in the attack stage group than those in the remission stage group and normal control group, and the difference was statistically significant

(P<0.05). However, the serum levels of IL-4, IL-5, IL-6, and IL-13 in the remission stage group were compared with those in the normal control group, the difference was not statistically significant (P>0.05). See Table 2.

**Table 1.** Comparison of TNF-α and IL-13 levels among the three group of children ($\bar{x}$±s)

| Groups | Number of cases | TNF-α | hs-CRP (mg/L) |
|---|---|---|---|
| Attack stage | 45 | 2418.36±1411.23ab | 2.67±1.45ab |
| Remission stage | 28 | 801.04±310.25 | 0.87±0.89 |
| Normal control | 32 | 710.12±280.24 | 0.35±0.21 |
| *t* | | 39.34 | 50.88 |
| *P* | | ＜0.001 | ＜0.001 |

Note: The same index was compared with the remission stage group, [a]P<0.05. The same index was compared with the normal control group, [b]P<0.05.

**Table 2.** Comparison of serum levels of IL-4, IL-5 and IL-10 among the three groups of children ($\bar{x}$±s)

| Groups | Number of cases | IL-4 (μg/L) | IL-5 (μg/L) | IL-6 (pg/mL) | IL-13 (pg/mL) |
|---|---|---|---|---|---|
| Attack stage | 45 | 44.23 ± 6.68ab | 16.87 ± 2.39ab | 24.47 ± 9.47ab | 61.25 ± 24.51ab |
| Remission stage | 28 | 27.45 ± 4.59 | 9.65 ± 1.24 | 11.25 ± 3.24 | 42.24 ± 8.47 |
| Normal control | 32 | 30.11 ± 7.42 | 8.24 ± 0.36 | 9.21 ± 2.15 | 39.45 ± 5.26 |
| *t* | | 74.27 | 285.85 | 62.12 | 19.06 |
| *P* | | ＜0.001 | ＜0.001 | ＜0.001 | |

Note: The same index was compared with the normal control group, bP<0.05.

## Comparison of T lymphocyte subsets among the three groups

$CD3^+$ and $CD8^+$ were compared among the three groups, but the difference was not statistically significant (P>0.05). CD4+ and CD4+/CD8+ were significantly higher in the attack stage group than in the remission stage group and normal control group, and the difference was statistically significant (P<0.05). However, $CD4^+$ and $CD4^+/CD8^+$ were higher in the remission stage group than in the normal control group, and the difference was not statistically significant (P>0.05). See Table 3.

## Comparison of humoral immunology indicators among the three groups

Serum levels of IgA, IgG, and IgM were significantly lower in the attack stage group than those in the remission stage and normal control group, while the level of IgE was significantly higher than that in the remission stage and normal control group. Moreover, the level of IgE was significantly higher than that in the control group. All differences were statistically significant (all P<0.05). However, the serum levels of IgA, IgG, and IgM in the remission stage group were compared with the normal control group. The differences were not statistically significant (P>0.05). See Table 4.

**Table 3.** Comparison of T lymphocyte subsets among the three groups o($\bar{x}$±s,%)

| Groups | Number of cases | $CD3^+$ | $CD4^+$ | $CD8^+$ | CD4+/ $CD8^+$ |
|---|---|---|---|---|---|
| Attack stage group | 45 | 60.21 ± 6.35[ab] | 40.37 ± 5.3[8ab] | 21.17 ± 5.34[ab] | 1.98 ± 1.54[ab] |
| Remission stage group | 28 | 61.23 ± 6.32 | 32.34 ± 4.21 | 22.41 ± 5.78 | 0.94 ± 0.87 |
| Normal control group | 32 | 61.89 ± 6.41 | 30.23 ± 3.46 | 23.54 ± 6.17 | 0.89 ± 0.45 |
| *t* | | 0.68 | 53.37 | 1.33 | 11.40 |
| *P* | | 0.510 | ＜0.001 | 0.270 | 0.000 |

Note: The same index was compared with the remission stage group, [a]P<0.05. The same index was compared with the normal control group, [b]P<0.05.

**Table 4.** Comparison of humoral immunology indicators among the three groups ($\bar{x}$±s)

| Groups | Number of cases | IgA (mg/L) | IgG (mg/L) | IgM (mg/L) | IgE (kU/L) |
|---|---|---|---|---|---|
| Attack stage | 45 | 1.15 ± 0.24 [ab] | 6.14 ± 1.35[ab] | 1.02 ± 0.11[ab] | 267.84 ± 44.65 [ab] |
| Remission stage | 28 | 2.45 ± 1.21 | 11.79 ± 3.85 | 2.65 ± 2.01 | 170.25 ± 34.41 |
| Normal control | 32 | 2.99 ± 1.87 | 12.98 ± 4.59 | 2.98 ± 2.15 | 78.35 ± 30.48 |
| *t* | | 23.45 | 46.65 | 17.24 | 233.14 |
| *P* | | ＜0.001 | ＜0.001 | ＜0.001 | ＜0.001 |

Note: The same index was compared with the remission stage group, [a]P<0.05. The same index was compared with the normal control group, [b]P<0.05.

## Comparison of serum level of IgG subgroups among the three groups

The difference in serum levels of $IgG_1$, $IgG_2$ and $IgG_3$ was not statistically significant (P>0.05) among the three groups. While the level of IgG4 subsets in the attack stage group was significantly higher than that in the remission stage group and normal control group, and the difference was statistically significant (P<0.05). See Table 5.

**Table 5.** Comparison of serum level of IgG subgroups between the two groups ($\bar{x}\pm s$)

| Groups | Number of cases | $IgG_1$ (g/L) | $IgG_2$ (g/L) | $IgG_3$ (g/L) | $IgG_4$ (g/L) |
|---|---|---|---|---|---|
| Attack stage | 45 | 6.18 ± 3.25 $^{ab}$ | 1.89 ± 0.14$^{ab}$ | 0.57 ± 0.78$^{ab}$ | 1.24 ± 1.26$^{ab}$ |
| Remission stage | 28 | 5.87 ± 2.84 | 2.17 ± 1.02 | 0.38 ± 0.25 | 0.48 ± 0.37 |
| Normal control | 32 | 5.41 ± 2.37 | 2.25 ± 1.25 | 0.32 ± 0.18 | 0.17 ± 0.04 |
| *t* | | 0.70 | 1.83 | 2.29 | 16.25 |
| *P* | | 0.501 | 0.166 | 0.167 | ＜0.001 |

CVA is a special type of asthma. The cough is the only or main clinical manifestation, with no other obvious symptoms or signs such as wheezing and shortness of breath, but with airway hyperreactivity. Most people are caused by continuous cough or recurrent attacks. Therefore, it will lead to the symptoms of breathing difficulties which seriously affect the normal life of the patients (8). If the CVA is not treated in time, it will gradually develop into more severe asthma. Currently, the pathogenesis of CVA is not clear. Most scholars believe that it is similar to the pathogenesis of typical asthma. It is chronic airway inflammation regulated by cytokines and involved with a variety of cells such as eosinophils (EOS). At the same time, it also involves the allergic inflammatory process mediated by IgE. Some studies have found that cytokines such as IL-4, IL-5 and IL-13 also play an important role in the pathogenesis of CVA in recent years. The main pathological manifestations of the CVA mainly include the spasms of bronchial smooth muscles, mucosal congestion and oedema, and infiltration of inflammatory cells, etc (9).

IL-4 is a cytokine secreted by type II helper cells (TH2 cells) and also known as B cell growth factor. Its main role is to stimulate the proliferation of B cells and plays an important role in regulating humoral immunity and adaptive immunity, especially in EOS infiltration at inflammatory sites in allergic diseases (10). IL-4 plays an important role in promoting IgE synthesis in B cells. Some studies have proved that IL-5 has an important regulatory role on B cells and EOS proliferation and differentiation. Meanwhile, it has a positive regulatory effect on the maintenance of the inflammatory response. However, EOS can secrete IL-5, which may lead to the emergence of chronic inflammation (11). IL-13 is an important factor in Th2 cells that mediate and cause an allergic reaction. An excessive increase in the level of IL-13 may induce the allergic inflammatory response and may be associated with increased adhesion factors and chemokines simultaneously. Therefore, IL-13 is considered to play an important role in the pathogenesis of asthma (12). As an inflammatory cytokine, TNF- is bioactive and produced by multiple cells. It may urge an increase in the expression level of endothelial cell adhesion factors and promote the infiltration and activation of inflammatory cells, thereby leading to the emergence of the inflammatory response (13). The hs-CRP is a cytokine that promotes the inflammatory response. It is mainly secreted by hepatocytes and its levels rise significantly when the inflammatory response occurs. It can be used as an index to reflect the degree of inflammation (14). This study has found that the serum levels of TNF-α and hs-CRP are significantly higher in the attack stage group than those in the remission stage group and normal control group. Serum levels of IL-4, IL-5, IL-6 and IL-13 are significantly higher in the attack stage group than those in the remission stage group and normal control group, indicating that the serum TNF-, hs-CRP, IL-4, IL-5, IL-6 and IL-13 are involved in the pathogenesis of the CVA and could be used as indicators for measuring the degree of remission.

T lymphocytes refer to lymphocyte stem cells derived from the bone marrow and have important immune effects. They are also important immune inflammatory cells involved in asthma. They may differentiate into $CD4^+$ and $CD8^+$ double-positive T cells, of which $CD4^+$ may regulate the maturation and activation of EOS through the secretion of IL-4 and IL-5 cytokines, while $CD8^+$ may activate $CD4^+$ cells and participate in airway inflammatory response in some diseases such as asthma (15). Some studies have reported that CVA patients may have an abnormal expression of $CD4^+$and $CD8^+$. In comparison, dysequilibrium of $CD4^+/CD8^+$ may lead to abnormal cellular immune function in patients. It may be one of the factors resulting in the development of CVA patients into asthma (16). This study shows that $CD4^+$ and $CD4^+/CD8^+$ are significantly higher in the attack stage group than those in the remission stage and normal control group, indicating that an abnormal cellular immune function emerges in CVA patients. The role of CVA in humoral immune function during the immune response is also a key point of the

research, and changes in humoral immunology indicators also have an important role in CVA diagnosis and treatment. IgA structures in monomeric and disomic forms can effectively inhibit microbial attachment to the respiratory tract, suppress viral reproduction, and have an important role in the immune barrier (17). IgG is the most important antibody in the body. It plays a role in antivirus, neutralizing virus, anti-bacteria and immune regulation and can be used to diagnose various diseases such as infection and immune deficiency (18). IgM is an immunoglobulin with the largest molecular weight. It has a potent bactericidal, immune conditioning and coagulating effect and may also participate in the pathological process of autoimmune response and hypersensitivity (19). Both IgE and $IgG_4$ are associated with an allergic reaction. Among them, the IgE level is significantly increased in the serum, indicating that the child is in an allergic state. It can be combined with the EOS receptor on the surface of IgE, resulting in cell activation, thereby changing target cell function and leading to the occurrence of the disease. While an increase in $IgG_4$ is associated with inflammation, such as the stimulation produced by chronic antigens. The results of this study show that the serum levels of IgA, IgG and IgM are significantly lower in the attack stage group than those in the remission stage group and normal control group, while the level of IgE is significantly higher than that in the remission stage group and control group. Moreover, the level of IgE in the two groups is significantly higher than that in the control group, indicating that the disease is relieved. Serum levels of IgA, IgG, and IgM are gradually increased, while the level of IgE is gradually decreased. This could effectively regulate the immune response and inhibit the allergic inflammatory response. The level of $IgG_4$ subsets is significantly higher in the attack group than that in the remission stage group and normal control group, indicating that the patients have developed respiratory infection and abnormal levels of $IgG_4$, which could lead to the onset of the CVA.

In conclusion, the levels of cytokines such as TNF-, hs-CRP, $CD4^+$ and $CD8^+$ cell immunity, and humoral immune indexes such as IgE are not in a normal range. Its combined test can be used to confirm the diagnosis of the CVA in the early clinical stage and avoid misdiagnosis, etc. However, due to the limited sample size for analysis and the short observation time in the study, the samples are needed to accumulate to further explore the clinical differences between cough variant asthma cells and humoral immunology indicators.

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Not applicable.

**Interest conflict**

The authors declare that they have no conflict of interest.

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