Dynamic changes and significance of sputum cells in bronchiolitis

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Abstract: Objective: To observe the cell composition and changes of sputum cells in children with bronchiolitis at different stages of disease, and to explore their role in the pathogenesis of disease. Methods: 75 children hospitalized in 2016 compliance with standard bronchiolitis were selected. The course of the disease was divided into acute attack period, the improvement period and remission period. The levels of sputum cells, such as sputum shedding epithelial cells, neutrophils, eosinophil, lymphocytes and other sputum cells were examined by light microscopy at different stages of acute exacerbation, disease progression and remission. The expression and proportion of cells were compared, and the differences of cell expression and clinical significance were compared. Results: In the early stage of acute bronchiolitis, the sputum cells were mainly neutral and exfoliated epithelium, lymphocytes and eosinophil were small. When the condition improved, the epithelial cells and the neutrophils decreased, while the lymphocytes and allergic family history of children with eosinophil correspondingly increased. During the remission period, neutrophils and sputum shedding epithelium continued to decrease, while the lymphocytes, eosinophil continued to increase. Conclusions: Epithelial cell shedding is a common phenomenon in the acute attack of bronchiolitis, which may be associated with the wheezing symptoms of children. During the acute stage and improvement phase, there is a significant increase in sputum neutrophils attributable to infection. Eosinophilia is associated with anaphylactic individual and family history, and increased eosinophilia is associated with high airway reactivity and prolonged wheezing. Lymphocytes play a role in maintaining chronic airway inflammation.

Key words: bronchiolitis; sputum; cells

Bronchiolitis is a common disease of infants and young children under 2 years of age, and the incidence is increasing in recent years. Clinical manifestations of cough, wheezing, respiratory syncytial virus is a common pathogen. Blood test generally showed the total number of white blood cells in the normal range. Lesions generally involve the bronchial,
alveolar wall, alveolar. Pathological manifestations of mucus secretion increased, cellulase blockage, epithelial cell necrosis, and bronchial lymphocyte infiltration. The aim of this study was to investigate the changes of airway cell composition and inflammation in children with bronchiolitis at different stages, and to explore the role of different cells in the pathogenesis of bronchiolitis.

1. Materials and Methods

1.1 Subjects

A total of 90 children who received hospitalized treatment of bronchiolitis in 2016 were recruited but excluding congenital respiratory dysplasia, bronchial foreign body, congenital heart disease and other diseases. Among them, 15 cases of children with sputum was removed as they did not meeting the quality requirements. Finally, a total of 75 cases were included in this study of which 9 cases have a personal or family history of allergy. All patients included in this study were diagnosed with bronchiolitis meeting diagnostic criteria. Paediatrics study covers those mostly in the winter and early spring 2 years old children, paroxysmal or persistent cough, accompanied by wheezing, some children have fever, lung auscultation sound and wheeze, white blood cells and classification in the normal range, and chest radiograph showing small dotted shadows. Allergy family history refers to the family of two relatives or compatriots have asthma, allergic rhinitis, and allergic dermatitis history. Personal allergic history refers to those suffering from eczema and allergic rhinitis.

Diagnosis was performed in accordance with the development process of bronchiolitis, the course of the division includes: 1. Acute attack period: course of 5 days, obvious signs of cough, nasal congestion, visible three palsy, lungs auscultation sound, wheezing and phlegm sounds can be heard; 2. Disease recovery period: the course of 5-7 days, alleviation of cough symptoms, dyspnoea symptoms disappeared, improvements made in the wheeze and a small amount of phlegm sounds; 3. Remission period: duration of more than 8 days, calm breathing , occasional cough or cough completely disappeared, lungs wheezing disappeared, and a small amount of phlegm sound.

1.2 Removing sputum

In the course of acute exacerbation, improvement and remission, the patients were administered sputum suction procedure. A sterile suction tube coming with negative pressure suction is applied at the nose and throat passage for duration of 10-15 seconds. If the sputum smear appears a large number of squamous epithelium and with no macrophages, it indicates that the sputum is from the upper respiratory tract, thus considered as unqualified sputum. Sputum screening criteria for sputum smear microscopic examination of squamous epithelium <10/low magnification and white blood cells> 25/low magnification or squamous epithelium/white blood cells ≤ 1: 2.5.

1.3 Analysis of sputum

The collected sputum specimens were applied to the slide. After drying, one smear was stained with HE and the nuclei were not included in squamous epithelial cells. Microscopic examination of sputum includes shedding of epithelial cells, neutrophils, eosinophil, lymphocytes and macrophages.

1.4. Statistical analysis

Statistical analysis was carried out using SPSS19.0 software. Normal distribution data show that the mean ± s revealed skewed distribution with the median [25 percentile, 75 percentile]. Count data were analysed using chi-square test. In the skewed distribution group, the rank sum test was used in the group, and the t-test was used for the normal distribution. p <0.05 showed statistically significant different. In comparing an experimental group with a number of experimental groups, there is a need to re-set the test level, correction α' = α/(k-1) = 0.017 (k for the experimental group
2. Results

Neutrophils, shedding epithelial cells, lymphocytes and eosinophil can be seen in sputum specimen. In different stages of disease development, a variety of cell expressions and proportions are different. In the acute onset of bronchiolitis, sputum cells are mainly neutrophils which accounted for about 70%, the majority of children can be seen about 40% of epithelial cells shed, a small amount of lymphocytes accounting for 2%, and eosinophil accounted for 0.6 %. When bronchiolitis condition improved, sputum neutrophils decreased by about 60%, epithelial also decreased by 28%, lymphocytes increased by 9% and eosinophil increased by 0.8%. During the remission stage, sputum neutrophils went down to 30%, shedding of epithelial cells down to 5%, lymphocytes rose to 20% and eosinophil increased to 1.2% (Table 1).

| Group               | Neutrophils | Epithelial Cells | Lymphocytes | Eosinophil |
|---------------------|-------------|------------------|-------------|------------|
| Acute Episode       | 72 (62, 83) | 43 (32, 56)      | 2 (1.8, 9)  | 0.6 (0.4, 1.0) |
| Rehabilitation Period | 61 (45, 78) | 28 (19, 38)      | 9 (6, 15)   | 0.8 (0.6, 1.8) |
| Remission of illness | 34 (18, 45) | 5 (2, 11)        | 20 (16, 49) | 1.2 (0.8, 3.2) |
| X² value            | 9.87        | 13.07            | 11.82       | 6.79       |
| P value             | 0.033       | 0.013            | 0.021       | 0.046      |

Note: a = comparison of neutrophils in the acute onset and remission of disease, b = comparison of neutrophils in the improvement of disease and remission of neutrophils, P = 0.017, 0.034. c = epithelial cell in the acute exacerbation period and remission period, d = epithelial cell in the disease improvement period and remission period, P = 0.016, 0.037. e = there was a statistically significant difference in the acute exacerbation and remission of lymphocytes, P = 0.002. f = eosinophil compared with acute exacerbation and disease remission, P = 0.027.

Comparison of sputum cells in different course of children with febrile disease. Sputum neutrophils in the course of the disease showed a gradual downward trend. There was no statistically significant difference between the acute onset and the improvement of the disease. The difference between acute exacerbation period and remission period, moderate improvement period and remission of neutrophils was statistically significant, P values were at 0.017, 0.034 and were less than 0.05. The number of exfoliated epithelial cells also showed a decreasing trend in the course of disease, but there was no statistically significant difference in acute exacerbation and disease progression (P = 0.075). In the acute onset and remission, the disease improvement period and the remission period was statistically significant (P value of 0.016 and 0.037). Lymphocytes showed an increasing trend in the course of disease. There was no significant difference in acute exacerbation period and condition improvement period, but there was significant difference between acute attack period and remission period (P = 0.002). Eosinophil also showed an increasing trend in the course of the disease, but mainly in children with a history of allergies or family history, in the acute onset and remission of the disease were statistically significant (P = 0.027). The results showed early onset of children with bronchiolitis, sputum cells to neutrophils granulocyte-based, suggesting that airway inflammation is mainly associated with infection, with personal allergies or family history of allergies in children. Convalescence inflammation gradually manifested as eosinophil increased, suggesting that after infection will be allergic inflammation, manifested as airway
hyper-responsiveness, and the course of the relative extension. In individuals with family history of allergic history, eosinophil in the progression of disease and remission stage increased significantly as compared to patients with no history of allergic history; the two were statistically significant, $P < 0.05$ (Table 2). Compared with the patients with no history of allergic history, the duration of diabetes mellitus in patients with allergy history was longer than that in patients without allergic history ($P < 0.05$) (Table 3).

### Table 2 Comparison of eosinophil in the presence of allergy history

| Group               | With history of Allergies | Without history of Allergies |
|---------------------|---------------------------|-----------------------------|
| Acute Episode       | 1.73 (0.48, 2.02)         | 0.54 (0.23, 1.21)           |
| Rehab. Period       | 2.02 (0.82, 3.68)         | 0.61 (0.28, 1.34)           |
| Remission of Illness| 4.52 (1.83, 6.12)         | 0.59 (0.22, 1.32)           |

| $X^2$ value | 12.45 |
| $P$ value   | 0.021 |

### Table 3 Comparison of the course of disease in the history of allergy history

| Disease Group | No. | Course of Disease | T Value | $P$ Value |
|---------------|-----|-------------------|---------|-----------|
| With history of Allergies | 9   | 14.23±3.31        | 317.5   | 0.022     |
| Without history of Allergies | 66  | 10.56±2.34        |         |           |

### 3. Discussion

Bronchiolitis lesion is located in the small airway and the overall cross-sectional area is big. Airflow speed is greatly slowed down as bacteria, viruses and harmful substances are easy to accumulate. Ciliated epithelium was damaged and destroyed to varying degrees. Mucous gland secretion increased giving rise to the difficulty of exclusion of sputum which remained in the bronchial, thus affecting ventilation. Studies have shown that 1/2–3/4 bronchiolitis were caused by RSV infection, mainly manifested by the alveolar space being widened and lymphocytes, plasma cells, macrophages exudation. In addition, we found that in the pathogenesis of bronchiolitis, sputum cells to neutrophils, followed by shedding of epithelial cells, lymphocytes and eosinophil, which is consistent with bronchiolitis caused by infection. Persson suggests that neutrophils can release chemokine and proliferative factors, and their increase may contribute to damage to the epithelial regeneration process. The observed neutrophil lifespan in this paper seems to support Persson's view that both may also play a beneficial role in promoting epithelial regeneration in asthma and contribute to the recovery from the disease.

Sputum cells also exist with a large number of shedding epithelial cells. With the disease gradually improved, epithelial cells gradually reduced. It was found that when the respiratory syncytial virus (RSV) and rhinovirus infected epithelial cells, epithelial cells shed, which produced cytokines such as IL-8, RANTES, MIP-1A, IL-6, GM-CSF and other cytokines, chemokine were up-regulated, and thus promoting mucus secretion, increasing vascular exudation and smooth muscle contraction.
Our experiments show that at the acute onset of the disease, there is a small amount of eosinophil in sputum cells. With the improvement of the disease, eosinophil count is found to be more than before for those with an individual or family history of allergies. However, the change is not that obvious for those without any history of allergies. At the same time, for those with a history of allergies the disease will prolong longer than those children with no history of hyperthyroidism; the difference was statistically significant, suggesting that allergic factors in the disease during the course also play a role in disease improvement and remission. Due to airway epithelial injury, subcutaneous nerve endings and airway smooth muscle being exposed, the airway naturally was affected by allergens or other stimuli that lead to airway hyper-responsiveness, which may be the contributing factor in the slow recovery of some children’s breathing problems.

With the improvement of the condition and remission of children, lymphocytes gradually increased. Studies have found that the activation of sputum lymphocytes indicators of soluble cell adhesion molecules 1% in the remission after 3 months still increased, suggesting that lymphocytes may play a role in maintaining chronic airway inflammation.

In the present study, we also found that sputum contains traces of macrophages, mast cells, basophils and other cells. As we use the composition ratio, the proportion of these cells were markedly less, thus not included in the study. Their clinical significance need further study.

Our studies found that bronchiolitis in different periods produce different expression of airway inflammatory cells. In the early stages of the disease, neutrophils and epithelial cells overshadowed the others in significance but as improvement of disease and remission sets in, lymphocytes and eosinophil significantly increased, which is related to the pathogenesis and pathogenesis of bronchiolitis. Therefore, the treatment of bronchiolitis entails anti-infection measures and protection of airway epithelium measures. For children with a history of allergies, additional measures such as anti-allergy and atomization treatment should be considered to reduce airway hyper-responsiveness.

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