Journal of Public Health International

Changes in Cellular Morphology in Bronchoalveolar Lavage Fluid of Children with Mycoplasma Pneumoniae Pneumonia

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Research Article

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Peer-Reviewed Article

DOI: 10.14302/issn.2641-4538.jphi-24-5044

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Keywords:

cytological morphology; bronchoalveolar lavage; Mycoplasma pneumoniae pneumonia; children.

Received: March 25, 2024

Accepted: March 31, 2024

Published: April 10, 2024

Academic Editor:

Sasho Stoleski, Institute of Occupational Health of R. Macedonia, WHO CC and Ga2len CC

Citation:

Kun Ma, Shujun Li (2024) Changes in Cellular Morphology in Bronchoalveolar Lavage Fluid of Children with Mycoplasma Pneumoniae Pneumonia. Journal of Public Health International - 7(1):45-51. https://doi.org/10.14302/issn.2641-4538.jphi-24-5044

Abstract

Objective: To study changes of cell morphology in BALF in children with Mycoplasma pneumoniae pneumonia（MPP）.

Methods: From December 2021 to May 2022, a group of 32 children diagnosed with Acute MPP and admitted for treatment in the Pediatrics Department and PICU of the First Affiliated Hospital of Xinxiang Medical University were selected for our study. These patients underwent bronchoalveolar lavage as part of their clinical assessment. For comparison, we included a control group comprising 10 children who were not infected but had bronchial foreign bodies. We investigated the cellular composition in the bronchoalveolar lavage fluid (BALF) using Wright-Giemsa staining and microscopic evaluation, aiming to understand the relationship between shifts in cell proportions and extra-pulmonary symptoms associated with MPP.

Results: In this study, a total of 42 cases were enrolled, with 32 cases in the study group and 10 cases in the control group. There were no statistically significant differences in gender, age, height, weight, and BMI between the two groups ($p > 0.05$). The study group exhibited significantly higher levels of neutrophil percentage (GRA%), CRP, D-dimer, and LDH in blood routine tests compared to the control group ($p < 0.05$). Furthermore, the proportions of neutrophils (%) and macrophages (%) in BALF were significantly higher in the study group compared to the control group ($p< 0.05$), while the proportion of lymphocytes (%) in BALF showed no statistically significant difference between the two groups ($p> 0.05$).

Conclusion: In the acute phase of MPP in children, BALF is predominantly composed of neutrophils. A lower proportion of lymphocytes in BALF is associated with a higher incidence of extra-pulmonary manifestations and longer hospitalization duration.

Introduction

Mycoplasma pneumoniae (MP) is one of the major causes of community-acquired pneumonia in children aged 5 years and older[1]. Mycoplasma pneumoniae pneumonia (MPP) is generally considered to be a mild and self-limiting disease, but it may also show more severe symptoms such as high fever, dry cough,

dyspnea and wheezing，among young patients, the mortality rate and incidence of complications are higher[2].It can also cause serious extrapulmonary complications of the circulatory, digestive, blood, urinary, neurological, musculoskeletal, cutaneous mucosal, and other systems [3]

Bronchoalveolar lavage (BAL) is a safe, easy to operate, minimally invasive and well tolerated technical means, is an important diagnostic tool for respiratory diseases, can promote the diagnosis of various lung diseases[4, 5]. By analyzing the bronchoalveolar lavage fluid（BALF）, the investigators can determine the cytological component profile and detect respiratory pathogens. Analysis of BALF facilitates the diagnosis of lung infection and provides cytological morphology and other findings that facilitate the diagnosis and management of multiple lung diseases, but the results of BALF analysis must be used in combination with other relevant clinical indicators, such as clinical presentation, medical history, physical examination and radiological findings, etc[6]. This paper aims to investigate the changes in cell morphology in BALF and its relationship with extrapulmonary manifestations in children with MPP.

Materials and methods

1. *Normal information:* As the study object, we selected children with acute MPP admitted to the pediatrics and PICU of Xinxiang Medical College from December 2021 to May 2022, and selected children with bronchial foreign bodies treated in the same period as the control group. This study was approved by the hospital ethics committee (approval number: 2021022), and all the parents or guardians of the participating patients gave the informed consent and signed the informed consent form.
2. Inclusion criteria: (1) refer to the relevant consensus and specifications[7, 8]Children with acute MPP who meets the diagnostic criteria for mycoplasma pneumoniae pneumonia; (2) the age limit is between 1 month and 14 years; (3) meets the indication of bronchoalveolar lavage[9].
3. *Exclusion criteria:* (1) cases with cardiovascular disease, chronic lung disease, bronchial asthma, and tuberculosis; (2) cases with bacterial and viral infection; (3) cases with congenital immune deficiency; (4) cases with incomplete clinical data collection; (5) disapproval from family members.
4. *Research methods*

(1) General clinical data

Clinical data of 42 included cases (32 patients in the study group and 10 patients in the control group), including general clinical data including age, sex, body weight, duration of fever, hospital stay, MP antibody, MP load in BALF (<500copies / ml negative), CBC, CRP, LDH, D-dimer, extrapulmonary manifestations, etc.

(2) Experimental method

After taking the lavage fluid sample, it was filtered with sterile gauze and placed in a 10ml centrifuge tube. The centrifuge tube was marked and put into the normal temperature centrifuge (Ronghua Instrument Manufacturing Co., LTD., China) for centrifugation. About 50μl of the bottom sediment was left for production. If it cannot be detected in time, the room temperature can be stored for 4 hours.

After mixing the residual sediment evenly, 5~10μl was put into the slide (SAIL BRAND, China). The Swiss-Jimsa staining solution produced by Beso Biotechnology Co., Ltd. of Zhuhai was dried after the push sheet and processed in strict accordance with the reagent instructions. Using an optical microscope

(Ningbo Yongxin Optics Co., LTD., China), the stained dry smear was observed, the uniform distribution of cells was selected, at least 200 cells (neutrophils, lymphocytes, macrophages, etc.) were counted, and the classification results were reported as a percentage.

5 *Statistical method*: The SPSS 26.0 software was used for statistical analysis. The chi-squared test was used to analyse the clinical characteristics of the groups. Qualitative data were expressed as percentages (%), and comparisons between groups were made using the chi-squared test. Quantitative data that conformed to the normal distribution and chi-squared test results were expressed as the mean±standard deviation using the independent samples t-test, and those that did not conform to the normal distribution were expressed as the median-quartile spacing using the nonparametric rank-sum test.A *P*-value <0.05 was considered a statistically significant difference.

Results

1 *Comparison of cell morphology in bronchoalveolar lavage fluid between the study group (32 cases) and the control group (10 cases)*

Between study and control groups: There was no statistically significant difference in gender, age, height, and weight between the two groups (*P*> 0. 05). The GRA%, CRP, D-dimer, and LDH in the blood of the study group were significantly higher than those in the control group, while the LM% in the blood routine was lower than that in the control group, and the differences were statistically significant (*P* <0.05). Significant differences in the proportion of BALF neutrophils and macrophages (%) in the study group (*P* <0.05), while the difference in he proportion of BALF lymphocyte (%) was not statistically significant (*P*> 0. 05). (Table 1).

Table 1. Comparison of clinical data and cell classification counts in BALF between study group and control children

Clinical characteristics	Study group (n=32)	Control group (n=10)	c^2	t/Z	*P*
Gender (male / female)	16/16	6/4	0.631		0.384
Age(years)	6.6±2.5	5.7±5.4		-0.653	0.528
Height(cm)	122.7±21.1	110.8±37.4		-1.229	0.227
Weight(kg)	20.8（18.80，27.53）	12（9.75，34）		-1.023	0.307
Proportion of BALF neutrophils (%)	46.00（38.00，55.00）	1.00（0.00，1.75）		-4.504	＜0.001
Proportion of BALF lymphocytes (%)	11.00（10.00，14.00）	11.50（9.75，14.00）		-0.235	0.817
Proportion of BALF macrophages (%)	43.00（33.87，51.00）	87.51（85.00，90.00）		-4.503	＜0.001
GRA% in blood	71.04（56.40，79.83）	46.51（34.50，62.45）		-2.400	0.016
LM% in routine blood	23.44（13.55，34.95）	43.22（33.10，58.55）		-2.667	0.008
CRP（mg/L）	17.48（4.86，37.65）	1.00（0.17，1.64）		-3.834	＜0.001
D-dimer (ug/ml)	1.75（0.80，4.23）	0.60（0.55，0.75）		-3.375	0.001
LDH （U/L）	342（269，482）	245（181，284）		-2.967	0.003

Note: GRA: percentage of neutrophils; LM: percentage of lymphocytes; CRP: C reactive protein; LDH: lactate dehydrogenase.

2 Comparison of the children with and without extrapulmonary manifestations in the study group

The difference in age between the two groups was not statistically significant ($P > 0.05$). The blood procalcitonin (PCT), LDH and azotransferase (AST) levels and the length of hospitalisation in the group with extrapulmonary manifestations were higher than those in the group without extrapulmonary manifestations, with a statistically significant difference ($P < 0.05$). However, the serum albumin level and the proportion of lymphocytes in the BALF in the group with extrapulmonary manifestations were lower than those in the group without extrapulmonary manifestations. The differences were statistically significant ($P < 0.05$),as shown in Table 2.

Table 2. Comparison of clinical data and proportion level of cell classification in groups with and without lung manifestations and BALF

Clinical characteristics	Group with extra pulmonary manifestations (n=8)	Group without extrapulmonary manifestations (n=24)	*Z*	*P*
Length of hospitalization(days)	19.00（18.00，22.00）	11.00（7.00，16.00）	-3.001	0.003
PCT（ng/ml）	0.74（0.42，3.59）	0.35（0.19，0.68）	-2.625	0.009
LDH（U/L）	697.0（469.0，1177.0）	296.0（255.0，417.0）	-3.163	0.002
AST（U/L）	54.0（42.0，109.0）	30.0（22.0，37.0）	-2.898	0.004
albumin (g/L)	33.9（27.3，40.2）	40.3（37.1，42.3）	-2.158	0.031
Proportion of BALF neutrophils (%)	45.00（37.50，59.00）	.50.0046（38，54.00）	-0.172	0.864
Proportion of BALF lymphocytes (%)	10.50（9.50，11.00）	13.00（10.00，14.50）	-2.220	0.026
Proportion of BALF macrophages (%)	44.00（31.00，52.00）	42.50（34.50，51.00）	-0.123	0.902

Note: PCT:calcitonin; LDH:lactate dehydrogenase; AST: glutamate aminotransferase.

Discussion

In recent years, the incidence of Mycoplasma pneumoniae pneumonia (MPP) has been gradually increasing among children and adolescents in China, accounting for 10%-40% of community-acquired pneumonia (CAP). Additionally, there is an increasing trend in refractory Mycoplasma pneumoniae pneumonia and severe Mycoplasma pneumoniae pneumonia [10]. Therefore, timely assessment and intervention of airway inflammation are of significant importance for improving the prognosis of affected children. Bronchoalveolar lavage (BAL), a non-invasive and well-tolerated method, can obtain samples from the lower respiratory tract. By analyzing bronchoalveolar lavage fluid (BALF), researchers can determine the cytological composition and detect respiratory pathogens. BAL plays a crucial role in the diagnosis of respiratory tract infections and can also be used to monitor lung allografts and evaluate pediatric lung diseases. Examination of BALF cells or non-cellular components through gene microarray technology or proteomics analysis may enhance BALF's diagnostic and management role in pulmonary diseases in the near future [11].

The airway inflammatory response in children with MPP is associated with levels of CRP, white blood cells, and neutrophils in the blood, reflecting the body's immune response to bacterial infection [12, 13]. Some studies suggest that these indicators can serve as a rapid method to differentiate between bacterial and viral pneumonia, provided that appropriate detection thresholds are set based on clinical presentation [14]. However, other research has found no significant differences in these indicators between bacterial and viral pneumonia, highlighting the limitation of relying solely on these markers to determine the etiology of pneumonia [15]. Timely identification of pneumonia etiology can improve clinical management, including decisions regarding antibiotic use. Most studies indicate that clinical signs and symptoms of bacterial pneumonia and suspected viral pneumonia overlap, and their specificity alone is insufficient to reliably distinguish between them [16]. To evaluate the airway inflammatory response in MPP children, this study collected bronchoalveolar lavage fluid (BALF) samples from 32 MPP patients and 10 patients with bronchial foreign body aspiration, analyzing their cellular characteristics. It was found that in MPP patients, the proportion of neutrophils in BALF significantly increased, while the proportion of alveolar macrophages significantly decreased, with no significant difference in lymphocyte proportion, suggesting that neutrophils may play a crucial role in the pathogenesis of MPP. Additionally, levels of CRP, IL-6, white blood cell count, lactate dehydrogenase, and D-dimer in the blood were significantly elevated in MPP patients. These results indicate a pronounced airway inflammatory response in MPP patients, consistent with previous research [17]. Recent evidence suggests that the pathogenesis of Mycoplasma pneumoniae infection is closely related to toll-like receptor stimulation of macrophages by releasing immunomodulatory and inflammatory cytokines and chemokines. Following infection, alveolar macrophages are locally attracted and activated, leading to the release of pro-inflammatory cytokines and chemokines, resulting in monocytic inflammation and airway hyperreactivity [18]. With the increasing severity of MPP, the proportion of pulmonary macrophages decreases, indicating a suppression of macrophage activity during MPP infection. This may be related to restricted migration or altered gene expression of macrophages during MPP [19].

In this study, it was found that MPP patients with extra-pulmonary manifestations had significantly longer hospitalization durations, higher levels of PCT, LDH, and AST, and lower levels of albumin and lymphocyte proportion in BALF compared to MPP patients without extra-pulmonary manifestations, with all differences being statistically significant ($p<0.05$). These results suggest that MPP patients with extra-pulmonary manifestations exhibit more severe systemic inflammatory responses and organ damage. The proportion of lymphocytes in BALF showed a negative correlation with the occurrence of extra-pulmonary manifestations, which may reflect the role of lymphocytes in inhibiting immune damage caused by MP infection. MP infection can result in various extra-pulmonary manifestations, primarily involving the gastrointestinal tract and skin, while neurological symptoms such as headaches are commonly observed in school-aged children. Other extra-pulmonary manifestations such as cardiac damage, renal impairment, and hematological disorders show no significant differences between pre-school and school-aged children,The observed extra-pulmonary manifestations in this study are consistent with the aforementioned findings[20].The immune damage caused by MP infection may be related to the similarity in antigenic composition between glycerophospholipids on the MP membrane and host cells, leading to the production of autoantibodies and activation of autoimmune reactions, thereby resulting in multi-system damage [21].Some studies have also found that LDH levels and hospitalization durations in MPP patients with extra-pulmonary manifestations are significantly higher than those without extra-pulmonary manifestations, consistent with the results of this study [22].

In summary, during the acute phase of MPP in children, BALF is predominantly characterized by neutrophils. A lower proportion of lymphocytes in BALF is associated with a higher likelihood of extra-pulmonary manifestations and longer hospitalization duration in MPP patients.

Declarations

Authors' contributions: Conception and design of the work: MK; Data collection: MK; Supervision: MK; Analysis and in terpretation of the data: MK; Statistical analysis: MK; Drafting the manuscript: MK; Critical revision of the manuscript: MK, LSJ; Approval of the final manuscript: all authors.

Data availability: All data generated or analyzed during this study are included in this published article.

Acknowledgement: Ethics approval and consent to participate. This study was conducted in accordance with the declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University(2021022), and with informed consent from the guardians.

Competing interests: All of the authors had no any personal, financial, commercial, or academic conflicts of interest separately.

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