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Original Article

Differences of CD64 on neutrophils in children with respiratory diseases

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Abstract: Objective: To investigate the application value of CD64, an infectious indicator, in children with common infectious diseases of respiratory system. Methods: A total of 1,425 patients with mycoplasma pneumonia, bronchial asthma, herpangina, bronchopneumonia and lobar pneumonia as well as normal control samples were collected. Flow cytometry was used to analyze the expression level of CD64 in neutrophils of the patients, so as to compare the differences of CD64 index among each group. Results: CD64 index ranked from low to high was as follows: normal control, mycoplasma infection, bronchial asthma, herpangina, lobar pneumonia and bronchopneumonia. Patients with mycoplasma pneumoniae infection had a lower index of CD64 expression, and those with bacterial infection had a high CD64 index. Conclusion: There is significant difference in the index of CD64 expression on neutrophils in patients with different diseases. The index of CD64 expression is of certain guiding value for the diagnosis of children with infectious diseases of respiratory system.

Keywords: CD64, respiratory infection, pneumonia

Introduction

Children have an immature immune system and poor immune function, which is easy to induce a variety of infectious diseases and allergic diseases [1, 2]. Bacteria, viruses, mycoplasma, etc. contribute to serious respiratory diseases in children. Infections caused by different pathogens have many similar symptoms in clinic and are usually hard to be distinguished by clinical symptoms [3, 4]. At present, the commonly used pathogen detection methods have limitations and fail to determine the pathogen accurately or timely.

As an important indicator of immune response, CD64 on neutrophils rapidly increases when immune function of the body is activated, while there is a difference in the increase amount of CD64 in the activation of immune function resulted from different pathogens and allergens [5-7]. Mycoplasma pneumonia, bronchial asthma, herpangina, lobar pneumonia and bronchopneumonia are the common respiratory diseases in children. They have significant pathogenic and pathological differences. At present, the diagnostic studies of CD64 in infectious diseases are mostly focused on bacterial infectious diseases [8, 9]. In this study, we collected samples from patients with mycoplasma pneumonia, bronchial asthma, herpangina, lobar pneumonia and bronchopneumonia who received treatment in our hospital from June 2014 to June 2016. The difference in the expression ratio of CD64 on neutrophils was analyzed to explore the application value of CD64 in these diseases.

Materials and methods

General data

This study has been approved by the Ethics Committee of Xuzhou Children's Hospital. A total of 137 patients with mycoplasma pneumonia, 85 patients with bronchial asthma, 379 patients with herpangina, 238 patients with lobar pneumonia and 513 patients with bronchopneumonia treated in our hospital from June 2014 to June 2016 were selected, and 73 children receiving physical examination were collected for control. All the diagnosis for

the patients complied with the diagnostic criteria for corresponding diseases [5]. Patients with combined complications were excluded. Pneumonia was grouped as follows: mycoplasma-positive pneumonia was included in the mycoplasma pneumoniae infection group, and mycoplasma-negative or pathogen-unknown pneumonia into the lobar pneumonia and bronchopneumonia group according to the pathology Informed consent has been obtained from parents of the children prior to subsequent drug treatment.

Instrument and reagent

Flow cytometry (type: BD FACSCantoII), monoclonal antibody CD64-PE and red blood cell lysate (FACSLysin) were purchased from BD Corporation in United States.

Methods

Sample collection: Intravenous blood (1-2 mL) was collected from patients with pneumonia in an EDTA (ethylene diamine tetraacetic acid) tube on the day of admission. A total of 100 μL anticoagulant peripheral blood was used for flow cytometry, and the remaining samples were used for blood routine examination and pathogen detection. Samples of healthy subjects were taken from children who received routine physical examination in our hospital.

Flow cytometry: Sample treatment: 20 μL monoclonal fluorescent antibody (CD64-PE) and 50μL anticoagulant whole blood were added to the labeled test tubes. They were mixed well, and incubated for 15 min at room temperature away from light. 1 mL 1 × FACSLysin was added to the test tubes and incubated for 10 min at room temperature away from light. The tubes were washed twice with 2 mL phosphate buffer, and the cells were suspended with 450 μL phosphate buffer saline. Finally, flow cytometry was conducted.

Test on the machine: Firstly, lymphocytes, monocytes and neutrophils in the forward scatter/side scatter (FSC/SSC) diagrams were circled respectively. Meanwhile, histograms were established respectively to demonstrate the mean fluorescence intensity (MFI) of the three groups of cells. Ten thousand cells were taken from each sample, and MFI of CD64 in each kind of cell was obtained.

Calculation of CD64 index: Calculation formula: CD64 index = (CD64 fluorescence intensity in neutrophils - CD64 fluorescence intensity in lymphocytes)/(CD64 fluorescence intensity in monocytes - CD64 fluorescence intensity in neutrophils).

Detection of mycoplasma: Mycoplasma was detected using mycoplasma pneumoniae-immunoglobulin M and mycoplasma pneumoniae-immunoglobulin G enzyme-linked immunosorbent assay. When the two results were not identical, the sample was tested again or discarded.

Statistical treatment

SPSS 19 and MedCalc 15 were used for analysis. A normality test was conducted on the data. The data conforming to normal distribution was expressed as mean ± standard deviation ($\overline{x}$ ± sd) and analyzed using one-way analysis of variance. Those data that did not conform to normal distribution were expressed as median (P25, P75) and analyzed using nonparametric test (Kruskal-Wallis test). A pairwise comparison was conducted using Mann-Whitney test to obtain the difference among each group. Categorical variable was expressed as ratio using X^2 test. $P<0.05$ suggested that the difference was statistical significant.

Results

Comparison of characteristics of clinical data of the patients

There was no statistical difference in the sex ratio of the children in each group (**Table 1**). The children in each group were divided into age subgroups according to the age <3 years old, 3-6 years old and >6 years old. The comparison showed that each kind of disease had great difference in different age groups. Most of the children with mycoplasma infection, herpangina and bronchopneumonia were under the age of 3, while children aged over 3 years old took up a high proportion in bronchial asthma and lobar pneumonia (**Table 1**).

Statistical comparison of CD64 index

The normality test of CD64 index in each group of samples showed that all the data rejected the hypothesis of normal distribution. Therefore,

Table 1. Comparison of composition of each group of samples

	Mycoplasma pneumonia	Bronchial asthma	Herpangina	Lobar pneumonia	Bronchopneumonia	Control group	P
Case (n)	137	85	379	238	513	73	
Gender (male/female)	89/48	63/22	230/149	160/78	322/191	49/24	0.214
Age (case)							<0.05
<3 years old	81 (59.12%)	5 (5.88%)	329 (86.81%)	44 (18.49%)	387 (75.44%)	38 (52.05%)	
3-6 years old	44 (32.12%)	50 (58.82%)	47 (12.40%)	119 (50.00%)	108 (21.05%)	19 (26.03%)	
>6 years old	12 (8.76%)	30 (35.30%)	3 (0.79%)	75 (31.51%)	18 (3.51%)	16 (21.92%)	

Table 2. Statistical difference of CD64 index among each group of samples

	Median (P25, P75)	Mycoplasma pneumonia	Bronchial asthma	Herpangina	Lobar pneumonia	Bronchopneumonia	Control group
Mycoplasma pneumonia	0.12 (0.08, 0.17)		0.02	<0.001	<0.001	<0.001	0.03
Bronchial asthma	0.14 (0.11, 0.20)	0.02		0.02	<0.001	0.00	<0.001
Herpangina	0.17 (0.12, 0.23)	<0.001	0.02		<0.001	0.11	<0.001
Lobar pneumonia	0.21 (0.14, 0.32)	<0.001	<0.001	<0.001		<0.001	<0.001
Bronchopneumonia	0.17 (0.12, 0.26)	<0.001	0.00	0.11	<0.001		<0.001
Control group	0.10 (0.08, 0.13)	0.03	<0.001	<0.001	<0.001	<0.001	
Total difference	<0.05						

non-parametric test was used to compare the differences of CD64 index in different diseases. The results showed that the difference was statistically significant (P<0.05). The results of pairwise comparisons among different groups showed that there was no significant difference between herpangina and bronchopneumonia (P = 0.11), while there were obvious differences among the remaining groups (**Table 2**).

CD64 index in each group ranked from low to high: control group (0.10 (0.08, 0.13)), mycoplasma pneumonia (0.12 (0.08, 0.17)), bronchopneumonia (0.14 (0.11, 0.20)), herpangina (0.17 (0.12, 0.23)), bronchopneumonia (0.17 (0.12, 0.26)) and lobar pneumonia (0.21 (0.14, 0.32)). See **Table 2** and **Figure 1**.

Discussion

CD64, also known as Fc-gamma receptor protein 1, is rapidly expressed on neutrophils after the body is infected by pathogens and activates neutrophils. Therefore, it is used as an indicator of infection [10, 11]. There is difference in the degree of activation caused by different pathogens. Studies have shown that such difference has certain ability to identify bacterial and viral infections [7, 12, 13]. Compared with infection indicators such as C reactive protein and procalcitonin, CD64 has higher sensitivity and specificity in the diagnosis of neonatal sepsis [14, 15]. Previous studies have shown that the expression level of CD64 on neutrophils can be assessed using monocytes and lymphocytes as a reference, and a formula that reflects the expression level of CD64 on neutrophils is proposed, which is called as CD64 index or ratio [16]. In this study, CD64 index was used to compare the difference in CD64 expression among patients with different diseases.

Five common pediatric respiratory diseases were selected as the objects of study. Among them, mycoplasma pneumonia is a definite mycoplasma pneumoniae infection taking up more than 10% of pneumonia in Children, and 60% in the epidemic phase [17, 18]. Herpangina is an acute infectious disease mainly caused by Coxsackie A virus. Bronchial asthma is a chronic inflammatory disease, which was caused by a variety of cells. In this study, lobar pneumonia and bronchopneumonia were grouped according to the pathology after mycoplasma pneumoniae infection was excluded. They can be caused by bacteria, viruses or mixed infections [19].

The difference in CD64 index showed that the increase in the expression of CD64 on the neutrophils caused by mycoplasma pneumoni-

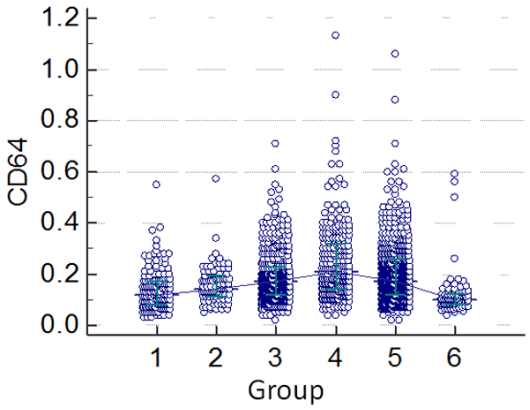

Figure 1. Scattergram of median of CD64 index in each group of samples. Group: 1-mycoplasma pneumonia, 2-bronchial asthma, 3-herpangina, 4-lobar pneumonia, 5-bronchopneumonia, 6-control.

ae infection was the lowest (0.12 (0.08, 0.17)), which was significantly lower than that caused by herpangina, lobar pneumonia and bronchopneumonia due to viruses or bacteria. It indicated that CD64 helps to distinguish mycoplasma infection from bacterial or viral infection.

A study of Pauksens et al. showed that the expression of CD64 on peripheral blood neutrophils in patients with influenza virus infection was significantly lower than that in patients with bacterial infection [20]. In this study, the expressions of CD64 on neutrophils in lobar pneumonia and bronchopneumonia were significantly higher than that in herpangina caused by Coxsackie A virus, which may prove the aforementioned conclusion, suggesting that CD64 can be used to distinguish bacterial infection from viral infection to a certain degree.

In each group of diseases, the expression of CD64 on neutrophils in bronchial asthma (a non-infectious disease) related to allergy, etc. was obviously higher than that in the normal control group, which indicated that the increase in the expression of CD64 on neutrophils is not only caused by the infection of pathogenic microorganisms, but also resulted from allergen and other factors that can cause inflammation, but the degree of increase is lower than that caused by bacteria and viruses [10].

The expressions of CD64 on neutrophils caused by mycoplasmas, viruses, bacteria and non-pathogenic microorganisms were compared in this study. However, due to the limitation in pathogen detection, specific mycoplasma, viruses, bacterial species and mixed infections failed to be refined. It cannot be excluded that mycoplasma pneumonia is mixed with infections of bacteria, viruses or other pathogens, and that lobar pneumonia and bronchopneumonia mixed with undetected mycoplasma infection [21, 22]. Therefore, further study involving more varieties of pathogenic diseases should be conducted in the future to reinforce the value of CD64 index in the study of these diseases.

In conclusion, CD64 indexes on neutrophils in children with mycoplasma pneumonia, herpangina, lobar pneumonia and bronchopneumonia are increased in turn, suggesting that CD64 index can provide a reference for the clinical diagnosis of mycoplasma pneumonia.

Disclosure of conflict of interest

None.

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