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Efficiency of interleukin 6 and interferon gamma in the differentiation of invasive pulmonary aspergillosis and pneumocystis pneumonia in pediatric oncology patients

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SUMMARY

Objective: Invasive pulmonary aspergillosis (IPA) and Pneumocystis pneumonia (PCP) are two types of pulmonary fungal infection that are not easy to differentiate. The purpose of this study was to investigate the role of inflammatory cytokines in the differential diagnosis of IPA and PCP.

Methods: A total of 227 pediatric oncology patients diagnosed with acute pneumonia were enrolled. They were divided into three groups: IPA, PCP, and 'others'. The cytokine levels in these groups were compared, including interferon (IFN)-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-10, IL-6, IL-4, and IL-2.

Results: Of the six cytokines, only IL-6 and IFN-γ levels were elevated in patients with acute pneumonia. IL-6 was comparable between patients with IPA and PCP (52.0 pg/ml vs. 25.8 pg/ml, $p = 0.092$), while IFN-γ was much higher in patients with PCP (19.9 pg/ml vs. 8.9 pg/ml, $p = 0.001$). The accuracy of IL-6 and the ratio of IL-6/IFN-γ in predicting IPA were 69.0% and 72.0%, respectively, while the accuracy of IFN-γ to predict PCP was 67.2%. IL-6 >140 pg/ml and IL-6/IFN-γ >9.0 presented specificities of 90% in predicting IPA, while IFN-γ >40 pg/ml presented specificity of 90% in predicting PCP.

Conclusions: IL-6 is predominantly elevated in IPA, while IFN-γ is significantly increased in PCP. These are helpful tools for the differential diagnosis of IPA and PCP.

1. Introduction

Pulmonary infection is a common cause of mortality among pediatric oncology patients, especially those with neutropenia. Fungi are common pathogens, with an increasing morbidity and high mortality.[1] For example, patients with hematological malignancies are the population most frequently affected by invasive aspergillosis,[2] and aspergillosis is the most common fungal infection in children, accounting for about 75% of invasive fungal disease.[3] *Pneumocystis jirovecii* is a ubiquitous fungus that can cause significant respiratory disease in immunocompromised hosts, with a mortality rate of 35–55% in HIV-negative patients.[4] Both of these fungi are common in pediatric oncology patients after intensive chemotherapy. However, due to the non-specific clinical manifestations and the poor sensitivity or specificity of most conventional laboratory tests and radiological imaging, the diagnosis is often difficult and delayed.[5,6]

Although these organisms can be identified using light microscopy, immunofluorescence, or molecular methods,[7] it is often difficult to obtain the necessary samples from these patients, such as bronchoalveolar lavage fluid, induced sputum, and lung biopsy specimens.[8,9] Recently, Samuel et al. reported that the PCR results for Pneumocystis pneumonia (PCP) using upper respiratory samples presented high consistency with those obtained using lower respiratory samples in children, which may make the diagnosis of PCP easier.[10] However, wider implementation of PCR on upper respiratory samples for the diagnosis of PCP is warranted to confirm its accuracy.

Inflammatory cytokines including interleukin (IL)-6, IL-10, tumor necrosis factor (TNF), and interferon (IFN)-γ are important biomarkers for distinguishing infections caused by various pathogens. A prospective study was previously conducted on the role of rapid cytokine profile determination by flow cytometry in pediatric hematology–oncology patients who had experienced a febrile illness since 2005. Previous studies have shown that these

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cytokines are very helpful in discriminating Gram-positive and Gram-negative bacteremia, viral and bacterial infections.[11,12] In order to investigate the role of inflammatory cytokines in differentiating pulmonary infections caused by Aspergillus and *P. jirovecii*, the clinical data of patients diagnosed with invasive pulmonary aspergillosis (IPA) and PCP were collected retrospectively and the cytokine profiles of the two entities were compared.

2. Patients and methods

2.1. Data collection

From January 2011 through June 2015, patients in the Department of Hematology–Oncology of the Children's Hospital of Zhejiang University School of Medicine diagnosed with acute pneumonia and with positive microbiological results were enrolled. The patients were divided into three groups according to the pathogen causing the pneumonia: IPA group, PCP group, and 'others' group. The 'others' group included patients with pneumonia caused by pathogens other than Aspergillus and *P. jirovecii*. Fifty pediatric oncology patients who underwent Th1/Th2 cytokine measurement at admission and who were without symptoms or signs of infection served as a control group. Information including patient demographic characteristics and clinical and laboratory findings at diagnosis was collected for analysis in this study.

2.2. Definition of IPA and PCP

The diagnosis of IPA was obtained when the following criteria were fulfilled: (1) host factors such as neutropenia, corticosteroid or immunosuppressant use, or chemotherapy; (2) clinical symptoms including cough, respiratory distress, dyspnea, and fever resistant to antibiotics for more than 72 h; (3) pulmonary computed tomography (CT) scan showing typical signs, such as nodular lesions with or without halo, cavities, and air crescent sign; (4) positive result for serum galactomannan (GM) antigen, direct stain, or culture as mycological evidence of IPA; (5) empirical therapy and definitive therapy with one or a combination of antifungal drugs was effective.[13]

The clinical diagnosis of PCP was retained when (1) at least two of the three following signs were present: non-productive cough or cough producing clear sputum, fever, and dyspnea increasing over time; (2) chest X-ray revealed the characteristic diffuse infiltrations in both lung fields, or a chest high-resolution CT scan showed diffuse frosted glass opacities and thickened alveolar sputum in both lungs; (3) a favorable outcome was obtained under co-trimoxazole therapy, provided that no other infectious agent was found.[14]

2.3. Th1/Th2 cytokine measurement

The Th1/Th2 cytokines were measured at diagnosis. Concentrations of IFN-γ, TNF-α, IL-10, IL-6, IL-4, and IL-2 were determined quantitatively using a cytometric bead array (CBA) kit (CBA Human Th1/Th2 Cytokine Kit II; BD Biosciences, San Jose, CA, USA), as described previously.[15]

2.4. Statistical analysis

Comparisons were performed using the Chi-square test or Fisher's exact test for categorical variables, and using the Mann–Whitney *U*-test (for two groups) or Kruskal–Wallis *H*-test (for three groups) for continuous variables. Receiver operating characteristic (ROC) curves were used to evaluate the discriminatory power (represented by the area under the curve) of IL-6, IFN-γ, and their ratios to differentiate IPA and PCP and to determine the cut-off values with optimal sensitivity and specificity. All statistical analyses were performed using IBM SPSS Statistics version 20.0 software (IBM Corp., Armonk, NY, USA). A p-value of <0.05 was considered to be statistically significant.

3. Results

3.1. Patient characteristics

A total of 277 pediatric oncology patients (227 patients with acute pneumonia and 50 control cases) were included in this retrospective analysis: 115 patients had IPA, 37 had PCP, 75 had pneumonia caused by other pathogens (50 by *Mycoplasma pneumoniae*, 15 by bacteria, and 10 by viruses and others), and 50 were control cases without symptoms or signs of infection. The demographic characteristics of the IPA, PCP, and other pneumonia groups are shown in Table 1. The age and sex distribution were comparable between the IPA and PCP groups. Acute lymphoblastic leukemia and acute myeloid leukemia were the major underlying diseases in this cohort. More than 70% of patients with IPA were in a neutropenic state when the diagnosis was made, while only about one-third of patients with PCP were neutropenic. On the other hand, more patients with PCP needed oxygen support

Table 1
Comparisons of demographic characteristics and laboratory findings between patients with IPA and PCP

Characteristics	IPA	PCP	Other pneumonia	p-Value[a]
Sex, male/female	66/49	24/13	45/30	0.421
Age, years, median (range)	7.3 (0.9–15.4)	5.3 (1.3–15.9)	4.0 (1.0–12.3)	0.072
Underlying disease				0.763
ALL	99 (86.1%)	32 (86.5%)	53 (70.7%)	
AML	14 (12.2%)	3 (8.1%)	11 (14.7%)	
Neutropenia	81 (70.4%)	13 (38.2%)	7 (9.3%)	<0.001
Oxygen support	18 (15.6%)	22 (59.5%)	5 (6.7%)	<0.001
Death	2 (1.7%)	2 (5.4%)	0 (0%)	0.084
IL-2 (pg/ml)	2.9 (1.0–5.7)	2.7 (1.0–6.0)	3.2 (1.0–7.1)	0.954
IL-4 (pg/ml)	3.0 (1.0–91.0)	3.0 (1.0–80.0)	3.3 (1.2–30.0)	0.507
IL-6 (pg/ml)	52.0 (2.5–2484.0)	25.8 (4.6–3738.1)	13.7 (1.3–552.9)	0.092
IL-10 (pg/ml)	6.7 (1.0–147.0)	7.7 (2.2–117.4)	4.9 (1.7–61.2)	0.701
TNF-α (pg/ml)	2.1 (1.0–63.0)	2.2 (1.0–52.3)	2.2 (1.0–48.0)	0.870
IFN-γ (pg/ml)	8.9 (1.0–1100.6)	19.9 (2.0–186.0)	10.2 (1.2–164.7)	0.001
CRP (mg/l)	52 (1–200)	34 (1–165)	7.0 (1–161)	0.827
PCT (μg/l)	0.22 (0.02–48.7)	0.19 (0.06–8.7)	0.11 (0.02–2.59)	0.903

IPA, invasive pulmonary aspergillosis; PCP, Pneumocystis pneumonia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; CRP, C-reactive protein; PCT, procalcitonin.

[a] Comparison between IPA and PCP groups.