Profiles and Predictive Value of Cytokines in Children with Human Metapneumovirus Pneumonia

Xiang Wen-qing
The Children's Hospital of Zhejiang University School of Medicine

Lin Li
The Children's Hospital of Zhejiang University School of Medicine

bing-han Wang
Zhejiang University School of Medicine

Ahmed Faisal Ali
The Children's Hospital of Zhejiang University School of Medicine

Wei Li (chweige@zju.edu.cn)
The Children's Hospital of Zhejiang University School of Medicine

Research Article

Keywords: Human metapneumovirus, Cytokines, Children, Pneumonia, Predictive value

Posted Date: August 3rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1903382/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background: Human metapneumovirus (HMPV) is an important cause of respiratory tract infections in young children. To evaluate the predictive value of Th1/Th2 cytokines which include IL-2, IL-4, IL-6, IL-10, INF-γ and TNF-α in pneumonia caused by HMPV.

Methods: A retrospective study was performed among 59 pneumonia pediatric patients with HMPV infection and 33 healthy children as the control cohort, which was detected by the immunofluorescence assay, and the Th1/Th2 cytokines were measured by flow cytometry.

Results: When compared with the healthy children, children who were infected with HMPV pneumonia had a significantly lower level of IL-2 (p<0.001) and higher levels of IL-4 ((p<0.001), IL-6 (p=0.001), IL-10 (p<0.001), and IFN-γ (p<0.001). Compared with patients diagnosed with influenza virus A (IVA) or influenza virus B (IVB) infections, HMPV-positive patients had significantly higher levels of IL-4 (p<0.001 and <0.001), IFN-γ (p<0.001 and <0.001), and TNF-α (p<0.001 and 0.016). Moreover, compared with IVA patients, HMPV-positive patients had a significantly lower level of IL-6 (p=0.033). Finally, when comparing cytokine levels among the patients with HMPV pneumonia, IL-6 and TNF-α levels were found to be significantly higher in the severe group than the mild group (p=0.027 and 0.049). The IL-6 and TNF-α were used to differentiate between mild and severe symptoms in children diagnosed with HMPV pneumonia with an AUC of 0.678 (95% CI, 0.526-0.829) and 0.658 (95% CI, 0.506-0.809), respectively.

Conclusions: Our study indicates that IL-2, IL-4, IL-6, IL-10, and IFN-γ may be a reference for auxiliary diagnose HMPV pneumonia in children. IL-4, TNF-α, and IFN-γ may serve as biomarkers to distinguish HMPV from IVA and IVB, whereas IL-6 may be able to differentiate between HMPV and IVA. IL-6 and TNF-α may be used to assessment of severity and prognosis of HMPV infection.

Introduction

Human metapneumovirus (HMPV) is a negative-sense, single-stranded RNA virus that belongs to Metapneumovirus genus within the family Pneumovirinae. It was first isolated from a pediatric patient in the Netherlands in 2001 [1]. It is also one of the most common viruses causing acute upper and lower respiratory tract infections in children, immunocompromised individuals, and elderly [2–5]. HMPV infection most commonly occurs in children below 2 years old [6]. The virus usually causes a series of severe diseases, such as pneumonia and bronchiolitis, and is associated with poor prognosis [7–10]. HMPV is mainly divided into two major groups, A and B, which are further subdivided into four subgroups, namely A1, A2, B1, and B2 [11, 12]. Recently, more HMPV subtypes were identified (A2c, A2b1, and A2b2) [13].

HMPV infection is one of the major causes of pneumonia in children that is associated with high morbidity and mortality [4, 14]. A rapid and correct diagnosis is crucial for the treatment of HMPV pneumonia. In clinical practice, real-time polymerase chain reaction (RT-PCR) is used to directly detect HMPV DNA in children with pneumonia due to high specificity and sensitivity of the test [15, 16]. However,
previous studies have shown that pneumonia caused by HMPV is often combined with other respiratory viruses, such as respiratory syncytial virus (RSV), influenza virus, etc. So the detection of HMPV alone is not sensible to establish the relationship between HMPV and pneumonia in case of co-infection [16, 17]. HMPV preferentially targets ciliated epithelial cells of the human respiratory tract and causes a broad spectrum of respiratory illnesses [18]. It is well-known that Th1/Th2 cytokines play an important role in anti-infection immunity after infection, and our previous study suggested that serum Th1/Th2 cytokines may be useful biomarkers in infectious patients [19–21]. Since serum Th1/Th2 profiles and levels are altered in infected patients, they can be used to quickly identify infectious exist in the early stage and determine the severity of the disease. In this article, we studied the clinical application value of Th1/Th2 cytokines as a reference of auxiliary diagnostic and assessment of disease severity and prognosis of HMPV infection in children with pneumonia.

Materials And Methods

Study design and patients

We conducted a retrospective study among the children who were diagnosed with pneumonia from May 2012 to June 2019. The study included 59 children with HMPV pneumonia and 33 healthy children as the control cohort. This study has been approved by the medical ethics committee of Children's Hospital of Zhejiang University School of Medicine. Written informed consents were obtained from parents or guardians of the patients involved in the study. Patients who met the following criteria were enrolled: (1) children under the age of 5 years; (2) primarily diagnosed with pneumonia [22]. At the onset of pneumonia, throat swab specimens and blood samples were taken for microbiological analyses and serum Th1/Th2 cytokines determination. According to guidelines for the management of community-acquired pneumonia in children of the People's Republic of China (2013 Edition) [23], the HMPV-positive patients were divided into 2 groups: mild and severe group.

Detection of HMPV

HMPV was detected by immunofluorescence assay (Diagnostic Hybrids INC, Ohio, USA) in throat swabs. All operations were conducted according to the manufacturer's instructions.

Detection of IVA and IVB

Influenza virus A (IVA) and influenza virus B (IVB) were detected by RT-PCR assay (liferiver, China) in throat swabs. All operations were conducted according to the manufacturer's instructions.

Measurement of Serum Cytokines

1 mL blood sample was collected from every child, and the blood samples were centrifuged at 1000 g for 20 min. The serum was carefully harvested, the Th1/Th2 cytokines were then measured by 320 flow cytometry. Concentrations of IL-2, IL-4, IL-6, IL-10, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) were quantitatively determined by the CBA kit-BDTM CBA Human Th1/Th2 Cytokine Kit II (BD
Biosciences, San Jose, CA). The minimal and maximum limits of detection for all six cytokines were 1.0 and 5000 pg/mL, respectively.

**Statistical analysis**

The measurement data were selected to test normality using the Shapiro-Wilk test or the Kolmogorov-Smirnov test depending on the sample size. The t-test or the Wilcoxon rank-sum test was used to test for differences in cytokines across influenza populations according to their normality. Non-parametric multiple group component differences were tested using the Kruskal-Wallis test and further adjusted for p-values for two-by-two comparisons using Holm’s method. The receiver operating characteristic curve (ROC) was used to analyze the results of mild and severe groups, and the sensitivity and specificity were calculated. The area under the receiver operating characteristic curve (AUC) was used to evaluate the diagnostic effect. The closer the AUC is to 1.0, the better the prediction. The AUC between 0.7 and 0.9 shows moderate accuracy of prediction. When the AUC is above 0.9, the accuracy is relatively high. Statistical analyses were completed using R 4.1.2. We reported 2-sided p values, and p < 0.05 was considered statistically significant.

**Results**

From May 2012 to June 2019, a total of 59 patients tested positive for HMPV, about 75% of the patients were below six months old, and 92.4% of the patients were below two years old.

The levels of six cytokines in the HMPV pneumonia group and normal control group are shown in Fig. 1. HMPV pneumonia group had lower IL-2 (median levels, pg/ml: 3.40 vs 5.80, p < 0.001), higher IL-4 (median levels, pg/ml: 3.00 vs 1.40, p < 0.001), IL-6 (median levels, pg/ml: 8.10 vs 4.10, p = 0.001), IL-10 (median levels, pg/ml: 9.20 vs 2.40, p < 0.001) and IFN-γ (median levels, pg/ml: 8.70 vs 4.60, p < 0.001), whereas no significant difference of TNF-α levels was found between the two groups (median levels, pg/ml, 2.40 vs 2.30, p = 0.160).

In order to confirm the differences of cytokines profiles between HMPV and influenza virus, 131 IVA- and 41 IVB-positive patients were enrolled in this study, and six cytokines were identified in IVA or IVB positive children. The cytokine levels in the HMPV, IVA, and IVB groups are shown in Fig. 2. Compared with IVA and IVB patients, HMPV-positive patients had significantly higher levels of IL-4, TNF-α, and IFN-γ (median levels, pg/ml, IL-4: HMPV = 3.00, IVA = 1.90 (p < 0.001), and IVB = 2.00 (p < 0.001); TNF-α: HMPV = 2.40, IVA = 1.90 (p < 0.001), and IVB = 2.10 (p = 0.016); IFN-γ: HMPV = 8.70, IVA = 5.55 (p < 0.001), and IVB = 5.20 (p < 0.001)). Compared with IVA patients, patients with HMPV had a significantly lower level of IL-6 (median levels, pg/ml, 8.10 vs 17.40, p = 0.033). In addition, compared with IVA patients, IVB-positive patients had significantly lower levels of IL-6 (median levels, pg/ml, 7.90 vs 17.40, p = 0.033) and IL-10 (median levels, pg/ml, 6.60 vs 11.00, p = 0.017). No difference was found in IL-2 among the three groups (median levels, pg/ml, HMPV = 3.40, IVA = 3.00, IVB = 3.00, p = 0.449).
Among the HMPV-positive patients, 39 patients comprised the mild group, and 20 patients comprised the severe group. As shown in Fig. 3, IL-6 and TNF-α were found to be significantly higher in the severe group than mild group (median levels, pg/mL IL-6: 15.35 vs 5.30, p = 0.027; TNF-α: 2.90 vs 5.30, p = 0.049). No significant differences of other inflammatory cytokines (IL-2, IL-4, IL-10, and IFN-γ) levels were found between these two groups (median levels, pg/mL, IL-2: severe = 3.25, mild = 3.70, p = 0.400; IL-4: severe = 3.10, mild = 2.90, p = 0.126; IL-10: severe = 9.95, mild = 7.90, p = 0.511; IFN-γ: severe = 10.25, mild = 8.10, p = 0.164).

To confirm the differential diagnostic value of inflammatory cytokine levels in the severity of HMPV pneumonia in children, we used ROC-analysis to compare the six cytokines (Fig. 4). The analysis results indicated IL-6 and TNF-α were effective biomarkers to differentiate between mild and severe symptoms in children diagnosed with HMPV pneumonia with an AUC of 0.678 (95% CI, 0.526–0.829) and 0.658 (95% CI, 0.506–0.809), respectively. IL-6 ≥ 7.20 pg/mL had a sensitivity of 85.0% and a specificity of 59.0% for severe HMPV pneumonia. TNF-α ≥ 2.50 pg/mL had a sensitivity of 70.0% and a specificity of 64.1% in differentiating between severe and mild HMPV pneumonia.

**Discussion**

Sensitive PCR tests are now widely used to detect respiratory viruses, and both the sensitivity and specificity are better than diagnostic methods used in the past. However, even after successful treatment, half of PCR tests are still positive, which results in an inability to assess disease progression and monitor it dynamically due to hysteresis [24]. In addition, molecular diagnostic methods such as RT-PCR still require standardization [25]. So, we aimed to evaluate the predictive value of Th1/Th2 cytokines in HMPV pneumonia.

To confirm the immune status of children with HMPV infection and predictive ability of Th1/Th2 cytokines in children with HMPV pneumonia, 59 children with a single infection of HMPV were enrolled in this study. Compared with the HMPV pneumonia group and healthy control group, the former had a significantly lower level of IL-2 and higher levels of IL-4, IL-6, IL-10, and IFN-γ. In addition, IL-6 and TNF-α showed increased expression with severe group. Unlike previous studies, our results showed that the level of IL-2 was significantly lower in the HMPV-infected group, which may because IL-2 is very important for maintaining and generating regulatory T cells, augmenting natural killer cells activity, and inhibiting the formation of granulocyte-macrophage colony. Hence, after infection, the level of IL-2 often decreases [20, 21]. IL-10 can deactivate the macrophage cells. The function and proliferation of T cells and natural killer cells can directly be inhibited by IL-10. Furthermore, the growth and differentiation of B cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells can be regulated by IL-10 [26, 27]. Alvarez et al. reported that up-regulation of IL-10 was found in mice with HMPV infection [28]. IL-6 is a major pro-inflammatory mediator that induces acute-phase responses and contributes to host defense against infection and tissue damage. Respiratory syncytial virus (RSV), which is closely related to HMPV. It had demonstrated a positive correlation between high IL-6 level and the severity of RSV [29]. In addition, there was a research showed HMPV induced a more severe disease in mice than that of RSV [30]. These
results were, to some extent, associated with higher level of IL-6. Consistent with our findings, IL-6 play an important role in HMPV pathogenicity, which may account for the level of IL-6 was significantly increased in the severe group [31]. The relationship between TNF-α and HMPV has rarely been mentioned in previous studies, but some studies have shown that the expression of TNF-α is increased in RSV-infected patients [32]. In our research, the expression level of TNF-α was significantly increased in the severe group, the mechanism may be similar to RSV, but needs to be further explored. TNF-α may be a predictor of the severity of HMPV. The previous research showed that excessive expression of TNF-α can activate the signal transducer and activator of transcription 3 (STAT3) pathway through NF-κB-mediated IL-6. Unconstrained TNF-α production leads to the excessive activation of inflammatory cytokines, forming a cytokine storm [33]. This may be the reason for the high expression of TNF-α in the SS group than that of MS group. Consistent with previous studies, our results indicated that IL-4 levels were elevated in HMPV-infected group. IL-4 is a potent inflammatory response activator. Previous studies have shown that IL-6 may increase IL-4 and TNF-α production to induce inflammatory responses during the Th2 differentiation process [34]. However, IL-4 secreted by Th2 helper cells may be inhibited when proinflammatory cytokine levels are elevated. On the other hand, increased IL-4 level may induce suppression of proinflammatory cytokines during the late immune response phase [31, 32]. This may lead to various sensitivities among cytokines that may be helpful to differentiate multiple respiratory viruses. Interestingly, it is speculated that a balanced Th1 type immunity may lead to the clearance of HMPV through activation of IFN-γ secreted by T cells [35].

The hospitalization rate of HMPV infection was similar to that of influenza, using cytokines as biomarkers may be able to differentiate between HMPV and IV. In the early period of influenza-virus infection, NK cells produce IFN-γ. In the subsequent immune response, T cells are the major producer of IFN-γ. Influenza-specific effector CD8+ T cells produce a series of cytokines, including IFN-γ and TNF-α through a variety of antigen-dependent pathways [36]. Due to less studies on the differences of cytokine profiles between HMPV and IV, our findings suggested that the levels of IFN-γ and TNF-α are higher than those of influenza virus may be the two viruses have different inflammatory mechanisms, which require further research. Apart from of IL-6, IL-1β, IFN-γ, TNF-α and IL-8, few studies reported increased levels of IL-4 after influenza virus infection [36, 37]. This rule consists with our findings that HMPV-positive patients had a significantly higher level of IL-4. According to a study on influenza A virus, the levels of IL-6 in the site of initial virus infection were increased persistently for 6 days, this may be related the level of IL-6 in HMPV-positive patients was lower than that of IVA-positive patients [37].

Our study also has a little limitation. The sample size is not large enough and the time span is long, so there is a controversy to accuracy of some specimens that have been placed for a long time. In further studies, we plan to increase the detection range and number of samples.

**Conclusion**

In conclusion, the majority of children diagnosed with HMPV were below six months old. Our study suggests that IL-2, IL-4, IL-6, IL-10 and IFN-γ are likely to be a reference for auxiliary diagnosis of HMPV
pneumonia in children. IL-4, TNF-α, and IFN-γ may serve as biomarkers to distinguish HMPV from IVA and IVB, whereas IL-6 may be able to differentiate between HMPV and IVA. IL-6 and TNF-α may also be used to assessment of severity and prognosis of HMPV infection. Finally, the aforementioned cytokines have a potential to be applied in clinical practice and can help doctors to diagnose HMPV pneumonia in the early stage.

Declarations

Author contributions

Wei Li conceived the idea and supervised this work. Wenqing Xiang wrote the manuscript. Lin Li and Binghan Wang performed data processing and analysis. Wenqing Xiang and Ahmed Faisal Ali collected data. All authors reviewed and approved the manuscript.

Funding:

This work was supported by the National Nature Science Foundation of China (81701535), the science and technology projects in Zhejiang Province (LGC21H200004 and 2019C03037) and the Medical Scientific Projects from the Health Department of Zhejiang Province (2018KY455).

Ethics Statement

The study was approved by the Committee on Ethics in the Children's Hospital, Zhejiang University School of medicine (2021-IRB-182).

Consent for publication

All authors are consent to its publication.

Conflicts of Interest:

None declared.

References


3. de Zwart AES, Riezebos-Brilman A, Alffenaar JC, van den Heuvel ER, Gan CT, van der Bij W, Kerstjens HAM, Verschuuren EAM. Evaluation of 10 years of parainfluenza virus, human metapneumovirus,


32. Nguyen TH, Maltby S, Simpson JL, Eyers F, Baines KJ, Gibson PG, Foster PS, Yang M. TNF-α and Macrophages Are Critical for Respiratory Syncytial Virus-Induced Exacerbations in a Mouse Model of


34. Cui AH, Zhao J, Liu SX, Hao YS. Associations of IL-4, IL-6, and IL-12 levels in peripheral blood with lung function, cellular immune function, and quality of life in children with moderate-to-severe asthma. Medicine Baltimore. 2017;96(12):e6265.


**Figures**
Figure 1

Serum cytokine levels in the control group and HMPV pneumonia group. *Control = healthy children group
Figure 2

Cytokine levels in children with IVA (N=131), IVB (N=41), and HMPV (N=59).
Figure 3

Cytokine levels in HMPV-positive children with mild (MS, N=39) and severe pneumonia (SS, N=20).
Figure 4

ROC curve of multiple cytokines prediction model for differentiating between mild (MS, N=39) and severe (SS, N=20) HMPV pneumonia.