The profile of leucocytes, CD3+, CD4+, and CD8+ T cells, and cytokine concentrations in peripheral blood of children with acute asthma exacerbation

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Abstract

Objective: To determine the leucocyte profile and cytokine concentrations in the peripheral blood of children with an acute asthma exacerbation (AAE).

Methods: This descriptive, cross-sectional study enrolled paediatric patients admitted to hospital for AAE. The severity of AAE was assessed using the paediatric asthma score (PAS). Peripheral blood samples were collected for automatic quantification of white blood cell counts, CD3+, CD4+, and CD8+ T cells populations by flow cytometry and cytokine concentrations by flow cytometry-assisted immunoassay.

Results: A total of 127 children with AAE and 30 healthy control subjects were included in the study. The proportion of paediatric patients with decreased CD3+, CD4+ and CD8+ T cells was significantly higher in those with severe AAE compared with those with mild-to-moderate AAE. The concentrations of interleukin (IL)-2, IL-8, IL-12, and IL-4 in paediatric patients with rhinovirus infection were significantly higher than in those without rhinovirus infection. IL-2, IL-4, IL-6, TNF-α and GM-CSF concentrations during AAE were significantly lower than control. IL-5 and IL-13 concentrations during AAE were significantly higher than control.

Conclusions: The decrease of CD3+, CD4+, CD8+ T cells and IL-2, IL-4, IL-6, TNF-α, and GM-CSF combined with the increase of IL-5 and IL-13, were associated with AAE in children with asthma.

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Introduction

Asthma is a chronic respiratory disease characterized by airway inflammation and bronchial hyperresponsiveness associated with reversible airway obstruction. In children, asthma is a common chronic respiratory disease.[1] In recent decades, the prevalence of asthma in children has increased constantly worldwide.[2] Increasing evidence suggests that it might be due to the interaction between different factors such as the environment (e.g. global urbanization and air pollution) and a genetic disorder.[3,4] Many inflammatory cells, cellular components, and signalling pathways are involved in the pathogenesis of asthma.[5] Among them, the role of T lymphocytes with their inflammatory cytokines has been discussed widely.[6–8]

Cytokines are preinflammatory and inflammatory peptides produced mainly from T lymphocytes and other cells such as eosinophils, macrophages, and fibrolasts.[9] Cytokines play a crucial role in modulating airway inflammation and in remodelling the airway structure of patients with asthma, especially in severe or refractory asthma.[10] The features of inflammatory cells and cytokines in the airways might define the clinical and pathological phenotypes of asthma patients.[8,11] Recently, the role of cytokines related to T lymphocytes in uncontrolled asthma, especially in viral infection-induced asthma exacerbation, has been demonstrated.[12,13] However, their role in children with acute asthma exacerbation (AAE) has not been well clarified.

This present study aimed to characterize the inflammatory cell profile and cytokine concentrations in the peripheral blood of children with asthma during AAE.

Patients and methods

Patient population

This descriptive, cross-sectional study consecutively enrolled children with AAE who were hospitalized in the Department of Respiratory Diseases, National Hospital of Paediatrics, Hanoi, Vietnam between February 2014 and April 2015. The inclusion criteria were as follows: (i) <17 years old; (ii) hospitalized for AAE where the diagnosis of AAE was based on the criteria recommended by the Global Initiative for Asthma.[14] The exclusion criteria were as follows: (i) concomitant presence of other severe cardiovascular or gastroenterological diseases, renal or hepatic dysfunction; (ii) concomitant presence of acute respiratory distress syndrome or severe pneumonia.

A group of healthy children in the same age group who received a routine health check at the National Hospital of Paediatrics were used as the control group.

This study was approved by the Medical Ethics Committee of the National Hospital of Paediatrics (no. 965B/BV.NTW-VNCSKTE). The parents or legal guardians of the children with AAE and the healthy control subjects provided written informed consent before the start of the study.

Diagnosis of asthma severity

The diagnosis of asthma was based on international recommendations for children ≥ 5 years old.[14] The severity of AAE was assessed according to the paediatric asthma score (PAS).[15,16] The examination consisted of five components: (i) respiratory rate; (ii) oxygen requirement; (iii) respiratory muscle retractions; (iv) auscultation; and (v) dyspnoea. Each component was scored from 1

to 3 according to the severity of the symptoms as follows: (i) respiratory rate: ≤ 34 (2–3 years), ≤ 30 (4–5 years), ≤ 26 (6–12 years), ≤ 23 (>12 years) = 1; 35–39 (2–3 years), 31–35 (4–5 years), 27–30 (6–12 years), ≤ 24–27 (>12 years) = 2; ≥ 40 (2–3 years), ≥ 36 (4–5 years), ≥ 31 (6–12 years), ≥ 28 (>12 years) = 3; (ii) oxygen requirements (peripheral capillary oxygen saturation [SpO_2]): >95% on room air = 1; 90–95% on room air = 2; <90% on room air or on any oxygen = 3; (iii) respiratory muscle retractions: none or intercostal = 1; intercostal and substernal = 2; intercostal, substernal and supraclavicular = 3; (iv) auscultation: normal breath sounds to end-expiratory wheeze only = 1; expiratory wheezing = 2; inspiratory and expiratory wheezing = 3; (v) dyspnoea: speaks in sentences = 1; speaks in partial or short sentences = 2; speaks in single words/short phrases/grunting = 3. The total scores ranged from 5–7 for mild asthma exacerbation, 8–11 for moderate asthma exacerbation and 12–15 for severe asthma exacerbation.

Quantifying white blood cells

Blood samples (2 ml) were collected through venipuncture and drawn into tubes containing 10% ethylenediaminetetra-acetic acid. All blood samples were kept refrigerated during a short storage period and immediate transportation to the laboratory. The white blood cells in peripheral blood samples were counted automatically using an ADVIA® 2120i Haematology System (Siemens Healthcare, Erlangen, Germany) by the Haematology Laboratory of the National Hospital of Paediatrics. The normal value of leucocytes in children classified by age group was: ≤ 2 years: $< 10.6 \times 10^3/mm^3$ (6–$17 \times 10^3/mm^3$); ≤ 4 years: $< 9.1 \times 10^3$ (5.5–$15.5 \times 10^3/mm^3$); ≤ 6 years: $8.5 \times 10^3/mm^3$ (5.0–$14.5 \times 10^3/mm^3$); ≤ 8 years: $8.3 \times 10^3/mm^3$ (4.5–$13.5 \times 10^3/mm^3$); ≤ 10 years: $8.1 \times 10^3/mm^3$ (4.5–$13.5 \times 10^3/mm^3$).

Quantifying CD3+, CD4+ and CD8+ cells by flow cytometry

Flow cytometry was used to determine the T lymphocyte subtypes (CD3+, CD4+ and CD8+) in peripheral blood by the Haematology Laboratory of the National Hospital of Paediatrics. A 50-µl sample of peripheral blood was mixed with 20 µl of fluorescent conjugated monoclonal antibodies (fluorescein isothiocyanate mouse anti-human CD3; phycoerythrin/Cy7 mouse anti-human CD4; allophycocyanin/Cy7 mouse anti-human CD8; Abcam, Cambridge, UK) and incubated at room temperature for 15 min. Then 450 µl of BD FACS™ Lysing solution 1 × (BD Biosciences, San Jose, CA, USA) was added to each sample, mixed and incubated at room temperature in the dark for 15 min. The fluorescent-labelled leucocytes were passed through a BD FACSCanto™ II flow cytometer (BD Biosciences). Based on the size, nuclear density and fluorescent colours, each individual cell population was identified and quantified. The normal value of the T lymphocyte subtypes in children, classified by age group, are: CD3+ (cells/mm^3): <1 year: ≥ 2500, 1–2 years ≥ 2100, >2 and ≤ 6 years: ≥ 1400, >6 and ≤ 12 years: ≥ 1200; CD4+ (cells/mm^3): <1 year: ≥ 1400, 1–2 years: ≥ 1300, >2 and ≤ 6 years: ≥ 700, >6 and ≤ 12 years: ≥ 650; CD8+ (cells/mm^3): <1 year: ≥ 500, 1–2 years: ≥ 620, >2 and ≤ 6 years: ≥ 490, >6 and ≤ 12 years: ≥ 370.

Quantifying cytokines by flow cytometry-assisted immunoassay

Venous blood samples (2 ml) from patients and control subjects were drawn into tubes without anticoagulant and transferred directly to the laboratory for analysis. Quantification of the cytokine concentration was performed in the Immunology Laboratory of the Military Medical Academy 103, Hanoi, Vietnam.

Monoclonal antibodies (Bio-Rad, Hercules, CA, USA) were used for eleven cytokines related to type 1 helper T (T_h1) cells (interleukin [IL]-2, IL-8, IL-12, interferon [IFN]-γ, and tumour necrosis factor [TNF]-α), regulatory T cells (T_{reg}; IL-10), and type 2 helper T (T_h2) cells (IL-4, IL-5, IL-6, IL-13, and granulocyte-macrophage colony-stimulating factor [GM-CSF]; Bio-Plex® human cytokines; Bio-Rad). The tests were conducted using a flow cytometry-assisted immunoassay provided by the Bio-Plex® Multiplex System (Bio-Rad). Blood samples without anticoagulant were placed in an incubator (37°C) for 30 min, then centrifuged at 1000 g for 5 min at 4°C (ScanSpeed 416; LaboGene, Lynge, Denmark). After removing the fibrin plug, the samples were centrifuged again at 4°C for 15 min at 10 000 g (ScanSpeed 416; LaboGene) and prepared for freezing. All samples were stored at −80°C until tested. Cytokines were detected by immunofluorescence using the sandwich technique on the surface of polystyrene microspheres in accordance with the manufacturer's protocol (Bio-Rad). The reduced immunoglobulin G was purified and coupled to the activated microspheres. Microspheres were incubated with biotinylated antibodies for IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IFN-γ, TNF-α and GM-CSF overnight with shaking at room temperature. Microspheres were then washed, resuspended in 1 × phosphate-buffered saline (PBS; pH 7.4), then transferred to tubes for acquisition on the flow cytometer (Bio-Plex® 200 System; Bio-Rad). Based on the density of the fluorescence emitted from the microspheres incubated with known concentrations of cytokines, the cytokines in the study samples were quantified using Bio-Plex Manager™ Software (Bio-Rad). For each cytokine, the curve fit parameters were established with international cytokine standards and stored in a post analysis database file. These master curves were used to analyse the immunoassay data.

Statistical analyses

All statistical analyses were performed using the SPSS® statistical package, version 22.0 (SPSS Inc., Chicago, IL, USA) for Windows®. Qualitative variables were expressed as numbers or percentages. Quantitative variables were presented as mean ± SD or median (min – max). The standard distribution was tested by using the Skewness–Kurtosis test. The Mann–Whitney U-test was used to evaluate the correlation between cytokine levels, CD cell counts, and the severity of asthma. The Z-test was used to compare the frequency between groups. The Kruskall–Wallis test was used for multiple comparisons. A P-value < 0.05 was considered statistically significant.

Results

The study enrolled 127 children with AAE with a mean ± SD age of 58 ± 38 months (range 7–165 months) who were hospitalized in the National Hospital of Paediatrics, Hanoi, Vietnam and 30 healthy control subjects with a mean ± SD age of 56 ± 44 months (range 1–168 months). There was no significant difference between the mean age or the age distribution between the two groups (data not shown). The clinical and demographic characteristics of the children with AAE are shown in Table 1. The majority of the patients with AAE were 2–5 years old, with a predominance of males. The serodiagnosis of rhinovirus was positive in 64 of 117 (54.7%) patients. Sixty-three of 127 (49.6%) patients had severe asthma exacerbation compared with 52 (40.9%) patients with moderate and 12 (9.4%) with mild asthma exacerbation (Table 1). There was no significant difference in the length of hospital stay between children with mild, moderate and severe AAE based on PAS.

The results of peripheral blood analysis showed that 103 of 127 (81.1%) patients had

Table 1. Demographic and clinical characteristics of children with acute asthma exacerbation (AAE) ($n = 127$) who participated in this study to characterize the inflammatory cell profile and cytokine concentration in the peripheral blood during AAE.

Characteristics		Frequency[a]	Proportion (%)
Age		$n = 127$	
	<2 years	24	18.9
	2–5 years	58	45.7
	>5 years	45	35.4
Sex		$n = 127$	
	Male	83	65.4
	Female	44	34.6
Atopic allergy		$n = 123$	
	Eczema	46	37.4
	Allergic rhinitis	86	69.9
	Food	12	9.8
	Others	13	10.6
Rhinovirus infection		$n = 117$	
	Positive	64	54.7
	Negative	53	45.3
PAS		$n = 127$	
	Mild	12	9.4
	Moderate	52	40.9
	Severe	63	49.6
Length of hospital stay Classified by days		$n = 127$	
	1–3 days	41	32.3
	>3–5 days	59	46.5
	>5 days	27	21.3
Classified by PAS		Days	Statistical significance[b]
	Mild	5.0 ± 1.5	NS
	Moderate	4.5 ± 2.0	
	Severe	5.0 ± 2.0	

Data presented as n of patients (%) or mean $\pm$ SD.
[a]Baseline data missing for some patients.
[b]Kruskall–Wallis test.
PAS, paediatric asthma score (mild 5–7, moderate 8–11, severe 12–15); NS, no significant between-group differences ($P \geq 0.05$).

increased leucocytes compared with 24 of 127 (18.9%) patients who had normal values (Table 2). A total of 38 of 122 (31.1%) patients had increased eosinophils compared with 84 of 122 (68.9%) patients who had normal values. A total of 84 of 127 (66.1%) patients had increased neutrophils compared with 43 of 127 (33.9%) patients who had normal values. The percentage of patients with increased leucocytes varied with the severity of AAE. It was highest in patients with severe asthma exacerbation in comparison with mild or moderate asthma exacerbation (87.3% [$n = 55$] versus 66.7% [$n = 8$] and 76.9% [$n = 40$], respectively), but it was only significantly different between the mild and severe asthma exacerbation groups ($P < 0.05$). The percentage of

Table 2. Leucocyte profile classified by the paediatric asthma score (PAS) for the acute asthma exacerbation.

Cell types		PAS				Statistical significance[a]
		Mild	Moderate	Severe	Total	
Leucocytes	Normal	4 (33.3)	12 (23.1)	8 (12.7)	24 (18.9)	NS*
n = 127	Increased[b]	8 (66.7)	40 (76.9)	55 (87.3)	103 (81.1)	P < 0.05** NS***
Eosinophils	Normal	7 (58.3)	35 (70.0)	42 (70.0)	84 (68.9)	NS*
n = 122[e]	Increased[c]	5 (41.7)	15 (30.0)	18 (30.0)	38 (31.1)	NS**
						NS***
Neutrophils	Normal	7 (58.3)	21 (40.4)	15 (23.8)	43 (33.9)	NS*
n = 127	Increased[d]	5 (41.7)	31 (59.6)	48 (76.2)	84 (66.1)	P < 0.01**
						P < 0.05***

Data presented as *n* of patients (%).
[a]Kruskall–Wallis test; *moderate versus mild; **severe versus mild; ***severe versus moderate.
[b]>10 × 10³/mm³; [c]>4%; [d]>45% for children <5 years and >65% for children ≥5 years.
[e]Data missing for five patients.
Paediatric asthma score: mild 5–7, moderate 8–11, severe 12–15.
NS, no significant between-group difference ($P \geq 0.05$).

patients with increased eosinophils was not significantly different between the three asthma exacerbation groups. The percentage of patients with severe asthma exacerbation and an increased neutrophil count was significantly higher than for patients with mild or moderate asthma exacerbation (76.2% [n = 48] versus 41.7% [n = 5] and 59.6% [n = 31], respectively; P < 0.01 and P < 0.05, respectively).

The proportion of patients with decreased CD3+ T cells in the severe AAE group was significantly higher than the mild-to-moderate AAE group (50.0% [n = 15] versus 23.8% [n = 5], respectively; P = 0.03) (Table 3). The proportion of patients with decreased CD4+ T cells in the severe AAE group was significantly higher than the mild-to-moderate AAE group (53.3% [n = 16] versus 35.0% [n = 7], respectively; P = 0.01). The proportion of patients with decreased CD8+ T cells in the severe AAE group was significantly higher than the mild-to-moderate AAE group (27.6% [n = 8] versus 4.8% [n = 1], respectively; P = 0.02).

The concentrations of IL-2 and IL-8 during AAE were not significantly different compared with those after the exacerbation period. The concentrations of IL-2 during and after AAE were significantly lower than that in the control subjects (P < 0.001; Table 4). The concentrations of IL-10, IL12 and INF-γ during and after AAE were not significantly different compared with control subjects. The concentration of TNF-α during AAE was lower than that after AAE and when compared with the control subjects, but a significant difference was only found in TNF-α concentrations during and after AAE compared with the control subjects (P < 0.05).

The concentration of IL-4 during AAE was significantly lower than after AAE and the concentrations of IL-4 during and after AAE were significantly lower compared with the control subjects (P < 0.001 for both comparisons; Table 4). The concentration of IL-6 during AAE was significantly lower than after AAE and compared with the control subjects (P < 0.05 for both comparisons). The concentration of IL-5 during AAE was significantly higher than after AAE and compared with the control subjects (P < 0.001 for both comparisons). The

Table 3. T lymphocyte profile classified by the paediatric asthma score (PAS) for the acute asthma exacerbation.

T lymphocyte subgroups		PAS			Statistical significance[a]
		Mild-to-moderate	Severe	Total	
CD3+	Normal	16 (76.2)	15 (50.0)	31 (60.8)	P = 0.03
n = 51	Decreased[b]	5 (23.8)	15 (50.0)	20 (39.2)	
CD4+	Normal	13 (65.0)	14 (46.7)	27 (54.0)	P = 0.01
n = 50	Decreased[c]	7 (35.0)	16 (53.3)	23 (46.0)	
CD8+	Normal	20 (95.2)	21 (72.4)	41 (82.0)	P = 0.02
n = 50	Decreased[d]	1 (4.8)	8 (27.6)	9 (18.0)	

Data presented as n of patients (%).
[a]Z-test; severe versus mild-to-moderate asthma exacerbation.
[b]Decreased CD3+ T cells: <2500 for <1 year, <2100 for 1–2 years, <1400 for >2 and ≤6 years, <1200 for >6 and ≤12 years, <100 for >12 years.
[c]Decreased CD4+ T cells: <1400 for <1 year, <1300 for 1–2 years, <700 for >2 and ≤6 years, <650 for >6 and ≤12 years, <530 for >12 years.
[d]Decreased CD8+ T cells: <500 for <1 year, <620 for 1–2 years, <490 for >2 and ≤6 years, <370 for >6 and ≤12years, <330 for >12 years.

Table 4. Profile of cytokine levels in patients during and after the acute asthma exacerbation compared with healthy control subjects.

Cytokines	Patients with acute asthma exacerbation		Control group n = 30	Statistical significance[a]
	During n = 127	After n = 59		
Cytokines related to type 1 helper T cells and regulatory T cells, pg/ml				
IL-2	0.16 (0.05–44.02)	0.16 (0.11–5.90)	0.51 (0.11–67.86)	NS* P < 0.001**
IL-8	5.07 (1.50–88.37)	4.11 (2.23–346.80)	5.07 (0.75–29.62)	NS*·**
IL-10	2.35 (0.01–399.78)	2.21 (0.32–10.27)	1.52 (0.30–43.00)	NS*·**
IL-12	0.01 (0.01–11.98)	0.01 (0.01–5.82)	0.01 (0.01–1.83)	NS*·**
IFN-γ	12.41 (0.21–1056.32)	12.41 (2.77–170.43)	12.40 (2.76–1477.20)	NS*·**
TNF-α	0.43 (0.21–249.91)	0.67 (0.10–393.92)	1.46 (0.32–44.46)	NS* P < 0.05**
Cytokines related to type 2 helper T cells, pg/ml				
IL-4	0.02 (0.01–2.80)	0.16 (0.11–5.9)	0.51 (0.11–67.86)	P < 0.001*·**
IL-5	1.49 (0.01–2.80)	0.02 (0.01–1.11)	0.08 (0.02–14.58)	P < 0.001*·‡
IL-6	0.30 (0.03–40.90)	0.97 (0.03–16.62)	1.03 (1.03–36.63)	NS¥ P < 0.05*·‡
IL-13	2.08 (0.07–33.88)	0.10 (0.10–5.87)	1.24 (0.09–5.89)	P < 0.001*·‡·¥
GM-CSF	0.91 (0.21–717.85)	0.91 (0.22–109.51)	5.73 (0.91–198.30)	NS* P < 0.001**

Data presented as median (min–max).
[a]*Kruskall–Wallis test; during versus after acute asthma exacerbation; **during and after acute asthma exacerbation versus control; ‡during versus control group; ¥after versus control; NS, no significant between-group difference (P ≥ 0.05).
IL, interleukin; IFN, interferon; TNF, tumour necrosis factor; GM-CSF, granulocyte-macrophage colony-stimulating factor.

Table 5. Cytokine concentrations in patients with or without rhinovirus (RV) infection.

	Patients with acute asthma exacerbation		
Cytokines	RV+ $n = 64$	RV− $n = 53$	Statistical significance[a]
Cytokines related to type 1 helper T cells and regulatory T cells, pg/ml			
IL-2	0.25 (0.10–44.02)	0.16 (0.05–38.78)	$P < 0.05$
IL-8	5.97 (2.08–88.37)	3.46 (1.50–21.96)	$P < 0.05$
IL-10	2.35 (0.03–48.61)	2.75 (0.01–399.78)	NS
IL-12	0.81 (0.01–11.98)	0.01 (0.01–5.59)	$P < 0.05$
INF-γ	12.41 (0.21–1056.32)	12.41 (2.43–642.50)	NS
TNF-α	0.43 (0.21–230.19)	0.63 (0.27–249.91)	NS
Cytokines related to type 2 helper T cells, pg/ml			
IL-4	1.14 (0.01–2.80)	0.02 (0.01–1.39)	$P < 0.01$
IL-5	2.11 (0.07–9.49)	0.90 (0.05–7.87)	NS
IL-6	1.03 (0.03–7.24)	0.06 (0.03–40.09)	NS
IL-13	3.01 (0.07–33.88)	1.67 (0.10–5.87)	NS
GM-CSF	5.73 (0.21–717.85)	0.91 (0.21–137.42)	NS

Data presented as median (min–max).
[a]Mann–Whitney U-test.
IL, interleukin; IFN, interferon; TNF, tumour necrosis factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; NS, no significant between-group difference ($P \geq 0.05$).

concentrations of GM-CSF during and after AAE were significantly lower compared with the control subjects ($P < 0.001$). The concentration of IL-13 during AAE was significantly higher than after AAE and compared with the control subjects ($P < 0.001$ for both comparisons). The concentration of IL-13 after AAE was significantly lower compared with the control subjects ($P < 0.001$).

The concentrations of IL-2, IL-8, IL-12, and IL-4 in patients with AAE and positive rhinovirus infection were significantly higher than the concentrations in patients without rhinovirus infection ($P < 0.05$ for all comparisons; Table 5). There were no significant differences in the concentrations of IL-10, INF-γ, TNF-α, IL-5, IL-6, IL-13 and GM-CSF between patients with AAE with and without rhinovirus infection.

The concentrations of cytokines related to T_h1 and T_{reg} (IL-2, IL-8, IL-10, INF-γ, and TNF-α) were not significantly different between patients with mild, moderate and severe AAE based on PAS (Table 6). The concentrations of cytokines related to T_h2 (IL-4, IL-5, IL-6, IL-13, and GM-CSF) were not significantly different between patients with mild, moderate and severe AAE based on PAS.

Discussion

The results of the present study showed that: (i) paediatric patients with AAE had significantly increased leucocyte counts with a predominance of high neutrophil counts especially in those with severe AAE based on the PAS; (ii) the proportion of paediatric patients with decreased CD3+/CD4+/CD8+ T cells in those with severe AAE was significantly higher than in paediatric patients with mild-to-moderate AAE; (iii) the changes in cytokine concentrations depended on AAE, rhinovirus infection, but not on AAE severity based on the PAS.

The results of this present study showed that nearly 55% of paediatric patients with

Table 6. Cytokine concentrations during acute asthma exacerbation classified by the paediatric asthma score (PAS) for the acute asthma exacerbation.

Cytokines	PAS		
	Mild $n = 12$	Moderate $n = 52$	Severe $n = 63$
Cytokines related to type 1 helper T cells and regulatory T cells, pg/ml			
IL-2	0.16 (0.05–13.86)	0.16 (0.05–44.02)	0.16 (0.10–15.99)
IL-8	5.20 (2.68–21.96)	5.01 (2.08–21.90)	5.07 (1.50–88.37)
IL-10	2.06 (0.07–78.07)	2.71 (0.01–57.97)	2.19 (0.03–48.61)
IL-12	0.45 (0.01–11.98)	0.01 (0.01–8.63)	0.02 (0.01–9.56)
INF-γ	12.41 (2.43–461.45)	22.41 (2.43–642.50)	12.41 (0.21–1056.32)
TNF-α	0.33 (0.32–95.70)	0.43 (0.27–249.91)	0.43 (0.21–230.19)
Cytokines related to type 2 helper T cells, pg/ml			
IL-4	0.01 (0.01–1.85)	0.02 (0.01–1.89)	0.02 (0.01–2.80)
IL-5	0.82 (0.28–8.17)	1.81 (0.07–6.85)	1.49 (0.05–9.49)
IL-6	0.53 (0.03–4.85)	0.06 (0.03–40.90)	0.72 (0.03–30.20)
IL-13	3.70 (0.19–4.87)	1.81 (0.60–7.04)	2.08 (0.07–33.88)
GM-CSF	0.22 (0.21–233.21)	0.91 (0.21–188.38)	0.91 (0.21–717.85)

Data presented as median (min–max).
[a]Kruskall–Wallis test; no significant between-group differences ($P \geq 0.05$).
Paediatric asthma score: mild 5–7, moderate 8–11, severe 12–15.
IL, interleukin; IFN, interferon; TNF, tumour necrosis factor; GM-CSF, granulocyte-macrophage colony-stimulating factor.

AAE had a rhinovirus infection, which was confirmed by a serodiagnostic test. Almost half of the paediatric patients had severe AAE. The present study also showed that 81.1% of patients had increased leukocyte counts, 66.1% had increased neutrophil counts and 31.1% had increased eosinophil counts during AAE. None of the 127 paediatric patients had severe pneumonia. These current results suggest that a high neutrophil count without pneumonia might be a characteristic of asthmatic children with AAE. It also suggests that asthmatic children with a rhinovirus infection might have increased leucocyte counts in the AAE period. Previous studies showed that rhinovirus infection increased lower airway responsiveness in allergic and asthmatic subjects.[17,18]

The present study showed that during AAE, the CD3+, CD4+ and CD8+ T cells were normal or decreased in all asthmatic paediatric patients. Fifty percent of paediatric patients with severe AAE had decreased CD3+ T cells, 53.3% had decreased CD4+ T cells, and 27.6% had decreased CD8+ T cells. The proportion of paediatric patients with decreased CD3+, CD4+ and CD8+ T cells in the group with severe AAE was significantly higher than that in the group with mild-to-moderate AAE. This result suggests that the reduction of CD3+, CD4+ and CD8+ T cells might be a good marker for AAE in asthmatic children. However, the T lymphocyte profile in asthmatic children during and after AAE has not been well clarified. A previous study found a reduction in the percentage of CD4+ producing IFN-γ in asthmatic children.[11] In contrast, another study demonstrated that the percentage of CD8+ IFN-γ cells was increased in the period after AAE.[19] Research demonstrated that the percentage of CD3+ cells producing related cytokines was not significantly different

between atopic, non-asthma and control subjects.[20] Similarly, another study demonstrated that the percentage of total CD3+ T cells in peripheral blood was similar in asthmatic patients and healthy control subjects.[21]

The pathophysiology of asthma is characterized by airway inflammation and its severity depends on the inflammatory response. Previous studies demonstrated the significant role of Th$_2$ cells producing IL-4, IL-5, and IL-13, in the pathology of allergic asthma.[22] The concentration of IL-4 during AAE was significantly lower than after AAE and the concentrations of IL-4 during and after AAE were significantly lower compared with the control subjects. The concentration of IL-6 during AAE was significantly lower than after AAE and compared with the control subjects. The concentration of IL-5 in asthma patients during AAE was significantly higher than that after AAE and compared with the healthy control subjects. This suggests that there was a dysregulation of these cytokines during AAE in children. IL-4 and IL-5 play an important role in mediating the progression of allergic airway inflammation and are involved in the differentiation of T cells.[23,24] In patients with allergic asthma, IL-4 is increased in the peripheral blood and bronchoalveolar lavage and it also plays a crucial role in airway hyperresponsiveness.[25–27] The role of IL-4 in children with asthma has been demonstrated by previous studies.[25,28] A previous study showed that, in children with asthma, the concentration of IL-4 depended on the severity of the disease.[29] However, the role of IL-4, IL-5, and IL-13 in AAE has not been clearly demonstrated.[30,31] The present study showed that the concentration of IL-13 was significantly increased in asthmatic children during the AAE period compared with after the AAE and compared with the control subjects. Hence, the discordant changes of IL-13 and GM-CSF during

asthma crisis might be a relevant marker for AAE in children. In the airway, IL-13 induces the contraction of smooth muscle cells and hypersecretion of sputum, especially in patients with asthma.[32,33] GM-CSF plays an important role in severe asthma exacerbation and drug resistance.[34,35]

The results of the present study showed that the concentration of TNF-α during and after AAE was significantly lower than that in control subjects. Previous studies showed that the concentration of TNF-α was significantly increased in children with severe and poorly controlled asthma;[36] and that TNF-α inhibitors could be a potential treatment for refractory asthma.[37,38] In the present study, the concentration of IFN-γ did not change during and after AAE and it was not different compared with the concentration in control subjects. In the present study, the concentrations of IL-2, IL-4, IL-8, and IL-12 were significantly higher in paediatric patients with rhinovirus infection compared with those patients without rhinovirus infection. This result was similar to previous studies.[17,39] The present study showed that the concentrations of all studied cytokines were not significantly different between mild, moderate and severe AAE as evaluated by PAS in asthmatic children.

In conclusion, AAE remains a challenge for respiratory care in children. The results of the present study showed that a reduction in CD+ T cells and IL-2, IL-4, IL-6, TNF-α, and GM-CSF concentrations, combined with an increase of IL-5 and IL-13 concentrations, were associated with AAE in children with asthma. However, more studies in this field are necessary to enrich our knowledge about the role of the inflammatory profile and pathways in children with asthma during acute exacerbation.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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