10.2478/chilat-2014-0009

ORIGINAL ARTICLE

Levels of Inflammatory Cytokines and Chemokines in Hospitalized Children with Sepsis and Pneumonia

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Summary
Introduction. Pneumonia is a common childhood lower respiratory tract infection, which accounts for large number of hospitalization and death among children; its diagnosis is based mainly on clinical signs.
Aim of the study was to measure inflammatory cytokine panels in children with pneumonia, and their correlation with clinically used inflammatory markers.
Materials and methods. We included 20 patients, hospitalized in Children's Clinical University hospital, with systemic inflammatory response syndrome (SIRS) and radiologically confirmed pneumonia from October 2011 to January 2013. In all patients cytokine and chemokine panels and clinical inflammatory markers were measured at the time of admission, after 24 hours and on the time of discharge.
Results. 12 different inflammatory cytokines were measured. sFAS, sVCAM1, IL-8, IL-10, TNF alpha, Eotaxin, G-CSF, IL1ra, IP10 and MCP1 showed statistically significant changes between levels of inclusion in the study and levels after 24 hours. G-CSF, IL-8, IFN gamma, TNF alpha and IL-10 showed also medium strong correlation with clinically used inflammatory markers (PCT, CRO, and IL-6).
Conclusions. Inflammatory cytokines show statistically significant changes during course of treatment, thus they could be used in diagnostics in septic patients with pneumonia, and also could show patients response to therapy.
Key words: Sepsis, systemic inflammatory response syndrome, SIRS, pneumonia, children.

INTRODUCTION

Pneumonia is a common childhood lower respiratory tract infection, it also accounts for large number of hospitalization and death among children below 5 years of age (1). The incidence of community-acquired pneumonia is reportedly 36–40 episodes per 1000 children per year in children younger than 5 years and 11–16 episodes per 1000 children per year in children 5–14 years. In Europe alone, 2.5 million cases of pneumonia occur annually (2). The etiological agents in children are variable, depending on age, in different studies they have been shown to be 7 - 65% bacterial, 37 – 45% viral, 1-23% mixed agents (8, 12). Also, the diagnosis of pneumonia is mostly based on clinical signs. Currently available guidelines state, that diagnosis of pneumonia should be considered if a child has persistent or repetitive fever >38.5 °C together with chest retractions and a raised respiratory rate (3). Radiologically confirmed pneumonia plays a significant role in clinical diagnosis of sepsis in children. According to guidelines by International Consensus Conference on Pediatric Sepsis (4), patient can be diagnosed as septic, if he or she has inflammatory response syndrome (SIRS) together with a confirmed source of infection; this confirmation can also be radiological. Concerning ancillary testing in pneumonia, guidelines state that acute-phase reactants, such as the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), or serum procalcitonin (PCT) concentration, cannot be used as a determinant to distinguish between viral and bacterial pneumonias, but they might be used in hospitalized children with more serious disease, to determinate course of disease and efficacy of treatment (5). Cytokine and chemokine response in children with pneumonia has been more studied during the outbreak of H1N1 influenza period, where in patients with pneumonia, the serum levels of interferon gamma (IFN gamma), interleukin-5 (IL-5), interleukin-6 (IL-6),interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-13 (IL-13), and monocyte chemoattractant protein-1 (MCP-1) were significantly higher than those in patients without pneumonia (7, 11); thus they might be investigated as novel diagnostic markers in children with pneumonia. In Latvia no studies of determining cytokine and chemokine levels in children with pneumonia were performed.

AIM OF THE STUDY

Was to determine inflammatory cytokine and chemokine panels, and their dynamical change in the course of treatment in hospitalized children with systemic inflammatory response syndrome (SIRS) and radiologically confirmed pneumonia, and their correlation with clinically approved inflammatory markers – CRP, PCT and IL-6.

MATERIALS AND METHODS

Patient recruitment took place from October 2011 to January 2013 in Children's Clinical University hospital, Riga, Latvia.

Inclusion criteria were:

1. Hospitalized child with SIRS and sepsis, as determined by International Consensus Conference on Pediatric Sepsis (4): at least two of below mentioned criteria, one of which must be abnormal temperature or leukocyte count:
   - Core temperature >38.5 or < 36.0 C.
   - Tachycardia, defined as a mean heart rate >2SD above normal for age; or for children <1yr old, bradycardia, defined as a mean heart rate <10th percentile for age.
   - Mean respiratory rate >2SD above normal for age.
   - Leukocyte count elevated or depressed for age or >10% immature neutrophils, and
2. Confirmed pneumonia by chest x-ray

Exclusion criteria were antibacterial therapy within the last 48 h, immunodeficiency, chronic liver or kidney illness, vaccination within 5 days before the start of the illness, congenital metabolic defects, chromosomal anomalies, and use of corticosteroids or immunosuppressant medications, Patients exclusion factors from study were obesity, diabetes mellitus, chronic inflammatory diseases, such as rheumatoid arthritis, systemic lupus erythematosus, vasculitis, inflammatory bowel disease, heart diseases, renal or liver diseases, or malignancies and other diseases which are known to be associated with significant changes of anti- and pro-inflammatory biomarkers, including surgery or trauma within the preceding 30 days.

In all patients CRP, PCT, IL-6 and inflammatory cytokine and chemokine panels (soluble apoptosis- stimulating fragment (sFAS), soluble vascular cell adhesion molecule (sVCAM-1), total plasminogen activator inhibitor type 1 (tPAI1), IL-8, IL-10, INF gamma, tumor necrosis factor alpha (TNF alpha), Eotaxin, granulocyte colony-stimulating factor (G-CSF), interleukin-1 receptor antagonist (IL-1ra), interferon-inducible protein-10 (IP10), MCP1) were measured on the time of inclusion, after 24 hours and on the discharge.

Data was analysed, using descriptive statistics, groups were compared, using chi-square, correlations were determined by Pearson correlation coefficient (correlation was considered strong, if r > 0,7, moderately strong if r >0,4); p values of less than 0,05 were considered statistically significant. Data analysis was performed on Statistix 9.

RESULTS

In total 20 patients were included – 11 girls and 9 boys. Median age of patients were 70 months, respectively, 5 years and 10 months (minimal age value 8 months, maximal 207 months (17 years and 3 months), mean age value 86,4). Patients were included in the study on the median 4,5th day of illness (minimal value 2, maximal 8 days, mean 4,75). 10% (2) of patients had severe sepsis, 10% (2) had septic shock, all these patients were treated in the intensive care unit (ICU), and one patient received artificial lung ventilation during ICU stay. 20% (4) patients had pleural effusion that required surgical drainage. All patients received intravenous rehydration therapy and antibacterial therapy – 30% (6) received one antibacterial preparation, 65% (13) received combined antibacterial therapy with two or more preparations, but in 5% (1) the antibacterial therapy was changed during the course of treatment.

The descriptive values of inflammatory cytokines and chemokines can be seen in Table 1

Table 1. Descriptive values of inflammatory markers, cytokines and chemokines, and their dynamics

	On the inclusion	24 hours after inclusion	On the discharge	P value
sFAS	3812,1 ± 1874,5 (95% CI 2934,8 – 4689,4)	4261,2 ± 1428,3 (95% CI 3592,7 – 4929,7)	4687,0 ± 1179,2 (95% CI 4118,6 – 5255,4)	< 0,001
sVCAM1	1290,0 ± 977,05 (95% CI 832,73 – 1747,3)	920,36 ± 647,62 (95% CI 617,26 – 1223,5)	616,19 ± 261,38 (95% CI 490,20 – 742,17)	< 0,001
tPAI1	101,86 ± 50,35 (95% CI 78,30 – 125,43)	104,78 ± 51,25 (95% CI 80,79 – 128,76)	125,78 ± 61,25 (95% CI 96,26 – 155,31)	0,84
IL-8	102.43 ± 277.02 (95% CI 31.01 - 235.95)	20.446 ± 26.89 (95% CI 7,86 – 33,03)	17,54 ± 28,86 (95% CI 3,18 – 31,89)	< 0,001
IL-10	474,84 ± 1617,0 (95% CI 304,53 – 1254,2)	240,57 ± 747,23 (95% CI 109,14 – 590,29)	41,21 ± 65.94 (95% CI 8.41– 74.0)	< 0,001
INF gamma	22,33 ± 32,48 (95% CI 6,67 – 37,98)	17,04 ± 27,75 (95% CI 4,06 – 30,03)	10,32 ± 22,02 (95% CI 0,63– 21,27)	0,20
TNF alpha	17,73 ± 15,79 (95% CI 10,11 – 25,33)	13,51 ± 9,27 (95% CI 9,17 – 17,84)	11,42 ± 9,09 (95% CI 7,04 – 15,81)	< 0,05
Eotaxin	92,68 ± 74,45 (95% CI 57,84 – 127,53)	80,82 ± 34,67 (95% CI 64,59 – 97,05)	108,30 ± 52,25 (95% CI 83,11 – 108,3)	< 0,001
G-CSF	2324,2 ± 3698,9 (95% CI 593,03 – 4055,3)	307,32 ± 552,05 (95% CI 48,95 – 565,68)	67,64 ± 80,81 (95% CI 28,68 – 106,59)	< 0,001
IL1ra	57.27± 126.25 (95% CI 1.81– 116.36)	43.68 ± 1617,0 (95% CI 9.19 – 96.55)	90.86 ± 231.49 (95% CI 20.72 – 202.43)	0,95
IP10	2650,8 ± 4676,5 (95% CI 462,15 – 4839,5)	1412,3± 2177,1 (95% CI 393,45 – 2431,2)	439,69 ± 259,69 (95% CI 314,53– 564,86)	< 0,001
MCP1	979,77 ± 1039,7 (95% CI 493,17 – 1466,4)	670,22 ± 903,66 (95% CI 247,30 – 1093,2)	797,63 ± 1256,1 (95% CI 192,23 – 1403,0)	< 0,001

There were several statistically significant moderate and strong correlations found among PCT, IL-6 and CRP. These correlations are depicted in Table 2.

Table 2. Correlations among clinical inflammatory markers and inflammatory cytokines and chemokines (correlation coefficient r, statistical significance)

	On inclusion		
	CRP	PCT	IL-6
TNF alpha	0,51 (p=0,03)	0,66 (p=0,002)	
G-CSF		0,57 (p=0,01)	0,52 (p=0,02)
IL-8		0,93 (p<0,001)	
	After 24 hours		
	CRP	PCT	IL-6
G-CSF	0,48 (p=0,03)	0,75 (p<0,001)	
IL-8			0,69 (p<0,001)
IFN gamma			0,52 (p=0,18)
	On discharge		
	CRP	PCT	IL-6
G-CSF	0,86 (p=0,01)	0,54 (p=0,02)	0,82 (p<0,001)
IL-10	0,82 (p<0,001)	0,66 (p=0,003)	
IL-8			0,64 (p=0,004)

There was also a moderately strong positive correlation between length of stay in the hospital and levels of TNF alpha (r=0,6, p=0,01), sFAS (r=0,59, p=0,01), sVCAM1 (r=0,50, p=0,04), MCP1 (r=0,53, p=0,03), IL-10 (r=0,52, p=0,03) at inclusion of the study.

Moderately strong positive correlations were found between age and sFAS (r=0,69, p=0,002), INF gamma (r=0,65, p=0,005) at inclusion of the study.

DISCUSSION

This study had several limitations – limited patient count and no control group. Several other studies have focused on changes of cytokine levels, grouped by etiological factors, for example, Takano et al. studied cytokine and chemokine response in children with H1N1 influenza pneumonia, where they found significantly higher elevated levels of IFN-γ, IL-6, IL-8, IL-10, IL-13, MCP-1 and IL-5 in patients with pneumonia than those without pneumonia (13). Another study, which included patients with H1N1 influenza, found that serum concentrations of INF –γ, TNF alpha, IL-4, and IL-2 were significantly lower in pneumonia patients with neutrophilic leukocytosis than in those without neutrophilic leukocytosis (7). Our study did not analyse cytokine levels in connection with leukocyte count, and there was just one patient group.

Ballin et al. studied inflammatory cytokine levels in children and adults with community acquired bronchopneumonia, where they found, that age was directly correlated with levels of IL-8 and G-CSF (1); whereas we did not find such kind of association in this study, positive correlation was found between age and levels of sFAS and INF gamma.

There have been several studies of inflammatory cytokines in adults with pneumonia and/or sepsis. Kellum et al. included 1886 patients with pneumonia and/or sepsis in a multicentre study, and measured levels of TNF alpha, IL-6 and IL-10. Patients were divided into 3 groups – patients who did not develop severe sepsis, patients with severe sepsis who died, and patients with severe sepsis who survived. Authors found, that in patients, who had severe sepsis, cytokine levels were higher than in those who had just pneumonia; also cytokine levels were higher in those patients, who had severe sepsis and died, than in those who survived (9). Also, Lee et al conducted a study with adult patients with severe community acquired pneumonia, who were treated in in ICU. Similarly, they found higher levels of TNF alpha, IL-6, IL-8, IL-10 in non-survivors than in survivors, also patients who developed acute respiratory distress syndrome had higher IL-6, IL-8 and IL-10 levels (10). Our study included patients with sepsis, but due to limited patient count we were not able to analyse cytokine levels between patient groups with sepsis and severe sepsis, but there was a correlation between inflammatory cytokine levels and length of stay in the hospital, which could indirectly note the severity of illness.

We found moderately strong correlation between G-CSF and PCT, and IL-6 on inclusion, which promotes the idea Horisberger et al., which have initiated a randomized controlled trial of G-CSF and IL-8 as markers for early diagnostics of sepsis in critically ill children (6).

CONCLUSIONS

sFAS, sVCAM1, IL-8, IL-10, TNF alpha, Eotaxin, G-CSF, IL1ra, IP10 and MCP1 showed statistically significant changes between levels of inclusion in the study and levels after 24 hours, indicating they could be used in diagnostics in septic patients with pneumonia, and also show patients response to therapy.

Also, G-CSF, IL-8 had moderately strong or strong correlations with clinically used inflammatory markers on different time points of the study, these markers should be further investigated as early diagnostic markers for children with sepsis.

Conflict of interest: None

Acknowledgement
This study was conducted as a part of State Research Program VPP BIOMEDICINE Project No 8.2 „Clinical, molecular-biological and morpho-functional research of diagnostics and treatment of inherited and acquired diseases of childhood".

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