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Evolution of cytokines/chemokines in cases with community-acquired pneumonia and distinct etiologies

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Abstract

Aim: To compare the systemic cytokines/chemokines levels over time during the evolution of children hospitalized with community-acquired pneumonia (CAP) with and without pneumococcal infection.

Methods: Children less than 5-years-old hospitalized with CAP were prospectively investigated in Salvador, Brazil. Clinical data and biological samples were collected to investigate 20 etiological agents and to determine serum cytokines/chemokines levels on admission and 2 to 4 weeks later. Cases with pneumococcal infection received this diagnosis irrespective of also having other etiologies.

Results: A total of 277 patients were enrolled, however, serum sample was unavailable for cytokine measurement upon admission (n = 61) or upon follow-up visit (n = 36), etiology was undetected (n = 50) and one patient did not attend the follow-up visit. Therefore, this study group comprised of 129 cases with established etiology. The median (interquartile range) age and sampling interval was 18 (9-27) months and 18 (16-21) days, respectively. Established etiology was viral (52.0%), viral-bacterial (30.2%), and bacterial (17.8%). Pneumococcal infection was found in 31 (24.0%) patients. Overall, median interleukin-6 (IL-6; 10.6 [4.7-30.6] vs 21.0 [20.2-21.7]; $P = .03$), IL-10 (3.5 [3.1-4.5] vs 20.1 [19.8-20.4]; $P < .001$), and CCL2 (19.3 [12.4-23.2] vs 94.0 [67.2-117.8]; $P < .001$) were significantly higher in convalescent serum samples, whereas median CXCL10 (83.6 [36.4-182.9] vs 14.6 [0-116.6]; $P < .001$) was lower. Acute vs convalescent levels evolution of IL-10, CCL2, and CXCL10 did not differ among patients with or without pneumococcal infection. However, IL-6 decreased (27.8 [12.3-48.6] vs 20.8 [20.2-22.6]; $P = .1$) in patients with pneumococcal infection and increased (9.0 [4.2-22.6] vs 21.0 [20.2-21.7]; $P = .001$) in patients without it.

Conclusion: The marked increase of IL-6 serum levels during the acute phase makes it a potential biomarker of pneumococcal infection among children with CAP.

KEYWORDS

acute respiratory infection, child, lower respiratory tract infection, lung disease, pneumococcal infection

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1 | INTRODUCTION

Community-acquired pneumonia (CAP) is one of the most frequent causes of death among children under 5 years, worldwide, being second to preterm birth complications.[1] In addition, CAP imposes a substantial burden on health care services, being a major cause of hospital referral and admission in children younger than 5 years.[2] In 2015, 294 000 deaths were estimated to occur in HIV-uninfected children aged 1 to 59 months due to pneumococcal infection, 81% of which had CAP.[3]

Streptococcus pneumoniae induces intense inflammation in the lungs with the release of cytokines/chemokines from innate immunity.[4] Accumulating evidence establishes IL-6 as a key player in the activation, proliferation and survival of lymphocytes during active immune responses.[5] Recently, it has been shown that IL-6 increase upon admission is independently associated with pneumococcal infection among children under 5 years hospitalized with CAP.[6] In another study, IL-6 was elevated among adult patients with bacteremic pneumococcal CAP, with sustained high levels during recovery, even after complete resolution of disease.[7] However, there is no information about cytokines/chemokines profiles during recovery among children with CAP. We aimed to fill this gap by comparing the systemic cytokines/chemokines levels over time during the evolution of children hospitalized with CAP with and without pneumococcal infection.

2 | PATIENTS AND METHODS

2.1 | Study design

This prospective cohort was conducted at the Federal University of Bahia Hospital Pediatric Emergency Department. Patients aged under 5 years with CAP diagnosed by the pediatrician on duty were evaluated. CAP diagnosis was based on: (a) respiratory complaints (cough or running nose) plus (b) report of fever/difficulty breathing (when the child breaths laboriously so that the caregiver realizes that the child is making effort to breath instead of breathing smoothly) plus (c) pulmonary infiltrate on the chest radiograph obtained upon admission. Exclusion criteria comprised: (a) chronic lung disease, except asthma, (b) underlying comorbidities, (c) other concurrent infections, (d) suspected or diagnosed immunodeficiency, or (e) transfer from other health-care units. Data were collected between September 2003 and May 2005.

2.2 | Patients

Patients came from the community and were thoroughly examined, when demographic and clinical data, blood samples and nasopharyngeal aspirates (NPA) were collected. Patients were re-evaluated 2 to 4 weeks later, when a second blood sample was collected for serological assays and comparison of specific immunoglobulin G (IgG) titers.

2.3 | Controls

A convenience sample of 30 asymptomatic healthy children under 5 years about to undergo elective surgery was enrolled. The blood sample was collected at the time of anesthesia induction after having received written informed consent from parents/legal guardians. Data on birth date, sex, surgery, and collection date were registered.

2.4 | Microbiological assays

Investigation of etiology comprised the performance of several tests to search for the same etiological agent; this procedure was carried out for every included case.

In short, respiratory viruses were investigated by searching for viral antigens in NPA by time-resolved fluoroimmunoassay with monoclonal antibodies and comparison of virus-specific paired serum IgG titers determined by enzyme-linked immunosorbent assay (ELISA; influenza A and B viruses, respiratory syncytial virus, parainfluenza viruses type 1, 2, and 3, and adenovirus), when diagnosis was based on a ≥3-fold antibody titers increase in paired serum samples.[8] Rhinovirus, enterovirus, and human metapneumovirus were searched for by reverse transcription-polymerase chain reaction (PCR) assays in the NPA.[9,10] Human bocavirus was investigated by quantitative PCR of NPA and serum, IgG increase determination in paired serum samples, and determination of IgM and IgG avidity by ELISA.[11] Bacterial infections caused by *S. pneumoniae*, nontypable *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and *Streptococcus pyogenes* were investigated by blood culture (Automated Bact/Alert Organon) before the beginning of antimicrobial treatment. Bacterial infection by *S. pneumoniae* was also sought by an in-house ELISA which measured IgG antibodies to pneumococcal pneumolysin and pneumococcal C-polysaccharide in paired serum samples when a ≥2 or ≥3-fold antibody titres increase, respectively, was considered diagnostic.[12] Pneumolysin-PCR was also used for the detection of *S. pneumoniae* DNA in blood buffy-coat collected upon admission.[13] Table 1 lists the laboratory tests along with the respective diagnostic criteria for diagnosing pneumococcal infection. For *H. influenzae* and *M. catarrhalis* infections, Ig (polyvalent) antibodies against whole bacterial cell antigens (a mixture of 10 different strains) were measured and a ≥3-fold antibody increase between paired serum samples was considered diagnostic.[12] An in-house microimmunofluorescence test was used to measure IgG, IgA, and IgM antibodies to *Chlamydia pneumoniae* and *Simkania negevensis*, using purified, formalized elementary bodies of strains Kajaani 6 in *C. pneumoniae*[14] and ATCC strain Z (VR-1471; American Type Culture Collection) in *S. negevensis* tests.[15] The diagnosis was based on a ≥4-fold increase in IgG or IgA antibodies between paired sera or on the presence of IgM antibodies (a titer of ≥10). *Mycoplasma pneumoniae* infection was investigated by testing for specific IgM by using a commercial ELISA Kit (Platelia; Bio-Rad, Marnes La Coquette, France).[16] *Chlamydia trachomatis* IgG antibodies were measured by a commercial, solid-phase ELISA (Ani

TABLE 1 Laboratory tests along with the respective diagnostic criteria for pneumococcal infection diagnosis

Laboratory test	Diagnostic criteria
Blood culture collected upon admission before antibiotic therapy commencement[a]	Isolation of pneumococcal strain
Pneumolysin-PCR in blood buffy-coat collected upon admission[12]	Detection of pneumococcal DNA
In-house ELISA for the quantitation of IgG antibodies to pneumococcal pneumolysin in paired serum samples[13]	≥2-fold antibody titres increase
In-house ELISA for the quantitation of IgG antibodies to pneumococcal C-polysaccharide in paired serum samples[13]	≥3-fold antibody titres increase

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; PCR, polymerase chain reaction
[a]Incubated in automated Bact/Alert Organon.

Labsystems Ltd, Vantaa, Finland). The laboratory diagnosis was based on signal to cut-off (S/CO) values, which were ≥1.4 S/CO.[17]

2.5 | Cytokines and chemokines assays

Serum concentrations of inflammatory and Th1/Th2 cytokines and chemokines were measured in the residual serum from the etiology tests which were kept frozen at −80°C, using the Cytometric Bead Array Human Inflammatory Cytokine Kit, Human Th1/Th2 Cytokine Kit, and Human Chemokine Kit, respectively (BD Biosciences Pharmingen, San Diego, CA). Flow cytometry (BD FACSArray) and the Software FCAP Array (BD Biosciences Pharmingen) were used to perform the acquisition and the analysis, respectively. The assays were performed by a technician blinded to etiological and clinical information. The same measurements and analyses were performed in the serum collected from healthy children (controls).

2.6 | Statistical analysis

For the purpose of analysis, the cases were grouped as viral, viral-bacterial, or bacterial infection. Patients with viral infection had all negative tests to investigate bacterial infection and at least one positive test to diagnose viral infection. Patients with bacterial infection had all negative tests to investigate viral infection and at least one positive test to diagnose bacterial infection. Patients with viral-bacterial infection had at least one positive test to diagnose viral infection and one positive test to diagnose bacterial infection. Subsequently, the cases were grouped as with or without pneumococcal infection. Cases with pneumococcal infection had at least one positive test to diagnose pneumococcal infection and cases without pneumococcal infection had all negative tests to investigate pneumococcal infection. Tachypnea was defined as respiratory rate ≥60 breaths/min in children aged <2 months, ≥50 breaths/min in children aged 2 to 11 months, and ≥40 breaths/min in children aged 12 to 59 months.[18] Fever was defined as axillary temperature of ≥37.5°C.[19] Respiratory rate was counted and axillary temperature was measured during physical examination. Nutritional evaluation was performed using the software Anthro, version 1.02 (Center for Disease Control and Prevention and WHO) and malnutrition and severe malnutrition were defined as Z-score for weight-for-age index under −2.00 or −3.00, respectively, using the National Center for Health Statistics standard.[20]

Cytokines serum levels were reported as median (interquartile range [IQR]) due to nonparametrical distribution. The bivariate analysis included the Wilcoxon test to compare cytokines serum levels measured upon admission and at follow-visit (paired samples), and the Mann-Whitney *U* test or the Kruskal-Wallis test to compare cytokines serum levels measured upon admission or at follow-up visit between two or three independent samples, respectively. The analysis was initially performed on the whole group and subsequently it was stratified within the distinct etiological subgroups in the SPSS software (version 9.0). We also performed a bivariate analysis on the frequency of each clinical characteristic of children with or without pneumococcal infection by using χ^2 or Fisher's exact test as appropriate; continuous variables were assessed by using the Mann-Whitney *U* test. All tests were two-tailed with a significance level of 0.05.

2.7 | Ethics approval

The study was approved by the Ethics Committee of the Federal University of Bahia. Written informed consent was signed by parents/legal guardians before enrollment.

3 | RESULTS

Out of the 277 patients enrolled, serum sample was unavailable for cytokine measurement upon admission (n = 61) or upon follow-up visit (n = 36), etiology was not detected (n = 50), and one patient did not attend the follow-up visit. Therefore, this study group included 129 patients, who each had every laboratory test performed to investigate etiology, and had probable etiology established. The median (IQR) age was 18 (9-27) months and 76 (58.9%) were male. The median (IQR) interval between blood collection upon admission and upon follow-up visit was 18 (16-21) days. The most frequent complaints were cough (99.2%), fever (96.1%), and difficulty breathing (85.3%); the most frequent findings were tachypnea (86.0%), crackles (66.4%), and chest indrawing (63.0%). Among the 30 healthy controls, the median (IQR) age was 41 (26-59) months and 23 (76.7%) were male. By comparing age distribution between cases and controls, it is possible to observe that controls were older than cases ($P < .001$). Surgeries comprised herniorrhaphy (56.7%), posthectomy

TABLE 2 Baseline characteristics of children hospitalized with community-acquired pneumonia and comparison between cases with or without pneumococcal infection

Characteristics	All cases (n = 129) n (%)[a]	Pneumococcal infection		P
		Yes (n = 31)[a]	No (n = 98)[a]	
Demographics				
Age (median [IQR] months)	18 (9-27)	19 (10-28)	17 (9-26)	.8
Male gender	76 (58.9)	20 (64.5)	56 (57.1)	.5
History				
Disease duration (median [IQR] days)	7 (5-15)	8 (5-15)	7 (4-14)	.2
Cough	128 (99.2)	31 (100)	97 (99.0)	1
Fever	124 (96.1)	31 (100)	93 (94.9)	.3
Difficulty breathing	110 (85.3)	25 (80.6)	85 (86.7)	.4
Vomiting	75 (58.1)	20 (64.5)	55 (56.1)	.4
Running nose	13 (10.1)	1 (3.2)	12 (12.2)	.2
Physical examination				
Tachypnea	111 (86.0)	28 (90.3)	83 (84.7)	.6
Crackles	85/128[b] (66.4)	17 (54.8)	68/97[d] (70.1)	.1
Chest indrawing	80/127[b] (63.0)	20/30[c] (66.7)	60/97[d] (61.9)	.7
Fever (axillary temperature ≥37.5°C)	74/128[b] (57.8)	19 (61.3)	55/97[d] (56.7)	.7
Chest recession	69/127[b] (54.3)	16/30[c] (53.3)	53/97[d] (54.6)	.9
Wheezing	58 (45.0)	12 (38.7)	46 (46.9)	.4
Rhonchi	41/125[b] (32.8)	12/30[c] (40.0)	29/95[d] (30.5)	.3
Malnutrition	10/128[b] (7.8)	0	10/97[d] (10.3)	.1

Abbreviation: IQR, interquartile range.
[a]Expressed as absolute number and percentage if not otherwise specified.
[b]The denominator was not 129 because there was missing information.
[c]The denominator was not 31 because there was missing information.
[d]The denominator was not 31 because there was missing information.

(16.7%), exeresis of thyroglossal cyst (9.9%), orchidopexy (6.7%), correction of polydactyly (6.7%), and exeresis of hemangioma (3.3%).

The probable etiological subgroups comprised viral infection (n = 67; 52.0%), viral-bacterial infection (n = 39; 30.2%), and bacterial infection (n = 23; 17.8%). Pneumococcal infection was diagnosed in 31 (24.0%) cases, out of which 8 (25.8%) had pneumococcal bacteremia, 3 (9.7%) had pneumococcal DNA detected in blood, and 20 (64.5%) had diagnosis based solely on serology. Table 2 shows the baseline characteristics of the whole group of patients and of cases with or without pneumococcal infection. No statistically significant difference was found when cases with or without pneumococcal infection were compared. Likewise, when the median (IQR) interval (days) between blood collection upon admission and upon follow-up visit was compared (18 [14-20] vs 18 [16-21]; $P = .5$).

Interleukin (IL)-1β, tumor necrosis factor-α, IL-12, IL-2, IL-4, IL-5, interferon-γ, CCL5, and CXCL9 did not have detectable levels in serum collected upon admission nor upon follow-up. IL-6, IL-10, IL-8, CCL2, and CXCL10 had serum levels detected upon admission and also upon the follow-up visit which could be compared. Table 3 compares the median (IQR) of these cytokines and chemokines at both times for the whole group. It is possible to observe that IL-6, IL-10, and CCL2 serum levels were significantly higher upon the follow-up visit. Conversely, IL-8 and CXCL10 serum levels were lower. Table 3 also compares the median (IQR) serum levels of these cytokines and chemokines measured in the serum samples collected upon admission and during convalescence from CAP cases and in the group of healthy controls. Notably, all cytokines were significantly higher during the convalescence phase in comparison to the healthy controls serum levels. On the contrary, chemokines CCL2 and CXCL10 were lower during convalescence in comparison with the healthy controls. On the other hand, IL-6 and IL-8 were significantly higher as well as IL-10 and CCL2 were significantly lower at admission in comparison to the healthy controls serum levels.

This analysis was repeated within subgroups stratified by the etiological classification: first, within the subgroups with viral, viral-bacterial, or bacterial infection and second within the subgroups with or without pneumococcal infection (Table 4). It is possible to observe that all three cytokines and two chemokines evolved similarly among cases with either viral, or viral-bacterial, or bacterial infection. However, when this comparison was performed among cases with or without pneumococcal infection, IL-8, IL-10, CCL2, and CXCL10 evolved similarly in these subgroups but IL-6 evolved differently: upon admission, IL-6 was higher among cases with pneumococcal infection yet lower among cases without pneumococcal infection. It means that IL-6 serum levels decreased among children with pneumococcal infection and increased among children without pneumococcal infection. Figure 1 shows the distribution of IL-6 measured in serum samples collected upon admission and during convalescence, stratified by pneumococcal infection, and in serum samples collected from healthy controls. When patients with (IL-6 median [IQR]: 27.8 [12.3-48.6] pg/mL) or without (IL-6 median [IQR]: 9.0 [4.2-22.6] pg/mL) pneumococcal infection were compared upon admission, $P = .001$.

We compared the median (IQR) serum levels of all three cytokines and two chemokines measured upon the follow-up visit among cases with viral, viral-bacterial, or bacterial infection and no significant difference was found (IL-6 $P = .08$, IL-10 $P = .8$, IL-8 $P = .8$, CCL2 $P = 1.0$, and CXCL10 $P = .3$). We also compared the median (IQR) serum levels of all three cytokines and two chemokines measured upon the follow-up visit between cases with or without pneumococcal infection and again, no significant difference was found (IL-6 $P = .8$, IL-10 $P = .9$, IL-8 $P = .9$, CCL2 $P = .7$, and CXCL10 $P = .08$).

Upon follow-up visit, 116 (89.9%) cases reported complete resolution of illness, 13 (10.1%) reported cough (median duration 5 [2-12] days), out of which 6 reported fever (median duration 1.5 [1-3.3] days). Upon admission, these 13 cases had viral (n = 6) or viral-bacterial (n = 7) infection, out of which 4 had pneumococcal infection. The whole analysis was repeated with the exclusion of the 13 cases who reported complaints during the follow-up visit and the results

TABLE 3 Comparison of median (IQR) serum levels (pg/mL) of cytokines and chemokines measured upon admission and during convalescence among 129 cases hospitalized with community-acquired pneumonia and between serum levels measured upon admission and during convalescence of cases and healthy controls

Cytokines/chemokines	Admission (Acute phase, n = 129)	Follow-up visit[a] (Convalescence, n = 129)	P^b	Healthy controls (n = 30)	P^c	P^d
IL-6	10.6 (4.7-30.6)	21.0 (20.2-21.7)	.03	4.5 (4.1-4.9)	<.001	<.001
IL-10	3.5 (3.1-4.5)	20.1 (19.8-20.4)	<.001	13.5 (12.2-14.8)	<.001	<.001
IL-8	84.7 (34.7-233.8)	68.0 (25.8-172.0)	.1	11.2 (3.4-89.6)	<.001	<.001
CCL2	19.3 (12.4-23.2)	94.0 (67.2-117.8)	<.001	106.4 (97.5-117.3)	<.001	.005
CXCL10	83.6 (36.4-182.9)	14.6 (0-116.6)	<.001	96.8 (82.4-99.4)	.3	.003

Abbreviation: IL, interleukin; IQR, interquartile range.
[a]Median [IQR] interval between blood collection upon admission and upon follow-up visit was 18 [16-21] days.
[b]Wilcoxon signed ranks test for two related samples.
[c]Mann-Whitney *U* test for two independent samples collected from cases upon admission and from healthy controls.
[d]Mann-Whitney *U* test for two independent samples collected from cases during convalescence and from healthy controls.

TABLE 4 Stratified comparison of median (IQR) serum levels (pg/mL) of cytokines and chemokines measured upon admission and during convalescence within distinct etiological subgroups of children hospitalized with community-acquired pneumonia

Cytokines/chemokines	Admission (acute phase)	Follow-up visit (convalescence)[a]	P^b	Median difference[c]
Bacterial infection (n = 23)				
IL-6	13.1 (5.9-48.6)	21.1 (20.3-22.6)	.9	−8.0
IL-10	3.5 (3.2-4.4)	20.1 (19.8-20.4)	<.001	−16.6
IL-8	155.0 (24.1-439.8)	73.6 (15.0-157.3)	.05	81.4
CCL2	20.2 (11.1-23.8)	102.3 (67.0-116.4)	<.001	−82.1
CXCL10	97.2 (31.8-150.5)	0 (0-117.0)	.09	97.2
Viral infection (n = 67)				
IL-6	11.8 (4.2-29.3)	21.1 (20.2-22.0)	.1	−9.3
IL-10	3.5 (3.0-4.7)	20.1 (19.8-20.4)	<.001	-16.6
IL-8	71.2 (36.3-151.4)	65.5 (26.7-208.3)	.8	5.7
CCL2	19.2 (12.3-23.6)	93.7 (66.5-117.5)	<.001	−74.5
CXCL10	80.4 (35.0-187.4)	19.4 (0-125.3)	.03	61.0
Viral-bacterial infection (n = 39)				
IL-6	9.2 (5.0-30.6)	20.7 (20.1-21.2)	.09	−11.5
IL-10	3.7 (3.1-4.5)	20.1 (19.8-20.3)	<.001	−16.4
IL-8	71.7 (31.2-195.6)	67.3 (26.1-167.9)	.6	4.4
CCL2	19.3 (14.2-22.4)	89.5 (69.7-123.2)	<.001	−70.2
CXCL10	91.4 (44.8-183.5)	0 (0-98.7)	<.001	91.4
With pneumococcal infection (n = 31)				
IL-6	27.8 (12.3-48.6)	20.8 (20.2-22.6)	.1	7.0
IL-10	3.4 (3.0-4.3)	20.1 (19.8-20.4)	<.001	−16.7
IL-8	117.1 (46.0-333.2)	69.3 (26.1-167.9)	.03	47.8
CCL2	20.2 (12.4-23.7)	89.5 (67.0-118.1)	<.001	−69.3
CXCL10	97.2 (46.9-182.9)	0 (0-116.1)	<.001	97.2
Without pneumococcal infection (n = 98)				
IL-6	9.0 (4.2-22.6)	21.0 (20.2-21.7)	.001	−12.0
IL-10	3.7 (3.1-4.5)	20.1 (19.8-20.4)	<.001	−16.4
IL-8	74.7 (32.8-215.7)	66.8 (25.2-180.4)	.6	7.9
CCL2	19.1 (12.5-23.1)	94.4 (67.2-119.2)	<.001	−75.3
CXCL10	75.8 (34.2-181.9)	21.1 (0-118.3)	.004	54.7

Abbreviation: IL, interleukin; IQR, interquartile range.
[a]Median [IQR] interval between blood collection upon admission and upon follow-up visit was 18 [16-21] days.
[b]Wilcoxon signed ranks test for two related samples.
[c]Median difference: admission—follow-up visit.

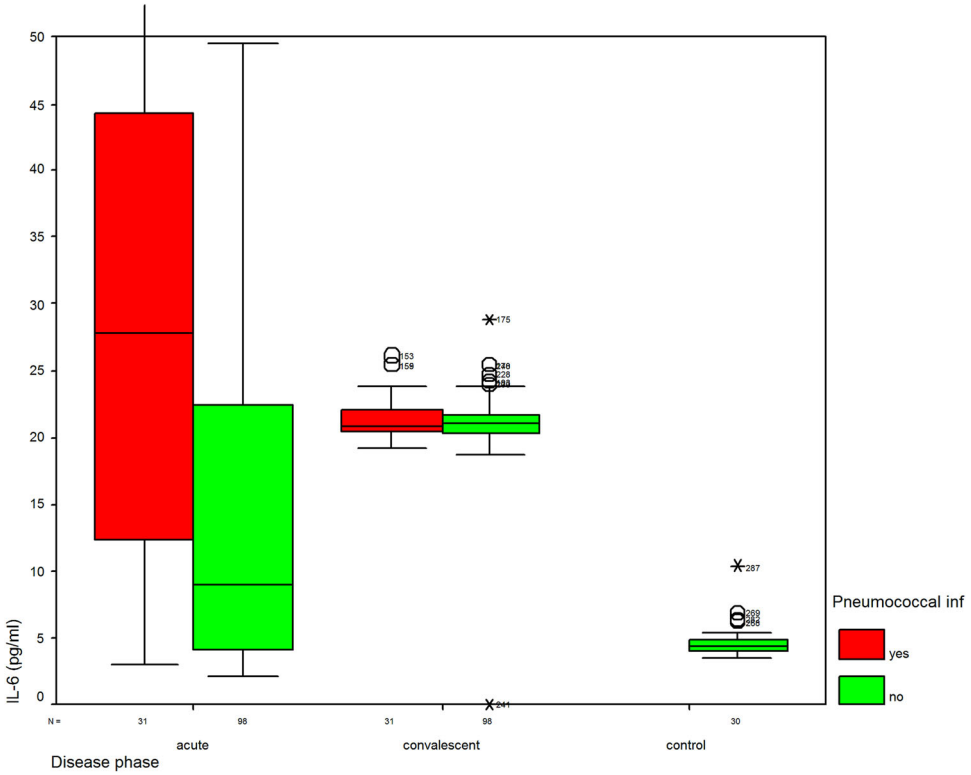

FIGURE 1 Distribution of IL-6 serum levels in samples collected upon admission and during convalescence of cases hospitalized with community-acquired pneumonia, stratified by pneumococcal infection, and in samples collected from healthy controls. IL, interleukin [Color figure can be viewed at wileyonlinelibrary.com]

are shown in Tables S1 and S2, which are similar to the ones found in the whole group. The median (IQR) serum levels of all three cytokines and two chemokines measured upon the follow-up visit were compared between cases with full recovery and cases with complaints and no difference was found (Table 5).

To address generalizability, the stratified analysis by pneumococcal infection was repeated with the inclusion of the 50 cases with undetected etiology in the group of cases without pneumococcal infection (Table 6). It is possible to note that similar results were found.

TABLE 5 Comparison of median (IQR) serum levels (pg/mL) of cytokines and chemokines measured during convalescence between cases hospitalized with community-acquired pneumonia and complete resolution of illness and cases with complaints upon follow-up visit

Cytokines/chemokines	Complete resolution of illness n = 116	Complaints upon follow-up visit[a] n = 13	P[a]
IL-6	21.0 (20.2-21.7)	21.1 (20.5-22.8)	.6
IL-10	20.1 (19.8-20.4)	20.2 (20.0-20.3)	.4
IL-8	72.3 (28.6-172.4)	31.6 (16.3-228.4)	.3
CCL2	94.1 (68.1-117.7)	74.6 (64.5-124.4)	.4
CXCL10	15.3 (0-119.5)	14.5 (0-66.2)	.4

Abbreviation: IL, interleukin; IQR, interquartile range.
[a]Mann-Whitney *U* test for two independent samples.

4 | DISCUSSION

We show herein that systemic levels of IL-6 evolved differently between cases with or without pneumococcal infection: IL-6 decreased among children with pneumococcal infection whereas it increased among children without pneumococcal infection. To the best of our knowledge, this is the first study to report this evolution. Conversely, systemic levels of IL-10, IL-8, CCL2, and CXCL10 did not differ between the acute and convalescent phases of children hospitalized with CAP, irrespective of the etiology. Besides that, IL-6 was markedly increased upon admission specifically among children with pneumococcal infection.

It is important to highlight that the same results were found when the analysis was repeated after having added cases with undetected etiology in the subgroup without pneumococcal infection (Table 6). It is well known that it is difficult to diagnose pneumococcal infection, particularly the noninvasive form.[21] Herein, the most important aspect is that pneumococcal infection diagnosis was mainly based on the specific serum IgG increase, which is a sensitive (92.3%) and specific (91.3%) test.[22] That means, cases with and without pneumococcal infection were reliably distinguished. Such distinction was made early during disease as IL-6 diagnostic increase was documented upon hospital admission. This finding may positively impact the management of children with CAP.

It is important also to emphasize that, in the pediatric population, there is only one previous investigation in which etiology of CAP was investigated by an expanded panel of tests and 15 different cytokines/

TABLE 6 Stratified comparison of median (IQR) serum levels (pg/mL) of cytokines and chemokines measured upon admission and during convalescence within the subgroup of children hospitalized with community-acquired pneumonia without pneumococcal infection (50 cases with undetected etiology were included)

Cytokines/ chemokines	Admission (acute phase)	Follow-up visit[a] (convalescence)	*P*[b]
IL-6	9.2 (4.2-22.7)	21.1 (20.2-22.7)	<.001
IL-10	3.6 (3.0-4.5)	20.1 (19.8-20.4)	<.001
IL-8	70.3 (32.3-191.1)	70.1 (29.1-188.1)	.5
CCL2	19.3 (9.6-23.9)	94.9 (66.7-123.1)	<.001
CXCL10	59.5 (32.2-131.6)	14.1 (0-108.9)	<.001

Abbreviation: IQR, interquartile range.
[a]Median [IQR] interval between blood collection upon admission and upon follow-up visit was 18 [16-21] days.
[b]Wilcoxon signed ranks test for two related samples.

chemokines were measured but only upon admission: no association was found between IL-6 and any specific etiology. Therein, cytokines/chemokines were measured using the Human Cytokine Multiplex Antibody Bead Kit. Indeed, the number of children studied was limited (n = 55) and, most important, the diagnosis of pneumococcal infection was based solely on positive or negative blood culture or pneumolysin-based PCR assays. In that study, the median (IQR) of IL-6 among 12 children with pneumococcal infection was 89 (43-203) pg/mL whereas it was 82 (50-381) pg/mL among 8 children with viral infection. Of note, all children had negative blood culture and 12 had positive pneumolysin-based PCR assays.[23] By comparing their results with ours, it is possible to infer that, therein, pneumococcal infection occurred in the subgroup labeled as viral infection and the pneumococcal infection was not identified. This finding may be due to the fact that those authors only searched for invasive pneumococcal infection. Therefore, they could not exclude cases with noninvasive pneumococcal infection among patients grouped in the viral infection category.

As we observed that IL-6 decreases between the acute and convalescent phases of CAP, specifically among children with pneumococcal infection, it is possible to infer that those cases have marked IL-6 increase upon admission. IL-6 plays a pivotal proinflammatory role in innate defense against pneumococcus.[4,24] It is a major mediator of the acute-phase plasma proteins by the liver and elevated serum glucocorticoid levels.[25] Marked IL-6 increase was described in adults with severe pneumococcal infection.[26] A recent study found that bacteremic (four pneumococcus and one *S. pyogenes*) lower respiratory tract infection in children had a higher concentration of IL-6 ($P < .0055$) on day 1.[27] We had previously shown that IL-6 increase upon admission is independently associated with pneumococcal infection among children under 5 years hospitalized with CAP.[6] This finding was detected when pneumococcal cases had either bacteremic or nonbacteremic pneumococcal infection.[6] Herein, our results support the potential role of IL-6 for diagnosing pneumococcal infection.

Interestingly, the cytokine/chemokine profile remained different 18 days after treatment's commencement, in regard to healthy controls (Tables 3 and S1). To the best of our knowledge, this is the first study to compare the cytokine/chemokine profile between acute and convalescent phases among children, and between patients in the convalescent phase vs healthy controls. In another study among adults, the evolution of systemic cytokine expression in pneumococcal pneumonia was assessed on days 0, 1, 2, 3, 5, and 7 and IL-6, IL-8, and IL-10 decreased rapidly in the first days after admission but no comparison with healthy controls was made.[28]

Our study has limitations. First, etiology was established based on probability yet supported by the most appropriate investigations available, as lung tissue was not studied for ethical reasons. We did perform a thorough investigation of etiology which was not solely based on the presence of viruses and bacteria in the nasopharynx; conversely, we investigated etiology by searching for specific host responses. Second, controls were older than cases, although in the same age range (<5 years). Thirdly, the decrease of IL-6 between admission and convalescing serum samples of 31 cases with pneumococcal infection was not statistically significant; on the contrary, the increase of IL-6 among the 98 cases without pneumococcal infection was. It is possible to infer that lack of statistical significance in the first subgroup may be due to sample size as it is easy to notice the distinct evolution of IL-6 among patients with or without pneumococcal infection (Figure 1). Finally, the study was performed in a single center.

In conclusion, IL-6 increases markedly during the acute phase of pneumococcal CAP and as such is a potential biomarker of pneumococcal infection among children with CAP.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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