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Systemic cytokines and chemokines on admission of children hospitalized with community-acquired pneumonia

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ABSTRACT

Community-acquired pneumonia (CAP) is the main cause of death in children under-5 years worldwide and *Streptococcus pneumoniae* is the most common bacterial agent. However, it is difficult to identify pneumococcal infection among children with CAP. We aimed to assess association between any cytokine/chemokine and pneumococcal infection in childhood CAP. Furthermore, we evaluated the diagnostic value of cytokine/chemokine for pneumococcal infection. This prospective study was conducted at an Emergency Room, in Salvador, Brazil. Children < 5-years-old hospitalized with CAP in a 21-month period were evaluated. On admission, clinical and radiological data were collected along with biological samples to investigate 20 etiological agents and determine serum cytokines (interleukin (IL)-8, IL-6, IL-10, IL-1β, IL-12, TNF-α, IL-2, IL-4, IL-5, γ-interferon), and chemokines (CCL2, CCL5, CXCL9, CXCL10) concentration. From 166 patients with etiology detected, pneumococcal infection was detected in 38 (22.9%) cases among which the median IL-6(pg/ml) was 31.2 (IQR: 12.4–54.1). The other 128 cases had other causative agents detected (*Haemophilus influenzae*, *Moraxella catarrhalis*, atypical bacteria and viruses) with the median IL-6 concentration being 9.0 (IQR: 4.1–22.0; $p < 0.001$). The area under the ROC curve for IL-6 to predict pneumococcal CAP was 0.74 (95%CI: 0.65–0.83; $p < 0.001$). By multivariate analysis, with pneumococcal CAP as dependent variable, IL-6 was an independent predictor for pneumococcal infection (OR = 5.56; 95%CI: 2.42–12.75, cut-off point = 12.5 pg/ml; $p = 0.0001$). The negative predictive value of IL-6 under 12.5 pg/ml for pneumococcal infection was 90% (95%CI: 82–95%). Independently significant difference was not found for any other cytokines/chemokines. Serum IL-6 concentration on admission is independently associated with pneumococcal infection among children under-5 years hospitalized with CAP.

1. Introduction

Community-acquired pneumonia (CAP) remains the main single cause of death and a frequent cause of hospital admissions in children under-5 years worldwide, with 1 million estimated deaths in 2015 and approximately 15 million estimated admissions in 2010 [1,2].

It is currently very difficult to establish the etiological diagnosis of CAP. This is mostly due to lack of rapid tests with sensitivity and specificity high enough to be appropriately employed in emergency rooms or primary health-care settings, particularly among children [3]. Among the several putative etiological agents of childhood CAP, *Streptococcus pneumoniae* has been identified as the most common bacterial agent [3,4]. Nevertheless, clinical and radiological parameters, as well as presently available laboratory biomarkers have been found to be useless to distinguish pneumococcal cases from non-pneumococcal cases [3,5].

S. pneumoniae induces an intense inflammation in the lungs with the release of cytokines and chemokines from innate immunity. This process activates alveolar macrophages and systemic neutrophils to promote the clearance of pneumococcal strains [6,7]. Some cytokines are recognized to play a pivotal role in innate defense against *S. pneumoniae*, such as tumor necrosis factor α (TNFα) and interleukin (IL)-1β, in the first moment of infection, being followed by IL-6, IL-8 and IL-10 [6–8].

Since the 1990's, several studies have been searching for association between inflammatory cytokines and bacterial CAP in adults [9,10].

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Few other studies have investigated this issue among children, but none has presented a practical and useful conclusion so far [11–13]. Thus, we aimed to evaluate further if there was association between serum cytokineś or chemokineś levels on admission and pneumococcal infection among children hospitalized with CAP.

2. Material and methods

2.1. Study design

This was a prospective study conducted at the Emergency Room of the Federal University of Bahia Hospital, in Salvador, Northeast, Brazil, from September 2003 to May 2005. Every child aged < 5 years hospitalized due to CAP was evaluated upon admission. The diagnosis was made by the pediatrician on duty. Diagnosis was based upon fulfillment of the following criteria: (1) respiratory complaints plus (2) fever or difficulty breathing plus (3) pulmonary infiltrates on the chest radiograph (CXR) taken at admission. Written informed consent was signed by parents/legal guardians before recruitment. The exclusion criteria were: (1) chronic lung disease, except asthma, (2) underlying co-morbidities, (3) other concurrent infections, (4) suspected or diagnosed immunodeficiency, or (5) transfer from other health-care units. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and it was approved by the Ethics Committee of the Federal University of Bahia.

2.2. Patients

Community-dwelling children were evaluated upon admission, when demographic and clinical data, blood samples and nasopharyngeal aspirates (NPA) were collected. At this moment, a chest radiograph was taken. Afterwards, an independent radiological evaluation was performed by a pediatric radiologist blinded to clinical and laboratory data. Two to four weeks later, the patients were invited to return for a follow-up visit, when a second blood sample was collected for serological assays and comparison of specific IgG titers.

2.3. Controls

We recruited a convenience sample of 30 asymptomatic healthy children. Eligibility requirements for controls included age < 5 years and performance of elective surgery. 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, gender, 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. Infections by the following pathogens were searched for: *S. pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Simkania negevensis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, rhinovirus, respiratory syncytial virus, influenza virus A and B, parainfluenza viruses types 1, 2 and 3, enterovirus, adenovirus, human metapneumovirus and human bocavirus. The investigation of these infections and the frequency of these etiological agents analyzed by age distribution have already been published [14–17].

In short, respiratory viruses investigation consisted of 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-immunoassay (ELISA) (influenza A and B viruses, respiratory syncytial virus, parainfluenza viruses type 1, 2, and 3, and adenovirus), when a ≥ 3-fold antibody titers increase in paired serum samples was considered diagnostic [18]. Reverse transcription-polymerase chain reaction (PCR) assays for the detection of rhinovirus, enterovirus, and human metapneumovirus were performed [16,19]. Human bocavirus was investigated by quantitative PCR of NPA and serum, IgG increase determination in paired serum samples, and searching for IgM and IgG avidity by ELISA [20]. Bacterial infections caused by *S. pneumoniae*, non-typable *H. influenzae*, *M. catarrhalis*, *S. aureus*, and *S. 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. A ≥ 2-fold or ≥3-fold increase, respectively, in antibody titres, was considered diagnostic [21]. 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 [21]. Pneumolysin-PCR was also used for the detection of *S. pneumoniae* DNA in blood buffy-coat collected upon admission [22]. An in-house microimmunofluorescence test was used to measure IgG, IgA and IgM antibodies to *C. pneumoniae* and *S. negevensis*, using purified, formalized elementary bodies of strains Kajaani 6 in *C. pneumoniae* [23] and ATCC strain Z (ATCC, Catalog No. VR-1471) in *S. negevensis* tests [24]. 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). *M. pneumoniae* infection was investigated by testing for specific IgM by using a commercial ELISA kit (Platelia, Bio-Rad, Marnes La Coquette, France) [25]. *C. trachomatis* IgG antibodies were measured by a commercial, solid-phase ELISA (Ani Labsystems Ltd., Vantaa, Finland). The laboratory diagnosis was based on signal to cut-off (S/CO) values, which were ≥1.4 S/CO [26].

2.5. Cytokines and chemokines assays

Serum concentrations of inflammatory cytokines (IL-8, IL-1β, IL-6, IL-10, TNFα and IL-12); Th1/Th2 cytokines (IL-2, IL-4, IL-5 and γ-interferon [IFNγ]); and chemokines (CCL2, CCL5, CXCL9, CXCL10) were measured in the residual serum from the etiology tests 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, USA). Flow cytometry (BD FACSArray) and the Software FCAP Array (BD Biosciences Pharmingen, San Diego, CA, USA) was used to perform the acquisition and the analysis, respectively. Lower limits of detection were: IL-8, 3.6 pg/ml; IL-1β, 7.2 pg/ml; IL-6, 2.5 pg/ml; IL-10, 3.3 pg/ml; TNFα, 3.7 pg/ml; IL-12, 1.9 pg/ml; IL-2, 2.6 pg/ml; IL-4, 2.6 pg/ml; IL-5, 2.4 pg/ml; IFNγ, 7.1 pg/ml; CCL5, 0.2 pg/ml; CXCL9, 2.5 pg/ml; CCL2, 2.7 pg/ml; CXCL10, 2.8 pg/ml. The maximum quantifiable level considered was supplied by the manufacturer: 10,000 pg/ml for all cytokines and chemokines. The assays were performed on two separate occasions by a technician blinded to etiological and clinical information. The residual serum samples from the etiology tests were kept frozen at −80 °C until cytokines and chemokines were measured. 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 classified into two groups: cases with pneumococcal infection or with non-pneumococcal infection. The former group comprised cases with bacteremic pneumococcal infection (that is, *S. pneumoniae* was isolated from blood culture) and cases with non-bacteremic pneumococcal infection (*S. pneumoniae* was not isolated from blood culture but pneumolysin-PCR was positive and/or IgG increase against pneumococcal pneumolysin and/or pneumococcal C-polysaccharide was found, according to the used cut-offs). The group with non-pneumococcal infection comprised