Systemic Inflammatory Responses in Children with Acute Otitis Media Due to *Streptococcus pneumoniae* and the Impact of Treatment with Clarithromycin

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This pilot study was designed to determine the serum cytokine profile of acute otitis media (AOM) due to *Streptococcus pneumoniae* and the impact of clarithromycin (Abbott Laboratories, Inc). Serum levels of interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α), IL-6, and IL-8 were measured at diagnosis and 3 to 5 days after start of antibiotic treatment in 10 patients (mean age, 18.5 ± 13.9 months) who had middle ear fluid culture positive for *S. pneumoniae*. The mean concentrations of all cytokines were elevated at diagnosis of AOM compared to levels in healthy controls, yet only IL-6 reached statistically significant (P = 0.05). IL-6 showed a statistically significant decrease in mean serum concentration at visit 2 (P = 0.03). IL-8 displayed a similar pattern to IL-6, but the difference between samples from day 1 and day 2 did not reach statistical significance. The cytokines IL-1β and TNF-α appear to be elevated in the serum of patients with *S. pneumoniae* AOM, but there was no significant change between mean serum levels obtained pre- and postinitiation of antibiotic treatment in the time frame studied. The results suggest a systemic inflammatory response as evidenced by increased IL-6. A significant decrease of IL-6 and improvement of clinical symptoms were observed. Determining cytokine levels, especially IL-6, in AOM could offer a powerful tool for objective assessment of response to treatment, minimizing unnecessary treatment of asymptomatic children who may still have some otoscopic findings suggestive of AOM at follow-up visits.

The impact of acute otitis media (AOM) on the health care system in the United States is significant. AOM was responsible for approximately 24.5 million office visits in 1990 (12) and constituted the most common diagnosis during pediatric office visits between 1993 and 1995 among 1 to 4 year olds (6). AOM is estimated to cost the public health care system more than $5 billion dollar annually (7) and accounts for more than 25% of all prescriptions for oral antibiotics (3). Although there is an up to 60 to 80% spontaneous cure rate for AOM at late follow-up (11), AOM due to *Streptococcus pneumoniae* is less likely to resolve without antibiotics (4, 13). *S. pneumoniae* is also the most common pathogen isolated in middle ear fluid from patients who have failed antibiotic therapy (10). These findings underscore the need for a stepwise approach to treatment of AOM aimed at documenting inflammation as it relates to acute infection, and they attempt to identify specific pathogens. An understanding of the interactions between pathogen and host defense, as well as individual differences in immune modulation, should help us to better explain the correlation between clinical and microbiological success or failure.

While extensive research has been done investigating local inflammatory processes in the middle ear, little is known about the systemic immune response during AOM.

Cytokines are glycoproteins synthesized by a variety of cells and are known to modulate cellular functions in inflammatory and immune reactions. Interleukin-1β (IL-1β) is known to induce synthesis of tumor necrosis factor alpha (TNF-α) (14) and the growth of osteoclasts and fibroblasts and to cause hemodynamic changes and fever. IL-6 is a potent inducer of C-reactive protein and has been shown in vitro and in animal models to inhibit TNF-α (1). IL-8, a potent chemoattractant, is produced predominantly by monocytes, macrophages, and endothelial cells in response to stimuli such as lipopolysaccharide and TNF-α (2). Numerous cytokines and growth factors have been detected in middle ear fluid of children with otitis media (9, 14). In a previous study (8), serum IL-6 levels were positively correlated with bacterial AOM, especially when caused by *S. pneumoniae*. Our pilot study was designed to investigate the systemic inflammatory response, and the impact of macrolide antibiotic therapy on this response, in children with AOM due to *S. pneumoniae*.

From September 1999 to January 2000, patients seen at our ambulatory care center with symptoms suggestive of AOM were screened for eligibility by the investigators. Patients were included if they had at least one clinical sign of inflammation (ear pain, fever >100.1°F or 37.8°C, redness of the tympanic membrane [TM], or bulging of the TM) and at least two signs of middle ear effusion (decreased TM mobility, visible air-fluid level, or yellow discoloration or opacification of the TM). Children with a history of chronic or recurrent AOM were excluded. Patients (n = 12) who met inclusion criteria and whose parents gave consent received a comprehensive clinical evaluation at enrollment, which included an AOM severity score previously described by Dagan et al. (5). Subsequently, middle ear fluid was collected by tympanocentesis and immediately
inoculated into brain heart infusion broth and sent for culture. Prior to the first dose of antibiotic, a venous blood sample was obtained.

To investigate the systemic inflammatory response in children with AOM, we measured serum levels of IL-1β, TNF-α, IL-6, and IL-8 at baseline. Study subjects were then started on clarithromycin, 15 mg/kg/day (first dose was given in clinic) in two divided doses, to complete 10 days of treatment. A follow-up visit was done 3 to 5 days after treatment was started, consisting of a limited clinical evaluation. The AOM severity score was again established. Patients (n = 10; mean age, 18.3 ± 13.9 months) who had middle ear fluid culture positive for S. pneumoniae underwent a second blood sampling for determination of serum cytokine levels. If the middle ear fluid isolate underwent a second blood sampling for determination of serum cytokine levels. If the middle ear fluid isolate underwent a second blood sampling for determination of serum cytokine levels.

| Patient group | Severity score | IL-1β (pg/ml) | TNF-α (pg/ml) | IL-6 (pg/ml) | IL-8 (pg/ml) |
|---------------|----------------|--------------|---------------|--------------|--------------|
| Visit 1       | 8.4 ± 1.8 (<0.001) | 1,201 ± 985 (0.32) | 13.5 ± 17.8 (0.15) | 54.5 ± 38.1 (0.05) | 45.5 ± 39.4 (0.47) |
| Visit 2       | 2.2 ± 2.2 (0.001)  | 1,530 ± 871 (0.51) | 15.2 ± 17.5 (0.76) | 15.9 ± 24.0 (0.03) | 28.5 ± 52.0 (0.69) |
| Visit 3       | 1.6 ± 2.1         | N/A*         | N/A           | N/A          | N/A          |
| Controls      | 0               | 737 ± 477    | 1.5 ± 2.22    | 9.7 ± 8.2    | 7.1 ± 8.6    |

* Results are means ± standard deviations (n = 9). P values for data from visit 1 are based on comparisons with controls; P values for data from visit 2 are based on comparisons with visit 1 data.

** Severity scores were determined according to the method of Dagan et al. (5). The clinical severity score by Dagan et al. was based on the temperature measured at the clinic, report of irritability and ear tugging by the parents, in response to specific questions addressed during visits 1 and 2, and the appearance (bulging) and redness of the eardrum observed by the examiner. The categories of irritability, tugging, redness, and bulging were classified as absent (0), mild (1), moderate (2), or severe (3). If the eardrum was perforated at the time of the second visit and pus was draining, this was scored by definition as “severe bulging.” We did not provide a definition for severity but let the parents and the examiner freely decide which level of severity to choose. Temperatures were scored as mildly (38.0–38.5°C), moderately (38.6–39°C), and severely (>39°C) elevated. The maximum score was 15 (when the temperature was >39.0°C and all other categories were judged “severe”), and the minimum score was 0 (when the temperature was <38.0°C and all other categories were judged “absent”).

* N/A, not applicable.
FIG. 1. Serum cytokine levels at diagnosis and early follow-up visit.
two sampling times, which could explain the highly variable levels noted at visit 2. Another possibility is that IL-6 might decrease in some patients on its own, or with spontaneous resolution of AOM. This is difficult to verify, as there was no untreated control group in this study. Although the sample size in this pilot study was small, our results highlight the importance of establishing a profile of inflammatory markers that could aid in the appropriate diagnosis and management of AOM in children. This pilot study allows us to specify the objectives of a larger study with this goal. Our study suggests a significant systemic inflammatory response in children with AOM due to *S. pneumoniae*. Measurement of IL-6 in serum of patients with presumed AOM may add information regarding the microbiological etiology of the infection, allowing for more objective criteria when choosing antibiotics. The results support the findings previously noted by Heikkinen et al. (8). While that study had a larger study population, it focused on the role of IL-6 in distinguishing certain bacterial and viral etiologies for AOM. By including more cytokine markers and by establishing profiles, we might be able to make more specific predictions and eventually identify a more specific marker or markers for AOM due to *S. pneumoniae*. Therefore, determining cytokine levels in AOM could offer a powerful tool for objective assessment of response to treatment, minimizing unnecessary treatment of asymptomatic children who may still have some otoscopic findings suggestive of AOM at follow-up visits. The in vivo test of cure has been advocated as the most reliable way to evaluate antibiotics for AOM by documenting microbiological success. Although scientifically sound, this approach involves a minimum of two tympanocentesis procedures to establish sterilization of the middle ear fluid, which is unacceptable in terms of time, cost, and discomfort, considering the large number of affected children. Serial measurements of IL-6 could potentially be used as a marker of acute infection in the middle ear, minimizing the discomfort to patients in double tympanocentesis studies. Our preliminary data do suggest that two measurements would be required to confirm effective treatment, because of the considerable overlap between acute and convalescent levels in different patients. Macrolide antibiotics are known to have an immune-modulating antiinflammatory effect. The changes noted in this study may reflect this to a certain degree. Further studies are necessary to evaluate the impact of other antibiotic classes on systemic inflammation in AOM due to *S. pneumoniae* and other organisms and the potential beneficial effect of macrolide-mediated attenuation of inflammation in AOM.

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