Etiology of Acute Otitis Media in Children Less Than 5 Years of Age

A Pooled Analysis of 10 Similarly Designed Observational Studies

Melissa K. Van Dyke, PhD,* Jean-Yves Pirçon, PhD,* Robert Cohen, MD;† Shabir A. Madhi, MD,‡‡ Pio Lopez, MD,§§ Laura Naranjo, MD, §§ Felix Pumarola, MD, §§ Nuntigar Sonsuwan, MD, || || and William P. Hausdorff, PhD*

Background: Acute otitis media (AOM) is an important cause of childhood morbidity and antibiotic prescriptions. However, the relative importance of the well-known otopathogens, Streptococcus pneumoniae (Spn) and Haemophilus influenzae (Hflu), remains unclear because of a limited number of tympanocentesis-based studies that vary significantly in populations sampled, case definitions and heptavalent pneumococcal conjugate vaccine use.

Methods: We conducted a pooled analysis of results from 10 AOM etiology studies of similar design, the protocols of which were derived from a common protocol and conducted in children 3 months to 5 years of age in different countries. Generalized estimating equations were used to account for within-study correlations.

Results: The majority, 55.5% (95% confidence interval: 47.0%–65.7%) of 1124 AOM episodes, were bacterial pathogen positive: 29.1% (24.3%–34.1%) yielded Hflu and 23.6% (19.0%–29.2%) Spn. Proportions of Hflu and Spn were higher and lower, respectively, in heptavalent pneumococcal conjugate vaccine–vaccinated children. Hflu and Spn were each isolated from 20% to 35% of children in every 1-year age range. Hflu was less commonly attributed to differences in study design (eg, case definition), access to care, local distribution of otopathogens and the specific vaccine formulation tested. Spn and nontypable Haemophilus influenzae (NTHi) have historically been the leading causes of AOM, with the former generally believed to be more associated with acute disease and the latter with recurrent cases as well as otitis media with effusion. Therefore, Spn and Hflu remain the leading otopathogens in all populations examined. While associated with overlapping symptoms and severity, they exhibit some differences in their likelihood to cause disease in specific subpopulations.

Key Words: otitis media, etiology, pediatric

Accepted for publication July 27, 2016.

From the *GSK Vaccines, Wavre, Belgium; †Association Clinique et Thérapeutique Infantile du Val de Marne (ACTIV), Saint-Maur-des-Fossés, CHI Créteil and UPEC, France; ‡Medical Research Council: Respiratory and Meningeal Pathogens Research Unit, and §Department of Science and Technology/National Research Foundation: Vaccine Preventable Diseases, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa; ¶Unidad de Otorrinolaringología, Hospital Dr Sotero del Rio, Puente Alto, Santiago, Chile; ¶¶Departamento de Infectología, Instituto Nacional de Pediatría de la Secretaría de Salud (SSA), Mexico City, Mexico; §§Otolaryngology Department, King Saud University & King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia; ††ENT-Center, Prinzenweg 1, 82319 Starnberg, Germany; ‡‡Centros de Estudios Infectología Pediátrica, Cali, Colombia; §§§GSK Biologicals, Ciudad Panama, Panama; §§Sección de ORL Pediátrica, Hospital Universitari Vall d’Hebron, Barcelona, Spain; and |||Otolaryngology Department, Faculty of Medicine Chiang Mai University, Chiang Mai, Thailand.

This work was supported by GlaxoSmithKline Biologicals SA. The funder was involved in the design and execution of the analyses, preparation of the manuscript and decision to publish.

The conflict of interest and funding statements of the authors are listed in the Acknowledgements.

Address for correspondence: William P. Hausdorff, PhD, Avenue JP Rullens 9, Brussels 1200, Belgium. E-mail: billhausdorff@hotmail.co.uk.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal’s website (www.pidj.com).

Copyright © 2016 The Author(s). Published by Wolters Kluwer Health, Inc.

© 2017 Wolters Kluwer Health, Inc. Unauthorized reproduction of this article is prohibited.
because of differences in study populations, PCV use and case definitions. Instead of performing a meta-analysis in which a range of studies may be considered for inclusion according to defined criteria, we sought to provide a more uniform assessment by conducting a pooled analysis of 10 similarly designed cross-sectional, observational studies that assessed AOM etiology in children in various countries,17–25 as each study’s protocol derived from a common protocol.26 This article presents the results of this pooled analysis.

METHODS

Study Design

This pooled analysis included 10 observational studies with similar protocols (eg, same case definitions, same age grouping, similar sample handling and laboratory procedures and identical plans for data collection to limit heterogeneity between studies). However, some modification was allowed to account for potential differences in local acceptability of performing tympanocentesis in different AOM subpopulations. These studies were conducted between 2007 and 2011 in children of 3–59 months of age. In each study, inclusion criteria required a bulging, diffused or localized inflamed tympanic membrane or spontaneous otorrhea of <24 hours since symptom onset, accompanied by one of the functional or general signs of otalgia/irritability, conjunctivitis or fever. The history of AOM cases enrolled, either sporadic or recurrent, could vary by country, depending on local clinical practice for management and treatment of disease. Middle ear fluid (MEF) samples were collected before antibiotic receipt at that visit by tympanocentesis or careful sampling of spontaneous otorrhea. Children receiving antibiotics before enrollment in the 72 hours before the visit as (1) systemic therapy for other otic receipt at that visit or (2) prophylactically for recurrent AOM were excluded, but AOM treatment failures were included. Further details about inclusion/exclusion criteria have been published previously.17–25

At the time of enrollment, a clinical examination was performed and medical history and general symptoms were collected by questionnaire. Severity of tympanic membrane inflammation was classified using the otoscopy scale-8 (described previously).27–29 An episode of AOM was classified as new if there was a ≥30-day AOM symptom-free period before onset. AOM cases were classified as treatment failures if symptoms persisted despite initiation of physician-prescribed antibiotics in the 48–72 hours between study enrollment. Recurrent AOM was defined as a reported history of ≥2 episodes within the past 6 months or ≥4 episodes within the past 12 months. A child was classified as vaccinated if he/she received ≥2 PCV doses before age 1 year or ≥1 PCV dose after age 1 year. Cases were classified as severe if a child had ear pain or an auxiliary temperature ≥38.5°C and/or if his/her tympanic membranes were scored as OS-6 (indicative of hyperemia—bulging rounded doughnut appearance of tympanic membrane) or OS-7 (indicative of hyperemia with bulla formation).

Laboratory Methods

MEF samples were first inoculated in Amies transport medium (with or without charcoal) and then cultured within 48 hours on blood agar, MacConkey and/or Sabouraud media supplemented with the appropriate antimicrobial agent to select the pathogens of interest.17–25 Only Spn, Hflu, Moraxella catarrhalis (Mcat) and Streptococcus pneumoniae (Spyo) isolated by bacterial culture were considered to be true otopathogens for further evaluation and characterization. Spn isolates were serotyped by Quellung reaction or polymerase chain reaction, and PCV7-CRM types include serotypes 4, 6B, 9V, 14, 18C, 19F and 23F as well as 6A.27,28 Hflu isolates were serotyped (a, b, c, d, e, f or nontypeable) by monovalent antisera and/or real-time polymerase chain reaction.29 Antibiotic susceptibility testing was performed using E-tests, agar dilution or microdilution, and nonsusceptibility was defined according to Clinical and Laboratory Standards Institute 2009 cut-offs.30 Antibiotic nonsusceptibility was defined to encompass both intermediate and resistant results. Beta-lactamase production was evaluated for Hflu and Mcat isolates using a nitrocefin test.31

Statistical Analysis

Because the studies were conducted in different countries, potential heterogeneity was tested using Cochran Q test based on inverse variance weights on the proportion of culture-positive samples to determine the need for a random effects model to account for within- and between-study heterogeneity in the pooled analysis, and reflected in the estimated variance used to build the confidence intervals (CIs). Generalized estimating equations were used to account for correlation between subjects from each study/country.12,23 All analyses were performed using SAS 9.2 (SAS Institute, Cary, NC). The generalized estimating equation models were fitted using the GENMOD procedure assuming an exchangeable correlation matrix. Finally, the analysis plan of the pooled analysis was defined when all the study databases were already frozen.

Individual patient data were pooled and analyzed according to episode status (first-reported episode or not), treatment status (untreated or treatment failure), sample collection type (otorrhea or tympanocentesis), PCV status (vaccinated or not), recurrent AOM (or not) and age group. For subgroup comparisons, pooled relative risks (RRs) with their 95% CIs were estimated. Each study RR was weighted by the inverse of the within-study variance plus the between-study variance. The basis of the pooled analysis is to combine the values derived from each intrastudy comparison. It is not a combination of all individual-level data from all studies. In some individual studies, no such comparisons were possible (eg, Saudi Arabia and PCV vaccination), so those data are not included. Therefore, subgroups should be and are compared using RR estimates as the proportions in each subgroup are not comparable from a statistical perspective. For analyses of etiology by serotype, serotype 6A was considered a PCV7-CRM type.

Ethical Considerations

All studies were conducted according to Good Clinical Practice, the Declaration of Helsinki and local rules and regulations of the country. All studies were approved by local institutional review committees and a parent/guardian provided written informed consent. Pooled analyses included only deidentified individual patient data, so additional consent was not required.

RESULTS

The pooled analysis included records from 1124 AOM episodes (Table 1). For each study, the median age of children enrolled was between 20 and 36 months and 46%–69% were male; additionally, 44%–80% of samples collected were culture positive, and 24%–95% of samples were obtained by tympanocentesis. The most predominant otopathogen reported was Hflu, closely followed by Spn. The proportion of children classified as PCV7-CRM vaccinated ranged widely from 0% to 83%, and the contribution of recurrent AOM varied from 0% to 98%. Because heterogeneity among studies in the proportions of culture-positive samples was statistically significant (P < 0.0001), the random effects pooling method was used to explore the relative contribution of each pathogen, adjusting for potential confounders such as age, vaccination status, AOM history, severity and history of antibiotic use. Figure 1 (left) presents the individual and pooled study proportions of Spn, Hflu, Spyo and Mcat.

Etiology by Age and Vaccination Status

Among all studies, 55.5% (95% CI: 47.0%–65.7%) of the 1124 AOM episodes were culture positive: 29.1% (24.8%–34.1%)
yielded Hfl, 23.6% (19.0%–29.2%) Spn, 3.7% (2.0%–6.8%) Spyo and 2.8% (1.4%–5.4%) Mcat (Fig. 1). These figures were not mutually exclusive, but only 2.7% (1.4–5.1%) reported any coinfection. Accordingly, Hfl, Spn, Spyo and Mcat represented 52.4%, 42.5%, 6.7% and 5.0%, respectively, of all culture-positive isolates. Of Spn episodes, 42.8% (29.0%–63.2%) were caused by PCV7-CRM serotypes, and 86.1% (71.7%–100%) of Hfl episodes were nontypable.

**TABLE 1. Overview of Included Studies**

| Country (GSK Study ID) | Data Collection | N  | Median Age, mo (Range) | Male (%) | PCV7-CRM Vaccinated (%) | Treatment Failure (%) | Recurrent Otitis Media (%) | Otorrhea (%) | Culture Positive (%) |
|------------------------|-----------------|----|------------------------|----------|-------------------------|-----------------------|--------------------------|--------------|---------------------|
| Chile (submitted)      | September 2009 to September 2010 | 164 | 24 (4–59) | 56 | 2 | 5 | 5 | 11 | 80 |
| Colombia (111397)     | February 2008 to January 2009 | 99 | 29 (5–55) | 55 | 18 | 0 | 10 | 16 | 62 |
| France (109369)      | May 2007 to April 2009 | 143 | 14 (4–47) | 53 | 83 | 73 | 27 | 18 | 61 |
| Germany (111640)     | November 2008 to April 2010 | 100 | 36 (3–59) | 56 | 72 | 3 | 23 | 76 | 44 |
| Mexico (110796)      | March 2008 to April 2009 | 111 | 23 (4–59) | 69 | 42 | 3 | 30 | 20 | 64 |
| Saudi Arabia (111357) | June 2009 to May 2011 | 66 | 20 (3–58) | 59 | 17 | 6 | 0 | 32 | 21 |
| South Africa (112135) | May 2009 to May 2010 | 182 | 15 (4–53) | 55 | 9 | 0 | 1 | 5 | 45 |
| Spain* (110513)      | June 2008 to March 2010 | 56 | 20 (5–35) | 64 | 63 | 18 | 98 | 25 | 61 |
| Thailand (110846)    | April 2008 to August 2009 | 112 | 36 (5–59) | 46 | 0 | 8 | 7 | 10 | 50 |
| Venezuela (111828)   | December 2008 to December 2009 | 91 | 26 (5–59) | 48 | 74 | 1 | 34 | 10 | 66 |

*In order to keep consistency with regard to the diverse case definitions, only a selection of the entire cohort from the study conducted in Spain was included in the analyses. N indicates number of AOM episodes; GSK, GlaxoSmithKline.

**FIGURE 1.** Bacterial etiology of AOM episodes. Left, etiology by individual study and pooled study proportions, expressed as percentages of all AOM episodes. Right, Spn and Hfl serotype distribution of AOM episodes according to PCV7-CRM vaccination status, expressed as percentages of Spn and Hfl episodes (upper), and as relative risk (lower). Error bars represent 95% CI, and 55.5% (47.0–65.7%) of all AOM episodes were culture positive (not including contaminants); the sum of individual pathogen pooled (GEE) proportions does not add up to 55.5% because of coinfections. *Spyo results were not available for France. Proportions of Spn (ST) serotypes are represented as percentage of all Spn cases (N = 170 in PCV unvaccinated and N = 83 in PCV vaccinated categories), and proportions of NTHi are represented as percentage of Hfl (N = 183 in PCV unvaccinated and N = 133 in PCV vaccinated categories). See statistical methods section for details related to RR calculation: Briefly, an RR <1 for a pathogen indicates that it comprises a smaller percentage of all pathogens isolated in PCV7-CRM–vaccinated compared with unvaccinated individuals; an RR <1 for a specific pneumococcal serotype or NTHi indicates that it comprises a smaller percentage of all pneumococci or of all Haemophilus isolated in PCV7-CRM–vaccinated than in unvaccinated individuals. 6A was included in PCV7 ST category.
The age distributions of Hflu and Spn were similar (Fig. 2), with Spn isolated from 20% to 29% and Hflu from 20% to 35% of AOM cases in each age group examined. The percentages were slightly lower (Spn) or higher (Hflu) in PCV7-CRM–vaccinated children compared with unvaccinated (Table 2 and Fig. 1, right), while Spyo appeared less likely to be identified in PCV7-CRM-vaccinees (RR: 0.28; 95% CI: 0.09–0.89; lower right Fig. 1).

Nonetheless, certain observations were consistent in both PCV7-CRM–vaccinated and unvaccinated children, and were significant in pooled analysis. Neither pathogen was more or less likely to be associated with the youngest age group (Table 2, Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C612) than with older ages. Hflu was less likely to be identified in the first episode (RR: 0.71; 0.60–0.84) than in subsequent episodes and more likely to be associated with recurrent rather than sporadic disease (RR: 1.40; 1.00–1.96). In contrast, Spn was less likely to be associated with recurrent disease (RR: 0.76; 0.61–0.97).

**Clinical Severity by Etiology**

Encapsulated Hflu isolates were more frequently associated with high severity of tympanic membrane bulging and inflammation (73% were OS-6/7), compared with Spn or NTHi or Mcat (30%–35% OS-6/7), whereas Spyo cases were only infrequently severe (8%–9%, Table, Supplemental Digital Content 2, http://links.lww.com/INF/C613). Conjunctivitis was more commonly identified in NTHi cases, but otherwise reported symptoms were similar between Spn and NTHi cases. Ear tugging and trouble sleeping were less frequently observed in cases caused by encapsulated Hflu than NTHi.

Both major otopathogens were thus associated with a range of disease severity (Table 2, Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C612): Hflu was more likely to be identified in cases with an OS-6 or 7 than in less severe cases (RR: 1.35; 1.06–1.71), and Spn was more likely to be identified in children with reported ear pain or a temperature ≥38.5°C (RR: 1.42; 1.01–2.01). Spyo was more likely (RR: 8.61; 4.74–15.63) and Mcat less likely (RR: 0.17; 0.04–0.69) to be found in MEF samples collected from otorrhea than by tympanocentesis.

**Observations According to Vaccination Status**

Some observations were statistically significant only in vaccinated or only in unvaccinated children, with little indication of a similar trend in the other group. For example, in PCV7-CRM–vaccinated children, Spn was more likely to be found in otorrhea rather than in tympanocentesis samples (RR: 1.66; 1.23–2.25) and in cases with severe tympanic membrane inflammation (RR: 3.58; 1.34–9.61). It was also more likely to be isolated from a treatment failure (RR: 1.63; 1.32–2.01) in vaccinated but not in unvaccinated children, and less likely to be identified in a first-reported episode in the youngest children (RR: 0.58; 0.37–0.91) (Table 2, Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C612).

In vaccinated children, Hflu was less likely to be isolated by otorrhea than by tympanocentesis (RR: 0.63; 0.47–0.85). In unvaccinated children, Hflu was less likely to be found in a child with severe symptoms such as ear pain or high fever (RR: 0.63; 0.43–0.91) than the one with milder symptoms (Table 2, Fig., Supplemental Digital Content 1, http://links.lww.com/INF/C612). However, in none of the above examples was a similar association apparent in the other group.

In unvaccinated children, the proportions of Spn and Hflu cases presenting with severe symptoms were 37.5% (30.6%–46.0%)
### TABLE 2. Acute Otitis Media Etiology According to PCV7-CRM Vaccination Status

|                | Spn % (95% CI) | RR (95% CI) | Hflu % (95% CI) | RR (95% CI) | N     | Spn % (95% CI) | RR (95% CI) | Hflu % (95% CI) | RR (95% CI) | N     |
|----------------|----------------|-------------|-----------------|-------------|-------|----------------|-------------|-----------------|-------------|-------|
| Overall        | 23.6 (19.0–29.2) | NA          | 29.1 (24.8–34.1) | NA          | 669   | 25.1 (20.4–30.9) | NA          | 27.0 (22.8–32.0) | NA          | 388   |
| 3–11 mo of age | 21.6 (22.2–34.4) | 1.17        | 33.6 (30.9–36.4) | 1.19        | 145   | 31.2 (25.2–38.6) | 1.31        | 32.8 (26.8–40.1) | 1.21        | 86    |
| 1st reported episode | 23.1 (17.5–30.4) | 1.18        | 23.6 (18.8–29.6) | 0.71        | 308   | 26.0 (20.3–33.2) | 0.94        | 22.4 (17.3–29.1) | 0.63        | 105   |
| 1st reported episode in 3–11 mo of age | 24.9 (21.0–29.6) | 1.14        | 32 (30.0–34.2) | 1.07       | 76    | 30.5 (29.3–31.7) | 0.91        | 20.2 (9.7–41.8) | 0.71        | 27    |
| Recurrent AOM  | 208 (15.3–25.4)  | 0.76        | 26.3 (20.1–31.9) | 1.4         | 77    | 20.3 (15.0–27.4) | 0.86        | 37.2 (30.1–46.1) | 1.56        | 129   |
| Otorrhea       | 223 (22.4–40.3)  | 1.34        | 30.2 (22.9–41.5) | 0.97        | 100   | 25.8 (17.0–39.2) | 0.98        | 33.1 (23.3–47.1) | 1.28        | 105   |
| Severe symptoms | 273 (25.7–42.1)  | 1.42        | 25.2 (17.9–35.4) | 0.8         | 125   | 37.5 (30.6–46.0) | 1.47        | 17 (10.1–28.6) | 0.63        | 135   |
| Severe OS     | 372 (21.4–30.7)  | 1.5         | 33.3 (27.4–40.5) | 1.35       | 217   | 27.9 (23.4–33.1) | 1.31        | 30 (23.2–38.8) | 1.11        | 134   |
| History of previous antibiotics | 310 (21.4–30.7) | 1.18        | 32.4 (28.7–36.5) | 1.15       | 129   | 31.6 (26.1–38.3) | 1.21        | 27.5 (19.1–39.7) | -          | 170   |
| Treatment failure | 143 (1.04–1.42) | 1.22        | 26.8 (20.0–35.9) | 0.81       | 42    | 31.7 (27.0–37.2) | 1.07        | 22.1 (10.0–45.5) | 0.69        | 93    |

Values in shaded cells are statistically significant. Pooled study proportions (%) and RR were computed using GEE model.

GEE indicates generalized estimating equations; N, number of episodes; NA, not applicable.
TABLE 3. Percentages of Spn and Hflu Isolates Nonsusceptible to a Specific Antibiotic

| Antibiotic                        | Spn Overall, % (95% CI) | RR (Vaccinated vs. Unvaccinated) (95% CI) | Hflu Overall, % (95% CI) | RR (Vaccinated vs. Unvaccinated) (95% CI) |
|-----------------------------------|-------------------------|------------------------------------------|--------------------------|------------------------------------------|
| Aminopenicillin                   | 18.5 (10.3–33.2)        | 1.27 (0.85–1.92)                         | 20.0 (11.8–33.7)         | 1.39 (0.99–1.92)                         |
| Cefotaxime                        | 9.8 (4.6–20.7)          | -                                        | 0.9 (0.3–2.9)            | -                                        |
| Azithromycin                      | 69.9 (45.2–100.0)       | 0.88 (0.76–1.02)                         | 6.2 (2.7–14.2)           | -                                        |
| Tetracycline                      | 37.2 (24.2–57.0)        | 0.99 (0.53–1.85)                         | 7.5 (5.1–10.8)           | 0.31 (0.11–0.92)                         |
| Trimethoprim/sulfamethoxazole     | 53.7 (36.6–75.6)        | 0.93 (0.57–1.49)                         | 47.8 (33.2–68.8)         | 1.37 (1.08–1.75)                         |
| Amoxicillin/clavulanate           | 0.5 (0.1–3.2)           | -                                        | 5.4 (2.6–11.3)           | 0.95 (0.25–3.57)                         |

Values in shaded cells are statistically significant. Pooled study proportions (%) and RR were computed using GEE model. GEE indicates generalized estimating equations.

and 17.0% (10.1%–28.6%), respectively, but in vaccinated children 28.3% (20.1%–39.9%) of the Spn cases and 33.8% (22.4%–50.9%) of the Hflu cases were severe.

Not surprisingly, serotype distribution also differed according to vaccination status (Fig. 1, right). PCV7-CRM types (including 6A) were less likely to be identified in PCV7-CRM–vaccinated children compared with unvaccinated [RR: 0.38 (0.24–0.61)]. Serotype 19A appeared to be more frequently identified in children who were PCV7-CRM vaccinated, although this did not achieve statistical significance (RR: 2.27; 0.93–5.56). In contrast, there was no obvious association of vaccination status with the proportion of serotype 3 (RR: 1.15; 0.58–2.27), that of the group of 1, 5 and 7F (RR: 0.70; 0.19–2.63), or the proportion of other non-PCV7-CRM types (RR: 1.08; 0.50–2.33).

**Antibiotic Nonsusceptibility**

Nonsusceptibility (Table 3) to first-line therapies such as aminopenicillin was 18.5% (10.3%–33.2%) among Spn isolates and 20.0% (11.8%–33.7%) for Hflu isolates. Nonsusceptibility to alternate first-line therapies differed: Spn isolates were rarely nonsusceptible to cefotaxime (9.8%; 4.6%–20.7%) but were frequently nonsusceptible to azithromycin (69.9%; 45.2%–100.0%). Small proportions of Hflu isolates were nonsusceptible to cefotaxime (0.9%; 0.3%–2.9%) and azithromycin (6.2%; 2.7%–14.2%). Spn and Hflu were also frequently nonsusceptible to second-line agents such as tetracycline and co-trimoxazole but were largely susceptible to amoxicillin/clavulanate. The percentage of Hflu isolates that were beta-lactamase negative was 74.6% (57.9%–96.0%), and 3.7% (1.7%–8.0%) were both beta-lactamase negative and ampicillin resistant. There were generally no significant differences in nonsusceptibility according to vaccination status, except for tetracycline nonsusceptible Hflu isolates, which were less likely to be found in vaccinated children [RR: 0.31 (0.11–0.92)], and trimethoprim/sulfamethoxazole nonsusceptible isolates, which were more likely to be observed in vaccinated children [RR: 1.37 (1.08–1.75)].

**DISCUSSION**

This analysis focused on 10 recent studies that had been implemented with similar study designs and characteristics so that a pooled analysis could assess the relative importance of different pathogens worldwide. It, thus, complements and extends previous reviews of pneumococcal serotype data from contemporaneous pediatric AOM studies as well as other reviews of bacterial AOM etiology. The finding that Spn and Hflu were the 2 major pathogens in the pooled analysis is consistent with results from each of the included studies and provides a more definitive confirmation of what has been reported in most other published single-country studies. The increased proportion of Hflu and moderately decreased proportion of Spn among cases who were PCV7-CRM vaccinated were also consistent with previous reports. Comparable results were observed within an eleventh individual study sharing the same design and conducted in Costa Rica; the results of that study were unfortunately not available at the time the pooling analysis was performed.

Because individual patient information was included in this pooled analysis, it was possible to explore more completely than usually possible in single studies the role that other covariates play in etiology. For example, Hflu is sometimes thought to cause only mild AOM but in this study, approximately 25% of Hflu AOM cases presented with more severe symptoms and almost one-third presented with severe inflammation of tympanic membranes. In addition, there appeared to be differences associated with vaccination status.

While in unvaccinated children the percentage of Hflu AOM cases presenting with severe symptoms (17%; 10.1%–28.6%) appeared lower than that of Spn cases (37.5%; 30.6%–46.0%), in vaccinated children the percentages were more similar [33.8% (22.4%–50.9%) and 28.3% (20.1%–39.9%), respectively], a finding for which we have no straightforward explanation. As previously reported, conjunctivitis was also particularly associated with NTHi AOM.

A noteworthy observation is that although Hflu was less likely to be reported as the cause of a first AOM episode than of later episodes and more likely to be identified as recurrent, it nonetheless still comprised 20.2% (9.7%–41.8%) of first-reported AOM in children younger than age 2 years implies that duration of protection would be an important attribute of any PCV in the prevention of childhood AOM.

The shift in pneumococcal serotype distribution toward non-vaccine types in AOM cases who were PCV7-CRM vaccinated was expected and similar to results seen in US studies. It is tempting to speculate that the predominantly non-PCV7-CRM types causing pneumococcal AOM in PCV7-CRM–vaccinated children, especially 19A and 3, are responsible for some of the other findings observed here—specifically, that only in PCV-CRM-vaccinated children was Spn more often found in otorrhea than in tympanocentesis samples, more often associated with severe tympanic membrane inflammation than mild, and more often from treatment failures than other cases.

The observation that Spyo is more likely and Mcat is less likely to be associated with spontaneous otorrhea rather than tympanocentesis is consistent with reports from other investigators. Spyo appeared less likely to be isolated in PCV7-CRM–vaccinated children.
than in unvaccinated children, but unless prior infection with the serotypes contained in PCV7-CRM selectively predisposes the child to a subsequent Spn infection, this appears a chance finding.

This pooled analysis is subject to limitations. First, there was statistically significant heterogeneity among the studies included, despite the use of similar protocols. For example, heterogeneity of PCV7-CRM use across the included studies limits broad interpretation of potential serotype and pathogen replacement. In an attempt to control for this, subgroup analyses were performed, the results were weighted, a random effect model was used and vaccination status was accounted for whenever possible when formulating the presented conclusions. Nonetheless, some countries had experienced a broad uptake of PCV7-CRM before the conduct of the study, and thus the contribution of herd effects was impossible to factor in. Evaluation after vaccination for a longer duration would be important.

As noted earlier, the pooling methodology relies solely on intrastudy comparisons, meaning that some pooled RR or proportion estimations did not include data from individual studies where such a comparison was not possible. It could, therefore, be argued that this introduces additional bias. However, the strength of the pooling methodology lies precisely in its focus on the consistency (or not) of the comparisons of the values obtained within each study. This should minimize variability that could otherwise arise from inclusion of values from only 1 group lacking a comparison set of value.

Second, despite efforts to standardize the design across studies, there were important differences in local guidelines for MEF collection. This is a recognized limitation of such prospectively planned pooled studies.26 One country required MEF to be collected only in cases that were considered to be recurrent. There are likely differences in care-seeking practices for AOM in other countries that could have affected the severity of cases enrolled. Nonetheless, a significant finding of this study is that despite these differences, no evidence suggested major setting-related differences in etiology.

Third, bacterial etiology was not determined with molecular techniques and the true contribution of these bacteria to AOM is likely higher than reported here (as previously described).19 It is also possible that culture-negative but polymerase chain reaction–positive samples may show different clinical severity or age tropisms compared with those described here.

Finally, although this analysis included 10 countries, most countries and regions are naturally not represented, including North America, most of Asia and sub-Saharan Africa, as well as native high-risk populations. Consequently, the generalizability of some of our findings may be limited to the health-care–seeking pediatric population in the countries included. However, as already noted, the identity of the most prominent etiologic pathogens as determined in the pooled analysis was largely consistent between the studies included, as well with other published reports, so those results may be considered to be more broadly applicable.

Despite differences in PCV7-CRM use and clinical management of AOM, this pooled analysis indicates that Spn and Hflu remain the leading causes of AOM in the different regions investigated. Both pathogens cause disease in the youngest and the oldest children, and it is known that early AOM cases are associated with more AOM cases/recurrence. Infants, thus, remain important targets for vaccination for either pathogen. Both pathogens are responsible for both mild and more severe AOM, as assessed by different measures and so are clinically significant. Finally, both pathogens are associated with antibiotic resistance. Although most serotypes in PCV7-CRM-vaccinated children now could be covered by 1 or both expanded serotype PCVs, one can note that already >20% of isolates were not covered by any currently licensed PCV, and this proportion will certainly increase as higher valency vaccines are fully implemented. The availability of next-generation PCVs should address some residual disease burden, but replacement and antibiotic resistance make elimination an elusive target. Continued evaluation of otopathogens is needed to support up-to-date treatment guidelines and inform decision making for new prevention strategies.

ACKNOWLEDGEMENTS

The authors wish to thank Cyrille Cartier (4 Clinics for GSK Vaccines) for his involvement in performing the statistical analyses and Thomas Déplanche (XPE Pharma & Science for GSK Vaccines) for his excellent suggestions and manuscript coordination.

R.C., S.A.M., A.R., M.M.P., K.A-M., G.G., P.L., L.N., F.P., and N.S. were primary investigators of the studies included in the pooled analysis reported in this article and contributed to the acquisition of data. M.K.V.D., J-Y.P., R.C., S.A.M. and W.P.H. participated in the conception/design/planning of the study. M.K.V.D., J-Y.P. and W.P.H. were involved in the assembling of the data and performed or supervised the analyses. M.K.V.D., J-Y.P., R.C., S.A.M., A.R., M.M.P., K.A-M. and W.P.H. contributed to interpretation of the results. All co-authors participated in drafting or revision of the submitted article. All authors approved the manuscript for the content and the submission and agreed to take responsibility for their contributions as presented in the manuscript.

W.P.H. was an employee of the GSK group of companies at the time of initial development of this article and own stocks/stock options of GSK group of companies. M.K.V.D. and J-Y.P. are currently employees of the GSK group of companies. R.C. declares having received money for consultancy and payment for lectures and his institution having received grants from GSK, Pfizer, Novartis, SP-MSD and AstraZeneca. S.A.M. declares his institution having received money for the conduct of the study in South Africa included in this pooled analysis. He reports personal fees for development of educational presentations from Medscape (about PCV, rotavirus and pertussis topics) consultancy in advisory board from GSK and Pfizer; payment for lectures from Sanofi Pasteur (in relation with Hexaxim), GSK (pneumococcal and rotavirus vaccines) and Pfizer (PCV), and his institution received grants from Novartis (GBS) and GSK (PCV). A.R. reports him and his institution having received grants for the conduct of the study in Chile included in this pooled analysis. M.M.P. reports grants to her institution for participation in protocols of trials from the following companies: Novartis, Sanofi Pasteur, Bayer, MSD (phase III), GSK (phase III and Pharmacoeconomics) and Pfizer (phase IIb and phase III). She declares having received consulting fees for lectures from Sanofi Pasteur; support for travel to meeting (Congresses) from GSK, Sanofi Pasteur, Pfizer and MSD, and fees for participation to advisory boards from GSK and Novartis. K.A-M. declares his institution having received grants for the conduct of the study in Saudi Arabia included in this pooled analysis; and he received support for travel to meeting from the GSK group of companies. PL. declares his institution having received a grant from GSK for the conduct of the study in Colombia included in this pooled analysis. L.N. declares her institution having received money for the conduct of the study in Venezuela included in this pooled analysis. She is currently an employee of the GSK group of companies. N.S. declares having received grant, consultancy fees, payment for lectures and support for travel to meetings during conduct of the study in Thailand included in this pooled analysis. W.P.H. is a co-holder of a patent for 13-valent PCV licensed to Pfizer/Wyeth, but receives no royalties as per industry practice. He is currently an independent consultant. R.C. (France), S.A.M. (South Africa), A.R. (Chile), M.M.P. (Mexico), K.A-M. (Saudi Arabia), G.G. (Germany).
REFERENCES

1. Monasta L, Ronfani L, Marchetti F, et al. Burden of disease caused by otitis media: systematic review and global estimates. PLoS One. 2012;7:e36226.

2. Lieberthal AS, Carroll AE, Chonmaitree T, et al. The diagnosis and management of acute otitis media. Pediatrics. 2013;132:e989.

3. Coco A, Vernacchio L, Horst M, et al. Management of acute otitis media after publication of the 2004 AAP and AAFP clinical practice guideline. Pediatrics. 2010;125:214–220.

4. O’Brien MA, Prosser LA, Paradise JL, et al. New vaccines against otitis media: projected benefits and cost-effectiveness. Pediatrics. 2009;123:1452–1463.

5. Kilpi T, Ahman H, Jokinen J, et al. Protective efficacy of a second pneumococcal conjugate vaccine against pneumococcal acute otitis media in infants and children: randomized, controlled trial of a 7-valent pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine in 1666 children. Clin Infect Dis. 2003;37:1155–1164.

6. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med. 2001;344:403–409.

7. Black S, Shenefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Pediatr Infect Dis J. 2000;19:187–195.

8. Fireman B, Black SB, Shenefield HR, et al. Impact of the pneumococcal conjugate vaccine on otitis media. Pediatr Infect Dis J. 2003;22:10–16.

9. Palmu AA, Jokinen J, Nieminen H, et al. Effect of pneumococcal Haemophilus influenzae protein D conjugate vaccine (PHD-CV10) on outpatient antimicrobial purchases: a double-blind, cluster randomised phase 3-4 trial. Lancet Infect Dis. 2014;14:205–212.

10. Tregnaghi MW, Sáez-Llorens X, Lopez P, et al. Efficacy of pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine (PHD-CV) in young Latin American children: a double-blind randomized controlled trial. PLoS Med. 2014;11:e1001657.

11. De Wals P, Erickson L, Poirier B, et al. How to compare the efficacy of conjugate vaccines to prevent acute otitis media? Vaccine. 2000;18:213–220.

12. Bluestone CD, Stephenson JS, Martin LM. Ten-year review of otitis media pathogens. Pediatr Infect Dis J. 1992;11(suppl):S7–S11.

13. Leibovitz E, Jacobs MR, Dagan R. Haemophilus influenzae: a significant pathogen in acute otitis media. Pediatr Infect Dis J. 2004;23:1142–1152.

14. Hausdorff WP, Yothers G, Dagan R, et al. Multinational study of pneumococcal serotypes causing acute otitis media in children. Pediatr Infect Dis J. 2002;21:1008–1016.

15. Coker TR, Chan LS, Newberry SJ, et al. Diagnosis, microbial epidemiology, and antibiotic treatment of acute otitis media in children: a systematic review. JAMA. 2010;304:2161–2169.

16. Johns Hopkins Bloomberg School of Public Health. International Vaccine Access Center (IVAC). Johns Hopkins Bloomberg School of Public Health. Vaccine Information Management System (VIMS) Global Vaccine Introduction Report, January 2015. Johns Hopkins Bloomberg School of Public Health. January 2016. Available at: http://www.jhsph.edu/research/centers-and-institutes/ivac/resources/maps.html. Accessed January 29, 2016.

17. Grevers G, Wiedemann S, Bohn JC, et al. Identification and characterization of the bacterial etiology of clinically problematic acute otitis media after tympanocentesis or spontaneous otorrhea in German children. BMC Infect Dis. 2012;12:312.

18. Sierra A, Lopez P, Zapata MA, et al. Non-typeable Haemophilus influenzae and Streptococcus pneumoniae as primary causes of acute otitis media in Colombian children: a prospective study. BMC Infect Dis. 2011;11:4.

19. Pumarola F, Marès J, Losada I, et al. Microbiology of bacteria causing recurrent acute otitis media (AOM) and AOM treatment failure in young children in Spain: shifting pathogens in the post-pneumococcal conjugate vaccination era. Int J Pediatr Otorhinolaryngol. 2013;77:1251–1266.

20. Naranjo L, Suarez JA, DeAntonio R, et al. Non-capsulated and capsulated Haemophilus influenzae in children with acute otitis media in Venezuela: a prospective epidemiological study. BMC Infect Dis. 2012;12:40.

21. Coulouigner V, Levy C, Francois M, et al. Pathogens implicated in acute otitis media failures after 7-valent pneumococcal conjugate vaccine implementation in France: distribution, serotypes, and resistance levels. Pediatr Infect Dis J. 2012;31:154–158.

22. Al-Mazrou KA, Shibli AM, Kandeil W, et al. A prospective, observational, epidemiological evaluation of the aetiology and antimicrobial susceptibility of acute otitis media in Saudi children younger than 5 years of age. J Epidemiol Glob Health. 2014;4:231–238.

23. Intakorn P, Sonsuwan N, Nakru S, et al. Haemophilus influenzae type b as an important cause of culture-positive acute otitis media in young children in Thailand: a tympanocentesis-based, multi-center, cross-sectional study. BMC Pediatr. 2014;14:157.

24. Parra MM, Aguilar GM, Echaniz-Aviles G, et al. Bacterial etiology and serotypes of acute otitis media in Mexican children. Vaccine. 2011;29:5544–5549.

25. Madhi SA, Gounder N, Dayal K, et al. Bacterial and respiratory viral interactions in the etiology of acute otitis media in HIV-infected and HIV-uninfected South African children. Pediatr Infect Dis J. 2015;34:753–760.

26. Blattert M, Sauerbrei W, Schlehofer B, et al. Traditional reviews, meta-analyses and pooled analyses in epidemiology. Int J Epidemiol. 1999;28:1–9.

27. Murray PR. American Society for Microbiology. Manual of Clinical Microbiology. 6th ed. Washington, DC: ASM Press; 1995.

28. Pai R, Gertz RE, Beall B. Sequential multiplex PCR approach for determining capsular serotypes of Streptococcus pneumoniae isolates. J Clin Microbiol. 2006;44:124–131.

29. Maaroufi Y, De Bruyne JM, Heymans C, et al. Real-time PCR for determining capsular serotypes of Haemophilus influenzae. J Clin Microbiol. 2007;45:2305–2308.

30. Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard. Vol M02-A10. 10th ed. Wayne, PA: Clinical and Laboratory Standards Institutes; 2009.

31. O’Callaghan CH, Morris A, Kirby SM, et al. Novel method for detection of beta-lactamases by using a chromogenic cephalosporin substrate. Antimicrob Agents Chemother. 1972;1:283–288.

32. Rotnitzky A, Jewell NP. Hypothesis testing of regression parameters in semiparametric generalized linear models for cluster correlated data. Biometrika. 1990;77:485–497.

33. Boos DD. On generalized score tests. Am Stat. 1992;46:327–333.

34. Rodgers GL, Arguedas A, Cohen R, et al. Authors’ reply to Prymula R. Re: “global serotype distribution among Streptococcus pneumoniae isolates causing otitis media in children: potential implications for pneumococcal conjugate vaccines.” Vaccine. 2007;27:5429–5430.

35. Tanur SO, Roth Y, Dalal I, et al. Changing trends of acute otitis media bacteriology in central Israel in the pneumococcal conjugate vaccines era. Pediatr Infect Dis J. 2015;34:195–199.

36. Casey JR, Kaur R, Friedel VC, et al. Acute otitis media otopathogens during 2008 to 2010 in Rochester, New York. Pediatr Infect Dis J. 2013;32:805–809.

37. Block SL, Hedrick J, Harrison CJ, et al. Community-wide vaccination with the heptavalent pneumococcal conjugate significantly alters the microbiology of acute otitis media. Pediatr Infect Dis J. 2004;23:829–833.

38. Casey JR, Pichichero ME. Changes in frequency and pathogens causing acute otitis media in 1995–2003. Pediatr Infect Dis J. 2004;23:824–828.

39. Abdelnour A, Arguedas A, Dagan R, et al. Etiology and antimicrobial susceptibility of middle ear fluid pathogens in Costa Rican children with otitis media before and after the introduction of the 7-valent pneumococcal conjugate vaccine in the National Immunization Program: acute otitis media microbiology in Costa Rican children. Medicine (Baltimore). 2015;94:e320.

40. Caemaeya L, Varon E, Levy C, et al. Characteristics and outcomes of acute otitis media in children carrying Streptococcus pneumoniae or Haemophilus influenzae in their nasopharynx as a single otopathogen after introduction of the heptavalent pneumococcal conjugate vaccine. Pediatr Infect Dis J. 2014;33:533–536.

41. Segal N, Givon-Lavi N, Leibovitz E, et al. Acute otitis media caused by Streptococcus pyogenes in children. Clin Infect Dis. 2005;41:35–41.

42. Brodies A, Dagan R, Greenberg D, et al. Acute otitis media caused by Moraxella catarrhalis: epidemiologic and clinical characteristics. Clin Infect Dis. 2009;49:1641–1647.

43. Palmu AA, Herva E, Savolainen H, et al. Association of clinical signs and symptoms with bacterial findings in acute otitis media. Clin Infect Dis. 2004;38:234–242.