Persistence of Immunity in Children Immunized with 13-Valent Pneumococcal
Conjugate Vaccine and Impact on Nasopharyngeal Carriage: A Cross-Sectional
Study

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SUPPLEMENTARY MATERIALS AND METHODS

Study Design

This cross-sectional study enrolled 500 children aged \( \leq 10 \) years of age who were previously administered one to four doses of PCV13 according to different schedules in 2016-2017. PCV13 (Prevnar 13®; Pfizer, USA) was stored refrigerated at 2-8 degrees C (36-46 degrees F) until used. A single blood sample was drawn from enrolled children and sera was separated from blood samples and frozen at −80°C until analysis.

The 500 children were divided into 4 groups based on each serotype studied: Catch-up (1-2 doses of each polysaccharide antigen received, n=257), 2+1 (n=60), 3+1 (n=164) and those without vaccination (n=19) (supplementary figure S1 and supplementary table S1). In the 2 + 1 schedule, 2 primary doses were given at 2 and 4 months of age, followed by a booster between 12-15 months of age. For 3 + 1 schedule, 3 primary doses were administered at 2, 4, 6 and a booster at 12-15 months. Catch-up immunization was done with a single dose of PCV13 to children 2 to 5 years of age or with 2 doses of PCV13 for children between 12 and 24 months of age. Before the enrollment in the study, children attending general pediatric clinics were screened for eligibility; vaccination status and dates were obtained from vaccination record book and confirmed by hospital electronic health record database. Nasopharyngeal swabs were collected from the 500 children immediately after blood sampling.

Immunogenicity Assessment and Opsonophagocytosis Assay

Immunoglobulin G (IgG) antibody titers specific for eight serotypes (3, 6A, 6B, 7F, 14, 19A, 19F and 23F) were determined by enzyme-linked immunosorbent assay (ELISA) using the international reference serum 007sp. Pneumococcal infections due to PCV13 serotypes 1, 4, 5, 9V and 18C are rare (<4 infections from 2012-2014) in this region;
therefore IgG concentrations of those five serotypes were not analyzed in this study. Approximately 5 mL of blood collected by venipuncture, separated and serum stored at −80°C. The standard ELISA was performed at the Molecular Infectious Diseases Research Center at Chang Gung Memorial Hospital. Briefly, each well of a 96-well medium binding microtiter plate (Corning, Inc., Corning, Taiwan) was coated with 100 μL of a serotype-specific pneumococcal polysaccharide antigen (American Type Culture Collection, Manassas, VA, USA) diluted to a predetermined concentration, and plates were incubated at 37°C for 5 h in a humidified chamber. The coated plates were washed with 1X Tris-buffered saline with 0.01% Brij 35 solution. Test sera were preabsorbed with cell wall polysaccharide (Statens Serum Institut, Copenhagen, Denmark) and 22F polysaccharide (ATCC), and the reference standard 007sp (provided by Carl Frasch, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA) was pre-absorbed with cell wall polysaccharide. Sera were serially diluted 2.5-fold in absorption solution and incubated at room temperature for 30 min. After incubation, the sera (50 μL) were transferred to the coated microtiter plates, and the plates were incubated for 2 h at room temperature. The plates were washed three times, and 100 μL of diluted alkaline phosphatase-conjugated goat anti-human immunoglobulin G (IgG; Southern Bio-tech, Birmingham, AL, USA) was added to each well. After a 2-h incubation, the plates were washed three times, and 100 μL of substrate solution (diethanolamine [Sigma, MO, USA] with 1 mg/mL of p-nitrophenyl phosphate powder [Sigma]) was added to each well. After a 2-h incubation at room temperature, 50 μL of 3 M NaOH was added to all wells to stop the enzyme reaction. After 30 minutes, read optical density values at 405 nm and 650 nm on a Spectramax plate reader, with Softmax Pro software (Molecular Devices, USA). Optical densities from SoftMax Pro software were converted to
antibody concentrations using Excel spreadsheet. The lower limit of assay quantification was 0·15 μg/mL and IgG concentrations of 0·35 μg/mL or higher were considered protective.

In a subset of 50 sera, opsonophagocytic activity was determined for serotypes 1, 3, 5, and 19A by multiplex Opsonophagocytosis assay (OPA) at the WHO Reference Laboratory, University College London Institute of Child Health (London, UK). Values are expressed as an opsonophagocytic assay titer (OPA, titer ≥1 : 8 considered positive), equivalent to the reciprocal of the serum dilution required to produce 50% killing of the relevant serotype.2

The immunogenicity endpoints for the study were the anti-pneumococcal polysaccharide IgG geometric mean concentrations (GMCs) and OPA geometric mean titers (GMTs) to 8 major PCV13 serotypes and proportion of subjects with IgG concentration of ≥0.35 μg/mL (responders).3

Statistical Analysis

All the analyses were performed using SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA) and SAS ver. 9.4 (SAS Institute Inc., Cary, NC, USA). GMCs were compared by a one-way analysis of variance (ANOVA) with Scheffe’s post hoc test and 2-sample t-test. Linear regression analyses was performed to assess the persistence of anti-CPS IgG post-PCV13 vaccination, correlation between the serum IgG (μg/mL) and time (years) was evaluated by a Pearson correlation coefficient (r).4,5 The association between nasopharyngeal point-prevalence and pneumococcal IgG concentration for each serotype studied was assessed from a logistic regression model using nasopharyngeal carriage rate reduction (dependent variable) as a function of the IgG concentrations (independent variable). Point-prevalence was defined as detection of a given serotype in vaccinated children; the NP point-prevalence rate describes the number of serotypes
detected relative to the total number of subjects. The predicted changes in point-
prevalence nasopharyngeal carriage rate corresponding to the antibody concentration
threshold of $\geq 0.35$ μg/mL and to selected percentiles of IgG concentration were
calculated for the total population. Predicted acquisition rates correspond to the 25th
to 90th percentiles of the observed IgG concentrations. Odds ratios (OR) and the
corresponding 95% confidence intervals (CI) were calculated from the logistic
regression models.

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