Superior Immune Response to Protein-conjugate vs. Free Pneumococcal Polysaccharide Vaccine in COPD

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Methods (844 words)

Study design: Randomized, open label trial that compared the safety and immunogenicity of PCV7 (1.0 mL) to PPSV23 vaccination in 120 subjects with moderate to severe COPD. The study was conducted by the 10 centers participating in the National Heart, Lung and Blood Institute's COPD Clinical Research Network (CCRN). Randomization was performed after linking to the CCRN coordinating center website and stratified by study center. The study was approved by the CCRN Protocol Review Committee, each of the participating center's Institutional Review Boards and by the Food and Drug Administration under an Investigational New Drug approval. The study was registered on-line as a clinical trial.

Study population: Subjects were men and women over age 40 with $\geq$ 10 pack-year cigarette smoking history with a clinical diagnosis of moderate to very severe COPD (as defined by post-bronchodilator $FEV_1/FVC < 70\%$ and $FEV_1 < 70\%$ predicted). Subjects were eligible if they had never received PPSV23 or if it was administered more than five years prior to randomization. Exclusion criteria included a diagnosis of asthma, sensitivity to pneumococcal vaccination, bleeding disorder or chronic anticoagulation, use of immunosuppressive medications other than corticosteroids, or the presence of conditions known to impair pneumococcal vaccine response (alcoholic cirrhosis, HIV, insulin-dependent diabetes, chronic renal failure requiring dialysis, nephrotic syndrome, malignancy, organ transplantation, immunodeficiency syndromes, or pregnancy). Subjects were also excluded if they had suffered an acute illness requiring antibiotics or steroids within the past month or were not expected to survive 12 months.

Vaccines and Administration: Each 1.0 mL dose of PCV7 contained 4 mcg of the capsular polysaccharide from serotypes 4, 9V, 14, 18C, 19F and 23F and 8 mcg of serotype 6B covalently linked to a total of 40 mcg of CRM197, a non-toxic diphtheria protein. The 1.0 mL dose of PCV7 also contained 0.250 mg of aluminum as aluminum phosphate. PPSV23 (Pneumovax®, Merck) was injected in the approved volume of 0.5 mL and contained 25 mcg of the capsular polysaccharide from each of the 23 serotypes included and 0.25% phenol. Vaccinations were given as a single intramuscular deltoid injection with a 1 inch needle. Neither subjects nor investigators were blinded to vaccine assignment.

Serologic Testing: Blood specimens were obtained immediately prior to and one month following vaccination and were shipped to the University of Alabama at Birmingham where serologic testing was performed. The capacity of each serum to opsonize *S. pneumoniae* for ingestion and killing by phagocytes was determined by incubating bacteria in serum and then exposing them *in vitro* to HL-60 cells (E1). The results are reported as an opsonophagocytosis killing index (OPK), which represents the reciprocal of the serum dilution that led to 50% uptake and killing of pneumococci during incubation at 37^{o} C for one hour. Total IgG antibody concentrations to the seven PCV7 serotypes were also measured using a WHO-recommended ELISA protocol (www.vaccine.uab.edu). To avoid measuring non-specific antibodies, the serum samples were pre-incubated with C-polysaccharide and capsular polysaccharide of serotype 22F. Laboratory personnel were masked to treatment assignments.

Safety: Local and systemic reactions to the vaccination were recorded in a seven day diary that was returned to the subject's clinical center. Each subject was also called

on day seven after vaccination to ask about adverse reactions. The diary provided graded levels of toxicity to the injection for each of several possible adverse events - grade I (present but easily tolerated), grade II (interfered with normal activity), and grade III (severe or incapacitating). Subjects were encouraged to contact the center if they experienced grade II toxicity on days 5-7 or grade III toxicity at any time during the first week. All subjects returned 1 month after vaccination, at which time information on any additional adverse events was recorded.

Statistics: Antibody levels (IgG) and OPK were transformed using natural logarithms for statistical analysis to account for their strongly skewed distributions and are reported as geometric means. A paired t-test was used to assess the increase in serotype-specific IgG and OPK from pre- to post- vaccination within study groups. An unpaired t-test was used for between group comparisons of post-vaccination IgG and OPK. In order to correct for differences in pre-vaccination IgG and OPK, we also compared the ratios of one month to baseline IgG and OPK between vaccine groups. Based on the data reported by Jackson describing PCV7 and PPSV23 responses in healthy elderly subjects (E2), we estimated that enrolment of 120 subjects would provide more than 90% power to detect a significant difference in the one month to baseline OPK ratio between the two study groups for each serotype. We performed univariate and multivariate linear regression to determine the relationship between age, gender, vaccine assignment, lung function impairment (FEV_1 % predicted), and prior vaccination status with vaccine responsiveness as measured by the number of serotypes to which a subject exhibited a ten-fold increase in OPK or a two-fold increase in IgG (E3). The proportion of subjects reporting systemic or local adverse reactions during the 7-day diary were

compared using Fisher's exact test. P values < 0.05 were considered significant. No adjustments were made for multiple comparisons.

References

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