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Stunting correlates with high salivary and serum antibody levels after 13-valent pneumococcal conjugate vaccination of Venezuelan Amerindian children

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ABSTRACT

Objective: To determine the impact of pre-vaccination nutritional status on vaccine responses in Venezuelan Warao Amerindian children vaccinated with the 13-valent pneumococcal conjugate vaccine (PCV13) and to investigate whether saliva can be used as read-out for these vaccine responses.
Methods: A cross-sectional cohort of 504 Venezuelan Warao children aged 6 weeks – 59 months residing in nine geographically isolated Warao communities were vaccinated with a primary series of PCV13 according to Centers for Disease Control and Prevention (CDC)-recommended age-related schedules. Post-vaccination antibody concentrations in serum and saliva of 411 children were measured by multiplex immunoassay. The influence of malnutrition present upon vaccination on post-vaccination antibody levels was assessed by univariate and multivariable generalized estimating equations linear regression analysis.
Results: In both stunted (38%) and non-stunted (62%) children, salivary antibody concentrations correlated well with serum levels for all serotypes with coefficients varying from 0.61 for serotype 3–0.80 for serotypes 5, 6A and 23F (all $p < 0.01$).

Surprisingly, higher serum and salivary antibody levels were observed with increasing levels of stunting in children for all serotypes. This was statistically significant for 5/13 and 11/13 serotype-specific serum and saliva IgG concentrations respectively.
Conclusion: Stunted Amerindian children showed generally higher antibody concentrations than well-nourished children following PCV13 vaccination, indicating that chronic malnutrition influences vaccine response.

Saliva samples might be useful to monitor serotype-specific antibody levels induced by PCV vaccination. This would greatly facilitate studies of vaccine efficacy in rural settings, since participant resistance generally hampers blood drawing.

The study was registered in a primary registry of the World Health Organization with identifier number RPCEC00000158.

1. Introduction

Protein-conjugated pneumococcal vaccines (PCVs) effectively induce antibody responses against the capsular polysaccharides. Although serum samples are the standard for antibody measurements, body fluid that can easily be taken using a less invasive procedure is strongly preferred, particularly in young children.

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Drawing blood is not always easy in Western countries and it is even more difficult in resource poor settings, also as a consequence of cultural differences and the sensitivity to drawing blood. An easily accessible sample is saliva in which antibodies against the pneumococcal capsular polysaccharides can be measured. There are limited data comparing salivary and serum antibody concentrations after PCV vaccination and only from Western world settings [1–3]. To our knowledge, this is the first study in which PCV induced antibodies are measured in saliva of children residing in a tropical rural region.

For PCVs, serotype-specific antibody concentrations $\geq$0.35 μg/ml are regarded as predictive of protection against invasive disease [4]. A recent study, however, suggests that the 0.35 μg/ml correlate of protection does not correctly predict the vaccine effectiveness for each pneumococcal serotype. For most serotypes, higher antibody concentrations seem to be needed for protection [5]. High antibody concentrations after vaccination are indeed associated with reduced acquisition of *Streptococcus pneumoniae* [6]. Despite the large number of studies evaluating the immunogenicity of PCVs in many settings, information on the factors determining the concentration of anti-pneumococcal antibodies is surprisingly scarce. Specific risk groups such as HIV-infected children and low birth weight infants have been studied and generally reach adequate antibody concentrations after PCV vaccination [7,8].

Indigenous children are a particularly vulnerable group with extraordinary high rates of pneumococcal carriage and related diseases [9,10]. In addition to infectious diseases, malnutrition is a major concern in indigenous communities with chronic malnutrition affecting up to over 50% of children under 5 years of age [10,11]. The devastating effects of malnutrition on the immune system may influence vaccine responses [12]. Despite the high burden of malnutrition among many indigenous populations, the potential influence of malnutrition on vaccine responses in these populations has not received much attention. Only a single published study described the influence of malnutrition in indigenous children (i.e. non-indigenous Spanish descendants and native Indian Guatemalan infants) on vaccine response. Asturias and co-workers measured significantly higher geometric mean concentrations against the *Haemophilus influenzae* type b and Hepatitis B vaccine antigens in Indian infants as opposed to nonnative infants. However, this was not related to nutritional status since malnourished infants responded equally well as healthy infants [13].

Approximately 50 million indigenous people belonging to more than 600 ethnic groups live in the Americas today, comprising more than 10% of the total population and 40% of the rural population in South America and the Caribbean [14]. Pneumococcal conjugate vaccines (PCVs) have been implemented in the National Immunization Programs of many South American countries, including Venezuela, during the past years. While this has led to increased access of indigenous children to PCVs, the influence of their impaired nutritional status on vaccine responses has not yet been investigated.

In this study we show for the first time that salivary PCV13 antibodies collected in a resource-poor setting correlate with serum antibody levels and thus may be used to measure vaccine-induced antibody concentrations. Additionally, we describe the influence of increased levels of stunting on serum and salivary antibody concentrations after PCV13 vaccination in Venezuelan Warao Amerindian children. Because nutritional status is known to be affected by helminth and protozoan infections in these children [15], the analyses were adjusted for the presence of intestinal parasites in fecal samples. Pneumococcal colonization rates in these villages are high with up to 76% of children under 2 years of age carrying *S. pneumoniae* [9]. Together with the high point-prevalence rates of acute respiratory tract infections of up to 50% [16], these data suggest that Warao children would benefit from pneumococcal vaccination. It is important to take into consideration the potential influence of the poor nutritional status of children on vaccine effectiveness in order to ensure PCV-related beneficial public health effects.

2. Methods

2.1. Ethical considerations

The study was approved by the ethical committee of the Instituto de Biomedicina, Caracas, Venezuela. In addition, written permission to carry out the study was obtained from the Delta Amacuro Indigenous Health Office and from community leaders of each included community. Children were enrolled if their parents or primary caregivers provided written informed consent.

2.2. Study setting

This study was performed in Antonio Diaz, the largest of four municipalities in the Orinoco River Delta in Venezuela from May to November 2012. The Warao people live in about 300 geographically isolated villages that are spread throughout the distributaries of the Orinoco River where they receive little medical attention and live under poor sanitary conditions. Warao children aged 6 weeks – 59 months from nine geographically dispersed communities in Antonio Diaz were included. Door-to-door visits were made to inform all parents of age-eligible children present in these nine communities during study visits. Exclusion criteria were: known immunosuppression, previous vaccination with any pneumococcal vaccine or major congenital malformations.

2.3. Sampling and vaccine schedule

Children were sampled just before the first vaccination and again at 1.5 months (median 6.7 weeks (IQR 6.4–6.9 weeks)) after the primary series. Nasopharyngeal swab samples were obtained with a flexible swab (Copan, Italia) in STGG medium [17]. Serum was obtained after collecting blood samples by venous puncture or, if unsuccessful, finger prick. Saliva samples were collected using cotton rolls and were stored in EDTA tubes [18].

All samples were refrigerated at 4 °C for $\leq$3 days, before being transported to a −20 °C freezer and, within four weeks, to a −70 °C freezer where they were stored until analyses took place. Serum and saliva samples were transported to The Netherlands on dry ice.

Children received a primary series of PCV13, i.e. children aged 6 weeks to 6 months received three vaccine doses whereas children aged 7-23 months and 24–59 months received two and one dose(s) respectively, following Centers for Disease Control and Prevention (CDC) guidelines [19]. PCV13 (Prevnar 13®, Pfizer) contains polysaccharide antigens corresponding to pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A, and 23F. Individual polysaccharide antigens were conjugated to a nontoxic diphtheria toxin (cross-reactive material 197). Each single-dose syringe contained 0.5 mL with 2.2 μg of each pneumococcal serotype, except for 4.4 μg of serotype 6B. Each dose was formulated in 5.0 mM succinate and 0.85% sodium chloride at pH 5.8 with 0.125 mg aluminum as aluminum phosphate and 0.02% polysorbate 80. Vaccine lot numbers used were 915893, 919692 and 920394. The vaccine was preservative-free and stored at 2–8 °C.

2.4. Laboratory methods

Determination of concentrations of pre- and post-vaccination serum and post-vaccination salivary immunoglobulin G (IgG) antibodies against all 13 serotypes was performed in the RIVM