Author's response to reviews

Title: A randomised trial to evaluate the immunogenicity, reactogenicity, and safety of the 10-valent pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) co-administered with routine childhood vaccines in Singapore and Malaysia

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Author's response to reviews: see over
Ms: 5705178781290279

Dear Dr. Ramos,

Please find attached the revised version of the manuscript entitled “A randomised trial to evaluate the immunogenicity, reactogenicity, and safety of the 10-valent pneumococcal non-typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) co-administered with routine childhood vaccines in Singapore and Malaysia” by Fong Seng Lim, Mia Tuang Koh, Kah Kee Tan, Poh Chong Chan, Chia Yin Chong, Yeo Wee Shung Yehudi, Yee Leong Teoh, Fakrudeen Shafi, Marjan Hezareh, Kristien Swinnen, and Dorota Borys.

This manuscript has been revised in response to the peer review comments. Changes made to the manuscript are detailed below and highlighted in yellow in the manuscript file.

We hope that this revised version of our manuscript is now acceptable for publication in BMC Infectious Diseases.

Yours sincerely,

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Changes in response to comments from Reviewer #1

**Suggestion 1.** Is the question posed by the authors well defined? Yes, the question proposed by the authors is clearly stated and defined. Title could be different being more concise in the comparison between 2 different pneumococcal conjugate vaccines that are equal in its composition to determine no differences between them.

**Suggestion 2.** Do the title and abstract accurately convey what has been found? Not at all, because the title remarks on a randomized new pneumococcal vaccine PHiD-CV that are co-administrated with routine vaccines and the main objective of the study is to compare a commercial and a phase III 11- serotype pneumococcal conjugate vaccine.

**Answer:** We agree with the Reviewer’s point of view that the comparison between the clinical and commercial lots of PHiD-CV was an important objective of this study. However, we have the feeling that rephrasing the title by putting more emphasis on the differences between lots would not correctly reflect all the manuscript content, since only the commercial lot was used in the booster phase of the study, which was only conducted in Singapore. Besides the study was performed to address a need to generate data in Asian population in Singapore and Malaysia. Taken this into account, we suggest to keep a more general title, which most optimally reflects and summarizes the objectives of the study. Please also note that the clinical phase III lot consisted of a 10-valent pneumococcal conjugate vaccine and not its 11-valent predecessor.

**Suggestion 3.** An important question to be assumed by the authors when they write: "Although it remains unclear why the magnitude of immune responses to pneumococcal conjugate vaccines varies in different populations, plausible explanations include genetic factors, early exposure to *S. pneumoniae*, or nasopharyngeal carriage of pneumococcal serotypes", is to justify why no blood or nasopharynx swab sample is obtained before the first primary vaccination before the age of 2 month-old. Some of these challenges would be able to have an answer including this action into the methodology design of the study. Taking blood before first vaccination will allow us to know which percentage of children were pneumococcal carriers before first vaccination and to know early exposures to them in this study population, which is likely different to other Western countries children.

**Answer:** We agree with the Reviewer’s comment that the analysis of blood samples taken before first vaccination would have provided an added scientific value to our manuscript. However, as specified in the protocol, blood samples were only taken from the study participants at one month post-primary vaccination, before and one month after the booster dose administration. No blood samples were collected at pre-vaccination, and therefore, we have no view on the antibody concentrations before primary vaccination.
Early exposure to S. pneumoniae in Asian children was evaluated in a previous study conducted in Taiwan, where the percentage of children with pre-vaccination antibody concentrations ≥0.2 µg/ml ranged from 11.5% to 42.5% for each PHiD-CV serotype, except serotype 14 (61%) (Lin et al., 2012). In this previous study, ≥95% of children had antibody concentrations ≥0.2 µg/ml at one month post-primary vaccination. A consistently lower percentage of children with pre-vaccination antibody concentrations ≥0.2 µg/ml was observed in a previous study conducted in Europe (Prymula et al., 2006), where it ranged from 0.8% to 33.9% of infants for each of the 10 PHiD-CV vaccine serotypes, except serotype 14 (≥58.3%) (unpublished data).

We suggest adding the following sentences in response to this comment: “Pre-vaccination antibody concentration, which is influenced by waning maternal antibodies and increasing adaptive immunity due to early exposure to S. pneumoniae, in Asian children was evaluated in a previous study conducted in Taiwan, where for each vaccine pneumococcal serotype the percentage of children with pre-vaccination antibody concentrations ≥0.2 µg/ml ranged from 11.5% to 42.5%, except serotype 14 (61%) [19].”

Changes in response to comments from Reviewer #2

Suggestion 1. The authors could explain why clinical study lot and commercial lot were compared.

Answer: In order to be able to produce PHiD-CV at a larger scale for commercial use, changes in the manufacturing process, which had no impact on the composition of the vaccine, were implemented. The purpose of this study was to demonstrate that these changes have no clinical impact and that the immune response induced by the commercial lot of PHiD-CV is non-inferior to that induced by the phase III clinical lot of PHiD-CV. In addition, the study was performed to address a need to generate data in Asian population in Singapore and Malaysia.

Suggestion 2. Please give more details on the ELISA for anti-prot D antibodies – or a Reference

Answer: We agree with the Reviewer’s comment that more details should be given on the ELISA for anti-protein D. IgG antibodies against NTHi protein D were measured by an in-house ELISA using a recombinant non-lipidated form of protein D as coating material. When dilutions of human sera are added to polystyrene plates with adsorbed protein D antigen, anti-protein D antibodies bind to the coated antigen. Then, the anti-protein D antibodies are detected using an anti-human-IgG peroxidase labelled antibody followed by the addition of tetramethylbenzidine substrate. The level of anti-protein D antibodies present in a sample is determined by comparison to a reference serum, which is a pool of human sera or plasma calibrated in arbitrary units. Anti-protein D antibody concentrations were expressed in ELISA units per mL (EL.U/mL) and the assay cut-off was 100 EL.U/mL.
The following sentence was rewritten in response to this comment: “Antibodies against NTHi protein D were measured by an in-house ELISA using a recombinant non-lipidated form of protein D as coating material. Anti-protein D antibodies, which are bound to protein D antigens adsorbed on polystyrene plates, are detected using an anti-human-IgG peroxidase labelled antibody followed by the addition of tetramethylbenzidine substrate. Anti-protein D antibody concentrations were expressed in ELISA units per mL (EL.U/mL), and the assay cut-off was 100 EL.U/mL.”

**Suggestion 3.** Are anti-protein D antibody concentrations connected to protection against NTHi infections?

**Answer:** A previous study has shown that the precursor 11-valent pneumococcal conjugate vaccine using protein D as carrier protein provided some efficacy against AOM due to NTHi (33.3% [95% CI: 0.3 to 55.4%]) (Prymula et al., 2006). Although a clear correlation between efficacy and anti-protein D antibody concentrations could not be established, it was reasonably assumed that the protein D carrier contributed to the induction of protection against NTHi. This previous study showed that three primary PHID-CV doses induced immune responses to protein D and immunological memory. These results were in line with those of another clinical trial conducted in Latin America, which suggested that the efficacy of PHID-CV against all episodes of NTHi AOM was 24.5% (95%CI: -38.3, 58.8%) (Tregnaghi et al., 2014). Of note, this study was not powered to assess efficacy against NTHi AOM.

The results of these studies on pneumococcal conjugate vaccines using protein D as carrier protein were in contrast with those of another study, where the incidence of AOM episodes caused by NTHi tended to increase following administration of two 7-valent pneumococcal conjugate vaccines not containing protein D (Eskola et al., 2001; Kilpi et al., 2003). Vaccine efficacy against NTHi AOM was -11% (95% CI: -34 to 8%) for the licensed 7-valent CRM197-conjugated pneumococcal conjugate vaccine and -9% (95% CI: -32 to 0%) for the 7-valent meningococcal outer membrane protein complex-conjugated pneumococcal vaccine.

Additionally, pre-clinical experiments using the juvenile chinchilla otitis media model confirmed that serum taken from vaccinees who had received primary immunisation with protein D-conjugated vaccine (either the 11-valent precursor pneumococcal conjugate vaccine or PHID-CV) had a biological activity against NTHi otitis media. As long as no surrogate marker is adequately established, these data are considered supportive of the protective impact of protein D in the vaccine, as they show that anti-protein D antibodies induced by PHID-CV have functional activities.

We suggest the following rewording in the discussion to respond to this comment: “Both PHID-CV lots induced antibodies against the NTHi protein D carrier, which could potentially provide protection against disease caused by NTHi. Although a clear correlation between efficacy and anti-protein D antibody concentrations has not been established, efficacy trials with the predecessor 11-valent NTHi protein D-
conjugated vaccine and PHiD-CV have suggested that the protein D carrier contributed to the induction of protection against acute otitis media due to NTHi [45;46].”