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Poor association between 13-valent pneumococcal conjugate vaccine-induced serum and mucosal antibody responses with experimental *Streptococcus pneumoniae* serotype 6B colonisation

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ABSTRACT

Background: Pneumococcal carriage is the primary reservoir for transmission and a prerequisite for invasive pneumococcal disease. Pneumococcal Conjugate Vaccine 13 (PCV13) showed a 62% efficacy in protection against experimental *Streptococcus pneumoniae* serotype 6B (Spn6B) carriage in a controlled human infection model (CHIM) of healthy Malawian adults. We, therefore, measured humoral responses to experimental challenge and PCV-13 vaccination and determined the association with protection against pneumococcal carriage.
Methods: We vaccinated 204 young, healthy Malawian adults with PCV13 or placebo and nasally inoculated them with Spn6B at least four weeks post-vaccination to establish carriage. We collected peripheral blood and nasal lining fluid at baseline, 4 weeks post-vaccination (7 days pre-inoculation), 2, 7, 14 and > 1 year post-inoculation. We measured the concentration of anti-serotype 6B Capsular Polysaccharide (CPS) Immunoglobulin G (IgG) and IgA antibodies in serum and nasal lining fluid using the World Health Organization (WHO) standardised enzyme-linked immunosorbent assay (ELISA).
Results: PCV13-vaccinated adults had higher serum IgG and nasal IgG/IgA anti-Spn6B CPS-specific binding antibodies than placebo recipients 4 to 6 weeks post-vaccination, which persisted for at least a year after vaccination. Nasal challenge with Spn6B did not significantly alter serum or nasal anti-CPS IgG binding antibody titers with or without experimental pneumococcal carriage. Pre-challenge titers of PCV13-induced serum IgG and nasal IgG/IgA anti-Spn6B CPS binding antibodies did not significantly differ between those that got experimentally colonised by Spn6B compared to those that did not.
Conclusion: This study demonstrates that despite high PCV13 efficacy against experimental Spn6B carriage in young, healthy Malawian adults, robust vaccine-induced systemic and mucosal anti-Spn6B CPS binding antibodies did not directly relate to protection.

1. Introduction

Streptococcus pneumoniae is a commensal bacterium that is commonly carried asymptomatically in the human nasopharynx but invades other sites and causes life-threatening diseases, including pneumonia, meningitis and sepsis, particularly in children aged under 5 years, immunocompromised individuals and older people [1,2]. Low-income countries are disproportionately burdened with pneumococcal disease [2].

Pneumococcal conjugate vaccines (PCV13) have reduced vaccine-type carriage and disease in most high-income countries and have provided herd immunity to unvaccinated populations [3,4]. In Malawi, a significant reduction in pneumococcal mortality rate followed the introduction of the 13-valent pneumococcal conjugate vaccine (PCV13) in 2011 [5]. However, vaccine-type pneumococcal carriage, especially in children and HIV-infected adults, has persisted, unlike in high-income

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countries [6]. Furthermore, PCV-13 vaccination in children did not lead to sustained population-level antibody immunity against carriage beyond the first year of life [7]. Since carriage is a prerequisite for pneumococcal disease and transmission [8], reducing carriage is critical in preventing pneumococcal disease among vulnerable populations in low-income countries.

The Controlled Human Infection Model (CHIM) of *Streptococcus pneumoniae* is a rapid, safe and accurate platform for studying pneumococcal carriage in the human nasopharynx [9,10]. Previous pneumococcal CHIM studies in the UK demonstrated that experimental pneumococcal carriage increased serum and nasal IgG antibody titers and was protective against homologous pneumococcal rechallenge [11]. They also demonstrated a 78 % efficacy of PCV13 against experimental *Streptococcus pneumoniae* serotype 6B (Spn6B) carriage in a UK population. We recently conducted a study using a similar approach in a Malawian adult population and demonstrated a 62 % efficacy of PCV13 against experimental Spn6B carriage [12]. However, the incidental natural carriage was significantly higher in Malawi (42 %) than in the UK (6 %). These observations suggest that immunological correlates of protection could be different in high transmission compared to low transmission settings.

There remains a knowledge gap on the PCV13-induced correlates of protection against pneumococcal carriage in a setting with high residual vaccine-type carriage and high background serotype-specific immunity, such as Malawi. Therefore, in this current work, we utilised the CHIM-PCV13 trial in Malawi [13] to describe humoral responses to PCV-13 vaccination and how they associate with protection against experimental nasopharyngeal carriage of Spn6B.

2. Materials and methods

Participant recruitment: Healthy non-smoking HIV-uninfected adults aged between 18 and 40 years were enrolled into a double-blinded, parallel-arm, randomised controlled trial investigating the efficacy of PCV13 or placebo (allocation ratio PCV13:placebo, 1:1) against experimental pneumococcal carriage of Spn6B) [13]. National Health Sciences Research Committee (16/07/2519) and Pharmacy Medicines and Regulatory Authority (PMRA/CTRC/III/10062020121) approved the trial in Malawi and the Liverpool School of Tropical Medicine (20–021) in the United Kingdom. We registered the primary trial with the Pan African Clinical Trials Registry (PACTR202008503507113).

Randomisation: We randomised volunteers to receive an intramuscular injection of either PCV-13 (Prevnar-13, Pfizer, New York; n = 98) or normal saline (n = 106). 28 days later, participants were inoculated intranasally with either 20 000 CFU/ml, 80 000 CFU/ml or 160 000 CFU/ml of SPN6B. We followed up at least 1-year post-inoculation, a subset of study participants who re-consented in both the PCV-13 vaccinated and placebo groups.

Sample collection: We collected peripheral blood and nasal lining fluid samples before vaccination, at 4 weeks post-vaccination, 2 days, 7 days, 14 days, and > 1 year post-inoculation. Nasal lining fluid was collected onto a Synthetic Absorption Matrix (SAM) using Nasosorption ^{TM} devices (Mucosal Diagnostics, UK) at all time points. We eluted nasal lining fluid from SAM with 200ul PBS containing 5 ng/μl CWPS Multi (SSI Diagnostica). We stored serum and nasal lining fluid samples at −80 °C. In addition, we collected nasal wash samples at all time points for microbiological culture to determine nasal carriage of Spn6B. Due to sample volume limitations in the nasal samples, we did not perform all assays on all individuals.

Pneumococcal anti-capsular polysaccharide antibody ELISA: Using the World Health Organization enzyme-linked immunosorbent assay (ELISA) protocol for the detection of anti-pneumococcal capsular polysaccharide antibodies[13], we measured the concentration of IgG or IgA antibodies to the capsular polysaccharide of Spn6B in sera and nasal lining fluid. In a subset of sera and nasal lining fluid, we measured the concentration of IgG antibodies to a non-vaccine pneumococcal serotype 15B.

Briefly, ELISA plates were coated with 5 μg/ml of serotype-6B/15B capsular polysaccharide and incubated at 2–8 °C overnight (9 to 15 h). Plates were washed three times with PBS-Tween 20 (Sigma) before adding samples or Human Anti-Pneumococcal capsule Reference Serum 007sp (National Institute for Biological Standards and Control) diluted in 5 ng/μl CWPS Multi (SSI Diagnostica). Four and seven 2.5-fold serial dilutions were performed for serum samples and standards, respectively and added to the ELISA plate in triplicate before incubation at room temperature for 2 h. Plates were washed 5 times with 0.05 % PBS-Tween 20 before adding Goat anti-Human IgG- or IgA-Alkaline Phosphatase (Southern Biotech) and incubated in the dark at room temperature for 2 hrs. After washing 5 times with PBS Tween 20, we added p-nitrophenyl phosphate substrate (Sigma) and incubated the plates at room temperature in the dark for 30 min. We measured optical densities at 405 nm and calculated concentrations using a four-parameter logistic curve in MyAssays online platform. We generated a separate 007sp standard curve for each ELISA plate.

Statistical analysis: We performed statistical analyses and graphical presentations in R version 4.0.1 using RStudio (2022.07.1 + 554). We used Spearman's correlation coefficient to test the association between anti-Spn6B CPS IgG titers in serum and nasal lining fluid. We compared antibody titers between groups using the Mann-Whitney *U* test, and between time points using a paired Wilcoxon signed-rank test. Differences with p-values less than or equal to 0.05 were considered significant.

3. Results

3.1. Demographics

All study demographics of the recruited participants are published with the main study, and there were no substantial differences between the PCV13 (n = 98) vaccinated and placebo recipients (n = 106) [13]. In brief, the median age of the study participants was 25·3 years (22·9–28·5), and 72 % were male. Furthermore, we successfully re-consented and followed up at least one year post-inoculation, 132 out of 204 study participants. Of the 132 participants, 56 were in the PCV13-vaccinated group, and 76 were in the placebo group.

3.2. PCV-13 induces systemic and nasal anti-Spn6B CPS-specific binding antibodies in young Malawian adults

First, we sought to assess the impact of PCV13 vaccination on serum and nasal antibody titers in healthy young Malawian adults living in a population with prior serotype-specific background immunity [10]. We measured the concentration of binding anti-Spn6B CPS IgG in serum and nasal lining fluid in the PCV13-vaccinated adults and placebo recipients' pre-vaccination, at 4 weeks post-vaccination and at 2-, 7- and 14-days post-inoculation. At the pre-vaccination time point, there were no differences between the two study groups (Fig. 1a-b). At all the post-vaccination time points, the binding anti-Spn6B CPS IgG titers in serum and nasal lining fluid were higher in the PCV13-vaccinated than the placebo recipients (Fig. 1a-b). In a subset of adults, matched for age, sex and natural carriage status, we measured the concentration of binding anti-Spn6B CPS IgA in nasal lining fluid at 4 weeks post-vaccination. We found that the binding anti-Spn6B CPS IgA titers in nasal lining fluid were also higher in PCV13-vaccinated adults than in placebo recipients (Fig. 1c).

The titers of binding anti-CPS IgG against a non-vaccine serotype 15 in serum (Fig. 1d) and nasal lining fluid (Fig. 1e) were similar between the PCV13-vaccinated adults and placebo recipients, confirming that the increase in binding anti-Spn6B CPS IgG and IgA was attributable to PCV13 vaccination. We observed a modest correlation between the binding anti-Spn6B CPS IgG titers in serum and nasal lining fluid at 4 weeks post-vaccination in the PCV13-vaccinated adults (Fig. 1f),

Figure 1

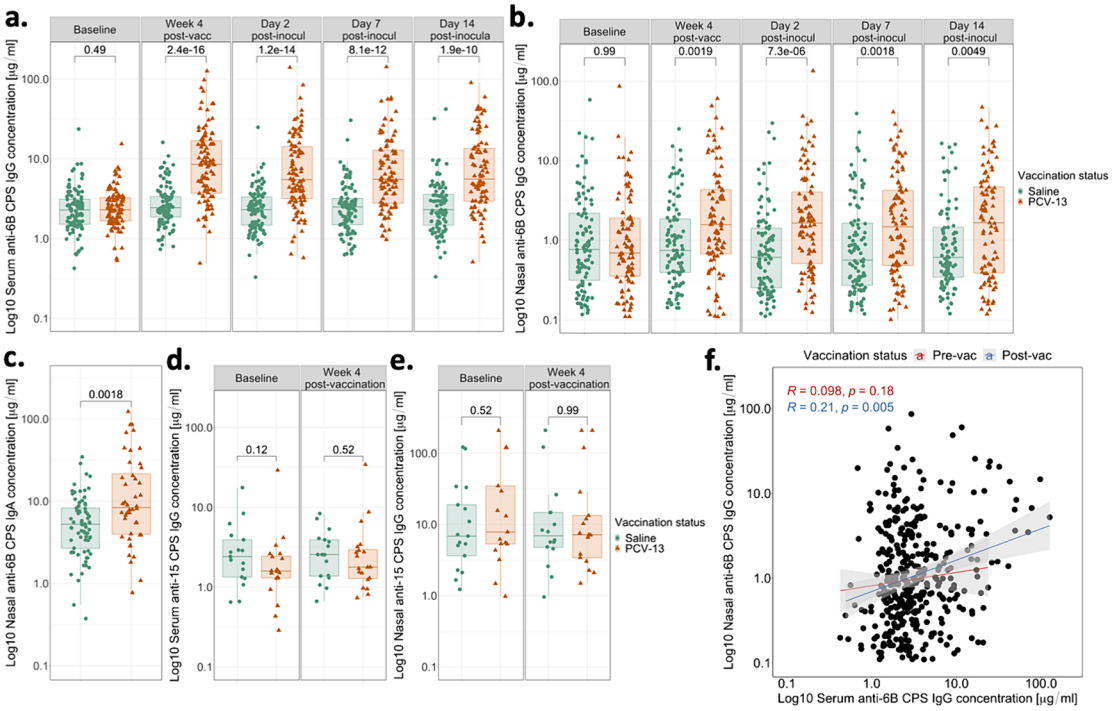

Fig. 1. Binding anti-Spn6B CPS antibodies in serum and nasal lining fluid before and following PCV13 vaccination. Titers of binding anti-Spn6B CPS antibodies in serum and nasal lining fluid were measured using an enzyme-linked immunosorbent assay (ELISA). The serum and nasal samples were obtained from volunteers vaccinated with PCV13 and those given a placebo as a control. All participants were inoculated with Spn6B approximately 4 weeks post-vaccination. The samples were obtained before (baseline) and after (4 weeks p.v., Day 2p.i., Day 7p.i. and Day 14p.i.) vaccination. **a)** Serum and **b)** nasal anti-Spn6B CPS IgG antibody titers before and after vaccination (Saline n = 106 vs. PCV13 n = 98). **C)** Nasal anti-Spn6B CPS IgA antibody titers after vaccination (Saline n = 73 vs. PCV13 n = 44). **D)** Serum and **e)** nasal anti-Spn15 CPS IgG antibody titers before and 4 weeks post-vaccination (Saline n = 17, PCV13 n = 20). **F)** Correlation between serum and nasal anti-Spn6B CPS IgG antibody titers, before (red) and 4 weeks post-vaccination (blue). The horizontal bars represent the median and interquartile range (IQR). Data were analysed using the Mann-Whitney *U* test, comparing PCV13 (green) with placebo (orange) within each time point, and correlation was determined using the Spearman test. P.v., post-vaccination; p.i., post-inoculation; CPS, capsular polysaccharide; IgG, immunoglobulin G; IgA, immunoglobulin A; Spn6B, *Streptococcus pneumoniae* serotype 6B. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

suggesting partial diffusion of antibodies from the blood into the nasal mucosa. The results show that PCV13 induces robust systemic and nasal anti-Spn6B CPS-specific binding antibodies despite high serotype-specific background immunity.

3.3. Persistent high titers of serum IgG and nasal IgG/IgA anti-Spn6B CPS-specific antibodies for at least a year post-PCV13 vaccination

Next, we sought to assess the longevity of PCV13-induced anti-Spn6B CPS-specific antibody responses. Serum and nasal anti-Spn6B CPS IgG binding antibodies increased following PCV13 vaccination and remained above baseline, and above placebo recipients at least one-year post-vaccination (Fig. 2a-b). Similarly, the binding anti-Spn6B CPS IgA titers in nasal lining fluid remained higher in PCV13 vaccinated adults than placebo recipients over a year following vaccination (Fig. 2c). The results show that PCV13-induced serum and nasal anti-Spn6B IgG binding antibodies persist at high titers for at least a year post-vaccination in healthy young Malawian adults.

3.4. Nasal challenge with Spn6B does not alter systemic or mucosal anti-CPS IgG antibody titers with or without experimental pneumococcal carriage

Experimental pneumococcal carriage augments nasal and serum binding anti-Spn6B CPS IgG titers in UK adults [14,15]. In our pilot work, experimental pneumococcal carriage did not induce serum binding anti-Spn6B CPS IgG titers in healthy young Malawian adults [10]. We, therefore, compared the fold change in nasal and serum anti-Spn6B CPS IgG binding antibodies pre- and post-inoculation between unvaccinated adults with or without experimental Spn6B carriage. The nasal and serum anti-Spn6B CPS IgG binding antibody fold change from pre- to post-inoculation was not different between Spn6B carriage^{pos} and carriage^{neg} at any time point (Fig. 3a-b). Furthermore, serum and nasal anti-Spn6B CPS IgG binding antibody fold change from pre- to post-inoculation was not different across post-inoculation time points (Fig. 3c-d). In addition, binding anti-Spn6B IgG/IgA in serum and nasal lining fluid were not different between individuals with and without natural carriage (Supplementary Fig. 1). The results demonstrate, at least in our dataset, that nasal challenge or experimental carriage with Spn6B does not significantly alter serum and nasal anti-CPS IgG binding antibody titers in healthy young Malawian adults.

3.5. Serum IgG and nasal IgG/IgA anti-Spn6B CPS antibodies are poorly associated with protection against experimental Spn 6B carriage in Malawian adults

PCV13-induced anti-Spn CPS IgG binding antibodies are associated with protection against carriage in children, with two-fold higher antibody concentrations required for protection in low-income relative to high-income countries [16]. However, in low-income settings with high residual vaccine-serotype carriage [6], such as Malawi, there remains uncertainty on the correlates of protection (CoP) against pneumococcal

Figure 2

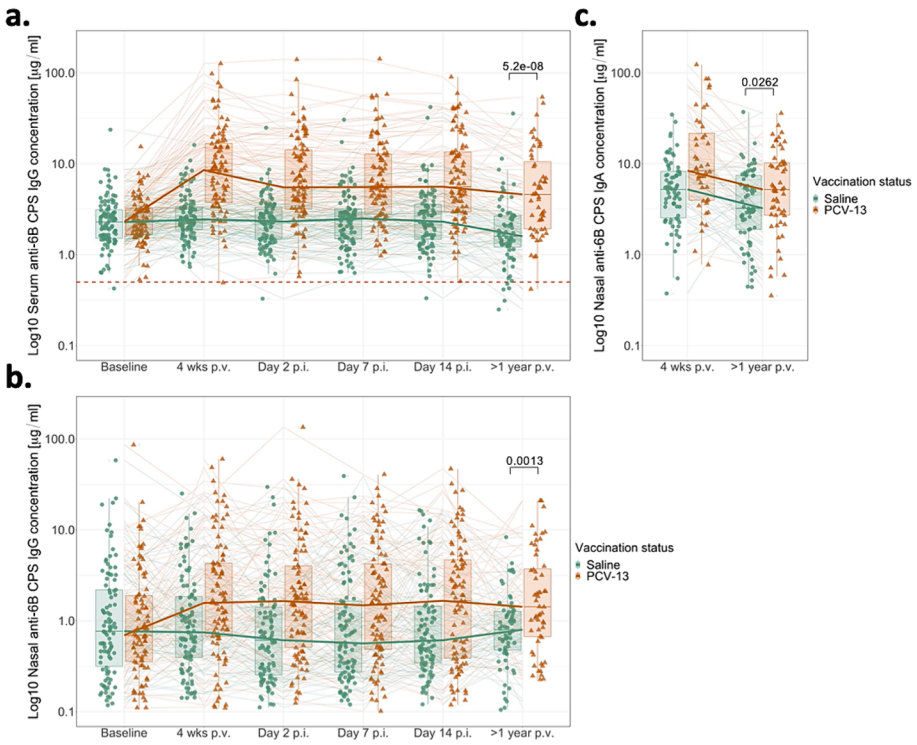

Fig. 2. Longevity of binding anti-Spn6B CPS antibodies in serum and nasal lining fluid following PCV13 vaccination. Titers of binding anti-Spn6B CPS antibodies in serum and nasal lining fluid were measured using an enzyme-linked immunosorbent assay (ELISA). The serum and nasal samples were obtained from volunteers vaccinated with PCV13 and those given a placebo as a control. All participants were inoculated with Spn6B approximately 4 weeks post-vaccination. The samples were obtained before (baseline) and after (4 weeks p.v., Day 2p.i., Day 7p.i., Day 14p.i. and > 1 yr p.v.) vaccination. a) Kinetics of a) serum and b) nasal anti-Spn6B CPS IgG antibody titers before and after vaccination (>1yr p.v., saline n = 76 vs. PCV13 n = 56; all other, Saline n = 106 vs. PCV13 n = 98). C) Kinetics of nasal anti-Spn6B CPS IgA antibody titers before and at least one year after vaccination (4wks p.v. Saline n = 73 vs. PCV13 n = 44; >1yr p.v., saline n = 71 vs. PCV13 n = 55). The horizontal bars represent the median and interquartile range (IQR). The red line is the published correlate to protection against Spn6B carriage of 0.50 µg/ml [16]. Data were analysed using the Mann-Whitney *U* test, comparing placebo recipients (green) with PCV13 vaccinated (orange) within each time point. p.v., post-vaccination; p.i., post-inoculation; CPS, capsular polysaccharide; IgG, immunoglobulin G; IgA, immunoglobulin A; Spn6B, Streptococcus pneumoniae serotype 6B. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

carriage. We, therefore, sought to establish the association between anti-Spn6B CPS IgG binding antibodies in serum and nasal lining fluid with experimental Spn6B carriage. The binding anti-Spn6B CPS IgG titers in serum and nasal lining fluid were similar between Spn6B carriage^{pos} and carriage^{neg} adults before and after inoculation in both the PCV13-vaccinated and placebo recipients (Fig. 4a-b). There was also no significant difference in the fold change in serum and nasal anti-Spn6B CPS IgG binding antibody concentrations following vaccination in Spn6B carriage^{pos} and carriage^{neg} adults (Fig. 4c-d).

Moreover, post-vaccination nasal anti-Spn6B CPS IgA binding antibody titers did not differ significantly between Spn6B carriage^{pos} and carriage^{neg} adults (Fig. 4e). However, further analysis based on carriage status alone, excluding vaccination status, revealed lower serum binding anti-Spn6B CPS IgG titers in Spn6B carriage^{pos} than carriage^{neg} adults, 7 days pre-inoculation and 2 days post-inoculation (Fig. 4f). In contrast, we did not observe differences in nasal anti-Spn6B CPS IgG binding antibody titers in Spn6B carriage^{pos} and carriage^{neg} adults before and after inoculation (Fig. 4g). Together, these findings indicate that PCV13-induced serum and nasal anti-Spn6B CPS IgG binding antibodies poorly correlate with protection against experimental pneumococcal carriage in adults living in a setting with high residual vaccine-serotype carriage and prior serotype-specific background immunity.

4. Discussion

The study investigated humoral responses to experimental pneumococcal challenge and PCV-13 vaccination and how they associate with protection against Spn6B carriage in a setting with high residual vaccine-type carriage and prior serotype-specific background immunity. We show that PCV13 vaccination induces robust serum IgG and nasal IgG/IgA anti-Spn6B CPS binding antibodies in healthy young Malawian adults, which persists at high titers for at least 1-year post-vaccination. We also found that PCV13-induced binding serum IgG and nasal IgG/IgA anti-Spn6B CPS titers did not strongly associate with protection against experimental Spn6B carriage following challenge.

Considering the 62 % protective efficacy of PCV13 vaccination against experimental Spn6B carriage observed in this cohort [13], it was surprising that we did not observe a strong association between systemic and mucosal anti-Spn6B CPS antibodies with protection against experimental carriage following Spn6B challenge. Moreover, pre-vaccination serum anti-Spn6B CPS IgG antibody titers in our study were half a log higher than the published correlate of protection threshold against Spn6B carriage in children [16,17]. However, they did not predict protection against experimental Spn6B carriage. This finding is consistent with observations from a similar study in the UK, which also could not show a direct relationship between PCV13-induced binding and functional antibody to experimental Spn6B carriage protection [18].

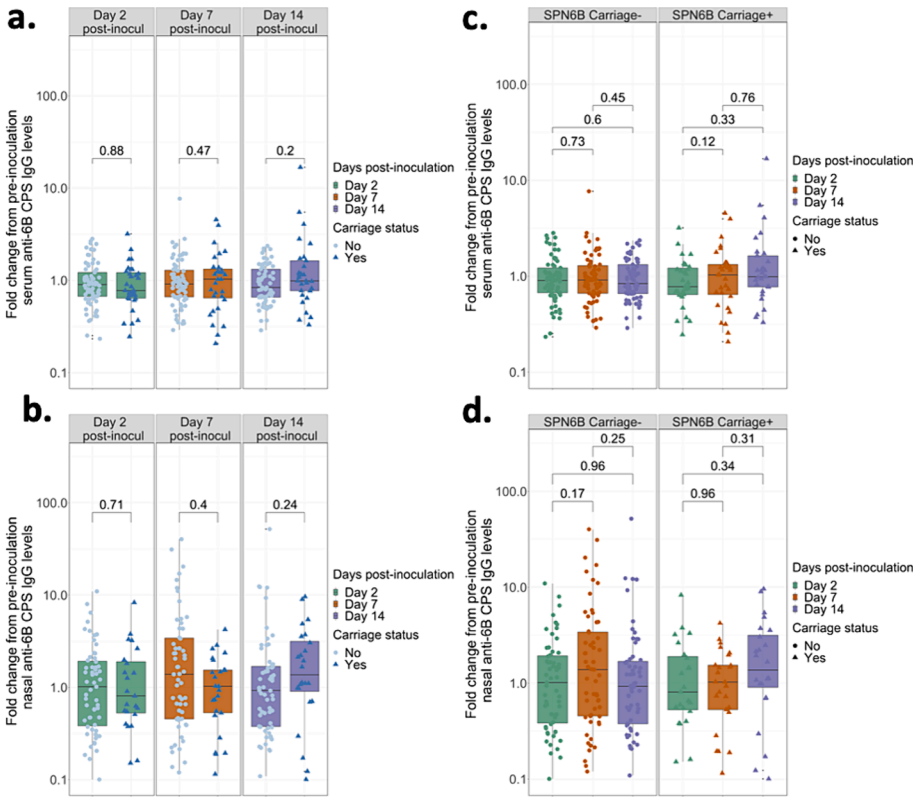

Fig. 3. Anti-Spn6B CPS IgG binding antibodies in serum and nasal lining fluid following Spn6B challenge. Titers of binding anti-Spn6B CPS IgG antibodies in serum and nasal lining fluid were measured using an enzyme-linked immunosorbent assay (ELISA). All participants were inoculated with Spn6B, and samples obtained before (Day −7) and after (Day 2p.i., Day 7p.i., and Day 14p.i.) inoculation were analysed. Fold change in **a)** serum and **b)** nasal anti-Spn6B CPS IgG antibody titers before and after (Day 2p.i., Day 7p.i., and Day 14p.i.) in experimental carriage positive compared to negative adults ($Spn6B^+$ n = 31 vs. $Spn6B^-$ n = 75). Comparison of fold change in **c)** serum and **d)** nasal anti-Spn6B CPS IgG antibody titers before and after, across different time points (all time points, $Spn6B^+$ n = 31 vs. $Spn6B^-$ n = 75). The horizontal bars represent the median and interquartile range (IQR). Data were analysed using the Mann-Whitney *U* test, comparing carriage positive (filled circles) with negative (filled triangles) within each time point. Comparisons across different time points were done using the Wilcoxon signed-ranked test. p.v., post-vaccination; p.i., post-inoculation; CPS, capsular polysaccharide; IgG, immunoglobulin G; Spn6B, *Streptococcus pneumoniae* serotype 6B.

However, there were some notable differences between the studies, including that the baseline serum anti-Spn6B CPS IgG binding antibody titers were 5 times higher in the Malawi cohort than in the UK cohort [14]. In addition, unlike in the UK adults [14], experimental pneumococcal carriage did not boost serum and nasal anti-Spn6B CPS IgG binding antibody titers in the Malawian adults. This observation is consistent with higher residual carriage of vaccine serotypes, including 6B [6], in Malawi than in the UK [12]. Hence, experimental pneumococcal carriage is unlikely to substantially boost serum and nasal anti-Spn6B CPS IgG binding antibody titers in Malawian adults with high pre-inoculation antibody titers from frequent natural exposure to Spn6B. Together, these findings highlight that the serum anti-CPS IgG CoP threshold against carriage in adults is likely different, especially in settings with high residual vaccine-type carriage, such as Malawi. However, we acknowledge that the CoP threshold may be more appropriate for large-scale population studies and may not be sufficient to predict individual susceptibility to carriage.

On the other hand, a single dose of PCV13 induced high titers of systemic and mucosal anti-Spn6B CPS antibodies in healthy young Malawian adults that persisted for at least a year post-vaccination. This finding is consistent with other studies showing that high opsonophagocytic activity (OPA) following PCV13 vaccination in older American adults (60–64 years) persisted for at least a year [19,20]. Moreover, others have also shown persistent anti-CPS IgG antibodies in European adults living with HIV on antiretroviral therapy, one year following PCV13 vaccination [21]. Consistent across these diverse populations is the ability of pneumococcal conjugate vaccines to induce robust humoral responses even with a single dose. This is due to the unique ability of conjugate vaccines to induce T cell help for B cell responses against polysaccharide antigens, resulting in long-term immunological memory and class-switched high-affinity antibodies [22]. Importantly, our study highlights the ability of PCV13 to also induce persistent mucosal CPS-specific IgG and IgA antibodies, even in a population with high serotype-specific background immunity.

Furthermore, we found a weak association between serum and nasal binding anti-Spn6B CPS IgG titers. This finding suggests that the increase in nasal antibody post-vaccination is likely due to local production by nasal-resident memory B cells [23] rather than simply by passive diffusion from systemic circulation. Moreover, circulating CPS-specific memory B cells have been shown to predict protection against experimental Spn6B carriage in UK adults [15]. Furthermore, in the experimental human challenge model of pneumococcal carriage in the UK, titers of nasal anti-Spn6B CPS IgG dropped post-inoculation, and in adults protected against experimental Spn6B carriage post-vaccination nasal samples exhibited higher agglutination than pre-vaccination samples [14]. In our Malawi study, however, the nasal anti-Spn6B CPS IgG and IgA titers did not significantly change post-inoculation in adults who successfully established experimental Spn6B carriage and those who did not. The potential reason for this difference in nasal antibody responses could be the frequent exposure to Spn6B in Malawi versus the UK population, resulting in antibody saturation in the nasal lining fluid of Malawian adults. It is also plausible that exposure to other related serotypes induces non-functional binding anti-CPS Spn6B cross-reactive antibodies due to Malawi's high pneumococcal carriage prevalence. Nonetheless, these findings indicate that nasal binding anti-Spn6B CPS IgG and IgA titers alone are unlikely sufficient to confer protection

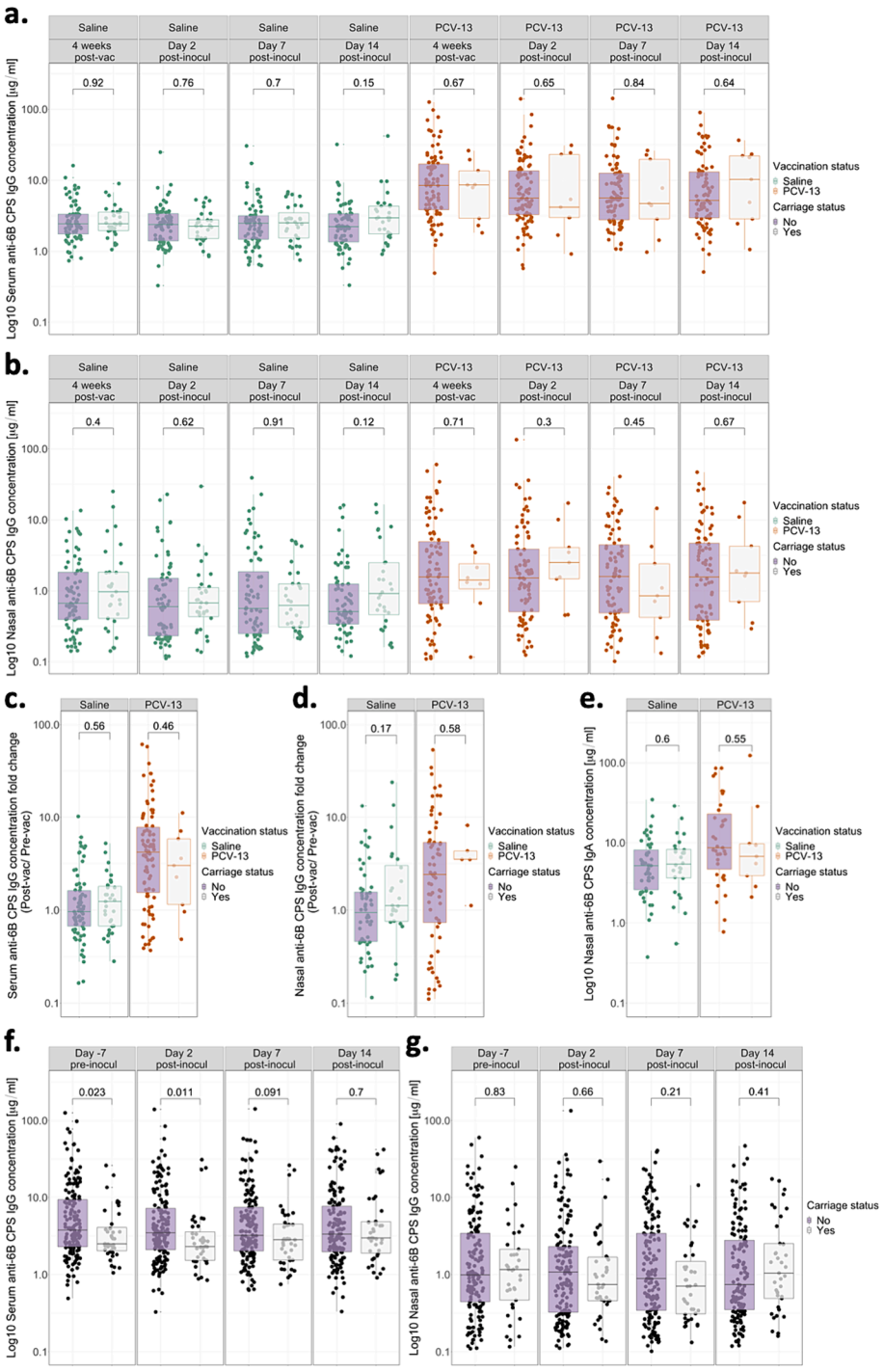

Fig. 4. Binding anti-Spn6B CPS antibodies in serum and nasal lining fluid in adults with or without experimental Spn6B carriage. Titers of binding anti-Spn6B CPS antibodies in serum and nasal lining fluid were measured using an enzyme-linked immunosorbent assay (ELISA). The serum and nasal samples were obtained from volunteers vaccinated with PCV13 and those given a placebo as a control. All participants were inoculated with Spn6B approximately 4 weeks post-vaccination. The samples were obtained before (baseline) and after (4 weeks p.v., Day 2p.i., Day 7p.i., Day 14p.i.) vaccination. **a)** Serum and **b)** nasal anti-Spn6B CPS IgG antibody titers before and after vaccination, comparing adults with or without experimental Spn6B carriage. Fold change in **c)** serum and **d)** nasal anti-Spn6B CPS IgG antibody titers before and 4 weeks post-vaccination, comparing adults with or without experimental Spn6B carriage. **e)** Nasal anti-Spn6B CPS IgA antibody titers after vaccination, comparing adults with or without experimental Spn6B carriage. **f)** Serum and **g)** nasal anti-Spn6B CPS IgG antibody titers before and after Spn6B inoculation, comparing adults with or without experimental Spn6B carriage. The horizontal bars represent the median and interquartile range (IQR). Data were analysed using the Mann-Whitney *U* test, comparing carriage positive (white) with negative (purple) within each time point. p.v., post-vaccination; p.i., post-inoculation; CPS, capsular polysaccharide; IgG, immunoglobulin G; IgA, immunoglobulin A; Spn6B, *Streptococcus pneumoniae* serotype 6B. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

against carriage in settings of high pneumococcal exposure and that the mechanistic correlates of protection are likely different in high compared to low pneumococcal transmission settings.

A limitation of this study is that it only focuses on binding-antibody titers and not functional antibody correlates, such as opsonophagocytic activity (OPA). However, published literature supports a significant correlation between serum binding anti-Spn6B CPS IgG titers and OPA following PCV13 vaccination in children and adults [24–26]. Moreover, the WHO ELISA that measures anti-Spn6B CPS IgG titers has been previously extensively used in the evaluation and licensure of pneumococcal conjugate vaccines, with a threshold of 0.35 μg/mL established for licensure in non-inferiority of serological responses [17,27–30]. On the other hand, in older adults (≥60 years), serum binding anti-Spn6B CPS IgG titers correlate poorly with OPA, as they could have normal anti-Spn6B CPS IgG titers while still susceptible to pneumococcal disease [31–33]. As such, OPA was used as a primary endpoint in clinical trials of PCV13 in adults, contributing to its approval and recommendation for use [34]. However, despite OPA being a gold standard mechanistic correlate of protection against pneumococcal disease, it might not be the primary mechanistic correlate of protection against pneumococcal carriage in the upper respiratory tract in high transmission settings. Hence, there is a need for a systems serology approach to elucidate potential under-appreciated mechanistic CoP against carriage in settings with high residual vaccine-type carriage, like that employed in this study [35].

In conclusion, our study demonstrates that PCV13 induces robust systemic and mucosal anti-CPS IgG antibodies in healthy young Malawian adults, which poorly associate with protection against experimental Spn6B carriage in a setting with high residual vaccine-type carriage and prior serotype-specific background immunity. The findings support the recalibration of serological vaccine-induced correlates of protection (CoP) thresholds in high transmission settings, such as Malawi, and further exploration of other measures of antibody function to enhance the public health relevance of CoPs across diverse populations.

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CRediT authorship contribution statement

G. Tembo: Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing, Formal analysis. **R. Kamng'ona:** Methodology, Writing – review & editing. **L. Chimgoneko:** Methodology, Writing – review & editing. **G. Chiwala:** Methodology, Writing – review & editing. **S. Sichone:** Methodology, Writing – review & editing. **B. Galafa:** Methodology, Writing – review & editing. **F. Thole:** Methodology, Writing – review & editing. **C. Mkandawire:** Methodology, Writing – review & editing. **A.E. Chirwa:** Methodology, Writing – review & editing. **E. Nsomba:** Methodology, Writing – review & editing. **V. Nkhoma:** Methodology, Writing – review & editing. **C. Ngoliwa:** Methodology, Writing – review & editing. **N. Toto:** Methodology, Project administration, Writing – review & editing. **L. Makhaza:** Methodology, Writing – review & editing, Data curation. **A. Muyaya:** Data curation, Writing – review & editing. **E. Kudowa:** Methodology, Writing – review & editing. **M.Y.R. Henrion:** . **D. Dula:** Investigation, Methodology, Writing – review & editing. **B. Morton:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. **T. Chikaonda:** Methodology, Supervision, Writing – review & editing. **S.B. Gordon:** Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **K.C. Jambo:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2024.03.055.

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