PERSISTENCE OF IMMUNITY FOLLOWING TWO-DOSE PRIMING WITH A 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE AT 6 AND 10 WEEKS OR 6 AND 14 WEEKS IN NEPALESE CHILDREN

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Abstract

Aim: The pneumococcal conjugate vaccine has had a substantial impact on invasive bacterial diseases. However, the incidence of childhood pneumococcal pneumonia remains high in under 5 year olds. Previously, we conducted a clinical trial in Nepal that compared immunity following the ten-valent pneumococcal conjugate vaccine (PCV10) administration at 6 and 10 weeks with administration at 6 and 14 weeks of age, both followed by a 9-month booster. In this study, we followed up those participants to evaluate the medium-term persistence of serotype-specific pneumococcal immunity at 2-3 years of age.

Method: Blood samples and nasopharyngeal swabs were collected.Serotype-specific IgG antibody concentrations were determined by ELISA, for the ten vaccine serotypes, at a WHO pneumococcal serology reference laboratory.

Results: We recruited 220 out of the 287 who completed the primary study. At 2-3 years of age, serum serotype-specific IgG greater than or equal to 0.35µg/mL was comparable for all PCV10 serotypes between the 6+10 and 6+14 week groups. Similarly, the geometric mean concentrations of serum serotype-specific pneumococcal IgG levels were similar in the two groups for all serotypes, except for serotype 19F which was 32% lower in the 6+10 group than the 6+14 group .

Conclusion: Our study results demonstrated that immunisation with PCV10 at 6+10 weeks or 6+14 weeks, with a booster at nine months in each case, results in similar persistence of serotype-specific antibody at 2-3 years of age.

Clinical significance: From the results, we would anticipate that protection from disease would be similar when either schedule is used.

Introduction

Streptococcus pneumoniae is a principal causative agent of bacterial pneumonia, meningitis, and sepsis in children.There is a very high disease burden observed in resource-limited countries.(1,2) The pneumococcal conjugate vaccine has been shown to have a substantial impact against these diseases, directly by inducing protective immunity in vaccinated infants and indirectly by inducing herd immunity through the reduction of nasopharyngeal carriage.(3-5) Long-term surveillance of invasive pneumococcal disease (IPD) at Patan Hospital, Nepal has shown pneumococcal serotypes 1, 5, and 14 to be the cause of the majority of IPD.All of these serotypes could be prevented by available pneumococcal conjugate vaccines(PCVs) (6, 7).The incidence of childhood pneumococcal pneumonia is high up to the age of 5 years.(8, 9) Thus, persistence of immunity beyond the first two years of life is of paramount importance in the direct protection against streptococcus pneumonia. The persistence of vaccine-related immunity also contributes to the development of herd immunity, which indirectly protects unvaccinated children and adults. In 2018, a study in Israel reported that declines in IPD rates among adults was most closely associated with reduced nasopharyngeal colonisation and increased vaccination coverage among children at 3-5 years of age. The association suggested that immunity in preschool children is more important than infant immunity for maintaining indirect protection in adults.(10)

In different countries there has been some variation in the PCV vaccine schedules which have been implemented.Research has been conducted to compare the immunogenicity of different PCV vaccine schedules(11). In 2010, our assessment of the immunogenicity of PCV10 at Patan Hospital demonstrated two-dose prime (6 and 14 weeks) with a 9-month booster (2+1) to be non-inferior to the conventional

three-dose priming schedule (3+0), without a boost,for serotype-specific IgG concentrations at 18 weeks. Added to this the two-dose prime-boost schedule resulted in higher IgG concentrations at ten months and 2-4 years of age than the three-dose prime schedule.(12)

In 2015, PCV10 vaccine was introduced into the routine immunisation schedule in Nepal, with a unique 2p+1 dosing schedule using 6 and 10 week priming doses with a booster at nine months of age. The timing was selected to avoid multiple injections at 14 weeks in the national immunisation schedule after the addition of the single inactivated poliomyelitis virus vaccine (IPV) at 14 weeks, which was believed to be a concern for parents and vaccinators. A 2p+1 schedule with only a 1month interval between the first two PCV10 doses has not previously been evaluated.In 2010, Goldblatt et al. trialled a schedule with priming doses of PCV7 at 2 and 3 months.However, the study had to be terminated due to poor immunogenicity seen in an interim analysis.(13) We previously reportedan open-label randomised non-inferiority trial of PCV10in 304 Nepalese infants to assess the immunogenicity of the novel Nepali schedule,when compared with the standard EPI schedule.(14) The study demonstrated that the schedule with two priming doses at 6 and 10 weeks of age and a 9-month booster was inferior to the schedule with two priming doses at 6 and 14 weeks of age and a 9-month booster for immunogenicity against some serotypes at 9 months (prior to boosting), but there were no significant differences at 10 months of age (one-month post boosting). To our knowledge, there are no prior studies that have directly compared the persistence of immunity, beyond the second year of life,in these two PCV schedules with different prime dose timings. We, therefore, undertook this study to examine the persistence of immunity in healthy

Nepalese infants at 2-3 years of age, following PCV10 immunisation at 6 and 10 weeks or 6 and 14 weeks of age, and by a boost at 9 months of age.

Methods

Study Design and Study Participants

We conducted a cross-sectional follow-up study at Patan Hospital among participants of our previous trial comparing different two-dose priming schedules (6+10 weeks versus 6+14) for PCV10.(14) Oxford Tropical Research Ethics Committee (OxTREC Ref no: 34-17) and Nepal Health Research Council (NHRCReg no: 459/2017) approved this study.

We approached, by telephone, 287 participants who completed the original study, and explained the details of the study to them. We invited interested participants to visit the Paediatric Research Department at Patan Hospital, where we provided them with an information sheet and discussed the study in more detail. The children of those parents/guardians who gave written consent to participate in the study were enrolled.

Procedures

Participants attended a single visit for both baseline assessment and sample collection. Blood samples and nasopharyngeal swabs were collected and the participant's demographics, medical history and information regarding the use of concomitant medication were recorded in the case record form.

Blood samples were centrifuged within 12 hours of collection and serum stored at -80°C prior to being shipped on dry ice to the World Health Organization (WHO) pneumococcal serology reference laboratory at University College London, UK.

Serotype-specific IgG antibody concentrations were measuredfor the ten vaccine serotypes by ELISA using 22F adsorption.(15)

Trained members of the research team collected the nasopharyngeal swab from each participant according to WHO guidelines(16) and samples were transferred to the laboratory in an insulated box within 4 hours of collection and placed in a tube of 0.5-1ml skim-milk-tryptone-glucose-glycerine (STGG). The swabs were cultured within 12 hours of collection on Columbia blood agar containing 5% sheep blood and gentamicin and incubated overnight at 35-37°C in 5% carbon dioxide. Morphologically distinct colonies were confirmed by optochin sensitivity test and then serotyped by Quellung reaction. All pneumococcal isolates were stored at -80°C. Quality control of the serotyping was performed at the University of Oxford, UK.

Outcomes

The primary outcome was the proportion of infants with serotype-specific IgG ≥0.35µg/mL against PCV10 serotypes at 2-3 years of age. The secondary outcomes were the geometric mean concentrations (GMC) of PCV10 serotype-specific IgG at 2-3 years of age for each of the two study groups, and serotype-specific pneumococcal carriage rates at 2-3 years of age.

Statistical analysis

Serotype-specific IgG values were log-transformed and summarized as geometric means with associated 2-sided 95% confidence intervals(CI).Observations below the threshold of detectionfor the assay were assigned a value of half the lower limit of detection before log transformation. The two groups were compared using the t-test.

The geometric mean change in IgG between the post-booster visit at ten months of age and the follow up at 2-3 years of age was derived from the exponent of the difference of log-transformed serotype-specific IgG for each group.

The proportion of children with serotype-specific IgG levels greater than or equal to 0.35(µg/mL) was calculated within each group and the CIs were computed using the binomial exact test. Chi-squared testwas used to compare the two groups.

Analyses were performed using SAS version 9.4, STATA version 14, and R Version 3.5.3.

Role of the funding source

The sponsor of the study was the University of Oxford. Investigators in Oxford and Nepal designed, conducted, analysed and interpreted the study. Gavi, The Vaccine Alliance, the funder of the research had no role in study design, data collection, data interpretation, or writing of the report and data analyses. The corresponding author had complete access to all data of the study and the authors had final responsibility for the decision to submit for publication.

Results

In the initial 2015 trial, a total of 304 children were enrolled and randomised 1:1 into either the 6+10 group or the 6+14 group, of which 287 completed the study. Both groups received a PCV10 booster at 9 months of age. For this follow-up study, between January 2018 and April 2018, we recruited 220 participants out of the total 287 (94%) who completed the primary study. Reasons for non-enrollment were: 12 participants had moved away, 11 were lost to follow-up, and 44 refused (Figure1). Children in the two arms of the study were similar in terms of age and sex distribution. The baseline characteristics of the participants at the time of enrolment

for the follow-up study are shown in Table 1. At 2-3 years of age, the proportion of children who had serum serotype-specific IgG greater than or equal to 0.35µg/mL was comparable for all PCV10 serotypes between the 6+10 and 6+14 groups (Figure 2A). At the same time point, the GMC of serum serotype-specific pneumococcal IgG levels in the 6+10 and 6+14 groups were similar for all serotypes except for serotype 19F. Antibody against serotype 19F was 32% lower in the 6+10 group than the 6+14 group (GMR 0.676,95%CI0.50–0.92,p=0.013) (Figure2B). Antibody levels induced by vaccination were expected to decay from the post booster visit at 10 months to the follow up at 2-3 years of age. However, some participants had antibody increases between the these two visits (Figure 3), in the absence of documented infection, indicating that there may have been an antibody responseto intercurrent nasopharyngeal carriage. Using a rise in antibody as a marker of pneumococcal exposure/carriage we found that the proportion of possible carriage acquisitions was highest for serotype 23F at 12.75%.

Nasopharyngeal swabs were collected from participants at 6 weeks, 10 months and at 2-3 years of age. The overall rate of pneumococcal carriage was 19.4% at 6 weeks, 59.2% at 10 months and 73.6% for those at 2-3 years of age. At 6 weeks of age, 5 (17.2%) of the 29 swabbed children in the 6+10 group carried PCV 10 serotypes compared with 2 (6%) of the 29 swabbed children in the 6+14 group. Likewise, at 10 months of age, 14 (17.2%) of the 81 swabbed children in the 6+10 group and 12 (13%) of the 92 swabbed children in the 6+14 group were carrying PCV10 serotypes. At 2-3 years of age, 2 (2.5%) of the 79 swabbed children in 6+10 group were carrying PCV10 serotypes compared with15 (16.4%) of the 91 swabbed children in 6+14 group.

At 2-3 years of age, the proportion of children with nasopharyngeal carriage of any PCV10 serotype in the 6+14 group was significantly higher than in the 6+10 group, (difference13.9%, 95%CI 5.5% to22.3%; p=0.0025). However, there was no difference between the groups in overall carriage of non-vaccine types (NVT). We also observed positive correlation between isolation of pneumococci and age.(Figure4)

Discussion

This is the first clinical trial to examine the persistence of immunity following immunisation with PCV10 according to Nepal's unique 2+1 dosing schedule of 6 and 10 week priming doses with a booster at 9-months compared with the WHO standard 2+1 schedule of 6 and 14 week priming doses with a 9-month booster. An important finding is that the immunogenicity of the Nepal 2+1 dosing schedule, with a four-week interval between the priming doses, is similarly immunogenic to the WHO standard 2+1 schedule, with an eight-week interval between the priming doses at 2-3 years of age.The overall GMC of the antibodies against most of the serotypes was similar in both groups.No significant difference was seen when proportion of children with IgG levels of at least 0.35µg/mL were compared between the two groups.The antibody levels had substantially decayed by the time of the follow-up visit, with levels for serotypes 1, 4 and 18C showing the greatest decline(GMCsless than 10% of the post-booster levels).Our dataprovide support for the WHO recommendation that, in situations where the standard 2+1 schedule cannot be implemented, a short interval priming schedule at 6 and 10 weeks can be used as an alternative.

In 2017, the WHO Strategic Advisory Group of Experts (SAGE) on Immunizations PCV Working Group reviewed the dosing schedule to examine how PCV

administered to healthy children in a 2+1 schedule compared with the 3+0 schedule, with respect to immune response in vaccinated children and impact on clinical outcomes.The review showed higher GMCs in children administered the 2+1 schedule compared with the children administered the 3+1 schedule. However, the proportions of participants who had an antibody response above the correlate of protection were similar in the two schedule groups. Studies have shown no significant difference in immunogenicity and response after the third dose between 2+1 and 3+0 schedule. Hence, SAGE has recommended the use of 3 vaccines, whether it be 2+1 or 3+0 schedule.(17) In Nepal's case, surveillance of IPD at Patan Hospital, Kathmandu indicates the majority of IPD occurs in late infancy and early childhood.(6, 7) For this reason, the National Immunisation Technical Advisory Group of Nepal preferred to use the 2+1schedule, with a booster dose at nine months, to protect Nepali children against IPD.

Around the world, countries are using different schedules for PCV. To our knowledge, the schedule with priming doses at 6 and 10 weeks of age, which is used in Nepal, is not currently used in any other locations. The majority of developed countries use a 2+1 schedule with an 8 week gap between priming doses and the booster at 9 months or later.(18, 19) In 2018, Goldblatt et al. conducted a randomised controlled trial in UK infants to compare the standard schedule of 2 primary and 1 booster dose with 1 primary and 1 booster dose. The study findings demonstrated equivalent or superior post-booster responses for nine of the 13 serotypes in PCV13 among infants primed with a single dose compared with the standard two priming dosing schedule.This study identified a 1+1 schedule as a possible option in countries with a mature PCV immunisation programme and established herd immunity. PCV was introduced in the UK in 2006 and there is a

mature PCV immunisation program in place, with established herd immunity for pneumococcus.Therefore, the UK has recently implemented the 1+1 schedule.(6, 7)

In 2016, a study was conducted in Poland to evaluate the long-term persistence of antibody after PCV10 administration in a 3+1 schedule, with vaccinations at 2, 3, 4 and 12-18 months of age. The study findings showed the importance of booster for persistence of immunogenicity.The study showed that the antibody persistence of PCV 10 may extend until at least 4 years after booster vaccination. Moreover, stronger immune response for all vaccine serotypes and vaccine-related serotypes was observed when an additional dose was administered in 6 year olds. (20)

An Australian team, Zimmerman et al.,studied persistence of immunity in children, at 13 months of age, immunised with PCV13 in a 3+0 schedule. The study findings showed a drop in GMC levels of antibody from 7 to 13 months of age, with many 13 month old infants having antibody levels below the correlate of protection threshold, with the lowest responses against serotypes 4,19A, 3, 6B and 23F.(21) The authors suggested that booster doses might be necessary in immunisation programmes for PCV to optimise protection against pneumococcal diseases.(21)

In 2016, Trück et al. studied the antibody response in children primed with PCV13 who were boosted with either PCV10 or PCV13. The geometric mean IgG concentrations and opsonophagocytic assay titres for most PCV13 serotypes were significantly superior in recipients of a PCV13 booster compared with a PCV10 booster. However, similar or inferior responses were seen for serotypes 4, 18C, and 19F. The superioriority of PCV13 may warrant the Nepalese health authorities to consider changing from PCV10 to PCV13 in the future.(22)

In general, we expect the antibody induced by vaccination to decay progressively post-vaccination. A late infancy booster dose does seem to have an important role in the persistence of immunity.Some participants had antibody levels which increased between post-booster and follow up visits, indicating a possible antibody response due to natural environmental exposure to the organism. In a time when many new vaccines are being developed andimmunisation schedules are becoming more crowded, our findings provide knowledge on immunogenicity after administration of PCV with only a 4 week gap between priming doses.Our results provide health authorities a proven and credible 2+1 alternative schedule for PCV vaccination, with priming doses at 6 and 10 weeks of age, and help manage congested immunization schedules.

To our knowledge, our study was the first and only study to demonstrate the long-term immunity of the PCV10 vaccine in Nepal. The fact that this study is a follow-up of a randomised trial adds to the strength of the study. However, the persistence of immunity beyond the third year of life after vaccination remains to be studied.

Conclusion

This study has shown that PCV10 immunisation at 6+10 weeks or 6+14 weeks, with a booster at nine months in each case, results in similar persistence of serotype-specific antibody at 2-3 years of age. From the results, we would anticipate that direct protection from disease at this age, and herd protection of the unvaccinated population, would be similar if either schedule is used.This suggests that a 2+1 PCV schedule, with a one month interval between priming doses, is a valid alternative to the current WHO PCVschedule.

Contributors

MG designed the study, coordinated the local study implementation, wrote and revised the report. PM recruited the participants and collected samples. ST, IA, GPS, SK, KOB, MV, DFK, DRM, AJP and SS designed the study, revised the report and conducted the research. PH and MV did the statistical analysis of the data and revised the report. GB led the analysis of serum for antibodies of the PCV10 antigens and anti-pneumococcal antibodies. All co-authors contributed to the interpretations of the data, reviewing and approving the final version of the report for submission.

Conflict of interests

AJP is Chair of the UK Dept. Health and Social Care's (DHSC) Joint Committee on Vaccination & Immunisation (JCVI) and is a member of the WHO's Strategic Advisory Group of Experts. The views expressed in this article do not necessarily represent the views of DHSC, JCVI or WHO. DFK receives salary support from the National Institute for Health Research Oxford Biomedical Research Centre. KOB has received a research grant from Gavi, the Vaccine Alliance, the Bill & Melinda Gates Foundation, GlaxoSmithKline and Pfizer. DG reports 7 grants from vaccine manufacturers GlaxoSmithKline, Sanofi Pasteur, and Merck, outside the submitted work. RK received the Robert Austrian Award in Pneumococcal Vaccinology, which was supported by Pfizer, at the 10th International Symposium on Pneumococci and Pneumococcal Diseases 2016.The remaining authors declare no conflicts of interest.

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TABLES

Table 1: Baseline characteristics of the 220 participants at the time of enrolment by group.

Baseline characteristics	***Group 6+10 (n=108)***	***Group 6+14 (n=112)***	***All (n=220)***
Mean Age,year(SD)	2.4 (0.2)	2.3 (0.2)	2.3 (0.2)
Sex			
Female	49 (45.4%)	49 (43.8 %)	98 (44.5%)
Male	59 (54.6%)	63 (56.3%)	122 (55.5%)
History of antibiotic taken in the last 4 weeks	6 (5.6%)	10 (8.9%)	16 (7.3%)
Hospitalization history within last 12 months	10 (9.3%)	3 (2.7%)	13 (5.9%)

FIGURES

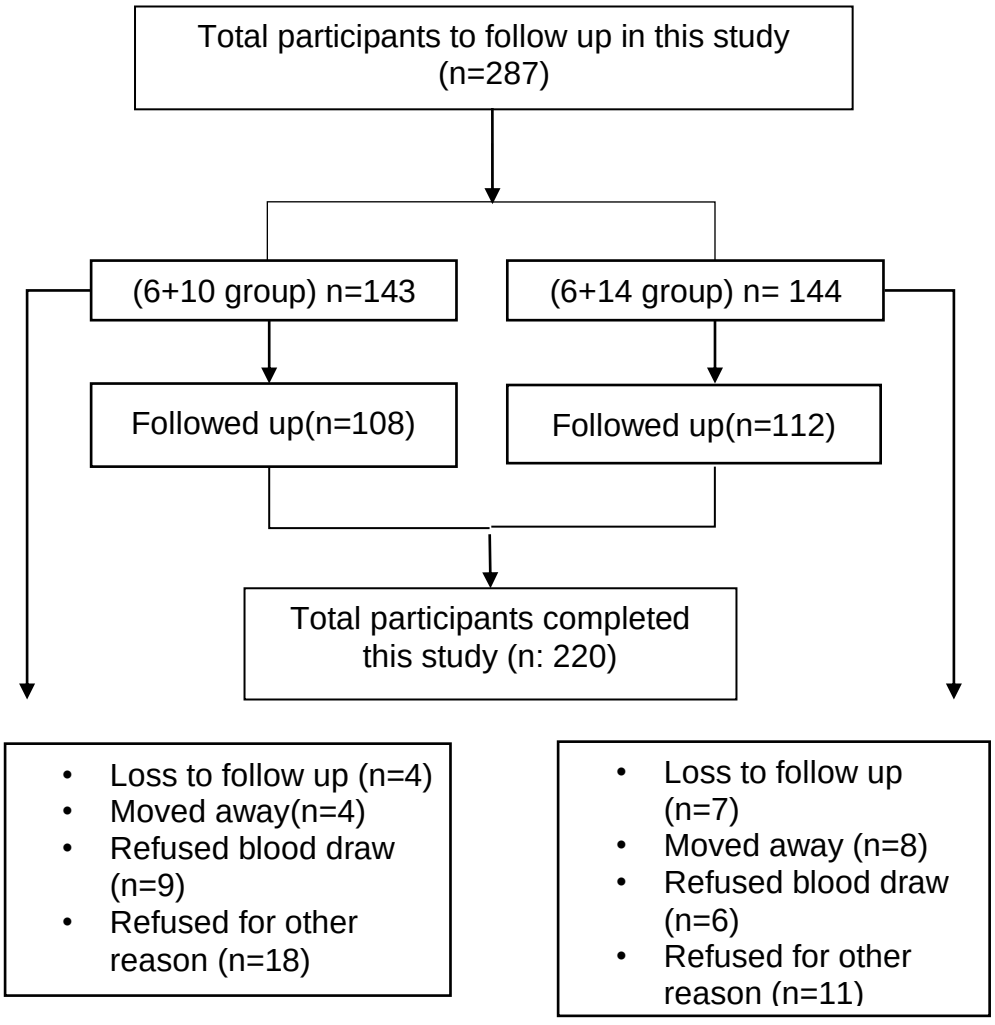
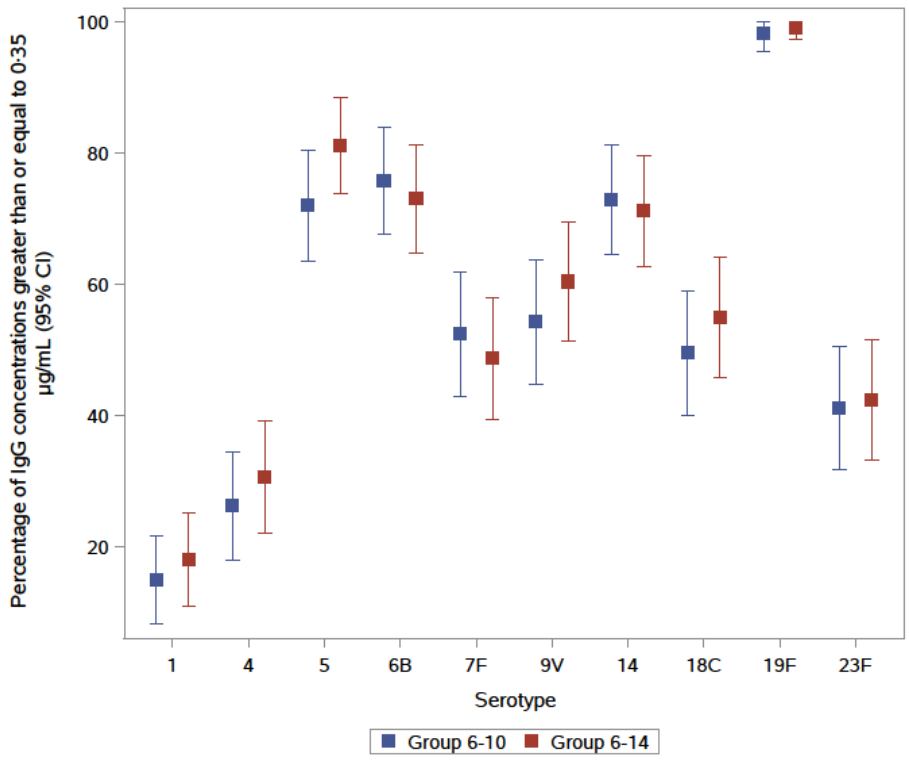


Figure 1: Studyprofile. In the initial trial Nepalese infants were randomised to receive PCV10 at 6 and 10 weeks of age followed by a 9-month booster or 6 and 14 weeks of age followed by a 9-month booster.These children were recruited for the current study between 2-3 years of age.

A


Percentage of IgG concentrations greater than or equal to 0·35 µg/mL (95% CI)
100
80
60
40
20
1
4
5
6B
7F
9V
14
18C
19F
23F
Serotype
Group 6-10
Group 6-14

B

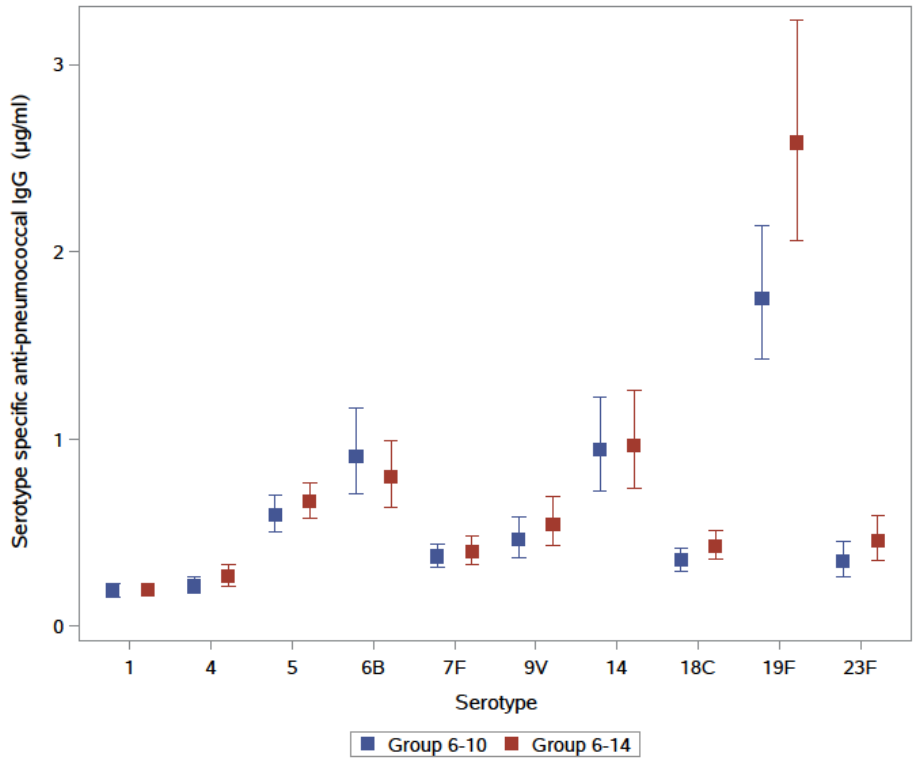

Serotype specific anti-pneumococcal IgG (µg/ml)
3
2
1
0
1
4
5
6B
7F
9V
14
18C
19F
23F
Serotype
Group 6-10
Group 6-14

Figure 2: Proportion of 2-3 year old Nepalese childrenwith serum pneumococcal serotype-specific IgG greater than or equal to 0.35 µg/mL (A) and GMCs of serum serotype-specific pneumococcal IgG at 2-3 years of age (B) following receipt of PCV10 at 6 and 10 weeks of age followed by a 9-month booster or 6 and 14 weeks of age followed by a 9-month booster.

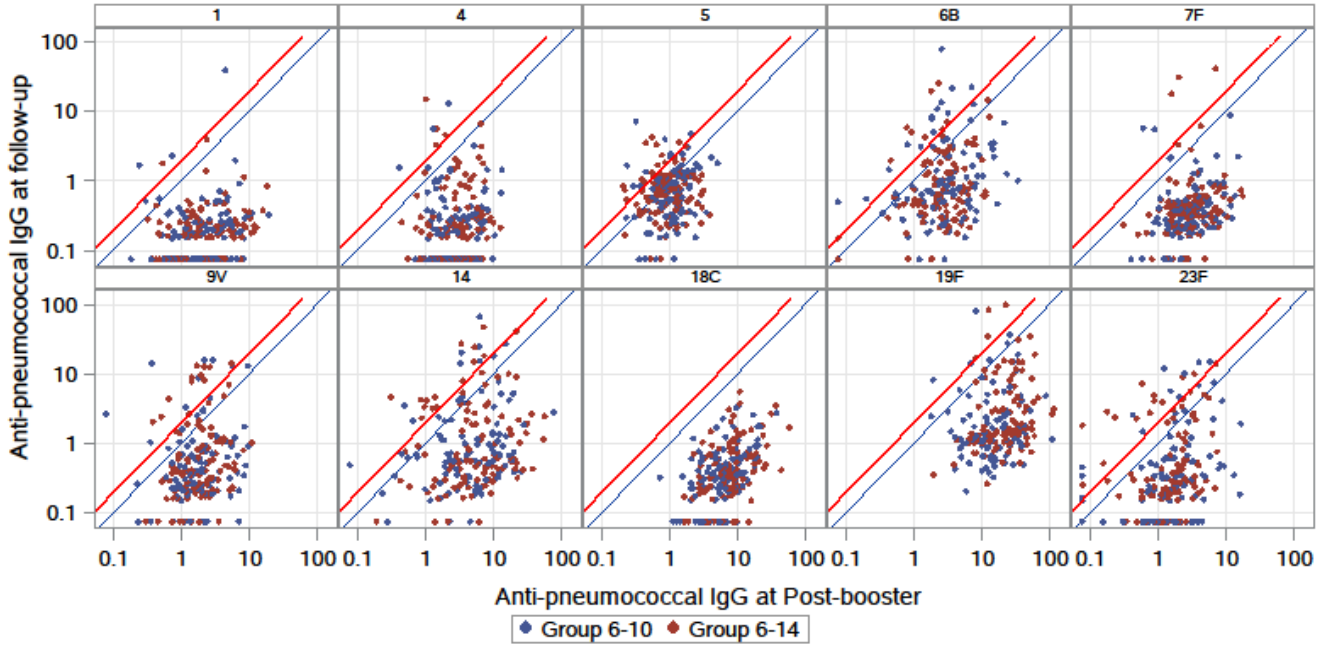


Figure 3: Log of anti-pneumococcal IgG at post-booster (10 months of age) compared with follow-up at 2-3 years of age. Diagonal line shows no change. Points above the line show participants with antibody levels that have increased between post-booster and follow-up visits. The red line shows 2-fold rise. (Serotype 23F has the highest proportion of carriage at 12.75%**)**

ü

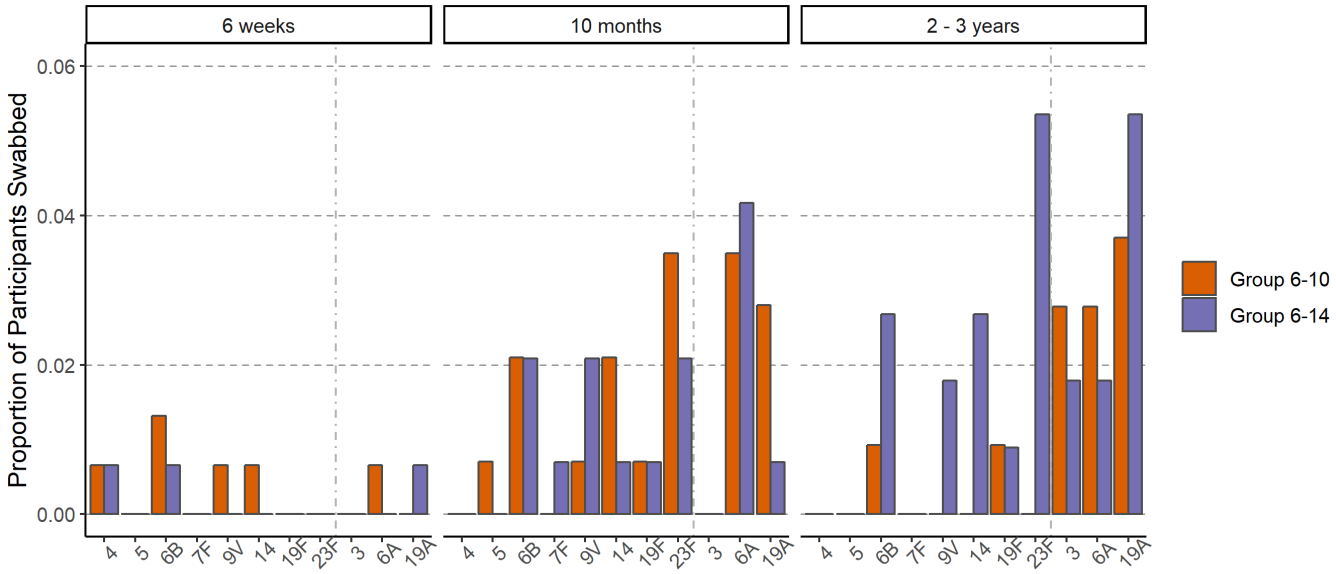


Figure 4: Nasopharyngeal carriage of serotypes of pneumococcus in children randomised to receive priming doses of PCV10 at 6 and 10 weeks or 6 and 14 weeks of age (Denominator: all participants swabbed)