13-valent Pneumococcal Conjugate Vaccine in children with acute lymphoblastic leukaemia: protective immunity can be achieved on completion of treatment

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SUMMARY (max. 40 words):

Children with acute lymphoblastic leukaemia can develop invasive pneumococcal disease. This study demonstrated that the immunogenicity of a 13-valent pneumococcal conjugate vaccine on completion of ALL treatment is equivalent to its immunogenicity when given six months later, thus providing a rationale for providing earlier protection.

Keywords: Acute Lymphoblastic Leukaemia, Pneumococcal Conjugate Vaccine, Immunisation, Immunocompromised

Running title: PCV13 in paediatric ALL

ABSTRACT (max: 250 words):

Background:

Children with acute lymphoblastic leukaemia (ALL) are at increased risk of invasive pneumococcal disease. This study describes the immunogenicity of 13-valent pneumococcal conjugate vaccine (PCV13) at three time points during and after chemotherapy to identify the earliest time point when protective immunity could be achieved.

Methods:

Children with ALL were allocated to study groups empirically and received a single dose of PCV13: Group 1 - during maintenance chemotherapy; Group 2 - end of chemotherapy; Group 3 - 6 months after completion of chemotherapy. A protective vaccine response was defined as at least 10 of 12 serotypes (or greater than 83% of serotypes with data) achieving post-vaccination serotype-specific IgG ≥ 0.35 μg/mL and ≥ 4 fold rise, compared to pre-vaccination at 1 and 12 months.

Results:

One hundred and eighteen children were recruited with 39, 40 and 39 children allocated to Groups 1, 2 and 3, respectively.

Only 12.8% (5/39; 95% CI 4.3% to 27.4%) of patients vaccinated during maintenance chemotherapy (Group 1) achieved the defined protective response at 1 month post-vaccination and none of these patients had a protective response at 12 months. For Group 2 patients, 59.5% (22/37; 95% CI 42.1% to 75.3%) achieved a response at 1 month and 37.9% (11/29; 95% CI 20.7% to 57.7%) maintained immunity at 12 months. For Group 3 patients, 56.8% (21/37; 95% CI 39.5% to 72.9%) achieved a protective response at 1 month and 43.3% (13/30; 95% CI 25.5% to 62.6%) maintained immunity at 12 months.

Conclusion:

This study demonstrated the earliest time point at which protective immunity can be achieved in children with ALL is on completion of chemotherapy; this is earlier than recommended in current guidelines and may ensure protection during a period when children are most susceptible to this infection.

FUNDING:

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CONFLICT OF INTEREST:

RB performs contract research on behalf of Public Health England for GSK, Pfizer and Sanofi Pasteur.

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Title: Immunogenicity of 13-valent Pneumococcal Conjugate Vaccine in children with acute lymphoblastic leukaemia: protective immunity achievable on completion of treatment

BACKGROUND:

Outcomes for children with acute lymphoblastic leukaemia (ALL) have steadily improved over time with recent trials demonstrating survival rates of > 90% in standard risk patients (1, 2). The excellent survival rates have been achieved through enhanced risk stratification with increased chemotherapy intensity and duration together with improvements in supportive care. However, the risk of treatment-related mortality remains a significant concern in the context of low relapse risk. The most common cause of treatment-related mortality is infection. During the UK ALL 2003 trial, for children in the low-risk group, deaths from infection were almost as common as death from ALL itself (3). As mortality from vaccine-preventable infections is potentially preventable, it is important to consider how vaccination may be optimised to minimise the risk to immunocompromised children both during and after completion of treatment.

The prolonged period of chemotherapy of up to three years for children with ALL renders them significantly immunosuppressed and at risk of infection. Studies in children with ALL have shown that they develop persistent deficits in their immune function that can last for months after completion of chemotherapy and that their immunity to vaccines received prior to diagnosis declines after completion of treatment (4-7).

One of these vaccine-preventable pathogens is *Streptococcus pneumoniae*, or pneumococcus. Invasive pneumococcal disease (IPD) is a major cause of hospitalisation and mortality in the immunocompromised child (8-11). For children with cancer, the risk of IPD is significantly higher than in healthy children and other immunocompromised groups such as those with human

immunodeficiency virus (12). Studies have reported a specific pneumococcal immune defect in children with ALL which persists after completion of chemotherapy (13, 14).

Traditional polysaccharide pneumococcal vaccines are ineffective in infants and in immunocompromised children (15, 16). However, pneumococcal conjugate vaccines (PCV) are much more immunogenic by eliciting a T-cell–dependent immune response. T cells provide the signals required for the generation of B-cell memory (17, 18). Conjugate vaccines have the potential to elicit a memory response on subsequent natural exposure to vaccine-type strains and PCV have been shown to be immunogenic in immunocompromised hosts (19-23) In the United Kingdom (UK), since the introduction of 7-valent PCV in 2006 and 13-valent PCV in 2010, the overall incidence of IPD has reduced by more than 50% (24).

Despite the known increased risk of IPD in children with ALL and the reported immunogenicity of PCV, there is no consensus as to when the optimum time for vaccination should be and international practice is variable (25-27). Current published guidelines recommend that children with cancer should receive pneumococcal vaccine 3 to 6 months after completion of treatment (28-32) (6 months in UK). However, delaying vaccination until 3 or 6 months after completion of cancer therapy potentially results in a prolonged period during which patients remain at risk of IPD. It is possible that PCV may be effective during chemotherapy or as soon as chemotherapy is complete. The aim of this study therefore was to examine the immunogenicity of PCV13 in children with ALL at 3 time points during / after their treatment, to identify the earliest time point when protective immunity can be generated. The study also provided an opportunity to characterise the immune function of children receiving, and recovering from, current ALL treatment regimens.

METHODS:

Study Design:

This non-randomised open-label study was conducted at 8 centres in the UK between September 2010 and July 2015. Children were allocated to study groups on the basis of their current time point in ALL treatment. The following study groups were used:

Study group 1: Vaccine administered during 3rd cycle of maintenance chemotherapy, 6 months from last intensive chemotherapy;
Study group 2: Vaccine administered 4 weeks after last oral maintenance chemotherapy, at the end of treatment;
Study group 3: Vaccine administered 6 months after last oral maintenance chemotherapy

Study population

Children between the ages of 2 to 18 years with ALL confirmed by immunophenotyping and receiving maintenance treatment or who had completed treatment within last 6 months as per UK ALL 2003 protocol were eligible for recruitment (1). Exclusion criteria included children with concomitant acquired or congenital immunodeficiency, those receiving immunosuppressive medication other than UK ALL 2003 chemotherapy, previous severe or anaphylactic reaction to PCV and/or diphtheria toxoid and any children with a contraindication to receipt of any vaccine as per UK guidelines (32). Receipt of PCV7 vaccines as part of the routine schedule prior to diagnosis was not an exclusion criteria.

Study vaccine

The vaccine used in the study was PCV13 (Prevenar-13™, Wyeth) which contains pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). A single 0.5 mL intramuscular dose of

vaccine was administered to each study patient as allocated to the study groups 1, 2 or 3 described above.

Safety and tolerability assessment

All patients were observed for 30 minutes following vaccine administration with appropriate medical treatment and supervision readily available in case of an anaphylactic event. Parents were given a report card to document any reactions to the vaccine.

Study procedures

Baseline blood samples were obtained pre-vaccination and follow-up samples were taken at 1 and 12 months post-vaccination. Serum concentrations of IgG anti-capsular polysaccharide antibodies to pneumococcal serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F were assayed by Public Health England, Manchester vaccination. Serotype-specific pneumococcal IgG antibodies were quantified for 12 serotypes using a multiplex microsphere assay as previously described (33). Opsonophagocytosis assays were tested at Institute of Child Health Immunobiology Reference Laboratory by multiplex assay ((further details here).

Immunogenicity assessment

In line with World Health Organization (WHO) guidance, both antibody concentrations and functional assays were used to define protective levels of anti-pneumococcal immunity at 1 and 12 months post-vaccination (33). IgG serotype-specific geometric mean concentrations (GMCs) and the

proportion of children with concentrations ≥ 0.35 μg/mL and with a ≥ 4 fold rise compared to pre-vaccination were calculated at 1 and 12 months post-vaccination for 12 of the 13 serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). A protective response was classified as having at least 10 out of 12 serotypes (or greater than 83% of serotypes with data) meeting this definition.

Functionality of anti-pneumococcal antibodies was assessed in 4 of the 13 serotypes (1, 4, 5 and 19A). Protective immunity was defined if all 4 serotypes achieved opsonophagocytosis assay titres ≥ 8 at 1 and 12 months post-vaccination (35). A patient was defined as having an overall composite response if they had an antibody response, as defined above, plus protective immunity, at 1 and 12 months.

Secondary assays collected from study participants were peripheral blood lymphocyte subsets, serum concentrations of total immunoglobulins and IgG subclasses and nasopharyngeal swabs for culture of pneumococci, serotyping and multi-locus sequence typing (MLST) of any pneumococcal isolates.

Statistical methods

Power calculations were based on the premise that ≥70% of children will develop protective levels of anti-pneumococcal antibodies following one dose of PCV. However, if a ≥ 50% response rate was observed then this would still be considered clinically useful for this group given their increased susceptibility to IPD.

The study required 40 participants per group (120 in total). With a response rate of ≥70% of subjects developing protective levels of anti-pneumococcal antibodies following one dose of PCV, the true response rate was estimated to within a confidence interval of ± 14% (i.e lower confidence interval of 56%), with 95% confidence.

Patient demographics and leukaemia and immunisation history information was summarised by study group. For the primary outcomes of overall response in concentrations of anti-pneumococcal antibodies at 1 and 12 months following a single dose of PCV13, point estimates (proportions) with 95% confidence intervals were calculated separately for the three study groups. Point estimates (proportions) with 95% confidence intervals were also calculated for the secondary outcomes of response in absolute concentration of anti-pneumococcal antibodies at 1 and 12 months post-vaccination, response in functionality of antibodies measured at 1 and 12 months post-vaccination, response in concentration of antibodies to each of the 12 serotypes measured at 1 and 12 months post-vaccination, and response in functionality of antibodies to each of the 4 serotypes measured at 1 and 12 months post-vaccination.

Logistic regression adjusting for leukaemia regimen and number of delayed intensifications was applied to the response data. Analysis of covariance (ANCOVA) was used to model the absolute concentration and absolute functionality data values of each serotype separately at 1 and 12 months post-vaccination.

Mann-Whitney tests were used to compare the concentration of anti-pneumococcal antibodies pre-vaccination and at 1 and 12 months post-vaccination respectively between PCV-7 subset and non-PCV-7 subset by serotype in PCV-7 for each study group.

Box and whisker plots were used to visualise the absolute values of the PCV-7 serotypes from the concentration assay at 1 and 12 months post-vaccination.

All statistical analyses were carried out using SAS 9.4 on records with complete data.

Ethical approval and compliance with guidelines.

The study was conducted in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice (as defined by the International Conference on Harmonisation), and United Kingdom regulatory requirements. Ethics approval was obtained from National Research Ethics Service (NRES) Committee South Central (REC reference number 09/H0504/112)

RESULTS:

Study population

A total of 224 children were screened, of which 118 (52.7%) were recruited to the study. Of the 118 children recruited, 39 (33.1%) were allocated to Group 1, 40 (33.9%) were allocated to Group 2, and 39 (33.1%) were allocated to Group 3 (Figure 1, Consort diagram). Details on baseline patient characteristics, leukaemia and immunisation history are shown in Table 1. There were 84 patients (71.8%) who had previously been immunised with PCV7, with the mean number of previous PCV-7 immunisations highest in study group 1, followed by study group 2 and study group 3 (2.5, 1.8 and 1.4, respectively). The mean age in years at the time of PCV13 vaccination was 6.3 years (standard deviation [SD] 4.13) in Group 1, 8.4 years (SD 3.95) in Group 2 and 9.3 years (SD 4.40) in Group 3.

Rates of seroprotection

At baseline, only 2/118 patients (1.7%) had protective antibody concentrations against at least 10 serotypes (all in study group 1 (2/39 [5.1%]). An overall protective response at 1 month post-vaccination (i.e. concentrations of serotype specific anti-pneumococcal antibody ≥ 0.35 μg/ml and 4-fold rise in ≥ 10 serotypes) was achieved in 12.8% of Group 1 (5/39; 95% CI 4.3% to 27.4%), 59.5% of Group 2 (22/37; 95% CI 42.1% to 75.3%) and 56.8% of Group 3 (21/37; 95% CI 39.5% to 72.9%) (Table 2). At 12 months post-vaccination, protective responses were achieved in 0% in Group 1 (0/37), 37% in Group 2 (11/29; 95% CI 20.7% to 57.7%) and 43.3% of Group 3 (13/30; 95% CI 25.5% to 62.6%) (Table 3).

The proportion of patients achieving protective responses to individual serotypes at 1 and 12 months post-vaccination is shown in Figures 2 and 3. These individual serotype responses were higher than the overall protective response proportions where the threshold was defined as 10 of 12 serotypes reaching ≥ 0.35 μg/ml and ≥4-fold rise. Protective responses were not maintained at 12 months in a proportion of patients, particularly those who had been vaccinated during chemotherapy.

Functional assay responses

The proportion of patients in each treatment group with an OPA titre ≥ 8 dilution at 1 and 12 months post-vaccination by serotype 1, 4, 5 and 19A and for all 4 serotypes is shown is Figures 4 and 5. For each treatment group, the proportion of patients who achieved a functional response was greater or equal to the number with concentrations of anti-pneumococcal antibodies considered to be protective both at 1 and 12 months post-vaccination.

Overall composite immune responses

Patients who had completed chemotherapy (Groups 2 and 3) had a significantly higher overall composite response rate compared to those children in Group 1, with no patients in Group 1 achieving the definition of both response in concentration of anti-pneumococcal antibodies and a response in functionality of anti-pneumococcal antibodies at 1 and 12 months (Table 4 and 5). Furthermore, logistic regression modelling confirmed no association between the intensity of treatment regimen (A, B or C) nor the number of delayed intensifications (1 or 2) and the response rate to immunisation (Tables 4 and 5).

The results of the immune function of the study population will be reported separately.

Impact of previous vaccination with PCV7

There were 84 patients (71.8%) who had received PCV7 vaccine prior to study entry. Of these, none had protective anti-pneumococcal antibody concentrations at baseline to the PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F and 23F).

There was no significant difference between the antibody response achieved after PCV13 vaccination in the PCV7 subset and the non-PCV7 subset at 1 and 12 months post-vaccination (Supplementary Data: Table 6 and 7).

Safety and tolerability

For the 7 days following vaccination, a significant fever (≥ 38°C but ≤ 40°C) was reported in 11/109 (10.1%) and there were no reports of fever duration longer than 2 days (Supplementary Data: Table 7). The incidence of pain and/or redness at the injection sites reported after vaccination is shown in

Table 8. In 4 of 118 (3.4%) of patients, fever or injection site reaction was significant enough to warrant hospital admission. In total there were 11 serious adverse events (SAE) in the 118 patients over the study period. The majority of these (7/11 [63.6%]) were considered to be unrelated or unlikely to be related to the vaccine.

There were no cases of proven pneumococcal disease reported during the study period.

Nasopharyngeal carriage of pneumococcal serotypes

Pneumococcal carriage was documented in 14 of 55 subjects (25.5%) at study entry and 8 of 43 (18.6%) at 12 months post-vaccination. The results of the molecular characterisation of these isolates will be reported separately.

DISCUSSION:

It is well-established that children with ALL are at particular risk of IPD during and after completion of treatment (9). This is the first immunogenicity study of PCV13 in a cohort of children with ALL. Our study demonstrates that while it is safe to administer PCV13 vaccine during therapy for ALL, immune responses are suboptimal. However, our data show that children can achieve protective immunity to PCV13 at the end of treatment which is equivalent to that achieved when PCV is given at 6 months after the completion of treatment, which is the current recommendation for such children.

Hung et al. recommend a single dose of PCV13 as soon as possible after cancer diagnosis followed by an additional dose of PCV13 after the completion of treatment (24). This study showed that children who had received previous PCV7 vaccination had similar rises in antibody titres for the 7 serotypes

when comparing children who were receiving chemotherapy with those who had completed chemotherapy and concluded that children receiving chemotherapy could benefit from a single dose of PCV13. This finding was not replicated in our study. This is perhaps because the Hung et al study recruited a number of children with solid tumours, including brain tumours, who do not have the same degree of immunosuppression as children with ALL.

Our study supports the safety and tolerability of PCV13, in keeping with published data in other immunocompromised populations with comparable rates of fever, pain and/or redness at the injection site. In 4 of 118 (3.4%) of patients fever or injection site reaction was significant enough to warrant hospital admission. This is higher than reported in healthy populations, but most likely reflects a lower threshold for triggering hospital admission in these children, rather than increased toxicity (35, 36). The majority of the 11 SAEs were considered to be unrelated to the vaccine and reflect the frequent hospital admission rate in this population of children while they are receiving chemotherapy.

The threshold for defining an overall protective response was high (10 out of 12 serotypes achieving WHO definition protection of ≥ 0.35 μg/mL and ≥4- fold rise). This definition is considered the serological correlate of protection against IPD. Of note, this WHO definition was derived from healthy infants and it therefore may not be appropriate to apply the same threshold to children who are immunosuppressed both from ALL itself and their immunosuppressive therapy.

Immunocompromised patients may potentially mount an adequate serological response but lack functional anti-pneumococcal protection. However, our study shows that the functional OPA assays performed demonstrated that the number of patients with functional protective immunity was

equal or greater to the number with concentrations of serotype specific IgG considered to be protective. This is reassuring in terms of interpreting the clinical implications of the results.

Although there were no documented cases of pneumococcal infection during the study period, a study limitation is that this surveillance did not extend further. While our data have shown the immunogenicity of PCV13, we were unable to demonstrate its clinical efficacy by comparing the incidence of pneumococcal infections before and after vaccination with PCV13.

Conclusion

This study demonstrates the vaccination with PCV13 in children with ALL at the end of chemotherapy is similar in effectiveness to vaccination at 6 months after completion of chemotherapy. We propose that relevant guidelines should now reflect this in order to maximally protect such children against pneumococcal disease.

Word count 2782 (max 3000)

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