Evaluation of the Immune Response to Vaccination Against Pneumococcus in Children Born Prematurely Including the Influence of Perinatal Factors

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Abstract

Background: The aim of this study was to assess the levels of antibodies after vaccination of heptavalent conjugate vaccine against *Streptococcus pneumoniae* (PCV7) in children born prematurely and to check whether there is a relationship between gestational age, birth weight, health condition at birth, and gender and immunogenicity of the PCV7. *Methods:* The study included 60 pre-term infants. Each child was vaccinated four times (2, 4, 6 and 16 months) with PCV7. Assessment of the level of antibodies was performed before vaccination, 4 weeks after primary series, before and 4 weeks after the booster dose. *Results:* Immunisation showed an increase of the average concentration of antibodies for all serotypes in most subjects; however, there were differences between the various serotypes. We found statistically significant relationship between fetal age and the concentration of antibodies related to serotypes 4, 9V, 14, 18C prior to dosing booster and to19F after it. In light of the relationship between health condition at birth and the level of antibodies, there was a marginal but significant correlation for serotypes 4, 6B, 23F (after a full course of vaccinations). We also found that girls had statistically higher concentration of antibodies after receiving all four doses of the vaccine. *Conclusions:* Heptavalent vaccine against *Streptococcus pneumoniae* has been proven to be highly immunogenic in preterm infants, even with extremely low birth weight. Among the perinatal factors, gender and health condition at birth clearly affect the level of post-vaccination response; less effect was noted in relation to gestational age.

Introduction

Streptococcus pneumoniae is the most common bacterial pathogen in the world, which can cause acute otitis media, sinusitis and bronchial pneumonia, and invasive infections – sepsis, bacterial meningitis, bacteraemia, pneumonia or peritonitis.[1] It is estimated that 7 of nearly 100 identified serotypes of the bacteria are responsible for 68.7% of cases

of invasive pneumococcal disease (IPD) in children under 5 years of age in Poland.[2]

It is known that the type and strength of response to vaccination depends not only on the vaccine itself, but it is also significantly determined by age, geographic region, time, health condition (including antigen presentation and activation of immune cells), genetic characteristics of the host, location of the tissue where an immune response occurs,[3-6] and also individual factors.[7-9] There are elements modifying immune response to a vaccine, too. These include: protective antibodies acquired from the mother or antibodies received by the administration of immunoglobulins being able to neutralise antigens present in a vaccine. It appears that the immune system of preterm babies effectively responds to immunisation[9,10] but induction of the immune response may be weaker in those children.[11]

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Until recently, heptavalent pneumococcal vaccine (PCV7) was used in infants only. Increase in the prevalence of infections caused by serotypes absent in the PCV7 vaccine − 1, 5, 6A, 7F and 19A[12] (in Poland those were types: 6A, 1, 3, 7F, 15B / C[13]), has led to the introduction of 10 and 13-valent vaccines. However, in Poland, the serotypes most frequently isolated in children under 5 years of age, are those included in PCV7: 14, 6B and 19F, that are responsible for 52.7% of IPD cases.[2] Moreover, in accordance with the criteria adopted by the World Health Organization (WHO) for assessment and registration of new vaccines, these vaccines are evaluated through comparisons of immunogenicity with PCV7.[14] Therefore, our results are still essential. Furthermore, the percentage of parents who decide to purchase the vaccine is still low.[15] At the same time, only few studies have been published on the use of this vaccine in the population of Polish children.[16,17] It seems important then to carry out these vaccinations, as it would lead to an extension of indication for free vaccinations.

The aim of the study was to evaluate the immune response before and after vaccination with heptavalent conjugate vaccine against *Streptococcus pneumoniae* (PCV7) in children born prematurely and to check whether there is a correlation between gestational age, birth weight, gender and health condition at birth and immunogenicity of the PCV7 vaccine.

Materials and Methods

In the period between January 2007 and December 2011, heptavalent conjugate vaccine against *Streptococcus pneumoniae* (PCV7) was given to 60 infants. The route of administration was intramuscular in the lateral part of the thigh, with 4 doses given at 2, 4, 6 and 16 months of age. Forty babies were vaccinated by the end of 2008, and preliminary results from that period were already published previously.[17] The following results are presented herein for all 60 vaccinated infants. The babies received the combination vaccine against diphtheria, tetanus, whooping cough (pertussis), polio and Haemophilus influenzae type b (DTaP-IPV-Hib). The study included babies born between 24th and 34th week of pregnancy (mean 29.9±2.61 weeks of gestation), with a birth weight ranging from 480 g to 2450 g (mean 1283.5 g±421 g), and in general, in a poor health state, i.e. with Apgar score of 5.5±1.47 points after one minute. Their clinical status assessed at the onset of vaccination on the basis of medical history, physical examination and evaluation of medical records was stable.

There were 48.3% (29/60) males and 51.7% females (31/60). The parents of the babies gave their consent to participation in the study and agreed to fulfil all the necessary recommendations during the research study. The analysis excluded preterm infants with known immunodeficiencies and diagnosed with severe chronic or progressive diseases.

For each patient antibody concentrations were determined four times − before the first dose of the vaccine, 4 weeks after primary series, immediately prior to the booster vaccination at 16 months, and 4 weeks later. Concentration of specific IgG antibodies against seven serotypes of *Streptococcus pneumoniae* (4, 6B, 9V, 14, 18C, 19F and 23F) contained in PCV7 was determined using a specific enzyme-linked immunosorbent assay (ELISA), and the measurements were carried out in the Statens Serum Institut in Denmark. According to the ELISA test used for these samples, the concentration of ≥0.35 µg/mL is protective against invasive pneumococcal disease.[18]

Two babies did not complete the study (no serum after booster vaccination was collected) because families left Poland permanently.

Statistica StatSoft 10.0 PL was used for statistical analysis. Variables expressed in quantitative scales were described using the geometric mean, median, standard deviation, and minimum and maximum values. Nominal-scale variables were represented by the number (n) and percentage of the study group. Normality of distribution of the analysed variables was tested with the Shapiro-Wilk test. As almost all variables were demonstrated to be significant (distributions differed significantly from the normal distribution), nonparametric tests were used for hypothesis testing. The nonparametric U Mann-Whitney test was used for comparison of antibody levels and for presentation of a correlation between gender and serum level. In order to demonstrate a correlation of birth weight, gestational age, and health condition at birth with the concentration of antibodies, the Spearman's rank correlation coefficient R was used. The null hypothesis was rejected when the computer-calculated level of significance met the assumption of $p<0.05$.

Results

Before the first dose of vaccine, the levels of antibodies were low for all serotypes. After primary vaccination we observed an increase in the average concentration of antibodies for all serotypes in the majority of babies, with

differences between various serotypes. The highest antibody concentration was observed for serotype 14 (4.1673 µg/mL), and the lowest for serotype 6B (1.0029 µg/mL). After further determinations of antibodies we observed variability in the immune response depending on the time of evaluation (Figure 1). Before administration of a booster dose, antibody concentrations significantly decreased in the majority of babies. Dynamics of decline in antibody levels were similar in all babies and serotype-dependent. The last vaccination was followed by a significant increase in the concentration of antibodies in all patients, and the results were higher than those achieved after three doses of the vaccine. That time, the highest values were once again observed for serotype 14 (14.4963 µg/mL), and the lowest for serotype 4 (2.6248 µg/mL).

Most of the babies in both groups - following the primary series as well as administration of the booster dose – achieved the protective antibody level of ≥0.35 µg/mL. Prior to the administration of the vaccine, one boy born at 31st week of pregnancy, with birth weight of 2000 g had a protective concentration of antibodies for all seven serotypes (for serotype 14 it was as high as 7.57 µg/mL), and three babies for six types. None of babies' mothers had ever received a pneumococcal vaccine. In a study conducted four weeks after the third dose of vaccine, 91% of babies had antibody levels considered to be protective, but for serotype 6B the concentration was the lowest - 71.7%. Prior

to the administration of the booster dose, the percentage of babies with antibody concentrations ≥0.35 µg/mL significantly decreased to a total of 76.4%. Finally, four weeks after the administration of the fourth dose of the vaccine, all babies achieved safe levels of antibodies.

Tables 1, 2, 3 and 4 summarise the assessment of a correlation of the duration of pregnancy, birth weight, health condition at birth assessed by Apgar score, and gender, with serum concentration of antibodies for all of the babies. In a vast majority of cases, we did not find any statistically significant correlation between the variable tested and the concentration of antibodies against a particular type of serological pneumococcal type of ≥0.35 µg/mL. A correlation was found for gestational age, but it was a weak correlation and related to serotypes 4, 9V, 14, and 18C before administration of the booster dose, and 19F following that dose. In addition, positive correlations were found (but also poor) between birth weight and the concentration of antibodies. The correlation was observed for three serotypes: 9V, 14, and 19F, but only prior to the administration of the vaccine. In subsequent periods, after the administration of the vaccine, those relationships were not statistically significant in all cases. It was also found that boys had statistically significantly higher levels of antibodies to all serotypes except for serotype 14 in the period before vaccination, while girls for serotype 6B, 14, and 19F one month after receipt of all four doses. Apart from the relationship between health condition at birth
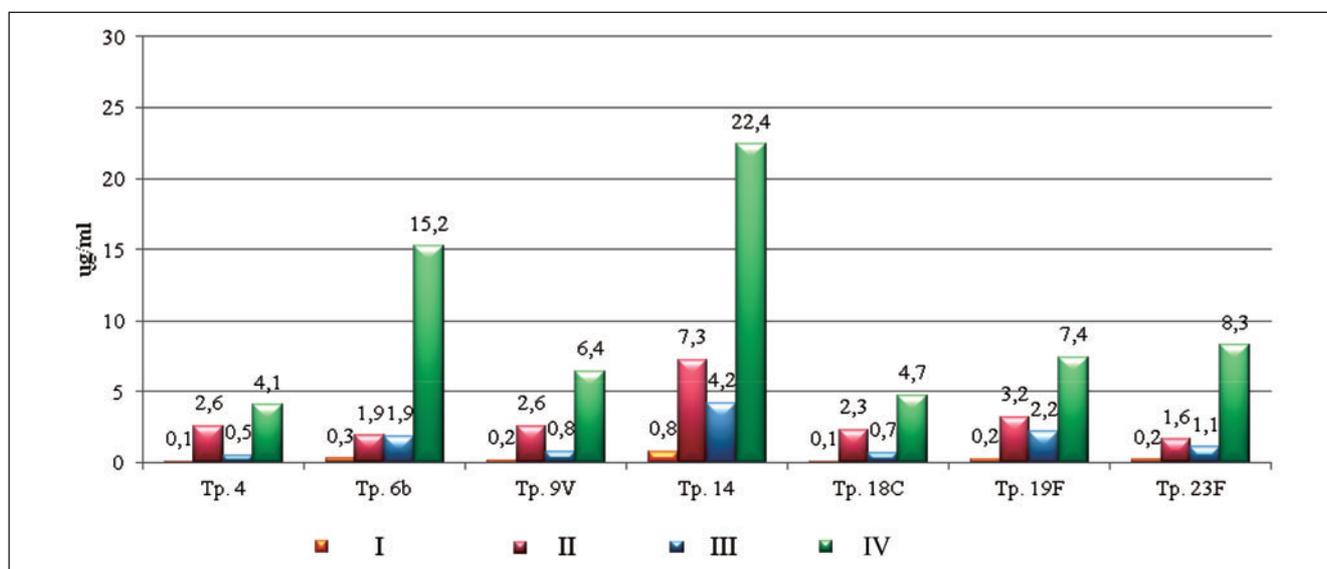

Figure 1　Geometric mean antibody concentrations for all serotypes, before the first dose of PCV7 (I), 4 weeks after the third dose (II), before the booster injection (III) and four weeks after the booster dose (IV).

Table 1   Assessment of the relationship between gestational age and the concentration of antibodies in the serum of all children (n=60), for each of serotypes and periods of study, using Spearman's rank correlation coefficient R; correlations statistically significant at p<0.05

Serotype	Before the 1st dose of PCV7		4 weeks after the 3rd dose of PCV7		Before the booster dose		4 weeks after the booster dose	
	R Spearmana	*p-value*	R Spearmana	*p-value*	R Spearmana	*p-value*	R Spearmana	*p-value*
4	0.05	0.6839	-0.16	0.2219	-0.34	0.0076	0.07	0.6198
6B	0.02	0.8893	-0.16	0.2365	-0.19	0.1444	0.18	0.1761
9V	0.16	0.2214	-0.14	0.2849	-0.29	0.0240	-0.02	0.8832
14	0.18	0.1761	-0.03	0.8143	-0.34	0.0087	0.12	0.3757
18C	0.01	0.9220	-0.15	0.2531	-0.27	0.0402	-0.02	0.8603
19F	0.16	0.2102	-0.07	0.5859	-0.10	0.4348	0.30	0.0201
23F	0.12	0.3538	-0.22	0.0874	-0.21	0.1127	0.05	0.7162

Table 2   Assessment of the relationship between birth weight and the concentration of antibodies in the serum of all children (n=60), for each of serotypes and periods of study, using Spearman's rank correlation coefficient R; correlations statistically significant at p<0.05

Serotype	Before the 1st dose of PCV7		4 weeks after the 3rd dose of PCV7		Before the booster dose		4 weeks after the booster dose	
	R Spearmana	*p-value*	R Spearmana	*p-value*	R Spearmana	*p-value*	R Spearmana	*p-value*
4	0.23	0.0772	-0.06	0.6538	-0.22	0.0933	-0.01	0.9482
6B	0.19	0.1566	-0.01	0.9346	-0.03	0.8466	0.10	0.4604
9V	0.41	0.0013	-0.13	0.3338	-0.12	0.3595	-0.03	0.8351
14	0.39	0.0022	-0.06	0.6331	-0.20	0.1206	-0.03	0.8208
18C	0.18	0.1724	-0.02	0.8956	-0.07	0.5742	-0.07	0.6120
19F	0.43	0.0007	0.13	0.3359	0.03	0.8011	0.18	0.1833
23F	0.24	0.0687	-0.09	0.5180	0.05	0.7144	0.07	0.6160

Table 3   Assessment of the relationship between gender and the concentration of antibodies in the serum of all of the children (n=60), for each type of serotypes and periods of study, using Spearman's rank correlation coefficient R; correlations statistically significant at p<0.05

Serotype	Before the 1st dose of PCV7		4 weeks after the 3rd dose of PCV7		Before the boosterb dose		4 weeks after the booster dose	
	R Spearmana	*p-value*	R Spearmana	*p-value*	R Spearmana	*p-value*	R Spearmana	*p-value*
4	2.47	0.0135	-0.86	0.3909	0.21	0.8302	-1.76	0.0789
6B	2.09	0.0370	-0.55	0.5842	1.21	0.2280	-2.81	0.0049
9V	2.48	0.0132	0.40	0.6896	0.94	0.3476	-1.86	0.0629
14	0.54	0.5892	0.02	0.9823	0.68	0.4962	-2.38	0.0174
18C	2.36	0.0183	0.84	0.3991	0.50	0.6202	-1.07	0.2833
19F	2.04	0.0412	0.64	0.5247	-1.13	0.2578	-2.47	0.0134
23F	3.11	0.0019	-0.14	0.8882	-0.44	0.6572	-1.94	0.0524

Table 4   To assess the relationship between Apgar score and the concentration of antibodies in the serum of all of the children (n=60), for each type of serological and periods of study, using Spearman's rank correlation coefficient R; correlations statistically significant at p<0.05

Serotype	Before the 1st dose of PCV7		4 weeks after the 3rd dose of PCV7		Before the boosterb dose		4 weeks after the booster dose	
	R Spearmana	*p-value*	R Spearmana	*p-value*	R Spearmana	*p-value*	R Spearmana	*p-value*
4	-0.05	0.258	0.05	0.7115	0.01	0.9635	0.26	0.0479
6B	-0.04	0.7771	-0.03	0.8487	-0.05	0.7011	0.27	0.0418
9V	0.00	0.9873	0.01	0.9119	0.12	0.3592	0.16	0.2501
14	0.13	0.3177	0.10	0.4403	0.07	0.6066	0.16	0.2280
18C	-0.14	0.2752	0.02	0.9062	0.09	0.4765	0.21	0.1055
19F	0.08	0.5564	0.18	0.1754	0.08	0.5558	0.23	0.0792
23F	0.10	0.4689	0.06	0.6727	0.22	0.0902	0.33	0.0127

determined by Apgar score and concentration of antibodies, there was also a marginal but significant correlation for serotypes 4, 6B and 23F (after receipt of a full course of vaccinations).

Eighteen of the 60 infants (30%) received glucocorticoids in the postnatal period. Broncho-pulmonary dysplasia (BPD) was diagnosed in seven of them. All of them received inhalatory steroids, at least for some time after the onset of vaccinations. Three babies received at least one dose of systemic corticosteroids after the first dose of vaccine. The lowest concentration of antibodies for serotype 6B was found in one of those babies after three doses of primary immunisation (data not shown).

Discussion

This research is a continuation of collective research conducted at our Clinic since 2007. The above results were obtained for 60 preterm infants. The first part of the data (for 40 infants) had been previously shown.[17] It is worth noting that although 10- and 13-valent vaccines were present on the market, they were (in contrast to PCV7) evaluated by comparing immunogenicity with heptavalent vaccines. In this study we prepared an accurate analysis of the influence of perinatal factors on immune response. Most of the so far published studies evaluating the immunogenicity after vaccination against pneumococcal concerned babies born at term.[19-21] Studies assessing premature babies included a number of children similar to those from our group.[22-24]

The concentration of antibodies, rated in our study for the first time, before PCV7 vaccine, was low for all serotypes with a few exceptions, which was consistent expected. It can be assumed that the antibody titers obtained before vaccination corresponds to the number of antibodies provided by the mother of the child in the transmission through the placenta.

The ability to respond to capsular polysaccharides is genetically determined and is inherited in autosomal dominant fashion. After vaccination some patients present a high level of IgG antibodies to all capsular polysaccharides. Others may show no response or poor response to most of them.[25] The immune response after vaccination may be affected by other factors, not only genetic, such as health condition during vaccination, gestational age, birth weight, gender, concentration of antibodies in the mother, race, geographic region, and even the time of year that vaccination is performed,[7,26-28] so we

decided that the analysis of the influence of various factors on the response rates among Polish babies would be important.

A recent report confirmed the need for not only quantitative determinations, but also qualitative tests, including the opsonophagocytosis assay (OPA), due to the functional role of the response induced by infection or vaccination.[29] Only quantitative methods were used in this study. However, a recommendation for using them for evaluation of immunogenicity, was included in the report on the meeting of an international group of experts, which was held in 2008 in Ottawa and in the recommendations of the WHO[30,31] after we started our research. In addition, despite some differences between the studies performed by ELISA and OPA, the method of ELISA may be used to determine post-vaccination antibody concentrations.[32]

As in other published papers, including the first post-registration survey,[33] we observed variability of immune responses depending on the period of determination of antibodies. Before vaccination, the level was very low. It increased after primary vaccination, but decreased significantly during the period before the booster dose. In Poland, the fourth dose of the vaccine is usually given at 16 months of age, as in our study. However, the most vulnerable to pneumococcal infection caused by serotypes included in the PCV7, are children between 6 and 17 months of age.[34,35] According to the Polish data, the highest incidence of IPD involved children between 6 and 12 months of age: 29.6/100000.[36] It is thus suggested that the best time to give a booster dose is the beginning of the second year of life.[37] After the last vaccination, antibody levels increased again in all of the children and remained, like in other research studies,[22-24,33] significantly higher than after the primary vaccination.

In 2005, WHO published serological criteria according to which the antibody concentration protective against invasive pneumococcal disease was at least 0.35 µg/mL four weeks after vaccination. This reference value was based on the results of three clinical trials on the efficacy of PCV7 in infants and children.[33,38-41] On the basis of those recommendations we evaluated a correlation of gestational age, birth weight, health condition at birth, and gender with antibody concentrations.

The most objective parameter in the evaluation of post-inoculation response is the gestational age.[42] We hypothesised that the antibody concentration is lower with a shorter gestational age. Neither our earlier study[17] nor this one confirmed that assumption. Although a positive correlation was found for serotypes 4, 9V, 14, 18C before

administration of a booster dose, and 19F, after that dose, it was marginal. We could not find such a correlation in available literature. As for birth weight, we noted that before vaccination some children with higher weights, presented slightly higher antibody titres for certain serotypes. However, the use of this vaccine resulted in an increase in antibody level in all children, also those with lower infant birth weight. In another study evaluating the immune response after PCV7 in children with very and extremely low birth weight, the authors showed such a relationship – therefore children with lower birth weights (<1000 g) had a lower concentration of antibodies for serotypes 6B and 23F.[24] Authors of that study demonstrated also that Caucasian race (all of our patients were Caucasian) is an independent predictor of lower antibody concentrations for serotype 6B and/or 23F. In this paper these serotypes were the least immunogenic. D'Angio et al did not find any correlation for gender, but female patients had higher post-vaccination levels of antibodies compared to males. However, the differences were not statistically significant. Gender is in fact a strong factor in the immune response to commonly used vaccines against infectious diseases.[26] We decided to check that in the population of Polish premature babies. It was found that, before vaccination, boys had significantly higher antibody concentrations for six vaccine serotypes (except for serotype 14). One of them, born in the 31st week of pregnancy, achieved the protective level of antibodies for all seven vaccine serotypes, and 3 others for six. Girls demonstrated higher antibody titres after a full cycle of vaccination (serotype 6B, 14, 19F). That is consistent with other reports showing that women produce higher levels of antibodies compared to men,[43-45] which may suggest that administered doses should be variable, depending on gender and age.[26] Stronger humoral response correlates with the severity of the most common vaccination-associated side effects (muscle and joint pain, headache).[45] In our study, parents of girls reported irritability, restlessness and fever following vaccination more frequently than parents of boys.

This study also found that lower levels of antibodies for some serotypes were present in children born in worse condition, assessed on the basis of the Apgar score. That observation does not confirm previous reports.[46] Moreover, findings of other authors also demonstrated an independent effect of postnatal administration of glucocorticoids on immunogenicity of the vaccine.[24,47-49] In our study that correlation related only to serotype 6B after three doses of vaccination.

Conclusions

1. Heptavalent vaccine against *Streptococcus pneumoniae* proved to be highly immunogenic in preterm infants, even those with extremely low birth weight.
2. Among the perinatal factors, gender and health condition at birth clearly affected the level of post-vaccinal response. The correlation was less pronounced for gestational age.

Acknowledgement

Declaration of Interest

None

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