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Haemophilus influenzae type b and pertussis vaccinations in preterm infants

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Abstract

Introduction: The aim of the study was to evaluate the serological efficacy of Hiberix and Infanrix-DTPa vaccines in preterm infants.

Materials and Methods: The results of the investigation of 61 preterm infants immunized three times (primary vaccination) with Hiberix and Infanrix-DTPa at 6-week intervals are presented. Of the 61 children, 17 were additionally immunized with a booster dose of these vaccines. Postvaccinal response to these immunizations was evaluated by means of an immunoenzymatic method.

Results: We observed a significant increase in protective postvaccinal antibody titers against *Haemophilus influenzae* type b (Hib) and *Bordetella pertussis* after the primary vaccination compared with the initial antibody levels ($p<0.05$). A significant increase ($p<0.0002$) in protective antibody titers after the booster dose of Hiberix compared with the primary vaccination was also noted. No correlations between birth weight, gestational age, and the achieved levels of postvaccinal anti-polyribosylribitol phosphate of Hib and of anti-pertussis toxin and anti-filamentous hemagglutinin of *B. pertussis* antibodies after the primary vaccination or booster dose were found. After the booster dose, all the preterm infants responded with the production of protective postvaccinal antibody titers against Hib and *B. pertussis*.

Conclusions: Due to the very good immunogenicity of the vaccines against Hib studied, inclusion of this immunization should be proposed in the obligatory vaccination schedule in Poland, especially in preterm infants. An additional immunization (i.e. a second booster dose) of Polish children with acellular pertussis (DTPa) vaccine is necessary to protect them from decreasing protective anti-pertussis antibody titers in early childhood.

Key words: Hib, pertussis, vaccination, preterm infants.

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INTRODUCTION

Due to immune incompetence of both humoral and cellular mechanisms, decreased immunological response and lower serological efficacy are observed after vaccinations in preterm infants with low or very low birth weights (<1500 g) [10, 11, 14, 16, 20]. These children belong to the group at high risk for developing sepsis, e.g. invasive *Haemophilus influenzae* type b (Hib) infections [6]. Some authors point out the increasingly important role of this pathogen as an etiologic factor for purulent meningitis [6, 18]. Invasive Hib infections in early childhood can endanger life, health, and further development. In the United States and Scandinavia, this infection is the most frequent cause of meningitis in children less than 5 years of age, whereas in Poland it runs second in this age group after *Neisseria meningitidis* [8]. Moreover, in Polish children the Hib carrier state prevails in the mucous membrane of the nasopharynx. Szenborn et al. [23] confirmed the presence of the above-mentioned bacteria in 29.8% of patients between 6 months and 5 years of age, while carriers were found more often among daycare attenders than in those who remained at home. Among the risk factors, apart from environmental constituents, involved in invasive Hib infection, immunity disorders and genetic aspects (high incidence in Native Americans (Navaho) and Alaskan Inuits) seem to be crucial [3, 17].

In Poland, like in other European countries, *Bordetella pertussis* infection is still a key issue. After the introduction of the whole-cell combined diphtheria-tetanus-pertussis vaccine (DTPw), the number of per-

tussis infections diminished continuously until 1989. However, after 1990 it began to increase and, moreover, changes in age distribution were noted, with increasing incidence amongst older children [24]. Similar epidemiological fluctuations in pertussis also appeared in other European countries [19]. The fluctuations in pertussis epidemiology were likely results of the reported adverse events (e.g. encephalopathy) following pertussis vaccinations. Moreover, the increase in pertussis incidence can result from changes in vaccination surveillance, a decrease in vaccine quality and its lower immunogenicity, or the appearance of *B. pertussis* strains resistant to vaccine activity (escape mutants) [24, 25]. In the case of risk groups (e.g. patients with neurological deficits, preterm infants), application of the acellular vaccine will allow us to reduce the number of children exempted from this vaccination and will contribute to an elevated level of population immunity. The US Advisory Committee for Immunization Practices and Committee on Infectious Diseases of the American Academy of Pediatrics recommend vaccination against pertussis also in children between 4–6 years of age [4]. The current Polish routine immunization schedule already includes vaccination in this age group.

The aim of this study was to determine Hib (anti-PRP) and *B. pertussis* (anti-PT&-FHA) antibody concentrations in preterm infants after primary vaccination and immunization with a booster dose of Hiberix and Infanrix-DTPa.

MATERIALS AND METHODS

Study site

This study was supported by grant from the Medical University of Łódź, Poland, and carried out at the Vaccination Clinic of Teaching Hospital No. 4 in Łódź from 2001 through 2003. Vaccination was performed in healthy preterm infants who were hospitalized prior to it due to prematurity and its complications at the Intensive Care Unit and in the Department of Pediatric Propedeutics and Bone Metabolic Diseases, Institute of Pediatrics, Medical University of Łódź. Written informed consent was obtained from the infants' parents before enrolment, and ethical approval was obtained from the Medical University Ethics Committee.

Subjects

The 61 preterm infants examined were immunized three times at 6-week intervals with Hiberix (SmithKline Beecham) at a dose of 10 μg and Infantrix-DTPa (SmithKline Beecham) at a dose of 0.5 ml intramuscularly (primary vaccination). Hiberix contains purified capsular polysaccharide in the form of the polyribosylribitol phosphate (PRP) of Hib chemically conjugated to tetanus toxoid (PRP-T), whereas Infanrix-DTPa is made from a minimum of 30 IU of diphtheria toxoid, a minimum of 10 IU of tetanus toxoid, 25 μg of pertussis toxoid (PT), 25 μg of filamentous hemagglutinin (FHA), and 8 μg of pertactin. Of the 61 preterm infants, 17 at ages of 16–18 months were additionally immunized with booster doses of Hiberix and Infanrix-DTPa vaccines. The vaccines used were mixed in one syringe and given in one injection in the anterolateral thigh. Of the 61 preterm infants, we obtained written consent in only 17 cases to continue immunization, i.e. to be boosted with the second dose of the vaccines under study.

Poland has been using whole pertussis vaccines in the primary immunization schedule (2, 3–4, 5 months) and as the first booster dose (16–18 months) since 1960, and acellular pertussis vaccines in routine immunization since 2003 as the second booster dose (at 6 years of age). Since 2004, Hib vaccination is part of the national immunization schedule only with regard to daycare attenders. However, there is a great campaign on the Polish market to inform parents of the possibility of immunizing their children against Hib, which may rescue them from the fatal outcome caused by this pathogen.

Collection of specimen

Blood (1.5 ml) was taken from the preterm infants by venepuncture into Microtainer Serum Separator tubes just before the first vaccination and 4 weeks after the third consecutive dose of Hiberix and Infanrix-DTPa. In 17 children, blood was additionally taken 4 weeks after the fourth dose. Sera separated from these samples were stored at –20°C until they were assayed for the evaluation of antibodies.

Methods

Detection of antibodies against Hib. Assessment of postvaccinal specific immunity after immunization with Hiberix was made by means of the enzyme-linked immunosorbent assay (ELISA) using commercially available test kits (BINDAZYME™ Human Anti-*Haemophilus influenzae* Enzyme Immunoassay Kit, The Binding Site, Birmingham, UK). Antibody concentrations were calculated according to the obtained standardized curves. In this assay, plates were coated with purified capsular polysaccharide-PRP of Hib. The achieved levels of postvaccinal antibodies were regarded as satisfactory (anti-PRP concentration >0.15 μg/ml and <1.0 μg/ml), good (anti-PRP concentration ≥ 1.0 μg/ml and <90 μg/ml) and very good (anti-PRP concentration ≥ 90 μg/ml).

Detection of antibodies against B. pertussis. IgG antibodies against pertussis (after immunization with Infanrix-DTPa) were detected by means of the ELISA using commercially available test kits (*Bordetella pertussis* ELISA Kit, Genzyme Virotech GmbH, Rüsselsheim, Germany) in accordance with the laboratory procedure recommended by the manufacturer. In this assay, plates were coated with a mixture of purified PT and FHA of

B. pertussis. The results of antibody concentrations were presented in Virotech units (VE), with a value >11.0 VE as positive, 9.0–11.0 VE as ±, and VE <9.0 VE as negative. The manufacturer recommended assessing all samples <11.0 VE as negative. Determination of antibodies by means of ELISA were carried out in the Department of Clinical Immunology, Teaching Hospital No 4. of Medical University in Łódź.

Study results were presented as geometric means for postvaccinal antibody concentrations. Because the variable distribution was not normal (based on the Shapiro-Wilk test), further statistical analysis followed the guidelines of non-parametric analysis. The dependent variables were compared with Wilcoxon's test. Analysis of variance (ANOVA) was applied because the children were divided into more than two groups. The relations between the measured data were determined with Spearman's correlation coefficient. The differences were considered significant at $p<0.05$.

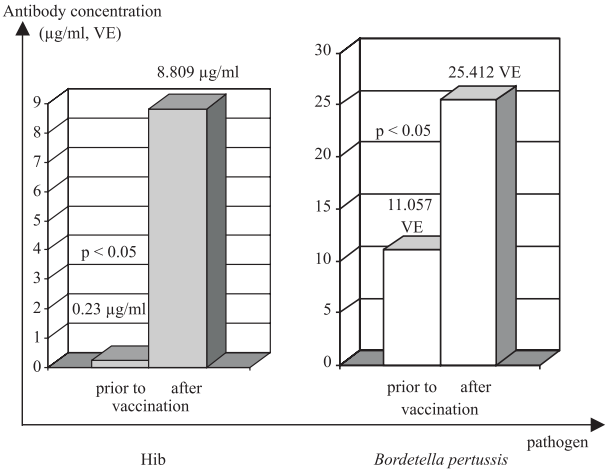

Fig. 1. Geometric means of antibody concentrations prior to and after the third dose of Hiberix and Infanrix-DTPa.

RESULTS

A total of 61 preterm infants born between 24 and 36 weeks' gestation with birth weights between 600 and 2500 g were recruited to the study. The median of age at the beginning of vaccination was 147 days (minimum: 70 days, maximum: 784 days; Table 1). None of the children had received post-natal steroids.

Analysis performed with Wilcoxon's test revealed a statistically significant increase in protective postvaccinal titers after primary immunization with Hiberix and Infanrix-DTPa. Geometric mean concentrations (GMCs) of antibodies prior to immunization compared with the levels achieved after 3 doses of vaccines were 0.23 μg/ml vs. 8.809 μg/ml for Hib ($p<0.05$) and 11.057 VE vs. 25.412 VE for *B. pertussis* ($p<0.05$; Fig. 1). Minimal concentrations of anti-PRP prior to immunization were 0.166 μg/ml and maximal 0.575 μg/ml, and after the third dose of Hiberix they were 0.19 μg/ml and 107.5 μg/ml, respectively. Accordingly, observed minimal concentrations of anti-PT&-FHA antibodies prior to immunization were VE and maximal 13 VE; as a result of triple immunization with Infanrix-DTPa the observed minimal concentration of postvaccinal anti-PT&-FHA antibodies was 0 VE (while four infants (6.6%) did not respond to vaccination) and the highest titers after primary immunization with Infanrix-DTPa were 46 VE.

Table 1. Characteristics of vaccinated preterm infants

The examined parameter	Median	Minimum and maximum values
Age at the beginning of vaccination (days)	147	70–784
Birth weight (g)	1100	600–2500
Gestational age (weeks gestation)	29	24–36

All children immunized against Hib presented initial anti-PRP concentrations >0.15 μg/ml even before vaccination. As a consequence of primary immunization with Hiberix, all the preterm infants produced protective postvaccinal anti-PRP titers (>0.15 μg/ml). Analysis of variance (ANOVA) confirmed that of this group, 16 patients (26.2%) showed protective antibody titers >0.15 μg/ml and <1.0 μg/ml (GMC=0.4392 μg/ml), 28 infants (45.9%) had higher protective antibody concentrations (≥1.0 μg/ml and <90 μg/ml, GMC=11.8339 μg/ml), and in the remaining 17 children (27.9%) the immunization was very good (GMC=91.1096 μg/ml). The observed differences between postvaccinal anti-PRP in these groups were statistically significant (Fig. 2). Moreover, we noted a significant correlation between birth weight, gestational age, and anti-PRP antibody concentrations prior to immunization (Spearman's correlation coefficient $r=0.5021$, $p=0.0179$ and $r=0.5820$, $p=0.0023$, respectively).

Among the preterm infants immunized with Infan-

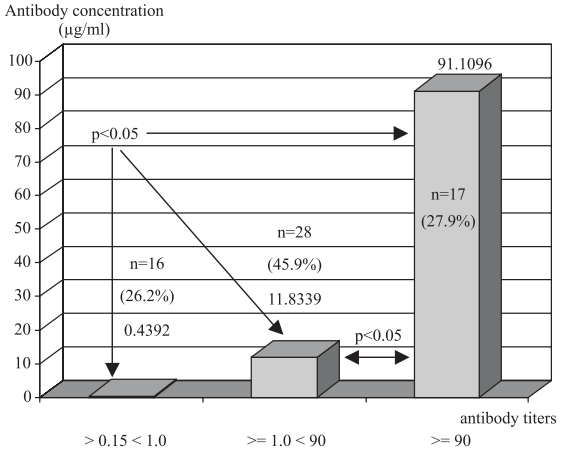

Fig. 2. Geometric means of postvaccinal anti-PRP antibody concentrations after the third dose of Hiberix.

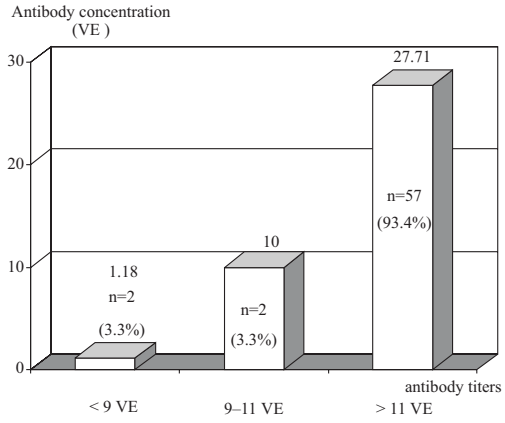

Fig. 3. Geometric means of postvaccinal anti-PT&-FHA antibody concentrations after the third dose of Infanrix-DTPa.

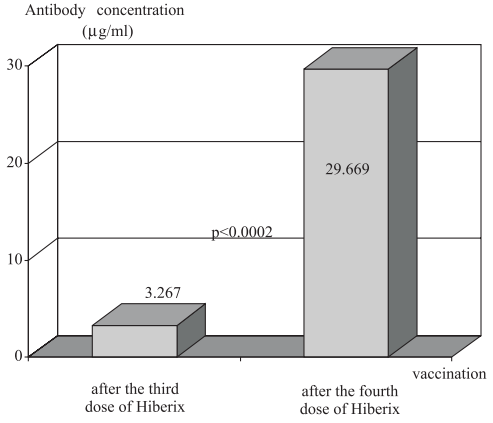

Fig. 4. Geometric means of postvaccinal anti-PRP antibody concentrations after the third and fourth dose of Hiberix.

rix-DTPa only, we found anti-PT and anti-FHA antibodies prior to vaccination in three children (including two patients with concentrations >11.0 VE). As a result of primary vaccination with Infanrix-DTPa, 57 of the premature infants (93.4%) responded by producing protective postvaccinal anti-PT and anti-FHA antibodies (>11.0 VE). Using analysis of variance (ANOVA) we showed that in the group immunized with the above-mentioned vaccination, two infants (3.3%) produced postvaccinal antibody titers <9.0 VE (GMC=1.18 VE), two (3.3%) presented antibody titers between 9.0–11.0 VE (GMC=10 VE), and 57 patients (93.4%) >11.0 VE (GMC=27.71 VE; Fig. 3).

Of the 61 preterm infants, only 17 took part in the later study and we decided to determine specific postvaccinal response after immunization with a fourth consecutive dose (booster dose) of Hiberix. A statistically significant increase in anti-PRP titers was noted compared with the antibody concentrations obtained after the primary vaccination (the third dose); GMC was 3.267 μg/ml (primary vaccination) vs. 29.669 μg/ml (after the fourth dose, $p<0.0002$, Wilcoxon's test; Fig. 4). Minimal anti-PRP concentration after the booster dose of Hiberix was 0.94 μg/ml and maximal 90 μg/ml. Comparing similar specific serologic response after immunization with Infanrix-DTPa, a slight increase in GMC of postvaccinal antibody concentrations after the booster dose was observed (27.116 VE vs. 31.985 VE), but it was not statistically significant. Individual analysis showed that despite immunization with the fourth dose of Infanrix-DTPa, in 9 infants (52.9%) a decline in anti-PT&-FHA antibody concentration was noted compared with the levels achieved after primary vaccination, but they did not decrease below the protective level. The observed minimal anti-PT&-FHA antibody concentrations after the booster dose amounted to 17.7 VE and the maximal to 52.6 VE.

Using Spearman's correlation coefficient, we did not note any significant correlations between birth weight, gestational age, and the obtained postvaccinal anti-PRP and anti-PT&-FHA antibody titers after primary vaccination or booster dose (Table 2). However, a statistically significant correlation between anti-PRP concentrations after primary vaccination and child's age at the beginning of immunization was observed ($r=0.5826$, $p=0.0023$). An individual analysis revealed that in children with postvaccinal antibody concentrations ≥90.0 μg/ml, the first dose of Hiberix was given after 180 days of life.

The serological response after the primary immunization of the vaccines turned out to be efficacious (100% for Hiberix and 93.4% for Infanrix-DTPa). However, after the booster dose of Hiberix and Infanrix-DTPa we found excellent serologic efficacy (all infants responded by producing postvaccinal antibodies at protective levels; Fig. 5).

Table 2. Correlations between birth weight, gestational age and the achieved levels of postvaccinal antibodies after primary vaccination and booster dose

Birth parameter	Anti-PRP antibody concentration (μg/ml) after primary vaccination		Anti-PT&-FHA antibody concentration (VE) after primary vaccination		Anti-PRP antibody concentration (μg/ml) after booster dose		Anti-PT&-FHA antibody concentration (VE) after booster dose	
	r	p	r	p	r	p	r	p
Birth weight (g)	–0.0310	0.8132	–0.1426	0.2728	0.1643	0.4765	–0.2409	0.2926
Gestational age (weeks gestation)	–0.0715	0.5839	–0.1186	0.3626	0.3706	0.3131	–0.0329	0.8871

r – Spearman's correlation coefficient.

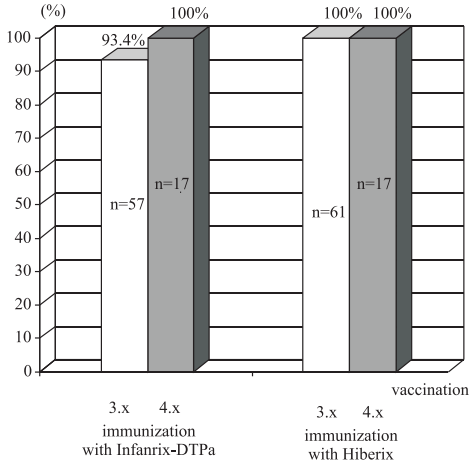

Fig. 5. Percentage of children with protective postvaccinal antibody titers after the third and fourth dose of Infanrix-DTPa and Hiberix.

DISCUSSION

Invasive Hib infections more often affect children with immunodeficiency than the remaining population of children and adolescents. Therefore, apart from those born prematurely, other patients at risk for these infections are those with lymphoma and leukemia, AIDS, those being treated with immunosuppressants, and HIV carriers. Research should be conducted on the antigenicity, immunogenicity, and reactivity of vaccines against Hib infections in the above-mentioned groups of patients. In the case of immunization against Hib, conjugation of the polysaccharide bacterial capsule with a protein carrier significantly improved its immunogenicity, which permitted its use in children [1, 5, 13]. Due to conjugation, the postvaccinal immune response was T lymphocyte dependent, which determined the specific cell-mediated anti-Hib response. Until now it has not been assessed what the protective Hib antibody levels are. It is assumed that short-term protection occurs at a minimal anti-PRP level of 0.15 μg/ml, whereas long-term protection is determined by concentrations exceeding 1.0 μg/ml [5, 9].

Taking into consideration the postvaccinal response to pertussis, it should be emphasized that the immunogenicity of acellular vaccines (DTPa) is comparable to whole-cell immunization (DTPw), while its reactivity is lower [2]. In the USA and Western Europe, DTPa is commonly used in the obligatory vaccination schedule. In Poland it was introduced to the routine immunization schedule in 2003 (as the second booster dose). We would like to point out that the use of DTPa could limit the number of children exempted from vaccination against pertussis in our country and contribute to increasing the immunization of children and adolescents. Zieliński et al. [25] carried out research on the efficacy of vaccination against pertussis in Poland during the epidemic in the 1997–1998, demonstrating a high efficacy of immunization against pertussis at the age of 2–5 years.

Studies evaluating postvaccinal immunity to Hib and *B. pertussis* in preterm infants have been described in literature [7, 11, 14, 16, 21]. Kirmani et al. [15] observed protective anti-PRP antibody titers (>1.0 μg/ml) lingering for 7 years in children vaccinated in early childhood; higher concentrations were presented in full-term infants than in preterm children. Similarly, Heath et al. [11] examining anti-PRP antibody concentrations in preterm infants at 2, 5, 12, and 64 months of age, demonstrated that premature infants developed lower antibody concentrations than term infants following Hib conjugate vaccination but, despite this, they achieved very high levels of clinical protection. Kirstensen et al. [16] assessed postvaccinal response after the conjugate vaccine HibCP-TT in 35 preterm infants at 2, 4, and 12 months of age. These authors revealed that immature infants might show an inadequate antibody response to the initial immunization, although many preterm babies could benefit from vaccination with HibCP-TT started at the same chronological age as term infants. According to Kristensen and other investigators the smallest preterm infants with complicated postnatal clinical course are more likely to have decreased, though protective, immune responses when completing a primary immunization series [14, 16].

In our studies we demonstrated in all the immunized preterm infants a significant increase in protective anti-PRP antibody titers after primary vaccination compared with the initial levels (Fig. 1). The majority of preterm infants showed an increase in protective anti-PT&-FHA antibody titers after primary immunization compared with the initial level (Fig. 3). The obtained results were to some extent consistent with the available medical literature. Hussey et al. [12], evaluating postvaccinal immunity 4 weeks after triple doses of TETRActHib (conjugated DTP and Hib vaccines) in children at 6, 10, and 14 months of age, demonstrated that in the majority of patients anti-PRP antibody concentrations were higher than 0.15 μg/ml and in 70% of the children higher than 1.0 μg/ml. According to these authors, protective antibody titers persisted at 9 and 18 months of age (45% of patients showed anti-PRP levels higher than 1.0 μg/ml).

In our study, protective anti-PRP and anti-PT&--FHA titers determined 4 weeks after the booster dose at 16–18 months of chronological age were detected in all 17 children immunized with Hiberix and Infanrix-DTPa (Fig. 5). More than half of the preterm infants demonstrated decreasing postvaccinal immunity to *B. pertussis* in a concurrent individual analysis of postvaccinal antibody levels (infants presented lower concentrations compared with the third dose of Infanrix-DTPa). However, such a decrease was not observed after the booster dose to Hib. Slack et al. [22] revealed that preterm infants with very low IgG responses to Hib conjugate vaccine mounted an adequate response to an early additional fourth dose of Hib. They concluded that all infants will benefit from a fourth dose of Hib, regardless of the age at which it is given.

Evaluating postvaccinal immunity to pertussis in preterm infants at the age of 5–6 years who had been vaccinated with DTPa-HBV at 3, 5, and 11 months of age, Esposito et al. demonstrated quantitatively and qualitatively worse response compared with full-term infants [7]. These authors pointed out the necessity of a booster dose given at the age of 5–6 years in term infants (in preterm children even earlier) in order to maintain an adequate pertussis-specific response. Schloesser et al. [21] conducted research on the immunogenicity of a two-component acellular pertussis vaccine in preterm infants; they immunized 50 preterm infants and 50 full-term infants, irrespective of their biological age and current weight. They concluded that immunization with an acellular pertussis vaccine was safe for preterm infants and the immune response was significantly lower compared with the control group of term infants, but the efficacy was high.

According to the recommendations of the American Academy of Pediatrics Committee on Infectious Diseases, medically stable preterm infants should receive full doses of acellular pertussis, diphtheria, tetanus, Hib, hepatitis B, poliovirus, and pneumococcal conjugate vaccines at a chronological age consistent with the schedule recommended for full-term infants. Vaccine dosages normally given to full-term infants should not be reduced or divided when given to preterm infants and those with low birth weight. Moreover, it was shown that all vaccines routinely recommended during infancy are safe for use in preterm infants and, although the immunogenicity of some childhood vaccines may be decreased in the smallest preterm infants, the antibody concentrations achieved usually are protective [20].

Our research demonstrated that the vaccines used against Hib and pertussis in preterm infants were highly immunogenic and should therefore be widely introduced into the obligatory vaccination schedule, especially in infants from high-risk groups. That is why we advocate the introduction of vaccination against Hib in preterm infants in Poland and support applying the second booster dose of DTPa in routine immunization in children at 6 years of age in order to prevent a further decline in immunity to pertussis.

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