A RANDOMISED TRIAL OF THE SAFETY AND IMMUNOGENICITY OF LOW DOSE HAEMOPHILUS CONJUGATE VACCINE IN HEALTHY INFANTS AT 6, 10, AND 14 WEEKS OF AGE

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A Research Report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Medical Microbiology

I. DECLARATION

I, Mark Patrick Nicol declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Medical Microbiology in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

Signed .....

On this the 1 day of SEPTEMBER, 2002.

II. PUBLICATION AND PRESENTATION ARISING FROM THIS STUDY

Publication

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III. ABSTRACT

Background Despite their proven efficacy, *Haemophilus influenzae* type b (Hib) conjugate vaccines are not given to most children in the developing world in the face of an estimated global Hib disease burden of nearly 2 million cases per annum. A major barrier to the introduction of the vaccine would be overcome by diluting the vaccine tenfold in DTP. We report a randomised trial comparing the use of Hib conjugate vaccine diluted tenfold in a multidose vial of DTP, with that of the full Hib dose.

Methods 168 infants were randomized to receive either the full-dose Hib polysaccharide tetanus toxoid (PRP-T) conjugate vaccine or a 1 in 10 dilution prepared by reconstituting the full dose in a 10-dose DTP vial. Infants were vaccinated at 6, 10 and 14 weeks of age and received a full-dose as a test of immunological memory at 9 months of age. Sera were collected at each visit and at one week following the booster dose. Serum anti-capsular PRP antibody concentrations were measured by ELISA.

Results Following the primary vaccination series, 95% of infants in the full-dose arm and 94% of infants in the 1/10 dose arm achieved protective anti-PRP IgG antibody concentrations of $\geq$ 1.0 μg/ml. Infants receiving the diluted vaccine had significantly higher levels of anti-PRP antibody in response to the booster dose (151.36 μg/ml versus 68.55 μg/ml, P = 0.009).

Conclusions The 1/10 dose of PRP-T was as immunogenic and safe as the full dose. The technique of diluting a single dose of PRP-T in a 10-dose DTP vial could potentially allow the widespread introduction of Hib vaccine in resource-poor countries currently unable to afford full dose Hib conjugate vaccine.

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1.0 INTRODUCTION

1.1 The Pathogen

Haemophilus influenzae is a small gram-negative, encapsulated bacillus. *In vitro* growth requires the presence of supplementary factors including 'X-factor' (haemin) and 'V-factor' (nicotinamide adenine dinucleotide). Encapsulated strains possess a polysaccharide outer capsule, which defines six antigenically and biochemically distinct types. In the pre-vaccine era 95% of all invasive disease caused by *H. influenzae* was due to the type b serotype (Hib) (Wenger et al., 1992). Hib is set apart from unencapsulated strains and from other capsular serotypes by the presence of a unique polyribosylribitol phosphate (PRP) capsule. This capsule endows Hib with singular pathogenic potential amongst strains of *H. influenzae* by inhibiting opsonophagocytosis and promoting invasion (Moxon and Murphy, 2000).

The organism is a relatively uncommon colonizer of the upper respiratory tract, with nasopharyngeal carriage occurring in 3-5% of children in the pre-vaccine era (Barbour, 1996). In an infant rat model (Moxon and Anderson, 1979), intranasal inoculation of Hib was followed by submucosal and subsequent blood stream invasion within minutes, suggesting that the outcome of exposure to Hib (i.e. either colonization or infection) is determined soon after contact with the respiratory mucosa.

The spectrum of invasive disease caused by Hib includes meningitis, pneumonia, epiglottitis, cellulitis, bacteraemia without localized disease and septic arthritis. Traditionally, meningitis has been thought to account for the largest proportion of morbidity due to Hib, however this view has been challenged by data suggesting that, in the developing world at least, pneumonia due to Hib may be the predominant clinical presentation (Mulholland et al., 1997). The focus in the literature however remains on those conditions more confidently ascribed to Hib, where the organism is isolated from a sterile body site. Non-encapsulated strains of *H. influenzae* are responsible for otitis media and other mild upper respiratory tract infections. These are not considered further here.

Meningitis due to Hib is the most serious acute manifestation. Prior to the introduction of vaccination, Hib was the most common cause of childhood meningitis in the developed world (Peltola, 2000). It usually affects children less than two years of age and is uncommon in adults. Peak attack rates occur at 6-7 months of age. The typical picture includes an antecedent upper respiratory tract illness followed by deterioration with the development of fever and signs of central nervous system involvement. The onset is usually insidious but progression may be fulminant. Complications are common and include subdural effusions and the development of permanent sequelae such as sensorineural deafness and motor deficit (Broome, 1987).

Epiglottitis is uncommon in the developing world, and presents with a dramatic onset of fever, sore throat, dyspnoea, drooling and dysphagia in the older child (2-7 years).

Cellulitis most frequently occurs on the cheek or in the periorbital region in young children and is frequently accompanied by bacteraemia. Amongst children 6-36 months of age bacteraemia without localized disease is most commonly caused by *Streptococcus pneumoniae*, with Hib the second most common cause of this syndrome. Septic arthritis caused by Hib is common in children less than two years of age. Hib infection is uncommon in adult life (Moxon and Murphy, 2000).

Pneumonia due to Hib is an important and often unrecognised childhood infection but probably represents the most common presentation of invasive Hib disease in the developing world (Peltola, 2000). Ascribing a specific aetiology to lower respiratory tract infections is problematic, but studies of lung aspirates from infants with pneumonia suggest that Hib pneumonia is far more frequent than previously believed (Greenwood, 1992).

1.2 Immunity to Hib – natural and vaccine induced

The observation that invasive disease due to Hib is predominant during the period in which maternal antibodies wane and prior to the development of acquired immunity suggests that specific antibodies mediate protection against Hib infection. Early work showed that the therapeutic administration of serum containing high titres of antibody against Hib capsular material enhances the phagocytosis of organisms in the cerebrospinal fluid (CSF) (Alexander et al., 1942). In addition, human hyperimmune globulin protected Apache infants from invasive Hib infection during infancy (Siber et al., 1992).

Exposure to cross-reactive epitopes (such as those of *Escherichia coli* K100) during the early years of life is thought to stimulate the development of natural immunity to Hib infection (Insel and Anderson, 1982). The majority of infants who develop invasive Hib infection have very low or undetectable titres of anti-PRP antibodies at the time of infection (Kayhty et al., 1981). Development of natural antibody to PRP, the antibody response to Hib infection and the antibody response to vaccination with purified Hib polysaccharide vaccine are all highly age dependent (Peltola et al., 1977). Young infants are unable to mount an effective antibody response to polysaccharide antigens, due to the T cell independent nature of these antigens.

T cell independent antigens are usually polysaccharides with multiple, identical, repeating epitopes and are common to bacteria. These antigens are internalised but not processed by T-cells, and consequently are not presented on the cell surface in the context of MHC molecules. The ability of such antigens to elicit an antibody response relates to their ability to directly activate B-cells by cross-linking antibody on the B-cell surface. Polysaccharide antigens are poorly immunogenic in young children, do not elicit the production of memory B-cells and stimulate the production of IgM and IgG2a antibodies (Eskola et al., 1993).

The conjugation (covalent linkage) of polysaccharide to a protein promotes antigen processing by T-cells and transforms the immune response to a T-cell dependent response. The conjugate antigen is immunogenic in infants, elicits a memory response,

and leads to affinity maturation of antibodies and production of antibodies of the IgG1 subclass. Protein conjugate technology forms the basis for the development of PRP-protein conjugate vaccines (Eskola et al., 1993).

1.2.1 Correlates of protection against invasive Hib disease

Levels of serum anti-PRP antibody are a widely accepted correlate of protection against invasive Hib infection. Based largely on passive immunization studies and data from polysaccharide vaccine trials, minimum serum concentrations of anti-PRP antibodies that afford protection against invasive infection have been estimated. Antibody concentrations of ≥ 0.15 μg/ml correlate with short-term protection whilst levels of 1 μg/ml are thought to be indicative of long-term protection (Kayhty et al., 1983). Total serum antibody levels may be measured by Farr-type radioimmunoassay (RIA) (Makela et al., 1977). IgG anti-PRP antibodies are measured by enzyme-linked immunosorbent assay (ELISA), which has largely replaced the RIA (Sutton et al., 1985).

Whilst antibody levels are useful correlates offering an acceptable surrogate marker of protection, care needs to be taken in interpreting antibody levels on their own. Additional markers such as the functional activity (avidity (Goldblatt et al., 1998), opsonophagocytic activity (Vernacchio et al., 2000)) and local mucosal immunity need to be considered. In addition, the ability to mount a rapid anamnestic response to repeat exposure to antigen (either in the context of repeat vaccination or natural infection) is likely to be critical in affording protection against disease (Granoff and Lucas, 1995). This is complicated by further evidence that suggests that the immunological priming of

infants without the presence of circulating antibody may be insufficient to protect against invasive Hib infection (Anderson et al., 2000). It is likely therefore that both circulating anti-PRP antibodies as well as the ability to elicit a booster response are critical to protection. The broad acceptance of these correlates of protection (serum anti-PRP titres and the anamnestic response) permits the initial assessment of vaccines against Hib by means of small-scale immunogenicity studies, rather than relying on large, expensive trials of vaccine efficacy.

1.3 The global burden of disease in the pre-vaccine era

In the pre-vaccine era, Hib was an important cause of life-threatening infection amongst infants worldwide. The burden of disease is most easily determined for those "classical" infections in which the organism can be isolated from a normally sterile body site (e.g. CSF, blood, joint fluid) and this data are most frequently used when citing the incidence of invasive Hib disease.

The incidence of meningitis shows significant geographic and ethnic variation, even in the pre-vaccine era (Peltola, 2000). In populations such as native inhabitants of Alaska, northern Canada and northern Australia, the annual incidence in children aged 0-4 years exceeded 150 per 100 000. In the United States, the annual incidence was approximately 50 per 100 000, twice the average for pre-vaccination Europe. Little reliable data are available for South Africa, however in Cape Town rates per 100 000 children were 112 and 34 for children <1 year and <5 years of age respectively (Hussey et al., 1994). The rate in black children was twice that of white and mixed race children. Mortality patterns

mirrored those of overall distribution, with case fatality rates of 5% in developed and >30% in developing countries.

Epiglottitis showed an unusual distribution, with peaks in incidence in European countries such as Switzerland (30 per 100 000) and Finland (13 per 100 000) in children <4 years old. In developing countries there was an inverse trend between meningitis and epiglottitis, with epiglottitis virtually absent from Australian Aborigine, Alaskan Inuit and African children (Peltola, 2001).

Cumulative averages, including all of the "classical" manifestations, varied from 1100 per 100 000 children <4 years amongst Australian Aborigines to 41 per 100 000 in Europe. In Cape Town the rates were 160 and 47 per 100 000 children <1 year and <5 years respectively (Hussey et al., 1997).

The incidence of pneumonia caused by Hib in infants is more difficult to establish, due to the difficulty in obtaining representative samples for bacteriological culture. A small study in Papua New Guinea showed a rate of 2860 per 100 000 in children <4 years (Lehmann et al., 1999). Data from The Gambia showed that *H. influenzae* was the second most common cause of pneumonia, with 75% of strains being type b (Greenwood, 1992). In the same country, a Hib conjugate vaccine reduced the incidence of severe childhood pneumonia by 21%, suggesting that up to a quarter of all severe childhood pneumonia may be due to Hib (Mulholland et al., 1997). This is of particular importance, since lower

respiratory tract infections remain the commonest cause of childhood mortality in the developing world.

The total number of cases Hib pneumonia worldwide may be 1 650 000 cases per annum if the Gambian estimations are correct. This is five times higher than the number of cases of Hib meningitis.

The disease burden is most heavy in the countries of the developing world. Based on the above data, a conservative estimate would show 2.1 million cases occurring annually in the developing world compared with 70 000 cases in the developed world in the pre-vaccine era (Peltola, 2000).

1.4 Vaccines against Hib

1.4.1 Polysaccharides and conjugates

The first vaccines to come into use against Hib infection consisted of the PRP capsular polysaccharide outer coat. These vaccines were shown to have an efficacy of 90% amongst children aged 18 to 71 months in Finland (Eskola et al., 1987), but were ineffective in younger children (Ward et al., 1990) in Alaska. Despite their shortcomings, these vaccines were licensed for use in children in the United States and Canada. The advent of conjugate vaccines in the late 1980's was a major advance. Four different protein conjugate vaccines rapidly became available. Diphtheria toxoid conjugate (PRP-D; ProHIBit) was followed by mutant diphtheria toxin (PRP-CRM_{197} or HbOC; HibTITER), meningococcal outer membrane protein (PRP-OMP; PedvaxHIB) and

tetanus toxoid (PRP-T; ActHIB, OmniHIB or Hiberix). The conjugate vaccines differ by protein carrier, polysaccharide size, method of chemical conjugation and the ratio of polysaccharide to protein. Table 1.1 summarizes the properties of these vaccines. PRP-D, PRP-CRM_{197} and PRP-T are given as a primary series of three doses, one to two months apart, with or without a booster dose at the age of 12-15 months. The PRP-OMP vaccine is given as two doses two months apart with a booster dose at 12-15 months of age. The United States Advisory Committee on Immunization Practices (1993) recommends primary vaccination at 2, 4 and 6 months of age, whilst South Africa follows the World Health Organisation accelerated schedule at 6, 10 and 14 weeks of age. The need for booster doses is controversial, with certain countries (such as the United States) recommending their use and others (such as the United Kingdom) electing to omit a booster dose.

Table 1.1 *Haemophilus influenzae* type b conjugate vaccines licensed for use among children

Vaccine	**Trade name (manufacturer)**	**Polysaccharide**	**Linkage**	**Protein carrier**
PRP-D	ProHIBiT(R) (Connaught)	Medium	6-carbon	Diphtheria toxoid
HbOC	HibTITER(R) (Lederle-Praxis)	Small	None	CRM_{197} (mutant *Corynebacterium diphtheriae* toxin protein)
PRP-OMP	PedvaxHIB(R) (Merck Sharp and Dohme)	Medium	Thioether	*Neisseria meningitidis* outer membrane protein complex
PRP-T	ActHIB™ OmniHIB(TM) (Pasteur Merieux Vaccines)	Large	6-carbon	Tetanus toxoid

1.4.2 Immunogenicity and efficacy of conjugate vaccines

The conjugate vaccines have clearly demonstrated their advantage over simple polysaccharide vaccines. They are highly immunogenic in young children, show a significant booster effect and are effective in reducing pharyngeal carriage of Hib. These vaccines are highly effective in preventing invasive Hib infection from the first months of life, with most studies demonstrating protection in excess of 90%. One notable exception is a study of PRP-D amongst Alaskan Inuits (a population with a high incidence of infection) in which the point estimate of efficacy was 35% (Ward et al., 1990). The same vaccine was shown to be 87% effective in Finnish infants (Eskola et al., 1987). Interestingly, vaccine immunogenicity was limited in both trials (less than 40% of infants achieving titres of greater than 1 μg/ml one month after the third dose). The reasons for the differences in efficacy between the two populations is unclear, but may be related to the age distribution of disease or the high levels of nasopharyngeal carriage amongst Inuit children. The PRP-D vaccine is widely regarded as inferior to the other conjugate vaccines and is infrequently used today.

Direct comparison of vaccines is complicated by differing vaccine and phlebotomy schedules as well as inter-laboratory variation in anti-PRP assays. There are however a number of important generalisations which can be made (Granoff et al., 1992). Firstly, the antibody response to PRP-D vaccine is lower than the other conjugate vaccines. Secondly, only the PRP-OMP vaccine induces substantial increases in antibody concentrations after the first dose, and may therefore be useful in those settings in which there is a high incidence of disease during early infancy (e.g., Alaskan Natives). The

immunogenicity of the PRP-T and PRP-CRM vaccines is generally comparable. Table 1.2 summarises immunogenicity data and Table 1.3 efficacy data from a number of clinical trials. Serum anti-PRP levels slowly decline following vaccination with any of the conjugate vaccines and are boosted by vaccination with any of the available vaccines or with native PRP (Decker et al., 1993).

Table 1.2 Immunogenicity of three *Haemophilus influenzae* type b vaccines among infants. Adapted from (1993).

Geometric mean titre, μg/mL (% >1 μg/mL) *					
Vaccine	**Study site**	**Before vaccination**	**After dose 1**	**After dose 2**	**After dose 3**
Study 1	**Tennessee**				
PRP-D		0.07	--	0.08	0.28 (29)
PRP-OMP @		0.11	0.83	0.84 (50)	1.14 (55)
HbOC		0.07	0.09	0.13	3.08 (75)
PRP-T		0.10	0.05	0.30	3.64 (83)
Study 2	**Alaska**				
PRP-D		0.06 (4)	0.04 (2)	0.06 (11)	0.55 (45)
PRP-OMP @		0.16 (14)	1.37 (57)	2.71 (79)	--
HbOC		0.15 (5)	0.07 (0)	0.59 (43)	13.70 (94)
PRP-T		0.18 (13)	0.06 (0)	0.32 (20)	2.46 (78)
Study 3	**Minnesota/ Missouri/ Texas**				
PRP-OMP @		0.18	2.69 (80)	4.00 (85) 5.21 (88)	
HbOC		0.17	0.11	0.45 (23)	6.31 (90)
PRP-T		0.25	0.19	1.25 (56)	6.37 (97)

* Measured by radioimmunoassay.

+ In all studies, vaccine was administered at 2, 4, and 6 months of age.

& Antibody level after one dose includes data from only one of four data collection sites in Tennessee.

@ Current recommendations require only two doses of PRP-OMP in the primary series. For studies 1 and 3, three doses were administered.

-- Data not available.

Table 1.3. Efficacy of *Haemophilus influenzae* type b conjugate vaccines among infants. Adapted from (1993).

Vaccine	Population	Design	Age (months)	% efficacy (95% CI *)
PRP-D	Finland	Open, randomised	3, 4, 6	89 (70- 96)
	Alaskan Native	Placebo-controlled, randomised	2, 4, 6	35 (-57- 73)
PRP-OMP	Navajo	Placebo-controlled, Randomised	2, 4	93 (53- 98)* 100 (67-100)&
PRP-CRM	California	Open, partially randomised	2, 4, 6	100 (67-100)
	Finland	Open	4, 6	97 @
PRP-T	California	Controlled, randomised	2, 4, 6	**
	North Carolina	Placebo-controlled	2, 4, 6	**
	United Kingdom	Open, controlled	2, 3, 4	++
	Finland	Historical controls	4, 6	++

* Confidence interval.

+ Includes cases that occurred before 18 months of age.

& Includes cases that occurred before 15 months of age.

@ Confidence intervals not reported.

** Studies evaluating efficacy of PRP-T vaccine in the United States were terminated before completion.

++ Point estimate and confidence intervals not reported.

1.4.3 Effect on nasopharyngeal carriage

In addition to their direct effect in reducing the risk of invasive Hib disease, conjugate vaccines are also effective in reducing nasopharyngeal carriage of Hib. This may contribute significantly to the overall effectiveness of these vaccines. The reduction in carriage persists until at least one year of age leading to a reduction in circulating pathogen and 'herd immunity' within a community (Barbour et al., 1995).

1.4.4 Combination vaccines

The combination of Hib conjugate vaccines with other vaccine components has become a popular strategy in order to reduce the number of injections required at each vaccine visit. Existing combinations included diphtheria toxoid, tetanus toxoid and whole cell pertussis vaccine (DTP) in combination with Hib conjugate. Further developments have included the use of acellular pertussis vaccine in the DTP component as well as combinations with hepatitis B and, recently, inactivated polio vaccines (Decker, 2001). A discussion of the various combination vaccines is beyond the scope of this review, apart from the observation that the combination of Hib vaccine with acellular pertussis (DtaP) elicits a lower anti-PRP levels when compared with Hib vaccine given alone (Eskola et al., 1999).

1.4.5 Side effects of vaccination

Vaccination is generally well tolerated, with minor side effects including fever, local erythema, swelling and tenderness. Less frequent complaints include sleepiness, irritability, prolonged crying, loss of appetite, diarrhoea, vomiting and skin rash. Fever (≥

38^0C) has been recorded in 7-30% of vaccines and induration in 1-50% of infants (Carlsson et al., 1994).

1.4.6 Effectiveness of vaccination of HIV-infected infants

Immune responses in HIV-infected children are significantly lower than in uninfected infants. Lower antibody levels (Kale et al., 1995) are reported. A study conducted in Soweto estimated the effectiveness of HbOC amongst HIV infected infants to be 50.9% compared with 96.5% in uninfected children (Madhi et al., 2002). In addition, three fully vaccinated HIV-infected children with antibody levels $\geq$ 0.15ug/ml developed invasive disease, suggesting possible functional impairment of antibody in this group of children.

1.4.7 Impact of conjugate vaccines

The introduction of routine vaccination with Hib conjugate vaccine has had a profound and predictable impact on the incidence of invasive Hib disease in the developed world with virtual elimination of disease in those countries offering vaccination. In the United Kingdom only 84 cases of invasive Hib disease occurred between 1992 and 1998 compared with an estimated 4803 cases should vaccination not have been introduced (Moxon et al., 1999). In addition, there was a 40% reduction in total (all causes) cases of bacterial meningitis. This pattern has been repeated wherever vaccination has been introduced. Indeed the possibility of elimination of invasive Hib infection in countries such as the United States is now being expounded as a real possibility (Santosham, 2000).

In contrast to the successes achieved in the developed world, little progress has been made in extending vaccine coverage to those parts of the world that would benefit most from vaccination. In Africa, South Africa, The Gambia and Botswana are alone in providing routine Hib vaccination. It has been estimated that less than 2% of invasive Hib disease worldwide is prevented by current vaccine coverage (Peltola, 2000). The reasons for failure to immunize in the developing world include underestimation of disease, vaccine supply and delivery issues and complex vaccine schedules. However, the primary reason for not including Hib vaccine is simply one of cost. In South Africa routine vaccination with PRP-T combined with DTP (CombAct-HIB) was introduced in June 1999. The projected costs for Hib vaccine for the country in 2002 are R61 million. This represents over 70% of total vaccine costs (R85 million) for South Africa (Personal Communication, K. Blackbeard, Pharmaceutical Services, Provincial Administration of the Western Cape).

1.4.8 Strategies to improve affordability of Hib vaccine

Clearly there is an imperative to extend vaccination to the developing world. A significant reduction in the cost of vaccine would be a major advance in this regard. Since vaccine manufacturers have been unwilling to decrease the price of their product, the alternative is to develop cost effective strategies for vaccine use. This may be achieved by using less vaccine to vaccinate the same number of children, either by reducing the number of doses required or by reducing the amount of vaccine delivered per dose.

Lagos et al (Lagos et al., 1998) conducted a trial comparing the immunogenicity of two-dose and fractional-dose regimens of PRP-CRM_{197} and PRP-T. Fractional doses of one-half or one third of the full-dose were derived by withdrawal of the appropriate fraction from a full-dose vial into an insulin syringe. The authors found that anti-PRP levels elicited by fractional dose PRP-T and PRP-CRM_{197} after a primary vaccine series, as well as those following boosting with PRP, were equivalent to those induced by the full-dose vaccine. Two dose schedules were also acceptable, however the two dose schedule of PRP-CRM_{197} gave the least acceptable response. The findings of another group (Fernandez et al., 2000) using the same dilution strategy in the Dominican Republic were similarly encouraging. Limitations of these studies included the practical difficulty in accurately withdrawing a small volume from a vial of vaccine, separate immunization with DTP as well as the danger in extrapolating results from a single batch of vaccine. Hussey et al (Hussey et al., 1995) compared the immunogenicity of a 1μg dose of PRP-OMP (PedvaxHIB, normal dose 15μg PRP) with full-dose HbOC. The proportion of children achieving protective anti-PRP concentrations after the primary vaccine series was the same in the two groups, with higher geometric mean concentrations in the PRP-OMP group.

Romero-Steiner et al (Romero-Steiner et al., 2001) evaluated the functional activity of antibodies elicited by fractional-dose (full, one-half or one-third dose) vaccination of infants with PRP-T. They performed assays of serum bactericidal activity and IgG avidity on serum of infants with anti-PRP IgG titres of 2μg/ml or greater. The fractional dose regimens elicited similar functional antibody activities to the full-dose vaccine with the

exception that the avidity index was significantly higher in the one-third dose group compared with the full-dose group.

Other authors have evaluated the immunogenicity of two dose schedules in which the first dose of PRP-T was given approximately one month following the first DTP dose (Campagne et al., 1998, Guimaraes et al., 2002). This strategy aims to exploit the carrier priming elicited by the tetanus toxoid component of DTP which might enhance the response to the PRP component. This approach elicits strong anti-PRP responses and may represent a useful alternative in areas that are unable to afford the full 3-dose regimen. The potential cost saving in this case is only one-third of the total vaccine costs.

1.4.9 The need for further investigation of low-dose Hib vaccine

Whilst the Lagos study represented a novel approach to reducing vaccine costs, several important questions remain to be answered. Firstly, is this work reproducible in different populations and with different batches of vaccine? Secondly, what is the lowest dose of Hib vaccine that is still able to elicit a satisfactory response? Finally, and importantly, is there a more practical method for administering low-dose vaccine on a large scale as part of a vaccine programme?

The present study aims to address these questions. A novel dilution technique, for point-of-delivery dilution of a single dose of PRP-T was developed. This allowed the use of a single dose of PRP-T for up to ten infants. Immune responses elicited by vaccination with the diluted vaccine were compared with full-dose vaccination.

2.0 METHODS

We conducted a randomised, controlled trial comparing the immunogenicity of full-dose Hib vaccine (PRP-T; ActHIB, Pasteur Merieux Connaught, now Aventis Pasteur, Lyon, France) with that of the same vaccine diluted 1 in 10 by reconstitution in a 10-dose DTP vial.

This study was conducted as part of a larger study evaluating the immunogenicity of two investigational Hib vaccines from other manufacturers as well as the two arms described here. The other investigational vaccines were given at doses of 10, 5, 2.5, 1.25 or 0.6 μg. (Data not shown here – analysis and serological assays as yet incomplete). At the time of conducting the study, the Expanded Programme on Immunization (EPI) in South Africa did not offer Hib vaccination. Enrolment was terminated at the time that Hib vaccination was introduced into the routine vaccination schedule in South Africa.

The study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand (M980211) and by the Medicines Control Council of South Africa. Written informed consent was obtained from the parent or guardian of each child.

2.1 Patients and eligibility

The study was conducted at four routine immunization clinics south of Johannesburg. Three clinics were situated in and served informal settlements south of Alberton (Zonkizizwe, Khumalo and Mluleki clinics) whilst one clinic in Bedfordview served an

affluent area with formal housing. These clinics were chosen because of their relatively stable communities, excellent records of EPI follow-up as well as established links with the local health authorities.

Healthy children aged 6 weeks (+/- 14 days) of age who had no contraindications to receiving DTP vaccine were eligible for enrolment. Parents were informed of the study by research nursing sisters and asked to read and sign the informed consent. Prior to study enrolment, nurses took a medical history and conducted an appropriate physical examination to ensure that they met all inclusion criteria and none of the exclusion criteria.

2.1.1 Subject eligibility

2.1.1.1 Inclusion criteria

1. Healthy male and female children 6 weeks +/- 14 days of age (defined as 28-56 days of age) whose parent(s) or guardian(s) agreed to vaccine administration following a detailed explanation of the study.
2. Children who completed a physical examination indicating they were in proper health for immunization.
3. Children who were expected to be available for the entire study period.

2.1.1.2 Exclusion Criteria

1. Children with known or suspected impairment of immunological function (including known HIV-infection) or those receiving immunosuppressive therapy.
2. Children with evolving neurological disorder or history of seizure.

3. Children with major congenital malformations or serious chronic disorders (e.g. Down's syndrome, diabetes, sickle cell anaemia).
4. Children on current antibiotic therapy for acute illness.
5. Children with previous anaphylactic reaction.
6. Children with previous severe vaccine associated adverse reaction.
7. Children with a history of *Haemophilus influenzae* infection of a normally sterile body site (e.g. meningitis, bacteraemia, pneumonia, epiglottitis)
8. Children with history of idiopathic thrombocytopenic purpura
9. Children for whom DTP or oral polio vaccine were contraindicated.

Note: Development of any of these conditions after enrolment resulted in exclusion from further doses of vaccine.

The following two conditions were considered temporary or self-limiting contraindications to vaccination and a subject was included once these conditions have resolved and no other exclusion criteria were met:

1. A current febrile (rectal temperature $>38^0$C) illness or other acute illness.
2. Received any vaccine within the past 4 weeks or gamma globulin within the past 3 months.

2.1.2 Withdrawal from the study

The following events occurring after a previous dose of study vaccine warranted withdrawal from further doses of study vaccine (these are adverse effects associated with the use of pertussis vaccination):

1. Encephalopathy within 7 days of vaccination;
2. A convulsion, with or without fever, occurring within 3 days of vaccination;
3. Persistent, inconsolable screaming or crying for 3 or more hours within 48 hours of vaccination;
4. An unusual, high-pitched cry within 48 hours of vaccination;
5. Collapse or shock-like state (hypotonic-hyporesponsive episode) within 48 hours of vaccination;
6. Temperature of greater than or equal to 40.5^0C unexplained by another cause, within 48 hours of vaccination;
7. An immediate allergic reaction (anaphylaxis)

2.2 Study groups and randomisation

Infants in the two arms reported here were randomised to receive either the full-dose PRP-T conjugate vaccine containing 10μg of PRP polysaccharide (ActHIB, Pasteur Merieux Connaught (PMC), now Aventis Pasteur, Lyon, France) or a one-in-ten dilution in DTP (PMC, Lyon, France) of this vaccine. Children randomised to the other 10 arms of the study received one of two investigational Hib vaccines from other manufacturers given at doses of 10, 5, 2.5, 1.25 or 0.6 μg. (Data not shown). All children received concurrent oral polio vaccine and hepatitis B vaccine. The randomisation scheme was

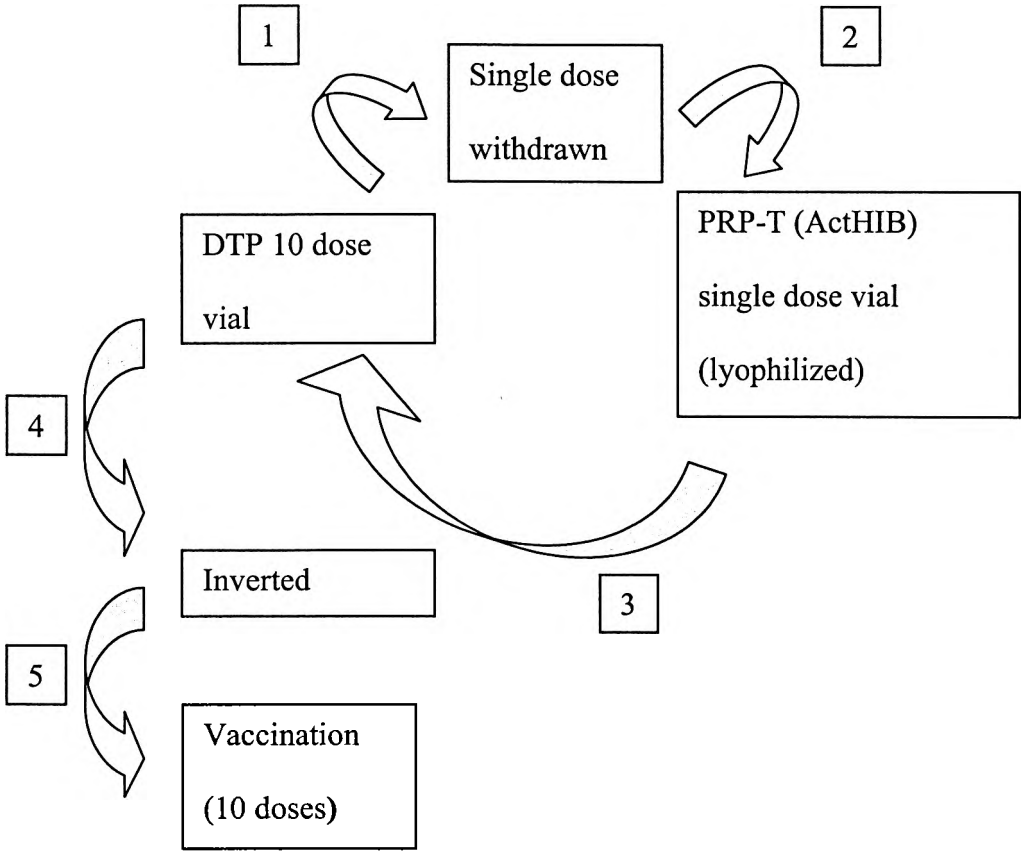
generated by a statistician and maintained in confidence from the investigators and from the clinical monitors.

2.3 Vaccine formulation and dilution technique

The full-dose vaccine contains 10μg PRP polysaccharide covalently conjugated to 24μg tetanus toxoid. The dilution was performed by reconstituting a single dose (0.5ml) of lyophilised Hib vaccine with DTP in a ten-dose DTP vial (Figure 1). This was done by removing 0.5 ml from the DTP vial, reconstituting the lyophilised Hib with this volume and then re-injecting the reconstituted DTP-Hib into the 10-dose DTP vial. The vial was inverted several times to mix the contents and a single dose of this vaccine was then used for each child (ten doses per vial).

Children receiving the full dose of PRP-T were vaccinated with DTP at a separate site. Reconstituted vaccine was kept refrigerated and vaccine remaining at the end of each day was discarded. Infants were vaccinated at 6, 10 and 14 weeks of age. A single injection of the full-dose Hib vaccine was given as a booster to all children at 9 months of age.

Figure 2.1 Method of deriving 1/10 dose by dilution in a 10-dose DTP vial



2.4 Collection of samples and serologic analysis

Heel-prick blood specimens were collected at each vaccine visit and at 18 weeks of age to assess the primary response to vaccination. In addition, specimens were collected at the 9-month visit and again one week later to assess the ability of the vaccines to elicit immunological memory.

The clotted blood was refrigerated and clarified by centrifugation within 24 hours after collection. Serum was stored in a -70^{0}C freezer. Anti-PRP IgG antibodies were measured by a standard quantitative enzyme immunoassay (Phipps et al., 1990) modified (Kayhty et al., 1987) using HboHA (NIBSC, Potters Bar, the UK) as antigen. The assays were performed on coded samples in a blinded fashion in the Pneumococcal Diseases Research Unit, Johannesburg, and whenever possible the sera before and after immunization were tested on the same plate. A subset of the same sera was also tested at the National Public Health Institute, Finland for quality control.

The primary outcome measures were the proportion of children achieving serum anti-PRP concentrations of 0.15μg/ml or greater and 1μg/ml or greater following the primary vaccine series.

2.5 Recording of adverse events

Following each vaccination, the subject was observed for approximately 30 minutes for any immediate significant reactions. Each subject also returned to the clinic at 24 hours after each vaccination when their body temperature was recorded as well as the size of

any induration or redness present at both the DTP and Hib injection sites. Hospital admission or any event requiring prescription medications, a physician visit within 7 days of vaccination, a hospitalisation at any time during the 8 month study period, or any event resulting in study termination at any period during the study was documented.

2.6 Statistical analysis

Data were entered into and analysed using EpiInfo Version 6. Chi-square and Mann-Whitney tests were used for comparison of antibody cut points and geometric mean titres, respectively. A p-value of ≤ 0.05 was considered statistically significant. Log transformed data were used for the calculation of the standard deviations.

Bias was minimised at the assay stage by the blinding of the technologist performing the ELISA assays. At the clinic level, due to the dilution technique, clinic sisters could not be blinded to vaccine formulation, but were educated as to the importance of the correct dilution technique.

3.0 RESULTS

3.1 Demographic details

A total of 168 infants were enrolled in these two arms. Of these, 83 received the full-dose vaccine whilst 85 received the 1/10 dose. Female children accounted for 55% of the full-dose arm and 47% of the 1/10 arm. The median age at enrolment in the full-dose arm was 47 days (range 26-121) and 46 days in the 1/10 arm (range 18-100). At 18 weeks of age 61 of those receiving the full-dose and 73 of those receiving the diluted dose were available for analysis. At nine months of age, 46 and 56 infants in each group respectively received the booster dose of vaccine.

3.2 Immunogenicity analysis

Both vaccine regimens resulted in excellent serological responses (Table 3.1). In both vaccine groups 100% of infants achieved anti-PRP responses of > 0.15 μg/ml following the primary immunization series. In the full dose arm 95% of infants and in the 1/10 dose arm 94% of infants achieved responses of > 1.0μg/ml Antibody concentrations of > 0.15 μg/ml were maintained by the majority of infants in both groups at the 9-month visit (100% in full-dose arm and 98% in diluted-dose arm). There were no significant differences between the two groups in the percentage of children achieving antibody concentrations > 0.15 μg/ml or > 1.0 μg/ml at any time. The loss to follow-up was similar in both groups. The geometric mean antibody concentrations after the primary

series were non-significantly lower in the 1/10 group, but were well above the 0.15 μg/ml level.

Both groups showed evidence of immunological memory with significant increases in antibody concentrations one week following the 9-month booster dose. In addition, amongst the children who completed the study, the mean post-boost antibody concentrations were significantly higher (p=0.009) in the group of 49 children who received primary immunization with the diluted vaccine (151.36μg/ml) than in the group of 41 children who received the full-dose vaccine (68.55μg/ml).

3.3 Adverse events

The incidence of local and systemic adverse reactions was not different between the two groups (Table 3.2). Two subjects receiving the diluted vaccine and one receiving the full-dose developed mild local induration following vaccination. Four children in the full-dose group were admitted to hospital during the course of the study with vaccine-unrelated illnesses (dysentery, bronchopneumonia, malnutrition, kwashiorkor) whilst one infant in the diluted-dose group was admitted with bronchopneumonia. There were no withdrawals from the study for vaccine-related adverse events.

Table 3.1. Serum PRP antibody concentrations following primary immunization series (18 weeks) and before and after booster dose of vaccine (9 months)

Time of measurement of anti - PRP concentrations	**Full-dose PRP-T**	**1:10 Dilution PRP-T**
18 weeks of age		
Number of infants	61	73
% ≥ 0.15μg/ml (95% CI)	100 (94 – 100)	100 (95 – 100)
% ≥ 1.0μg/ml (95% CI)	95.1 (86.9 – 98.4)	94.4 (86.7 – 97.8)
Geometric mean anti-PRP concentrations in μg/ml (95% CI)	19.40 (12.90-29.17)	14.13 (10.02-19.91)
9 months of age (pre-boost)		
Number of infants	46	56
% ≥ 0.15μg/ml	97.8 (89.1 – 99.6)	100 (93.6 – 100)
% ≥ 1.0μg/ml	87.0 (89.1 – 99.6)	78.6 (72.8 – 92.8)
Geometric mean anti-PRP concentrations in μg/ml	5.30 (3.30-8.52)	3.55 (2.50-5.04)
One week following 9 month booster		
Number of infants	41	49
% ≥ 0.15μg/ml	100 (91.6 – 100)	100 (92.7 – 100)
% ≥ 1.0μg/ml	100 (87.7 – 100)	100 (92.7 – 100)
Geometric mean anti-PRP concentrations in μg/ml	68.53 (44.88-104.17)	151.28 (94.92-241.04)

Table 3.2 Summary of adverse events associated with Hib vaccination (all clinic visits).

	Number of children experiencing adverse reaction	
	Full-dose group	**1/10 dilution group**
Immediate reaction	1*	0
Induration (24 hours)	1	2
Redness (24 hours)	0	0
Tenderness (24 hours)	0	0
Hospital admissions	4[&]	1[@]

*Infant restless, cried - treated with paracetamol

[&]dysentery, bronchopneumonia, malnutrition, kwashiorkor

[@] bronchopneumonia

4.0 DISCUSSION

4.1 Immunogenicity of the full-dose and 1/10 vaccines

4.1.1 Antibody concentrations after the primary vaccine series

The antibody responses achieved by both vaccine arms in this study after the primary immunization series were excellent and comparable to the best responses achieved in other trials of the immunogenicity of the ActHIB vaccine (Mulholland et al., 1997). More than 94% of infants in both groups achieved antibody levels correlating with long-term protection against invasive Hib infection. Importantly there was no difference between the two groups with respect to the percentage of infants achieving protective levels. Whilst the geometric mean concentration (GMC) of antibody after the primary series was non-significantly lower in the 1/10 arm, the GMC in this arm (14.13 μg/ml) was far in excess of the 1 μg/ml thought to correlate with long-term protection.

4.1.2 Memory responses to booster vaccine

As discussed above, the ability of conjugate vaccines to elicit a booster response to subsequent antigenic exposure sets them apart from pure polysaccharide vaccines. Some authorities have argued that this ability to prime is as appropriate a marker of protection as the concentration of antibody concentration following the primary vaccine series (Granoff et al., 1993). We measured antibody levels one week following a 9-month booster dose of vaccine as a marker of immunological memory. Both the full-dose and 1/10 vaccine elicited a significant booster response in 100% of subjects. All the infants in

both groups achieved protective antibody concentrations of > 1 μg/ml one week following a booster dose of vaccine.

Of note was the fact that the booster response in the 1/10 arm was significantly stronger than in the full-dose arm (GMCs of 151.28 μg/ml and 68.53 μg/ml respectively).

4.1.3 Possible explanations for the stronger booster response in the 1/10 group

There is accumulating evidence that increasing antigen load of the tetanus toxoid protein carrier appears to affect the humoral immune response to both the hapten and protein carrier. Dagan et al (Dagan et al., 1998) have shown that infants receiving pneumococcal vaccine and Hib vaccine both conjugated to a tetanus toxoid carrier showed significantly lower anti-PRP responses than those receiving a pneumococcal vaccine conjugated to diphtheria toxoid. Antibody concentrations to the tetanus component were also affected adversely with increasing tetanus toxoid content of the co-administered vaccines. Similarly, low doses of pneumococcal polysaccharide vaccine conjugated to tetanus toxoid induced better booster responses than did higher doses of the same vaccine (Ahman et al., 1999).

The reasons for the inhibitory effect of high concentrations of tetanus toxoid are incompletely understood, but several explanations have been proposed. It appears that development of an immune response against the carrier can interfere with the response to the polysaccharide component (Barington et al., 1993). This may be the result of dominance of B cell clones specific to the carrier component. Carrier-specific clones are

present at a much higher frequency than polysaccharide-specific clones, and so the extent of the decrease in the response to the polysaccharide will be proportional to the intensity of the response to the carrier (Dagan et al., 1998). A second possible explanation is that the presence of high concentrations of antibodies to the protein carrier could interfere with antigen capture or presentation of the saccharide component(Peeters et al., 1991, Ahman et al., 1999). Maternal antibodies to tetanus toxoid have previously been shown to interfere with the induction of anti-PRP titres in vaccinated infants(Barington et al., 1993). Finally, the high dose of tetanus toxoid may induce T cell tolerance during the primary series(Ahman et al., 1999).

Whatever, the explanation for the improved booster response of low-dose vaccine, the finding carries important implications. Firstly, the co-administration of multiple polysaccharide vaccines bearing the same protein carrier may result in reduced antibody responses. Secondly, the currently licensed dose of PRP-T conjugate vaccine may be too high to elicit an optimal response. Thirdly, the 1/10 vaccine, as used in this study, may provide superior immunity to the full-dose vaccine (using the booster response as correlate of protection).

These findings extend previous work by showing that a one-in-ten fraction of Hib vaccine conjugated to tetanus toxoid (PRP –T, Aventis Pasteur, Lyon, France) is as immunogenic as a full dose regimen. Since this finding has to date been restricted to vaccines conjugated to tetanus toxoid, the serological response to the dilution of this

vaccine may therefore not be applicable to similar dilutions of other Hib vaccines, particularly those in which tetanus toxoid is not used as the carrier protein.

4.1.4 Effect of Hib vaccine on the immunogenicity of other vaccine components

As described earlier in this report, the concomitant administration of Hib vaccine together with acellular pertussis vaccine has been associated with a reduction in antibody response to the PRP component (Eskola et al., 1999). The same is not true of the combination of single-dose DTP containing whole cell pertussis with PRP-T. Miller et al compared single injection PRP-T plus DTP vaccine with separate injections of the two vaccines and found similar antibody responses to all vaccine components in the two groups (Miller et al., 1995). There is no evidence to suggest that the combination used in this study (whole cell pertussis was used) should result in reduced antibody responses to the DTP component.

Further, the vaccine manufacturer suggests that the lyophilized PRP-T vaccine can be reconstituted with single-dose DTP. It is therefore unlikely that the addition of lyophilized PRP-T to a 10-dose DTP vial, as described in this study, would result in a reduction in the immunogenicity of the DTP component. This was not, however specifically tested in this study and should perhaps be formally assessed prior to the widespread use of the diluted vaccine.

4.2 Safety of the low-dose vaccine

Both vaccines were well tolerated. There were no vaccine related severe adverse events in either vaccine group. In addition, minor events (swelling and redness) were uncommon and no different between the two groups. Since the low-dose vaccine contains the same formulation as the full-dose (albeit in a lower dose) there is no reason to suspect that the low-dose should be more likely to cause an increased frequency of adverse events..

Bacterial and viral contamination of multi-dose vials has previously been described, including an outbreak of Group A streptococcal and staphylococcal skin sepsis associated with multi-dose DTP vaccine (Simon et al., 1993). In the present study, emphasis was placed on strict aseptic technique during the dilution process and with each withdrawal from the vial. At the time of the study, the use of multi-dose DTP vaccine was routine in clinics. There were no cases of local injection-site sepsis in this study. Clearly this issue would need to be closely monitored if widespread vaccination using this technique was to be introduced.

A further practical point that would need to be addressed, should such a method be used on a wider scale outside a study setting, would be the need for clear labeling to distinguish the PRP-T-containing DTP vials from those containing DTP only.

5.0 CONCLUSION

There is an ethical as well as medical imperative to make Hib vaccine available in those areas of the world that, despite suffering the highest burden of Hib disease, are currently unable to afford vaccine. The method of dilution employed here, using a single dose of Hib vaccine reconstituted in a 10-dose DTP vial, is simple and convenient, and it provides a practical method for utilising fractionated doses on a large scale. The potential ten-fold reduction in vaccine costs brings the cost of Hib conjugate vaccine in line with that of other EPI vaccines and within the reach of many developing countries.

Further work is needed to demonstrate the stability of the reconstituted diluted vaccine on overnight storage, to reduce wastage, although the availability of up to 10 doses for use in a single day is already a significant advance over current practice. Lot consistency analyses and further studies in other countries of the immunogenicity of the dilution in DTP of this Hib conjugate vaccine are suggested. Finally, although the immunological correlates of protection of Hib vaccine are well established, the clinical effectiveness of the low-dose vaccine in preventing invasive Hib disease needs to be demonstrated. Ideally this would take the form of a large randomised, controlled trial but given the cost and logistic implications and the doubtful support of vaccine manufacturers for such a trial, this is unlikely to be feasible. Alternatively, 1/10 dose vaccine could be introduced into an area where the current full-dose vaccine is unaffordable. If good baseline data on disease prevalence as well as a sound surveillance system were in place in such an area, then the effectiveness of the introduction of 1/10 dose vaccine could be demonstrated.

The recent focus on vaccine delivery in the developing world and the earmarking of funds for this purpose has highlighted the need to expand vaccination coverage to areas of the world where vaccine-preventable diseases are still common. The strategy employed here provides one potential avenue for the cost-effective deployment of such funds.

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