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Evaluation of Vaccines for the Prevention of Pneumonia in Children in Developing Countries

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INTRODUCTION

Despite the increasing availability and use of antibiotics for the treatment of acute respiratory infections (ARI) in children, global mortality from ARI, mainly due to pneumonia, remains high, since many children in developing countries do not have access to appropriate health care. The current strategy advocated by the World Health Organization relies on improved case management utilizing simple clinical signs for the diagnosis of pneumonia and inexpensive oral antibiotics for treatment (1). This has been successful in some areas, but such programs are difficult to sustain, and ultimately control of the problem will rest on prevention. Improvements in nutrition, housing, and indoor air quality can all be expected to reduce the incidence of pneumonia, but these improvements will depend upon economic development, which has been progressing slowly in many developing countries and has a disturbing tendency to exclude the poorest communities.

Vaccination offers the possibility of preventing pneumonia in a shorter time frame and in a manner that reaches rich and poor children equally. The widespread use of measles and pertussis vaccines has substantially reduced the incidence of and mortality from respiratory infections associated with measles and pertussis in most parts of the world, but the development of vaccines against other causes of childhood pneumonia has proven to be more difficult. Pneumonia is a syndrome caused by many pathogens, so vaccines must be directed sequentially against the leading pathogens. Over the last two decades, many studies of the etiology of childhood pneumonia in developing countries have been conducted (2–9). The results have been remarkably consistent. The leading bacterial causes are *Streptococcus pneumoniae* (pneumococcus) and *Haemophilus influenzae*, both type b and nonencapsulated strains, while the most important viral cause is respiratory syncytial virus (RSV). Variability in the apparent importance of RSV reflects the time-limited nature of many of these studies, which may or may not have included an RSV epidemic. In all studies, there remains a significant group of cases of unknown etiology, the size of which varies according to the population under study, the methods used, and the prevalence of antibiotic use in the community. Until recently, no vaccines were available for protecting young children against the three leading pathogens, although a study of the use of pneumococcal polysaccharide vaccine in Papua New Guinea children (10) showed evidence of protection against death from pneumonia in children over 6 months of age (see below). The development of protein polysaccharide conjugate vaccines against *H. influenzae* type b (Hib) has provided a vaccine capable of preventing bacterial pneumonia in young infants, and vaccines against pneumococcus and RSV are under development.

This paper addresses the evaluation of vaccines designed to protect children from pneumonia, with particular emphasis on pneumococcal vaccines, and draws from the experience of two large Hib vaccine trials conducted recently in The Gambia (11) and Chile (12). The measurement of vaccine efficacy is discussed, as well as the potential of vaccine trials to provide information about the etiology of pneumonia and the need for antibiotic treatment for various categories of ARI. The special requirements of regulatory agencies in the conduct of clinical trials for the purposes of licensure in industrialized countries are beyond the scope of this paper and are not discussed.

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Abbreviations: ARI, acute respiratory infections; DTP, diphtheria-tetanus-pertussis; Hib, *Haemophilus influenzae* type b; RSV, respiratory syncytial virus; PRP-T, polysaccharide-tetanus protein conjugate.

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HIB VACCINES

Since 1977, when the Hib polysaccharide vaccine was shown to be effective in preventing invasive Hib disease in Finnish children over 18 months of age but ineffective in younger children (13), research efforts have been directed towards developing a vaccine that would be effective in young infants. This resulted in the development of protein-polysaccharide conjugate vaccines that were licensed for use in young infants in the United States in 1990. Studies of the efficacy of these vaccines were carried out initially in industrialized countries, where most cases of invasive Hib disease are meningitis and Hib pneumonia is an uncommon finding (14–16). This is in contrast to the situation in developing countries, where pneumonia is the most important manifestation of Hib disease (8, 9). In contrast to meningitis, the pathogenesis of Hib pneumonia does not necessarily involve blood-borne spread; therefore, prior to these studies, it was conceivable that Hib vaccines might have been ineffective in preventing pneumonia, seriously impairing their usefulness in developing countries. For this reason, and because of the poor performance of one of the Hib vaccines under conditions resembling those of developing countries (17), Hib vaccine efficacy studies were conducted in two very different developing countries, The Gambia and Chile.

The Gambian Hib vaccine trial

The Gambian Hib vaccine trial was designed to assess the efficacy of a Hib polysaccharide-tetanus protein conjugate (PRP-T) vaccine for the prevention of Hib pneumonia and other invasive Hib disease in Gambian infants (11). The primary endpoints were bacteriologically proven Hib pneumonia, meningitis, or other invasive Hib disease. Among the 42,848 infants individually randomized to receive either PRP-T mixed with diphtheria-tetanus-pertussis (DTP) vaccine or DTP alone at 2, 3, and 4 months of age, 1,821 episodes of pneumonia were investigated, producing only 17 cases of Hib pneumonia proven by positive blood or lung fluid culture. Not all episodes of pneumonia among children in the vaccine cohort are likely to have been investigated, because of problems with access to and utilization of health care services, which are typical in developing countries. Ten cases of proven Hib pneumonia were detected in children who had received at least two doses of DTP alone, while none were detected in children who had received at least two doses of DTP plus PRP-T. This yielded a point estimate of vaccine efficacy of 100 percent (95 percent confidence interval: 55, 100).

Analysis of the 449 children with definite radiologic evidence of pneumonia showed that the PRP-T recipients had received 21 percent protection from pneumonia defined in this way, and that among children vaccinated with PRP-T, about 50 cases of pneumonia had been prevented. Some pneumonia cases would have been missed, 30 percent of the cases investigated were not x-rayed, and protection against pneumonia was probably less than 100 percent, so it could be estimated that at least 100 Hib pneumonia cases actually occurred in control children. Since 21 cases of Hib meningitis occurred in the same group, and cases of meningitis are less likely to have been missed than cases of pneumonia, it follows that the ratio of Hib pneumonia to Hib meningitis in that community is approximately 5:1. In the absence of a vaccine, approximately 250 of every 100,000 Gambian infants contract Hib meningitis in their first year of life (18). Therefore, the incidence of Hib pneumonia must be at least 1,250 per 100,000 infants per year, and the total incidence of invasive Hib disease must be at least 15 per 1,000 infants per year (figure 1). Since most Hib disease in The Gambia occurs in the first year of life, it can be estimated that, in the absence of a vaccine, 1.5–2 percent of Gambian children born will contract invasive Hib disease. This is about 10 times the risk borne by children in industrialized countries before the introduction of the vaccine (19).

Earlier studies of the etiology of pneumonia which had been carried out in the same community had estimated that Hib caused 5–10 percent of cases (8), yet the trial suggested that 21 percent of cases of pneumonia with definite radiologic consolidation in that community were caused by Hib. The estimated number of pneumonia cases that were presumed to be due to Hib in the control group (100 cases) seems small for a pop-

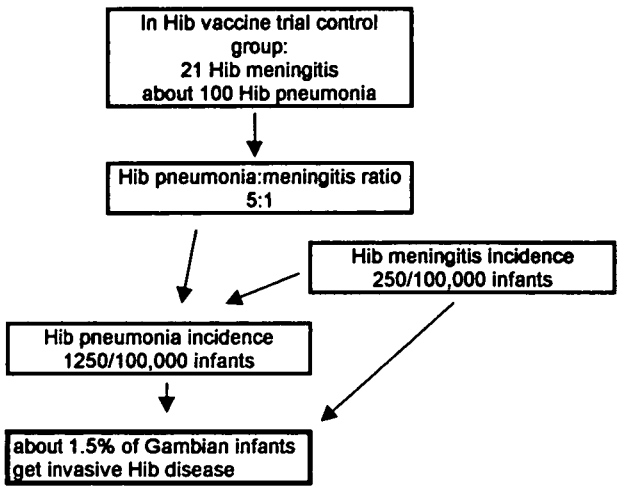

FIGURE 1. Use of *Haemophilus influenzae* type b (Hib) trial data to estimate the incidence of Hib disease in the community, The Gambia (11, 18).

ulation of 21,358 children. From the above incidence estimates, one would have expected approximately 250 cases of Hib pneumonia in the study population (and 50 cases of Hib meningitis rather than the 21 observed). This discrepancy could be explained by a combination of herd immunity (vaccine-induced reduction in disease incidence in the unvaccinated control population) and incomplete case detection.

These incidence estimates are consistent with extrapolations from mortality studies (figure 2). In areas of The Gambia where access to health services is poor, the infant mortality rate is approximately 100 per 1,000 live births, and about half of those 100 deaths occur in infants over 2 months of age (20). Previous studies from The Gambia have suggested that half of the deaths in children aged 2–12 months are due to ARI (20). If 20 percent of those deaths are due to Hib, then Hib pneumonia should be responsible for about five deaths for every 1,000 live births. In an area with poor health services, this is consistent with the incidence estimate of 12.5 per 1,000 infants derived above, suggesting that in poorly served areas, Hib pneumonia that occurs in the community carries a high level of mortality.

These estimates also provide insights into the etiology of ARI at the community level. A community-based ARI study from The Gambia, in which 500 children under age 5 years living in a rural area were studied for 1 year, found 450 episodes of pneumonia as defined by the World Health Organization (figure 3) and 165 episodes of radiologically proven pneumonia per 1,000 children per year (21). The Hib vaccine trial suggests that 12.5 cases of Hib pneumonia occur for every 1,000 infants. If, as studies suggest, pneumococcal pneumonia occurs in about three times as many infants as Hib pneumonia, it would seem that together Hib and pneumococcal pneumonia affect less than 5 percent of Gambian children in their first year of life. This is a small proportion of those children classified by the World Health Organization as having pneumonia requiring antibiotics, and it raises important questions about the etiology of most ARI episodes treated, and therefore about the specificity of the case management strategy promoted by the World Health Organization. Pneumonia incidence in the Gambian community study appeared to be higher than that in the vaccine trial, as would be expected in a more rural area. The Hib:pneumococcus ratio used for these estimates may be incorrect if pneumococcus proves to be a more important contributor to less severe forms of ARI. This will be evaluated in the current phase 3 pneumococcal conjugate vaccine trials (see below).

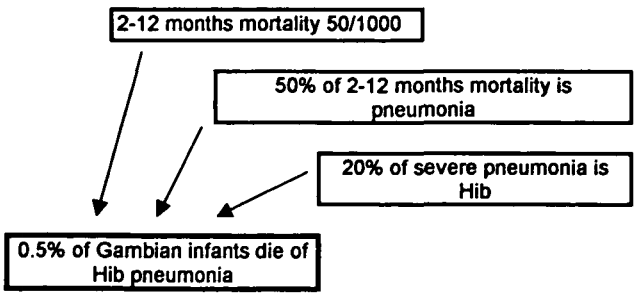

FIGURE 2. Use of *Haemophilus influenzae* type b (Hib) trial data to estimate mortality due to Hib pneumonia in the community, The Gambia (11, 20).

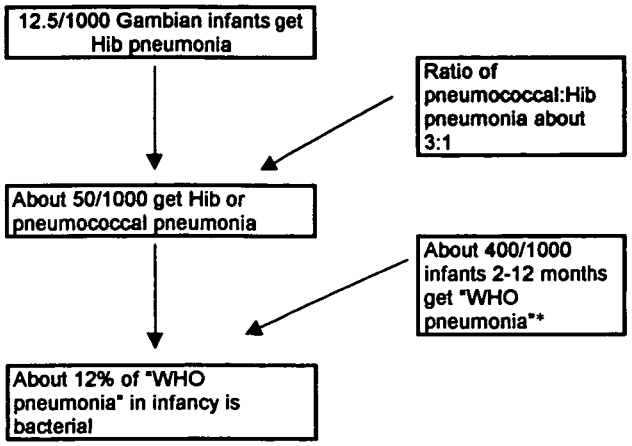

FIGURE 3. Use of *Haemophilus influenzae* type b (Hib) trial data to estimate the proportion of pneumonia at a community level that is bacterial in origin, The Gambia (11, 18, 21). (*"WHO pneumonia" refers to children under 5 years of age who present with cough or difficult breathing and are found to have either fast breathing (>50 breaths/minute for infants aged 2–12 months and >40 breaths/minute for older children) or lower chest wall indrawing. The World Health Organization (WHO) recommends that these children be treated as having pneumonia.)

Thus, while examination of children with severe radiologic pneumonia who presented to the hospitals involved in the Gambian study revealed that a surprisingly high proportion of this type of pneumonia was due to Hib, the study also provided valuable insights into the burden of Hib pneumonia at a community level. Not all episodes of pneumonia in study children were investigated, so we must build into the Hib pneumonia:meningitis ratio that is central to these estimates a factor estimating the number of episodes missed. We have assumed an Hib pneumonia:meningitis ratio of 5:1. If the true figure were higher, for example 10:1, Hib burden estimates would almost double. That could be true, but it would imply that the case fatality rate of Hib pneumonia in poorly served areas is lower; this may be the case, since antibiotics are available to a limited extent in many of these areas.

If some of these issues had been addressed beforehand, the Gambian Hib trial could have been improved

by the establishment of representative cohorts among whom all ARI episodes were documented. This would have enabled the investigators to determine the true incidence of ARI according to various categories in the study population, and the proportion of ARI in each category due to Hib. This would, in turn, have allowed the development of much more accurate estimates of the incidence of Hib pneumonia on a population basis. Most importantly, the Hib pneumonia:meningitis ratio would have been a measure rather than an estimate.

The Chilean Hib vaccine trial

For determination of the efficacy and effectiveness of PRP-T vaccination under programmatic conditions, the 71 urban health centers in Santiago, Chile, were randomized to deliver DTP vaccine alone or PRP-T vaccine with DTP to infants presenting for DTP vaccination at 2, 4, and 6 months of age for a 1-year period (12). The primary objective of the study was to evaluate protection against culture-confirmed invasive Hib diseases such as meningitis, sepsis, and bacteremic pneumonia. The study, which included more than 95,000 infants, showed that three doses of PRP-T vaccine provided 92 percent protection against bacteriologically confirmed invasive Hib disease. This included 80 percent efficacy against bacteremic pneumonia (there were 10 cases among children who received DTP versus two among those who received PRP-T).

After the Gambian data described above were reported (11), the effectiveness of PRP-T vaccine in preventing hospitalized pneumonia in Santiago was assessed by a retrospective study. The primary objective of this subsequent analysis was to determine the amount of radiologically confirmed pneumonia requiring hospitalization that could be prevented by PRP-T vaccination. A case definition was developed to approximate closely that used in the Gambian study. Thus, the study was designed to identify hospitalized infants aged 4–23 months with radiographic evidence of pneumonia or other indicators of likely bacterial pneumonia. For this analysis, surveillance was limited to three of the six health regions in Santiago. These three regions included >21,000 infants, who provided >35,000 child-years of observation (21a).

In this analysis, two or three doses of PRP-T vaccine did not reduce significantly the overall incidence of pneumonia requiring hospitalization in children aged 4–23 months (16.8 cases/1,000/year vs. 17.8 cases/1,000/year in PRP-T and control groups, respectively). However, there was a 22 percent reduction in the incidence of pneumonia with radiographic consolidation or pleural effusion (3.9 cases/1,000/year vs. 5.0 cases/1,000/year in PRP-T and control groups, respectively ($p = 0.12$)), an outcome measure similar to that used in The Gambia but one that misses some cases of bacterial pneumonia. Other indicators of likely bacterial pneumonia were also evaluated. An analysis of likely bacterial pneumonia (defined as pneumonia associated with radiologic consolidation, pleural effusion, bronchial breath sounds, or an erythrocyte sedimentation rate >40 mm/hour) showed a significant reduction in those who had received the Hib vaccine (7.2 cases/1,000/year vs. 9.7 cases/1,000/year in PRP-T and control groups, respectively; vaccine efficacy = 26 percent (95 percent confidence interval: 8, 54)).

Despite the lower rates of radiographically confirmed pneumonia, the proportion of these events attributable to Hib in Chile was remarkably consistent with the results obtained in The Gambia (12). These findings suggest that, in developing countries where Hib is prevalent, this bacterium is probably responsible for about 20 percent of severe pneumonia episodes in infants aged 2–11 months. Pneumonia deaths throughout the world occur in areas where treatment facilities are poor or nonexistent. Thus, it would not be unreasonable to suggest that, in those areas, 20 percent of pneumonia deaths in this age group are probably due to Hib.

PNEUMOCOCCAL VACCINES

Three approaches to pneumococcal vaccine development have been explored: polysaccharide vaccines, protein-polysaccharide conjugate vaccines, and vaccines based on common protein antigens.

Polysaccharide vaccines were first tried in 1911, but it was not until 1977 that a polysaccharide vaccine covering 14 pneumococcal serotypes (14-valent) was licensed for use in the United States (22). In 1983, a 23-valent vaccine was licensed, and today 23-valent pneumococcal polysaccharide vaccines (covering the 23 most important of the 90 pneumococcal serotypes) are available throughout the world and are used to a variable extent in high risk groups and the elderly (23). Since it is a composite of 23 vaccines, the efficacy of the 23-valent vaccine has been difficult to establish, but the novel method of indirect cohort analysis developed by Broome et al. showed significant protection against invasive pneumococcal disease in adults (24, 25). However, efficacy against nonbacteremic pneumonia in adults has been difficult to demonstrate; different studies show conflicting results. In one study of South African gold miners (26), 6- or 12-valent pneumococcal polysaccharide vaccines were effective in preventing 80 percent of pneumococcal pneumonia episodes, but the total number of pneumonia episodes prevented was actually less than the number of pneumococcal pneumonia episodes prevented. This sug-

gests that little of the pneumococcus-negative pneumonia was pneumococcal and/or that some of the observed reduction may have been artifactual, possibly because of the conversion of bacteremic pneumonia to nonbacteremic pneumonia.

A recent small study carried out in Sweden suggested that 23-valent pneumococcal vaccine, given to adults at the time of discharge following hospitalization for pneumonia, had no effect on the overall rate of pneumonia recurrence (27). This study was interpreted as showing limited vaccine efficacy, but it is probably more accurate to say that the study showed that very little pneumonia, by the investigators' definition in their population, was pneumococcal in origin. Some of the diagnostic criteria used lack specificity. When only blood culture-positive cases were considered, there was some protection observed, but the numbers were small.

In Papua New Guinea, in a study that vaccinated children between 6 months and 5 years of age with a pneumococcal polysaccharide vaccine, Riley et al. (10) found a 25 percent reduction in mortality among children under 2 years of age and a 19 percent mortality reduction in all children under age 5 years. In addition, there was a 28 percent reduction in moderate-to-severe lower respiratory tract infection (28). In the same study, antibody responses to most serotypes measured were present in children older than 6–9 months, while responses to the poorly immunogenic serotypes such as 6B and 19F were seen only after 12 months (29). These findings have not been widely accepted, partly because the results reported represented a compilation of three different trials carried out at different sites using different vaccines and different designs, and partly because the serotypes causing disease in Papua New Guinea children contained an unusually high proportion of serotypes usually associated with adult disease. Plans to conduct a similar study in The Gambia in 1989 were shelved when it became clear that pneumococcal conjugate vaccines would soon be available. Pneumococcal polysaccharide vaccines are not currently used in children in any part of the developing world, and no attempt has been made to reproduce Riley et al.'s findings.

At the time of this writing, there are three pneumococcal conjugate vaccines in advanced stages of development. The most advanced product is the conjugate vaccine produced by Wyeth Lederle Vaccines (West Henrietta, New York), which uses the mutant diphtheria toxin CRM197 as the carrier protein. This is bound covalently to the capsular polysaccharide from the serotypes chosen for inclusion in the vaccine. At present, phase 3 trials using a 7-valent vaccine (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) are under way in the United States and Finland, and recently a phase 3 trial of a 9-valent vaccine (incorporating serotypes 1 and 5, which are of particular importance in developing countries) was started in South Africa. This vaccine is immunogenic in young infants and reduces nasopharyngeal carriage of serotypes included in the vaccine (30, 31). An 11-valent pneumococcal conjugate vaccine, using both ditheria and tetanus toxoid proteins as carriers, is being developed by Pasteur Mérieux Connaught (Lyon, France). An earlier version of this vaccine has been shown to affect nasopharyngeal carriage (32). Another 11-valent pneumococcal conjugate vaccine is being developed by Smith Kline Beecham (Rixensaart, Belgium) using a protein derived from *H. influenzae* as a carrier.

The main problem with these capsular approaches is that each serotype must be considered separately, and if there is competition between serotypes, this may reduce the effectiveness of the vaccines. To overcome this problem, researchers have been working on an approach that identifies conserved protein epitopes. At least three groups of vaccine candidates are currently being investigated. The most advanced of these is pneumococcal surface protein A (PspA), which in animal models has shown protection following both parenteral and oral administration (33, 34). This class of vaccines is still in an early stage of development.

It is important that vaccine trials be designed to evaluate new vaccines with the dose regimen they are likely to be used in. In the case of trials designed to prevent disease in infancy, the schedule should reflect the disease burden. Pneumococcal vaccines are currently being evaluated according to infant DTP schedules, in the same manner that Hib conjugate vaccines were (ages 6, 10, and 14 weeks; 2, 3, and 4 months; and 2, 4, and 6 months). This is appropriate for the prevention of otitis media in North American infants, but it ignores the substantial burden of severe pneumococcal disease that occurs during the first 3 months of life. A recent multicenter study performed by the World Health Organization in four developing countries showed pneumococcus to be one of the three important bacterial pathogens in infants under 3 months of age and the most important cause of meningitis in that age group (35, 36). A recent meeting of the World Health Organization discussed the two approaches to this problem: neonatal immunization and maternal immunization (37, 38). These alternative strategies are beyond the scope of this paper, but studies in both areas are proceeding.

RSV VACCINES

Since the discovery of "chimpanzee coryzal agent," later renamed respiratory syncytial virus (RSV) in

1956 (39), RSV has been shown to be the most important viral cause of acute lower respiratory tract infection in young infants in both developed and developing countries (40–42). In most settings where it has been studied systematically, RSV has been associated with annual epidemics, appearing during the winter in temperate climates and (usually) during the wet season in the tropics. While RSV causes disease in all age groups, severe disease is mainly seen in infants aged 1–6 months (43). Curiously, infants under 1 month of age are rarely affected. Natural infection does not provide effective lasting immunity, and repeated infections are common, even during the same epidemic (44). Severe RSV infection in infancy is known to be associated with considerable respiratory morbidity in later childhood, but whether or not this is a causal relation is unknown.

The first vaccine against RSV was developed in the 1960s soon after identification of the virus. It was based on virus cultured in African green monkey kidney cells, formalin-inactivated, precipitated in alum, and concentrated by centrifugation. The vaccine underwent trials in North American infants, but the results were catastrophic (45–48). Vaccinated infants developed complement-fixing antibodies against RSV but only low levels of neutralizing antibody. After exposure to the wild virus, almost half of the vaccinated infants needed to be hospitalized because of severe infection; several required prolonged ventilation, and two died.

Since then, RSV vaccine development has proceeded along two parallel paths: the subunit vaccine approach and the live attenuated vaccine approach (49). A subunit vaccine based on the fusion (F) protein has been developed and has been shown to be effective in previously exposed children (50). However, it has not been administered to nonimmune infants, and many believe that its main use will be in the elderly (51). A live attenuated vaccine is currently being evaluated and has already been tried in seronegative infants (49). Like all live attenuated vaccines, this vaccine will need to balance protective efficacy against virulence. This may be a particularly difficult task in the case of RSV, since immunity following natural infection is short-lived at best (44).

Whichever RSV vaccine proves to be the best option for infants, its evaluation in infants will be more complex than that of other pneumonia vaccines, since it will need to address the prevention of both acute disease and long term effects. The association between RSV infection in infants in industrialized countries and subsequent wheezing is well established, although it is still unclear whether RSV damages the respiratory tract or simply selects for more severe illness those infants with a predisposition to wheezing (52). A recent study from Africa suggested that in developing countries with a low incidence of asthma, RSV causes recurrent lower respiratory tract infections rather than recurrent wheezing (M. Weber, World Health Organization, personal communication, 1999). The prevention of recurrent lower respiratory tract infections could multiply the beneficial effects of a vaccine, and researchers will need to evaluate this outcome carefully in phase 3 trials in developing countries.

DESIGN OF FIELD TRIALS FOR CHILDHOOD PNEUMONIA VACCINES

Several phase 3 trials of pneumococcal protein-polysaccharide conjugate vaccines are currently under way, and others are being planned. The key decisions that face investigators contemplating such studies are the choices of vaccine (both study vaccine and control vaccine, if used), trial site, randomization system, and endpoints to be investigated. Similar considerations will have to be taken into account when RSV vaccines enter large-scale field trials. The following discussion will focus on pneumococcal vaccine trials, since these are imminent, but many of the points considered are relevant to trials of RSV vaccines as well.

In vaccine trials in children, it is ethically difficult to justify giving a placebo injection to a child. There are two ways to avoid this. If the vaccine can be given as a combined vaccine, participants can receive an injection of either the combined vaccine or the original vaccine mixed with a placebo. For example, Gambian infants received either DTP vaccine mixed with PRP-T or DTP vaccine mixed with placebo. The alternative approach is to use a control vaccine. This needs to be a vaccine that does not affect the endpoints being measured, looks identical to the study vaccine, can be given according to the same regimen, and is beneficial for the child. In the Finnish pneumococcal vaccine trial, hepatitis B vaccine is being used as a control, while in most of the other studies a group C meningococcal conjugate vaccine is being used.

Trial site

Children recruited into a pneumococcal vaccine trial should be representative of the populations likely to benefit most from a successful pneumococcal vaccine. While the prevention of acute otitis media will be of value to families and physicians in industrialized countries, the true life-saving potential of pneumococcal conjugate vaccines will be realized in developing countries. Because the population at risk includes virtually all children in developing countries, the choice of site is likely to be determined mainly by the exis-

tence of an infrastructure capable of sustaining a large trial and by the feasibility of measuring the endpoints of importance. This usually involves some trade-offs, since the populations at highest risk are generally found in areas where the infrastructure is insufficient to support a large trial. Building up the infrastructure of a poorly served area is likely to reduce the pneumococcal disease burden to some extent. In the end, these issues will be resolved by practical compromise.

The situation is slightly different for RSV vaccine trials. Although RSV disease has now been identified in all parts of the world, vaccine trials should be conducted in those areas where the epidemiology, clinical impact, and long term sequelae have been well studied (M. Weber, World Health Organization, personal communication, 1999). Because of the problems with earlier vaccine trials, in which recipients developed severe disease upon exposure to the wild virus, the availability of adequate health services is a key requirement. Therefore, these studies will have to be performed in better served areas, at least until the safety of the vaccine has been demonstrated beyond doubt.

Randomization system

For vaccine trials in which some of the endpoints are subjective (e.g., severe pneumonia), the argument for a double-blind trial design is compelling. The choice then lies between individual randomization and randomization in clusters (table 1). Individual randomization is the classical means of evaluating a vaccine and gives the best measure of individual protection, but it does not allow good measurement of herd immunity likely to result from reduced nasopharyngeal carriage among infants in the community (pneumococcus) or reduced transmission between infants (RSV). Since the impact of pneumococcal conjugate vaccines on carriage has been demonstrated consistently in the small studies already undertaken, this effect may prove to be of considerable importance (30–32). If herd immunity occurs in an individually randomized vaccine trial, it will effectively reduce the power of the study, and it may lead to an underestimate of efficacy by reducing the incidence of disease in the control group. However, herd immunity may not be as marked as that which has been observed in the case of Hib vaccines, because in many developing countries a high proportion of adults and older children, who will not have been vaccinated, carry pneumococcus and may act as potential sources of infection. Within an individually randomized trial, it may be possible to study herd immunity by conducting a case-control study of pneumonia cases occurring among siblings of trial participants.

TABLE 1. Comparison of individual randomization and cluster randomization for the conduct of pneumococcal conjugate vaccine trials

	Individual randomization	Cluster randomization
Potential biases	• Herd immunity	• Variable access to health care • Potential for pneumococcal disease clustering • Potential for clustering of acute lower respiratory infections caused by other agents
Power	• Maximal (may be reduced by herd immunity)	• Slightly reduced (depending on the number of clusters)
Accuracy of efficacy measure	• Maximal	• Efficacy and herd immunity combined
Practical value of results	• Predicts individual protection only • Underestimates impact on antimicrobial resistance	• Predicts effect of vaccine use in community • Predicts true value of the vaccine for reducing levels of antimicrobial resistance

Cluster randomization, randomization by village or district, gives a combined measure of individual and herd immunity, but it does not allow the two effects to be distinguished. Since herd immunity is likely to vary from place to place, this limits the generalizability of the findings. Another problem with cluster randomization arises from the variable access to health care services that is found in most developing country settings. There is a risk that by chance one group or the other may have better access to services and therefore be found to apparently have more episodes of ARI. Furthermore, an outbreak of disease caused by a single serotype or a confounding viral agent (e.g., influenza) could be restricted to part of the study area, potentially affecting one group more than the other, thereby introducing bias. These risks of cluster randomization can be reduced by ensuring that an adequate number of clusters are used. For each study, the advantages and disadvantages of each system must be evaluated.

Endpoints under evaluation

The choice of primary endpoints is the most critical aspect of trial design, and endpoints must be carefully specified for each study. There is more at stake here

than the simple measurement of vaccine efficacy. Disease burden and treatment issues should also be addressed.

Microbiologic endpoints. Conventionally, vaccines are evaluated in terms of the protection they provide against disease proven to be due to the pathogen in question. In situations where the disease may be caused by a variety of pathogens, as is the case with pneumonia and meningitis, the tendency has been to look for protection against disease which is microbiologically proven to be due to the organism in question. Thus, for Hib vaccines the primary endpoint was invasive Hib disease, usually meningitis, and for pneumococcal vaccines the tendency is to focus on invasive pneumococcal disease. In developing countries, where most invasive pneumococcal disease is pneumonia, this becomes a search for pneumococcal pneumonia caused by one of the serotypes included in the vaccine and proven by positive blood culture or positive lung aspirate culture. In the case of RSV, the endpoint is likely to be bronchiolitis or pneumonia associated with RSV isolation from the nasopharynx.

While there is no doubt that these endpoints should be evaluated during pneumonia vaccine trials in developing countries, the shortcomings of this approach in the case of pneumococcal vaccines should be recognized. Most pneumococcal pneumonia is blood culture-negative, so the impact of the vaccine assessed only on blood culture-positive pneumococcal disease might be misleading, since efficacy against culture-negative pneumococcal pneumonia might be lower. In addition, there is some evidence to suggest that vaccine-induced reduction in carriage of vaccine serotypes of pneumococci leads to an increase in the carriage of nonvaccine serotypes (30, 31). If serotypes normally compete for the same ecologic niche, then the removal of a serotype by the vaccine may leave the way open for colonization by less common serotypes. Early studies, using mouse inoculation techniques, have shown that colonization by second and third serotypes is common but that these bacteria are usually present only in small numbers (53, 54). Thus, what appears to be replacement of a serotype eliminated by the vaccine may actually be unmasking of minority serotypes. Either way, this could lead to increased disease due to nonvaccine serotypes of pneumococcus in situations where the incidence of invasive pneumococcal disease is determined more by factors facilitating invasion, such as an associated viral infection, than by characteristics of the pneumococcus. In such circumstances, a vaccine could show satisfactory efficacy against the vaccine serotypes but have little impact on overall pneumonia rates. Indeed, it is possible that comparatively rare serotypes will replace those that currently colonize the nasopharynges of most young children in developing countries, and which are represented in the present generation of vaccines. Because we know little about these serotypes, it is not possible to predict their pathogenic potential, and therefore the extent to which they might erode the effectiveness of the vaccine.

Careful evaluation of the impact of a vaccine on pneumococcus carriage is therefore an essential component of pneumococcal vaccine trials. Investigators need to evaluate nasopharyngeal pneumococcus carriage in controls and vaccinees, a specified amount of time after vaccination. If possible, studies should be quantitative and should include techniques for identifying colonization with multiple serotypes. These studies will be important for the interpretation of unexpected increases in invasive disease due to particular serotypes. They also allow for a realistic measure to be made of the impact of vaccination on the prevalence of pneumococci resistant to penicillin and other antibiotics.

Because pneumococcal conjugate vaccines are likely to disturb the usual bacterial ecology of the nasopharynges of young infants (30, 31), it is possible that vaccine use will increase the rates of carriage of other potentially pathogenic bacteria, although changes of this type have not been observed following the widespread use of Hib vaccines. These other bacteria should be searched for in carriage studies, as well as among invasive isolates from blood and cerebrospinal fluid.

Blood cultures obtained from children with pneumococcal pneumonia are positive in only a minority of cases. It is not known whether this is a question of chance or whether blood culture-positive infants are a pathologically distinct group. If they are a distinct group, it is possible that vaccine efficacy in this group may differ from efficacy in children with blood culture-negative cases of pneumococcal pneumonia. There is some evidence that this is the case with pneumococcal polysaccharide vaccine, which has been shown to prevent bacteremic pneumococcal disease in adults but not nonbacteremic pneumonia (22). More troubling, though, is the possibility that vaccine-induced antibodies may suppress bacteremia, or simply reduce the probability of the bacterium's growing in culture, while not affecting pneumococcal pneumonia rates. This seems to have happened in one of the pneumococcal polysaccharide vaccine studies (26). Such an effect would lead to an overestimate of the true efficacy of a vaccine. For example, in the Gambian Hib trial, it could have been argued that the finding of no bacteremic Hib pneumonia cases in vaccinees was due to in vitro suppression of blood cultures, had the effect on overall pneumonia rates not been demonstrated. These possibilities emphasize the need to measure overall pneu-

monia rates as a primary outcome and to pay particular attention to trends in serotype distribution, both among carriage strains and among strains causing disease. The true purpose of pneumococcal and Hib vaccines in infancy is to prevent potentially fatal pneumonia and meningitis in children. Bacteriologic endpoints are useful but are imperfect surrogate measures for this.

Indirect tests of pneumococcal etiology are one step further removed from the outcomes of public health importance. Such tests are acceptable in vaccine trials only if their specificity is nearly perfect. In most trials of pneumococcal vaccines, blood culture-positive pneumococcal disease will be found in only ≤5 percent of children with lower respiratory tract infection whose cases are investigated. Thus, a test with 95 percent specificity (in children with nonpneumococcal acute lower respiratory infection) will produce similar numbers of false positives and true positives, which is inadequate for a vaccine trial. This is compounded by the impossibility of definitively demonstrating the test's specificity in children with nonpneumococcal acute lower respiratory infection (because their nonpneumococcal status cannot be proven) and the likelihood that many of the false positives will be caused by pneumococcal carriage, which will itself be affected by the vaccine. It is unlikely that any of the currently available indirect pneumococcal diagnostic tests will be useful in pneumococcal vaccine trials. Pneumococcal vaccine trials can be used to help validate indirect diagnostic tests by examining the reduction in test-positive pneumonia cases among vaccinees, but again this will be confounded by a vaccine effect on pneumococcal carriage, which would reduce the number of carriage-related false positives in vaccinees.

In the case of RSV, etiologic diagnosis is simpler. Using modern techniques, RSV can be identified in the great majority of cases of severe lower respiratory tract infection due to the virus. However, during RSV epidemics, most infants in a community are affected over a period of several months, and samples taken from many healthy infants in the community will also yield RSV (55). Thus, the presence of RSV in a child with a lower respiratory tract infection does not necessarily prove that the child's illness was due to RSV, although it is likely that this is the case. Furthermore, studies with RSV immune globulin have shown a reduction in the rate of RSV-associated severe disease but no significant impact on the rate of RSV infection (56). It is likely, then, that the benefits of a successful RSV vaccine will be most evident at the severe end of the clinical disease spectrum, as has been seen with another vaccine directed against a mucosal viral disease, rotavirus (57). Again, it is the measurement of the impact on severe disease that will be most important, rather than the microbiologic endpoint.

Mortality. Since the public health objectives of pneumococcal and Hib vaccination are reductions in mortality and morbidity due to pneumonia and meningitis, it should be possible to evaluate a vaccine in terms of its impact on mortality. There are two major problems with the design of such a trial. First, the sample size required will inevitably be very large. In The Gambia, where the infant and neonatal mortality rates are approximately 100 and 40 per 1,000 live births, respectively, it is estimated that about 50 percent of infant deaths outside the neonatal age group are due to pneumonia (20). At most, two thirds of these deaths could be pneumococcal, and 75 percent of those could be due to vaccine serotypes. If these assumptions are true, a 100 percent effective vaccine would reduce the postneonatal infant mortality rate from 60 per 1,000 live births to 45 per 1,000. Evaluating an 80 percent effective vaccine under such circumstances would require very large numbers. Verbal autopsy or postmortem questionnaires may improve statistical power by using ARI-specific mortality as the endpoint. However, this will create problems in malaria-endemic areas, where it is difficult to differentiate between deaths due to malaria and deaths due to pneumonia using this method. One approach to this problem would be to exclude deaths occurring among children who, for a variety of reasons (such as age at death or season of death), are likely to have malaria. For example, in The Gambia, where most malaria deaths occur in children over 12 months of age and between the months of August and November, an analysis restricted to the other eight months of the year and to children under 12 months of age might have adequate power to detect an effect of the vaccine on mortality (20). However, interpretation of the results would be qualified; one might simply conclude at the end of the study that there was a significant effect on mortality of unknown magnitude, and the possibility of "replacement deaths" (children being saved from pneumonia but dying from another cause) could not be ruled out. Since it is well documented that the proportion of deaths due to pneumonia increases with increasing infant mortality rate (58), a study with ARI mortality as an endpoint would be more difficult to carry out in an area with a lower infant mortality rate, and would be easier in areas with higher infant mortality rates.

The other main problem associated with the use of mortality as an endpoint is the ethical problem of conducting an expensive study in an area with relatively poor health services, where the outcome of interest is death from an easily treatable cause. As the health infrastructure is bolstered to support the vaccine trial,

infant mortality, particularly the component due to pneumonia, should fall. To conduct the study without allowing this to happen would be ethically unacceptable, so the act of setting up the study may make this endpoint unattainable. Unlike *S. pneumoniae*, RSV may not be a major cause of mortality in developing countries. If that is the case, the benefits of vaccination would be limited to savings in morbidity, and a mortality endpoint would be inappropriate (43). However, not everyone agrees with this position; some argue that the effect of an RSV vaccine on mortality could be substantial, citing the observed seasonality of pneumonia in some settings as evidence. Such possibilities should be taken into account when designing trials.

Clinical pneumonia. ARI in children represent a continuum. At one end are the vast majority of episodes that are benign and short-lived, producing upper respiratory tract symptoms with or without cough. These episodes are predominantly viral, as is shown by studies identifying viral causes and by the proven uselessness of antibiotics in these cases (59). Further down the continuum, and involving fewer children, are the illnesses characterized by cough and fast breathing. Many of these illnesses show minor, nonspecific abnormalities upon chest radiography. This is the group that the World Health Organization says should receive antibiotics, based on studies which showed that they are likely to have crepitations upon auscultation or chest radiograph abnormalities (60). However, this is not a homogeneous group. At the level of the hospital outpatient department, where most of the studies were performed, this group contains many children with complicated pneumonia. At the community level, most children with cough and fast breathing detected by active surveillance in the home have mild ARI, and their parents may not have sought medical attention. In some ARI intervention studies, these children have been identified and treated with antibiotics. The etiologic identity of this group of infections is open to debate. Certainly many episodes are viral in origin, and in most studies (but not all) the blood culture isolation rate has been very low (21). Some argue that most children with cough and fast breathing but no other signs of pneumonia do not need antibiotic treatment; yet the World Health Organization strategy, based on treating this group, has reduced overall mortality in several studies (1).

The next level in the continuum comprises children with more severe pneumonia. This can be defined in terms of clinical findings, radiographic findings, or pulse oximetry. The World Health Organization advocates the use of lower chest wall indrawing as the sign indicating pneumonia severe enough to warrant hospital admission. However, this sign has some limitations. It is most pronounced in children with wheezing, not necessarily severe wheezing, or upper airways obstruction (61, 62). Radiography is probably the most suitable way of identifying the children with more severe pneumonia, although it must be noted that fatal pneumonia may be associated with only vague radiologic signs (63). In the Gambian Hib vaccine trial, a definition designed to identify only definite cases of radiologic pneumonia with clear alveolar consolidation excluded many radiographs with minor degrees of consolidation, yet in the analysis it appeared to have included almost all children with Hib pneumonia. The use of pulse oximetry to define a group of severe cases selects a group heavily weighted towards RSV, since RSV infections in young infants progress to hypoxemia more frequently than other common respiratory infections. This group did not appear to include many Hib pneumonia cases in the Gambian study, but it will be an important group to study in pneumococcal or RSV vaccine trials.

The careful evaluation of clinical and radiologic endpoints in pneumococcal vaccine trials will serve three related functions. First, by defining the ability of the vaccine to prevent severe pneumonia, a trial will provide an indication of the vaccine's potential to prevent death due to pneumonia in areas with poor health services. Since the most severe pneumonia cases are the most likely to be bacterial, this endpoint should be the most discriminating endpoint for the estimation of vaccine efficacy, and therefore the one most likely to show a statistically significant result if the vaccine is effective. Second, by measuring the impact of the vaccine on the total burden of World Health Organization-defined pneumonia (see figure 3), the trial will provide an estimate of the total ARI burden attributable to pneumococci of the vaccine serotypes in that community. This will give a better indication of the true worth of the vaccine, which would be greatly increased by a significant effect on less severe pneumonia, which is a highly prevalent condition. Third, by subdividing the documented ARI episodes along clinical and radiologic lines, it will be possible to see to which categories of ARI pneumococci make a significant contribution. The definition of categories of ARI that do not contain significant numbers of Hib or pneumococcal cases will open the way to a more critical evaluation of which children with ARI truly need antibiotics. It is likely that many children who currently receive antibiotics do not benefit from them, or the benefit may be minor. Findings in this area will have to be interpreted with caution, since smaller vaccine effects in the milder disease categories could be due to lower efficacy against these forms of disease rather than a lesser pneumococcal contribution, although this seems unlikely.

Acute phase reactants such as C-reactive protein have been proposed as a means of differentiating ARI of bacterial origin from ARI of viral origin, yet studies done in children have been inconclusive (64). Measurement of these parameters in the context of a vaccine trial would be a suitable way of evaluating their use in this regard, and might provide a useful secondary analysis if, for example, a significant reduction in nonbacteremic pneumonia associated with an elevated C-reactive protein was observed in vaccinees.

As was discussed above, RSV vaccines may have no impact on overall RSV infection rates yet may provide protection against more severe disease. Thus, it is imperative in RSV studies to include full and complete clinical evaluation of cases, including pulse oximetry and radiology. Children in RSV trials should be followed prospectively for a number of years to evaluate the impact of vaccination on long term morbidity. This will create problems in terms of blinding, but the information will be necessary for judgement of the potential (positive or negative) impact of the vaccine.

DESIGN IMPLICATIONS FOR PNEUMOCOCCAL VACCINE TRIALS

All pneumococcal vaccine trials will endeavor to determine efficacy against a bacteriologic endpoint. All will try to demonstrate an effect on carriage and on the prevalence of penicillin-resistant pneumococci. Carriage studies will employ sensitive methods to detect the presence of small numbers of second and third serotypes of pneumococci, to distinguish unmasking from true replacement, and to better understand accompanying trends in serotypes causing invasive disease. Where studying a mortality endpoint is feasible, every effort should be made to detect it, but in most settings this will be impossible. The most difficult area is the determination of clinical and radiologic endpoints, yet this is the most important area. Both of the key functions of the trial, vaccine efficacy and disease burden, must be considered. For vaccine efficacy, a representative sample of each disease category will suffice, while for disease burden enough information must be collected to allow for extrapolation to the disease burden of the whole population.

It is useful to consider those cases that may be missed in a trial carried out along lines similar to those of the Gambian Hib trial. At the mild end of the spectrum, many case children do not present at all to health care facilities, and other cases are managed at first-level health facilities that are not ordinarily part of the case detection system. The milder cases that are seen and recorded are those occurring among children whose parents self-refer them to the larger clinics and hospitals. Of all the children with cough and fast breathing, these are probably the children with the most severe cases, followed by those whose cases are managed at the peripheral level and those who stay at home. However, some of the children in the last group may progress to more severe disease.

At the other end of the spectrum is the more troubling group of children with severe, life-threatening disease who, because of poor access to medical services, parental reluctance to use the services, or failure on the part of health care personnel to recognize their illness, fail to receive adequate treatment. This often results in the death of the child. An understanding of both groups will be necessary in order to fully understand the disease burden. This could be achieved by the identification of a well defined, representative population for careful documentation of the milder forms of ARI and another, similar population for identification and investigation of all deaths. If the mortality endpoint is an objective for the study, the latter group could be the entire study population.

Pneumococcal vaccine trials offer our best hope for understanding the true nature of pneumococcal disease among children in developing countries, as well as the potential public health utility of vaccines for its prevention. It is essential that the opportunities offered by the trials currently under way or being planned be fully exploited by careful and appropriate trial design. RSV vaccine trials will be complex and long. Sites in developing countries at which RSV disease is currently being studied should be considering now whether or not they are potential vaccine trial sites. If they believe that they are, they should be conducting epidemiologic studies designed to form the framework on which a vaccine trial can be built.

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