Combined Effects of Antenatal Receipt of Influenza Vaccine by Mothers and Pneumococcal Conjugate Vaccine Receipt by Infants: Results from a Randomized, Blinded, Controlled Trial

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A 2 × 2 factorial trial was performed to determine the efficacy of antenatal influenza vaccination of mothers plus pneumococcal conjugate vaccination of their infants against respiratory illness during early infancy. The efficacy of trivalent inactivated influenza vaccine (TIV; delivered to mothers) plus 7-valent pneumococcal vaccine (PCV7; delivered to infants) was higher than the efficacy of TIV alone or PCV7 alone. During the period of the study in which influenza was circulating, the efficacy of TIV plus PCV7 was 72.4% (95% confidence interval, 30.2%–89.1%) against febrile respiratory illness and 66.4% (95% CI, 14.3%–68.9%) against medically attended acute respiratory illness. Clinical Trials registration NCT00142389.

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Influenza virus infection predisposes individuals to infection with bacterial pathogens such as Streptococcus pneumoniae [1]. Influenza virus is frequently found with S. pneumoniae in dual infections, and influenza virus infection has been implicated in as many as 22% of cases of community-acquired pneumonias [1, 2]. In a mouse model, McCullers et al demonstrated that, in influenza virus and S. pneumoniae dual infections, the sequence of each infection matters: infection with influenza virus first was more lethal than simultaneous infection and infection with S. pneumoniae first [3].

A strategy that considers synergies between influenza virus infection and pneumococcal infection would optimally deliver influenza vaccine with or before pneumococcal vaccine. Currently, there is no approved influenza vaccine for infants aged <6 months. However, immunizing pregnant women against influenza and vaccinating their infants against S. pneumoniae infection may be an effective alternative strategy to reduce respiratory illness in early infancy. Evaluating the combined efficacy of influenza vaccine in mothers and pneumococcal vaccine in infants is particularly important for decision making in developing countries, where introduction of pneumococcal conjugate vaccine is a major priority of the World Health Organization (WHO). WHO’s Strategic Advisory Group of Experts on Immunization recently designated pregnant women as the most important risk group for inactivated seasonal influenza vaccination.

We are not aware of a trial to evaluate the combined effects of influenza and pneumococcal vaccines in mothers and infants, respectively. In this study, we evaluated the combined effects of influenza vaccine delivery to pregnant women and pneumococcal conjugate vaccine delivery to their infants in preventing acute respiratory illness during early infancy.

METHODS

We conducted a randomized, double-blind, controlled 2 × 2 factorial trial in Dhaka, Bangladesh, in collaboration with facilities that provide maternal healthcare and WHO Expanded Programme on Immunization services. Eligibility for participation was restricted to women in their third trimester of pregnancy who had a normal medical and obstetric history, had a literacy level that allowed them to complete the consent forms, and were aged 18–40 years. Women were excluded if they did not plan to deliver their infants in Dhaka and remain in the Dhaka area for 5 months after delivery. The study excluded women who had a history of systemic disease, complicated...
Enrolled women were randomized to receive either trivalent inactivated influenza vaccine (TIV; Fluarix, which contained strains for the 2004 influenza season) or 23-valent pneumococcal polysaccharide vaccine (PPSV23; Pneumovax) in their third trimester. Infants of women in each group were further randomized to receive either the 7-valent pneumococcal conjugate vaccine (PCV7) or Haemophilus influenzae type b (Hib) conjugate vaccine at 6, 10, and 14 weeks of age (Figure 1). Mothers, families, and study staff who collected data were unaware of the identity of specific vaccines delivered to mothers and infants [4].

Infants were followed weekly through 24 weeks of age. Blood was collected for serologic assessment at birth (cord blood); at 6, 10, 14, and 18 weeks of age; and between 22 and 24 weeks of age. All infants received the local routine childhood immunizations at 6, 10, and 14 weeks of age. Infants received 3 doses of PCV7 or Hib conjugate vaccine at 6, 10, and 14 weeks of age from clinic staff not involved with study outcome assessments. Digital thermometers were given to mothers, who were taught to record the temperatures of their infants. Mothers were interviewed once per week from the time of infant birth up to 24 weeks of age, to assess illness in mother or child. Mothers of ill infants were encouraged to bring their child to the study clinic for evaluation and treatment, which was provided free of charge [4]. The study end points were respiratory illness with fever and medically attended acute respiratory illness (MAARI).

We previously documented influenza virus circulation in Dhaka from January through October 2005 [4]. Similarly, Brooks et al reported results from a contemporaneous, independent influenza surveillance study from Dhaka that identified cases of laboratory-confirmed influenza from early 2004 through December 2007 [6]. In this independent study, laboratory-confirmed influenza cases were identified only sporadically between September 2004 and January 2005, similar to our previously reported data [6]. A few influenza virus isolates were reported during February and March 2005; from April through June 2005, substantial numbers of influenza A virus subtype H3N2 and influenza B virus were isolated [6]. Therefore, on the basis of influenza virus circulation data and serologic influenza virus data [7], we classified the period after 31 January 2005 as the period of influenza circulation. In addition to our analysis of data for the full study period, we performed a subgroup analysis for the period of influenza circulation.

The original sample size was computed to detect a difference in pneumococcal antibody titers, assuming an attrition rate of 25% among participating infants [4]. The study had a power of 80% to detect a difference of ≥30% in the illness rates [4]. Poisson regression was used to compute incidence rate ratios (IRRs) among infants aged >10 weeks (ie, 4 weeks after receipt of the first PCV7 dose). The percentage efficacy was calculated as follows: [(1−IRR) × 100]. The confidence interval (CI) bounds for efficacy were derived from the CI bounds for IRRs, using the same approach as that used for the point estimate of efficacy. A synergy index was used to evaluate the magnitude and direction of interaction (ie, whether the combined effects of the 2 vaccines were greater than their individual effects). The synergy index measures the excess risk of an outcome from the 2 exposures being evaluated in the presence of interaction versus the excess risk of the outcome from the 2 exposures in the absence of interaction [8]. We used the following formula to compute the values of the

Figure 1. Flow diagram of mother enrollment and vaccine assignment. Abbreviation: Hib, Haemophilus influenzae type b.
synergy index for the interaction:

$$\frac{\text{IRR}_{11} - 1}{(\text{IRR}_{10} - 1)(\text{IRR}_{01} - 1)}$$

where IRR$_{11}$ is the IRR in the presence of both exposures (ie, vaccines in this study) and IRR$_{10}$ and IRR$_{01}$ are IRRs when only 1 of the 2 exposures is present. A negative value of the synergy index indicates a protective effect of the combined interventions (ie, protective interaction). A test of homogeneity was used to evaluate the statistical significance of the interaction. This test uses the following formula:

$$X^2_{k-1} = \sum_{i=1}^k \frac{(\log \text{IRR}_i - \log \text{IRR})}{\text{var}(\log \text{IRR}_i)}$$

where IRR$_i$ denotes stratum-specific IRRs and IRR denotes the pooled IRR [9, 10]. Associations were considered statistically significant at an $\alpha$ level of 0.05.

The institutional review boards at the International Centre for Diarrheal Disease Research (Dhaka) and the Bloomberg School of Public Health at Johns Hopkins University (Baltimore, MD) reviewed and approved the project protocol.

**RESULTS**

Recruiting and immunization occurred from August 2004 through May 2005. Of the 823 pregnant women screened, 340 met the inclusion criteria and agreed to participate in the study (Figure 1). The demographic characteristics of mothers and infants were similar between the 2 study groups [4]. The cohorts of mother-infant pairs were observed through November 2005, with observation durations of 1651 person-months for infants and 2165 person-months for mothers.

Mothers who received the PPSV23 vaccine and their infants who received the Hib conjugate vaccine were considered the reference group for efficacy estimates. During the full study period, for the end point of respiratory illness with fever, the maternal PPSV23 plus infant PCV7 combination had an efficacy of 4.5% (95% CI, −34.8% to 32.3%), maternal TIV plus infant Hib conjugate vaccine had an efficacy of 36.5% (95% CI, 9.3%–62.1%; Table 1). For MAARI, the maternal PPSV23 plus infant PCV7 combination had an efficacy of 2.7% (95% CI, −48.2% to 36.1%), maternal TIV plus infant Hib conjugate vaccine had an efficacy of 41.7% (95% CI, 5.0%–64.3%), and maternal TIV plus infant PCV7 had an efficacy of 45.5% (95% CI, 8.7%–67.5%).

During the influenza circulation period, the efficacy against respiratory illness with fever was −35.3% (95% CI, −145.8% to 25.5%) for maternal PPSV23 plus infant PCV7, 31.6% (95% CI, −55.0% to 69.8%) for maternal TIV plus infant Hib conjugate vaccine, and 72.4% (95% CI, 30.2%–89.1%) for maternal TIV plus infant PCV7 (Table 1). During the same period, for the MAARI end point, the maternal PPSV23 plus infant PCV7 combination had an efficacy of −50.0% (95% CI, −178.3% to 19.1%), maternal TIV plus infant Hib conjugate vaccine had an efficacy of 37.7% (95% CI, −45.6% to 73.3%), and maternal TIV plus infant PCV7 had an efficacy of 66.4% (95% CI, 14.3%–86.9%). There was statistical evidence of interaction/synergy between TIV and PCV for both respiratory illness with fever (synergy index, −19.6; $P = .02$) and MAARI (synergy index, −5.4; $P = .02$) during the period of influenza circulation.

**DISCUSSION**

In this 2×2 factorial analysis, the combination of maternal TIV plus infant PCV7 had the highest efficacy against respiratory illness with fever and MAARI in infants. The impact of maternal TIV plus infant PCV7 on the study end points was higher during the period of influenza circulation, compared with the full study period.
There is biological plausibility of the observed findings. Influenza virus infection can cause narrowing of airways by disrupting surfactant, reducing ciliary function, and generating an inflammatory response. Influenza virus damages the respiratory epithelium, providing an increase in the number of attachment sites for pneumococci \[11, 12\]. Moreover, the virus may alter the immune system by decreasing the ability of the host to clear \textit{S. pneumoniae} or by accentuating the inflammatory cascade, thus increasing the likelihood of severe disease after co-infection. \textit{S. pneumoniae} plays its role in the co-infection with the influenza virus by boosting inflammation and by increasing the virulence of the virus through mechanisms that haven’t been well described \[2, 13\].

We previously documented the positive impact of TIV administered during pregnancy on birth outcomes, such as preterm birth and birth weight, in the Mother’s Gift trial \[14\] and elsewhere \[15\]. Therefore, another potential reason for the observed effects could be that the infants born to mothers receiving TIV were healthier and therefore experienced enhanced effects of PCV7. In other words, the impact of TIV on birth outcomes could be part of a causal pathway in which maternal TIV modifies the effects of PCV7 in infants.

A limitation of this study is that the control group vaccines (ie, PPSV23 and Hib conjugate vaccine) might have prevented some respiratory illness, resulting in bias due to nondifferential misclassification. This bias is likely to have produced somewhat conservative estimates of efficacy. Moreover, this was a relatively small trial. The small number of subjects and the relatively low frequency of the outcomes may have contributed to limiting the effect size. Therefore, our findings will need to be replicated.

In this South Asian setting, the combination of maternal TIV plus infant PCV7 takes advantage of the biological interaction between influenza virus and \textit{S. pneumoniae}. In the context of pneumococcal conjugate vaccine introduction in developing countries, maternal influenza vaccination may be a useful strategy for preventing clinical respiratory illness during early infancy.

**Notes**

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**Potential conflicts of interest.** M. C. S. served as a consultant for Novartis, GSK, and Merck during the year before manuscript submission. In 2009, S. B. O. was awarded the Maurice R. Hilleman Early-Stage Career Investigator Award by the National Foundation for Infectious Diseases (NFID). The award was funded by an unrestricted educational grant to the NFID from Merck; however, S. B. O. had no direct interaction with Merck. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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