Pneumococcal Coinfection with Human Metapneumovirus

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Background. Infection with the newly discovered human metapneumovirus (hMPV) may lead to hospitalization of children with lower respiratory tract infection (LRTI), although the pathogenesis thereof remains to be elucidated.

Methods. This hypothesis-generating study involved a cohort of children randomized to receive 9-valent conjugate pneumococcal vaccine or placebo and who were tested for hMPV infection when hospitalized for LRTI. By use of a nested reverse-transcription polymerase chain reaction assay targeted at amplifying a fragment of the hMPV fusion (F) protein gene, 202 such infections were identified among 2715 episodes of LRTI in children.

Results. Among human immunodeficiency virus (HIV)–uninfected children who had received 3 doses of conjugate pneumococcal vaccine, the incidence of hMPV-associated LRTI was reduced by 45% (95% confidence interval [CI], 19%–62%; \( P = .002 \)) and the incidence of clinical pneumonia was reduced by 55% (95% CI, 22%–74%; \( P = .003 \)). Similarly, in fully vaccinated HIV-infected children, the incidence of hMPV-associated LRTI was reduced by 53% (95% CI, 3%–77%; \( P = .035 \)), and that of clinical pneumonia was reduced by 65% (95% CI, 19%–85%; \( P = .020 \)).

Conclusions. The pathogenesis of hMPV-associated LRTI that results in hospitalization of both HIV-infected and -uninfected children involves bacterial coinfection with pneumococcus, and a significant proportion of these hospitalizations may be prevented by vaccination with pneumococcal conjugate vaccine.
tion and that pneumococcal coinfection in children with virus-associated pneumonia requiring hospitalization could be delineated by using a pneumococcal conjugate vaccine as a probe [13].

In the present study, we aimed to determine whether the prevention of pneumococcal pneumonia by vaccination with a 9-valent pneumococcal polysaccharide–protein conjugate vaccine (PCV) had any effect on the incidence of hospitalization for hMPV-associated LRTI. We therefore used the vaccine as a probe to define the role of pneumococcal coinfection in the pathogenesis of hMPV-associated LRTI in this hypothesis-generating study.

SUBJECTS AND METHODS

Study population. Our study population consisted of children participating in a phase 3 study conducted in South Africa, the primary objectives of which were to determine the efficacy of a 9-valent PCV to prevent invasive pneumococcal disease and radiographically confirmed pneumonia. Details of the study have been published elsewhere [13–15]. Briefly stated, recruitment of 39,836 children was started on 1 March 1998, and enrollment of all subjects was completed by October 2000, with the last child being immunized in December 2000. The first dose of study vaccine was administered at a mean ± SD age of 6.6 ± 1.2 weeks, and 2 further doses of study vaccine were administered, one at 11.2 ± 2.5 weeks and the other at 15.9 ± 3.8 weeks. No booster dose of PCV was given. Surveillance for study outcome cases was hospital based and continued until 15 November 2001, at which stage the data were analyzed for the primary objectives of the study. Thereafter, investigators and laboratory staff remained blinded to the randomization arm of the individual subjects, and surveillance continued until October 2005. All children who were hospitalized were clinically evaluated by one of the study doctors, who used a standardized form for documenting signs and symptoms.

Testing for hMPV. Nasopharyngeal aspirate samples were obtained for respiratory viral studies from children hospitalized with LRTI [13]. Aliquots of nasopharyngeal aspirate samples obtained from the study subjects were archived at –70°C from January 2000 onward. Those samples obtained between 1 January 2000 and 31 December 2002 were tested for the presence of hMPV, using a nested reverse-transcription (RT) polymerase chain reaction (PCR) assay targeted at amplifying a fragment of the hMPV fusion (F) protein gene to detect hMPV. Details of the methods used for processing the samples and testing for hMPV were reported in our initial exploratory study on the prevalence of hMPV in infants during a single winter season [5].

HIV testing. The HIV infection status of individual subjects who were hospitalized was determined using 2 HIV ELISA tests (Axsym and Murex HIV 1+2; Murex Diagnostic Limited). An HIV PCR (Roche Amplicor version 1.5) test was used to confirm the infection status of children <18 months of age if the ELISA test was reactive or if any child had a nonreactive HIV ELISA test result despite the presence of symptoms of AIDS.

Other tests. C-reactive protein (CRP) tests were performed using immunoturbidimetry (717 Automated Analyzer; Boehringer Mannheim/Hitachi) at the National Health Laboratory Service, Johannesburg, South Africa. Either samples were sent for testing by the attending physician at the time of admission of the child to hospital or serum samples that were obtained within 12 h of admission and stored at –70°C were retrospectively analyzed for CRP, when available.

Study-specific definitions. The definitions used to diagnose clinical pneumonia, bronchiolitis, LRTI, and World Health Organization (WHO)–defined severe/very severe pneumonia have been described elsewhere [13, 15]. Briefly, all children hospitalized with a study physician diagnosis of pneumonia or bronchiolitis, irrespective of symptoms found by clinical examination or chest radiography (CXR), were considered to have LRTI. Children with LRTI were categorized as having clinical pneumonia if they had evidence of alveolar consolidation on CXR (CXR-AC) or if they fulfilled the clinical diagnosis of LRTI without wheeze on chest auscultation but had rales and/or bronchial breathing. Children were categorized as having bronchiolitis in the presence of wheezing on chest auscultation performed by one of the study doctors and in the absence of documented alveolar consolidation on chest radiograph or bronchial breathing on chest wall auscultation. CXR-AC was evaluated on the basis of criteria agreed on by the Vaccine Trialist Working Group, coordinated by the WHO [16]. A clinical diagnosis of WHO severe/very severe pneumonia was made if the child had a cough <14 days in duration and lower chest wall in-drawing and/or any of the following signs and symptoms of severe pneumonia: feeding difficulties, convulsions, central cyanosis, or encephalopathy [17].

Statistical analysis. Data were analyzed using STATA (version 8.0; StataCorp) and Epi Info (version 6.04d; Centers for Disease Control and Prevention). Vaccine efficacy (VE) was calculated using the vaccine efficacy calculation function in Epi Info for cohort studies. This is based on the formula VE (%) = [(incidence rate in the unvaccinated – incidence rate in the vaccinated)/incidence rate in the unvaccinated] × 100. All children who were randomized were included in the intent-to-treat (ITT) analysis from the day that they received their first dose of study vaccine. Children were considered to be fully vaccinated and included in the per protocol (PP) analysis if the LRTI event occurred >14 days after the third dose of study vaccine and the child received all of the study vaccines as per the planned schedule. Only the first episode of any clinical syndrome was included in the VE calculation for an individual study participant. Similarly, the age group subanalysis included the first episode that occurred in an individual child during the relevant age group period. Proportions were compared us-
Figure 1. Summary of children hospitalized for lower respiratory tract infection (LRTI) who were investigated for all episodes (first and subsequent) of human metapneumovirus (hMPV) infection. LRTI episode, total no. of LRTIs (bronchiolitis or pneumonia); NPA done, nasopharyngeal aspirate performed to test for respiratory viruses other than hMPV; RT-PCR done, no. of NPA samples that were available for the reverse-transcription polymerase chain reaction assay to detect hMPV.

- **Table:**
  - **Study group**
  - **Overall**
    - **LRTI episode**
      - Vaccine recipients: n = 1533
      - Placebo recipients: n = 1643
    - NPA done: n = 1476 (96.3%)
    - RT-PCR hMPV done: n = 1306 (88.5%)
    - hMPV identified: n = 78 (5.8%)
  - **HIV Uninfected**
    - Vaccine recipients: n = 958
    - Placebo recipients: n = 973
    - NPA done: n = 933 (97.4%)
    - RT-PCR hMPV done: n = 835 (89.5%)
    - hMPV identified: n = 60 (7.2%)
  - **HIV Infected**
    - Vaccine recipients: n = 551
    - Placebo recipients: n = 635
    - NPA done: n = 524 (95.1%)
    - RT-PCR hMPV done: n = 456 (87.0%)
    - hMPV identified: n = 16 (3.5%)

**RESULTS**

**hMPV in children hospitalized with respiratory tract illness.**

Figure 1 illustrates the proportion of nasopharyngeal aspirate samples that were available to test for the presence of hMPV in children hospitalized for LRTI. There was no difference between vaccine and placebo recipients in the proportion of children from whom a nasopharyngeal aspirate was taken or in the proportion of those samples that were available for hMPV testing (figure 1). Overall, samples were available for hMPV analysis for 2715 (88.5%) of the 3069 episodes of LRTI for which nasopharyngeal aspirate samples were collected. The proportion of samples available for hMPV testing that were obtained during the hMPV epidemic period was similar to that of the entire study period (data not shown). Children from whom nasopharyngeal aspirate samples were unavailable for hMPV testing (354 [11.5%] of 32715; P < .00001) than were those from whom nasopharyngeal aspirate samples were available for hMPV testing. These differences were evident in HIV-infected as well as HIV-uninfected children; however, there were no other clinical or demographic differences observed regarding LRTI episodes for which nasopharyngeal aspirate samples were unavailable for hMPV testing (data not shown).

**Ethical issues.** The phase 3 efficacy study and subsequent study involving testing samples for hMPV were approved by the Ethics Committee for research on Human Subjects of the University of the Witwatersrand. Signed informed consent was obtained from the parent/legal guardian of the children at the time of enrollment into the phase 3 study.

**Effect of pneumococcal conjugate vaccine on the incidence of hospitalization for hMPV-associated pneumonia.** In fully vaccinated children, the incidence of hospitalization for at least 1 episode of hMPV-associated LRTI was reduced by 46% (P < .0002) overall, by 45% (P < .002) in HIV-uninfected children, and by 53% (P < .035) in HIV-infected children (table 1). The ITT estimates (table 2) of VE for most of the outcomes were not significantly different from the estimates in the PP analysis. Although there was no difference in VE estimates by age group for hMPV-associated LRTI in the ITT analysis in HIV-uninfected children (P = .51, \( \chi^2 \) test for trend) or in HIV-infected children (P = .98, \( \chi^2 \) test for trend), there was a trend toward a lesser effect of the vaccine in reducing the incidence of hMPV-associated LRTI in vaccine recipients < 6.0 months of age, compared with that in older children (table 3).

There was a significant reduction in the incidence of clinical pneumonia among vaccine recipients overall (58%; P = .0001), in HIV-uninfected children (55%; P = .003), and in HIV-infected children (65%; P = .02). In addition, when the WHO criteria for severe/very severe pneumonia were used as an outcome, a 44% (P = .003) reduction in incidence was observed overall, a 40% (P = .02) reduction was observed in HIV-uninfected children, and a 53% (P = .04) reduction was observed.
<table>
<thead>
<tr>
<th>hMPV-associated outcome</th>
<th>Overall</th>
<th>HIV uninfected</th>
<th>HIV infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccine recipients</td>
<td>Placebo recipients</td>
<td>Efficacy (95% CI), %</td>
</tr>
<tr>
<td>LRTI</td>
<td>52</td>
<td>97</td>
<td>46 (25 to 62)</td>
</tr>
<tr>
<td>Clinical pneumonia</td>
<td>26</td>
<td>62</td>
<td>58 (34 to 73)</td>
</tr>
<tr>
<td>Bronchiolitis</td>
<td>26</td>
<td>35</td>
<td>26 (−23 to 55)</td>
</tr>
<tr>
<td>WHO severe pneumonia&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41</td>
<td>73</td>
<td>44 (18 to 62)</td>
</tr>
<tr>
<td>CRP ≥40 mg/dL&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9 [31]</td>
<td>26 [59]</td>
<td>65 (26 to 84)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of cases, unless otherwise indicated. Values in brackets are total nos. of the LRTI episodes for which the test was performed. CI, confidence interval.

<sup>a</sup> hMPV-associated LRTI associated with alveolar consolidation on chest radiograph.

<sup>b</sup> World Health Organization clinically diagnosed LRTI.

<sup>c</sup> hMPV-associated LRTI with C-reactive protein (CRP) level ≥40 mg/dL.
Table 2. Percentage efficacy of pneumococcal conjugate vaccine by intent-to-treat analysis in the prevention of human metapneumovirus (hMPV)–associated lower respiratory tract infections (LRTIs).

<table>
<thead>
<tr>
<th>hMPV-associated outcome</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccine recipients</td>
<td>Placebo recipients</td>
<td>Efficacy (95% CI), %</td>
</tr>
<tr>
<td>LRTI</td>
<td>72</td>
<td>123</td>
<td>42 (22 to 56)</td>
</tr>
<tr>
<td>Clinical pneumonia</td>
<td>38</td>
<td>78</td>
<td>51 (28 to 67)</td>
</tr>
<tr>
<td>Bronchiolitis</td>
<td>34</td>
<td>45</td>
<td>25 (−18 to 52)</td>
</tr>
<tr>
<td>WHO severe pneumonia(^b)</td>
<td>56</td>
<td>93</td>
<td>39 (16 to 57)</td>
</tr>
<tr>
<td>CRP ≥40 mg/dL(^c)</td>
<td>14 [42]</td>
<td>32 [74]</td>
<td>56 (18 to 77)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of cases, unless otherwise indicated. Values in brackets are the total no. of LRTI episodes for which the test was performed.

\(^a\) hMPV-associated LRTI associated with alveolar consolidation on chest radiograph.

\(^b\) World Health Organization clinically diagnosed LRTI.

\(^c\) hMPV-associated LRTI with C-reactive protein (CRP) level ≥40 mg/dL.

Table 3. Percentage efficacy of pneumococcal conjugate vaccine by intent-to-treat analysis in the prevention of human metapneumovirus–associated lower respiratory tract infections, by age group at time of hospitalization.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Overall</th>
<th>HIV uninfected</th>
<th>HIV infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccine recipients</td>
<td>Placebo recipients</td>
<td>Efficacy (95% CI), %</td>
</tr>
<tr>
<td>&lt;6.0 mo</td>
<td>19</td>
<td>22</td>
<td>14 (−59 to 53)</td>
</tr>
<tr>
<td>6.1–12.0 mo</td>
<td>17</td>
<td>35</td>
<td>52 (14 to 73)</td>
</tr>
<tr>
<td>12.1–24.0 mo</td>
<td>23</td>
<td>38</td>
<td>40 (−1 to 64)</td>
</tr>
<tr>
<td>&gt;24.0 mo</td>
<td>17</td>
<td>31</td>
<td>45 (1 to 70)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. of cases, unless otherwise indicated.
in HIV-infected children. The impact of PCV on the incidence of hMPV-associated pneumonia was also compared, using outcomes that may be more specific for bacterial pneumonia—that is, radiologically confirmed pneumonia (CXR-AC) and LRTI associated with an elevated CRP level (≥40 mg/dL).

Chest radiographs were available for 176 (90.3%) of the 195 children with hMPV-associated LRTI overall, 134 (89.9%) of 149 HIV-uninfected children, and 39 (90.7%) of 43 HIV-infected children, with no significant difference in the number tested between vaccine and placebo recipients (tables 1 and 2). In fully vaccinated children, there was a 56.0% (P = .02) overall reduction in the incidence of hMPV pneumonia associated with CXR-AC, as well as an 80% reduction in the incidence of hMPV pneumonia associated with CXR-AC in HIV-infected children (P = .04) (table 1). The reduction in the incidence of radiologically confirmed pneumonia in HIV-uninfected children was not significant (40%; P = .31), but the power of the study to detect a significant difference of this magnitude was only 17%.

Measurements of CRP level were available for 116 (59.5%) of 195 hMPV-infected children overall, 88 (59.1%) of 149 HIV-uninfected children, and 27 (62.8%) of 43 HIV-infected children, with no difference in the proportion of children tested between PCV and placebo recipients (tables 1 and 2). There was a 65% (P = .007) reduction in the incidence of hMPV pneumonia associated with a CRP level of ≥40 mg/dL in fully vaccinated PCV recipients overall, as well as a 61% (P = .05) reduction in HIV-uninfected children (table 1).

There was a nonsignificant reduction in the incidence of hospitalization for hMPV-associated bronchiolitis among vaccine recipients in the entire study population (VE, 25%; P = .21) and in HIV-uninfected children (VE, 25%; P = .12) and a nonsignificant increase in HIV-infected children (4 vs. 3 cases).

S. pneumoniae was isolated from samples collected during 4 (2.1%) of the 189 episodes of hMPV-associated LRTI for which blood was cultured for bacteria. All of the episodes of S. pneumoniae bacteremia in children with LRTI occurred in HIV-infected children, including 1 (6.3%) of 16 in vaccine recipients and 3 (11.1%) of 27 in placebo recipients.

Overall, among children investigated for all episodes (first and subsequent) of hMPV-associated LRTI, the prevalence of coinfection with other respiratory viruses was 4.1-fold (95% CI, 1.1–18.8) greater in vaccine recipients (9 [11.8%] of 76) than placebo recipients (4 [3.2%] of 126) (P = .02). Similarly, HIV-uninfected vaccine recipients with hMPV-associated LRTI were 3.5-fold (95% CI, 0.9–16.4) more likely to be coinfected with other respiratory viruses (8 [13.3%] of 60) than were placebo recipients (4 [4.3%] of 94) (P = .06). In addition, there was 1 HIV-infected vaccine recipient with hMPV-associated LRTI in whom a viral coinfection was identified.

**DISCUSSION**

Animal-model and in vitro studies show that respiratory viral infections may increase susceptibility to bacterial pneumonia [18]. The absence of sensitive tools to diagnose bacterial pneumonia has, however, been a major impediment to defining the role of bacterial coinfection in humans. Experimental tools aimed at improving the sensitivity of diagnosis of bacterial pneumonia indicate that approximately one-third of children with RSV-associated pneumonia may have pneumococcal coinfections [19, 20]. Validating the sensitivity and specificity of these experimental assays is problematic in the absence of a reference standard against which they can be evaluated.

Recently, we showed that 3 doses of PCV were able to reduce the incidence of hospitalization for pneumonia associated with influenza A virus by 39%, pneumonia associated with parainfluenza virus types 1–3 by 44%, and pneumonia associated with RSV by 32% in HIV-uninfected children [13]. These data suggested that these respiratory viral infections predispose to pneumococcal coinfection in the lung and that bacterial-viral coinfections are important in the pathogenesis of virus-associated pneumonia in children.

The results of the present study suggest that bacterial coinfections, particularly pneumococcal infections, are an essential part of the pathogenesis of most severe hMPV infections progressing to pneumonia. The estimate of a 58% overall reduction in the incidence of clinical pneumonia in vaccine recipients is only a conservative estimate of the prevalence of pneumococcal coinfection in children with hMPV-associated pneumonia. The true prevalence of pneumococcal coinfection in children with hMPV-associated pneumonia may be even higher than what we have inferred. Factors that may have resulted in an underestimation of the importance of pneumococcal coinfections in children with hMPV-associated pneumonia include the following: (1) the PCV used in our study includes only 9 of 90 different pneumococcal serotypes, although it does include those most commonly responsible for invasive disease; (2) PCV efficacy against nonbacteremic pneumococcal pneumonia may be lower than that observed against invasive disease (83%–98%) [14, 21]; and (3) there may be an excess of nonbacteremic pneumonias caused by nonvaccine pneumococcal serotypes in the vaccine recipients. Limitations of this study include differences in the ages of subjects and in the prevalence of other respiratory viruses in those LRTI episodes for which nasopharyngeal samples were unavailable for hMPV testing. It is, therefore, possible that the VE in younger children may be lower than that observed in the overall population, as suggested by the age group subanalysis in table 3. Possible explanations for this lesser effect include the following: (1) hMPV is an independent cause of severe LRTI in very young infants; (2) PCV is less able to protect against pneumococcal pneumonia in very young infants, since some may not have completed their full series of primary vaccination; and
(3) coinfections with other respiratory viruses—especially RSV, which most commonly infects infants <6.0 months of age—may also result in severe LRTI [8, 12]. That the latter may occur independently of pneumococcal coinfection is indirectly supported by our findings that, in children hospitalized with hMPV-associated LRTI, the prevalence of coinfection with other respiratory viruses was greater among PCV recipients than placebo recipients, suggesting that viral coinfections may have been involved in the pathogenesis of severe LRTI among these vaccine recipients. Since some specimens from younger children were unavailable for hMPV testing, the bias introduced by the exclusion of those samples may have led us to a conservative estimate of the association between hMPV and other viral pneumonias.

The present study provides insight into the pathogenesis of pneumonia that is caused by this newly discovered virus. It would appear that coinfection with pneumococci is an important event in the clinical course of hMPV infections that result in children >6 months of age progressing to develop pneumonia that necessitates hospitalization. An implication of this observation is that children hospitalized for hMPV-associated pneumonia should be treated with antibiotics. In addition, viral coinfections may also play a role in hMPV infections progressing to severe LRTI in young children.

Although the role of CRP in discriminating between viral and bacterial infections is controversial [22], much of the confusion may be related to the lack of sensitive tools for the identification of bacterial infections. We have reported the usefulness of CRP for improving the specificity of chest radiographs in diagnosing pneumococcal pneumonia [23]. In the present study, we show that PCV reduced the incidence of hMPV-associated pneumonia when the CRP level was ≥40 mg/dL. These data suggest that the findings of Dollner et al., who reported that children with hMPV-associated pneumonia had high CRP levels (median, 105.5 mg/dL [range, 5.9–281 mg/dL]), most likely reflected undiagnosed bacterial coinfections in those children [3]. In that study, children with hMPV-associated LRTI had high fevers (mean, 39.9°C), which is a known predictor of bacterial infections [24]. In the same study by Dollner et al. [3], as well as in that by Jarreti et al. [25], CRP levels were found to be low in children with bronchiolitis (median, 9.0–9.5 mg/dL), providing indirect evidence that bacterial coinfections may be less important in children with bronchiolitis.

Although molecular mechanisms have been described that enhance the human host’s susceptibility to pneumococcal infection after infection by influenza virus, parainfluenza virus type 3, and RSV [18], our data suggest that such mechanisms should also be sought for hMPV.

Acknowledgment

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