MAJOR ARTICLE

Dual Infection of Infants by Human Metapneumovirus and Human Respiratory Syncytial Virus Is Strongly Associated with Severe Bronchiolitis

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The association between severe bronchiolitis and dual infection by human metapneumovirus (hMPV) and human respiratory syncytial virus (hRSV) was investigated in <2-year-old infants with bronchiolitis who were admitted to the hospital during the 2001–2002 winter season. hMPV in nasopharyngeal aspirate and/or cells and fluid collected by nonbronchoscopic bronchoalveolar lavage was detected by reverse transcriptase–polymerase chain reaction (RT-PCR). hRSV was detected in nasopharyngeal aspirate and/or cells and fluid collected by nonbronchoscopic bronchoalveolar lavage by enzyme immunoassay, tissue culture, and RT-PCR. Dual infection with hMPV and hRSV confers a 10-fold increase in relative risk (RR) of admission to a pediatric intensive-care unit for mechanical ventilation (RR, 10.99 [95% confidence interval, 5.0–24.12]; $P < .001$, by Fisher exact test). Dual infection by hMPV and hRSV is associated with severe bronchiolitis.

Bronchiolitis is the clinical description of the commonest and most severe lower-respiratory-tract infection during early childhood. It is an endemic disease with an epidemic peak that occurs during winter in temperate countries. Typically, there is a prodromal coryzal period of 2–3 days, followed by tachypnea, subcostal recession, and/or bilateral inspiratory crackles lasting 5–7 days. There is a spectrum of severity. In the vast majority of cases, there is mild disease with rhinorrhea, mild fever, and a wet cough. These mildly affected infants are often nursed at home. In 2.5% of cases—those with moderate disease—hospital admission is required because mucus obstruction and respiratory distress interferes with feeding. In the most severely affected infants, there is hypoxia and respiratory distress that may require mechanical ventilation [1].

Approximately 75%–85% of cases of bronchiolitis can be attributed to infection by human respiratory syncytial virus (hRSV), and 10%–20% can be attributed to infection by rhinovirus or parainfluenza virus. Rarely, human influenza virus, adenovirus, and *Bordetella pertussis* are detected. In 10% of cases, no pathogen is detected. Human metapneumovirus (hMPV) is now recognized as 1 of several viral pathogens that can cause bronchiolitis in the absence of other pathogens [2]. Preliminary epidemiological data have described a prevalence of 1.5%–8% for hMPV infection in children with respiratory-tract infections who were admitted to the hospital [3, 4].

We recently described, in a study of infants with bronchiolitis who were admitted to a pediatric intensive-care unit (PICU) for mechanical ventilation during the 2000–2001 winter season, a high (70%) frequency of dual infection by hMPV and hRSV [5]. We suspected that there was an association between dual infection and severe disease. To test this hypothesis, we compared the case incidence of dual infection by hMPV and hRSV in infants with bronchiolitis who were admitted, during the subsequent winter season (2001–2002), to either

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the general wards or the PICU of the same hospital.

SUBJECTS AND METHODS

Study design. The study was conducted in 2 stages. Stage 1 was a retrospective case-incidence study of the detection of virus in samples of respiratory secretions; stage 2 was a linkage of limited clinical information—specifically, gestation status at birth, history of complex congenital cardiac disease, the site of the hospital stay (as directed by a "clinical pathway," as described below), and requirement for either supplemental oxygen or mechanical ventilation—and detection of virus.

Because the aim of this study was to determine the effect of viral infection in previously healthy infants, infants who either had a history of complex congenital cardiac disease or had previously been admitted to the hospital were excluded from analysis. Ethical approval was granted by the Liverpool Children's Local Research Ethics Committee.

Subjects and clinical method. Bronchiolitis was diagnosed by attending pediatricians at Alder Hey Children's Hospital, Liverpool, when infants (children <2 years old) presented with tachypnea (>50 breaths/min), subcostal recession, and bilateral inspiratory crackles on auscultation. The regional PICU is also located at Alder Hey Children's Hospital. As part of the diagnostic process and for surveillance of community-acquired respiratory infections, nasopharyngeal aspirate (NPA) was routinely collected from infants with bronchiolitis who were admitted during 13 November 2001–15 March 2002.

Infants were managed by use of a clinical pathway. Infants were admitted to the general wards, for feeding support and/or oxygen therapy. Criteria for admission to the general wards include oral intake <75% of normal and oxygen saturation at <93% in air. The pathway directs that oxygen be administered only to maintain oxygen saturation at >92%. Infants with respiratory failure who required mechanical ventilation were then admitted to the PICU. Infants were also transferred to the PICU from regional pediatric units.

The clinical severity of bronchiolitis was defined as described elsewhere [6]. In this system, mild disease is defined as no need for hospital admission. Moderate disease is defined as admission to the hospital, because of feeding difficulties, but no need for supplemental oxygen at any time during the hospital stay. Severe disease is defined as admission to the hospital and a need for supplemental oxygen, including mechanical ventilation, at any time during the hospital stay.

The association between dual infection and severity of disease was investigated by 2 methods. In the first method, the site of the hospital stay was used as a surrogate for the severity of disease, infants were divided into 2 groups according to the site of their hospital stay, and the prevalence of dual infection in the groups was compared. In the second method, we used the previously described definition of severity of bronchiolitis to divide infants into 2 groups, and the prevalence of dual infection in the groups was compared.

Although premature birth is a recognized risk factor for severe bronchiolitis [7], full-term infants accounted for the majority of admissions to the PICU. We performed a retrospective analysis of a subgroup of patients receiving mechanical ventilation in the PICU, and we divided them into 2 groups, on the basis of gestation status at birth—with prematurity being defined as birth at <37 weeks of gestation and term being defined as birth ≥37 weeks of gestation—and cross-tabulated this information with virus(es) detected.

NPA was collected by nursing staff, usually during the process of admission and always within 24 h of admission. NPA was collected into a conical trap (Maersk Medical) by direct suction (<20 mm of Hg) without lavage. NPA samples were analyzed by researchers who had no prior knowledge of the patients' clinical status.

As part of a large clinical study of hRSV pathogenesis and bronchiolitis, bronchoalveolar lavage (BAL) samples from 25 infants receiving mechanical ventilation for severe bronchiolitis and from 10 infants receiving mechanical ventilation who did not have infections were collected during the same winter period [8]. BAL samples were collected by 1 of the investigators (M.G.S. or P.S.M.) or by 1 of 2 specialist respiratory physiotherapists, in accordance with the European Respiratory Society 2000 guidelines [9]. Written informed consent was obtained from the parents of infants who underwent BAL.

Of the 25 BAL samples from infants with bronchiolitis receiving mechanical ventilation, 20 were collected within 24 h of the infants' admission to the PICU, and 5 were collected within 48 h of the infants' admission to the PICU. BAL sampling was delayed in these 5 patients because 2 of them had been admitted to general wards but deteriorated and required mechanical ventilation within 48 h of admission to the hospital and because BAL samples taken from 3 of them were insufficient for analysis; samples taken on day 2 were sufficient for analysis. On the first morning after the infants' admission to the PICU, BAL samples were collected from 10 control infants receiving mechanical ventilation in the PICU who did not have infections.

Detection of hRSV and hMPV. NPA samples were diluted with 3 mL of 0.9% saline, and the solution was mixed thoroughly; BAL samples were not diluted further. One milliliter of sample was used for rapid detection of hRSV antigen by EIA (BD Directigen RSV; Becton Dickinson), and, if the results were negative, another 1 mL of sample was sent for tissue culture. Residual sample was stored at −80°C until analyzed by reverse transcriptase–polymerase chain reaction (RT-PCR).

Nucleic acid was extracted from 140 μL of sample by use of the QIAamp Viral RNA Mini Kit (Qiagen). Fifteen microliters of purified nucleic-acid solution was subjected to RT-PCR, as described elsewhere [5, 10], to test for the presence of hRSV

Table 1. Site of the hospital stay of infants with bronchiolitis, cross-tabulated with virus detected.

Virus detected	Site of the hospital stay	
	PICU (n = 25)	General wards (n = 171)
hMPV and hRSV[a]	18 (72)	15 (10)
hMPV only	0	4 (2)
hRSV only[a]	7 (28)	134 (78)
No virus detected	0	18 (11)

NOTE. Data are no. (%) of infants. hMPV, human metapneumovirus; hRSV, human respiratory syncytial virus.

[a] Included in calculation of relative risk (RR) (RR, 10.99 [95% confidence interval, 5.0–24.12]; $P < .001$, by Fisher exact test).

nucleoprotein (N) gene and hMPV matrix (M), fusion (F), and nucleoprotein (N) genes. Detection of at least 2 different hMPV gene amplicons in any sample (NPA or BAL) was taken as evidence of hMPV infection.

RESULTS

During the 4-month 2001–2002 winter season, 196 infants with bronchiolitis who were eligible for study were admitted to the hospital—171 to the general wards, and 25 to the PICU for mechanical ventilation.

Detection of hMPV. hMPV was detected, by RT-PCR, in samples from 37 (19%) of the 196 infants with bronchiolitis who were admitted to the hospital—in the NPA from 22 infants, in the BAL samples from 14 infants, and in both the NPA sample and the BAL sample from 1 infant. hMPV was the only virus detected in the NPA samples from 4 (2%) of these 196 infants; these 4 were admitted to the general wards, and all required supplemental oxygen.

Detection of hRSV. hRSV was detected in samples from 174 (89%) of 196 infants with bronchiolitis who were admitted to the hospital; it was detected in the NPA samples from 172 infants (in 122 infants by EIA, in 41 by tissue culture, and in 9 by detection of the N gene by RT-PCR) and, by both EIA and RT-PCR, in the BAL samples from 2 infants receiving mechanical ventilation whose NPA was negative for hRSV by both EIA and RT-PCR. hRSV was the only virus detected in samples from 141 (72%) of 196 infants with bronchiolitis.

NPA and BAL specimens were taken on the same day from 20 infants receiving mechanical ventilation. EIA detected hRSV in 20 NPA samples but in only 17 BAL samples. hRSV was detected in the 3 discordant BAL samples by RT-PCR. Neither hMPV nor hRSV was detected in the NPA samples from 18 (9%) of the 196 infants.

Control cases. Neither hMPV nor hRSV was detected in the BAL samples from 10 control infants receiving mechanical ventilation in the PICU for reasons unrelated to respiratory infections.

Detection of hMPV and hRSV in paired samples of NPA and BAL. In 6 of 11 hRSV-infected infants receiving mechanical ventilation whose BAL and NPA samples were collected at the same time, RT-PCR detected hRSV in both samples (RT-PCR detected hRSV in a total of 8 NPA samples and 9 BAL samples).

In contrast, in 1 of 9 hMPV-infected infants receiving mechanical ventilation whose BAL and NPA samples were collected at the same time, RT-PCR detected hMPV in both samples (RT-PCR detected hMPV in a total of 1 NPA sample and 9 BAL samples). Overall, RT-PCR detected hMPV infection in BAL samples from 15 of 18 infants receiving mechanical ventilation and in NPA samples from 4 of these 18 infants.

Dual infection and site of the hospital stay. Dual infection by hMPV and hRSV was demonstrated in 15 (10%) of 149 hRSV-infected infants admitted to the general wards and in 18 (72%) of 25 hRSV-infected infants admitted to the PICU (table 1), suggesting a significant association between dual infection and admission to the PICU. Relative risk (RR) comparing dual infection and infection by hRSV alone was 10.99 (95% confidence interval [CI], 5.0–24.12; $P < .001$, by Fisher exact test).

Dual infection and severity of disease. Complete clinical information was available for 136 of 178 infants with hMPV and/or hRSV infection. Dual infection was detected in 3 (14%) of 21 infants with moderate disease and in 30 (26%) of 115 infants with severe disease (table 2); the level of dual infection in infants with severe disease did not achieve statistical significance (RR, 1.11 [95% CI, 0.96–1.28]; $\chi^2 = 1.5$; $P = .2$).

Gestation. A retrospective analysis was performed on the 25 infants receiving mechanical ventilation in the PICU. Infants were subgrouped by gestation status (premature or term) at birth and were cross-tabulated on the basis of virus detected (table 3). Infants born at term were more likely to have dual infections, but, because only a few infants were born prema-

Table 2. Severity of disease in infants with bronchiolitis, cross-tabulated with virus detected.

Virus detected	Severity of disease	
	Severe (n = 115)	Moderate (n = 21)
hMPV and hRSV[a]	30 (26)	3 (14)
hMPV only	4 (3)	0
hRSV only[a]	81 (70)	18 (86)

NOTE. Data are no. (%) of infants. hMPV, human metapneumovirus; hRSV, human respiratory syncytial virus.

[a] Included in calculation of relative risk (RR) (RR, 1.11 [95% confidence interval, 0.96–1.28); $\chi^2 = 1.5$; $P = .2$).

Table 3. Infants receiving mechanical ventilation for severe bronchiolitis, cross-tabulated by gestation status at birth and virus detected.

Virus detected	Gestation status	
	Term (n = 20)	Premature (n = 5)
hMPV and hRSV	15	3
hRSV only	5	2

NOTE. Data are no. of infants. hMPV, human metapneumovirus; hRSV, human respiratory syncytial virus. Relative risk is 1.16 ([95% confidence interval, 0.70–1.96]; P = .6, by Fisher exact test).

turely, statistical significance was not achieved (RR, 1.16 [95% CI, 0.70–1.96]; P = .6, by Fisher exact test).

DISCUSSION

The impact of dual infection upon severity of disease was assessed by 2 methods. In the first method, the site of the hospital stay was used as a surrogate for the severity of disease. This is valid for Alder Hey Children's Hospital because infants with bronchiolitis are managed there according to a clinical pathway that defines intervention on the basis of physiological measurements and that directs the site of the hospital stay accordingly. Infants are admitted to the general wards only if they meet strict entry criteria and are admitted to the PICU only for mechanical ventilation.

In the second method, we used a previously described definition of severity of disease—that is, whether supplemental oxygen was required. Infants received supplemental oxygen only when their oxygen saturation was at <93% in air.

Dual infection by hMPV and hRSV was least frequent (14%) in infants with moderate disease (i.e., those with feeding difficulties only) who were admitted to the general wards, was more frequent (26%) in infants with severe disease who were admitted to the general wards (i.e., those who required supplemental oxygen), and was most commonly (72%) observed in infants with severe disease who were admitted to the PICU for mechanical ventilation.

Infants born prematurely are recognized to be at risk of severe disease when infected by hRSV. Immunological and physiological mechanisms explain the susceptibility of prematurely born infants to severe disease. However, in our study and in general, infants born at term without any preexisting disease still represent the majority of PICU admissions with severe bronchiolitis. In this study, we found that more infants born at term who were admitted to the PICU had dual infections than did infants born prematurely, but we could not demonstrate statistical significance. Larger studies, which pool data from several winter seasons, are necessary to investigate this phenomena.

We have noted that, when the NPA and BAL samples were taken at the same time from hMPV-infected infants receiving mechanical ventilation, detection of hMPV was discordant between the 2 samples, with hMPV being detected more often in BAL samples. This discordance raises the possibility that bronchiolitis caused by hMPV is more prevalent than is currently recognized and that it has been underreported because the virus has not been found in the nasopharynx. This possibility contrasts with what was observed for bronchiolitis caused by hRSV, in which the virus is usually detected in both the upper and lower respiratory tracts. Collection of BAL samples would therefore appear to be the preferred sampling method for the detection of hMPV in infants receiving mechanical ventilation. For infants who do not require mechanical ventilation, nasopharyngeal secretions will remain the only acceptable source for sampling.

The majority of hMPV infections found in infants in the PICU were diagnosed by RT-PCR analysis of BAL samples (18 cases were detected on the basis of BAL samples, 3 on the basis of NPA samples, and 1 on the basis of both). This raises the possibly that hMPV infection during endemic seasons may be more common than is currently recognized and that it has been undetected because, for pragmatic and ethical reasons, sampling from the lower respiratory tract is not possible on infants who do not require mechanical ventilation. However, the baseline case incidence of unrecognized hMPV infection of the lower respiratory tract would need to be ~70% for our finding of an association with disease severity to be coincidental.

We believe that it is unlikely that the high frequency of hMPV infection in infants in the PICU was due to nosocomial infection. All 25 BAL samples taken from infants in the PICU were collected within 48 h of admission to the PICU—20 during the first morning after admission to the PICU and 5 within 48 h of admission to the PICU—and we believe that it is most unlikely that, within 48 h of admission to the PICU, these latter 5 intubated infants could have become nosocomially infected and could have started to shed virus from the lower respiratory tract.

Cases of dual infection in the PICU occurred during 25 November–19 February, with a peak on 5–6 January, when 5 dual-infected infants were receiving mechanical ventilation. There were 4 periods during this season (a total of 18 days) when no infants with dual infection were identified, although there were infants infected with hRSV who were receiving mechanical ventilation. Such temporal scatter does not support the hypothesis that nosocomial transmission accounts for the high rate of hMPV infection in infants in the PICU. Furthermore, during the same winter season, we could not detect hMPV (or hRSV) in 10 infants receiving mechanical ventilation in the same PICU for reasons unrelated to respiratory infections.

In the PICU, infants stay in cubicles, and 1 nurse cares for 1 infant for the duration of each shift, with 1 identified nurse

providing relief for breaks, and nurses working in the cubicles are not moved from one cubicle to another. Nurses, doctors, and physiotherapists are required to put on disposable aprons and to wash their hands when entering the cubicles and to remove aprons and to rewash their hands when leaving cubicles.

This is the second consecutive season in which we have observed a similarly high frequency of dual infection by hMPV and hRSV in infants receiving mechanical ventilation for severe bronchiolitis. Dual infection by rhinovirus and hRSV, in association with a 5-fold increased risk of severe disease, has been described elsewhere [11]. A high case incidence (52%) of hMPV infection has been described in association with hospital admission, in Hong Kong, of patients with severe acute respiratory syndrome (SARS) [12]. hMPV was detected in NPA samples from 30 of 48 patients with SARS, and the case incidence of dual isolation, from NPA samples, of both hMPV and SARS coronavirus virus was 20%.

The case incidence of hMPV infection during endemic seasons appears to vary greatly from year to year within a given region, and endemic seasons of hMPV infection and hRSV infection may not coincide [13, 14], and this may explain the observed variations in severity of bronchiolitis, which, until now, could not be accounted for by the detection of hRSV only.

CONCLUSIONS

hMPV must be viewed as a significant contributor, both in its own right and in its synergistic pathology with hRSV and other viruses, to morbidity in infants with bronchiolitis. Nonbronchoscopic collection of BAL samples would appear to be the preferred sampling method for the detection of hMPV in infants receiving mechanical ventilation. The prevalence of hMPV infection in cases of bronchiolitis may be underreported because of the pragmatic limitations to the collection of BAL samples from infants who do not require mechanical ventilation. Several clinical studies are currently investigating the immunopathogenesis of severe bronchiolitis caused by hRSV. In any given bronchiolitis season there may be several different endemic viruses. Severe disease in an individual can be understood to be a function of host response to a particular virus. To improve our understanding of host response, we must now search for multiple viral pathogens and examine the timing and sequence of viral infections. We would be wise to seek consent to archive samples, to allow for retrospective investigations of the influence of presently unknown pathogens that may confound current studies of bronchiolitis.

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