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Short communication

Association between high nasopharyngeal viral load and disease severity in children with human metapneumovirus infection

Samantha Bosis [a], Susanna Esposito [a], Albert D.M.E. Osterhaus [b], Elena Tremolati [a], Enrica Begliatti [a], Claudia Tagliabue [a], Fabiola Corti [a], Nicola Principi [a,*], Hubert G.M. Niesters [b]

[a] *Institute of Pediatrics, University of Milan, Fondazione IRCCS "Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena", Via Commenda 9, 20122 Milan, Italy*

[b] *Department of Virology, Erasmus Medical Center, University Medical Center, Rotterdam, The Netherlands*

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Abstract

Background: Previous studies have shown that viral genotype and viral load may play a significant role in the pathogenesis of viral infections.
Objectives: The aim of this study was to evaluate these aspects of hMPV infections in children and their household contacts.
Study design: Between 1 November 2003 and 31 March 2004, we prospectively studied 2060 children attending our Emergency Department for acute reasons. Nasopharyngeal swabs were collected upon enrolment and then tested with real-time PCR assays for the major viral causes of respiratory illness.
Results: Sixty children (2.9%) were infected by hMPV: 24 (1.2%) by hMPV A, 14 (0.7%) by hMPV B, 11 (0.5%) by untyped hMPV, and 11 (0.5%) by hMPV and an additional respiratory virus. There were no differences in disease presentation or in clinical or socioeconomic impact in relation to viral genotypes. HMPV viral load was significantly higher in children with lower respiratory tract involvement ($p < 0.05$), hospitalised children ($p < 0.05$), and the prevalence of secondary cases of a similar disease in the household of index cases ($p < 0.05$).
Conclusion: A high hMPV viral load correlated with disease presentation, whereas the overall clinical and socioeconomic burden caused by the two hMPV genotypes was similar.

Keywords: Human metapneumovirus; Epidemiology; Respiratory tract infections; Respiratory viruses; Children

1. Introduction

Human metapneumovirus (hMPV) is a major cause of acute respiratory infections worldwide (Van den Hoogen et al., 2001; Crowe, 2004; Broor and Bharaj, 2007). The diseases associated with hMPV infection clinically resemble those due to respiratory syncytial virus (RSV) (Greensill et al., 2003; Peiris et al., 2003; Van den Hoogen et al., 2004a; Hamelin and Boivin, 2005; Wolf et al., 2006; Williams et al., 2006; García-García et al., 2007; Manoha et al., 2007), whereas the socioeconomic impact of hMPV appears similar to that of influenza viruses (Principi et al., 2004a,b; Bosis et al., 2005).

Genetic analyses of hMPV isolates have identified two major serotypes A and B (Biacchesi et al., 2003; Van den Hoogen et al., 2004b; Chano et al., 2005; Ludewick et al., 2005; Agapov et al., 2006; Principi et al., 2006), but little is known relating genotype to their epidemiological and clinical characteristics. Previous studies of some viral infections have shown that viral load plays a greater pathogenetic role than genetic characteristics (Templeton et al., 2006; Van der Eijk et al., 2006; Gorzer et al., 2007; Schutten et al., 2007). There are few studies that evaluated the role of viral load in hMPV infection (Sarasini et al., 2006; Do Carmo Debur et al., 2007) and none of these examined the

Abbreviations: hMPV, human metapneumovirus; RSV, respiratory syncytial virus; hCoV, human coronavirus.

* Corresponding author. Tel.: +39 02 57992498; fax: +39 02 50320206.
E-mail address: Nicola.Principi@unimi.it (N. Principi).

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relationship between hMPV viral load and disease severity.

The aim of this study was to evaluate the role of genetic characteristics and viral load in hMPV infections affecting children and their households.

2. Material and methods

We prospectively studied children attending the Emergency Department of the Institute of Pediatrics, University of Milan, Italy, on Wednesdays and Sundays between 1 November 2003 and 31 March 2004. The inclusion criteria were an age of <15 years, any medical diagnosis other than surgical disease or trauma and attendance of Emergency Room no later than 24 h after disease's onset; subjects with a chronic disease increasing the risk of the complications of viral respiratory infections and those who attended the Emergency Room 24 h after disease's onset were excluded.

At enrolment the patients' demographic characteristics and medical history were recorded using standardised written questionnaires (Principi et al., 2004a,b; Bosis et al., 2005; Esposito et al., 2005). After a complete physical examination, the children were classified on the basis of the final diagnosis using well-established criteria (Behrman et al., 2004).

Nasopharyngeal samples were collected upon enrolment (within 24 h of disease onset as defined above) using Virocult swabs, and tested at the Department of Virology, Rotterdam, The Netherlands, by means of previously described real-time PCR assays for hMPV types A and B, influenza virus types A and B, RSV types A and B, adenovirus, parainfluenza viruses types 1–4, rhinoviruses, and human coronaviruses (hCoV) types 229E, OC43, NL63 and HKU1 (Van den Hoogen et al., 2001, 2003; Zambon et al., 2001; Heim et al., 2003; Fouchier et al., 2004; Maertzdorf et al., 2004; Ward et al., 2004; Watzinger et al., 2004; Bosis et al., 2005; Bosis et al., 2007; Woo et al., 2005; Templeton et al., 2006). HMPV RNA was relatively quantified in nasopharyngeal swabs. In order to standardise the quantification of hMPV RNA, a recombinant plasmid was used in serial dilutions covering a range of 6 logs. The criteria for a positive reaction were a cycle threshold of <40 cycles and a fluorescence count of >0.5. The minimum genome viral load that would allow reproducible quantification was 10 copies per reaction, corresponding to 500 copies/mL of nasopharyngeal aspirate specimen. Virologic results were not available to the clinicians.

The medical history of each child was re-evaluated 5–7 days after enrolment and every 2–3 days thereafter until the resolution of their illness, by means of interviews and clinical examinations by trained investigators using standardised questionnaires (Esposito et al., 2005). On the basis of findings at clinical examinations, illness was considered resolved when the signs and symptoms observed at enrolment were absent and when there was no additional evidence of acute infection. During these evaluations, information was also obtained regarding acute illnesses and related morbidity in their households. The children's parents or legal guardians answered questions regarding the outcome of their child's disease (e.g., final diagnosis, administered medication, hospitalization, duration of signs/symptoms, medical visits, examinations, and number of lost school days) and the involvement of other family members (e.g., diseases in households, medication, hospitalization, medical visits, number of working days lost by parents to care for themselves and their children, and the number of domestic help days required to care for the ill children). All the data were verified from medical records.

The study was approved by the Institutional Review Board of the University of Milan, Italy, and written informed consent was obtained from the parents or legal guardians of all the enrolled children.

The parametric data were compared using analysis of variance (ANOVA), and abnormally distributed or non-parametric data were analysed using the Kruskal–Wallis test. The categorical data were analysed by means of contingency analysis and the Chi-squared or Fisher's test.

3. Results

A total of 2060 children (1112 males; mean age ± S.D.: 3.46 ± 3.3 years) were enrolled. Sixty patients (2.9%) were infected with hMPV: 24 (1.2%) with hMPV A; 14 (0.7%) with hMPV B; 11 (0.5%) with untyped hMPV; and 11 with hMPV co-infection with other respiratory viruses (0.5%: hMPV A in 6 cases; hMPV B in 4; untyped hMPV in 1). Both serotypes were isolated throughout the study period. In addition to hMPV, the co-infections involved hCoV in 4 cases (36.3%), RSV in 3 (27.3%), adenovirus in 2 (18.2%), influenza virus in 1 (9.1%), and rhinovirus in 1 (9.1%). There were no differences between hMPV A, hMPV B, and untyped hMPV in the co-infection rates. For purposes of comparison, influenza viruses were detected in a total of 235 cases (11.4%), RSV in 171 (8.3%), adenovirus in 136 (6.6%), rhinoviruses in 130 (6.3%), hCoVs in 79 (3.8%), and parainfluenza viruses in 29 (1.4%).

Table 1 shows the demographic characteristics, clinical presentations, diagnostic methods, therapeutic approaches, clinical outcomes and household impact of the children with different hMPV genotypes and hMPV co-infections. hMPV isolates were predominately from children aged less than 5 years, and were mainly associated with lower respiratory tract infections. There were no correlations between hMPV genotype and clinical syndrome, clinical outcome, or the clinical and socioeconomic impact of the infection on subsequent infection in the households of infected children.

Table 2 summarises the relationship between the nasopharyngeal hMPV viral load and clinical data. Lower respiratory tract involvement was associated with a significantly higher hMPV viral load than upper respiratory tract disease ($p < 0.05$). Viral load was also significantly higher among the hospitalised children than those managed as outpatients

Table 1
Demographic characteristics, clinical presentations, diagnostic methods, therapeutic approaches, clinical outcomes, and impact on households of children with different hMPV genotypes and hMPV co-infections

Characteristics	hMPV-A ($n=24$)	hMPV-B ($n=14$)	Untyped hMPV ($n=11$)	hMPV-Co-infected ($n=11$)
Demographic data				
Gender, males (%)	16 (67)	7 (50)	5 (45)	4 (45)
Mean age ± S.D. (years)	2.2 ± 1.6	2.1 ± 1.4	2.3 ± 1.8	2.8 ± 1.4
Clinical presentation				
Axillary temperature ≧ 38 °C (%)	19 (79)	9 (64)	6 (55)	10 (91)
Common cold (%)	0 (0)	2 (14)	0 (0)	0 (0)
Pharyngitis (%)	6 (25)	3 (21)	2 (18)	5 (46)
Acute otitis media (%)	2 (8)	3 (21)	1 (9)	0 (0)
Acute bronchitis (%)	4 (17)	2 (14)	3 (28)	2 (18)
Bronchiolitis (%)	4 (17)	1 (8)	2 (18)	1 (9)
Pneumonia (%)	4 (17)	2 (14)	2 (18)	2 (18)
Gastroenteritis (%)	2 (8)	1 (8)	1 (9)	0 (0)
Fever without source (%)	2 (8)	0 (0)	0 (0)	1 (9)
Clinical outcome				
Hospitalisation (%)	4 (17)	1 (7)	1 (9)	1 (9)
Lost school days, median (range)	8 (1–15)	5 (1–10)	5 (1–10)	7 (1–15)
Pharmacological treatment				
Antibiotics	12 (50)	8 (57)	5 (45)	6 (55)
Antipyretics	15 (63)	9 (64)	5 (45)	9 (82)
Inhaled bronchodilators	5 (21)	4 (29)	2 (18)	2 (18)
Inhaled steroids	1 (4)	0 (0)	1 (9)	0 (0)
Oral steroids	2 (8)	0 (0)	1 (9)	1 (9)
Household contacts				
Disease similar to that of infected child (%)	12/85 (14)	4/47 (8)	3/39 (8)	5/41 (12)
Additional medical visits (%)	7/85 (8)	5/47 (11)	1/39 (3)	2/41 (5)
Antipyretic prescriptions (%)	8/85 (9)	4/47 (8)	3/39 (8)	5/41 (12)
Antibiotic prescriptions (%)	2/85 (2)	2/47 (4)	1/39 (3)	2/41 (5)
Hospitalisation (%)	0 (0)	0 (0)	0 (0)	0 (0)
Working days lost by parents, median (range)	3 (1–7)	3 (1–5)	2 (1–4)	3 (1–5)
School days lost by siblings, median (range)	2 (1–5)	2 (1–3)	2 (1–3)	3 (1–5)

hMPV, human metapneumovirus. No significant between-group differences.

Table 2
Relationships between hMPV viral load and clinical data in children with hMPV infection

Variable	Viral load (copies/mL)
HMPV genotype	
hMPV-A	$4.43 \times 10^5 \pm 1.50 \times 10^6$
hMPV-B	$5.16 \times 10^5 \pm 1.75 \times 10^6$
Untyped hMPV	$3.47 \times 10^5 \pm 3.58 \times 10^5$
HMPV-co-infection	$5.56 \times 10^5 \pm 1.59 \times 10^6$
Diagnosis	
URTI	$3.27 \times 10^3 \pm 5.54 \times 10^3$*
LRTI	$1.42 \times 10^6 \pm 3.40 \times 10^6$
Clinical outcome	
Hospitalised	$4.81 \times 10^6 \pm 5.46 \times 10^6$*
Not hospitalised	$7.41 \times 10^4 \pm 1.15 \times 10^5$
Impact on households	
Similar disease to that of study child	$1.76 \times 10^6 \pm 3.73 \times 10^6$*
No disease among households	$9.72 \times 10^3 \pm 1.61 \times 10^4$

URTI, upper respiratory tract infections; LRTI, lower respiratory tract infections. Mean values ± standard deviation. *$p<0.05$ *vs.* LRTI, not hospitalised, no disease among household; no other significant between-group differences.

($p<0.05$), and among those who had household contacts with a similar disease than those who did not ($p<0.05$).

4. Discussion

This study shows for the first time that nasopharyngeal viral load plays a greater role than viral genotype in the severity of hMPV infection in children, thus providing new information concerning the pathogenesis of diseases due to this emerging virus.

Our finding that hMPV A and B co-circulated in Italy during the study period, with a slight predominance of type A, is in agreement with previous findings showing that hMPV A and hMPV B can circulate simultaneously in the same winter season (Ludewick et al., 2005; Sarasini et al., 2006).

From a clinical point of view, like Agapov et al. (2006), but unlike Biacchesi et al. (2003), we found that the prevalence of upper and lower respiratory diseases in our study population, their clinical outcomes, and their drug prescriptions did not depend on the type of hMPV. Our results also indicate that both types of hMPV have a similar societal impact,

resulting from medical and/or socioeconomic costs from secondary respiratory illnesses in the households of the index cases. Like Van den Hoogen et al. (2004a,b) and Biacchesi et al. (2003), but not Greensill et al. (2003), we found that hMPV co-infections had the same clinical and socioeconomic impact as the infections due to a single hMPV strain. Furthermore, our data showing a significant correlation between nasopharyngeal hMPV load and disease severity are in line with findings of an hMPV immune response in an animal model (Huck et al., 2007), and suggest that a high viral load plays a role in clinical presentation.

A limitation of this research is that it was performed during one winter season and the study period included only 5 months. Further 12-month studies that included more than 1 year of surveillance and more hMPV positive cases should be performed to confirm our results and to establish the utility of viral load is evaluating an individual case. Moreover, patients attending an Emergency Room may not be fully representative of the disease in the community, and may select for more severe disease. Furthermore, since we determined a relative quantification of viral load, and considering that the efficiency of this method was not 100%, our measures of viral load could be underestimates. However, these limitations do not modify the observed relation between high viral load and clinical severity. In addition, there were cases in which the PCR results did not permit hMPV classification into type A or B because viral load was not high enough to obtain a good amplification product for sequencing. In our study untyped cases were 12 out of 60 (20%). However, the absence of differences between hMPV A, hMPV B, untyped cases with or without co-infections support our conclusions. Finally, although we observed a relationship between high hMPV load and presence of secondary cases among household members, which were similar to the disease in our index cases, secondary cases were not virologically studied. Consequently, these cases could have many other causes, including the co-pathogens when they were present.

In conclusion, a high viral load seems to be central in hMPV disease's presentation, whereas the overall clinical and socioeconomic burdens of the different hMPV genotypes seem to be similar.

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