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Human metapneumovirus viral load is an important risk factor for disease severity in young children

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ABSTRACT

Background: The role of viral load in human metapneumovirus (HMPV) disease severity has not yet been clearly determined.
Objective: We evaluated the importance of viral load along with other factors in HMPV disease severity among children aged <3 years old.
Study design: HMPV-positive cases were selected from a cohort of outpatients and hospitalized children with lower respiratory tract infections. HMPV groups (A or B) and viral loads were determined in their nasopharyngeal aspirates. Disease severity was defined by assessing risk for hospitalization and by using two validated clinical severity scores.
Results: Of the 118 HMPV cases detected over 4 years for which viral load could be determined, 60 belonged to genotype A and 58 to genotype B. Baseline characteristics were similar in HMPV-A and HMPV-B mono-infected patients. In multivariate analysis, HMPV hospitalization was associated with viral load ≥1000 copies/10^4 cells (OR, 3.2; 95%CI, 1.4–7.4), age <6 months (OR, 3.1; 95%CI, 1.2–8.6) and presence of ≥3 children in the household (OR, 2.7; 95%CI, 1.04–6.9). A high HMPV viral load was also associated with pulmonary rales ($p = .03$), use of bronchodilators ($p = .02$) and inhaled corticosteroids ($p = .01$).
Conclusion: HMPV viral load is associated with disease severity in young children along with young age and household crowding.

1. Background

Respiratory tract infections (RTIs) are the second leading cause of death in children under the age of 5 years worldwide [1]. The majority of RTI are thought to be caused by viruses, among which human metapneumovirus (HMPV) figures prominently [2,3]. Indeed, HMPV accounts for 5–15% of all respiratory viral infections requiring hospitalization in children compared to 40–50% for respiratory syncytial virus (RSV) [4]. The clinical presentations of HMPV and RSV infections are indistinguishable and mainly include bronchiolitis and pneumonia in infants [4]. Based on phylogenetic analysis of the F and G genes, two main genetic groups (A and B) and 5 lineages of HMPV circulate in humans [3,5]. Whether these HMPV lineages are associated with different clinical outcomes remains unresolved [6]. In that regard, several groups found no evidence for differential severity between groups [7,8], whereas others suggested more severe clinical disease associated with HMPV-A [9] or HMPV-B [10] strains. Our group previously reported that, in children aged <3 years, HMPV-B was an independent risk factor for severe disease among hospitalized patients, along with female sex and prematurity [11]. However, the influence of HMPV viral load (VL), a factor possibly involved in disease severity [12,13], was not investigated in those previous studies.

Abbreviations: RTI, respiratory tract infection; HMPV, human metapeumovirus; HMPV-Ac, human metapneumovirus group A infected cohort; HMPV-Bc, human metapneumovirus group B infected cohort; RSV, respiratory syncytial virus; PCR, polymerase chain reaction; CSS, clinical severity score; NPA, nasopharyngeal aspirate; LNA, locked nucleic acid probes; RR, relative risk; OR, adjusted odd ratio; CI, confidence interval; VL, viral load; LBW, low birth weight.

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2. Objective

We aimed to evaluate the role of VL, along with other factors, in HMPV disease severity among children aged <3 years old enrolled in a prospective study, in Quebec City, Canada.

3. Study design

3.1. Population and samples

All HMPV cases that tested positive in nasopharyngeal aspirates (NPA) by a multiplex polymerase chain reaction (PCR)/DNA microarray hybridization assay (infiniti RVP assay, Autogenomics, Carlsbad, CA) were selected from a previously described prospective study [11]. This study was approved by the Centre Hospitalier Universitaire de Québec Research Ethics Board. Participants were <3 years of age and either received medical attention at an outpatient pediatric clinic or were hospitalized at a pediatric center for acute RTI during 4 winter seasons (2006–2010), in the same district of Québec City, Québec, Canada. Eligible outpatients were required to manifest signs/symptoms of lower RTI, defined as the presence of cough and either fever (≥38 °C) or suggestive findings on auscultation (rales/wheezing). Clinical data were prospectively collected at study entry and after 1-month follow-up. Determination of HMPV groups (A or B) was confirmed by sequencing PCR products targeting the F and G genes [5]. Data regarding baseline characteristics, clinical manifestations, medical management and disease severity were extracted from standardized questionnaires. Disease severity was defined first by assessing risk for hospitalization and also by using two previously published clinical severity scores [11,14,15]. Trained nurses obtained standard NPA samples.

3.2. Clinical Severity Scores (CSS)

The severity of illness was assessed by two different clinical scores, CSS1 and CSS2. CSS1 is a scoring system initially described by Gern et al. [14], that was validated for both outpatients and inpatients. The adapted CSS1 had a maximum of 23 points (instead of 31 points in the original version) distributed as follows: 1 point each for fever, hoarseness of voice, cough, duration of illness >4 days; 2 points for rhinorrhea; 3 points for apnea; and 5 points each for wheezing, retractions and tachypnea. CSS2 is a severity index for hospitalized children adapted from Papenburg et al. [11], which assigns 1 point to each of the following items: use of supplemental oxygen (fraction of inhaled $O_2 \geq 0.3$), admission to the pediatric intensive care unit, and hospital stay >5 days. A score ≥1 indicates greater disease severity.

3.3. Determination of viral loads

To take into account several variables that can influence the quantification of HMPV in NPA, HMPV A and B VL were standardized to a fixed amount of nasopharyngeal cells. Briefly, the HMPV VL was normalized to that of RPL32, a housekeeping gene shown to be stable in the respiratory tract [16]. HMPV and RPL32 copy numbers were determined by designing two separate TaqMan™ real-time PCR (qPCR) assays using external standards on the Roche LightCycler 480™ instrument. For HMPV, a recently described RT-qPCR protocol targeting the N gene of both groups was used [17]. The lower limit of quantification was 5 copies for both HMPV groups with a linear range ≥7 log10 copies. For RPL32, fluorescent labeled Locked Nucleic Acid probes and primers were selected using the probe Finder assay design tool [16]. PCR reactions and thermal cycling conditions were performed according to the manufacturer's instructions using the LightCycler® 480 RNA Probes Master (Roche Diagnostics, Laval, Quebec, Canada). VL results were reported as number of HMPV copies per 10^4 human cells (corresponding to 10^4 copies of RPL32).

3.4. Statistical analysis

Proportions and distributions were compared using the X^2 test or the Fisher exact test whereas continuous values were analyzed using the Student's *t* test or the Wilcoxon rank-sum test. Univariate and multivariate logistic regression analyses were performed to examine the association between risk factors and disease severity. Variables with a univariate *p* value of ≤.2 and potential confounding factors were considered for inclusion in multivariable logistic regression models. Analyses were performed using Statistical Analysis Systems software, version 9.2 (SAS, Cary, NC).

4. Results

A total of 127 HMPV-positive cases were identified in the tested children (127/1039 or 12.2%). Of those, 9 cases (6 HMPV-B and 3 HMPV-A) were excluded from the analysis due to insufficient material for one and inconclusive viral loads for 8. Of the 118 remaining HMPV cases, 60 belonged to group A and 58 to group B. The co-infection rate was 18.6% (22/118) for the total HMPV cohort: while it was higher in HMPV-B cohort (HMPV-Bc) than HMPV-A cohort (HMPV-Ac), the difference was not statistically significant (15/58 [25.9%] vs 7/60 [11.7%], respectively, $p = .06$).

4.1. Baseline characteristics, disease severity and viral load according to HMPV group

Baseline characteristics of mono-infected HMPV-Ac and HMPV-Bc patients were remarkably similar (Table 1) except that the former group had a higher number of children in the household ($p = .02$). When looking at the clinical manifestations and medical management of infected children, a few differences were observed between HMPV-A and B infections (Table 2). First, signs of lower respiratory tract involvement (i.e., pulmonary rales; 74.4% vs 34%; $p < .0001$) were more frequent in HMPV-Bc patients. In keeping with this finding, HMPV-Bc received more inhaled (41.9% vs 22.6%; $p = .05$) and systemic (23.3% vs 7.5%; $p = .04$) corticosteroid therapy. However, no differences were noted between HMPV groups concerning hospitalization rate (51.2% vs 58.5%; $p = .54$) and discharge diagnoses, including pneumonia (25.6% vs 15.1%; $p = .21$) and bronchiolitis (62.8% vs 60.4%; $p = .84$). The mean CSS1 score was higher in HMPV-B than HMPV-A mono-infected children but the difference was not statistically significant (15.4 vs 14.1; $p = .13$). The same trend was noted for CSS2, with more HMPV-B infected hospitalized patients having a score ≥1 points (77.3% vs 54.8%; $p = .15$). Notably, HMPV-B mono-infected patients had a higher median VL (3.6 log vs 1.21 log copies/10^4 cells; $p = .0007$) (Table 2). In multivariate analysis, having no siblings at home (OR, 4.09; 95%CI, 1.04–6.81), VL ≥1000 HMPV copies/10^4 cells (OR, 2.62; 95%CI, 1.03–6.7) and rales on auscultation (OR, 5.19; 95%CI, 2.0–13.5) were associated with HMPV-B infection.

4.2. Risk factors for HMPV disease severity

In univariate analyses of the HMPV-infected children total cohort, the presence of heart disease, having ≥3 children in the household and a higher VL were more frequently seen among hospitalized subjects whereas daycare attendance (associated with older age) was associated with presentation to the pediatric clinic (Table 3). In the HMPV-Ac, daycare attendance was more frequent in the clinic patients and, in the HMPV-Bc, heart disease was more frequent in hospitalized children. Risk factors for CSS2 ≥1 in total