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Patient characteristics and severity of human rhinovirus infections in children

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ABSTRACT

Background: It is increasingly recognized that human rhinoviruses (HRV) can be associated with severe infections. However, conflicting results have been reported on the relative prevalence and severity of the three HRV species.
Objectives: The relative prevalence and clinical characteristics of HRV-A, B and C, in children attending a South London teaching hospital were investigated retrospectively.
Study design: Children aged <16 years with episodes of respiratory tract infections and detectable entero/rhinovirus RNA in respiratory samples between November 2009 and December 2010 were investigated. Retrospective case review was performed and patients' characteristics recorded.
Results: Entero/rhinoviruses were the commonest viral pathogens (498/2316; 21.5%). Amongst 204 infection episodes associated with entero/rhinovirus, 167 were typed HRV, HRV-C was the most prevalent (99/167, 59.3%) followed by HRV-A (60/167; 35.9%) and HRV-B (8/167, 4.8%). The severity spectrum of HRV-A and HRV-C infections were similar and affected all parts of the respiratory tract. Co-pathogens were observed in 54 (26.5%) episodes. Severity was increased in patients with non-viral co-pathogens and those with an underlying respiratory condition. Univariate and multiple regression analyses of potential prognostic variables including age, co-pathogens and underlying respiratory illnesses showed that mono-infection with HRV-C, as compared with other HRV species, was associated with more severe disease in young children <3 years.
Conclusions: HRV-C was the most prevalent species and on its own was associated with severe disease in children <3 years. The association between infection with HRV species and clinical presentation is complex and affected by many confounding factors.

1. Background

Human rhinoviruses (HRV) are respiratory picornaviruses under the extended genus of enterovirus. They have been most frequently implicated as the causative agent of common cold. However, there were recent suggestions that HRV could be associated with severe respiratory tract infections (RTI), acute asthma exacerbations [1,2], recurring wheeze [3] and lower RTI (LRTI) [4–7]. Dependent on the study, the reported incidence of HRV infections in children ranges from <10% in RTI [8] to 90% in acute asthma [9].

There are three HRV species: HRV-A, HRV-B and the newly identified HRV-C, each with multiple types (77 HRV-A, 26 HRV-B and 63 HRV-C) [10]. New types continue to be described, especially for the novel HRV-C, and typing of the new species remains challenging since very few full genomes for the proposed types are available for comparison. The results of many clinical and epidemiological studies on HRV are contradictory with no consensus on the significance of its role.

2. Objectives

In this study, we retrospectively investigated episodes of RTI with respiratory samples positive for entero/rhinoviruses over a

Abbreviations: 5′NCR, 5′noncoding region; HDU, High Dependency Unit; HMPV, human metapneumovirus; HRV, human rhinovirus; ICU, Intensive Care Unit; IQR, interquartile range; LRTI, lower respiratory tract infection; PIV, parainfluenza-viruses; RSV, respiratory syncytial viruses; RTI, respiratory tract infection; URTI, upper respiratory tract infection.

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one-year period to determine the clinical characteristics of RTI associated with different HRV species in children.

3. Study design

3.1. Patients

Respiratory samples were obtained routinely from all paediatric patients (<16 years of age) with respiratory symptoms attending the Evelina Children's Hospital in London, UK. Samples submitted between November 2009 and December 2010 were investigated for viral pathogens using a multiplex nucleic acid amplification panel (ResPlex II v2, Qiagen, November 2009–June 2010 or xTAG RVP FAST v1, Luminex, from July 2010). The nucleic acid targets of the multiplex panel consisted of influenza A and B viruses, parainfluenzaviruses 1–4 (PIV), respiratory syncytial viruses A and B (RSV), human metapneumovirus (HMPV), adenoviruses, coronaviruses, bocavirus and entero/rhinoviruses. Respiratory specimens used include nasopharyngeal aspirate, nasal swab, throat swab and bronchoalveolar lavage. Samples that tested positive for entero/rhinovirus RNA were further classified into individual enterovirus and HRV types by direct sequencing.

RTI were classified as an upper RTI (URTI) when only upper respiratory tract symptoms were present with no radiological evidence of LRTI; as an airway disease when the predominant clinical finding was of an obstructive airway disease such as asthma or bronchiolitis without evidence of pneumonia on the chest radiographs; and as LRTI when symptoms were associated with evidence of radiological changes in the chest X-ray. Disease was classified as severe when the respiratory condition warranted admission to Intensive Care Unit (ICU) or High Dependency Unit (HDU), and not severe when admitted to the general paediatric wards. To avoid clinician bias, the case note of each patient was reviewed by the authors retrospectively before the entero/rhinovirus typing results were known. Cases in which the contribution of RTI to disease severity was uncertain were further reviewed and categorized after extensive discussions between the authors. Chronic respiratory diseases, chronic heart conditions, neurological conditions, prematurity, immunosupression and other underlying medical conditions were recorded. Bacterial and fungal organisms detected in respiratory samples by culture or immunofluorescence were considered as co-pathogens if case review concluded that they were significant and not due to colonization. Organisms detected in normally sterile sites, such as blood or cerebrospinal fluid, were considered as significant unless specimen contamination was suspected. Co-infection was defined as the detection of a co-pathogen 7 days before or after the entero/rhinovirus positive sample.

3.2. Molecular analysis

RNA extraction and cDNA synthesis was performed as previously described [11]. Samples were screened by PCR with the HotStarTaq Master Mix Kit (Qiagen) targeting the 5′noncoding region (5′NCR) using primers DK001 [12] and DK004 [13] under the following conditions: 15 min at 95 °C, 30 s at 94 °C, 30 s at 53.4 °C, 30 s at 72 °C (45 cycles) and 10 min at 72 °C. Amplification of the VP4/VP2 region for typing was performed as above with primers VP4/2 F and VP4/2 R [14] or RCV556F (ACT ACT TTG GGT GTC CGT GTT TC) and RCV886R (TTT CCR ATA GTG ATT TGC TTK AGC C) with 60 °C annealing and 40 cycles or 52 °C and 40 cycles, respectively. Bidirectional sequencing was performed by LGC Genomics GmbH (Berlin, Germany) or in house (PCR product cleaning with microClean (Microzone), cycle sequencing with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on the automated sequencer 3130xl Genetic Analyzer (Applied Biosystems)). Sequence analysis was performed using the software BioEdit (version 7.0.9) and MEGA5 (version 5.03).

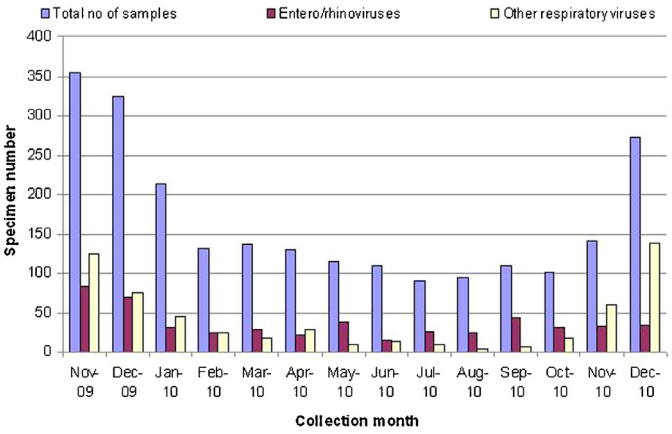

Fig. 1. Respiratory samples received during the study period and samples positive for entero/rhinoviruses and other respiratory viruses.

3.3. Statistical analysis

We used generalized linear models with binomial errors and logit link function for univariate and multiple logistic regression analysis of binary response data. The association between 11 independent variables representing clinical and demographical patient characteristics and presence of severe infection, HRV-C or co-infection (viral, non-viral or either) was explored by means of univariate analyses. We conducted multiple regression analysis to assess the significance of age (< or ≥3 years), underlying respiratory conditions, virus type (HRV-C or not) and the interaction between any two independent terms as potentially prognostic variables of severity. The significance of model terms was assessed through deletion tests. All analyses were performed in R version 2.13.2 [15]. In order to determine the importance of HRV species, all enteroviruses and untyped entero/rhinovirus episodes were excluded from statistical analyses. A P-value of <0.05 was considered as statistically significant.

4. Results

Of 2316 respiratory specimens tested in the study period (1510 ResPlex II, 806 RVP FAST), at least one respiratory virus was detected in 1065 (46.0%). The most commonly detected virus was entero/rhinoviruses (498, 21.5%), followed by RSV (267, 11.5%), influenza A viruses (88, 3.8%), bocaviruses (85, 3.7%), PIV (83, 3.6%), HMPV (71, 3.1%), coronaviruses (54, 2.3%), adenoviruses (47, 2.0%) and influenza B viruses (13, 0.6%). While most seasonal viruses like RSV and influenza A and B viruses were found mainly during winter months, entero/rhinoviruses were detected in similar numbers throughout the study period (Fig. 1).

Of the 498 entero/rhinovirus positive respiratory specimens, 248 (50%) had residual sample available for typing. In several instances multiple specimens were collected from one patient. In order to account for this, specimens belonging to the same infection episode (defined as identical virus by sequencing) in a patient were excluded. This resulted in a total of 204 episodes of entero/rhinoviral infection in 195 patients (median age 0.98 year, interquartile range (IQR) 1.79 years) of which 163 (79.9%) were in children under the age of 3 (median age 0.67 year, IQR 0.91 years). The main virus was HRV-C with 99 infection episodes (48.5%) followed by 60 HRV-A (29.4%), 15 enteroviruses (7.3%) and 8 HRV-B (3.9%). In 22 episodes (10.8%), the sequencing of VP4/VP2 failed despite the use of degenerate primers. Although 5′NCR sequences were available, they were not suitable for typing [16] and were