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Clinical and molecular epidemiology of human rhinovirus infections in patients with hematologic malignancy

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ABSTRACT

Background: Human rhinoviruses (HRVs) are common causes of upper respiratory tract infection (URTI) in hematologic malignancy (HM) patients. Predictors of lower respiratory tract infection (LRTI) including the impact of HRV species and types are poorly understood.
Objectives: This study aims to describe the clinical and molecular epidemiology of HRV infections among HM patients.
Study design: From April 2012–March 2013, HRV-positive respiratory specimens from symptomatic HM patients were molecularly characterized by analysis of partial viral protein 1 (VP1) or VP4 gene sequence. HRV LRTI risk-factors and outcomes were analyzed using multivariable logistic regression.
Results: One hundred and ten HM patients presented with HRV URTI ($n=78$) and HRV LRTI ($n=32$). Hypoalbuminemia (OR 3.0; 95% CI, 1.0–9.2; $p=0.05$) was independently associated with LRTI, but other clinical and laboratory markers of host immunity did not differ between patients with URTI versus LRTI. Detection of bacterial co-pathogens was common in LRTI cases (25%). Among 92 typeable respiratory specimens, there were 58 (64%) HRV-As, 12 (13%) HRV-Bs, and 21 (23%) HRV-Cs, and one Enterovirus 68. LRTI rates among HRV-A (29%), HRV-B (17%), and HRV-C (29%) were similar. HRV-A infections occurred year-round while HRV-B and HRV-C infections clustered in the late fall and winter.
Conclusions: HRVs are associated with LRTI in HM patients. Illness severity is not attributable to specific HRV species or types. The frequent detection of bacterial co-pathogens in HRV LRTIs further substantiates the hypothesis that HRVs predispose to bacterial superinfection of the lower airways, similar to that of other community-acquired respiratory viruses.

1. Background

Human rhinoviruses (HRVs) are traditionally considered "common cold" viruses, but they also play a substantial role in lower respiratory tract infections (LRTIs) in children, elderly, and immunocompromised hosts. HRVs are classified taxonomically within the enterovirus (EV) group and subdivided into three species: A, B and C. Across the three species are more than 100 different serotypes. Since identification and designation of HRV-C species in 2006 [1], several studies have demonstrated increased rates of asthma exacerbations and LRTIs among children with HRV-C versus HRV-A or HRV-B infections [2–4]. However, more recent data have failed to observe an association between HRV species and illness severity [5,6].

Patients with hematologic malignancy (HM) are at risk for complications of respiratory viral infections including prolonged illness, progression to LRTI, and bacterial superinfection. Understanding predictors and outcomes of HRV LRTI in HM patients including the

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effect of HRV species and type may aid in risk-stratification and empiric antibiotic use in patients with acute respiratory illness.

2. Objectives

This study aims to describe the clinical and molecular epidemiology of HRV infections among HM patients engaged in care at a New York City (NYC) hospital over a 12-month period.

3. Study design

3.1. Study population and data collection

At New York-Presbyterian/Weill Cornell Medical Center (NYP/WCMC), all symptomatic HM patients are tested by molecular methods for respiratory viruses via nasopharyngeal (NP) swab or bronchoalveolar lavage (BAL). From April 2012 to March 2013, we reviewed the NYP/WCMC Clinical Microbiology Laboratory records daily to identify adult HM patients (age $\geq$ 18 years) testing positive for HRV. We excluded patients with HM that was in remission and who had not received chemotherapy in the previous year. All hematopoietic stem cell transplant (HSCT) recipients were included if transplant occurred within the previous 12 months and/or chronic graft-versus-host disease was present. HRV-positive respiratory specimens were frozen and shipped to Wadsworth Center at the New York State Department of Health in Albany, NY for molecular characterization. Clinical data corresponding to each episode of HRV infection were abstracted from the electronic medical record.

3.2. Clinical microbiologic evaluation

During the study, the NYP/WCMC Clinical Microbiology Laboratory performed molecular testing for 17 respiratory viruses by multiplex real-time PCR (FilmArray Respiratory Panel [RP], BioFire Diagnostics, Inc., Salt Lake City, UT). All three HRV and four EV species are detected by the RP. Due to sequence homology, the RP assay does not reliably distinguish HRV from EV. For the purposes of this study, patients testing positive for the HRV/EV target on the RP assay were assumed to have HRV infection, which was confirmed by molecular testing at Wadsworth. In addition to the RP, the following diagnostic microbiology studies were performed on BAL fluid from immunocompromised patients at NYP/WCMC using standard procedures: Gram stain and bacterial culture, calcofluor white potassium hydroxide stain and fungal culture, acid-fast bacillus stain and mycobacterial culture, PCRs for *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Bordetella pertussis*, *Legionella* direct fluorescent antibody (DFA) and culture, *Pneumocystis jirovecii* DFA, and *Aspergillus* galactomannan.

3.3. Virologic methods

An aliquot of 350 μl from each respiratory sample was extracted and eluted into 110 μl on the bioMérieux easyMAG (bioMérieux, Durham, NC). Reverse transcription was performed on 10 μl of RNA using the Quanta cDNA kit with random primers (Quanta, Gaithersburg, MD). The viral protein 1 (VP1) gene sequence of HRV was initially targeted using PCR primers designed at the Centers for Disease Control and Prevention (CDC) (Unpublished, kindly provided by Dr. Dean Erdman). If this assay failed to produce a band for sequencing, a further assay with primers targeting a different region of the VP1 gene was used [7]. If the VP1 gene assays failed, the VP4 gene sequence was amplified with primers described in Coiras et al. [8]. PCR products were visualized on a 1.5% TAE gel, and purified using Affimetrix ExoZapIt on samples displaying the appropriate size products (Affimetrix Santa Clara, CA). Sequencing was performed using the PCR primers from each of the assays.

Sequences from either the VP1 or VP4 genes were first BLAST analyzed to determine the HRV type. Sequences obtained using the CDC unpublished VP1 assay were aligned to representative VP1 sequences from species A, B and C imported from GenBank using the Clustal W multiple alignment program in MEGA 6.0 [9]. The maximum likelihood phylogenetic tree was constructed using MEGA 6.0 with the neighbor-joining method for HRV-A and -B, and HRV-C. Since the sequences obtained using the Nix et al. assay (VP1) [7] and Coiras et al. assay (VP4) [8] target different regions, they were not used in the phylogenetic analysis.

3.4. Definitions

URTI was defined as having rhinorrhea, pharyngitis, or cough without clinical or radiographic evidence of lower respiratory involvement or hypoxia. LRTI was defined as having cough, dyspnea, sputum production, fever or hypoxia and new radiographic pulmonary infiltrates. LRTI was further sub-classified as (1) proven HRV LRTI when HRV was detected in BAL fluid and (2) possible HRV LRTI when bronchoscopy was not performed and HRV was detected in a NP swab. A separate URTI or LRTI episode required at least a two-week symptom-free period between episodes.

3.5. Statistical analysis

Given the conflicting literature about the effect of HRV species on illness severity, we sought statistical power to detect a difference in LRTI rates between HRV-A/HRV-B versus HRV-C if one truly existed. We hypothesized that HRV-C is associated with LRTI more often than HRV-A or HRV-B. Based on prevalence studies in other geographic locations, [3,10–13], we estimated a 1-to-1 ratio of HRV-A and HRV-B versus HRV-C infections. We predicted that 40% of HM patients would have HRV LRTI [14,15], and we estimated an absolute difference of 30% in LRTI rates between HRV-A/-B and HRV-C infections [3,4,12,15]. Therefore, a sample size of 41 subjects was needed in each group to detect a 30% difference in rates of LRTI with a 5% level of significance and 80% power.

Recognizing that HRV illness severity may also be driven by host, geographic, or temporal events, we evaluated other factors associated with HRV LRTI. We excluded patients co-infected with other respiratory pathogens from this analysis because patients with LRTI are likely to undergo more thorough microbiologic evaluation, a potential confounder. Univariable analysis was conducted using chi-square and Fisher's exact tests, as appropriate, for categorical variables; a P-value ≤ 0.05 was considered significant. Variables with a P-value ≤ 0.1 on univariable analysis were subsequently analyzed by multivariable logistic regression. Patients were followed for six months after the first HRV infection. Data were analyzed using STATA 12.1 (College Station, TX).

4. Results

4.1. Study population and respiratory co-pathogens

From April 2012 to March 2013, 110 HM patients had HRV-associated respiratory illness. HRV was the most common respiratory virus detected in symptomatic HM patients, followed by influenza H3N2 ($N = 53$), respiratory syncytial virus ($N = 37$), parainfluenza virus 3 ($N = 30$), coronavirus OC43 ($N = 24$), and human metapneumovirus ($N = 18$).

Patients were diagnosed with HRV during routine or acute outpatient visits (37%), or during inpatient admissions for chemotherapy or HSCT (16%), fever and/or respiratory symptoms (40%), or other acute reasons (6%). Forty-nine (45%) patients were