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Human rhinovirus infections of the lower respiratory tract in hematopoietic stem cell transplant recipients

S.E. Jacobs, R. Soave, T.B. Shore, M.J. Satlin, A.N. Schuetz, C. Magro, S.G. Jenkins, T.J. Walsh. Human rhinovirus infections of the lower respiratory tract in hematopoietic stem cell transplant recipients.
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Abstract: *Background.* Human rhinoviruses (HRVs) are a common cause of upper respiratory infection (URI) in hematopoietic stem cell transplant (HSCT) recipients; yet, their role in lower respiratory illness is not well understood.
Methods. We performed a retrospective chart review of HSCT recipients with HRV infection from the time molecular detection methods were implemented at our institution in 2008. Factors associated with proven or possible HRV pneumonia at the first HRV detection were evaluated by univariate and multivariate analysis. We then characterized all episodes of proven and possible HRV pneumonia from the initial HRV infection through a 1-year follow-up period.
Results. Between 2008 and 2011, 63 HSCT recipients had ≥1 documented HRV infections. At first HRV detection, 36 (57%) patients had HRV URI and 27 (43%) had proven or possible HRV pneumonia; in multivariate analysis, hypoalbuminemia (odds ratio [OR] 9.5, 95% confidence interval [CI] 1.3–71.7; $P = 0.03$) and isolation of respiratory co-pathogen(s) (OR 24.2, 95% CI 2.0–288.4; $P = 0.01$) were independently associated with pneumonia. During the study period, 22 patients had 25 episodes of proven HRV pneumonia. Fever (60%), cough (92%), sputum production (61%), and dyspnea (60%) were common symptoms. Fifteen (60%) episodes demonstrated bacterial ($n = 7$), fungal ($n = 5$), or viral ($n = 3$) co-infection. Among the remaining 10 (40%) cases of HRV monoinfection, patients' oxygen saturations ranged from 80% to 97% on ambient air, and computed tomography scans showed peribronchiolar, patchy, ground glass infiltrates.
Conclusions. HRV pneumonia is relatively common after HSCT and frequently accompanied by bacterial co-infection. As use of molecular assays for respiratory viral diagnosis becomes widespread, HRV will be increasingly recognized as a significant cause of pneumonia in immunocompromised hosts.

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Key words: human rhinovirus; viral pneumonia; hematopoietic stem cell transplantation; HSCT; lower respiratory infections

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The human rhinoviruses (HRVs) are most commonly associated with upper respiratory infection (URI); however, HRV also plays an important role in lower respiratory tract infection (LRTI). *In vitro* studies have established that HRVs are capable of infecting human respiratory epithelial cell lines and inducing pro-inflammatory cytokine and chemokine release (1). In healthy volunteers inoculated with HRV by intranasal aerosol insufflations, HRV is detected in bronchial biopsy specimens by *in situ* hybridization (2). HRVs are now recognized to have a major impact on asthma pathogenesis, including asthma development and

exacerbations (3–5). Studies of hospitalized pediatric and adult patients demonstrate that HRV infection is associated with bronchiolitis and pneumonia (6–10). Furthermore, among elderly nursing home residents, HRVs have been implicated in outbreaks of severe acute respiratory illness leading to hospitalization and even death (11, 12).

Among hematopoietic stem cell transplant (HSCT) recipients, HRVs are the most common cause of URI (13); yet, their role in LRTI is not well understood. A recent prospective study of HRV and coronavirus infections in allogeneic HSCT recipients determined that rates of progression of HRV URI to pneumonia were low; however, 2 patients died of respiratory failure with HRV as the most likely etiologic agent (13, 14). In a retrospective study of HSCT recipients with acute pulmonary infiltrates, HRVs were detected in 8 bronchoalveolar lavage (BAL) fluid specimens from 6 patients, representing 6% of all BALs performed in HSCT recipients during the study period (15). To understand further the role of HRVs in LRTI in HSCT recipients, we conducted a review of all HRV infections in our institution's HSCT population since the implementation of molecular detection methods beginning in 2008.

Methods

Study population and data collection

We identified all adult (age >17 years of age) allogeneic, syngeneic, and autologous HSCT recipients testing positive for HRV at New York–Presbyterian Hospital/Weill Cornell Medical College in New York City from March 6, 2008 to April 20, 2011. Patients who tested positive for HRV during pre-HSCT conditioning were also included. Clinical, laboratory, and radiographic data were abstracted from the existing electronic medical records. When available, lung tissue specimens from patients who underwent biopsies during episodes of proven HRV pneumonia were reviewed by one of the co-authors (C.M.). The study was approved by the Institutional Review Board at Weill Cornell Medical College.

Specimen collection and detection of HRV

At our institution, HSCT recipients with URI or LRTI symptoms are routinely screened by culture and/or molecular methods for viral infection via nasopharyngeal (NP) swab or bronchoscopy with BAL. Beginning in March 2008, our laboratory implemented reference molecular testing for respiratory viruses by the xTAG Respiratory Viral Panel (RVP) (Luminex Molecular Diagnostics, Toronto, Canada) conducted by ViraCor Laboratories (Lee's Summit, Missouri, USA). The RVP assay comprises a multiplex real-time polymerase chain reaction (PCR), followed by a multiplex target-specific primer extension step (16, 17). The RVP assay detects 10 respiratory viruses (human metapneumovirus, influenza A, influenza B, parainfluenza viruses [PIV] 1–3, respiratory syncytial viruses [RSV] A and B, adenovirus, and HRV) and 2 additional subtypes (influenza A subtypes H1N1 and H3N2). In addition to the RVP assay, all BAL specimens from HSCT recipients with suspected pneumonia are evaluated by the following microbiologic studies: bacterial Gram stain and culture, fungal Calcofluor potassium hydroxide stain and culture, acid-fast bacillus stain and culture, *Legionella* culture, *Pneumocystis jirovecii* direct fluorescent-antibody testing, and respiratory virus culture including cytomegalovirus, influenza, PIV, RSV, and adenovirus.

Definitions

URI was defined as clinical symptoms including rhinorrhea, nasal congestion, pharyngitis or cough without clinical or radiographic evidence of lower respiratory involvement or hypoxia. Pneumonia was defined as new radiographic pulmonary infiltrates in patients with signs and symptoms of LRTI, including cough, dyspnea, sputum production, and fever. A separate, subsequent pneumonia episode required at least a 2-week symptom-free period between episodes. Pneumonia was further classified as (i) *Proven HRV pneumonia* if HRV was isolated from BAL fluid, (ii) *Possible HRV pneumonia* if HRV was isolated from an NP swab and no bronchoscopy was performed, and (iii) *Non-HRV pneumonia* if HRV was not detected in the NP swab or BAL fluid during the episode of pneumonia. Neutropenia was defined as an absolute neutrophil count $\leq$500 cells/μL, and lymphopenia was defined as an absolute lymphocyte count $\leq$200 cells/μL occurring within 1 week before HRV infection. Hypoalbuminemia was defined as serum albumin $\leq$3.2 mg/dL. Invasive fungal infections were defined according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group definitions (18).

Statistical analysis

Descriptive statistics were expressed as median values, range, and interquartile range (IQR). We evaluated

factors associated with proven or possible HRV pneumonia at first detection of HRV post transplant by univariate analysis using chi-square and Fisher's exact tests as appropriate for categorical variables and Mann–Whitney *U*-test for continuous variables; *P*-value ≤0.05 was considered significant. Variables with a *P*-value ≤0.1 on univariate analysis were subsequently analyzed by multivariable logistic regression. Data were analyzed using STATA 12.1 (College Station, Texas, USA). Patients were followed up for 1 year after the first HRV infection.

Results

Study population

Between March 2008 and April 2011, 444 patients underwent HSCT (239 autologous, 202 allogeneic, and 3 syngeneic). Fifty-four (12%) patients had 1 or more HRV infections at a median of 73.5 days (IQR 14–258) post transplant. An additional 9 patients transplanted before March 2008 also had 1 or more HRV infections during the study period. Therefore, a total of 63 HSCT recipients were included. Forty-two (67%) patients received allogeneic HSCT, 20 (32%) patients received autologous HSCT, and 1 (2%) patient received syngeneic HSCT. The conditioning regimen was myeloablative in 42/63 (67%) patients. Additional patient demographics, transplant characteristics, and comorbidities are presented in Table 1. HRV infections, including URIs and proven and possible HRV pneumonias, occurred year-round with peak infection rates observed in October, March, and April (Fig. 1).

Clinical features at initial detection of HRV

The median age at initial detection of HRV post HSCT was 55 years (range 22–71). Seventeen (27%) patients were inpatients admitted for HSCT, 19 (30%) were inpatients admitted for an acute reason, and 27 (43%) were outpatients. Thirty-six (57%) and 27 (43%) patients met the clinical criteria for URI and pneumonia, respectively. None of the patients had a history of pneumonia within 30 days prior to HRV infection. Eleven (17%) patients had concurrent bacteremia.

According to the pre-specified definitions, the 27 episodes of HRV pneumonia were further sub-classified as proven HRV pneumonia (n = 14) and possible HRV pneumonia (n = 13). Nine of the 14 patients with proven HRV pneumonia and 4 of the 13 patients with possible HRV pneumonia had respiratory co-infection

Baseline characteristics of hematopoietic stem cell transplant (HSCT) recipients with ≥1 post-transplant human rhinovirus (HRV) infections

Variable	*N* = 63 (%) patients
Female	25 (40)
Median age at HSCT (range)	55 (21–71)
Primary hematologic disorder	
Acute leukemia	26 (41)
Lymphoma	20 (32)
Multiple myeloma	10 (16)
Chronic myelogenous leukemia	3 (5)
Other	4 (6)
Transplant type	
Allogeneic	42 (67)
Matched-related donor	19/42 (45)
Matched-unrelated donor	14/42 (33)
Mismatched-unrelated donor	1/42 (2)
Cord blood	8/42 (19)
Autologous	20 (32)
Syngeneic	1 (2)
Conditioning regimen	
Myeloablative	42 (67)
Reduced-intensity	21 (33)
Graft-versus-host disease (GVHD)[1,2]	21/42 (50)
Comorbidities	
Chronic obstructive pulmonary disease/Asthma	6 (10)
Other post-transplant lung disease[3]	7 (11)
Tobacco use current[4]	8 (13)
Tobacco use ever	27 (43)
Diabetes	10 (16)
History of pneumonia within previous 30 days	0
Visit type at first HRV infection	
Inpatient	36 (57)
Admission for HSCT	17 (27)
Admission for acute reason	19 (30)
Outpatient	27 (43)

[1]Includes patients with acute GVHD grade 2–4 and/or chronic GVHD.
[2]An additional 10 subjects were diagnosed with GVHD during the 1-year follow-up period.
[3]Bronchiolitis obliterans (*N* = 5), Idiopathic pulmonary fibrosis (*N* = 1), Interstitial pneumonitis (*N* = 1).
[4]Defined as tobacco use within the previous 12 months.

Table 1

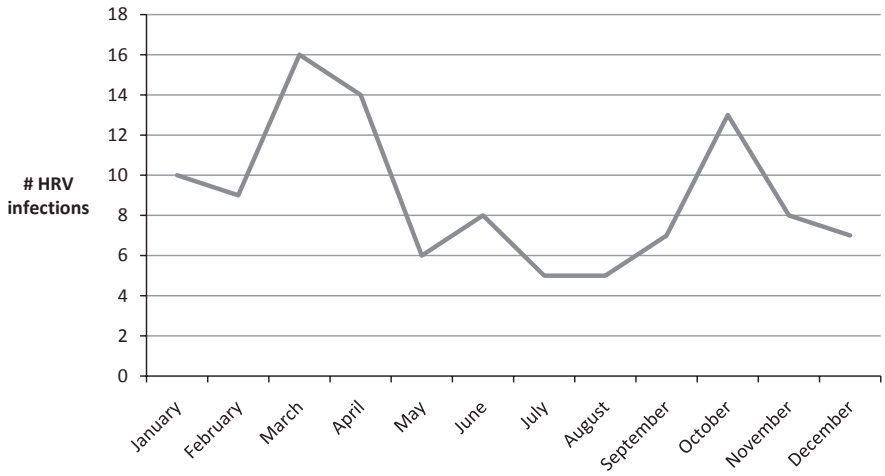


Fig. 1. Seasonal epidemiology of human rhinovirus (HRV) infections among hematopoietic stem cell transplant recipients at New York Presbyterian Hospital/Weill Cornell Medical College, 2008–2011.

with bacterial, fungal, or viral pathogens. In univariate analysis, the following variables were associated with proven or possible HRV pneumonia at the first HRV detection: neutropenia (odds ratio [OR] 4.7, 95% confidence interval [CI] 1.3–17.2; $P = 0.02$), hypoalbuminemia (OR 12.7, 95% CI 3.7–43.6; $P < 0.001$), receipt of ≥24 h of antibiotic therapy within the previous 7 days (OR 7.1, 95% CI 2.3–21.8; $P = 0.001$), and isolation of respiratory co-pathogen(s) (OR 32.5, 95% CI 3.9–272.5; $P = 0.001$). In multivariate analyses, hypoalbuminemia (OR 9.5, 95% CI 1.3–71.7; $P = 0.03$) and isolation of respiratory co-pathogen(s) (OR 24.2, 95% CI 2.0–288.4; $P = 0.01$) remained independently associated with proven or possible HRV pneumonia at the first detection of HRV post transplant (Table 2). Transplant type and intensity of conditioning regimen were not associated with proven or possible HRV pneumonia.

Subsequent episodes of pneumonia

Figure 2 outlines all episodes of pneumonia and HRV association during a 1-year period following initial detection of HRV post HSCT. Among the 36 patients who presented with HRV URI at the first HRV detection, 6 (17%) had ≥1 subsequent episodes of proven or possible HRV pneumonia, and 9 (25%) had ≥1 subsequent episodes of pneumonia in which HRV was not detected or not tested. The median time from HRV URI to the first episode of pneumonia was 61 days (range 22–310) in patients with proven or possible HRV pneumonia and 167.5 days (range 27–303) in patients with pneumonia in whom HRV was not detected or not tested.

Among the 27 patients who presented with proven or possible HRV pneumonia at the first HRV detection, 8 (30%) had ≥1 subsequent episodes of proven or possible HRV pneumonia, and 5 (19%) had ≥1 subsequent episodes of pneumonia in which HRV was not detected or not tested. The median time to the second episode of pneumonia was 53 days (range 41–253) among patients with proven or possible HRV pneumonia, and was 201 days (38–546 days) among patients with pneumonia in whom HRV was not detected or not tested.

In total, 25 episodes of proven HRV pneumonia and 26 episodes of possible HRV pneumonia occurred among 33 HSCT recipients during the study period (Table 3). One or more additional respiratory co-pathogens were detected in 47% of pneumonia episodes (bacterial $n = 12$, fungal $n = 7$, viral $n = 5$).

Proven HRV pneumonia

During the study period, 22 patients had 25 episodes of pneumonia in which HRV was detected in BAL fluid. Among these 22 patients with proven HRV pneumonia, 82% were allogeneic HSCT recipients, of which 61% had graft-versus-host disease (GVHD), and 84% had received antibiotics within the previous 7 days. Fever (60%), cough (92%), sputum production (61%), and dyspnea (60%) were common symptoms. In 8 proven HRV pneumonia episodes, an upper respiratory tract sample was analyzed within the previous 2 weeks, and HRV was detected in all specimens.

Another respiratory pathogen was identified in 12 (48%) of 25 BALs. Three additional patients met criteria

Factors associated with proven or possible human rhinovirus (HRV) pneumonia at first post-hematopoietic stem cell transplant (HSCT) HRV detection

Variable	HRV URI only, *N* = 36 (%)	HRV pneumonia[1], *N* = 27 (%)	Univariate odds ratio (95% CI)	Univariate *P*-value	Multivariate odds ratio (95% CI)	Multivariate *P*-value
Median age in years at HSCT (range)	54 (25–70)	55 (21–65)	–	0.9		
Female	16 (44)	9 (33)	0.6 (0.2–1.8)	0.4		
Transplant type						
Allogeneic	25 (69)	17 (63)	1.0	–		
Autologous	11 (31)	9 (33)	1.2 (0.4–3.5)	0.7		
Syngeneic	0	1 (4)	–	0.4		
Donor status						
Matched-related	14/25 (56)	6/18 (33)	1.0	–		
Matched-unrelated	8/25 (32)	6/18 (33)	1.8 (0.4–7.3)	0.5		
Mismatch-unrelated	0	1/18 (6)	–	0.3		
Cord blood	3/25 (12)	5/18 (28)	3.8 (0.7–21.7)	0.2		
Conditioning regimen						
Myeloablative	23 (64)	19 (70)	1.0	–		
Reduced-intensity	13 (36)	8 (30)	0.8 (0.3–2.2)	0.8		
Graft-versus-host disease (GVHD)[2]	11 (31)	10 (37)	1.3 (0.5–3.8)	0.6		
Corticosteroid use	9 (25)	12 (44)	2.4 (0.8–7.0)	0.1	4.5 (0.9–24.1)	0.08
Relapsed disease	3 (8)	3 (11)	1.4 (0.3–7.4)	0.7		
Comorbidities						
COPD/asthma	4 (11)	2 (7)	0.6 (0.1–3.8)	0.6	0.4 (0.03–5.8)	0.5
Tobacco use within past 12 months	7 (19)	1 (4)	0.2 (0.02–1.4)	0.1		
Tobacco use ever	15 (42)	12 (44)	1.1 (0.4–3.1)	0.8		
Diabetes	6 (17)	4 (15)	0.9 (0.2–3.4)	0.8		
HRV infection within 100 days of HSCT	19 (53)	12 (44)	0.7 (0.3–1.9)	0.5		
Neutropenia (<500 cells/μL)	4 (11)	10 (37)	4.7 (1.3–17.2)	**0.02**	0.8 (0.1–10.0)	0.8
Lymphopenia (<200 cells/μL)	7 (19)	11 (41)	2.8 (0.9–8.8)	0.07	1.2 (0.1–12.7)	0.9
Albumin <3.2 mg/dL	14 (40)	22 (82)	12.7 (3.7–43.6)	**<0.001**	9.5 (1.2–71.7)	**0.03**
Concurrent bacteremia[3]	5 (14)	8 (30)	2.6 (0.7–9.2)	0.1	0.8 (0.1–6.1)	0.9
Antibiotic therapy within previous 7 days	9 (25)	19 (70)	7.1 (2.3–21.8)	**0.001**	2.6 (0.4–15.6)	0.3
Co-infection with ≥1 additional respiratory co-pathogens[4]	1 (3)	13 (48)	32.5 (3.9–272.5)	**0.001**	24.2 (2.0–288.4)	**0.01**

Bold values are significant.
[1]Includes 14 cases of proven HRV pneumonia and 13 cases of possible HRV pneumonia.
[2]Includes patients with acute GVHD grade 2–4 and/or chronic GVHD.
[3]Coagulase-negative staphylococcal bacteremia was defined as 2 positive blood cultures drawn within 72 h of each other.
[4]Respiratory co-pathogens: *Stenotrophomonas maltophilia* (2 cases); Influenza A; *Escherichia coli; S. maltophilia, Acinetobacter baumannii;* Vancomycin-resistant *Enterococcus faecium; Haemophilus influenzae;* Parainfluenza virus 3 (3 cases); Possible fungal pneumonia; Proven fungal pneumonia; Respiratory Syncytial Virus A; *Pseudomonas aeruginosa.*
URI, upper respiratory infection; CI, confidence interval; COPD, chronic obstructive pulmonary disease.

Table 2

for proven, probable, and possible fungal pneumonia. Although the numbers were small, compared with patients with HRV alone detected in BAL fluid, patients with bacterial, fungal, or viral respiratory co-infection were not significantly different in terms of age at HSCT, transplant type, conditioning regimen, GVHD, or

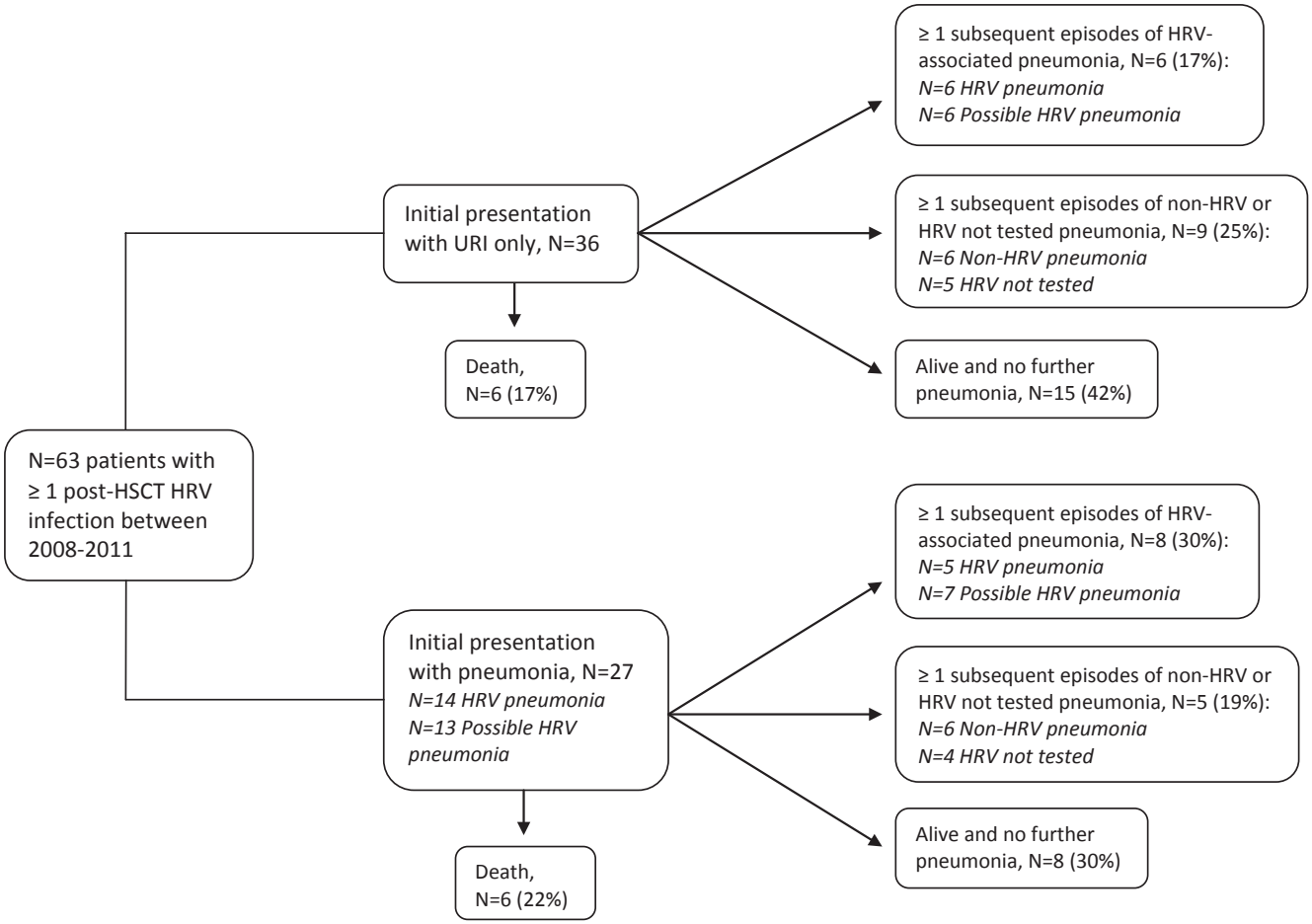


Fig. 2. Flow diagram of all pneumonia episodes and human rhinovirus (HRV) association in patients with ≥1 post-hematopoietic stem cell transplant (HSCT) HRV infection. URI, upper respiratory infection.

receipt of antibiotics within the previous 7 days (data not shown).

Computed tomography images and radiology reports from 8 of the 10 patients with HRV monoinfection in BAL fluid were reviewed by 2 of the study investigators (S.E.J. and T.J.W.). In all 8 patients, pulmonary infiltrates were bilateral and involved 3 or more lung segments. The most common infiltrate appearance was patchy ground glass opacities (n = 5 cases); in 4 of the 5 cases, some of these opacities became more confluent to form consolidative lesions (Fig. 3). Small nodular consolidations with surrounding ground glass were the predominant appearance of HRV pneumonia in 2 additional cases. In all of the above 7 cases, infiltrates aggregated in a peribronchiolar distribution. Finally, 1 patient who presented from home to the emergency department with acute respiratory distress after a week of fever and upper respiratory symptoms had dense bilateral multilobar consolidations. Among all 10 patients, oxygen saturations ranged from 80% to 97% on room air, with 7 patients having an oxygen saturation <93% and 1 patient requiring mechanical ventilation.

During the study period, 5 tissue specimens obtained via transbronchial biopsy during episodes of proven HRV pneumonia were available for review (Fig. 4). Bacterial co-infection was present by culture in BAL fluid in 2 cases (*Escherichia coli* and *Staphylococcus aureus*), fungal co-infection was present in 1 case (*Pneumocystis* pneumonia), viral co-infection was present in 1 case (RSVA), and the fifth case had no evidence of bacterial, fungal, or viral co-infection. The 2 cases of HRV and bacterial co-infection showed an acute neutrophil-rich necrotizing bronchitis and interstitial pneumonitis, with cytopathic changes including effaced nuclear chromatin with preserved nuclear membrane and ciliocytophthoria primarily observed in bronchial epithelium. The case of HRV and *Pneumocystis*

Characteristics of subjects with proven or possible human rhinovirus (HRV) pneumonia

Variable	Proven HRV pneumonia, *N* = 22 (%)[1]	Possible HRV pneumonia, *N* = 19 (%)[2]
Median age in years (range) at HSCT	50 (22–65)	55 (24–66)
Median days HSCT to first HRV detection (IQR)	34 (10.5–337)	100 (11–292)
Median days HSCT to HRV pneumonia (IQR)	106 (20–346)	154 (21.5–313)
Transplant type		
Allogeneic	18 (82)	12 (63)
Autologous	4 (18)	7 (37)
Conditioning regimen		
Myeloablative	16 (73)	15 (79)
Reduced-intensity	6 (27)	4 (21)
GVHD at time of HRV detection	11/18 (61)	8/12 (67)
Relapse of underlying hematologic malignancy	2 (10)	3 (16)
COPD/Asthma	1 (5)	2 (11)
Antibiotic therapy within previous 7 days	21/25 (84)	15/26 (58)
Signs and symptoms[3]		
Fever (≥38.0°C)	15 (60)	15 (58)
Cough	22/24 (92)	26 (100)
Sputum production	14/23 (61)	16/22 (73)
Dyspnea	16 (64)	16 (62)
Hypoxia (oxygen saturation <95% on room air)	15 (60)[4]	8 (31)[4]
Laboratory markers[3]		
Neutropenia (<500 cells/μL)	5 (20)	10 (38)
Lymphopenia (<200 cells/μL)	12 (48)	10 (38)
Albumin <3.2 mg/dL	24 (96)	21 (81)
CT scan characteristics in patients with HRV mono-infection[5]		
Multilobar (≥2 lung segments)	8 (100)	12 (80)
Interstitial infiltrates	1 (13)	0
Patchy ground glass infiltrates	5 (63)	5 (33)
Peribronchiolar infiltrates	7 (88)[4]	5 (33)[4]
Consolidation with air bronchograms	5 (63)	10 (67)
Nodular infiltrates with surrounding ground glass	2 (25)	2 (13)
Respiratory co-pathogen(s) detected[3]	15 (60)[4]	8 (31)[4]
Bacterial co-pathogens	*Stenotrophomonas maltophilia* (2 cases); Methicillin-resistant *Staphylococcus aureus; Escherichia coli; S. maltophilia, Acinetobacter baumannii; Corynebacterium pseudodiphtheriticum*	*Pseudomonas aeruginosa*; *Escherichia coli*, *P. aeruginosa*; *P. aeruginosa*; *Haemophilus influenzae*; Vancomycin-resistant *Enterococcus*
Viral co-pathogens	Parainfluenza virus 3 (2 cases); Respiratory syncytial virus A	Parainfluenza virus 3; Influenza A

Table 3 Continued

Variable	Proven HRV pneumonia, $N = 22$ (%)[1]	Possible HRV pneumonia, $N = 19$ (%)[2]
Fungal co-pathogens	*Pneumocystis jirovecii* (2 cases); *Aspergillus calidoustus;* Proven fungal pneumonia;[6] Probable fungal pneumonia;[7] Possible fungal pneumonia	*Hormographiella aspergillata*

[1]A total of 25 episodes of proven HRV pneumonia in 22 patients.
[2]A total of 26 episodes of possible HRV pneumonia in 19 patients.
[3]During $N = 25$ and $N = 26$ episodes of proven and possible HRV pneumonia, respectively; denominator indicated where data are missing.
[4]$P = 0.05$, 0.03, and 0.05 for comparison of hypoxia, peribronchiolar infiltrates, and co-pathogen(s) detected, respectively, using Fisher's exact test.
[5]Among 10 episodes of proven HRV pneumonia and 18 episodes of possible HRV pneumonia in which HRV was the sole pathogen detected, there were 8 and 15 CT scans available for review, respectively.
[6]Proven fungal pneumonia criteria met: bilateral pulmonary infiltrates, skin biopsy with angioinvasive septated hyphal forms.
[7]Probable fungal pneumonia criteria met: positive result for *Aspergillus* antigen in ≥2 blood samples, lower respiratory tract fungal disease (dense, well-circumscribed lesions without a halo sign).
HSCT, hematopoietic stem cell transplantation; IQR, interquartile range; GVHD, graft-versus-host disease; COPD, chronic obstructive pulmonary disease; CT, computed tomography.

Table 3

co-infection showed a type II pneumocyte hyperplasia with some cellular atypia and an interstitial pneumonitis. Multiple aggregates of characteristic *Pneumocystis* organisms were also seen in the alveolar spaces. The case of HRV and RSVA co-infection demonstrated subacute interstitial pneumonitis with type II pneumocyte hyperplasia unassociated with a significant inflammatory host response. In the fifth case, where HRV alone was isolated in BAL fluid, histopathologic features included slightly prominent pneumocytes and interstitial fibrin deposition, suggesting capillary injury. Cytoplasmic inclusions, multinucleation, and megaloblastic changes were not present in any of the 5 specimens.

Mortality

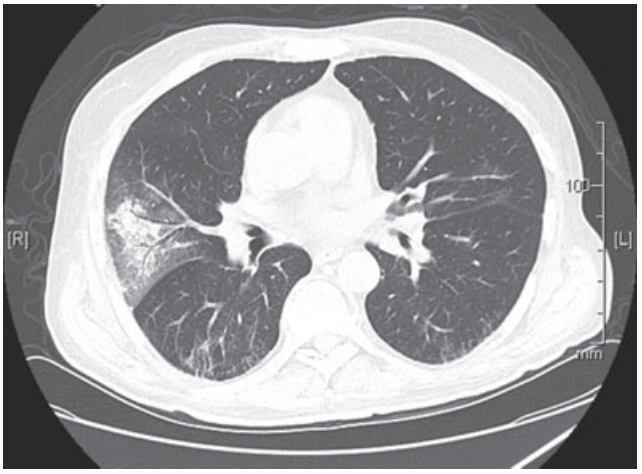
Overall mortality in the total cohort of 63 patients was 38% during the 1 year following the first HRV infection. Among patients who presented with proven HRV pneumonia ($n = 14$), possible HRV pneumonia ($n = 13$), and HRV URI ($n = 36$) at the first HRV detection, 3-month and 1-year mortality was 21% and 50%, 38% and 38%, and 8% and 33%, respectively. Among patients whose first pneumonia was non-HRV or HRV not tested pneumonia ($n = 10$), 3-month and 1-year mortality was 0% and 20%, respectively. Pneumonia was the immediate cause of death in 3 patients; microbiologic data for these patients were as follows: HRV, *Stenotrophomonas maltophilia, Acinetobacter baumannii* detected in BAL fluid ($n = 1$), HRV detected in NP swab, and bloodstream infection with *A. baumannii* (bronchoscopy not performed) ($n = 1$), and HRV detected in NP swab and vancomycin-resistant *Enterococcus faecium* detected in BAL fluid (BAL fluid not sent for RVP testing) ($n = 1$).

Outcomes in patients with pre-transplant HRV infection

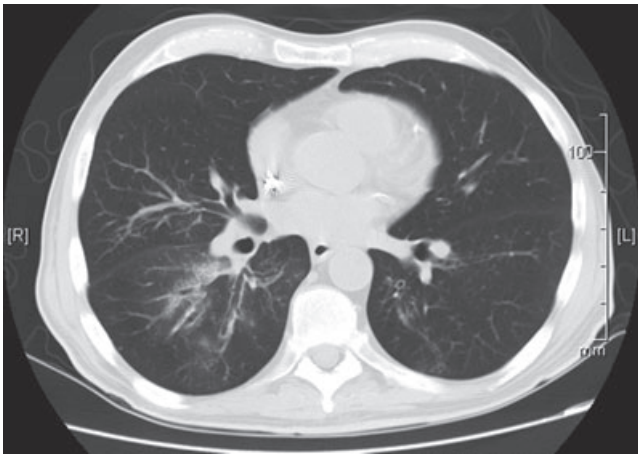
During the study period, 3 allogeneic HSCT recipients had symptomatic HRV infection during pre-transplant conditioning. One patient progressed to proven HRV pneumonia 16 days after HSCT. Her symptoms resolved within 2 weeks; ultimately, she died 6 months post HSCT from relapsed acute myelogenous leukemia. One patient had persistent URI symptoms and HRV positivity for 3 months post HSCT; he was alive at 1 year after the first HRV infection. The third patient resolved his pre-HSCT URI symptoms and had no further episodes of URI or pneumonia during the next year.

Discussion

Since molecular methods of respiratory virus testing were implemented at our institution in 2008, a total of 63 HSCT recipients have had at least 1 HRV-associated acute respiratory illness, including 51 episodes of



Patient 4



Patient 19

Fig. 3. Representative computed tomography scans from 2 patients with pneumonia and human rhinovirus alone detected in bronchoalveolar lavage fluid. Infiltrates are bilateral, focal, and peribronchiolar.

proven or possible HRV pneumonia. HRV infection rates peaked during the fall and early spring, consistent with the seasonal epidemiology observed in other studies of temperate climates (19). Furthermore, 4% of all patients undergoing HSCT between March 2008 and April 2011 were diagnosed with HRV infection during their inpatient transplant admission; nearly half of these patients had pneumonia. To our knowledge, this is the largest described cohort of HSCT recipients with HRV-associated LRTI. We suspect that as use of multiplex PCR assays for diagnosis of respiratory viral infections becomes widespread, HRV will be increasingly recognized as a significant cause of pneumonia in immunocompromised hosts.

In univariate analysis, we found that markers for increased immunosuppression and illness severity including neutropenia, hypoalbuminemia, receipt of antibiotics, and infection with respiratory co-pathogen(s) were associated with pneumonia at first post-transplant detection of HRV. Notably, in multivariate analyses, the presence of 1 or more additional respiratory pathogens remained independently associated with pneumonia. Furthermore, among all 25 episodes of proven HRV pneumonia, 60% demonstrated bacterial, fungal, or viral co-infection. These findings are consistent with previous reports of HSCT recipients with pneumonia and HRV detection in BAL fluid that have also identified bacterial or fungal co-infection in the majority of cases (15, 20). Among immunocompetent adults and children, HRVs have been detected in up to 17% of episodes of community-acquired pneumonia using PCR (21, 22), often with concomitant bacterial infection. Several potential mechanisms through which HRV increases susceptibility to bacterial infection have been demonstrated *in vitro*. For example, HRVs stimulate *Streptococcus pneumoniae* adhesion to human tracheal epithelial cells via increases in platelet-activating factor-receptors (23), promote *S. aureus* internalization into non-fully permissive cultured pneumocytes (24), and disrupt epithelial cell barrier function by dissociation of zona-occludens 1 from the tight junction complex, thereby facilitating transmigration of bacteria (25). Therefore, HRVs may be similar to influenza in predisposing to bacterial superinfection. Other mechanisms by which influenza increases susceptibility to bacterial infection, including airway epithelial damage, surface receptor changes, and altered neutrophil function, warrant further study in models of HRV infection.

Viral co-infection was uncommon in this study, occurring in 5 (9%) episodes of proven or possible HRV pneumonia (Influenza A [$n = 1$], PIV3 [$n = 3$], RSVA [$n = 1$]). This is notable because all patients were tested for a panel of 10 respiratory viruses using multiplex PCR during each HRV infection, and therefore, the likelihood of missed cases of viral co-infection was low. Our findings are consistent with those of Greer et al. (26), who showed a statistically significant reduction in viral co-detection compared with other respiratory viruses, perhaps because of mediation of interferon-stimulating genes, thus inducing a protective antiviral state.

Our study also identified 10 cases of pneumonia in which HRV was the sole pathogen identified in BAL fluid. To date, little research has been performed on the pathogenesis of HRV pneumonia. However, studies focused on the mechanism of HRV-induced exacerbations of chronic lung disease, including asthma (27–29)

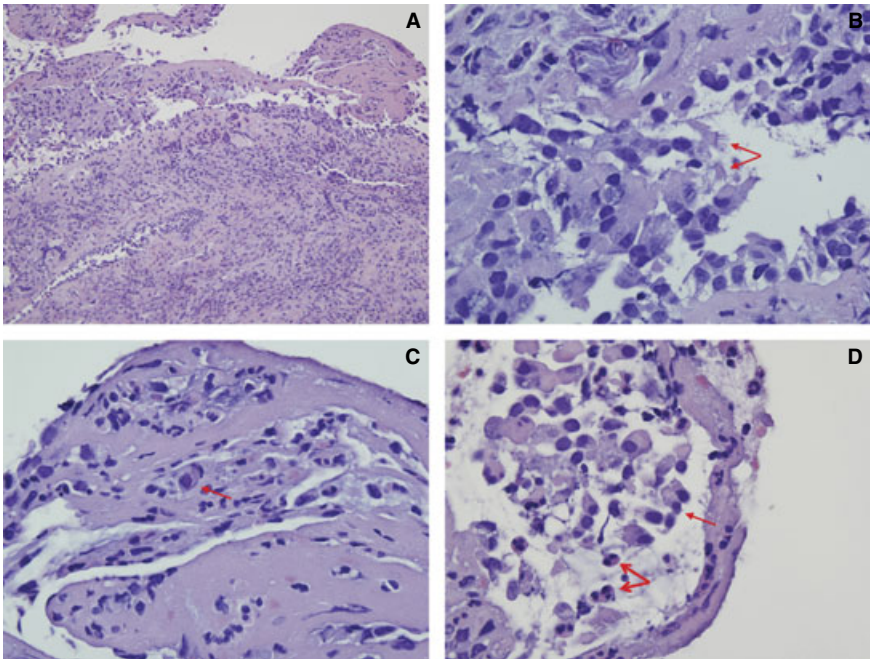


Fig. 4. Trans-bronchial biopsy specimens during 5 episodes of human rhinovirus pneumonia were reviewed. (A) There is marked inflammation in the bronchial wall with degenerative epithelial changes. The dominant inflammatory cell infiltrate is neutrophilic in nature (hematoxylin-eosin [H-E] stain, original magnification ×200). (B) There is ciliocytophthoria characterized by detached tufts of cilia separated from the remainder of the bronchial cell, a finding suggestive of a viral infection (34) (arrows) (H-E stain, original magnification ×1000). (C) The chromatin is effaced and appears eosinophilic. These cytopathic changes are consistent with viral infection (arrow) (H-E stain, original magnification ×400). (D) There is marked bronchial inflammation. The bronchial epithelial cells are detached (thin arrow). The chromatin is largely effaced. The nuclei have a homogeneous amphophilic quality likely indicative of viral effect (thick arrows) (H-E stain, original magnification ×1000).

and chronic obstructive pulmonary disease (COPD) (30–32), may provide insights into the pathophysiology of HRV pneumonia. For example, in patients with asthma, impaired innate and adaptive immune responses, including deficient interferon production (29, 33) and augmented T-helper (Th)2 or impaired Th1 or Interleukin-10 immunity (27), have been implicated in increased bronchial inflammation and hyperreactivity. Through a similar mechanism identified in patients with asthma and COPD, HRV may potentially cause parenchymal destruction through direct cell injury as well as altered immune responses in immunocompromised hosts.

Although a limited number of our patients had lung tissue obtained during episodes of proven HRV pneumonia, the predominant histopathologic findings are associated with viral, rather than bacterial or fungal, infection. HRV infection appears to have 2 histological components: a necrotizing bronchitis and an interstitial process. These findings included a striking necrotizing bronchitis including ciliocytophthoria of bronchial epithelial cells (34) and an effaced nuclear chromatin amidst bronchial epithelial cells, although without unequivocal intranuclear and/or cytoplasmic inclusions. Similar nuclear changes were not seen within the alveolar pneumocytes. In the few reported cases of HRV LRTI with human histology, HRV is capable of causing both interstitial and alveolar processes. In these reports, pathologic findings included bronchiolitis obliterans with organizing pneumonia (14), interstitial pneumonitis (20), acute and chronic inflammation with fibrinopurulent alveolar debris (35), and hyperplasia and desquamation of alveolar cells (36). These findings collectively contribute to our understanding of the pathogenesis of pulmonary HRV infection.

This study has several limitations. First, as our data are observational and rely on clinical specimens, some patients with HRV-associated acute respiratory illness may have been missed if they were not tested for HRV. Missed cases of HRV-associated acute respiratory illness would be more likely to occur with mild URI than pneumonia, because most HSCT recipients with pneumonia are hospitalized and undergo thorough

microbiologic evaluation. Therefore, we cannot infer the relative frequency with which HRV causes pneumonia compared to URI in the HSCT population. Concern may be raised that HRV detection in the lower airways reflects colonization rather than infection. However, the same argument could be made for other respiratory viruses, such as influenza A, PIV3 and RSV, which are known to cause both URI and LRTI. Several studies have demonstrated HRV replication in primary human airway epithelial cells (37, 38). Moreover, the ability of HRVs to replicate in bronchial epithelium after experimental upper airway infection of healthy human volunteers has been demonstrated using *in situ* hybridization (2). Another limitation is that molecular testing was not routinely performed for certain viral (coronavirus, PIV4, and human bocavirus) and atypical pathogens (*Chlamydophila pneumonia* and *Mycoplasma pneumonia*) during the study period, potentially missing additional cases of HRV co-infection.

Of note, owing to sequence homology between human enterovirus (HEV) and HRV, the RVP molecular methods may not reliably distinguish HEV and HRV. However, because tests of clinical performance characteristics of the RVP assay found that 42 of 43 specimens testing positive for HEV/HRV were confirmed to be HRV (personal communication with the manufacturer), the specimens identified as HRV in our patients also have a high probability of being HRV. This conclusion is also consistent with 2 recent studies in which 100% of clinical specimens (n = 164) positive for HRV/HEV by RVP were subsequently confirmed positive for HRV and negative for HEV upon further molecular testing (39, 40).

There may be other risk factors for HRV pneumonia that we were unable to ascertain in this study. In 2006, molecular methods showed a significant genotypic variation in HRV and identified a novel HRV species named HRV-C (41). In hospitalized patients, HRV-C may be associated with wheezing, pneumonia (10, 42), and viremia (43) more often than HRV-A and HRV-B. However, rates of LRTI vary considerably among the 3 HRV species owing to different patient populations, primary outcomes, and geographic study locations (44, 45). In addition, HRV viral load may correlate with symptom severity in hospitalized, immunocompetent pediatric and adult patients, asthmatics, and lung transplant recipients (27, 46, 47). Because of unavailability of specimens and limitations of the testing methodology, we were unable to determine the HRV strain or quantify viral load in the current study to evaluate whether these factors may identify patients at risk for severe or recurrent infection.

In conclusion, this study provides definitive evidence that HRVs are a significant lower respiratory tract pathogen in HSCT recipients. Given the high morbidity and mortality associated with pneumonia in HSCT recipients, a better understanding of the molecular epidemiology and distinguishing clinical features of HRVs is needed in patients with underlying hematologic malignancies. Knowledge that certain strains and species of HRV are associated with severe respiratory infection in patients with hematologic malignancy may aid in risk stratification of patients with HRV URI before transplantation and improve infection control practices in hospitalized patients. A study of the molecular epidemiology of HRV infection in patients with hematologic malignancy and HSCT recipients is ongoing at our institution.

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