Prospective Evaluation for Respiratory Pathogens in Children With Sickle Cell Disease and Acute Respiratory Illness

Ashok Srinivasan, MD,[1,2]* Winfred C. Wang, MD,[2,3] Aditya Gaur, MD,[2,4] Teresa Smith, BS,[5] Zhengming Gu, PhD,[6] Guolian Kang, PhD,[7] Wing Leung, MD, PhD,[1,2] and Randall T. Hayden, MD[6]

Background. Human rhinovirus (HRV), human coronavirus (hCoV), human bocavirus (hBoV), and human metapneumovirus (hMPV) infections in children with sickle cell disease have not been well studied. **Procedure.** Nasopharyngeal wash specimens were prospectively collected from 60 children with sickle cell disease and acute respiratory illness, over a 1-year period. Samples were tested with multiplexed-PCR, using an automated system for nine respiratory viruses, *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and *Bordetella pertussis*. Clinical characteristics and distribution of respiratory viruses in patients with and without acute chest syndrome (ACS) were evaluated. **Results.** A respiratory virus was detected in 47 (78%) patients. Nine (15%) patients had ACS; a respiratory virus was detected in all of them. The demographic characteristics of patients with and without ACS were similar. HRV was the most common virus, detected in 29 of 47 (62%) patients. Logistic regression showed no association between ACS and detection of HRV, hCoV, hBoV, hMPV, and other respiratory pathogens. Co-infection with at least one additional respiratory virus was seen in 14 (30%) infected patients, and was not significantly higher in patients with ACS ($P = 0.10$). Co-infections with more than two respiratory viruses were seen in seven patients, all in patients without ACS. Bacterial pathogens were not detected. **Conclusion.** HRV was the most common virus detected in children with sickle cell disease and acute respiratory illness, and was not associated with increased morbidity. Larger prospective studies with asymptomatic controls are needed to study the association of these emerging respiratory viruses with ACS in children with sickle cell disease. Pediatr Blood Cancer 2014;61:507–511.
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Key words: children; respiratory virus; sickle cell disease

INTRODUCTION

The primary causes of acute chest syndrome (ACS) have been reported as pulmonary fat embolism, infarction, and single- or mixed-pathogen infections [1]. In a study by the National Acute Chest Syndrome Study group, 27 different infectious pathogens were identified in 671 episodes of ACS in 538 patients with sickle cell disease [1]. *Chlamydophila pneumoniae* was the most frequent, followed by *Mycoplasma pneumoniae*, respiratory syncytial virus (RSV), coagulase-positive *Stapyhylococcus aureus*, *Streptococcus pneumoniae*, *Mycoplasma hominis*, and parvovirus. *C. pneumoniae* [2,3], *M. pneumoniae* [4], and influenza [5], have been well recognized as pathogens associated with ACS in other studies. However, these studies did not use molecular methods of detection to study infections by human rhinovirus (HRV), human coronavirus (hCoV), human bocavirus (hBoV), human enterovirus (hEV), and human metapneumovirus (hMPV). HRV [6] and hCoV [7] infections are common in children, and associated with hospitalization for acute respiratory illness. However, little is known about the clinical impact of infections from these and other emerging respiratory viruses in children with sickle cell disease.

We hypothesized HRV to be the most common respiratory virus detected in this population, and that co-infection with multiple viruses predisposed patients to ACS. To evaluate this, we prospectively analyzed respiratory samples from children with sickle cell disease diagnosed with an acute respiratory illness for HRV, hCoV, hBoV, hEV, human adenovirus (hADV), hMPV, parainfluenza virus (PIV), RSV, influenza, *C. pneumoniae*, *M. pneumoniae*, and *Bordetella pertussis* using multiplexed-polymerase chain reaction (PCR).

PATIENTS AND METHODS

Children ≤18 years with sickle cell disease, diagnosed with an acute respiratory illness from the in-patient units and out-patient clinics at St. Jude Children's Research Hospital (SJCRH) were eligible for enrollment in this prospectively conducted cohort study. Clinical characteristics of patients with and without respiratory virus, and clinical characteristics and distribution of respiratory viruses in patients with and without ACS were evaluated. The duration of the study was for one year from October 2010 through September 2011. The study was approved by the SJCRH institutional review board and a waiver of consent obtained.

Nasopharyngeal wash samples were collected as ordered by the treating physician based on the presence of symptoms of acute respiratory illness at the time of initial presentation. Nasopharyngeal wash was obtained using a pre-saline filled syringe aspiration kit (N-Pak, Annandale, MN). A minimum of 0.5 ml of aspirate was collected and transported immediately to the SJCRH microbiology

laboratory on ice. Diagnostic studies were performed with a respiratory panel which included testing for PIV 1–3, RSV, influenza A, influenza B, hADV, and hMPV. The results were available to the physicians after reporting. An aliquot from the sample remaining after completion of clinical diagnostic testing (aliquot A) was tested by multiplexed-PCR as previously described [8] after removal of patient identifiers. Physicians were blinded to the results of testing on aliquot A. Data from the research study were not used for clinical care.

Testing on aliquot A was performed using an automated broadly multiplexed PCR system (FilmArray, BioFire Diagnostics, Inc., Salt Lake City, UT), integrating specimen processing with nested multiplexed-PCR. This system enables simultaneous detection of HRV, hCoV-229E, hCoV-HKU1, hCoV-OC43, hCoV-NL63, hBoV, hEV, PIV 1–4, RSV, influenza A, influenza AH1, influenza AH12009, influenza AH3, influenza B, hADV, hMPV, *M. pneumoniae*, *C. pneumoniae*, and *B. pertussis*.

Definitions

Acute respiratory illness was defined as the presence of two or more of the following symptoms: fever, rhinorrhea, cough, nasal congestion, otitis media, and pharyngitis. Patients with chest pain, temperature more than 38.5°C, tachypnea, wheezing, or cough, and a new pulmonary infiltrate on chest radiography involving at least one complete lung segment that was consistent with the presence of alveolar consolidation but excluding atelectasis, were diagnosed as having ACS. Patients without ACS had no lung findings on examination. The day of infection onset was defined as the day when the first positive sample was collected. Patients were considered infected by a respiratory virus if it was detected by multiplexed-PCR.

Clinical Analyses

Clinical data were abstracted from a prospectively collected database that included patient demographics, past medical history of ever having suffered from vaso-occlusive crises, ACS or asthma, use of hydroxyurea, results of the most recent hemoglobin electrophoresis, and presence or absence of ACS, radiological findings, co-pathogens, need for oxygen therapy, treatment received, hemoglobin and white blood cell count at presentation, duration of in-patient stay, and admission to the intensive care unit with the acute respiratory illness.

Statistical Analyses

Descriptive statistics of patients with and without respiratory virus, and patients with and without ACS were provided. The prevalences of respiratory viruses in patients with and without ACS were included. Fisher's exact test and Mann–Whitney's test were used to compare patients with and without respiratory virus, and with and without ACS for the categorical and continuous variables, respectively. Fisher's exact test was used to study the association of individual viruses and co-infections with ACS. Exact McNemar test was used for comparison of the frequency of HRV and the other eight viruses. The first sample that tested positive for any virus was used for this purpose. The first episode of acute respiratory illness was used for analysis, whether or not a virus was isolated. Univariate logistic regression analysis was performed to determine

TABLE I. Demographics and Clinical Characteristics of Patients With Sickle Cell Disease

| Characteristic | Overall (n = 60) | Patients without virus (n = 13) | Patients with virus (n = 47) | P-value[†] | Acute chest syndrome | | P-value[‡] |
					No (n = 38)	Yes (n = 9)	
Age in years							
Median	6.1	9.8	6	0.4	6	6.1	0.95
Range	0.5–18	1.5–17	0.5–18		0.5–18	2–15	
Male %	48	62	45	0.4	39	67	0.26
Genotype %				0.5			0.66
SS	70	62	72		71	78	
SC	23	38	19		21	11	
Sβ^0 thalassemia	3	0	4		3	11	
Sβ$^+$ thalassemia	1	0	4		5	0	
Hydroxyurea %	32	38	30	0.7	24	56	0.10
Chronic transfusion (%)	10	15	9	0.6	11	0	0.57
Past history %							
VOC	22	38	17	0.13	16	22	0.64
ACS	35	31	36	1.00	32	56	0.25
Asthma	8	0	11	0.58	13	0	0.57
Median temperature	38.8	38.5	38.8	0.5	38.8	38.9	0.6
Inpatients, N (%)	26 (43)	9 (69)	17 (36)	0.06	8 (21)	9 (100)	<0.0001
Median duration (days)	3.5	5	3	0.11	3	3	0.79
Hospital stay (range)	1–13	2–13	1–6		2–6	1–5	
Median WBC (×10^3/μl)	14.6	14.6	14.3	0.4	13.1	17.8	0.04
Median ALC (×10^3/μl)	3.6	4.2	3.3	0.1	2.8	4.0	0.11
Median hemoglobin (g/dl)	8.5	8.3	8.8	0.8	9.1	8.1	0.57

VOC, vaso-occlusive crises; ACS, acute chest syndrome; WBC, white blood cell count; ALC, absolute lymphocyte count; N, number of inpatients. Data are *percentages* of patients, unless otherwise indicated. [†]P-value calculated for patients with and without respiratory virus. [‡]P-value calculated for patients with and without acute chest syndrome.

the association between ACS and clinical variables including age as a continuous variable, gender, and the nine respiratory viruses detected. All reported *P*-values were 2-sided and considered significant if <0.05. SAS version 9.2 (SAS Institute, Cary, NC) and StatXact (Cytel Corporation, Cambridge, MA) Windows version 8 was used for statistical analyses.

RESULTS

There were 60 patients with an acute respiratory illness who were eligible and all were enrolled on study; a respiratory virus was detected in 47 (78%) patients. Demographic and clinical characteristics of the patients are detailed in Table I.

Of the 60 patients, 9 (15%) had ACS; a respiratory virus was detected in all of the latter. Patients with ACS were all treated as inpatients, had a longer length of hospitalization ($P < 0.0001$), and had a higher white blood cell count ($P = 0.04$) compared to patients without ACS.

Presenting symptoms and signs in patients with ACS included cough and fever in all, tachypnea and chest pain in 6 (67%), and shortness of breath in 2 (22%) patients. Two patients had an oxygen requirement by nasal cannula for 2 days. No patient required admission to the intensive care unit. Presenting symptoms in patients with respiratory virus and without ACS included cough 35 (92%), nasal congestion 19 (50%), fever 29 (76%), and pharyngitis in 3 (8%) patients, respectively. No patient had clinical signs or radiologic evidence of lower respiratory tract involvement.

HRV was the most common respiratory virus detected in comparison with hADV ($P < 0.0001$), hCoV ($P < 0.0001$), influenza ($P = 0.002$), PIV ($P = 0.001$), hBoV ($P = 0.0001$), hEV ($P < 0.0001$), hMPV ($P < 0.0001$), and RSV ($P < 0.0001$). It was detected in 7 (78%) patients with ACS and 22 (58%) patients without ACS. Co-infection with at least one additional respiratory virus was seen in 14 (30%) infected patients, and was not significantly higher in patients with ACS ($P = 0.10$; Table II). *M. pneumoniae*, *C. pneumoniae*, and *B. pertussis* were not identified in this cohort of patients. Bacterial pathogens were not identified from the blood cultures obtained in febrile patients. Co-infections with more than two respiratory viruses were seen in seven patients, all in patients without ACS. Three patients had co-infection with three viruses, two with four viruses, and two with five viruses. All were managed as outpatients, except for the patient with RSV + HRV + hCoV infection who presented with croup, was treated with racemic epinephrine and dexamethasone, and discharged after 3 days.

Univariate logistic regression showed no association of patients with ACS and age ($P = 0.37$; odds ratio 1.07; 95% CI, 0.91–1.26), male gender ($P = 0.14$; odds ratio 3.4; 95% CI, 0.74–15.91), HRV ($P = 0.45$; odds ratio 2.55; 95% CI 0.47–13.91), hCoV ($P = 0.32$; odds ratio 2.43; 95% CI 0.37–15.95), hBoV ($P = 0.41$; odds ratio 0.31; 95% CI 0.03–2.75), hEV ($P = 1.0$; odds ratio 1.46; 95% CI 0.13–15.92), PIV ($P = 1.0$; odds ratio 0.92; 95% CI 0.16–5.25), RSV ($P = 0.57$), influenza ($P = 0.09$), hADV ($P = 1.0$), and hMPV ($P = 0.19$). There were no patients with ACS who had RSV, hADV or influenza virus detected. Only one patient with ACS had detectable hMPV.

Of the 47 patients with respiratory virus detected, all except one patient with ACS had upper respiratory tract infection. There were 35 (76%) patients with an upper respiratory tract infection and 5 (56%) patients with ACS October through March, and 11 (24%)

TABLE II. Respiratory Viruses Detected in Patients With Sickle Cell Disease With and Without Acute Chest Syndrome

Virus	Overall (n = 47)	Acute chest syndrome No (n = 38)	Yes (n = 9)	*P*-value
HRV %	62	58	78	0.45
hCoV %	13	11	22	0.32
hBoV %	26	29	11	0.41
hEV %	9	8	11	1.00
PIV %	23	24	22	1.00
PIV 1	6	3	22	
PIV 2	11	11	11	
PIV 3	2	3	0	
PIV 4	4	3	11	
RSV %	11	13	0	0.57
Influenza%	23	29	0	0.09
Influenza A	4	5	0	
Influenza B	13	17	0	
hADV %	4	5	0	1.00
hMPV %	2	0	11	0.19
1 virus %	55	58	44	0.49
HRV %	19	32	50	
hCoV %	4	5	25	
PIV %	11	18	25	
RSV %	6	14	0	
Influenza %	13	27	0	
hADV %	2	5	0	
Co-infection[a] % 2 viruses	30	24	56	0.10
HRV + PIV %	6	22	20	
HRV + hBoV %	13	56	20	
Co-infection % >2 viruses	15	18	0	0.32

HRV, human rhinovirus; hCoV, human coronavirus; hBoV, human bocavirus; hEV, human enterovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus; hADV, human adenovirus; hMPV, human metapneumovirus. Data are percentages of patients, unless otherwise indicated. [a]Only combinations with more than one virus in each group are shown. No bacterial pathogens were identified with the co-infections.

patients with an upper respiratory tract infection and 4 (44%) patients with ACS April through September, respectively. The difference in the distribution of patients with upper respiratory tract infections was significant ($P = 0.0007$). Respiratory viruses were detected in 36 (77%) patients from October through March, and 11 (23%) patients from April through September (Fig. 1). There were 21, 10, and 5 patients with HRV, hBoV, and hCoV infections detected from October through March, compared to 8, 2, and 1 patients, respectively, from April through September. The difference was statistically significant for HRV ($P = 0.03$) and hBoV ($P = 0.04$) but not for hCoV ($P = 0.22$). Influenza and RSV infections were only detected from October through March. PIV infections were detected year-round.

DISCUSSION

Acute respiratory infections are a major cause of morbidity in patients with sickle cell disease and contribute to the etiology of
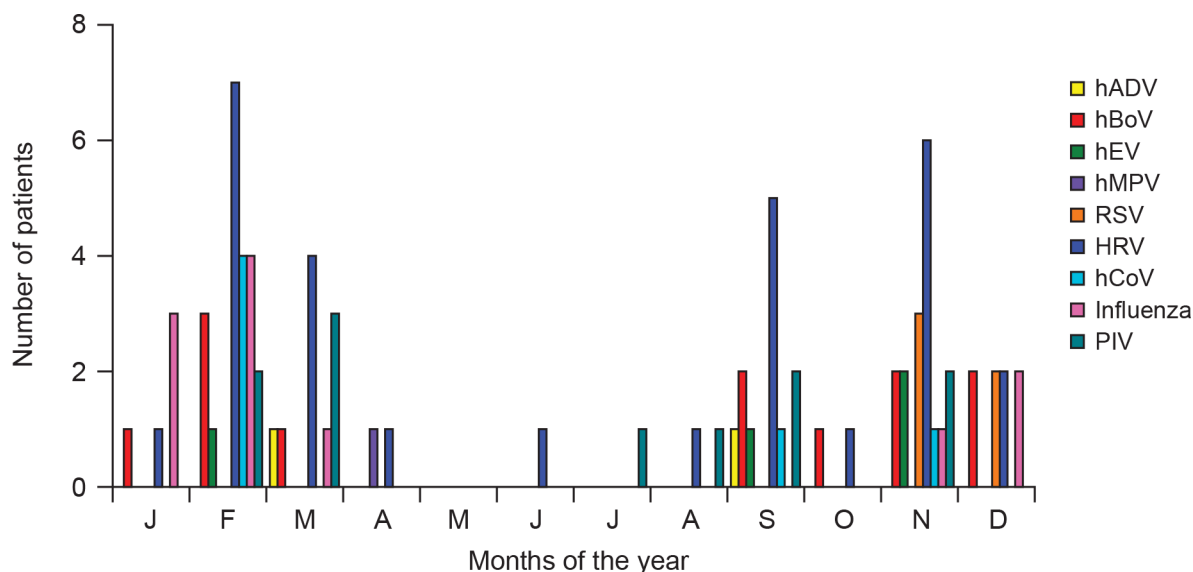
Fig. 1. Seasonal distribution of respiratory virus infections by months of the year. HRV, human rhinovirus; hCoV, human coronavirus; hBoV, human bocavirus; hEV, human enterovirus; PIV, parainfluenza virus; RSV, respiratory syncytial virus; hADV, human adenovirus; hMPV, human metapneumovirus.

ACS. In the past few years newly discovered viruses including coronaviruses, human metapneumovirus, bocavirus, enterovirus, and rhinovirus have been implicated in the causation of lower respiratory tract disease in both immune-competent [6,7] and immune-compromised children [9]. However, their role in patients with sickle cell disease has not been defined. To our knowledge, this is the first prospective study of HRV, hCoV, hBoV, hEV, and hMPV respiratory infections in this population.

HRVs are members of the family *Picornaviridae*, and are well recognized to cause upper respiratory tract illness. Recently, several studies have reported that HRV may be associated with more severe lower respiratory tract illness and hospitalization in otherwise healthy children [6,10]. Although it was the most common respiratory virus detected in our population, HRV was not associated with increased rates of hospitalization, morbidity, or ACS.

Coronaviruses cause upper respiratory illness, and lower respiratory tract disease in infants [11], and immunocompromised patients [12]. Only two patients in our study had hCoV detected as a sole pathogen, one with ACS. The prevalence of hCoV infections may also not be significantly higher in immune-competent children, compared to asymptomatic controls [13].

hBoV was only associated with co-infections in our study and not as a sole pathogen. Similar rates of detection of hBoV have been shown in immune-competent patients with pneumonia who did not have co-infection with other respiratory viruses, compared with controls [14]. The role of this virus as a respiratory pathogen presently remains unproven. Human enterovirus was associated with co-infections in our study mostly in patients with upper respiratory tract infection. However, human enterovirus has been reported in a case series as a cause of severe pneumonia in recipients of stem cell transplantation [15]. Respiratory infections by hMPV were shown to be associated with a substantial burden of hospitalizations and outpatient visits in young mostly previously healthy children [16].

None of the patients with influenza infection developed ACS, perhaps due to early initiation of therapy with oseltamivir and infection with seasonal strains other than 2009 H1N1. Infection with the pandemic H1N1 strain has been shown to be associated with ACS and requirement for mechanical ventilation in contrast to infection with seasonal influenza strains [5].

Some studies have shown a positive association between viral co-detection and worse clinical outcomes. Infants with lower respiratory tract infection where RSV and HRV were co-detected had longer duration of hospital stay needing supplemental oxygen compared to those with RSV alone [17]. Infants with bronchiolitis and viral co-infection with hMPV and RSV were more likely to require intensive care admission for mechanical ventilation [18]. However, co-infection of hMPV with other respiratory viruses did not result in increased disease severity in another study [19]. Multi-viral infections were not associated with ACS in our study although the sample size was small.

A major limitation of the study was a lack of asymptomatic control patients with sickle cell disease. Healthy asymptomatic immune-competent patients can shed HRV and hCoV, and it is not known if this is true for patients with sickle cell disease. The cohort size and the number of patients with ACS were small and these limit the conclusions that can be made on the demographic characteristics. Duration of shedding was not determined. A larger prospective study with asymptomatic control patients should be considered to study the association of these emergent respiratory viruses with ACS. Since broncho-alveolar lavage was not performed, co-pathogens may have either not been detected or over-represented. Hydroxyurea has been shown to reduce the frequency of ACS episodes [20]. This was not seen in our study perhaps due to the small sample size.

Our study had the advantage of being prospectively designed, using multiplexed molecular diagnostics to detect newly described or emerging respiratory viruses in a unique population where this has not been examined. No association was found between ACS and

HRV, hCoV, hBoV, and hEV infections in this population. These viruses were detected predominantly in the respiratory season rather than year around. Multi-viral infection, also increasingly recognized with the use of newer diagnostic methods, was not shown to be associated with ACS.

PCR-based assays for these emerging respiratory viruses have only recently become commercially available. Further studies are needed to determine the clinical utility of this testing, and study the effects of these viruses on long-term pulmonary function, ACS, and the healthcare utilization between patients with or without respiratory virus detected. Pleconaril has been shown to significantly improve recovery from HRV infection [21]. Accurate diagnosis of these infections may hence have implications for both infection control and treatment.

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