

Respiratory Viral Detection in the Pediatric Hematopoietic Stem Cell Transplant Population

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Abstract

Respiratory viral infections (RVI) are a frequent cause of morbidity and mortality in hematopoietic stem cell transplant (HSCT) patients. We examined clinical characteristics and respiratory viral detection in asymptomatic pediatric HSCT pre-transplant patients and symptomatic post-transplant patients. Coxsackie/echovirus (most common virus detected pre and post-transplant), rhinovirus, and coronavirus were detected pre-transplant and at the first post-transplant event suggesting persistent detection. None of the clinical characteristics examined were associated with viral detection and there was no increase in mortality noted with asymptomatic viral detection.

Keywords: Pediatrics; Hematopoietic stem cell transplant; Respiratory virus.

follow-on post-transplant period and it has been suggested that patients with asymptomatic viral detection do not have increased mortality and those with symptomatic viral detection do have increased mortality [4]. Screening for respiratory viral infections may impact a physician's decision of timing for HSCT and transplant may be delayed until viral clearance is documented [5,6].

Studies on respiratory viruses in the pediatric HSCT transplant population have identified rhinovirus as a common virus found in the pre-transplant period and rhinovirus, coronavirus, parainfluenza virus, respiratory syncytial virus and adenovirus in the post-transplant period [4,7-11]. We evaluated asymptomatic pediatric HSCT patients for the presence of respiratory virus in the immediate pre-transplant period and symptomatic patients for up to 90 days post-transplant to examine epidemiology and clinical characteristics associated with viral detection.

Study Design

Allogeneic and autologous hematopoietic stem cell transplant (HSCT) candidates between 1 month and 17 years of age were enrolled in this single-site, prospective study. There were two enrolment periods; Phase 1 between December 1st, 2008 and December 1st 2009, and Phase 2 between November 1, 2011 and September 30, 2012. A total of 45 subjects were enrolled. The study was approved by the Duke University Institutional Review Board for Clinical Investigations (Duke IRB 00011275). Parents provided written informed consent and all volunteers >6 years of age assented to study participation.

Introduction

Infections, including respiratory viral infections (RVI), are one of the leading causes of morbidity and mortality in hematopoietic stem cell transplant recipients as a direct result of the viral infection and non-infectious sequelae including graft *versus* host disease and allo-immune lung syndromes [1-3]. Few studies in pediatric HSCT patients have examined the presence of viruses in the immediate pre-transplant period through the

Routine clinical virology testing

All patients scheduled to undergo HSCT had a nasopharyngeal wash (NPW) for viral testing as part of clinical care prior to hospital admission. During the Phase 1 of the study, viral testing for clinical care was performed using standard tissue culture and antigen detection techniques (respiratory syncytial virus, influenza, parainfluenza, and adenovirus).

Due to the improved sensitivity of newer diagnostic techniques, during Phase 2 of the study, the routine clinical testing transitioned to a multiplex RT-PCR assay capable of detecting eight viruses (adenovirus, human meta-pneumovirus, parainfluenza 1, 2, 3, influenza A and B, and respiratory syncytial virus).

After transplant, when patients presented with fever and/or symptomatic respiratory events, NPW samples were collected for viral testing as part of routine clinical care. Signs and symptoms of respiratory events included: fever, cough, tachypnea, headache, malaise, sore throat, nasal congestion, poor feeding, abnormal lung exam and/or increase in oxygen requirement. Study patients were followed up to day 90 after HSCT. As with the pre-transplant specimens, routine clinical testing during Phase 1 was performed using viral culture and antigen detection and during Phase 2 testing was performed using the commercial 8-pathogen multiplex RT-PCR.

Supplemental Virology Testing

Patients were enrolled in the study within 72 h of hospital admission for HSCT and additional baseline NPW samples were collected for an expanded target, multiplex viral RT-PCR. In addition to clinical testing described above, samples collected during respiratory events were also stored for later testing using the expanded testing.

The research samples were processed using the BioRobot EZ1 and the EZ1 Virus minikit (Qiagen, Hilden, Germany). Molecular testing was performed using the research Qiagen Resplex II V2.0 RT-PCR kit. The kit detects: respiratory syncytial virus A and B, influenza A and B, parainfluenza virus 1, 2, 3, 4, human metapneumovirus; coxsackie/echovirus, rhinovirus, coronavirus 229E, OC43, NL63, HKU1, bocavirus, and adenovirus B and E. Both clinical and research testing results were included in the analysis. If either testing modality was positive, it was considered a positive result. Clinical information was collected from the chart by study personnel. Analysis was performed using JMP PRO®, Version 11 SAS Institute Inc., Cary, NC.

Results

A total of 45 subjects were enrolled over both Phase 1 (N=20) and Phase 2 (N=25). The mean age of participants was 6.8 years (range: 0.7-17.8), with a median of 5.5 years. Patients were split equally by gender (51.1% male) and were predominantly Caucasian (62.2%), with a large African American minority (28.9%). Study patients were enrolled in the study for a mean of 106 days and 43 of 45 patients (95.6%) were followed 90 days post-HSCT.

The most common underlying conditions leading to HSCT were inborn errors of metabolism (31.1%) and hematologic malignancy (26.7%), followed by other conditions (20%), solid tumors (13.3%), and myelodysplastic syndrome (8.9%). All patients were to receive their 1st HSCT, including 28 unrelated cord blood transplants, 10 autologous transplants, and 6 matched, related bone marrow transplants. Mean time of hospitalization was 60 days ± 29 days (continuous days from pre-transplant admission to post-transplant discharge).

All 45 patients had baseline samples collected at the time of enrollment. Of these, 10 (22.2%) baseline samples revealed viral detection including coxsackie/echovirus [5], rhinovirus [2], adenovirus [2], coronavirus HKU1 [1], coronavirus NL63 [1] and parainfluenza 3 [1]. Two children had co-infection (more than one virus isolated) at baseline (adenovirus with coxsackie/echovirus [2]). Negative testing occurred in 77.8% of subjects. No samples tested positive for respiratory syncytial virus, influenza A and B, parainfluenza virus 1, 2, 4, human meta-pneumovirus, coronavirus NL63, HKU1, or bocavirus. The children with positive samples at baseline did not differ from those with negative samples in respect to age, sex, race, or underlying disease requiring transplant.

Of the 45 enrolled patients, 26 (57.8%) developed 49 febrile and/or respiratory events prompting NPW collection. Of these 26 children, 12 children had only one event, while 7 had two events, and seven had 3 or more events (range 1-4 events, median 2, mean 2.74). A respiratory virus was detected in 25/49 (51.0%) of samples collected during acute respiratory illness. Viruses detected during the acute events included coxsackie/echovirus [9], bocavirus [6], rhinovirus [4], coronavirus NL63 [2], coronavirus HKU1 [2], human metapneumovirus [2], parainfluenza 3 [2], adenovirus 1, and parainfluenza 4 [1]. Coinfection was found in four samples collected during acute respiratory events (bocavirus with coronavirus HKU1, bocavirus with coxsackie/echovirus, coronavirus HKU1 with parainfluenza virus 4, and coxsackie/echovirus with human metapneumovirus). No pathogenic respiratory virus was identified in 49.0% of the symptomatic subjects. Respiratory syncytial virus, influenza A and B, parainfluenza virus 1 and 2, coronavirus 229E, and OC43 were not detected in any samples. The sample collection time points do not allow clear determination of how many of these infections were hospital vs. community acquired. Of the 10 subjects with positive baseline samples, 7 subjects (70%) developed a subsequent respiratory event; 4 of whom had the same respiratory virus detected from the pre-transplant sample as the first symptomatic post-transplant event. These viruses included coxsackie/echovirus [2], coronavirus NL63, and rhinovirus.

Five children experienced infections with multiple viruses over the acute illness follow up period. Remarkably, 28 children did not have a virus isolated at any time point in the study.

Clinical manifestations during respiratory events were generally mild; cough and nasal congestion were the most common (**Table 1**). No patients required intensive care or intubation. Two children died over the course of the study; one from metastasis of their underlying malignancy and the other

from respiratory failure attributed to adenovirus, aspergillus, and toxoplasmosis.

Table 1: Acute respiratory event signs and symptoms compared for those who had a positive viral detection compared those who had a negative viral detection during an acute respiratory event.

Sign/symptom	N (%) virus positive	N (%) virus negative	N (%) total
	25 (51.0)	24 (49.0)	49
Nasal congestion	15 (60.0)	12 (50.0)	27 (55.1)
Fever	12 (48.0)	13 (54.2)	25 (51.0)
Cough	9 (36.0)	11 (45.8)	20 (40.8)
Tachypnea	7 (28.0)	13 (54.2)	20 (40.8)
Mucositis	6 (24.0)	9 (37.5)	15 (30.6)
Acute Graft Versus Host Disease	7 (28.0)	7 (29.2)	14 (28.6)
Poor feeding	6 (24.0)	5 (20.8)	11 (22.4)
New O ₂ requirement	4 (16.0)	5 (20.8)	9 (18.4)
Sore throat	1 (4.0)	3 (12.5)	4 (8.2)
Wheezing	1 (4.0)	1 (4.2)	2 (4.1)
Mean temperature ± 1 Standard Deviation	37.9 ± 0.9	38.3 ± 1.2	38.1 ± 1.1

Discussion

RVI's are one of the leading causes of morbidity in HSCT recipients and may result in death; with one recent study demonstrating a case-fatality rate of 10% in the pediatric HSCT population [9]. Clinical clues to diagnose respiratory infection can be unreliable in immunocompromised patients, although clinical scoring systems have been proposed [12]. Our study did not identify any specific demographic, sign, or symptom associated with viral detection in pre and post-transplant patients. We identified coxsackie/enterovirus as the most common viral etiology detected both in the asymptomatic pre-transplant period and the symptomatic post-transplant period. Bocavirus was also frequently identified during the post-transplant period. Other studies examining the pediatric HSCT population have identified rhinovirus as a common virus found in the pre-transplant period and rhinovirus, coronavirus, parainfluenza, respiratory syncytial virus, and adenovirus in the post-transplant period [4,7-11]. It is interesting that these studies identified patients with respiratory syncytial virus and influenza in their study population but neither virus was found in patients in our study despite enrollment during typical respiratory seasons.

In our study, a number of viruses (coxsackie/echovirus, rhinovirus and coronavirus NL63) were detected at baseline and repeatedly during acute respiratory events, suggesting the possibility of asymptomatic infection pre-HSCT resulting in prolonged shedding, perhaps as a result of immunosuppression. Furthermore, subclinical respiratory viral infection has been

documented and is considered transmissible [13-15]. A recent study by Campbell et al., looking at clinical outcomes in patients with pre-HSCT viral detection and post-transplant viral detection found no increased mortality with asymptomatic viral detection including those with asymptomatic pre-transplant viral detection but did find increased mortality with symptomatic viral detection including those with symptomatic pre-transplant viral detection [4]. We also found no increased mortality associated with asymptomatic pre-transplant viral detection.

The major limitation of the study was the small sample size. It would have also been interesting to perform viral sequencing and continue to test pre-transplant patients with positive viral detection on an on-going basis to determine the duration of viral persistence and more closely examine its impact on patient outcome. However, important strengths are the prospective nature of this study, as well as the use of internal controls during RT-PCR testing that verified the integrity of the archival specimens.

While we can now identify these respiratory viruses in this population, their role in ultimate clinical outcome and the degree of nosocomial transmission remains unclear. There are increasing numbers of patients undergoing HSCT with increasing complexity. However, there also are newer drugs being studied for the treatment of viral infections (i.e., adenovirus, parainfluenza virus, and respiratory syncytial virus) in this population and they offer a potential to suppress or eliminate these viruses throughout the peri-transplant continuum [16-18]. Further prospective studies will be needed to determine the clinical impact and optimal prevention and treatment strategies for RVI's in this population.

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References

1. Boeckh M (2008) The challenge of respiratory virus infections in hematopoietic cell transplant recipients. *Br J Haematol* 143: 455-467.
2. Weigt SS, Gregson AL, Deng JC, Lynch JP, Belperio JA (2011) Respiratory viral infections in hematopoietic stem cell and solid organ transplant recipients. *Semin Respir Crit Care Med* 32: 471-493.
3. Versluys AB, Rossen JWA, van Ewijk B, Schuurman R, Bierings MB, et al. (2010) Strong association between respiratory viral infection early after hematopoietic stem cell transplantation and the development of life-threatening acute and chronic alloimmune lung syndromes. *Biol Blood Marrow Transplant* 16: 782-791.
4. Campbell AP, Guthrie KA, Englund JA (2015) Clinical outcomes associated with respiratory virus detection before allogeneic hematopoietic stem cell transplant. *Clin Infect Dis* 61: 192-202.
5. Tomblyn M, Chiller T, Einsele H (2009) Guidelines for preventing infectious complications among hematopoietic cell

- transplantation recipients: a global perspective. *Biol Blood Marrow Transplant* 15: 1143-1238.
6. Neemann K, Freifeld A (2015) Respiratory Syncytial Virus in Hematopoietic Stem Cell Transplantation and Solid-Organ Transplantation. *Curr Infect Dis Rep* 17: 490.
 7. Choi JH, Choi EH, Kang HJ (2013) Respiratory viral infections after hematopoietic stem cell transplantation in children. *J Korean Med Sci* 28: 36-41.
 8. Lee JH, Jang JH, Lee SH (2012) Respiratory viral infections during the first 28 days after transplantation in pediatric hematopoietic stem cell transplant recipients. *Clin Transplant* 26: 736-740.
 9. Hutspardol S, Essa M, Richardson S (2015) Significant Transplantation-Related Mortality from Respiratory Virus Infections within the First One Hundred Days in Children after Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant* 21: 1802-1807.
 10. Lo MS, Lee GM, Gunawardane N, Burchett SK, Lachenauer CS, et al. (2013) The impact of RSV, adenovirus, influenza, and parainfluenza infection in pediatric patients receiving stem cell transplant, solid organ transplant, or cancer chemotherapy. *Pediatr Transplant* 17: 133-143.
 11. Srinivasan A, Gu Z, Smith T (2013) Prospective detection of respiratory pathogens in symptomatic children with cancer. *Pediatr Infect Dis J* 32: e99-e104.
 12. Ferguson PE, Gilroy NM, Sloots TP, Nissen MD, Dwyer DE, et al. (2011) Evaluation of a clinical scoring system and directed laboratory testing for respiratory virus infection in hematopoietic stem cell transplant recipients. *Transpl Infect Dis* 13: 448-455.
 13. Kassis C, Champlin RE, Hachem RY (2010) Detection and control of a nosocomial respiratory syncytial virus outbreak in a stem cell transplantation unit: the role of palivizumab. *Biol Blood Marrow Transplant* 16: 1265-1271.
 14. Peck AJ, Englund JA, Kuypers J (2007) Respiratory virus infection among hematopoietic cell transplant recipients: evidence for asymptomatic parainfluenza virus infection. *Blood* 110: 1681-1688.
 15. de Lima CRA, Mirandolli TB, Carneiro LC (2014) Prolonged respiratory viral shedding in transplant patients. *Transpl Infect Dis* 16: 165-169.
 16. Florescu DF, Pergam SA, Neely MN (2012) Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. *Biol Blood Marrow Transplant* 18: 731-738.
 17. Mazur NI, Martínón-Torres F, Baraldi E (2015) Lower respiratory tract infection caused by respiratory syncytial virus: current management and new therapeutics. *Lancet Respir Med* 3: 888-900.
 18. Shah DP, Shah PK, Azzi JM, Chemaly RF (2016) Parainfluenza virus infections in hematopoietic cell transplant recipients and hematologic malignancy patients: A systematic review. *Cancer Lett* 320: 358-364.