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Short communication

Prolonged respiratory viral shedding in transplant patients

C.R.A. de Lima, T.B. Mirandolli, L.C. Carneiro, C. Tusset, C.M. Romer, H.F. Andreolla, L.F. Baethgen, A.C. Pasqualotto. Prolonged respiratory viral shedding in transplant patients.
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Abstract: Respiratory viral infections are frequent causes of morbidity in transplant patients. We screened symptomatic adult transplant recipients for respiratory viruses in a cohort of patients attending a referral medical center in Brazil. The duration of viral shedding and the prevalence of viral codetections were also determined. During a 1-year period (2011–2012), swabs were obtained from 50 patients. An in-house polymerase chain reaction panel designed to detect 10 viruses was used. Viruses were identified in 19 (38%) patients, particularly parainfluenza III (32%) and the respiratory syncytial virus (20%); multiple viruses were identified in 26% of patients. Prolonged viral shedding was observed with 60% of individuals excreting viruses for >10 days. The clinical and epidemiologic relevance of prolonged viral shedding remains to be determined.

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Respiratory viral infections are usually associated with significant morbidity following organ transplantation (1). Transplant patients could be infected by the same respiratory viruses that affect the overall population, including adenovirus (ADV), human bocavirus (hBOV), coronavirus, human metapneumovirus (hMPV), parainfluenza viruses (PIV), and respiratory syncytial virus (RSV) (2). However, because of the state of immunosuppression, the rate of disease complication may be increased in the transplant population. The duration of viral shedding is an important determinant of viral infectivity and transmissibility; factors that provide vital information for effective infection control (3–8). In the transplant population, limited data are available regarding the duration of viral shedding following a respiratory tract infection. Here, we investigated the distribution of viruses causing acute respiratory syndrome in transplant patients, to determine the outcome of these infections and the duration of viral excretion.

Methods

This was a prospective cohort study conducted between August 2011 and August 2012. The study was performed at Santa Casa de Misericordia de Porto Alegre, a reference transplant center located in Southern Brazil. Patients were included in or excluded from this research based on the Center for Disease Control and Prevention recommendations. Patients were studied if they presented fever, cough, and headache, plus at least 1 of the following symptoms: rhinorrhea, coryza, arthralgia, myalgia, prostration, sore throat, chest pain, abdominal pain, or nasal congestion (9).

Individuals were excluded in the presence of signs/symptoms lasting >14 days. Informed written consent was obtained from all patients, and the study was approved by the local Ethics Committee (542/09).

Respiratory samples were obtained as recommended by the World Health Organization (10). Briefly, rayon swabs were used to collect samples from nasopharyngeal and oropharyngeal sites. Swabs were transported in saline solution to the laboratory. Samples were stored at 4°C for a few hours until processing.

Total nucleic acids were extracted with PureLink® Viral RNA/DNA Mini Kit (Invitrogen™; Life Technologies, Grand Island, New York, USA) and stored at −80°C. The cDNAs of influenza A H3N2 (FluA), pandemic influenza A/H1N1 (pH1N1), influenza B (FluB), RSV, PIV I, II and III), and hMPV were synthesized by SuperScriptIII® First-Strand Synthesis SuperMix (Invitrogen™) with the following conditions: 10 min at 25°C, 50 min at 50°C, and 5 min at 85°C. Table 1 shows a list of the primers used in the study. Real-time polymerase chain reaction (PCR) assay was performed using Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen™).

The real-time PCR conditions for all RNA viruses except for RSV were as follows: 2 min at 50°C, 5 min at 95°C, 45 cycles of 15 s at 95°C, and 30 s at 64°C, followed by 15 s at 95°, 1 min at 60°C, and 30 s at 95°C. For RSV, the condition was 2 min at 50°C, 5 min at 95°C, 45 cycles of 15 s at 95°C, 30 s at 56°C, and 15 s at 72°C. ADV and hBOV were detected by conventional PCR with Platinum Taq DNA Polymerase (Invitrogen™). The conditions used to amplify ADV and hBOV were 10 min at 95°C, 38 cycles of 30 s at 95°C, 30 s at 56°C, and 30 s at 72°C.

To determine the duration of viral shedding, patients with positive PCR tests were retested every 3–5 days, until a negative PCR test was obtained.

Patients' charts were reviewed in an attempt to obtain clinical and demographic information. To compare the distribution of respiratory viruses infecting the overall

Oligonucleotide sequence of respiratory viruses analyzed by real-time polymerase chain reaction

Primer	Sequence	Annealing temperature (°C)	Fragment size
FluA-F	TACCCATTGCCTTCYCTTCC	60.8	133 bp
FluA-R	CTGCTTCTCCAAGCGAATCT	63.9	
FluB-F	CTGATGTCCATCAAGCTCCA	62.4	174 bp
FluB-R	CCTTTGACATCTGCATCACG	63.2	
pH1N1-F	GGGAAACAAATCGTGGAATG	64.3	95 bp
pH1N1-R	GTCAGAAAGGTAGCGCGAAG	64.6	
PIV I-F	GGGAAAACAAWTAGTTCATATTGGTC	66.3	98 bp
PIV I-R	TGCATTGTTGTTGCAATCAGT	63.7	
PIV II-F	TGATGGAATCAATCGCAAAA	63.8	125 bp
PIV II-R	GAAAGCAGTCTCAGTTCAGCTA	61.2	
PIV III-F	AATCGAGAGTRAACCCAGTCATAA	63.3	122 bp
PIV III-R	TGTTATAGTGTGTAATGCAGCTYGT	60.8	
RSV-F	TGGGAGAGGTAGCTCCAGAA	63.6	107 bp
RSV-R	CAGATCTRTCCCCTGCTGCT	62.8	
hMPV-F	ATGGCAAAGCATTAGGCTCA	65.2	110 bp
hMPV-R	TGTTGGATGACCTGGCAAT	62.4	
ADV-F	ATGACTTTTGAGGTGGATCCCATGGA	73.6	133 bp
ADV-R	GCCGAGAAGGGCGTGCGCAGGTA	83.2	
hBOV-F	GTCCAGAAAGAGGGGAGAGG	64.1	125 bp
hBOV-R	GCTGATTGGGTGTTCCTGAT	63.4	

FluA, influenza A H3N2; F, forward; R, reverse; FluB, influenza B; pH1N1, pandemic influenza A/H1N1, PIV, parainfluenza virus; RSV, respiratory syncytial virus; hMPV, human metapneumovirus; ADV, adenovirus, hBOV, human bocavirus; bp, base pairs.

Table 1

population with those in transplant patients, data from 254 non-transplant patients who had positive PCR tests for respiratory viruses in the same study period were also reviewed.

Results

A total of 50 transplant patients were studied. They were predominantly male (62%), with a median age of 50.5 years (range 21–71 years). Most patients have undergone kidney transplantation (76%), whereas others had received liver (12.0%), lung (8%), and stem cell transplants (4%). Patients were included after a median time of 3 years post transplant (6 days–15 years). A total of 36% of study patients reported recent contact with individuals with respiratory symptoms, and 42% had received a conjugated influenza A/H1N1 vaccine. The main clinical manifestations were cough (29.2%), coryza (18.5%), and fever (17.8%). The frequency of hospitalization was 80%, and the mean length of hospital stay was 29.2 days. Four patients died during the study (8.0%); the median time of death was 38.5 days after hospitalization (range 25–65 days), and the cause of death in all patients was disseminated infection.

Respiratory viruses were detected in 19 patients (38.0%), mostly in fall and winter months. Multiple viruses were identified in 5 individuals (26.3%), including 1 patient who had 3 viruses detected. The most prevalent virus was PIV III (32%), followed by RSV (20%), hBOV (12%), hMPV (12%), pH1N1 (8%), ADV (8%), FluA (4%), and FluB (4%). The viral distribution differed from what was observed for the overall population, in which RSV was the predominant agent (54.5%). The prevalence of viral codetection in the general population was 35%, with 5 patients in the group showing concomitantly 4 respiratory viruses.

Table 2 presented the duration of viral shedding per patient studied. Viral shedding ranged from 6 days (hBOV; $n = 1$) to 44 days (hMPV; $n = 1$). The average duration of viral shedding was 10 days for pH1N1 ($n = 2$), 17 days for PIV III ($n = 3$), and 13 days for RSV ($n = 3$).

Viral distribution, viral shedding, and symptoms presented by transplant patients with acute respiratory illness in Southern Brazil, over a 1-year period (2011–2012)

Patients	Virus	Symptoms	Viral shedding (days)
1	hMPV	Fever, myalgia, runny nose, and cough	0
2	RSV	Fever and cough	23
3	PIV III	Fever, runny nose, cough, and nasal congestion	23
4	hMPV	Fever, sore throat, and cough	0
5	PIV III	Myalgia, runny nose, sore throat, and cough	0
6	hMPV and FluA	Fever and cough	44
7	PIV III	Myalgia, runny nose, nasal congestion, and cough	21
8	PIV III	Cough and runny nose	0
9	ADV	Fever, myalgia, runny nose, sore throat, and cough	0
10	ADV	Fever, myalgia, and cough	0
11	PIV III	Fever and cough	6
12	RSV and hBOV	Runny nose, nasal congestion, and cough	12
13	PIV III	Runny nose and cough	0
14	PIV III	Fever and cough	0
15	pH1N1, RSV and PIV III	Fever, myalgia, and cough	3
16	FluB	Fever, myalgia, sore throat, and cough	0
17	pH1N1 and hBOV	Fever, myalgia, runny nose, and cough	17
18	hBOV and RSV	Runny nose and cough	0
19	RSV	Coryza, runny nose, sore throat, and cough	0

hMPV, human metapneumovirus; RSV, respiratory syncytial virus; PIV III, parainfluenza III virus; FluA, influenza A H3N2; ADV, adenovirus; hBOV, human bocavirus; FluB, influenza B; pH1N1, pandemic influenza A/H1N1.

Table 2

Discussion

This prospective study revealed that 38% (19/50) of transplant patients who presented with acute respiratory syndrome were diagnosed with a viral infection. A total of 8 viruses were detected in this cohort, and 5 patients (26.3%) were found to harbor >1 virus. The detection of multiple viruses has been reported in 22% of Brazilian transplant patients (11). Recent studies reported a frequency of 21.4% and 10% of dual respiratory viral infection in children with bronchiolitis after molecular assays were added to the diagnostic list of clinical laboratories (12, 13). Therefore, when using modern and sensitive methods, such as real-time PCR, in the diagnosis of respiratory tract infections, even higher rates of viral codetection are anticipated to be found in the transplant setting (11). As shown before (11), the detection of multiple respiratory viruses in transplant recipients was not associated with a poor prognosis in these patients.

In our study, PIV III was the most frequent virus detected in transplant patients (31.6%), followed by RSV (20%), hBOV (12%), hMPV (12%), pH1N1 (8%), and ADV (8%). In previous investigations, using conventional diagnostic methods (i.e., direct immunofluorescence and viral culture), the incidence of PIV III infection following transplantation varied from 2–9% (4, 14). On the other hand, studies based on PCR revealed a higher frequency of PIV III infection, as high as 14.0% (4). In contrast to transplant patients, in whom PIV III was the predominant virus, RSV was the main cause of respiratory infections in the non-transplant population (54.5%), referred to the same hospital. The high RSV frequency detected in non-transplant patients suggests that most of these patients were probably children, which is in accordance with the literature that suggests that RSV is one of the most common causes of severe lower respiratory disease in young children (15). Human rhinoviruses and coronaviruses are frequently associated with the common cold and are generally considered to replicate principally within the upper respiratory tract (16). Despite the high frequency of these viruses in the population, the real importance of these agents in the transplant population remains to be determined (16).

In this study, hBOV, hMPV, ADV, FluA, and FluB were detected in 12%, 12%, 8%, 4%, and 4% of transplant patients, respectively. These frequencies were in agreement with the literature data (17–23). In contrast, pH1N1 was detected in only 8% of transplant patients. The low percentage of pH1N1 detection in this study may be justified by the massive FluA/pH1N1 immunization campaign that had occurred in Brazil few months before the study was initiated.

Information about the duration of viral shedding is important to establish effective infection control measures in the hospital environment. In this work, we reported a prolonged shedding in this cohort of transplant patients, with 60% of individuals excreting viruses for >10 days. This finding is in agreement with the literature, in which a prolonged shedding has been reported for PIV III (6–42 days), RSV (5–39 days), and hMPV (7–24 days) infections (3–8). Even though a previous study showed prolonged hBOV shedding in non-transplant children (7), only 1 patient with hBOV infection was included in our study, and therefore, we were not able to properly evaluate hBOV shedding in our transplant cohort. The current data on pH1N1 shedding are in agreement with a previous report, which suggested that the presence of pH1N1 was a significant risk factor for prolonged viral shedding (median of 27 days) (8).

Limitations of this work included the inability to test for rhinovirus in this cohort of patients, and the difficulty to follow these individual for a prolonged period of time. Another limitation was the small number of transplant patients studied. We only included patients who presented with acute respiratory illness (symptoms lasting for <14 days, as based on the CDC recommendations). Because viral shedding is prolonged in these patients (as demonstrated by the current study), this criterion could have reduced the number of patients enrolled in our study (9). Furthermore, it is already known that transplant patients have fewer and milder symptoms. Because of this fact, we could have missed some patients with mild to no symptoms, which could affect the estimated duration of shedding.

This work demonstrated that respiratory viruses are a significant cause of morbidity in transplant patients. The use of molecular tools in clinical practice may facilitate the management of such patients, particularly when infection control measures are being considered. Transplant patients have demonstrated prolonged viral shedding – the clinical relevance of that, and the potential for intrahospital dissemination of disease remain to be determined.

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References

1. Renaud C, Campbell AP. Changing epidemiology of respiratory viral infections in hematopoietic cell transplant recipients and solid organ transplant recipients. Curr Opin Infect Dis 2011; 24 (4): 333–343.
2. Fischer AS. Emerging viruses in transplantation: there is more to infection after transplant than CMV and EBV. Transplantation 2008; 86 (10): 1327–1339.
3. Ryoo SM, Kim WY, Sohn CH, et al. Factors promoting the prolonged shedding of the pandemic (H1N1) 2009 influenza virus in patients treated with oseltamivir for 5 days. Influenza Other Respir Viruses 2013; 7 (5): 833–837. Epub 2012 Dec 26.
4. Peck AJ, Englund JA, Kuypers J, et al. Respiratory virus infection among hematopoietic cell transplant recipients: evidence for asymptomatic parainfluenza virus infection. Blood 2007; 110 (5): 1681–1688.
5. Debiaggi M, Canducci F, Terulla C, et al. Long-term study on symptomless human metapneumovirus infection in hematopoietic stem cell transplant recipients. New Microbiol 2007; 30 (3): 225–258.
6. Nichols WG, Erdman DD, Han A, Zukerman C, Corey L, Boeckh M. Prolonged outbreak of human parainfluenza virus 3 infection in a stem cell transplant outpatient department: insights from molecular epidemiologic analysis. Biol Blood Marrow Transplant 2004; 10 (1): 58–64.
7. Martin ET, Fairchok PM, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. J Infect Dis 2010; 201 (11): 1625–1632.
8. Choi SM, Boudrealt AA, Xie H, Englund JA, Corey L, Boeckh M. Differences in clinical outcome after 2009 influenza A/H1N1 and seasonal influenza among hematopoietic cell transplant recipients. Blood 2011; 117 (19): 5050–5056.
9. CDC. Update: novel influenza A (H1N1) virus infection – Mexico, March-May, 2009. MMWR Morb Mortal Wkly Rep 2009; 58 (21): 585–589. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5821a2.htm (Accessed 19 November 2013).
10. World Health Organization. Manual for the national surveillance of antimicrobial resistance of *Streptococcus pneumoniae* and *Haemophilus influenzae*: epidemiological and microbiological methods; August 1994. Available at: http://www.who.int/chd/publications/ari/bact/bact.htm (Accessed 19 November 2013).
11. Machado CM, Vilas Boas LS, Mendes AVA, et al. Low mortality rates related to respiratory virus infections after bone marrow transplantation. Bone Marrow Transplant 2003; 31 (8): 695–700.
12. Andreoletti L, Lesay M, Deschildre A, et al. Differential detection of rhinoviruses and enteroviruses RNA sequences associated with classical immunofluorescence assay detection of respiratory virus antigens in nasopharyngeal swabs from infants with bronchiolitis. J Med Virol 2000; 61 (3): 341–346.
13. Ong GM, Wyatt DE, O'Neill HJ, et al. A comparison of nested polymerase chain reaction and immunofluorescence for the diagnosis of respiratory infections in children with bronchiolitis, and the implications for a cohorting strategy. J Hosp Infect 2001; 49 (2): 122–128.
14. Schiffer J, Kirby K, Sandmaier B, Storb R, Corey L, Boeckh M. Timing and severity of community acquired respiratory infection after myeloblative versus non-myeloblative stem cell transplantation. Haematologica 2009; 94 (8): 1101–1108.
15. Mendes ET, Ramos J, Peixoto D, et al. An outbreak of respiratory syncytial vírus infection in hematopoietic stem cell transplantation outpatients: good outcome without specific antiviral treatment. Transpl Infect Dis 2013; 15 (1): 42–48.
16. Milano F, Campbell AP, Guthrie KA, et al. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. Blood 2010; 115 (10): 2088–2094.
17. Garbino J, Soccal PM, Aubert JD, et al. Respiratory viruses in bronchoalveolar lavage: a hospital-based cohort study in adults. Thorax 2009; 64 (5): 399–404.
18. Schenk T, Strahm B, Kontny U, Hufnagel M, Neumann-Halfelin D, Falcone V. Disseminated bocavirus infection after stem cell transplant. Emerg Infect Dis 2007; 13 (9): 1425–1427.
19. Souza JS, Watanabe A, Carraro E, Granato C, Bellei N. Severe metapneumovirus infection among immunocompetent and immunocompromised patients admitted to hospital with respiratory infection. J Med Virol 2013; 85 (3): 530–536.
20. Álvarez AM, Catalán P, Alba A, Zubieta M. Profilaxis de infección por vírus respiratórios em niños y adultos sometidos a transplante de órganos sólidos y precursores hematopoyéticos. Rev Chilena Infectol 2012; 29 (1): 33–36.
21. Choi JH, Choi EH, Kang HJ, et al. Respiratory viral infections after hematopoietic stem cell transplantation in children. J Korean Med Sci 2013; 28 (1): 36–41.
22. Soccal PM, Aubert JD, Bridevaux PO, et al. Upper and lower respiratory tract viral infections and acute graft rejection in lung transplant recipients. Clin Infect Dis 2010; 51 (2): 163–170.
23. Liu M, Worley S, Arrigain S, et al. Respiratory viral infections within one year after pediatric lung transplant. Transpl Infect Dis 2009; 11 (4): 304–312.