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Comparison of conventional and molecular detection of respiratory viruses in hematopoietic cell transplant recipients

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Abstract

Background—Sensitive detection of respiratory viruses is important for early diagnosis of infection in patients following hematopoietic cell transplantation (HCT). To evaluate the relative sensitivity of respiratory virus detection in specimens from HCT recipients, we compared the results of conventional and quantitative molecular methods.

Methods—We tested 688 nasal wash samples collected prospectively from 131 patients during the first 100 days after HCT by viral culture, fluorescent antibody staining (FA), and real-time quantitative reverse transcription-polymerase chain reaction (PCR) assay for detection of respiratory syncytial virus (RSV), influenza virus types A (FluA) and B (FluB), and parainfluenza virus types 1 (PIV1) and 3 (PIV3). Testing for human metapneumovirus (MPV) was performed only by PCR. Data regarding 10 respiratory symptoms were collected with each sample.

Results—By any method 37 specimens were positive for a respiratory virus; 34 were positive by PC, 15 by culture, and 6 by FA. Four specimens were positive by all 3 methods (3 RSV, 1 FluA). One specimen was positive for PIV1, and 2 were positive for rhinovirus by culture alone. Specimens positive by PCR alone included 2 RSV, 2 PIV1, 8 PIV3, and 8 MPV. In 10 specimens positive for RSV, PIV, or influenza virus collected from patients reporting no respiratory symptoms, 9, 4, and 1 specimen were positive by PCR, culture, and FA respectively. Overall, specimens positive only by PCR had significantly fewer viral copies/mL (mean log₁₀=4.32) than specimens positive by both PCR and culture (mean log₁₀=5.75; $p=0.002$) or PCR and FA (mean log₁₀=6.83; $p<0.001$).

Conclusions—FA testing alone did not detect a significant proportion of respiratory virus positive samples in HCT recipients, especially in patients with no respiratory symptoms and patients with PIV detection. PCR increased the yield of positive specimens 2 times relative to culture and more than 4 times relative to FA. Detection of respiratory viruses by PCR alone was associated with lower virus quantities and with fewer reported respiratory symptoms compared with concomitant detection by both PCR and conventional methods, indicating that PCR may be important to detect asymptomatic or mildly symptomatic stages of respiratory viral infections.

Keywords

transplantation; respiratory viruses; polymerase chain reaction; molecular detection; hematopoietic stem cell transplant

Laboratory detection of respiratory viral pathogens, which cause severe respiratory illnesses and death in patients following hematopoietic cell transplantation (HCT), is essential for the diagnosis of respiratory illness in these patients, because clinical syndromes in the immunocompromised host are often nonspecific. Respiratory virus detection using highly sensitive methods may facilitate early diagnosis, so that isolation and treatment of the patient can be initiated promptly. In addition, identification of persons with respiratory viruses in nasopharyngeal secretions is critical to effective intervention during nosocomial outbreaks of respiratory infections. Thus, highly sensitive methods are needed to detect respiratory virus infections in patients with few or no symptoms, where the quantity of virus shedding is likely to be low but transmission may still occur.

Polymerase chain reaction (PCR) assays have been shown to be more sensitive than culture or antigen testing for the detection of some respiratory viruses in specimens from various populations, including both immunocompetent and immunosuppressed children (1–5) and adults (2,6–8), and to detect respiratory viruses earlier in the disease course (3,9). Few studies have comprehensively compared the relative sensitivities of 2 commonly used conventional methods, viral culture and direct fluorescent antibody staining (FA), and molecular testing with real-time reverse transcription (RT)-PCR for the detection of respiratory infections in patients after HCT. We tested nasal wash specimens collected from HCT recipients regardless of the presence of respiratory symptoms as part of a prospective, longitudinal study. A previous analysis from this study described the patterns of respiratory virus infections and assessed the spectrum of clinical symptoms from evaluable patients with longitudinal follow-up (10). In this analysis, we include specimens collected from all study participants who were tested by all 3 methods to specifically evaluate the relationship of viral load and assay sensitivity. We also examined the performance of traditional assays for detecting virus in nasal wash samples relative to the number of reported respiratory symptoms.

Patients and methods

Specimen collection

The specimens included in this analysis are a subset of the approximately 1200 samples collected from 157 patients during a prospective, longitudinal surveillance study of patients following HCT (10) that was approved by the Institutional Review Board at Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. The study began in December 2000 and continued for four winter/spring seasons (between the months of November and June) until June 2004. Nasopharyngeal (NP) wash (or NP swab) and oropharyngeal (OP) swab samples were collected at weekly or longer intervals from each subject for up to 100 days after HCT. NP washes were collected using 10 mL of saline (5 mL/nostril) for adults and 5 mL (2.5 mL/nostril) for children and combined into 1 sterile vial with the OP swab. The samples were tested for respiratory viruses by PCR and by 1 or 2 conventional methods (culture and FA) depending on specimen volume. The subset of 688 specimens from 131 patients that were tested by all 3 methods was analyzed in this study. Subjects completed surveys that asked them to report the presence of any of 10 respiratory symptoms, including rhinorrhea, shortness of breath, sputum production, cough, wheezing, sore throat, watery eyes, sinus congestion, nasal congestion, and sinus headaches.

Respiratory virus detection

Specimens were stored and transported to the laboratory on ice and split into multiple aliquots within 4 to 8 h of collection. One aliquot was immediately cultured for respiratory viruses by conventional tube culture on rhesus monkey kidney, buffalo green monkey kidney, HL (transformed HeLa), and HDF (human foreskin fibroblast) cell lines (11), which

allowed for isolation of respiratory syncytial virus (RSV), influenza virus type A (FluA), influenza virus type B (FluB), parainfluenza viruses (PIV), adenoviruses (AdV), and rhinoviruses (RhV). An aliquot was tested by a direct FA assay for RSV, FluA, FluB, PIV types 1–3, and AdV (1).

A third aliquot was frozen at -80°C for subsequent analysis by PCR. The samples were thawed, extracted, and analyzed for the presence of RSV, FluA, FluB, PIV types 1 and 3, and human metapneumovirus (MPV) RNA by 6 separate, quantitative, real-time RT-PCR assays as previously described (1). For the quantitative assays, the threshold cycles of clinical samples were compared to standard curves generated in each RT-PCR run by amplification of known numbers of specific RNA transcripts containing the viral targets. Results were expressed as viral copies per mL of original sample. Each assay reliably detected 10 viral copies per reaction, providing a sensitivity of 1000 copies/mL (10 μL of specimen added per reaction) and the assays were shown to be specific for their intended targets. Specimens with positive results of <10 copies/reaction were repeated to confirm positivity. Interassay standard deviations and coefficients of variation (CV) were calculated between virus-specific RT-PCR runs to evaluate assay reproducibility. The CV for viral quantification was $<10\%$ for each respiratory virus RT-PCR assay when estimated using the results of the low positive control.

Results

Qualitative virus detection

Over the 4 winter/spring seasons, 688 specimens collected from 131 patients were analyzed by all 3 methods. Overall, 37 specimens (5.4%) from 26 patients (24 adults and 2 children) had one of the respiratory viruses detected. Thereby, 34, 15, and 6 were positive by RT-PCR, culture, and FA, respectively. Four of the 37 specimens positive for a respiratory virus by any method were positive by all 3 methods, including 3 specimens positive for RSV and 1 positive for FluA. Of the 20 specimens positive by RT-PCR alone, 2 were positive for RSV, 2 for PIV type 1, 8 for PIV type 3, and 8 for MPV. Neither culture nor FA was designed to detect MPV. One specimen was positive for PIV type 1, and 2 for RhV by culture alone. Defining any positive result as a true positive (i.e., each method is considered 100% specific), the sensitivity of RT-PCR, culture, and FA for the detection of respiratory viruses was 91.9%, 40.5%, and 16.2%, respectively. Excluding positive results for MPV and RhV, which were not targeted by all 3 assays, the sensitivity of RT-PCR, culture, and FA for the detection of the other respiratory viruses was 96.3%, 48.1%, and 22.2%, respectively.

The number of specimens positive for each virus type is shown in Table 1 by detection method. AdV were not detected in any specimen by FA or culture and were not included as targets in the RT-PCR assay. There were no discrepancies between methods in the type of virus detected in individual specimens.

Among the 27 specimens positive for influenza viruses, RSV, or PIV, 10 were from patients who reported none of 10 respiratory symptoms at the time of specimen collection, 4 were from patients who reported 1 or 2 symptoms, and 13 were from patients who reported 3 or more symptoms. The number and proportion of specimens collected from patients reporting 0, 1 or 2, and 3 or more respiratory symptoms that were positive by each of the 3 methods are shown in Table 2. The conventional assays detected a respiratory virus in 11 (64.7%) of 17 symptomatic and 4 (40%) of 10 asymptomatic specimens. In contrast, the RT-PCR assays detected a respiratory virus in all 17 symptomatic specimens and in 9 (90%) of 10 asymptomatic specimens. The sensitivity of PCR, culture, and FA was 90% (9 of 10), 40% (4 of 10), and 10% (1 of 10), respectively, for detection of influenza viruses, RSV, or PIV in specimens from asymptomatic patients.

Quantitative virus detection

The quantity of virus (log₁₀ copies/mL) detected in each of the 26 specimens positive by RT-PCR for RSV, FluA, FluB, or PIV is shown in Figure 1A classified according to the results of the two conventional detection methods and the specific respiratory virus type. Of 13 specimens with viral loads <10⁵ copies/mL, including 9 of 17 PIV, 2 of 6 RSV, and both FluB-positive specimens, all were negative by FA and 10 were negative by culture, demonstrating that the conventional methods failed to detect low quantities of virus. In addition, culture and FA identified PIV in only 2 (11.8%) and 5 (29.4%) specimens, respectively, of the 17 specimens positive for PIV by RT-PCR, regardless of viral load.

The mean log₁₀ viral copies/mL quantified by RT-PCR in all 26 RT-PCR-positive specimens is shown in Figure 1B by detection method. Specimens positive only by RT-PCR had significantly fewer viral copies/mL than specimens positive by both RT-PCR and culture ($P = 0.002$) and by both RT-PCR and FA ($P < 0.001$). The difference in the quantity of viral copies detected in specimens positive by RT-PCR alone and those positive by RT-PCR and either of the conventional detection methods was similar when specific viral types were examined. The mean virus quantity (log₁₀ copies/mL) in 6 specimens positive for RSV and 15 specimens positive for PIV type 3 by RT-PCR is shown in Figure 1C by detection method. The mean log₁₀ copies/mL were significantly lower in specimens positive by PCR alone for both the RSV ($p = 0.001$) and PIV type 3 ($p = 0.008$) positive specimens. The mean log₁₀ copies/mL of MPV in 8 specimens positive by RT-PCR was 6.44.

Discussion

In this study of respiratory specimens collected from patients with and without respiratory symptoms following HCT, RT-PCR testing was significantly more sensitive than either culture or FA for detection of respiratory viruses, providing an increased yield of positive specimens 2 times relative to culture and more than 4 times relative to FA. Excluding specimens positive for MPV, which the FA method was not designed to detect, FA testing alone missed a significant proportion (23 (79%) of 29) of respiratory virus detections in specimens from the HCT recipients. Importantly, RT-PCR was markedly more sensitive than both conventional methods for detecting patients with asymptomatic viral shedding. Inclusion of rhinovirus in the RT-PCR testing panel and of human metapneumovirus in the FA panel for this study would likely have increased the sensitivity of both RT-PCR and FA.

A number of studies comparing PCR with viral culture and/or FA for respiratory virus detection have shown significantly higher sensitivity of the PCR method. In this study, the increased detection of respiratory viruses by RT-PCR compared to FA (greater than 4-fold) was higher than the 1.4 to 2.7-fold increases reported by others (1,2,4,7). However, these previously published studies were performed on specimens collected mostly from immunocompetent children with respiratory symptoms. Our study population consisted mainly of immunocompromised adults, who shed lower quantities of virus compared to immunocompetent children (12). In addition, we collected from patients regardless of the presence of symptoms. These factors likely led to the increased proportion of samples from this study that was positive only by the more sensitive PCR assay. The findings in our study are similar to those of previous studies comparing detection methods for respiratory viruses in specimens from HCT recipients (3,6,8). In one study (6), in which specimens from adult recipients of stem cell transplants were tested for 7 respiratory viruses by culture and PCR, a virus was detected in 21% and 63% of episodes of respiratory illness, and in 1% and 9% of specimens from asymptomatic patients by culture and PCR, respectively. In another study, positive specimens collected from subjects without respiratory symptoms contained fewer virus copy numbers than specimens collected from ill patients (10), accounting for some of the increased detection by PCR alone. These data have important implications both for

detection of asymptomatic viral shedding and also for implementation of appropriate isolation measures for patients with subclinical viral respiratory infections. Our data indicate that PCR-based assays should be utilized for optimal and early detection of respiratory viruses, including detection of nosocomial viral respiratory infections.

In this study, the specific respiratory viruses most often not detected by the conventional detection methods were PIVs, which accounted for 10 (50%) of the 20 specimens positive by RT-PCR alone, and MPV, which accounted for 8 (40%) of the 20 specimens positive by RT-PCR alone. The FA and culture techniques used in our laboratory were not designed to detect MPV, so this result was not surprising. Although FA and particularly culture have previously been considered the “gold standard” for detection of respiratory viruses (13–15), our results clearly indicate that RT-PCR is superior for detection of all respiratory viruses and especially of PIV in upper respiratory samples from HCT recipients.

Viral infections detected by RT-PCR alone had significantly lower virus copy numbers compared with infections detected by both RT-PCR and conventional methods. It is possible that some specimens had low levels of virus due to suboptimal specimen collection. Quantitative results for nasal wash specimens may vary due to differences between patients in specimen collection volumes. Nasal wash recovery volumes were recorded for each specimen in this study and varied from 2 mL to 9.7 mL. In a previous analysis of these specimens (10), adjustment of viral load for specimen volume did not significantly change the viral load results, suggesting that the differences we observed are truly related to the detection methods used by each of the assays and not specimen collection. Other investigators reported an association between low viral quantity as estimated by the PCR threshold cycle values and increased detection by PCR alone compared with detection by both PCR and FA and/or culture (2,3,7), although the viruses were not quantified as in the present study. The presence of low levels of respiratory virus nucleic acid by PCR may not always reflect an association with infectious virus production and transmission or progression to more severe illness in the patient. Clinical studies are warranted to address these issues. PIV, in particular, has been documented to cause prolonged nosocomial outbreaks among HCT recipients even with proper infection control measures (16,17). Since infection control programs generally emphasize symptoms, this suggests that asymptomatic infection may perpetuate ongoing transmission.

In conclusion, this study further extends our understanding of how respiratory virus quantity in nasal wash samples from immunosuppressed patients determines the ability of different diagnostic techniques to detect the viruses and how different diagnostic techniques perform relative to the presence of respiratory symptoms. The use of RT-PCR to analyze samples from an HCT recipient population may facilitate early detection of respiratory viruses, even prior to onset of symptoms when viral loads are likely to be low. Although it is not known whether respiratory viruses detected by PCR in asymptomatic patients may lead to respiratory virus transmission, optimized sensitivity may be important for identification of patients who need to be placed in isolation. For symptomatic patients, PCR testing provides a sensitive diagnostic approach to identifying the etiology of respiratory symptoms and appropriate isolation of the ill patient. Additionally, quantitative RT-PCR assays can be used to initiate appropriate treatment and monitor changes in viral load during therapy. For these reasons, we propose that molecular diagnostic methods should be the standard method for detection of respiratory viruses in severely immunocompromised patients.

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Fig. 1.
A. Quantity of virus (log₁₀ copies/mL) in 26 real-time polymerase chain reaction (RT-PCR)- positive specimens according to result of conventional detection method and respiratory virus type: respiratory syncytial virus (RSV) (◆), parainfluenza virus (PIV) (■), influenza virus type A (FluA) (▲), influenza virus type B (FluB) (●). **B.** Mean quantity of virus (log₁₀ copies/mL) quantified by RT-PCR in 12 specimens positive for any respiratory virus by RT-PCR alone, 12 positive by both RT-PCR and culture, and 6 positive by both RT-PCR and fluorescent antibody staining. **C.** Mean quantity of virus (log₁₀ copies/mL) quantified by RT-PCR in 6 specimens positive for RSV (open bars) and 15 specimens positive for PIV type 3 (solid bars) by method of detection.

Detection of specific respiratory viruses by real-time polymerase chain reaction (RT-PCR), culture, and direct fluorescent antibody staining (FA) in 688 specimens from hematopoietic stem cell transplant patients

Table 1

Method	Number of specimens with respiratory virus detected						
	Any	RSV	FluA	FluB	PIV	MPV	RhV
RT-PCR	34	6	1	2	17	8	ND
Culture	15	4	1	2	6	ND ^I	2
FA	6	3	1	0	2	ND	ND
Total	37	6	1	2	18	8	2

RSV, respiratory syncytical virus; FluA, influenza virus type A; FluB, influenza virus type B; PIV, parainfluenza virus; MPV, metapneumonia virus; RhV, rhinovirus; ND, not done.

^I Assay was not designed to detect this virus.

Table 2

Number and proportion of samples positive by real-time polymerase chain reaction (RT-PCR), culture, and direct fluorescent antibody staining (FA) for respiratory syncytial virus, parainfluenzavirus, or influenza viruses, according to the number of respiratory symptoms reported by the patient when the specimen was collected

Method	Number (%) of positive specimens by number of reported respiratory symptoms		
	0 (n = 10)	1 to 2 (n = 4)	3 to 10 (n = 13)
RT-PCR	9 (90)	4 (100)	13 (100)
Culture	4 (40)	2 (50)	9 (69.2)
FA	1 (10)	0 (0)	5 (38.5)