MAJOR ARTICLE

Polymerase Chain Reaction Is More Sensitive than Viral Culture and Antigen Testing for the Detection of Respiratory Viruses in Adults with Hematological Cancer and Pneumonia

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We retrospectively analyzed the value of polymerase chain reaction (PCR) for the detection of respiratory viral infections in 43 patients with hematological cancer whose bronchoalveolar lavage (BAL) samples had been stored. In addition, 17 nose-throat (NT) swabs and 29 blood samples had been obtained. PCR was performed to detect parainfluenza viruses 1–3, respiratory syncytial virus, rhinovirus, influenza viruses A and B, enteroviruses, and coronaviruses. Viral cultures or antigen testing of BAL samples revealed 9 respiratory viruses in 8 patients. By use of PCR, 8 more respiratory viruses were detected in another 7 patients, increasing the rate of identification from 19% to 35% ($P < .0005$). Available NT swabs yielded the same results with PCR as did BAL samples. We conclude that PCR is more sensitive than viral culture or antigen or serologic testing for detection of respiratory viruses in patients with hematological malignancies, and that it offers the possibility for early, more rapid diagnosis.

Pneumonia is one of the most common infectious complications of stem cell transplantation (SCT) and cytotoxic treatment for hematological malignancies. Traditionally, pulmonary infections in patients who undergo SCT or who receive cytotoxic agents have been mostly attributed to bacteria, fungi, and herpesviruses. During the past decade, respiratory viruses have increasingly been recognized as important causes of severe lower respiratory disease in these patients [1–5]. Respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses, adenoviruses, and picornaviruses have all been identified as significant pathogens of community-acquired and nosocomial infections.

At present, viral culture is the "gold standard" for laboratory diagnosis of respiratory virus infections. However, it is not suitable as a rapid diagnostic test, because culture usually takes 2–10 days to yield results, and, therefore, its clinical value is limited. To overcome these limitations, more rapid diagnostic techniques, such as direct viral antigen detection, have been introduced in the routine laboratory setting. These techniques provide results faster, but they are generally considered to be less sensitive and specific than is conventional cell culture. Also, they are not suitable for detection of all respiratory viruses; for

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example, antigen testing for rhinoviruses is not possible, because too many subtypes exist and cocirculate at the same time [6, 7]. Although it has been studied in several patient groups, the role of respiratory virus infections as the cause of severe pulmonary complication in patients receiving cytoreductive therapy or undergoing SCT is not yet clarified and may have been underestimated in previous studies, particularly in studies that have relied on virus culture.

PCR, either in single or multiplex format, has proven to be an extremely specific and sensitive method for the detection of respiratory viruses [8, 9]. In our hospital, nested reverse-transcriptase PCR (RT-PCR) techniques have been developed to detect the following respiratory viruses: parainfluenza viruses 1–3, RSV, rhinoviruses, influenza viruses A and B [9], enteroviruses, and coronaviruses.

In this study, we investigated the value of PCR for the detection of respiratory virus infections in 43 adults with hematological cancer who also had signs of pneumonia to further establish the role of respiratory viruses in these patients.

PATIENTS AND METHODS

Patients. The University Medical Center at Utrecht, The Netherlands, is a referral center for treatment of hematological malignancies in adults. Every year, ~75 patients undergo either autologous or allogeneic SCT.

From October 1997 through May 2000, all patients from the hematology ward and the hematology outpatient clinic who underwent bronchoalveolar lavage (BAL) were selected for study through the database of the Department of Virology. Since October 1997, BAL samples obtained from patients with hematological malignancies have been routinely stored at the hospital's diagnostic virology laboratory. For this retrospective study, 43 adults with hematological cancer who also had signs of pneumonia and radiographic pulmonary abnormalities and whose BAL samples had been stored were considered assessable. We reviewed the patients' charts to obtain the following information: underlying disease and therapy; antimicrobial treatment; additional bacterial, fungal, and viral culture data or antigen testing results; serologic data; clinical features; and outcome. A total of 43 BAL specimens from these patients had been investigated routinely for the following pathogens: bacteria, mycobacteria, fungi, herpesviruses, and respiratory viruses (influenza viruses, RSV, parainfluenza viruses, picornaviruses, and adenoviruses). Nose-throat (NT) swabs had also been obtained from 17 of these 43 patients within 1 week of the BAL sample. These NT swabs had also been stored after conventional testing for respiratory viruses. In addition, paired serum samples had been obtained from 29 patients for detection of atypical bacterial (e.g., *Mycoplasma pneumoniae*, *Chlamydia* species, *Legionella* species) and respiratory virus pathogens.

The stored BAL samples and NT swabs were subsequently analyzed by use of PCR techniques for the detection of respiratory viruses.

SCT regimens, infection prophylaxis, and infection-prevention measures. Patients with an expected duration of neutropenia of >7 days received antibacterial prophylaxis with orally administered ciprofloxacin (500 mg twice per day) and orally administered antifungal prophylaxis with amphotericin B tablets (200 mg 4 times per day) and fluconazole (50 mg once per day). The antimicrobial regimen was continued until the granulocyte count had increased to $>0.5 \times 10^9$ cells/L. For prevention of bacteremia caused by α-hemolytic streptococci, patients received clindamycin (300 mg 3 times per day) while they had neutropenia in case of high-dose cytarabine ($\geqslant$500 mg/m^2). Patients undergoing SCT received intravenous cephalothin (1 g 6 times per day) after transplant while they had neutropenia. Patients who underwent allogeneic SCT routinely received valacyclovir (500 mg twice per day) and cotrimoxazole (480 mg once per day) during the first 12 months after transplantation. In addition, patients who had a positive result of a cytomegalovirus pp65 test during the first 3 months after they underwent allogeneic SCT received preemptive therapy with ganciclovir [10]. Hospitalized patients were cared for in single rooms with free entry for staff and visitors. Careful hand washing and the use of low-microbial-count food were the only preventive measures used for these patients. Pulmonary infections were considered to be hospital acquired if symptoms developed $\geqslant$4 days after admission.

Diagnostic methods for the routine detection of respiratory virus pathogens. Nasopharyngeal and throat swabs, which were placed in the same viral transport media, and BAL samples, which were placed in a tube containing virus transport medium, were obtained for viral culture; they were either transported to the laboratory immediately or stored at 4°C for a maximum of 24 h. The material was divided: some of it was frozen and stored at −70°C for further analysis by PCR, and some was directly used for viral culture. These cultures were performed by inoculating HEp-2C, R-HELA, and tertiary monkey kidney (t-MK) cells with 100 μL of each clinical sample for the detection of respiratory viruses (adenoviruses, parainfluenza viruses, RSV, influenza viruses, and picornaviruses). The cultures were examined for cytopathic effect twice per week for 10 days. In positive cultures, virus was identified by immunofluorescence with commercial monoclonal antibodies (Dako Imagen) for influenza A and B viruses, RSV, parainfluenza viruses 1–3, and adenoviruses. Rhinoviruses were distinguished from enteroviruses by means of acid-lability testing.

Rapid antigen testing was performed after 1–2 days of culture, usually before a cytopathic effect could be noticed. Immunofluorescence microscopy that used virus-specific monoclonal an-

tibodies (Dako Imagen) was used to detect RSV, parainfluenza viruses 1–3, influenza A and B viruses, and adenoviruses.

Only paired serum samples were used for serologic detection of respiratory viral illness, and a positive diagnosis was defined as a 4-fold increase in virus-specific antibody titers. The standard serologic test complement fixation was used for RSV, influenza A and B, parainfluenza virus 1–3, and adenovirus infection. In addition, the indirect immunofluorescence assay was used to detect RSV and influenza A and B.

RNA extraction from clinical specimens and nested PCR. PCR was performed to detect influenza A and B virus, parainfluenza viruses 1–3, picornaviruses (rhinovirus and enterovirus), RSV, and coronaviruses on the stored BAL samples obtained from all 43 patients and on the NT swabs obtained from 17 of these patients; the NT swabs had been obtained within 1 week of the BAL.

Primers were obtained from literature or selected from GenBank on conserved regions of the genes of the matrix protein for influenza A virus, of the hemagglutinin gene for influenza B virus [9], the 5′ noncoding region for the picornaviruses [11], the nucleocapsid protein for RSV A and B, the hemagglutinin-neuraminidase glycoprotein for parainfluenza 1–3 [12], and the nucleocapsid protein for coronavirus 229E and OC43 [13]. Nucleic acid extraction was performed from 100 μL of patient material in accordance with the method of Boom et al. [14]. For all PCR reactions, a 1-tube RT-PCR was followed by a nested PCR, essentially as described by Nijhuis et al. [15]. Modifications of this method consisted of optimization of each separate PCR reaction by serial dilution of $MgCl_2$ and primer concentrations. PCR was performed on a PE 9600 Thermocycler (ABI). Rhinoviruses were identified by *Bgl*I digestion of the picornavirus RT-PCR amplicons [16]. PCR products were visualized on an ethidium bromide–stained agarose gel by use of ultraviolet illumination.

Statistical analysis. Descriptive statistics were expressed as median values. χ^2 Analysis was performed to determine the degree of significance between the various variables.

RESULTS

Patient characteristics. The demographic characteristics, underlying disease, conditioning therapy, use of prophylaxis, and immunologic status of the patients are shown in table 1. The majority of patients presented with signs and symptoms of respiratory disease. Fever (in 30 [70%] of 43 patients), cough (in 28 [65%]), and shortness of breath (in 23 [53%]) were the most common complaints. Ten (23%) of 43 patients developed signs and symptoms of pneumonia at the time of hospital admission, and 33 patients (77%) developed community-acquired pneumonia. Twenty-eight (65%) of 43 patients had undergone SCT; the median duration from transplantation until the onset of symptoms of respiratory disease was 4 months (range, 0–28 months). Twenty-six (60%) of 43 patients developed pneumonia during the winter season (October–March).

Detection of respiratory viruses. By means of culture, antigen testing, or both, 9 respiratory viruses were identified in 8 patients, of which 4 were RSVs, 3 were rhinoviruses, and 2 were influenza A viruses. The same 9 respiratory viruses were detected by nested RT-PCR. One of the patients had an infection with a respiratory virus twice. Initially, this patient was admitted with pneumonia caused by RSV, which subsided spontaneously within 10 days. Then, the patient, who was still an inpatient at the hospital, developed nosocomial pneumonia again 1 week later, which was caused by culture-proven influenza A. An additional 8 respiratory viruses were detected by PCR in another 7 patients (table 2).

One patient had a dual infection with rhinovirus and parainfluenza 1 virus. In total, 17 respiratory viruses were detected by PCR in 15 (35%) of 43 patients, compared with 9 respiratory viruses (19%) in 8 patients detected by culture, antigen testing, or both ($P < .0005$). Paired serum samples were available for 29 patients. Serologic testing showed a 4-fold increase in RSV-specific IgG antibody titer in only 4 patients and a 4-fold increase in titer for adenovirus in only 3 patients.

A combined NT swab was obtained from 17 of 43 patients within 1 week of the BAL sample. In 7 patients with respiratory virus disease for whom samples of both NT and BAL were available, the nested RT-PCR on NT samples always yielded the same results as the BAL samples (table 2).

Other causes of pneumonia. In 10 patients (23%), no cause of pneumonia was found (table 3). Respiratory virus pathogens could be detected in 15 patients (35%). In 6 (40%) of 15 patients in whom a respiratory virus pathogen was detected, another cause of pulmonary infection or lung injury was clinically probable. Two of these patients were thought to have pneumonia caused by both a bacterium and a respiratory virus (*Staphylococcus aureus* and rhinovirus in one patient, and *Haemophilus influenzae* and rhinovirus in the other); of another 2 patients, 1 had a proven *(Aspergillus fumigatus)* and 1 had a probable pulmonary fungal infection together with an infection with enterovirus and influenza A virus, respectively. Another patient had a posttransplantation lymphoproliferative disease with pulmonary involvement after receiving a stem cell transplant from a matched, unrelated donor, in combination with an enterovirus infection. In 1 patient, coronavirus was detected in addition to a bronchiolitis obliterans. Four (9%) of 43 patients had pneumonia probably caused by 1 (in 3 patients) or 2 (in 1 patient) bacteria. *Enterobacter* species, *Pseudomonas* species, *H. influenzae,* and *Stenotrophomonas maltophilia* were isolated from these patients. A total of 9 patients (21%) had a proven (in 4 patients) or probable (in 5) pulmonary infection with fungi. Five patients had other causes of pulmonary disease.

Table 1. Characteristics and outcomes of 43 adults with hematological cancer for whom abnormalities were visible on a chest radiograph.

Characteristic	All patients (n = 43)	Patients with respiratory virus (n = 15)	Patients without respiratory virus (n = 28)
Age, median years (range)	46 (17–66)	45 (18–65)	43 (17–66)
Sex, no. male/no. female[a]	28/15	8/7	20/8
Underlying disease			
Acute myelogenous leukemia	10	4	6
Acute lymphoblastic leukemia	6	3	3
Chronic myelogenous leukemia	6	—	6
Multiple myeloma	7	4	3
Non-Hodgkin's lymphoma	6	2	4
Myelodysplastic syndrome	4	1	3
Other	4	1	3
Treatment			
Stem cell transplantation[a]	28	11 (73)	17 (61)
Allogeneic	24	9	15
Autologous	4	2	2
Cytotoxic therapy	15	4 (27)	11 (39)
Granulocytopenia[a,b]	18	5 (33)	13 (46)
Received immunosuppressive therapy[a]	24	9 (60)	15 (54)
Clinical signs and symptoms[a]			
Fever	30	10 (67)	20 (71)
Cough	28	12 (80)	16 (57)
Dyspnea	23	8 (53)	15 (54)
Malaise	18	8 (53)	10 (36)
Rhinitis	3	—	3 (11)
Pharyngitis	1	1 (7)	—
Type of specimen obtained			
BAL fluid	43	15	28
BAL fluid and nose-throat swab	17	7	10
No. cases nosocomial/no. cases community-acquired respiratory disease[a]	10/33	3/12	7/21
Time between transplantation and pulmonary abnormalities, median months[a]	4	5	3
Acquired infection during winter months[a,c]	26	11 (73)	15 (54)

NOTE. Data are no. (%) of patients, unless otherwise indicated. BAL, bronchoalveolar lavage.

[a] None of the differences between the groups were significant.

[b] Granulocyte count, $\leq 0.5 \times 10^9$ cells/L

[c] October–March.

One patient had a progressive Epstein-Barr virus–associated posttransplantation lymphoproliferative disease with pulmonary involvement after receiving a stem cell transplant from a matched, unrelated donor; 2 patients had bronchiolitis obliterans; another patient had a cytomegalovirus pneumonitis; and 1 patient developed toxic lung injury after transplantation.

Treatment. Two of the 5 patients with RSV pneumonia were treated with aerosolized ribavirin (2 g 3 times per day, for a minimum of 7 days). At the start of ribavirin treatment, these 2 patients had had symptoms of upper respiratory tract infection for 1 week. Both patients recovered completely after 1 week of treatment. Another 2 patients also recovered from RSV pneumonia, but without administration of ribavirin. One patient died. This patient had contacted RSV pneumonia during a recurrence of acute myelogenous leukemia shortly after receiving an allogeneic stem cell transplant from a matched, unrelated donor; also, the patient did not receive treatment, because the diagnosis was made by a positive PCR result for RSV only after death.

Comparison between patients with pneumonia with or

Table 2. Detection of respiratory viruses by culture, antigen testing, or both, and by PCR of paired serum samples and either bronchoalveolar lavage (BAL) samples or nose-throat (NT) swabs.

Virus	BAL samples		NT swabs		Paired serum samples
	Culture and/or antigen testing	PCR	Culture and/or antigen testing	PCR	
Respiratory syncytial virus	4	5	4	4	4
Human rhinovirus	3	5	0	0	ND
Parainfluenza viruses 1–3	0	2	0	0	0
Human coronaviruses	0	1	0	0	ND
Influenza viruses A and B	2	2	1	2	0
Enteroviruses	0	2	0	1	ND
Adenoviruses	0	ND	0	ND	3
Total	9	17	5	7	7

NOTE. BAL samples were tested for 43 patients, NT swabs were tested for 17 patients, and 29 patients underwent serologic testing. ND, not done.

without respiratory virus. Patients with pneumonia caused by a respiratory virus were compared with patients who had pneumonia that was not caused by a respiratory virus with regard to the following characteristics: underlying disease, treatment, immune status, use of immunosuppressives, signs and symptoms, type of specimen obtained, presence of nosocomial or community-acquired respiratory disease, time of transplantation, and the period of the year that they acquired their infection (table 1). There was no significant difference in parameters between the 2 groups, although there seems a tendency toward more male patients, use of immunosuppressives, and the presence of neutropenia in the group of patients who had pneumonia that was not caused by a respiratory virus. The majority (11 [73%] of 15) of the cases of respiratory virus–associated pneumonia occurred during the winter season (October–March), whereas the occurrence of pneumonia without detection of respiratory virus was spread equally throughout the year (15 [54%] of 28 cases occurred during the winter months vs. 13 [46%] of 28 cases during the summer months).

DISCUSSION

For 43 patients with hematological cancer and pneumonia, stored BAL samples yielded significantly more respiratory viruses when a nested RT-PCR was performed, as compared with standard culture, rapid culture, or both. Serologic testing was only of value in 4 cases of acute RSV infection and in 3 cases of adenovirus infection. These results indicate that previous studies relying on viral culture, antigen testing, or both to determine the incidence and role of respiratory viruses in this patient group may have underestimated the true incidence [1–5].

During the past decade, respiratory viruses have been increasingly recognized as causative agents of respiratory tract infections in severely immunocompromised patients [1–5]. High frequencies of nosocomial acquisition, persistence of infection beyond the time periods reported for immunocompetent patients, and a high frequency of pneumonia and death have been found in association with respiratory virus infections in immunocompromised patients [4]. As in some other studies, by PCR, we found a relatively high incidence of respiratory virus–associated pneumonia in immunocompromised patients. Reported incidences of respiratory virus infections were 26%–36% in adult bone marrow transplant recipients with acute upper and lower respiratory illnesses; for immunocompromised patients, the rate was 19% [3, 17, 18]. However, we cannot confirm some of the reported high frequencies of nosocomial acquisition, nor did we find a high rate of deaths due to respiratory virus–associated pneumonia.

Table 3. Causes of pulmonary abnormalities in 43 adult patients with hematological malignancies.

Pathogen or other cause of radiographic abnormality	No. of cases (no. proven/ no. probable)
Bacteria	4
Bacteria and respiratory virus	2
Respiratory virus	9
Respiratory virus plus fungi	2 (1/1)
Fungi	9 (4/5)
Other[a]	5
Other[a] plus virus	2
Unknown	10

[a] Other causes were bronchiolitis obliterans (in 2 patients), Epstein-Barr virus–associated posttransplantation lymphoproliferative disease (in 2), acute toxic lung injury (in 1), and cytomegalovirus pneumonia (in 1).

Overall, in studies published elsewhere, RSV accounted for the majority of respiratory virus infections with high mortality rates [4, 18, 19]. RSV-related mortality rates as high as 83% have been reported in hospitalized adult patients with leukemia, and the rates have been as high as 78% in persons who undergo SCT [19]. Prompt therapy of RSV infections with aerosolized ribavirin with or without intravenous immunoglobulin appears to impact favorably on the frequency of progression to pneumonia and death in some studies, but randomized controlled studies are lacking [20, 21]. Data from our study are consistent with those of previous studies that have shown that RSV is the most prevalent respiratory virus in persons with respiratory virus–associated pneumonia. The mortality rate in our study, however, was only 20%.

Influenza and parainfluenza viruses have also been reported frequently in immunocompromised patients during community-outbreak periods of these respiratory viruses [22–25]. In particular, parainfluenza virus infection may be an important cause of life-threatening pneumonia in patients who undergo SCT or who have received treatment for leukemia, with mortality rates of up to 66% [22, 23]. The incidence and severity of pneumonia in immunocompromised patients caused by influenza virus varied in several studies. In one study [24], influenza was isolated in 29% of persons who underwent SCT and who had an acute respiratory illness, and it had been complicated by pneumonia in 75% of these patients; the pneumonia-associated mortality rate was 17% in this study. Other researchers have concluded that influenza A virus in immunocompromised patients only occasionally causes severe complications, and that it is often mild and self-limiting [25]. We found 2 patients with influenza A virus–associated pneumonia and 2 patients with parainfluenza virus infections. None of these patients received antiviral therapy, and all recovered without sequelae.

Recently, rhinoviruses have also been identified as pathogens with the potential to infect the lower respiratory tract. Ghosh et al. [26] described 22 cases of rhinovirus-associated infections in myelosuppressed adult blood and bone marrow transplant recipients early after transplant, 7 of whom developed fatal pneumonia. In this study, rhinoviruses were detected by means of conventional methods. Of interest, with the use of molecular diagnostics, we documented an increased involvement of 30% of rhinoviruses in virus-associated pneumonia, indicating that rhinoviruses may play a serious role as a cause of pneumonia in immunocompromised patients.

We detected coronavirus in a sample obtained from one of our immunocompromised patients. Our observation is in line with the findings of another study, which demonstrated that pneumonia caused by coronavirus occurred in a patient who had received an autologous bone marrow transplant to treat breast cancer [27]. We also analyzed our samples for the presence of enterovirus, although the lower respiratory tract is not the usual site of enterovirus infection. However, in immunocompromised patients with pneumonia, presence of enterovirus in BAL specimens was demonstrated in several studies [28–30]. These results were confirmed in our own study, in which 2 patients revealed the presence of an enterovirus and thereby demonstrated that these infections should be considered as a cause of pneumonia in severely immunocompromised patients.

Although a method for the detection of adenovirus by PCR has been described elsewhere [31], we only detected adenovirus infections by serologic testing. We found a 4-fold increase in serum titer of adenovirus in 3 patients. No adenoviruses were detected by standard culture or antigen detection; a generic PCR for adenovirus infection was not yet available at our laboratory.

There are still some limitations to be considered when PCR is used as diagnostic tool for the detection of respiratory viruses. First, we cannot completely rule out contamination of the BAL sample from the upper respiratory tract. As stated before, we found 100% concordance between PCR results for BAL and NT samples. Thus, we cannot rule out the possibility of contamination. However, this uncertainty is intrinsically related to the use of BAL. Second, we cannot rule out the possibility of positive PCR results having little clinical significance, because severely immunocompromised patients are known to shed virus for long periods of time [32]. Therefore, the exact meaning of a positive PCR result still needs to be determined in prospective studies.

Because different antiviral agents are now available or under development for treatment of RSV, influenza virus A, and rhinovirus infections [33–35], it is important to improve methods to rapidly diagnose respiratory illness that are caused by respiratory viruses in immunocompromised patients. A rapid and sensitive method for detecting respiratory viruses is essential to implement prompt measures, both to start treatment as soon as possible in patients who are at risk of developing pneumonia caused by respiratory viruses and to prevent or limit nosocomial spread of infection. We have showed that PCR might be an important tool to accomplish this goal.

In conclusion, we have shown that molecular diagnostic techniques significantly increase the detection rate of respiratory viruses in patients with hematological cancer and pneumonia, as compared with traditional methods. However, prospective surveillance studies are still necessary to further establish the clinical value of these techniques.

References

1. Englund JA, Sullivan CJ, Jordan MC, et al. Respiratory syncytial virus infection in immunocompromised adults. Ann Intern Med **1988**; 109: 203–8.
2. Wendt CH, Hertz MI. Respiratory syncytial virus and parainfluenza virus infections in the immunocompromised host. Semin Respir Infect **1995**; 10:224–31.

3. Whimbey E, Champlin RE, Couch RB, et al. Community respiratory virus infections among hospitalized adult bone marrow transplant recipients. Clin Infect Dis **1996**; 22:778–82.
4. Whimbey E, Englund JA, Couch RB. Community respiratory virus infections in immunocompromised patients with cancer. Am J Med **1997**; 102(3A):10–18.
5. Ljungman P. Respiratory virus infections in bone marrow transplant recipients: the European perspective. Am J Med **1997**; 102(3A):44–7.
6. Doller G, Schuy W, Tjhen KY, et al. Direct detection of influenza virus antigen in nasopharyngeal specimens by direct enzyme immunoassay in comparison with quantitating virus shedding. J Clin Microbiol **1992**; 30:866–9.
7. Schmidt ML, Kudesia G, Wake S, et al. Prospective comparative study of culture specimens and methods in diagnosing influenza in adults. BMJ **1998**; 316:275.
8. Osiowy C. Direct detection of respiratory syncytial virus, parainfluenza virus, and adenovirus in clinical respiratory specimens by a multiplex reverse transcription–PCR assay. J Clin Microbiol **1998**; 36:3149–54.
9. Van Elden LJ, Nijhuis M, Schipper P, et al. Simultaneous detection of influenza viruses A and B using real-time quantitative PCR. J Clin Microbiol **2001**; 39:196–200.
10. Verdonck LF, Dekker AW, Rozenberg-Arska M, et al. A risk-adapted approach with a short course of ganciclovir to prevent cytomegalovirus (CMV) pneumonia in CMV-seropositive recipients of allogeneic bone marrow transplants. Clin Infect Dis **1997**; 24:901–7.
11. Andeweg AC, Besteboer TM, Huybreghs M, et al. Improved detection of rhinoviruses in clinical samples by using a newly developed nested reverse transcription–PCR assay. J Clin Microbiol **1999**; 37:524–30.
12. Echevarria JE, Erdman DD, Swierkosz EM, et al. Simultaneous detection and identification of human parainfluenza viruses 1, 2, and 3 from clinical samples by multiplex PCR. J Clin Microbiol **1998**; 36:1388–91.
13. Myint SH, Johnston SL, Sanderson G, et al. Evaluation of nested polymerase chain methods for the detection of human coronaviruses 229E and OC43. Mol Cell Probes **1994**; 8:357–64.
14. Boom R, Sol CJ, Salimans MM, et al. Rapid and simple method for purification of nucleic acids. J Clin Microbiol **1990**; 28:495–503.
15. Nijhuis M, Boucher CA, Schuurman R. Sensitive procedure for the amplification of HIV-1 RNA using a combined reverse-transcription and amplification reaction. Biotechniques **1995**; 19:178–80, 182.
16. Papadopulos NG, Hunter J, Sanderson G, et al. Rhinovirus identification by *Bgl*1 digestion of picornavirus RT-PCR amplicons. J Virol Methods **1999**; 80:179–85.
17. Ljungman P, Gleaves CA, Meyers JD. Respiratory virus infection in immunocompromised patients. Bone Marrow Transplant **1989**; 4: 35–40.
18. Bowden RA. Respiratory virus infections after marrow transplant: the Fred Hutchinson Cancer Research Center Experience. Am J Med **1997**; 102(3A):27–30.
19. Whimbey E, Couch RB, Englund JA, et al. Respiratory syncytial virus pneumonia in hospitalized adult patients with leukemia. Clin Infect Dis **1995**; 21:376–9.
20. Sparrelid E, Ljungman P, Ekelöf-Andström E, et al. Ribavirin therapy in bone marrow transplant recipients with viral respiratory tract infections. Bone Marrow Transplant **1997**; 19:905–8.
21. Ghosh S, Champlin RE, Englund J, et al. Respiratory syncytial virus upper respiratory tract illnesses in adult blood and marrow transplant recipients: combination therapy with aerosolized ribavirin and intravenous immunoglobulin. Bone Marrow Transplant **2000**; 25:751–5.
22. Wendt CH, Weisdorf DJ, Jordan MC, et al. Parainfluenza virus respiratory infection after bone marrow transplantation. N Engl J Med **1992**; 326:921–6.
23. Lewis VA, Champlin R, Englund J, et al. Respiratory disease due to parainfluenza virus in adult bone marrow transplant recipients. Clin Infect Dis **1996**; 23:1033–7.
24. Whimbey E, Elting LS, Couch RB, et al. Influenza A virus infections among hospitalized adult bone marrow transplant recipients. Bone Marrow Transplant **1994**; 13:437–40.
25. Ljungman P, Andersson J, Aschan J, et al. Influenza A in immunocompromised patients. Clin Infect Dis **1993**; 17:244–7.
26. Ghosh S, Champlin R, Couch R, et al. Rhinovirus infections in myelosuppressed adult blood and marrow transplant recipients. Clin Infect Dis **1999**; 29:528–32.
27. Folz RJ, Elkordy MA. Coronavirus pneumonia following autologous bone marrow transplantation for breast cancer. Chest **1999**; 115:901–5.
28. González Y, Martino R, Badell I, et al. Pulmonary enterovirus infections in stem cell transplant recipients. Bone Marrow Transplant **1999**; 23: 511–3.
29. Rabella N, Rodriguez P, Labeaga R, et al. Conventional respiratory viruses recovered from immunocompromised patients: clinical considerations. Clin Infect Dis **1999**; 28:1043–8.
30. Gonzalez Y, Martino R, Rabella N, Labeaga R, Badell I, Sierra J. Community respiratory virus infections in patients with hematologic malignancies. Haematologica **1999**; 84:820–3.
31. Echavarria M, Kolavic SA, Cersovsky S, et al. Detection of adenoviruses (AdV) in culture-negative environmental samples by PCR during an AdV-associated respiratory disease outbreak. J Clin Microbiol **2000**; 38:2982–4.
32. Gubavera LV, Matrosovich MN, Brenner MK, et al. Evidence for zanavamir resistance in an immunocompromised child infected with influenza B virus. J Infect Dis **1998**; 178:1257–62.
33. The MIST Study Group. Randomised trial of efficacy and safety of inhaled zanamivir in treatment of influenza A and B virus infections. Lancet **1998**; 352:1877–81.
34. Nicholson KG, Aoki FY, Osterhaus ADME, et al. Efficacy and safety of oseltamivir in treatment of acute influenza: a randomised controlled trial. Neuraminidase Inhibitor Flu Treatment Investigator Group. Lancet **2000**; 355:1845–50.
35. Schiff GM, Sherwood JR. Clinical activity of pleconaril in an experimentally induced coxsackievirus A21 respiratory infection. J Infect Dis **2000**; 181:20–6.