bjh review

The challenge of respiratory virus infections in hematopoietic cell transplant recipients

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Summary

Respiratory virus infections in hematopoietic cell transplant (HCT) recipients are a major cause of morbidity and mortality. While respiratory syncytial virus (RSV), human metapneumonvirus, parainfluenzaviruses, and influenza viruses are well known for their potential to cause fatal pneumonia, information has only recently emerged regarding the significance of the newly discovered viruses, such as human coronaviruses NL63 and HKU1, and human bocavirus. Lymphopenia seems to be the most important risk factor for progression to lower respiratory tract disease. Airflow obstruction is another complication of respiratory virus infections after HCT, and data to date indicate this complication may occur following parainfluenza virus and RSV infection. Infection control procedures are key for prevention. Unfortunately, there are no randomized treatment studies, which make the interpretation of the literature on interventions difficult. This article reviews the spectrum of pathogens, epidemiology, risk factors and clinical manifestations of infection, as well as recent advances in diagnostic and clinical management.

Keywords: hematopoietic cell transplantation, respiratory viruses, respiratory syncytial virus, parainfluenzaviruses, influenzaviruses.

In immunocompetent persons, respiratory viruses cause a wide range of syndromes. These syndromes span from the mere common cold episodes to incidences of croup, bronchiolitis, and airflow obstruction in children. Moreover, respiratory viruses may lead to exacerbation of asthma or chronic obstructive pulmonary disease, and frank pneumonia in adults (Kim *et al*, 2007). While direct mortality is rarely associated with these viruses in immunocompetent people, the impact on quality of life and the economic losses are substantial. In contrast, the clinical spectrum of disease is much more severe in immunocompromised patients. Respiratory viruses can cause fatal pneumonia and trigger a late airflow obstruction syndrome, which is associated with significant morbidity and mortality (Whimbey *et al*, 1993, 1996; Ljungman, 2001; Nichols *et al*, 2001a; Chemaly *et al*, 2006; Erard *et al*, 2006). This review will summarize the current status of respiratory virus diagnostics, disease associations, and management strategies.

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Epidemiology

Most of the available information focuses on respiratory syncytial virus (RSV), parainfluenza viruses, and influenza viruses, probably due to the fact that these viruses are readily identified by traditional virological detection methods, such as viral cultures and immunofluorescence-based methods. The more recently described human coronaviruses (HCoV), human metapneumovirus (HMPV), human bocavirus (HBoV), as well as human rhinoviruses (HRhV) require molecular detection methods for optimal detection; thus, information on their clinical importance has only begun to emerge.

The infection epidemiology or respiratory viruses in HCT recipients usually parallels that observed in the community, as these viruses circulate in immunocompetent individuals (including health care personnel and family members).

Importance of diagnostics

Non-molecular methods available for testing include: standard viral cultures (results available in several days), shell vial centrifugation cultures using specific monoclonal antibodies (results after 1–3 d), direct fluorescent antibody (DFA) tests (2 h), and enzyme immunoassays (2 h). On tissue sections from lung biopsy or autopsy specimens, virus-specific monoclonal antibody staining, viral cultures, or polymerase chain reaction (PCR) can be used.

For non-molecular methods, and to a lesser extent, for molecular detection methods, specimen acquisition techniques and handling are important for maximal diagnostic yield. Nasal wash or swab specimens should be placed on ice or in the refrigerator immediately and transported to the laboratory

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without delay (Englund *et al*, 1996). For non-molecular methods, specimen set-up in the laboratory should occur within 2–4 h.

There has been a significant shift towards molecular detection techniques in recent years. Indeed, these techniques offer the potential for multiplex testing platforms (Lee *et al*, 2007; Mahony *et al*, 2007; Nolte *et al*, 2007). This is important because of the non-specific clinical presentation of these infections. This ''syndromic'' nature of respiratory viral infections will ultimately require a multiplex testing platform for comprehensive detection of these pathogens. Several assays have been described in the literature. The Hexaplex^{TM} assay detects seven respiratory viruses and has shown excellent performance characteristics in various clinical settings (Hindiyeh *et al*, 2001; Kehl *et al*, 2001). Another multiplex platform (MultiCode-PLx system, EraGen Biosciences, Inc., Madison, WI) detected 17 respiratory viruses simultaneously and showed significantly increased diagnostic yield compared to DFA or culture methods. This was mainly caused by improved detection of influenza A virus and viruses not readily detected by standard virological methods, including HMPV, HcoV, and HRhV (Nolte *et al*, 2007). A 20-respiratory virus microbead-based assay also showed excellent sensitivity and specificity, as well as an increased yield for detection of viruses that are difficult to detect by culture or DFA (Mahony *et al*, 2007). Microarray and nanotechnologies are also being explored in order to develop large-scale and efficient viral detection platforms (Liu *et al*, 2006; Chiu *et al*, 2007; Fournier-Wirth & Coste, 2007).

Respiratory syncytial virus

Significance and risk factors

In patients with hematological malignancies, including HCT recipients, RSV causes upper respiratory infection (URI), which may progress to fatal pneumonia (Harrington *et al*, 1992). RSV lower respiratory tract infection has also been linked to late airflow obstruction, a debilitating condition of accelerated loss of lung function after HCT (Erard *et al*, 2006). During the respiratory virus season, the incidence may be as high as 10%, and both allogeneic and autologous transplant recipients may be infected (Nichols *et al*, 2001a; Small *et al*, 2002) (Table I, Fig 1). Acquisition of RSV may occur at any time after transplantation, but is most common when patients are in the outpatient setting.

In one large cohort study, winter season, male gender, and use of bone marrow as a stem cell source were identified for the acquisition of RSV in HCT recipients (Nichols *et al*, 2001a). URI precedes pneumonia in 80–90% of patients, and approximately 30–40% of patients with RSV URI progress to pneumonia after a median of 7 d. However, in some patients with RSV pneumonia, URI is not present, is very mild, or occurs only concurrently with the onset of pneumonia (Nichols *et al*, 2001a). Strongest risk factors for progression to pneumonia are older age and lymphopenia (Nichols *et al*, 2001a). During the first 3 months after HCT, patients receiving non-myeloablative conditioning regimens seem to be protected from progression to lower respiratory tract disease (Schiffer *et al*, 2006). Following reduced intensity conditioning, regimens may also have a somewhat lower risk of progression and fatal disease (Chakrabarti *et al*, 2002), however, larger studies are needed to better define the risk of progression. Most studies indicate that asymptomatic shedding of RSV is not a common phenomenon (Ljungman *et al*, 1989; Adams *et al*, 1999; Peck *et al*, 2007).

Treatment and prevention

Without treatment, RSV pneumonia is almost uniformly fatal in highly immunosuppressed HCT recipients (Harrington *et al*, 1992). However, recovery from RSV lower respiratory tract disease without specific treatment has been reported (Abdallah *et al*, 2003). These differences are probably due to less intense immunosuppressive regimens and whether lymphopenia is present at the time of diagnosis (Chemaly *et al*, 2006). Pulmonary copathogens are detected in up to one third of the patients with RSV pneumonia and require aggressive treatment. No adequately powered controlled trials exist for the treatment of RSV infection and

Table I. Respiratory virus infections after HCT: comparison of RSV, Parainfluenzavirus 3, and influenzaviruses.

Virus	Incidence of infection (%)	Progression from URI to pneumonia (%)	Time from URI to pneumonia (median, d)	Proportion of pneumonia without URI (%)	Pulmonary copathogens in cases with pneumonia (%)	Overall mortality at 1 month after diagnosis of pneumonia (%)
RSV	1·8–6*	40	7	20–50	2·5–33	45
Parainfluenza virus 3	4–7	18–44	7	31	53	35–37
Influenzaviruses A and B	1·3–2·6†	18	11	18	50	25–28

*During the winter season, the incidence may be as high as 10%.

†Maybe significantly higher during outbreaks (Martino *et al*, 2003).

HCT, haematopoietic cell transplantation; RSV, respiratory syncytial virus; URI, upper respiratory infection.

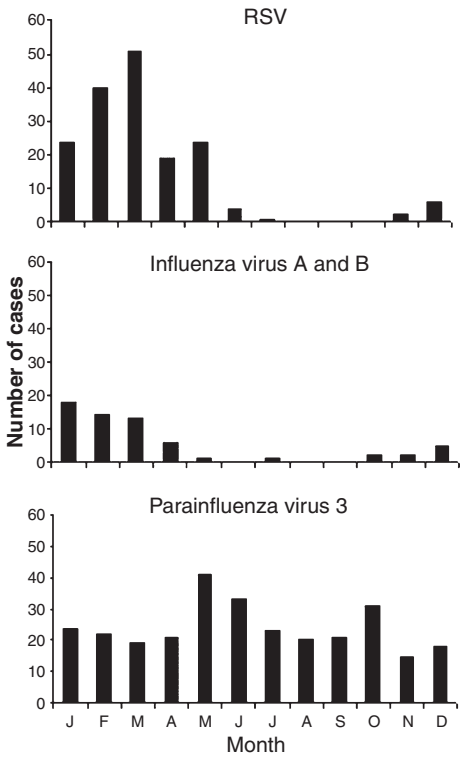

Fig 1. Monthly distribution of respiratory syncytial virus (RSV), parainfluenza virus 3, and influenza infections after HCT in the 1990's at the Fred Hutchinson Cancer Research Center [from Boeckh (2004)]. With permission from Blackwell Publishing, Oxford, UK.

pneumonia in the HCT setting. Available evidence comes from small uncontrolled cohort studies and one small randomized trial (Boeckh *et al*, 2007). The data suggest that treatment of early pneumonia (i.e. prior to mechanical ventilation) is associated with improved outcome. Intermittent short duration (2 g, over 2 h, three times per day) or continuous aerosolized ribavirin is considered the treatment of choice for RSV pneumonia. With this regimen, the 30-d all-cause mortality is approximately 40% (Table I). In a large multivariate analysis, RSV pneumonia (but not URI) was independently associated with mortality (Nichols *et al*, 2001a). Systemic ribavirin alone does not seem to be effective for the treatment of pneumonia (Lewinsohn *et al*, 1996; Sparrelid *et al*, 1997); better response rates were reported for treatment of RSV URI, however, the small sample size limits the strength of the data (Schleuning *et al*, 2004). There are no data on combined oral and aerosolized ribavirin.

The role of concomitant intravenous immunoglobulin, or RSV-specific immunoglobulin, or palivizumab, an RSV-specific monoclonal antibody directed at the F protein of RSV, for the treatment of RSV pneumonia is poorly defined. Uncontrolled data suggest that high-titer antibody preparations or palivizumab may be required if such adjunctive therapy is given (DeVincenzo *et al*, 2000; Boeckh *et al*, 2001; Small *et al*, 2002). There are several factors that may account for differences in outcome in the available cohort studies. One important factor appears to be the timing of initiation of therapy. Several studies suggest that, when treatment is started after respiratory failure has occurred, it is almost uniformly unsuccessful (Whimbey *et al*, 1995). Other factors that may explain the differences in outcome between studies include: the presence of lymphopenia at the time of diagnosis, the presence of co-pathogens (e.g. invasive moulds), and the use of immunosuppressive agents (Ljungman, 2001; Boeckh *et al*, 2004; Chemaly *et al*, 2006). Available studies are too small to control for these parameter, making the interpretation of results of pharmacological interventions difficult. Most centers now agree that aerosolized ribavirin should be given to HCT patients with RSV lower respiratory tract disease. There is no consensus on the role of antibody preparations, however, the author uses palivizumab for radiographically and virologically proven RSV pneumonia.

Due to the high mortality of RSV pneumonia, much interest has focused on prevention. A recent small controlled study of preemptive, antiviral therapy of aerosolized ribavirin, given at the time of RSV URI, showed a decline in viral load, but no significant difference in RSV pneumonia; the study was prematurely stopped due to slow accrual(Boeckh *et al*, 2007). A larger retrospective study suggests that this strategy may be effective (Chemaly *et al*, 2006). Whether ribavirin is indicated for RSV URI remains controversial; some centers (including the author's) administer it in the presence of lymphopenia (lymphocyte count $<0{\cdot}3 \times 10^9$/l) when the risk of progression is particularly high (Boeckh *et al*, 2004; Chemaly *et al*, 2006). Lymphopenia is a highly significant risk factor for progression to lower tract disease during the first 3 months after HCT (Ljungman *et al*, 2001; Chemaly *et al*, 2006). There is no data on the risk of progression later after HCT; anecdotal experience suggests that other factors, such as poor lung function, may be more important in patients with prolonged immunosuppression; further studies are needed to define the risk factors in this setting. Whether palivizumab given preemptively to patients with RSV URI to prevent progression to lower respiratory tract disease is effective has not been definitively determined (Chavez-Bueno *et al*, 2007; Khanna *et al*, 2008).

Prophylactic measures recommended throughout the respiratory virus season include: isolation of infected patients, hand washing before and after every patient contact, educational efforts targeted at health care personnel and family members, and avoiding patient contact of health care personnel and family members with uncontrolled secretions (Boeckh *et al*, 2004). No studies exist on pharmacological prophylaxis for RSV acquisition.

For pretransplant infections with RSV, parainfluenzavirus or influenzavirus, most transplant centers postpone the transplant procedure until resolution of symptoms and cessation of viral shedding, especially when a myeloablative allogeneic HCT is planned. This approach was supported by a recent study of

pretransplant RSV infection, which showed rapid progression to RSV pneumonia in patients in whom the transplant procedure was not postponed (Peck *et al*, 2004). However, there is evidence that low-risk autologous transplant patients may be transplanted without adverse outcome (Aslan *et al*, 1999). Additionally, the outcome of respiratory virus infections appears to be less severe with non-myeloablative or reduced-toxicity conditioning regimens (Schiffer et al, 2006).

Human metapneumovirus

Significance and risk factors

Human metapneumovirus is a newly discovered negative-sense non-segmented RNA paramyxovirus (van den Hoogen *et al*, 2001). Structurally, the virus is closely related to RSV. By the age of 5 years, virtually all children are seropositive. The virus can cause upper and lower tract infection during the winter season (Boivin *et al*, 2002). Serious lower respiratory tract disease associated with HMPV has been reported in immunocompromised patients, but no risk factors for acquisition have been described (Table II). HMPV infection occurs in up to 5% of HCT recipients (Fig 2) (Peck *et al*, 2007). Whether asymptomatic shedding occurs is controversial (Debiaggi *et al*, 2007; Peck *et al*, 2007). The progression rate to lower respiratory tract disease has not been determined. Among HCT recipients undergoing bronchoalveolar lavage (BAL) for clinical and radiographic pneumonia, 3–4% had HMPV detected (Englund *et al*, 2006). Most of these patients were previously classified as having idiopathic pneumonia syndrome (Englund *et al*, 2006). HMPV disease in HCT recipients immediately post-transplant presented with upper respiratory symptoms, including: fever, nasal congestion, and cough. Once pneumonia developed, rapidly progressive pulmonary infiltrates, frequently accompanied by hypotension, septic shock, or both, were observed (Englund *et al*, 2006). Radiographic findings ranged from diffuse bilateral alveolar and interstitial infiltrates to emphysema without infiltrate. A high viral load was observed in the bronchoalveolar lavage fluid. Histological assessment showed that diffuse alveolar damage with hyaline membrane formation, foci of bronchiolitis obliterans and organizing pneumonia, and diffuse alveolar hemorrhage are common (Englund *et al*, 2006).

Treatment and prevention

To date, there is no established treatment for HMPV disease, which may be fatal in HCT recipients (Englund *et al*, 2006). Although IVIG has been shown to effectively neutralize HMPV *in vitro*, its effect *in vivo* is unknown (Wyde *et al*, 2003). The role of steroids is also not defined. Ribavirin has been shown to have *in vitro* antiviral activity against HMPV (Wyde *et al*, 2003), however, there are no reports on treatment with ribavirin. Infection control measures similar to those outlined for RSV are the mainstay of prevention.

Table II. Seasonality, symptoms, and diagnosis of respiratory viruses in immunocompetent subjects and HCT recipients [adapted from Nichols *et al* (2008), with permission from American Society for Microbiology].

		Disease manifestations in immunocompetent persons						Disease in HCT recipients			Diagnosis			
Virus	Seasonality	URI	Otitis media	Croup	Bronchiolitis	Pneumonia	AFO and/or wheezing	URI	Pneumonia	AFO	Culture	EIA	FA rapid cultures	PCR
RSV	++	+++	++	+	+++	+++	+++	+++	+++	++	+	+	++	+++
HMPV	++	+++	++	+	++	+++	++	+++	+++	++	Research	NA	Research	+++
PIV 1	+	+++	+	+++	+	++	++	+++	+++	+++	+	NA	+	+++
PIV 2	+	+++	+	++	+	++	++	+++	+++	+++	+	NA	+	+++
PIV 3	–	+++	+	++	++	++	+++	+++	+++	+++	+	NA	+	+++
PIV 4	–	+++	+	+	+	+	+	+++	+++	ND	+	NA	++	+++
Influenza A, B	++	+++	+	+	+	++	+	+++	+++	+	+	+	++	+++
Adenoviruses	+	+++	+	+	++	+	–	+	+++	–	+	++	++	+++
Rhinoviruses	+	+++	+	+	+	+	+++	+++	+	++	+	NA	NA	++
Coronaviruses	+	+++	+	++	+	+	++	ND	ND	ND	Research	NA	NA	+++
Bocavirus	+	+++	ND	+	+	+	+	ND	ND	ND	NA	NA	NA	+++

HCT, haematopoietic cell transplantation; RSV, respiratory syncytial virus; HMPV, human metapneumovirus; PIV, parainfluenza virus; URI, upper respiratory infection; AFO, airflow obstruction; EIA, enzyme immunoassay; FA, fluorescent antibody; PCR, polymerase chain reaction; ND, no data; NA, not available.

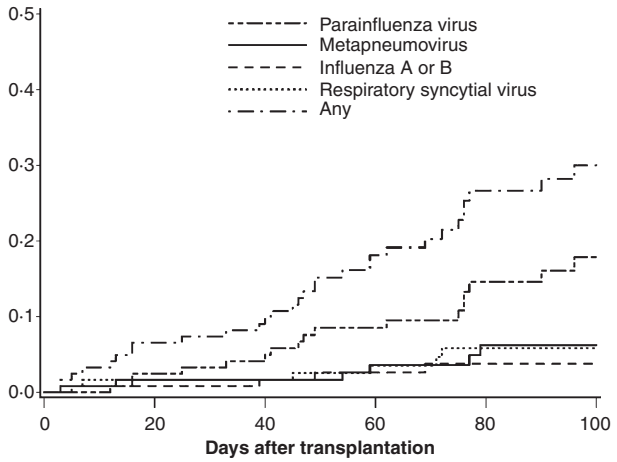

Fig 2. Time to first respiratory virus infection after haematopoietic cell transplantation. This research was originally published in *Blood*. Peck *et al* (2007). © the American Society of Hematology.

Parainfluenza viruses

Significance and risk factors

Parainfluenza viruses are classified into four serotypes. Of the four types of parainfluenza viruses, parainfluenzavirus 3 is the most common (approximately 90%), followed by serotypes 1 and 2. The incubation time is 1–4 d. Parainfluenzavirus infections remain detectable throughout the summer season (Fig 1). Even with standard virological methods, the incidence of parainfluenzaviruses is higher than that of RSV in different cancer centers (Nichols *et al*, 2001a). PCR detection significantly increases the diagnostic yield (Peck *et al*, 2007).

Other than HCT from an unrelated donor, no risk factor for acquisition of the parainfluenzavirus has been found (Nichols *et al*, 2001a). In T cell-depleted patients, CD4 lymphopenia has been reported to increase the risk of all respiratory virus infections, including parainfluenzavirus infections (Chakrabarti *et al*, 2002).

Similar to RSV and HMPV, URI is the predominant clinical presentation (Table II). Progression to pneumonia seems to be less common than with RSV (Nichols *et al*, 2001a). Late airflow obstruction has been linked, not only to parainfluenzavirus pneumonia, but also to URI (Erard *et al*, 2006). The most important risk factors for the progression from URI to pneumonia is use of systemic corticosteroids and lymphopenia.(Nichols *et al*, 2001b; Chakrabarti *et al*, 2002). During the first 3 months after HCT, recipients of non-myeloablative conditioning regimens appear to have a small risk of progression to lower respiratory tract disease (Schiffer et al, 2006). Reduced intensity conditioning regimens may also have a somewhat lower risk of progression and fatal disease (Chakrabarti *et al*, 2002), however, larger studies are needed to better define the risk of progression. Although the overall progression rate to pneumonia is only 18%, in allograft recipients the risk is 40% if 1 mg/kg of prednisone is given (for treatment of graft-versus-host disease, GvHD), and 65% with 2 mg/kg (Nichols *et al*, 2001b). Parainfluenzavirus 3 pneumonia may also occur after autologous transplantation, however, mainly in the setting of CD34 selection or use of high-dose steroids (Nichols *et al*, 2001b). Parainfluenzavirus 3 pneumonia is associated with serious pulmonary co-pathogens, such as *Aspergillus fumigatus*, in approximately half of the cases. Factors associated with poor outcome after pneumonia include presence of co-pathogens and mechanical ventilation (Nichols *et al*, 2001b). Thus, aggressive diagnostic intervention (i.e. BAL) and therapy are indicated in patients with suspected parainfluenzavirus pneumonia. In a large retrospective analysis both URI and pneumonia, due to parainfluenzavirus 3, were associated with overall mortality in multivariable models (Nichols *et al*, 2001b).

Treatment and prevention

Mortality of pneumonia is approximately 35% in recipients of allografts following myeloablative conditioning (Nichols *et al*, 2001b). Outcome appears to be somewhat better in patients who received reduced toxicity conditioning regimens (Chakrabarti *et al*, 2002). In a retrospective analysis, neither aerosolized ribavirin nor intravenous immunoglobulin led to improved outcome of pneumonia or a reduction in viral shedding following pneumonia (Nichols *et al*, 2001b). Systemic ribavirin has only been reported in case reports (Chakrabarti *et al*, 2000a). Randomized treatment studies have not been performed. No definitive assessment can be made as to whether earlier antiviral treatment (i.e. preemptive treatment for URI) is effective in the prevention of pneumonia or late airflow obstruction (Chakrabarti *et al*, 2000a). The association of high-dose steroid treatment with progression to disease might suggest that reduction on immunosuppression may be useful (Nichols *et al*, 2001b), however, such an approach has not been tested. The role of immunoglobulin in the treatment of parainfluenzavirus infections is also poorly defined. One retrospective analysis did not find a benefit of pooled immunoglobulin given for parainfluenzavirus pneumonia (Nichols *et al*, 2001b). However, antibody content in pooled preparations is highly variable. The highest titers can be obtained from RSV-specific immunoglobulin that also contains high titer of antibodies directed against parainfluenzavirus (Cortez *et al*, 2002).

Infection control is the mainstay of prevention strategies. Unfortunately, current infection control practices seem to be far from perfect in keeping parainfluenza virus out of HCT units, as indicated by the high incidence figures and repeated outbreaks (Zambon *et al*, 1998; Cortez *et al*, 2001; Nichols *et al*, 2004b). Possible explanations for the difficulty to prevent parainfluenzavirus from entering HCT units include: lack of a vaccine for health care workers and close contacts, very mild or

lacking symptoms in immunocompetent individuals in combination with prolonged shedding, prolonged asymptomatic shedding in infected patients, and persistence of the virus on environmental surfaces (Peck *et al*, 2007).

Influenzaviruses

Significance and risk factors

Influenza is classified into three major types, of which type A is most common, followed by type B (Ljungman *et al*, 1993; Whimbey *et al*, 1996; Bowden, 1997). Influenza type C is very uncommon, even in the immunocompetent population; there are no reports of influenza C in HCT recipients. Influenza virus infections seem to be less common than RSV and parainfluenza virus infections (Table I, Fig 1).

Acquisition of influenza is increased in patients with high-risk underlying disease (Nichols *et al*, 2004a). Progression to severe pneumonia can occur similar to RSV and parainfluenzavirus (Bowden, 1997), however, progression rates appear to be lower and risk factors for progression differ (Table II). In contrast to parainfluenzavirus infection, where corticosteroids are an important risk factor for the development of pneumonia, influenza virus lower tract disease appears to be less common in patients treated with corticosteroids (Nichols *et al*, 2004a; Machado *et al*, 2005). Also, patients receiving non-myeloablative conditioning regimens appear to be less likely to develop pneumonia (Schiffer et al, 2006). Interestingly, the clinical presentation in HCT often lacks myalgia and high fever, which are commonly seen in immunocompetent individuals (Fig 3).

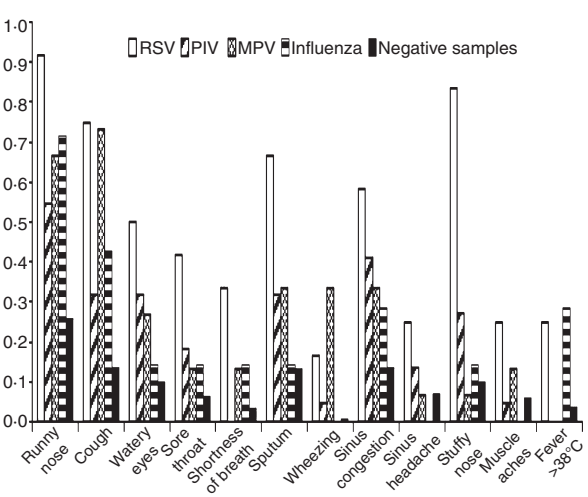

Fig 3. Symptoms associated with respiratory virus infection after haematopoietic cell transplantation. This research was originally published in *Blood*. Peck *et al* (2007). © the American Society of Hematology. RSV, respiratory syncytial virus; PIV, parainfluenza virus; MPV, metapneumovirus.

Treatment and prevention

Effective prevention is available for influenza, which may explain the lower incidence. Health care personnel, family members, and visitors are advised to get vaccinated against influenza early in the season. Antiviral therapy is available for influenza virus infection; however, available agents have not been studied systematically in HCT recipients. Due to the recent emergence of resistance against the M2-inhibitors, amantidine and rimantidine, these agents should not be used to treat or prevent influenza A (Bright *et al*, 2006). Neuraminidase inhibitors (i.e. zanamavir, oseltamivir) are now available (Hayden *et al*, 1997; Nicholson *et al*, 2000) and are active against influenza A and B. Uncontrolled studies suggest that preemptive therapy with neuraminidase inhibitors (i.e. oseltamivir, zanamivir) is effective in preventing progression to lower tract disease (Johny *et al*, 2002; Nichols *et al*, 2004a; Chemaly *et al*, 2006). Widespread chemoprophylaxis for susceptible immunosuppressed patients in outbreak situations has been recommended [Centers for Disease Control; Prevention; Infectious Diseases Society of America; American Society of Blood; Marrow Transplantation (CDC/IDSA/ASBMT), 2000], and a case-control study of oseltamivir suggests that this approach is safe in HCT recipients (Vu *et al*, 2007).

Human rhinoviruses and enteroviruses

Significance and risk factors

Human rhinovirus is classified as a picornavirus and is characterized by small, naked, single-stranded RNA. There are approximately 100 serotypes, which usually cause mild upper respiratory tract symptoms in immunocompetent hosts. Using culture techniques, rhinovirus is infrequently reported as a cause of respiratory infection in HCT recipients. However, several reports found the organism in patients with pneumonia. Ghosh *et al* (1999) reported lower tract disease in seven of 22 patients with rhinovirus infection while Bowden (1997) documented lower tract disease in only one of 29 infected patients. Rhinovirus appears to occasionally cause lower respiratory tract disease (Gutman *et al*, 2007), although a clear causative role is sometimes difficult to ascertain because of the presence of co-pathogens (Ghosh *et al*, 1999; Ison *et al*, 2003). Surveillance studies using reverse transcription (RT)-PCR are ongoing in order to more precisely define the role of HRhV in HCT recipients. Enteroviruses are also picornaviruses. They are transmitted by the fecal-oral and respiratory route. Respiratory infections can occur, but limited evidence exists as to their significance as respiratory pathogens.

Treatment and prevention

There are presently no drugs or vaccines available for the treatment or prevention of HRhV infections. Pleconaril, a capsid-binding compound, was the first anti-rhinovirus

antiviral to be submitted to the US food and Drug Administration for regulatory approval, but was not approved for licensure for the treatment of colds in adults, primarily based on drug interactions and modest treatment effects. Other antiviral agents, including protease inhibitors, are active *in vitro* but whether these compounds will be developed is uncertain (Patick, 2006). Zinc salts and preparations of Echinacea are also weakly active *in vitro*. Meta-analyses have shown no benefit for zinc salts (Jackson *et al*, 1997) and have displayed a moderate effect for *Echinacea* in immunocompetent subjects, although results are not consistent (Turner *et al*, 2005) (Linde, *et al* 2006). No studies have been performed in immunosuppressed patients with these compounds.

Human coronaviruses

Significance and risk factors

Human coronaviruses are RNA viruses that can cause URI, croup, wheezing, and pneumonia in immunocompetent individuals. Since 2004, several new strains have been described, including NL63 and HKU1, in addition to the previously described OC43 and 229E strains. In a recent study, 5·7% of 823 patients admitted to the hospital had HCoV detected; HCoV infection occurred in 8·8% of immunocompromised patients, compared with 4·5% of immunocompetent patients. Fourteen of the 47 HCoV-infected patients (30%), including three HCT recipients, were also infected with significant co-pathogens (Gerna *et al*, 2006). The four HCoVs have also been detected in solid organ transplant recipients, patients with underlying malignancies, and HIV-infected individuals (Kumar *et al*, 2005). HCoV infections have also been associated with long-term decline in FEV1 (forced expiratory volume in 1 s) in lung transplant recipients (Kumar *et al*, 2005). Surveillance studies are ongoing to define the incidence and disease associations of the HCoVs in HCT recipients.

Treatment and prevention

There is no specific treatment for HCoV. Infection control measures are recommended, similar to those for other respiratory viruses.

Human bocavirus

Significance and risk factors

Human bocavirus is a newly identified human parvovirus, originally identified by random PCR amplification/cloning technique from hospitalized children in Sweden (Allander *et al*, 2005). HBoV has been detected worldwide (Chung *et al*, 2006; Foulongne *et al*, 2006; Manning *et al*, 2006), and appears to be common in young children. Symptoms associated with HBoV infections include rhinorrhea, cough, fever, wheezing, hypoxia, and diarrhea (Arnold *et al*, 2006). To date, there is only limited information on the clinical significance of HBoV infection in immunocompromised patients (Schenk *et al*, 2007). Surveillance studies are ongoing to better define the incidence and disease associations of HBoV in HCT recipients.

Treatment and prevention

There is no specific treatment for HBoV. The infection control measures that are recommended are similar to those for other respiratory viruses.

Adenoviruses

Significance and risk factors

Adenoviruses are categorized in subgroups A to F, and there a presently 51 human serotypes known. Serotypes from all five subgroups can cause disease in both immunocompetent and immunocompromised subjects, although the disease spectrum associated with strains may differ (Horwitz, 2001; Bruno *et al*, 2003). After primary infection, adenovirus establishes latency in adenoidal tissues (Horwitz, 2001) with lifelong persistence of specific antibodies.

Transmission of adenovirus occurs by either respiratory droplet or the oral-fecal route. Adenovirus enters the mucosa and infects epithelial cells, resulting in inflammation and necrosis. Subsequent viremia may lead to disseminated disease. After HCT, reactivation from latency is probably more problematic than exogenous infection (Bruno *et al*, 2003), however, transmission via the respiratory route can occur.

Adenovirus infections are common after HCT and some recent reports suggest that they may be increasing, possibly related to transplantation practices (e.g. T cell-depletion) (Flomenberg *et al*, 1994; Bruno *et al*, 2003). However, the incidence of adenovirus disease is dependent on the degree of immunosuppression and seems to be increasing only in recipients of T cell-depleted grafts (Flomenberg *et al*, 1994). Clinical manifestations include pneumonia, hepatitis, gastrointestinal disease, nephritis, cystitis, and eye infections (Cox *et al*, 1994; Flomenberg *et al*, 1994; Bruno *et al*, 2003, 2004).

Risk factors for adenovirus asymptomatic infection after HCT include: GvHD, unrelated donor status, use of total body irradiation, T cell depletion, and younger age (Flomenberg *et al*, 1994; Baldwin *et al*, 2000; Bruno *et al*, 2003). The degree of T cell depletion and post-transplant immunosuppression directed at T cell function appears to be particularly important (Lion *et al*, 2003). Almost all studies have reported a higher incidence in children (Wasserman *et al*, 1988; Bruno *et al*, 2003).

Adenovirus end-organ disease is most commonly seen following conditioning regimens that include *in vivo* or *ex vivo* T cell depletion (Flomenberg *et al*, 1994). Risk factors for adenovirus disease include: younger age (Howard *et al*, 1999), viremia and shedding from more than two sites (Howard *et al*, 1999; Baldwin *et al*, 2000; Schilham *et al*,

2002), total body irradiation (Hale *et al*, 1999), GvHD (Flomenberg *et al*, 1994), and transplantation from an unrelated donor (Baldwin *et al*, 2000). Plasma viremia is an important risk factor for disease (Erard *et al*, 2007). Adenovirus disease is associated with a fatality rate of 30–50% (Kim *et al*, 2007). Response to treatment seems to be particularly poor in patients with pneumonia or disseminated disease (Ljungman *et al*, 2003). A recent multivariate analysis in non-T cell-depleted HCT recipients indicated that adenovirus infection was independently associated with mortality (Bruno *et al*, 2003).

Prevention and treatment

There are no controlled treatment studies for adenovirus infection in immunocompromised patients. Intravenous ribavirin has been used, but results are conflicting (Liles *et al*, 1993; Chakrabarti *et al*, 1999; Bordigoni *et al*, 2001; Gavin & Katz, 2002). Recently, cidofovir has been shown to have *in vitro* activity, and several uncontrolled case series showed promising results (Legrand *et al*, 2001; Ljungman *et al*, 2003; Yusuf *et al*, 2006; Neofytos *et al*, 2007; Sivaprakasam *et al*, 2007). Ganciclovir has *in vitro* activity against adenovirus and has a moderate effect in the prevention of adenovirus infection in non-T cell-depleted patients (Bruno *et al*, 2003). However, ganciclovir is not recommended for the treatment of adenovirus disease. The antiretroviral drugs, zalzitabine (DDC), alovudine, and stavudine, have activity *in vitro* (Naesens *et al*, 2005; Uchio *et al*, 2007). Differences in *in vitro* susceptibility and treatment outcome results between studies may be due to strain and serotype differences, which often were not reported in these studies. Specific and non-specific T cell therapy have been reported in small series (Chakrabarti *et al*, 2000b; Regn *et al*, 2001; Feuchtinger *et al*, 2006; Leen *et al*, 2006).

In high-risk settings, such as haploidentical transplantation or cord blood transplantation, weekly PCR surveillance for adenovirus viremia and preemptive treatment with cidofovir is now used at several centers (Lion *et al*, 2003; Yusuf *et al*, 2006). However, this strategy does not completely eliminate adenovirus disease in highest-risk patients (Yusuf *et al*, 2006) even if cidofovir is initiated at any viral load. In T cell-replete transplant recipients, plasma DNAemia of greater than 1000 copies/mL correlated well with the presence of adenovirus disease (Erard *et al*, 2007). At what level preemptive antiviral therapy should be initiated depends both on the PCR assay performance characteristics and the underlying immunosuppression. The latter determines the *in vivo* viral dynamics. At this time, no definitive threshold has been established, however, repeated testing after 2–3 d of a low viral load test can identify patients with a rapid increase in viral load. The use of adenovirus-specific T cells for prevention and treatment of adenovirus disease are an area of active research (Feuchtinger *et al*, 2006). Randomized trials are needed to evaluate prevention strategies.

Isolation practices

Recommendations have been summarized by the CDC/IDSA/ASBMT (2000); a revision of this document is expected to be published early in 2009. Hand washing is the single most effective way of preventing spread of respiratory viruses, and should be performed after each patient contact by all health care personnel and visitors. Respiratory isolation is used for HCT recipients with documented respiratory virus infections, as well as upper respiratory symptoms during the diagnostic work-up period (masks, gowns, gloves, and eye protection). One area of uncertainty is when to lift isolation in patients who have become asymptomatic, but have persistent low-level shedding which is only detected by molecular methods. To what extent these patients are contagious is presently unknown. For the viruses that are known to cause serious disease (i.e. RSV, parainfluenzaviruses, influenzaviruses, HMPV), it appears prudent to isolate patients that are positive by PCR, even if they are asymptomatic. Whether this is necessary for viruses with an extremely uncommon or unclear disease association (e.g. HRhV, HCoV), is presently an area of active research.

The ubiquitous use of masks among health care workers, family members, and asymptomatic patients remains controversial. One non-randomized study suggests a benefit of masks (Raad *et al*, 1997). However, questions remain on the type of mask, frequency of changing the mask, and who should wear masks. Not all transplant centers have a universal mask policy. Recent CDC guidelines recommend using masks during patient transport [CDC/IDSA/ASBMT (2000)]. Another approach that is likely to reduce transmission is to restrict health care workers and family members from patient contact if they have URI and systemic symptoms, such as rhinorhea, watery eyes, sneezing, fever, and myalgia (Boeckh *et al*, 2004). Such a strategy will only be effective for infections that present with significant drainage and symptoms, such as RSV, rhinovirus, HMPV, and influenza infections. Parainfluenzavirus infections, which may present with only mild symptoms, may be missed by this approach (Nichols *et al*, 2004a; Peck *et al*, 2007). Most centers restrict small children from direct patient contact during the respiratory virus season, due to their predisposition to URIs and prolonged high-titer shedding [CDC/IDSA/ASBMT (2000)].

Future perspective

Respiratory viruses are now recognized as causes of pneumonia and late airflow obstruction after HCT. Although major progress has been made in understanding disease associations and in the development of diagnostic tests, major gaps remain. One such gap is the availability of a sensitive, specific, and inexpensive multiplex diagnostic platform that detects all relevant respiratory viruses, and optimally, important bacterial pathogens, as well. Fortunately, the development of such testing methods is advancing. The establishment of these

methods will enable the study of disease associations, and will also be essential for the development of more therapeutic options, not only in the immunosuppressed, but also in immunocompetent subjects. One key reason for the slow progress in drug development is the absence of widespread office-based testing (Nichols *et al*, 2008). A systematic and comprehensive evaluation of the disease burden of these infections is also needed. Historically, only pneumonia and death were reported, however, data on airflow obstruction, health care utilization including duration of hospitalization, intensive care, and mechanical ventilation are important as well.

Another area of need is that of disease pathogenesis. Although several studies point towards lymphopenia as an important risk factor for severe manifestations of disease, the exact host defence mechanisms that lead to the devastating consequences of respiratory virus infections in HCT recipients are virtually unknown. Studies of the importance of virus load, T cell and humoral immunity, as well as cytokine and chemokine expression, and genetics, are needed. To date, most studies focus on the early period after transplantation. More information is needed on the risk of progression and outcome, late after HCT (i.e. after the first 3 months), when lymphopenia is rare. There may be a birectional relationship between respiratory viruses and lung function late after transplantion; viral infections may lead to prolonged airflow obstruction, and preexisting airflow obstruction may predispose individuals to progression of lower tract disease and poor outcome.

There is also very limited knowledge of what determines the risk of virus acquisition. Studies are ongoing to determine whether there are genetic predispositions. Furthermore, major progress is needed in regards to treatment options for respiratory viruses.

With the exception of treatment for influenza virus, there are no potent and easy to administer drug treatments. The development of novel treatment and prevention options for RSV has the highest priority. Existing options (ribavirin, palivizumab, polyclonal antibody preparations) are of only moderate efficacy, complicated to give, and/or prohibitively expensive–especially when given to adult patients. Several lead compounds are currently being evaluated (Nichols *et al*, 2008), and a recently established volunteer infection model could speed up drug and vaccine development (Devincenzo *et al*, 2007). In the past, it has been difficult to conduct randomized clinical trials for RSV in HCT recipients (Boeckh *et al*, 2007). However, the failure to complete these trials was directly linked to the level of complexity required to give aerosolized ribavirin (Boeckh *et al*, 2007). With novel antiviral compounds that are easy to administer, do not require hospitalization, and lack serious side effects it should be highly feasible to conduct clinical trials in the HCT setting.

Another unmet medical need is a treatment option for parainfluenzavirus, the most prevalent of the respiratory viruses with known disease associations (Nichols *et al*, 2008). Also, well designed prospective observational studies are needed to define the disease burden of recently discovered respiratory viruses, such as novel coronaviruses, human bocavirus, and polyomaviruses. Finally, even today we see symptomatic patients in whom we are unable to make a virological diagnosis despite multiplex PCR testing. Thus, we will probably see additional, yet to be discovered, respiratory viruses in the future.

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