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21.1 Introduction

Common respiratory viruses are probably among the leading causes of community-acquired pneumonia [1, 2] but, oddly enough, have long been underrecognised in patients with haematologic malignancies (HMs), perhaps in part due to the lack of rapid and sensitive diagnostic methods and of effective treatments. Over the last 2 decades, the expanding use of high-dose chemotherapy and haematopoietic stem cell transplantation (HSCT) in various malignancies has improved the prognosis of patients with HMs at the cost of increased opportunities for severe infections [3–5]. Combined with the development of new diagnostic methods and introduction of antiviral drugs, this increased infection rate has rekindled interest in the effects of common respiratory viruses in patients with HMs. These viruses are now recognised as true opportunistic respiratory pathogens in immunocompromised hosts.

21.2 Common Respiratory Viruses

There is no clear definition of common respiratory virus. Viruses that consistently produce respiratory manifestations are considered respiratory viruses. These include members of the following families of RNA viruses: Orthomyxoviridae (human influenza viruses), Paramyxoviridae (respiratory syncytial virus [RSV], parainfluenza viruses [PIV], and human metapneumoviruses [MPV]), Coronaviridae (human respiratory coronaviruses), and Picornaviridae (human rhinoviruses). Other viruses that cause respiratory manifestations in many, but not all, patients are considered respiratory viruses because they are often associated

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with seasonal respiratory events. Examples include Adenoviridae (adenoviruses) and some Picornaviridae (non-poliomyelitic enteroviruses). A separate chapter of this book focuses on newly discovered and emerging respiratory viruses (avian influenza viruses, newly discovered coronaviruses, polyomaviruses KI and WU, human bocavirus, and mimivirus). CMV and Herpes pneumonia will also be addressed by Dr. Chemaly and colleagues in two different chapters in this book.

Human influenza viruses are enveloped, negative-sense, single-stranded RNA viruses. They are classified into three antigenic types, A, B, and C [6]. The envelope of influenza A and B viruses carries two major antigenic proteins, haemagglutinin (HA) and neuraminidase (NA). HA allows the virus to bind to sialic acid-conjugated glycoproteins at the surface of respiratory epithelium cells. NA plays a crucial role in viral dissemination by releasing newly formed virions from host cells. The influenza C envelope supports a unique protein sharing the properties of both HA and NA and known as haemagglutinin-esterase-fusion factor [6]. Influenza B and C viruses can infect humans and some mammalian species, whereas influenza A also infects most avian species [6].

RSV and PIV types 1–4 are enveloped, negative-sense, single-stranded RNA viruses. RSV belongs to the *Paramyxovirus* genus, and PIV 1–4 to the *Pneumovirus* genus, of the Paramyxoviridae family [7]. Their envelopes support two major antigenic surface proteins, one for binding and one for fusion to the cell membrane. Attachment to host cell receptors is mediated by the G protein of RSV and by a haemagglutinin neuraminidase for PIV [7, 8]. The fusion protein F is common to both genera and allows the virus to penetrate within the host cells after binding [8]. Antigenic variations in the G protein determine two major RSV groups, A and B. Antigenic variations also occur in PIV but have less immunological impact [7]. The natural hosts of RSV are humans and chimpanzees, whereas PIV infects only humans [9, 10]. Human MPV is a recently discovered virus belonging to the Paramyxoviridae family [11]. The human MPV genome and structure are very similar to those of RSV, and both viruses display the same surface proteins [12]. Available data indicate that human MPV infects only humans, although this virus is believed to originate from birds [13].

Human rhinoviruses and non-poliomyelitic enteroviruses are non-enveloped, single-stranded RNA viruses belonging to the Picornaviridae family. The

Rhinovirus genus consists of more than 100 different serotypes divided into three species (A, B, and the newly described C species) based on antigenic variations in the three proteins VP1, VP2, and VP3 found on the capsid surface [14, 15]. Enteroviruses are divided into four species (A, B, C, and D) [16, 17]. They share the same structure as other Picornaviridae. Humans are the only natural host of these viruses [14, 16].

Coronaviruses are enveloped, positive-sense, single-stranded RNA viruses belonging to the Coronaviridae family [18]. Their envelope supports two main proteins, the haemagglutinin-esterase protein and the antigenic protein S [18]. Human coronaviruses 229E and OC43 were the predominant human respiratory coronavirus strains before the recent identification of the new human coronaviruses NL63, HKU1, and SARS [19–21].

Adenoviruses are non-enveloped, double-stranded DNA viruses that form the Adenoviridae family. Six subgenera (A–F) and 51 human serotypes of adenoviruses have been described [22]. Adenoviruses have an icosahedral capsid made of 250 protein subunits called capsomeres [23]. Humans are the only natural hosts of these viruses.

21.3 Epidemiology

Common respiratory viruses are ubiquitous pathogens that share a number of epidemiological features. Most of them are responsible for outbreaks that occur with a remarkable seasonal pattern. These seasonal variations in viral activity occur only in temperate climates and tend to disappear in equatorial zones. This phenomenon is incompletely understood and may involve not only specific viral characteristics, but also seasonal changes in living conditions [6]. Influenza, RSV, human MPV, and Coronavirus activities peak during the winter months [6, 7, 18]. PIV1 and 2 cause outbreaks in the fall [7]. Seasonality is less pronounced for PIV3 and Picornaviridae, which exhibit year-round activity with peaks in the spring for PIV3, spring and fall for human rhinoviruses, and summer and fall for non-poliomyelitic Enteroviruses [7, 14, 16]. Little is known about the epidemiology of PIV4, since this virus is responsible for mild infections and is therefore rarely identified [7].

Common respiratory viruses typically cause infections in early childhood. Influenza C usually causes

mild infections that lead to the acquisition of protective antibodies capable of preventing recurrences [24]. Influenza A and B are considerably more common and can cause disease of greater severity, most notably in high-risk groups, such as elderly people and patients with chronic respiratory or cardiovascular conditions, chronic renal failure, diabetes mellitus, or immune deficiencies [6]. It is estimated that about 5% of adults and 20% of children experience an influenza episode every year throughout the world [25]. Although most influenza episodes are mild, the high occurrence rate results in a huge burden of disease that has a severe socioeconomic impact [6]. Influenza viruses are believed to be the seventh cause of death in the USA [26]. Influenza A and B infections start in childhood and continue to occur throughout the life span, because antigenic variations affecting HA and, to a lesser extent NA, hamper the acquisition of protective immunity. Two mechanisms of antigenic variations have been described, antigenic drift and antigenic shift [6]. Antigenic drift probably results from the accumulation of point mutations that occur continuously in influenza viruses, with new variants appearing annually or every few years. These new variants replace pre-existing ones by immunological selection and are only incompletely recognised by immunity resulting from exposure to earlier strains. Antigenic shift is a considerably rarer event that occurs only in influenza A and results in a virus with a completely new HA or NA [6]. The result is an influenza pandemic due to the absence of protective immunity against the new virus. Progressive acquisition of immunity against this new strain attenuates the intensity of the epidemic over the following years. The exact genetic mechanisms of antigenic shift are incompletely known, and the emergence of pandemic strains is not predictable. Pandemic strains originate from the recombination of influenza strains circulating in different animal species, including birds, pigs, and humans [6]. Five pandemics occurred in the twentieth century, and the 2009 “Mexican flu” pandemic due to the new H1N1 influenza virus is the first pandemic in the twenty-first century [6, 27]. RSV and PIV are typically infections of early childhood [7]. RSV and, to a lesser extent, PIV also undergo antigenic changes that lead to recurrences throughout the life span. These recurrences may be severe, especially in patients with chronic respiratory conditions and in the elderly. The burden of RSV-related diseases may be comparable to that of influenza [28].

Few data are available on the specific epidemiological characteristics of common respiratory viruses in patients with HMs. Reported incidence rates vary widely, from 1.8% to 30% [29, 30]. This variability may be explained by disparities in the type of patients enrolled, reasons for screening patients for respiratory viruses and, most importantly, diagnostic methods used. Whatever the exact incidence, one can reasonably assume that attack rates of common respiratory viruses in immunocompromised patients are closely related to the prevalence of respiratory viruses in the community, which serves as the viral reservoir for immunocompromised patients [31]. This has been confirmed in recent studies showing that the occurrence of viral respiratory diseases in the enrolled immunocompromised patients paralleled the concomitant viral activity in the community [32, 33]. Accordingly, influenza viruses and RSV are the viruses most commonly identified in the largest cohorts of HM patients, each accounting for about one-third of viral respiratory events [34–36]. PIV, Picornaviridae, and adenoviruses come next. It must be pointed out that influenza viruses accounted for a much higher proportion (up to 75%) of viral respiratory events in the study by Martino et al. conducted in the setting of a major influenza outbreak in the community [32].

Adenovirus epidemiology in patients with HM deserves special attention. Similar to other common respiratory viruses, adenoviruses cause primary infections in childhood, usually during the first few years of life. Adenoviruses may account for 5% to 10% of viral respiratory infections in children [23], and also cause outbreaks in closed and semi-closed populations of young adults such as the military [37, 38]. A distinctive feature of adenoviruses is the ability to cause chronic latent infection by persisting in lymphoepithelial tissues, most notably in the nasopharynx [23]. The epidemiology of adenoviruses in patients with HM has not been the focus of specific studies. Nevertheless, exogenous contamination, by the respiratory or oro-faecal route, may be less important than reactivation of the latent virus [39]. Thus, adenoviral infections in patients with HM may be related chiefly to the level of immunosuppression and, perhaps, to unknown viral factors driving reactivation, rather than to the level of viral activity in the community.

The epidemiologic characteristics of influenza, RSV, and PIV are particularly well known, perhaps in part because infections with these viruses are considerably more common compared to those due to other

viruses. However, the availability of simple and rapid diagnostic tools for these three viruses probably played a great role. Since Picornaviridae, Coronaviridae, and the newly discovered human MPV are mainly diagnosed using reverse-transcriptase polymerase-chain-reaction technology (rt-PCR), data on their epidemiology are still limited. Recent studies using rt-PCR detection of respiratory viruses in patients with HM revealed a high incidence of human MPV infections, similar to that of RSV infections [30, 36]. In the near future, studies using molecular biology tools may help to clarify the epidemiology of common respiratory viruses and may lead to a radical change in current concepts.

21.4 Clinical Manifestations

Common respiratory virus infections have been studied chiefly in patients receiving high-dose chemotherapy and haematopoietic stem cell transplantation (HSCT). Compared with immunocompetent patients, several distinctive features have been reported. Viral shedding lasts longer and progression to pneumonia is more common [31]. Late airflow obstruction has been reported in HSCT recipients [40]. Some respiratory viruses are responsible for extra-respiratory manifestations. Although rare in immunocompetent hosts, these manifestations might be more common in patients with HM.

Common respiratory viral infections in patients with HM are characterized by prolonged viral shedding. A median duration of 2 weeks was found in most studies [30, 32, 33, 36]. However, viral shedding may last for months in some cases [41, 42]. In addition, the amount of virus shed per day may be greater in HM patients than in immunocompetent individuals [43]. Similarly to findings in immunocompromised patients, children shed more viruses over a longer period than do adults [31]. This prolonged high-load viral shedding is of critical importance, since it may enhance both viral dissemination [43, 44] and the emergence of resistant strains via prolonged exposure to antiviral drugs [41, 42].

As stated above, common respiratory viruses have long been underrecognised in patients with HM. In the 1990s, the first reports of common respiratory virus infections focused on influenza and RSV [45–50]. In

these studies, progression to pneumonia occurred in about 75% of patients infected with influenza viruses and 50% of those infected with RSV, and was associated with mortality rates of 25% for influenza and about 80% for RSV. One possible explanation for these striking observations may be a surge of interest in respiratory viral infections in HM patients that led to the publication of the most severe cases. Not surprisingly, these studies had small numbers of patients and high rates of nosocomial infections.

More recently, studies of large patient cohorts led to better characterization of influenza-related pneumonia in patients with HM [29, 32–34]. About one-third of patients develop lower respiratory tract infection (LRTI). Although less common than in previous reports, influenza-related pneumonia remains a severe condition with a 15–30% mortality rate. As in immunocompetent hosts, upper respiratory tract infection (URTI) is almost always present and often precedes LRTI [32, 49, 51, 52]. URTI can be considered a useful argument supporting the diagnosis of respiratory viral infection in patients with HM investigated for pneumonia [53]. However, the identification of a common respiratory virus does not exclude an associated bacterial or fungal infection. These co-infections are very frequent, occurring in 12–25% of cases of influenza-related pneumonia [32, 34, 50]. There are no reports of extra-respiratory manifestations in patients with HM, although myocarditis, pericarditis, myositis, meningo-encephalitis, Guillain-Barré syndrome, and Reye syndrome have been described in immunocompetent patients [6, 54].

RSV-related pneumonia is now believed to complicate about 30–40% of RSV infections in patients with HM [32, 34, 47]. Again, mortality remains high, ranging from 15% to 30%. URTI is a common clinical manifestation. No clear data on the incidence of concomitant bacterial or fungal infections are available in the literature, and RSV-related pneumonia in patients with HM are often ascribed to viral infection [53]. However, bacterial co-infections were present in as many as 30% of patients in a large study of RSV infections in immunocompetent individuals [55]. Bacterial or fungal co-infection may be more common in patients with HM and must be carefully sought. Non-sustained viraemia may accompany RSV infection, and RSV was associated with cardiac arrhythmias and neurological disorders in two case reports [8, 56]. However, no extra-respiratory manifestations of RSV

infection have been described in immunocompromised patients.

PIV infections have also been studied in patients with HM [34, 57]. PIV type 3 was the cause in 90% of reported cases. In these studies, PIV-related pneumonia was comparable to influenza-related pneumonia, with about one third of patients being affected, a high rate of concomitant URTI, and a 15–30% mortality rate. Co-infections were found in as many as 53% of the patients studied by Nichols et al. [57]. However, in a recent study using rt-PCR for routine respiratory virus detection in HSCT recipients, PIV was found in respiratory samples of asymptomatic patients [30], whereas influenza, RSV, and human MPV were not detected. This finding suggests that studies using conventional diagnostic methods may have overestimated the true severity of PIV in patients with HM. Some reports suggested a role for PIV3 in the occurrence of neurologic manifestations (such as meningitis, encephalitis, and Guillain-Barré syndrome) and myocarditis [58]. Such manifestations have not been reported in immunocompromised patients.

Case reports suggest that the recently discovered human MPV may induce severe pneumonia in patients with HM [59–61]. To our knowledge, two retrospective cohorts [62, 63] and three prospective cohorts [30, 36, 64] describing human MPV infections in patients with HM have been published. The small number of patients and highly variable results (pneumonia in 0–43% of patients with a 0–40% mortality rate) preclude definitive conclusions about human MPV-related pneumonia in patients with HM. Although debated, the occurrence of asymptomatic viral shedding may suggest lesser severity of human MPV-related pneumonia compared to influenza and RSV [30, 65]. Two cases of encephalitis with human MPV detected in

cerebrospinal fluid in immunocompetent paediatric patients have been published [66, 67]. No extra-respiratory manifestations have been reported in patients with HM.

Since the identification of Picornaviridae and Coronaviridae relies chiefly on molecular biology tools, only limited data are available about their contribution to respiratory viral infections in patients with HM. Epidemiological evidence supports an association of human rhinovirus with LRTI in immunocompetent children and elderly people [68, 69]. Despite growing evidence that human rhinovirus is able to replicate in lower respiratory tract cells in vitro and in vivo [70, 71], whether this virus can cause pneumonia remains debated [72]. Several reports in patients with HM link human rhinovirus to LRTI [73–77]. However, these retrospective studies in small patient populations with high rates of pulmonary co-pathogen identification fail to demonstrate a clear role for human rhinovirus in the development of pneumonia. Non-poliomyelitic enteroviruses are chiefly responsible for URTI [16]. Only a few studies with small numbers of patients have focused on these viruses in patients with HM, and no conclusions can be drawn from their results [77–80]. Coronaviridae are also principally responsible for URTI but may occasionally cause pneumonia [81]. No studies have evaluated the role of Coronaviridae in patients with HM, and only two case reports have been published [82, 83].

Although it is now clear that most of the common respiratory viruses are responsible for an increased rate of pneumonia in patients with HM (Table 21.1 and Fig. 21.1–21.3), the exact nature and causative mechanisms of pneumonia in this situation remain unknown. The only risk factor for virus-related pneumonia found consistently in studies is lymphocytopenia, defined

Table 21.1 Clinical manifestations of influenza, respiratory syncytial virus (RSV), parainfluenza virus (PIV), human metapneumovirus (hMPV), and adenovirus in patients with haematological malignancies

	Influenza	RSV	PIV	hMPV	Adenovirus
Pneumonia rate (%)	33	30–40	Up to 30 ^a	Up to 40 ^a	NA
Coinfection rate (%)	12–25	NA	Up to 50 ^a	NA	50
Mortality rate (%)	15–30	15–30	15–30 ^a	Up to 40 ^a	50–80
Asymptomatic shedding	No	No	Yes	Yes	No

NA not available

^aThe occurrence of asymptomatic viral shedding may indicate lesser severity than previously thought

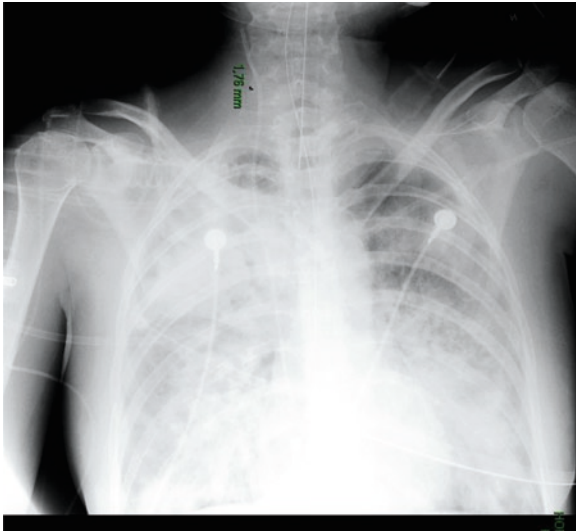


Fig. 21.1 Diffuse alveolo-interstitial pneumonia in a PIV3-infected patient

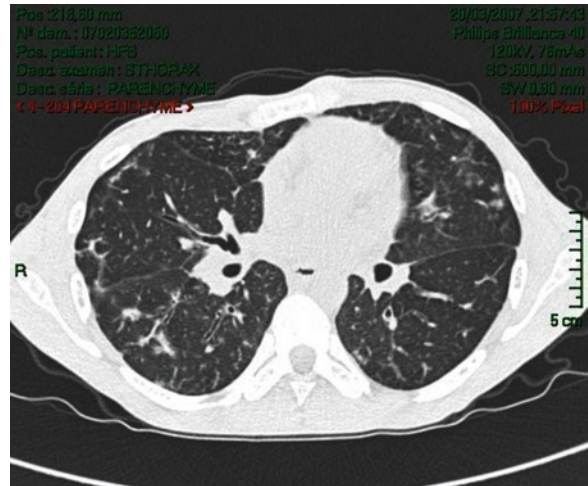


Fig. 21.3 Thoracic CT scan showing bronchiolitis pattern (bronchial wall thickening associated with ground-glass attenuation, interlobular septa thickening and tree-in-bud opacities) in an influenza-infected patient



Fig. 21.2 Diffuse alveolar consolidations in a patient co-infected with influenza and *Streptococcus pneumoniae*

using a variety of cutoffs [29, 32–34]. One study found that lymphocytopenia (absolute lymphocyte count less than 200 cells/mL) was independently associated with mortality in patients with influenza-related pneumonia [34]. A higher incidence and greater severity of virus-related pneumonia have been noted in HSCT recipients compared with other patients with HM. This finding may reflect deeper lymphocyte depletion. However, in a study by Martino et al., pneumonia rates were comparable in patients with and without lymphocytopenia [32]. The possible higher incidence and greater severity

of virus-related pneumonia within the first few days after HSCT, compared with infections occurring later on, may reflect deeper lymphocyte depletion [53]. A similar mechanism may underlie the surprising protective effect of corticosteroid therapy in the study by Nichols et al., since this treatment is often given to treat graft-versus-host disease (GVHD) after lymphoid engraftment [33].

Common respiratory viruses increase mortality in patients with HM mainly by causing severe pneumonia, but they may also contribute to a long-term decline in pulmonary function. RSV, PIV, and influenza infections have been linked to late airflow obstruction in HSCT recipients [40]. In a study focusing specifically on the role of respiratory viruses in late airflow obstruction, PIV LRTI was the strongest risk factor, followed by PIV URTI [84]. Pneumonia caused by RSV was a borderline-significant risk factor. The exact causative mechanisms remain unknown, but inflammation resulting from prolonged viral persistence and pneumonia-induced post-acute-phase inflammation have been suggested as possible factors [84, 85].

A distinctive feature of adenoviruses in HM patients is that they may either be acquired via exogenous respiratory or oro-faecal contamination, or arise via reactivation of latent viruses [39, 86]. Adenoviruses cause a wide spectrum of clinical manifestations ranging from benign conjunctivitis to rapidly fatal disseminated disease. In patients with HM, the risk of severe adenoviral

disease seems related to the level of immunosuppression. Adenoviral disease has been reported in 3–27% of HSCT recipients within the first 100 days after transplantation [87–89]. Higher rates may occur in paediatric patients compared with adults [88, 90]. In vivo and ex vivo graft T-cell depletion [86, 87, 91], lymphocytopenia [86], GVHD [87, 89], viral shedding from more than one site [86, 88, 90], and adenovirus viraemia [86, 92–94] have been reported as risk factors for severe adenoviral disease. Adenovirus viraemia consistently preceded viral shedding from more than one site in the study by Chakrabarti et al. [86]. This risk factor is of critical importance since it guides pre-emptive antiviral therapy. Adenoviral disease has been chiefly reported in myeloablative HSCT recipients, but has also been described in non-myeloablative HSCT recipients, autologous transplant recipients, and patients with chronic lymphocytic leukaemia treated with fludarabine combined with CD52 monoclonal antibodies [88, 89]. In allogeneic transplant recipients, the most frequent manifestations of adenoviral infection are gastrointestinal disease and haemorrhagic cystitis [95, 96]. Although haemorrhagic cystitis is a benign localised event, it is associated with high morbidity [90]. It must be pointed out that in a patient with febrile haematuria and adenovirus urinary excretion, the onset of acute renal failure with flank pain may reveal adenovirus nephritis [97]. Gastrointestinal manifestations include gastroenteritis and colitis, which often manifest as febrile haemorrhagic diarrhoea [95, 98]. The most severe manifestations of adenoviral infection are hepatitis, encephalitis, and pneumonia [86, 89, 90]. Adenovirus-related pneumonia occurred in 15 of the 85 patients with adenovirus infection in the study by La Rosa et al. [89]. The pneumonia was isolated in 11 patients and a manifestation of

disseminated disease in 4 patients. Bacterial and fungal co-infections were found in 50% of patients, in keeping with other studies [90]. The mortality rate was 73% overall, 50% in patients with isolated pneumonia, and 80% in patients with disseminated disease [89].

21.5 Diagnosis

Respiratory viral infections are the second most common cause of community-acquired pneumonia [2, 99]. They were long considered a simple differential diagnosis, without practical consequences for the patient. Recently, however, the diagnosis of viral respiratory infection has become increasingly relevant for medical care [100, 101]. First, RSV, influenza, PIV, and adenoviruses have been identified as significant causes of community-acquired and nosocomial respiratory infections [29, 102]. Second, new nucleic acid-based assays have been shown to be more sensitive than conventional techniques. Third, specific antiviral drugs are now available for some respiratory viruses, including influenza and adenovirus. Several diagnostic tests have been introduced for the detection of respiratory viruses. The main characteristics of the most widely used assays are shown in Table 21.2.

High-quality clinical specimens are needed to ensure the accurate detection of respiratory viruses. Indeed, respiratory virus detection requires a large number of epithelial cells, since viruses are intracellular pathogens. Nasopharyngeal aspirates and nasopharyngeal washes are recognised to be superior to other sample types for detecting respiratory viruses [103, 104]. Because the collection process is unpleasant and

Table 21.2 Methods for detecting common respiratory viruses

Test	Sensitivity	Specificity	Time to result	Viral targets	Cost
Immunofluorescence staining	++	++	<3 h	<8	15–25 Euros
Rapid antigen testing (immunochromatography)	+	+/++	<1 h	Only available for influenza and RSV	10–15 Euros per test
Cell culture	+	+++	Days to weeks	Depending on the cell lines used	NA
Monoplex NASBA or real-time PCR assay	+++	+++	<12 h	1 or 2 per reaction	10–20 Euros per target
Multiplex molecular assay	+++	++/+++	6 h–24 h	12–20 per reaction in 1 or 2 tubes	50–80 Euros per panel

time-consuming, causes patient discomfort, and requires a suction device, flocked swabs were recently introduced for respiratory sample collection. Flocked swabs were found to improve the collection and release of epithelial cells [105, 106], and were recently recommended for the diagnosis of the new pandemic H1N1 influenza strain. The number of cells collected is lower with flocked swabs than with nasopharyngeal aspirates, but seems sufficient for respiratory virus detection by immunofluorescence [105, 106]. Flocked swab collection is sensitive, specific, easy to use, and better tolerated by patients, and may therefore constitute a good alternative to nasopharyngeal aspiration. Because most respiratory viruses have high replication rates in the upper respiratory tract, nasopharyngeal secretions are usually sufficient to diagnose respiratory viral infections. However, asymptomatic respiratory virus shedding in the upper respiratory tract may occur even in immunocompromised patients, and URTI may be followed by LRTI, particularly during influenza infections. Thus, virus detection in lungs via bronchoalveolar lavage may be useful to assess the causative role for the virus.

Over the past 2 decades, virus isolation and antigen detection have been the mainstay of clinical laboratory testing for respiratory virus infections. Virus isolation required several cell lines and was mainly performed on shell vial cultures. The diagnosis was based on the presence of the cytopathic effect, which requires several days or weeks to develop. To shorten the time to diagnosis, specific monoclonal antibodies were introduced to enable the detection of specific viral antigens within 1–2 days. Direct or indirect fluorescent antibody staining of cells from respiratory specimens (mainly nasopharyngeal swabs or nasopharyngeal aspirates [NPA] and bronchoalveolar lavage fluid) is commonly used to detect viral antigens. In many laboratories, the first-line test is immunofluorescence staining, which requires very little material and provides a result within about 3 h. However commercial monoclonal antibodies are available for a limited number of targets, including influenza A and B; RSV; adenovirus; PIV 1, 2 and 3; and human MPV. Thus, rhinoviruses and coronaviruses, the most frequent causes of URTI and the most recently discovered agents, cannot be detected using monoclonal antibodies.

Nucleic acid amplification tests have been proven to be rapid, very sensitive, and specific. The early tests, including PCR, nucleic acid sequence-based

amplification (NASBA), and real-time PCR assays, were developed in monoplex format. Despite the gain in sensitivity and the ability to test for most respiratory viruses, the need to carry out as many PCR tests as targets has limited the use of this method. None of the current PCR assays has sufficiently high throughput to handle large numbers of samples containing multiple targets. Over the last few years, assays have been introduced in multiplex format. Different technologies have been used. In addition, sensitivities may be lower compared to monoplex real-time RT-PCR. Multiplex PCR procedures are difficult to optimise, because each amplification target corresponds to a set of primers characterized by a unique combination of optimal annealing conditions. Multiple primers included in a single tube may also result in primer-primer interference and in nonspecific nucleic acid amplification.

Currently, several multiplex assays can detect up to nine respiratory viruses in a single reaction. Real-time multiplex assays detect up to five targets in a single reaction, depending on the number of channels available in the real-time PCR machine [107–110]. Existing multiplex PCR assays that use agarose gel electrophoresis or capillary electrophoresis as the detection system or multiplex PCR assays combined with an enzyme-linked immunosorbent assay can detect five to nine targets per reaction [111–113].

More recently introduced techniques can detect up to 20 targets in one or two tubes. These include the four techniques detailed below.

1. *Low-density micro-array* based on cDNA spots that are immobilised on a polymer-coated slide and that hybridise with specific DNA sequences previously amplified from the sample using PCR. Hybridisation is detected based on the production of an insoluble product on the microarray at the sites where amplified DNA products are captured by the probes [114].
2. *Multiplex detection based on Luminex* technology. The PCR products are coupled with spectrally distinct fluorescence-labelled micro-beads, which are detected by flow cytometry [115, 116].
3. *Multiplex ligation-dependent probe amplification (MLPA)* involves specific viral amplification followed by amplification after hybridisation and binding of virus-specific probes to the PCR product. Identification is accomplished by PCR fragment size analysis using gel electrophoresis methods [117, 118].

4. *Mass spectrometry* assays for respiratory virus detection are based on the analysis of base compositions in RT-PCR amplicons [119].

Most assays exhibited specificities comparable to those of cell culture and monoplex real-time reverse transcription (RT)-PCR, and sensitivities similar to those of monoplex (RT)-PCR assays.

Although molecular assays, particularly RT-PCR, provide same-day results, they require a nucleic acid extraction step. This step increases the time to results compared to antigen detection by fluorescent staining. Molecular assays should be performed as often as possible to improve patient care by shortening the hospital stay, curtailing or preventing antibiotic therapy, preventing nosocomial spread, and allowing specific antiviral therapy. Therefore, and given the cost of the equipment needed (RT-PCR machine, sequencer, luminex, nucleic acid extractor, DNA chip reader, mass spectrometer), molecular assays will probably be chiefly performed in laboratories receiving large numbers of samples per day.

Few data are available on the exhaustive and sensitive detection of respiratory viruses in HM patients. Recent data show that using molecular assays increases the virus detection rate twofold compared to cultures and fourfold compared to immunofluorescence staining [120]. Two prospective studies of molecular assays in

HSCT recipients documented persistent asymptomatic respiratory shedding of PIV and human MPV [30, 65]. These data emphasise the need for prospective studies of the molecular detection of respiratory viruses to elucidate factors associated with symptomatic or asymptomatic respiratory viral infection and to help in the interpretation of positive assays in HM patients.

21.5.1 Antiviral Treatment

A chapter from Dr. Sandherr is dedicated to antiviral therapy in this book. Regarding common viruses, we will briefly describe available treatments on influenza and RSV. Two classes of antiviral drugs are available for the treatment of influenza: M2 ion channel inhibitors and neuraminidase inhibitors. The M2 ion channel inhibitors, amantadine and rimantadine, act by inhibiting the M2 protein needed for viral RNA release within the host cells. They are active only against influenza A strains. Their clinical effect is limited, being not significantly different from that of a placebo [121], and is further hampered by the worldwide emergence of resistant influenza A strains [122]. The neuraminidase inhibitors (Table 21.3), oral oseltamivir and inhaled zanamivir, are sialic acid analogues that competitively inhibit the influenza neuraminidase. Randomised controlled trials

Table 21.3 Antiviral treatments for common viral pneumonia

	Oseltamivir	Zanamivir	Ribavirin	Cidofovir
Therapeutic class	Neuraminidase inhibitor	Neuraminidase inhibitor	Nucleoside analogue	Nucleoside analogue
Route	Oral	Inhaled	Inhaled	Intravenous
Regimen	75 mg <i>bid</i>	10 mg <i>bid</i>	Up to 2 g <i>tid</i> ^a	5 mg/kg weekly ^a
Duration	5 days	5 days	10 days ^a	Variable ^b
Side effects	Rash	Rash bronchospasm	Haemolytic anaemia ^d bronchospasm	Nephrotoxicity ^c myelosuppression retinitis
Indication	Influenza	Influenza	RSV ^c	Adenovirus ^c
Evidence supporting use	Cohort studies	Cohort studies	Cohort studies	Cohort studies

NA not available, *bid* twice daily, *tid* three times daily

^aThe therapeutic regimens are those described in the available literature

^bCidofovir is given in a dose of 5 mg/kg weekly for 2 weeks then every 2 weeks according to the clinical response

^cHyperhydration and probenecide may help to prevent cidofovir nephrotoxicity

^dHaemolytic anaemia may be readily controlled by blood transfusion

^eThese drugs have not been approved by regulating authorities in these indications

showed moderate decreases in symptom intensity and duration with both drugs [123–125]. Neuraminidase inhibitors may also limit bacterial super-infection of the lungs [125, 126]. Early administration of these drugs seems mandatory to obtain a clinical benefit [127]. A more recent cohort study suggested a diminution of 15-day mortality with neuraminidase inhibitors [128]. This result must be interpreted with caution, especially given the study design. There is growing concern about the emergence of oseltamivir-resistant influenza A strains [129]. This phenomenon is not surprising given the high mutation rate in the influenza genome. It should lead clinicians to reconsider the indications of neuraminidase inhibitors and their use as monotherapy to treat influenza [130]. No randomised controlled trials have evaluated the efficacy of neuraminidase inhibitors in patients with HM. The apparently lower mortality in descriptive cohort studies compared with historical studies must be interpreted with caution [33–35, 131]. Many of the patients in these cohorts had bacterial and fungal co-infections, whose specific treatment probably contributed to decreasing mortality. Furthermore, the decision to treat patients was left to clinician discretion, which may have introduced a bias toward selection of patients with greater disease severity. Given both the absence of strong evidence supporting beneficial effects of neuraminidase inhibitor therapy and the rapid emergence of resistant strains, we believe that neuraminidase inhibitor therapy cannot be recommended for HM patients with influenza. Randomised controlled trials are needed to determine the efficacy, safety, and ecological impact of neuraminidase inhibitors in these patients.

Ribavirin is the only available antiviral drug for Paramyxoviridae infection (Table 21.3). The efficacy of aerosolized ribavirin was variable in uncontrolled cohorts of patients with RSV-related pneumonia [34, 46, 132]. Again, the results must be interpreted with caution. Early ribavirin therapy, introduced before the onset of respiratory failure, seemed associated with lower mortality in the study by Whimbey et al. [132]. This observation led some authors to evaluate pre-emptive ribavirin therapy, characterised by ribavirin treatment of RSV URTI to prevent progression to pneumonia [133, 134]. The study by Boeckh et al. is the only randomised controlled trial evaluating pre-emptive ribavirin therapy in patients with RSV infection [133]. Unfortunately, the trial did not enrol a sufficient number of patients to produce conclusions.

Intravenous ribavirin has also been evaluated and found unhelpful [29, 135]. Combining ribavirin with intravenous immunoglobulins or RSV-specific immunoglobulins produced variable levels of efficacy in cohort studies [35, 132, 136, 137]. Palivizumab, an RSV-specific monoclonal antibody, was evaluated only in a small cohort [138].

In vitro, ribavirin exhibits mild antiviral activity against PIV [58]. No clear efficacy of aerosolized ribavirin was found in small, uncontrolled cohorts of patients with HM [34, 139]. Early initiation of ribavirin therapy has been proposed to enhance efficacy [140]. No studies have evaluated intravenous immunoglobulins or RSV-specific immunoglobulins, which contain high titres of PIV-specific immunoglobulins.

An in vitro study suggests that ribavirin may be of interest for treating human MPV-related pneumonia, but this drug has not been evaluated in patients with HM [141].

To date, there is no strong evidence supporting the use of ribavirin, either alone or combined with immunoglobulins, to treat Paramyxoviridae-related pneumonia in patients with HM.

No controlled randomised study has evaluated the treatment of adenoviral disease in patients with HM. Ribavirin and cidofovir have been suggested for the treatment of adenoviral infections, but only uncontrolled cohort studies are available to support their use. Intravenous ribavirin therapy failed to demonstrate benefits in available studies [89, 142–147]. Cidofovir therapy (Table 21.3) seemed to result in better outcomes in small, uncontrolled series [148–153]. Ganciclovir and antiretroviral drugs, such as zalcitabine, alovudine, and stavudine, exhibit in vitro activity against adenoviruses, but have not been specifically evaluated in clinical settings [95, 154]. Considering the lack of specific antiviral drugs, decreasing the level of immunosuppression seems to be a rational treatment approach [155]. T-cell therapy has also been reported in some small series [155, 156]. Early cidofovir treatment seems associated with a better outcome [151]. Weekly PCR screening for adenovirus viraemia has been suggested to guide pre-emptive cidofovir therapy [91–94, 153, 157]. The exact threshold above which pre-emptive cidofovir therapy should be initiated is unknown. Randomised controlled trials are needed to better define the optimal viraemia threshold for antiviral therapy and to evaluate the benefits from this treatment.

The greater severity of common respiratory viral infections in HM patients and the lack of clearly proven effective antiviral drugs emphasise the critical importance of preventive strategies.

21.6 Prevention

The occurrence of common respiratory virus infections in patients with HM is closely related to viral activity in the community. However, nosocomial outbreaks have been described in haematologic wards, often within a few days after an outbreak in the community [31, 43, 44, 46, 158–160]. Preventing nosocomial transmission of common respiratory viruses requires a multifaceted approach (Table 21.4) that targets not only the modes of transmission, but also the source of the viruses [43]. Nosocomial outbreaks are believed to originate from infected patients and from infected health-care workers. Infection control measures include screening symptomatic patients for common respiratory viruses, early isolation of infected patients, screening health-care workers and visitors for respiratory symptoms, and avoiding contact of symptomatic individuals with patients. Most haematologic wards do not allow

children to visit patients, as children are highly prone to common respiratory virus infections and may exhibit viral shedding long after symptom resolution [39]. Continual reinforcement of standard hygiene measures, especially hand hygiene before and after contact with patients, is also strongly recommended. Awareness of the usefulness of these measures, and therefore adherence to the measures, are low, but can be enhanced by educational programs [161]. Most of the common respiratory viruses are transmitted by droplets or direct contact of infected secretions with the nasal mucosa or conjunctiva. Indirect transmission via contaminated fomites or devices is also possible, since these viruses may survive several hours on these surfaces [6, 7, 58]. Consequently, contact isolation of infected patients combined with droplet precautions is mandatory [162]. A gown, gloves, and a mask with eye protection must be worn for all contacts with infected patients. Airborne transmission of influenza is suspected [163, 164] but not yet proven, and two recent studies suggest that surgical masks may be as effective in preventing influenza transmission as masks with greater filtration capacity [165, 166]. Infection control measures have not been evaluated in randomised control trials, but a before-and-after study suggests they may help to prevent the transmission of common respiratory viruses [43]. It must be pointed out that asymptomatic viral shedding, when present, limits the effectiveness of infection control measures [30, 65]. The optimal duration of isolation is unknown. Whether isolation should be prolonged in asymptomatic patients with persistent low-titre viral shedding detected only by molecular methods remains unclear. Since adenovirus acquisition is known to result also from endogenous reactivation, adenoviral infections may be chiefly related to the level of immunosuppression [39, 86] and, therefore, may not be effectively prevented by standard infection control measures.

To date, influenza is the only common respiratory virus for which a vaccine is available. Immunisation remains the cornerstone of influenza prevention [6]. The immunogenicity of the inactivated influenza vaccine is lower in patients with HM compared with immunocompetent controls [167–172]. A second dose of influenza vaccine has been proposed to enhance immunogenicity, but this strategy was not beneficial in an open-label randomised study [169, 170]. The timing of influenza immunisation may be important. Immunogenicity may be improved by giving the vaccine at least 6 months after HSCT or at least 7 days

Table 21.4 Infection control measures for common respiratory viruses

Specific infection control measures
Screening patients for respiratory virus infections
Contact isolation with droplet precautions for infected patients
Screening visitors and healthcare workers for URTI symptoms
Prohibiting visits by individuals with URTI symptoms
Prohibiting work for healthcare workers with URTI symptoms
Prohibiting visits by children
Enhanced standard precautions
Reinforced hand hygiene before and after contact with patients
Annual influenza immunisation
Patients with HM
Family contacts of patients
Health-care workers

after intensive chemotherapy [168, 171]. The efficacy of influenza immunisation has not been evaluated in randomised control trials. However, a retrospective cohort study suggested benefits from influenza immunisation in HSCT recipients [173]. Influenza immunisation appears safe in patients with HM, and lifelong seasonal administration of the influenza vaccine is therefore recommended in these high-risk patients [174]. Importantly, family contacts should be immunised also to prevent transmission within the household. Of critical importance is the issue of health-care worker immunisation, which is known to prevent nosocomial acquisition of the virus [175].

Pharmacological interventions have been proposed to prevent common respiratory virus infections. Intravenous immunoglobulins and palivizumab have been suggested for RSV infection prevention, but have not been evaluated in patients with HM. Prophylactic post-exposure oseltamivir therapy is currently recommended in patients with HM [176]. This preventive measure has not been evaluated in randomised controlled trials, but seems safe based on a recent retrospective study in HSCT recipients [177]. Considering the lack of proven benefits of oseltamivir therapy and the growing concern about the emergence of oseltamivir-resistant influenza strains, oseltamivir therapy should be carefully evaluated before it is suggested for use on a large scale [130].

Another way to prevent morbidity and mortality related to common respiratory viruses in patients with HM is to limit the risk of progression to pneumonia. The pre-emptive treatment of viral URTI has been discussed above. Given the severity of common respiratory virus pneumonia in patients with HM, it appears logical and prudent to postpone HSCT in patients with documented viral infections. A retrospective cohort study suggested that this measure might prevent RSV-related pneumonia in patients undergoing HSCT [178]. It is unclear whether this measure might be applied to all types of HSCT, to intensive chemotherapy courses, and for all respiratory viruses.

21.7 Conclusion

Common respiratory viruses are now recognised as true opportunistic respiratory pathogens in patients with HM. In these patients, they constitute a common

cause of potentially severe pneumonia. However, areas of uncertainty remain, indicating a need for further investigations. The epidemiology of common respiratory virus infections in patients with HM has not been extensively studied. Large epidemiological studies of common respiratory virus infections in patients with HM based on molecular biology tools may lead to radical changes in knowledge. Although it is now clear that common respiratory viruses are responsible for increased pneumonia rates in patients with HM, the exact nature and causative mechanisms of these pneumonia cases remain unknown. Studies are warranted to investigate the role of viruses in the development of these LRTIs. The virus might directly injure alveolar epithelium or promote lung super-infection by causing bronchial epithelium damage. These studies would also help to determine the potential benefit of pre-emptive or curative antiviral therapy. Finally, randomised controlled studies of antiviral therapy in patients with HM are urgently needed.

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