New Respiratory Viruses and the Elderly

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Abstract: The diagnostics of respiratory viral infections has improved markedly during the last 15 years with the development of PCR techniques. Since 1997, several new respiratory viruses and their subgroups have been discovered: influenza A viruses H5N1 and H1N1, human metapneumovirus, coronaviruses SARS, NL63 and HKU1, human bocavirus, human rhinoviruses C and D and potential respiratory pathogens, the KI and WU polyomaviruses and the torque teno virus. The detection of previously known viruses has also improved. Currently, a viral cause of respiratory illness is almost exclusively identifiable in children, but in the elderly, the detection rates of a viral etiology are below 40%, and this holds also true for exacerbations of chronic respiratory illnesses. The new viruses cause respiratory symptoms like the common cold, cough, bronchitis, bronchiolitis, exacerbations of asthma and chronic obstructive pulmonary disease and pneumonia. Acute respiratory failure may occur. These viruses are distributed throughout the globe and affect people of all ages. Data regarding these viruses and the elderly are scarce. This review introduces these new viruses and reviews their clinical significance, especially with regard to the elderly population.

Keywords: Bocavirus, coronavirus, elderly, influenza virus, metapneumovirus, polyomavirus, respiratory infection, torque teno virus.

INTRODUCTION

Life expectancy has increased globally over the past two centuries by almost 30 years [1] and only over the last five decades by almost 20 years. This very recent phenomenon emerged as a consequence of improvements in nutrition, hygiene, antimicrobial therapy and vaccinations [2, 3]. The development of antiviral therapy, has, however, lagged behind. In the elderly, respiratory viral infections still cause significant morbidity and mortality: up to 40% of non-pneumonic lower respiratory illnesses have been linked to respiratory viral infection, and in USA alone, an estimated 54,000 annual deaths have been attributed to the influenza and respiratory syncytial viruses (RSV) [4-11].

A milestone in the diagnostics of respiratory viral infections was the discovery of influenza A virus in 1933 [12]. After the discovery of the coronaviruses in 1965, no new respiratory viruses or significant virus strains were identified in 32 years. The development of polymerase chain reaction (PCR) techniques in the 1990s initiated a new wave in viral diagnostics. The first avian flu epidemic in humans caused by influenza virus H5N1 struck in 1997 and alerted healthcare professionals by its severity [13]. In the same year, a virus family never seen in humans before, was identified: the anellovirus, the torque teno virus (TTV) as a signature virus, was found, but their link to human illnesses has not been clarified [14-16]. In 2001, human metapneumovirus (MPV) was found followed by the discoveries of other new and significant respiratory viruses and virus strains: the coronaviruses SARS, NL63 and HKU1, the human bocavirus (HBoV), new human rhinovirus (HRV) strains (HRV-C and HRV-D), influenza A virus H1N1, and, as potential respiratory pathogens, the polyomaviruses KI and WU [17-28]. This review introduces these new viruses and reviews their clinical significance, especially with regard to the elderly population.

SEARCH STRATEGY AND SELECTION CRITERIA

We made systematical searches through the PubMed data base for articles published before March 5, 2011 and indexed with the following search terms: elderly; and influenza H5N1 virus; influenza H1N1 virus; metapneumovirus; coronavirus SARS, NL63, or HKU1; bocavirus; rhinovirus C or D; polyomavirus KI or WU; or torque teno virus. We reviewed only articles published in English.

INFLUENZA VIRUSES H5N1 AND H1N1

The most severe epidemics have been caused by the influenza A virus. In 1918-1919 the H1N1 virus pandemic resulted in an estimated 50 to 100 million deaths [29]. The mortality was surprisingly high among young adults. The most recent pandemics have usually been caused by the influenza A virus strain H1N1 and H3N2. The 2009 H1N1 virus was highly contagious from human to human [30, 31]. In USA, the prevalence of H1N1-associated deaths was 12 deaths per 100 000 population. Of these, only 9% occurred

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in persons aged ! 65 years [31]. Similarly, H5N1 which had its source in birds, has infected humans since 1997, and has, by March 2011, been associated with high mortality; of 528 patients with confirmed disease, 311 (59%) have died [13, 30, 32, 33]. The mortality rate associated with H5N1 infection has been 89% among children aged <15 years [32]. Elderly are often protected by pre-existing antibodies from previous illnesses, maybe even decades back [28, 30, 31]. On the other hand, the risk of severe illness is markedly increased by underlying medical conditions, especially chronic obstructive and other pulmonary diseases, immunosuppression, diabetes, obesity, or chronic heart conditions, which often accompany old age.

Influenza viruses typically cause mid-winter epidemics. The typical respiratory symptoms (cough, fever and sore throat), however, are poorly associated (43%) with confirmed influenza illnesses in older adults [34, 35]. Hypoxia and chest radiographs consistent with the acute respiratory distress syndrome are characteristic of patients requiring intensive care [34]. If death occurs, it follows approximately more than one week after the onset of symptoms and mortality correlates with high virus titers. The cause of death is usually progressive cardiopulmonary failure. The influenza-related morbidity in the elderly is closely related to the prevalence of influenza virus infections among children (reservoir) [36]. The diagnosis is mainly based on antigen detection and PCR of respiratory specimen, but culture and serology are also available. Treatment options are neuraminidase inhibitors (oseltamivir and zanamivir) or adamantane derivatives (amantadine and rimantadine) [37]. Systemic corticosteroids are not effective and may, in fact, increase the risk of hospital-acquired pneumonia and superinfections [38]. Strain-specific influenza vaccination is usually available after 6 to 12 months after emergence of pandemic and has usually high immunogenicity among elderly subjects [39].

HUMAN METAPNEUMOVIRUS

Human metapneumovirus (MPV) causes upper and lower respiratory infections in patients of all ages, but mostly in children aged less than 5 years [17]. In healthy elderly subjects, MPV-infection is rare: one large study showed MPV RNA in nasal specimens in 1-2% of symptomatic and in 0-2% of asymptomatic elderly subjects [40]. In another study, MPV was only found in 2% of patients with acute exacerbation of chronic obstructive pulmonary disease (COPD) [41]. The prevalence of symptomatic MPV infection is higher (up to 4-7%) in residents of long-term care facilities [40]. During outbreaks, up to 72% of elderly institutionalized persons may fall ill, 31% may develop radiographically confirmed pneumonia and 50% may die [42, 43].

In adults and elderly, MPV typically causes influenza-like symptoms, such as rhinitis, cough and sore throat, but elderly subjects are more prone to lower respiratory symptoms such as wheezing and dyspnea [40, 42-44]. Overall, in the elderly, MPV infections are likely to be less severe than RSV infection and influenza [40]. The risk factors for severe illness, in addition to old age and institutionalization, include immunosuppression and chronic cardiopulmonary illness. MPV usually causes mid-winter epidemics. Asymptomatic MPV infections are rare. There seem to be two proposed genotypes, A and B, and several subgenotypes of MPV [45, 46], and thus it is unlikely that infection by either genotype of MPV confers cross-protective immunity. The diagnosis of MPV infections is based on PCR, but immune fluorescence assays are also available. Several vaccine candidates are being investigated [47, 48].

SARS-ASSOCIATED CORONAVIRUS

The pandemic caused by SARS-CoV (SARS = severe acute respiratory syndrome) initiated in November 2002 in Guangdong province, China. It affected more than 8000 patients and caused 774 (approximately 10%) deaths of all ages on 5 continents during an approximately 12 month period [20, 49, 50]. Infection by SARS-CoV was almost exclusively symptomatic resembling influenza with initial symptoms of fever, myalgia, malaise and chills or rigor [49]. Cough was common, but dyspnea was prominent only later in the course of the illness. Death was usually due to respiratory failure or a sepsis-related syndrome. Advanced age and co-morbidities increased markedly the risk of severe illness [50]. PCR tests have been rapidly developed and serology is also available [19, 20, 49]. No effective treatment is available, but interferons and ribavirin seem to inhibit virus replication [49]. Since the inflammation is part of the pathogenesis of this disease, corticosteroids may also be helpful if combined with antiviral medication [49]. Several new antivirals and vaccine candidates are being investigated.

CORONAVIRUSES NL63 AND HKU1

In addition to coronaviruses 229E and OC43, the new coronaviruses NL63 and HKU1 were identified in samples of patients with respiratory symptoms in 2005-2006 [18, 21, 22, 44, 51, 52]. Like other coronaviruses, NL63 and HKU1 can be detected in a small percentage of individuals of all ages [53]. These viruses have primarily been associated with mild upper respiratory tract infections, but severe lower respiratory tract infections have also been reported [51, 52]. Diarrhea and abdominal pain may also occur, but symptoms and signs relate primarily to the respiratory tract [53-56]. Chronic underlying conditions and advanced age increase the susceptibility and disease severity of CoV infections, and mortality occurs [44, 53]. In a study of community-acquired pneumonia, most of the HKU1-positive patients were old (median age 72 years) and had significant underlying diseases, especially of the respiratory and cardiovascular systems [18]. One study reported an outbreak of acute respiratory infection in a personal-care home, where CoV NL63 was identified in 7 of 8 patients aged >50 years [57]. One elderly patient died 5 days after the onset of HCoV-NL63 infection. CoV-NL63 and CoV-HKU1 are distributed throughout the globe and throughout the year, although they usually peak in the winter time [54] and cause irregular epidemics every 2–3 years [58]. Since these viruses are difficult to culture, diagnostic tests other than PCR are not available. There is neither specific antiviral therapy nor vaccine available for HCoV infections [59].

HUMAN BOCAVIRUS

The prevalence of HBoV DNA in respiratory specimens ranges from 1.5-19% [23, 60, 61] and the most typical age

for a primary HBoV infection is 6-48 months [62]. HBoV occurs world wide and throughout the year. HBoV has been associated with upper and with lower respiratory tract infections in children [62-66], but very little is known about HBoV infections among elderly people. HBoV has also been detected in the feces, in 1-9% of small children with or without gastrointestinal or respiratory symptoms [67-70] as well as in river and sewage water [71, 72], but whether it is a true enteric pathogen is not known.

Although HBoV infections are usually diagnosed with PCR, serological studies have shown that the mere presence of HBoV DNA in the respiratory tract is not proof of an acute primary HBoV infection [62, 73-75]. Studies on consecutive NPA samples have indeed shown that HBoV DNA can persist in the nasopharynx for several months [76, 77]. Among adults, over 94% have antibodies to HBoV, indicating that they have encountered this virus during their lives [78, 79]. The prevalence figure is high and shows that HBoV infections are extremely common. Only a limited number of HBoV DNA-positive adults have been reported, mainly among immunosuppressed subjects, an observation that is in line with the high seroprevalence of HBoV [80-84]. This age pattern was, however, interestingly contradicted by a Canadian study that did not find differences in the prevalence of HBoV among different age groups [85]. In adults, HBoV-DNA positivity seems to be associated with symptoms, and therefore, HBoV cannot be considered simply an innocent bystander virus [81, 83, 86]. Since the discovery of HBoV, three other related bocaviruses (HBoV2, 3 and 4) have been identified in human stool samples [69, 87, 88].

HUMAN RHINOVIRUS, GROUPS C AND D

Human rhinovirus, the common cold virus, is the most common respiratory pathogen in all age groups [12]. In the two last decades of the last millennium it was thought that two major genetic groups, A and B, and 99 HRV serotypes exist. Novel PCR-based techniques, however, have identified additionally two groups, C and D, and possibly over 150 different HRV strains [24, 89, 90]. Moreover, PCR has markedly increased the detection rates of HRV infections. HRV-infection is often associated with other pulmonary morbidity and is the most common virus (up to 60-70%) associated with exacerbations of asthma of all ages and with COPD in adults and the elderly [91-96]. In long-term care facilities HRV may cause serious morbidity and mortality, and goes often unrecognized. Louie *et al.* (2005) reported an epidemic of respiratory illness in a long-term care facility, which caused a mortality rate of 21% (12/56 affected residents died) [10]. Seven of 13 respiratory specimens were culture-positive for rhinovirus [10]. Hicks *et al.* (2006) reported two nursing home outbreaks of respiratory illness that caused the death of 7 residents out of 294 (2.4%). Of the 29 collected samples, 10 (34%) were positive for rhinovirus [11]. There is an overall paucity of data on HRV-infections in the elderly.

Rhinovirus may in exceptional instances cause chronic lung infections which may have a duration of more than 12 months. Such prolonged infections may occur in immunocompromized subjects with lung transplants or hypogammaglobulinemia [96, 97]. The recently identified group C HRV appears to be related to high morbidity. This virus has circulated at a rate similar to those of the HRV-A and -B groups [24, 98, 99] and is the cause for almost half of all HRV-associated hospitalizations in children [100, 101]. Different HRV strains circulate in the community throughout the year, but HRV epidemics typically peak in fall and spring. Diagnosis is based on PCR since these viruses are difficult to culture and serology is not feasible.

KI AND WU POLYOMAVIRUSES

In addition to the previously known polyomaviruses, BK and JC, seven new human polyomaviruses have been identified in rapid sequence in 2007-2011. Two of them, detected in the respiratory tract samples, have been named by the institutes where they have been found: KI (Karolinska Institute) polyomavirus (KIPyV) and WU (Washington University) polyomavirus (WUPyV) [25, 26]. Two have been named by the diseases in association with which they were detected, MCPyV from a skin cancer called Merkel-cell carcinoma and TSPyV from a skin disease called *trichodysplasia spinulosa* [102, 103]. The remaining three polyomaviruses were also detected in skin samples, and named by numbers, PyV6, 7 and 9 [104-106]. The prevalence of the respiratory KI- and WU-polyomaviruses is 2-7% in patients with respiratory symptoms [25, 26]. Most patients with KI- or WUPyV DNA in their upper airways, are young children with symptoms of rhinitis, cough, bronchiolitis and even pneumonia. Serologic studies show seroprevalences of 50 to 80% for KI- and WUPyVs in healthy children and adults [108-110].

Data on the occurrence of these viruses in the elderly are lacking but are urgently needed, since PyVs are potentially oncogenic and can persist in human tissues [103, 111]. KI- and WUPyV become reactivated at similar frequencies as the BK and JC viruses during immunosuppression [111, 112]. Diagnostics is based on PCR and serology [107, 109].

TORQUE TENO VIRUS

Torque teno virus DNA has been recovered from many tissues and secretions but whether this observation is causally related to clinical symptoms or not has not been demonstrated [14, 113-115]. TTV is possibly able to replicate in airway tissues [116, 117] and many other tissues e.g. liver and bone marrow [118]. The airways might be the primary route of transmission. TTV is very often detected in blood; the prevalence of TTV DNA in the blood of healthy individuals is approximately 70-90% [119]. A single TTV infection may persist for years and cause chronic viremia [120-122]. Simultaneous infections by different TTV variants may also occur. TTV might also aggravate the symptoms caused by other respiratory viruses, or then TTV may be an indicator of the disease process as implied by the findings that TTV concentrations in nasal secretions or plasma have a positive correlation with markers of eosinophilic inflammation and a negative correlation with pulmonary function in asthma [15]. Also, the severity of bronchiectasis and of idiopathic pulmonary fibrosis correlate with high TTV concentrations [115]. The association between TTV and disease, could be based on a direct viral effect or be mediated by inflammatory processes that predispose to virus replication. Indeed, TTV replication kinetics have been used as a marker of immune

reconstitution after suppression [123]. Although multiple TTV variants cause problems in detection, the diagnosis of TTV infections is based on PCR; serology will apparently not be developed in the near future [124].

DIAGNOSIS

Making a clinical diagnosis of a respiratory viral illness for elderly patients poses a challenge. The clinical picture is much more blurred in comparison to the typical upper respiratory infection, seen in children and young adults [35, 125-127]. Viral infections are usually due to reinfection, and elderly adults usually have some degree of immunity [128]. Because of pre-existing systemic and mucosal antibodies, elderly adults have probably lower amounts of respiratory secretions and lower viral loads as compared to children. Among elderly patients respiratory viral illness may accompany symptoms of lower respiratory tract involvement, pulmonary and cardiac failure, and nonspecific or atypical symptoms such as confusion, anorexia, dizziness, falls and lack of fever [125-128]. Finally, some elderly may also be unable to articulate their symptoms clearly, something they have in common with infants [128].

A further challenge to the diagnostics of viral illness is optimal sampling. Nasopharyngeal swabs, aspirates or washes are traditionally used in children but they are not well tolerated in older adults or older people. The best way and time to take samples for viral diagnostics are not known for the elderly. Although nasopharyngeal swab sampling is a sensitive and sufficient method for children [129, 130], this simplest sampling method may be difficult to apply to adults [131]. To obtain a sufficient sample and viral load, optimal sampling probably requires both nasopharyngeal and oropharyngeal sampling. Taking swab samples is probably the quickest way and causes the least discomfort while nasopharyngeal washing may collect more viruses [128, 131].

Of the conventional diagnostic methods available for these new respiratory viruses, serology is available for the influenza virus, MPV, HBoV and PyVs [62, 73, 109, 132, 133]. However, serology is not often practical in the acute phase. Of the other conventional methods, a rapid antigen detection test is available for the influenza virus. Some reports suggest that a rapid antigen detection test is relatively sensitive for detection of the influenza virus in elderly patients; during outbreaks up to 77% are detected by rapid antigen testing of culture positive samples [133, 134]. Other studies have reported much lower sensitivities (38-43% compared with PCR) [135, 136]. The sensitivity may be only 8-22% in patients ! 80 years of age [137]. Despite a poor sensitivity, the rapid antigen detection test is highly specific for detecting influenza viruses in the elderly

All new respiratory viruses can be diagnosed by sensitive PCR methods [44]. When diagnosing acute HBoV or SARS-CoV infection, PCR needs to be complemented with serology. Currently, up to 85-95% of all viruses in respiratory samples of children with respiratory symptoms may be detected [61, 138-142]. PCR is the best choice also for the elderly since it is the most sensitive method for detection of viruses in this age group as well [136, 143-147], although the detection rates, probably due to the difficulties in sampling, decline with age. The detection rates among elderly patients have remained below 40% even in exacerbations of chronic pulmonary disease [91, 92, 95, 148] and viral pneumonia [149]. The rates in the intermediate age groups, i.e., adults with exacerbations of COPD or asthma have been up to 64% [150-152]. The actual prevalences of the new viruses among the elderly population are not known [5, 7, 10, 11, 148, 153].

The interpretation of positive PCR results is complicated by multiple co-existing viruses especially in symptomatic children (up to 43%) and by high virus detection rates in asymptomatic subjects (up to 40-68% in young children) [139, 154-157]. In a review of the literature that goes back to 1965 and stretches to 2008, the prevalence of viruses in 15000 samples from asymptomatic subjects was higher by PCR than by conventional methods [158]. This casts some doubt on the clinical significance of PCR-positive viral findings overall. Several studies have, on the other hand, demonstrated that positive PCR results are clinically relevant at least as far as HRV is concerned. Identification of HRV correlates with respiratory symptoms, dual HRV infections are rare and overall, the prevalence of recurrent or persistent respiratory viral infections (excluding TTV and HBoV) is low (3-4%) [96, 158-160]. Positive findings with PCR correlate with systemic or local immune responses in children and in adults [161-163]. These findings, which mainly apply to HRV and not to HBoV, suggest that HRV-PCR positivity probably reflects a true, current respiratory infection with or without symptoms, rather than residual nucleic acids from some other distant infection. Of course, any findings in upper airway samples do not necessarily reflect the situation in lower airways [164]. Multiple PCR analyses of single samples (multiplex PCR) may sound attractive, but the sensitivity for identification of individual viruses may be lost compared to single virus PCR [165]. Of note, most of these data are from studies on children and adults, and data on new respiratory viruses in the elderly are scarce.

IMMUNOSENESCENCE

The term immunosenescence describes the deleterious age-associated changes in the immune system that render elderly individuals susceptible to infectious disease and increases morbidity and mortality [3, 166]. With age, all components of immunity are affected, but the T cells are the most susceptible [167]. Although the adaptive function of immunity appears to be more seriously affected than the innate immune system, the increased susceptibility to lower respiratory tract viral infections relates particularly to defective innate immunity [163, 168]. The weakening immune responses could be linked to the over-all long-term poor outcome in the elderly [166]. Immunosenescence is a multifactorial process and is associated with thymic involution, chronic antigenic stimulation (predominantly attributable to persistent infections), signal transduction changes in immune cells, and protein-energy malnutrition [169]. There is a paucity of accurate data on the link between the causes of death of elderly and the age-associated changes in the immune system.

TREATMENT

With the exception of the influenza viruses, there are no specific treatments or vaccines available to combat the new

viruses. In this sense, there is no clinical need for a viral diagnosis. Viral detection may still have practical importance with regard to isolating practices of infected patients in hospitals or in long-term care settings to prevent transmission of disease [128] and for proper supportive treatment, including avoidance of unnecessary antibiotic treatments [146].

An increased susceptibility to viral infections could be a marker of a pulmonary inflammatory processes, and indicate a need for intensified treatment of chronic pulmonary illness. For example, TTV and AdV infections are associated with a chronic inflammatory state of the lungs [15, 170-173]. In children, there is a link between susceptibility to HRV-induced wheezing and the development of asthma [174-178], and in adults, HRV is the most important trigger of exacerbations of COPD [91, 95].

Current knowledge on bacterial-viral coinfections in the elderly is very limited. In community-acquired pneumonia of adults, there is evidence of mixed viral-bacterial infection in up to 15% of cases and in children up to 45 % of cases [179]. The most frequent combinations have been *Streptococcus pneumoniae* with influenza A virus or HRV. Bacterial and viral infections may act deleteriously through synergistic mechanisms. There may be destruction of the respiratory epithelium by the viral infection, which may increase bacterial adhesion; virus-induced immunosuppression may cause bacterial superinfections; and the inflammatory response to viral infection may up-regulate the expression of molecules that are suitable for bacteria as receptors [180].

Vaccines are being developed against these new viruses. The most promising preclinical results have been reported for vaccine candidates for MPV and SARS-CoV, but their efficacy have not been studied in humans [181, 182].

CONCLUSIONS

The new respiratory viruses or viral strains include influenza A virus H5N1 and H1N1, MPV, SARS-, NL63- and HKU1-CoV, HBoV, HRV-C and –D and the possible respiratory pathogens, KI- and, WU-PyV and TTV [13, 14, 17-28]. All these new viruses are distributed throughout the globe and affect people of all ages, but data on these viruses and the elderly are scarce. These new viral infections can be diagnosed by sensitive PCR methods. The viruses may be detectable in the airways for varying periods of time also after the acute phase and this leads to a diagnosis of several concomitant viruses. The classical predisposing factors to viral infections include advanced age, chronic illnesses and poor immune responses. The elderly often have partial immunity and chronic illnesses; these circumstances modify their responses to viruses and thus respiratory viral infections may manifest themselves as atypical symptoms or as exacerbation of chronic illnesses. Serious outbreaks have been reported in long-term care facilities. Vaccination is the most effective way to prevent serious disease, but it is only available for the influenza virus. Virus-specific treatment is also available only for the influenza virus. Early identification of a viral pathogen through improved viral diagnostics is crucial for successful treatment of viral illnesses. Preventive measures are also important, such as vaccinations, hand-washing and isolation of the affected individuals in hospitals and long-term care facilities. The ultimate clinical significance of the new respiratory viruses is still poorly unknown in the elderly population but probably these infections are greatly underestimated.

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