SPECTRUM OF VIRAL INFECTIONS IN PATIENTS WITH CYSTIC FIBROSIS

H. Frickmann^{1,2*}, S. Jungblut^{3*}, T. O. Hirche⁴, U. Groß⁵, M. Kuhns^{5**} and A. E. Zautner^{5**, ***}

¹ Fachbereich Tropenmedizin am Bernhard-Nocht-Institut, Bundeswehrkrankenhaus Hamburg, Hamburg, Germany

² Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Universitätsklinikum Rostock, Rostock, Germany

³ Praxis Dr. Jungblut, Frankfurt am Main, Germany

⁴ Abteilung für Pneumologie, Deutsche Klinik für Diagnostik (DKD), Wiesbaden, Germany

⁵ Institut für Medizinische Mikrobiologie, Universitätsmedizin Göttingen, Göttingen, Germany

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This review explores the extensive influence of viral infections leading to chronic deterioration of lung function in patients with cystic fibrosis (CF). The mechanisms how viral agents affect the pathogenesis as well as the inflammatory and immune response of CF are discussed. Viral infections of the upper and lower respiratory tract due to viruses in CF patients and methods for diagnosis of respiratory viruses are described in detail. The importance of respiratory and non-respiratory viral agents for the pathogenesis, especially for the exacerbation of bacterial lower respiratory tract infections and course of CF, is stressed, especially emphasizing respiratory syncytial virus, influenza virus, rhinovirus, and human herpes viruses. Possible harmful effects of further viruses like adenovirus, bocavirus, coronavirus, metapneumovirus, parainfluenzavirus on the lung function of CF patients are discussed. The potential use of adenovirus-based vectors for somatic gene therapy is mentioned.

Keywords: cystic fibrosis, mucoviscidosis, virus, infection, immune response, inflammation, respiratory syncytial virus, human rhinovirus

Introduction

Cystic fibrosis (CF) is an autosomal-recessive genetic disease. More than 60,000 people worldwide suffer from this illness. The CF gene, the cystic fibrosis transmembrane conductance regulator (CFTR) gene, was found in 1989. More than 800 mutations have been sequenced in the mean time [1]. The life expectancy for patients with CF has remarkably improved during the last decades. However, the progressive deterioration of pulmonary function continues [2].

Pulmonary infections represent the major causes of morbidity and mortality. *Staphylococcus aureus* and non-typable *Haemophilus influenzae* are the most common infectious agents in the lung of CF patients in the early stages of the disease [3]. This spectrum changes in the course of the illness. By 18 years of age, 80% of patients harbour *Pseudomonas aeruginosa* and 3.5% are infected with *Burkholderia cepacia* complex [1]. Especially *P. aeruginosa* often grows in mixed bacterial flora with anaerobic bacteria like *Prevotella* spp., *Veillonella* spp., and *Propionibacterium* spp. that can aggravate the inflammatory reaction [4]. Further bacterial species de-

scribed in association with severe deterioration of respiratory symptoms in CF patients are *Pandora* spp. [5, 6] and multi-drug resistant *Inquilinus limosus* [7–9]. However, also other less common bacteria, non-tuberculous mycobacteria, fungi and last but not least viruses regularly threaten the health of CF patients [1].

The absence of fever, neutrophilia, and systemic symptoms suggests that non-bacterial factors play an important role for exacerbations of these bacterial pulmonary infections [10]. Some authors have suggested respiratory viruses as main suspects [2]. This review deals with virus-triggered infections in patients suffering from CF.

Viral respiratory infections in cystic fibrosis patients

Viral respiratory infections play an important role in the deterioration of lung function of CF patients [11, 12] and produce severe respiratory morbidity in children with CF [13] (see Table 1). Respiratory illnesses occur significantly more often in CF patients than in the genetically healthy control

*These authors contributed equally to this work.

**These authors contributed equally to this work.

***Corresponding author: Andreas Erich Zautner, M.D.; Institut für Medizinische Mikrobiologie, Universitätsmedizin Göttingen, Kreuzberggring 57, D-37075 Göttingen, Germany; Phone: +49-551-39-5758; Fax: +49-551-39-5861; E-mail: azautne@gwdg.de

subjects, although the frequency of proven viral infections is identical [14]. School-aged patients with CF are not significantly more susceptible to viral infections than genetically healthy controls. However, the majority of hospital admissions for pulmonary exacerbations of CF patients are preceded by viral infections. Respiratory viruses are important causes of hospitalization in infants suffering from CF and are associated with exacerbations in CF. In particular rhinovirus, influenza A and influenza B virus and can be often detected in the course of respiratory exacerbations [15].

Viral respiratory infections show pronounced and long-lasting effects on patients with CF, resulting in significant declines in FVC (forced vital capacity), FEV-1 (forced expiratory volume in 1 second) and significant increases of both the frequency and duration of hospitalizations [16]. Especially the frequency of viral respiratory infections is closely associated with pulmonary deterioration in patients with CF [14]. Significant correlations can be found for the annual incidence of viral infections and the disease progression in patients with CF concerning the percentage of ideal weight for height and the forced mid-expiratory flow rate. Viral respiratory infections are associated with an increase in morbidity at short and long term. They have a greater impact on CF patients compared to non-CF controls and result in increased respiratory symptoms, decline of the Shwachman and radiological scores, increased use of antibiotics and a higher frequency of exacerbations at follow-up [17]. However, the CF patients with the highest frequency of viral infections seem to be the younger ones who have the lowest rate of decline in lung function and in severity score [18]. Obstruction resulting from induced or aggravated reactive airway disease is a factor contributing to the worsening of lung function in patients suffering from viral exacerbations of CF. Anti-obstructive pharmaceutical agents could be helpful under such circumstances [19]. Adults with CF have a lower incidence of respiratory viral infections associated with pulmonary exacerbations requiring intravenous antibiotics compared to children and infants with CF. The presence of viral infection in association with a pulmonary exacerbation do not adversely affect lung function or inflammatory markers in the short term in this population.

The annual incidence of hospital admissions per adult patient associated with viral infection is about 5% [20].

Typical viral infectious agents in CF patients are respiratory syncytial virus (RSV), influenza A and B virus, parainfluenza virus, cytomegalovirus (CMV), human rhinovirus and adenovirus [21]. Deleterious effects on patients with CF have been reported for most viruses studied but the effects of RSV and influenza virus seem to have the severest impact [22]. There is circumstantial evidence that respiratory virus infections may facilitate bacterial infections, particularly *P. aeruginosa* [17, 23]. However, there is only a weak association between viral seroconversion and the isolation of *P. aeruginosa* from sputum [21]. Viral infections do not necessarily precipitate bacterial infection or lead to a change of the colonizing flora in children with CF [24]. In the absence of bacteria, viral infections in CF patients show an acute onset of respiratory distress and an uncomplicated clinical course. While viral infections are often self-limited, admission to hospital is associated with early acquisition of *P. aeruginosa* and persistent respiratory symptoms [22]. New bacterial colonization and increased anti-pseudomonal antibody levels are typical for episodes of viral respiratory infections. Little is known about the interactions between viruses and bacteria in CF lung disease yet [17]. Viral respiratory exacerbations in CF can occur independently from bacterial infections [15]. However, interaction between viruses and bacteria in CF is suggested [17]. The synergistic interaction with bacteria is counteracted by the practice of aggressive antimicrobial therapy [19].

If they are present, upper respiratory symptoms are strong predictors for the presence of viral agents [15]. However, clinical symptoms fail to indicate the type of viral infection having caused symptomatic disease. Thus, routine surveillance for viral infections seems advisable in patients with CF (Table 1) [24].

Methods to diagnose respiratory viruses

The nature and timing of lower respiratory infections in infants with CF is largely unknown because infants usu-

Table 1. Effects of viral infections on the pulmonary status of cystic fibrosis patients

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- Deterioration of lung function
 - Increased respiratory symptoms
 - Increased frequency and duration of hospitalization
 - Declines in FVC and FEV-1
 - Reduced mid-expiratory flow rates
 - Decreased weight for height
 - Decline of the Shwachman and radiological scores
 - Increased use of antibiotics
 - Higher frequency of exacerbations at follow-up
 - Obstruction resulting from induced or aggravated reactive airway disease
 - Facilitation of bacterial infections
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Table 2. Procedures to diagnose respiratory viruses in patients with cystic fibrosis

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- Induction of sputum
 - Performance of broncho-alveolar lavage
 - Performance of (reverse transcription) real-time PCR
 - Immunofluorescence assays (DFA), enzyme immunoassays (EIA), chromatographic and optical immunoassays for RSV and influenza virus
-

ally do not produce sputum and swab cultures taken from the upper respiratory tract may fail to predict lower respiratory tract pathogens [25]. Broncho-alveolar lavage (BAL) is the method of choice to determine lower respiratory tract infection and inflammation in this patient group [25]. It is very difficult to detect viruses in viscous sputum specimens of CF patients even in cases of characteristic sputum production. Multiplex real-time polymerase chain reaction (PCR) assays (in the case of RNA-viruses reverse transcription real-time PCR) combined with colorimetric amplicon detection shows good results in detecting respiratory viruses in the sputa of CF patients. The real-time PCR method carried out on sputum may provide a convenient method of investigating the role of virus infection in respiratory exacerbations of CF patients [26]. The short time-to-result and the potential to facilitate clinical decisions, e.g. concerning the use of anti-viral drugs and administration of antibiotics, are the main advantages of real-time PCR (Table 2) [15]. To date, a wide range of different (reverse transcription) real-time PCR assays have been developed. Table 3 gives a comprehensive overview of the established PCR-assays for the different viruses associated with respiratory infections.

Alternatively, there is a variety of antigen detection assays including direct immunofluorescence assays (DFA), enzyme immunoassays (EIA), chromatographic and optical immunoassays especially for the rapid detection of RSV [41–43] and influenza viruses [42, 44]. The advantages of these tests are their availability and their practicability. Sensitivity and specificity of these tests are usually only sufficient if used during the peak of the virus season. However, the value and reliability of these tests for diagnosis is not completely resolved.

Viral infections of the upper respiratory tract in cystic fibrosis patients

Viral infections of the upper respiratory tract, like common colds show severe effects on lower respiratory morbidity, are associated with pulmonary function abnormalities as well as disease progression and predispose to secondary bacterial infection and colonization in CF [45]. Rhinoviruses and Enteroviruses play an important role in these reversible airway diseases and are detectable with the help of nuclear acid sequence-based amplification [36, 46]. Pulmonary dysfunction is similar following Picornavirus and non-Picornavirus infections [45]. The mean decrease from baseline in forced expiratory volume in one

second (FEV-1) is about –15% and about –10% at 1–4 days and 21–24 days, respectively, after onset of a cold. Children who experience more colds than average regularly show evidence of disease progression with reduction in Shwachman score, increasing Crispin–Norman score and greater deterioration in FEV-1 *per annum* [45].

Human Rhinovirus (HRV) is the most commonly detected virus in children with CF [46, 47] but it seems to have relatively little impact on the course of CF. The acute reduction of FEV-1 due to infections with HRV is relatively low compared with other viral agents [24]. The viruses do not lead to additional deterioration in the clinical status of CF patients. They can be detected in nasopharyngeal aspirates during respiratory exacerbations. Detection of HRV at a high viral load is associated with severe upper and lower respiratory tract infections. In contrast, HRVs may represent only bystander viruses if detected at a low to medium viral load [35]. However, they are regularly associated with prolonged applications of intravenous antibiotics (Table 4) [49].

Human Coronaviruses (HCoV) seem to have comparably little impact on the course of CF in children, similar to HRV. There were broadly varying prevalence data for HCoV in children with CF. In one study, HCoV was the second most prevalent respiratory virus in a 6-month winter period after HRV. These prevalence data were comparable to a cohort of age-matched healthy children [46]. Other studies, performed during a whole year period including the summer season, detected HCoV only at a percentage of 0.8%. This indicates a merely marginal role of this virus in CF patients [47].

The clinical importance of infections with the parainfluenza virus types 1–4 (HPIV 1–4) in CF patients is not well described. Prevalence rates in CF patients vary from 0.3% to 17% [47–50]. HPIV are predominantly detected in upper airway samples [47]. Their influence on an exacerbation of CF should be considered as low.

Viral infections of the lower respiratory tract in cystic fibrosis patients

CF patients are four times more likely to develop a lower respiratory tract infection (LRTI) compared with genetically healthy control subjects [50]. Patients, suffering from CF incurring respiratory virus infections, are at significant risk for LRTI, for hospitalization and for deterioration in lung function that persists months after acute illness [50].

Respiratory syncytial virus (RSV) is an important cause of early acute respiratory tract morbidity in young

Table 3. PCR primer and RT-PCR probe sequences for the detection of respiratory viruses

Virus	Target gene	Amplicon size (bp)	Oligonucleotide function	Oligonucleotide sequence	Reference
Human Adenovirus A (hAdV A)	Hexon group A	74	Forward primer A-F	CCGGKCTGGTGTCAATTCG	[27]
			Reverse primer A-R1	CGATCCACGGGCACAAA	
			Probe	CCACGGACACCTACTTCACCCCTGGG	
Human Adenovirus B (hAdV B)	Hexon group B	72	Forward primer B-1	TGGACATGACYTTTGAGGTGGAT	[27]
			Reverse primer B-2	CGTCGAARACTTCGAARAGAAGA	
			Probe B	CCATGGATGAGCCACCCCTGCTTT	
Human Adenovirus C (hAdV C)	Penton group Cw	78	Forward primer C-F	TCGACACCACCCGTGTGTAC	[27]
			Reverse primer C-R	TGCTGTGGTCGTTCTGTGTAGTT	
			Probe C	TGGACAACAAGTCAACGGATGTGGCA	
Human Adenovirus D (hAdV D)	VA RNA	143	Forward primer D-F	AAAAACGAAAGCGGTTGAGC	[28]
			Reverse primer D-R	CGGGTCGAGACGGGAGT	
			Probe D	FAM-CCAATACCACGTTAGTCGCGGCT-TAMRA	
Human Adenovirus E (hAdV E)	Hexon group E	79	Forward primer E-1	TTAACCAACCACCGCAATGC	[27]
			Reverse primer E-2	TGGATGTGGAATGGCACGTA	
			Probe E	TACCGCTCCATGCTCCTGGGCA	
Human Adenovirus F (hAdV F)	Hexon group F	75	Forward primer F-1	TGTTYGAAAGTTTTTCGACGYGT	[27]
			Reverse primer F-2	SAGGTAGACGGCCTCGATGA	
			Probe F	CGCATCCACCAGCCSCACC	
Human Bocavirus (HBoV)	NS1	88	Forward primer	TGCAGACAACGCYTAGTTGTTT	[29]
			Reverse primer	CTGTCCCGCCCAAGATACA	
			Probe	CCAGGATTGGGTGGAACCTGCAAA	
NP-1	NP-1	81	Forward primer	AGAGGCTCGGGCTCATATCA	[29]
			Reverse primer	CAC TTGGTCTGAGGTCTTCGAA	
			Probe	AGGAACACCCAATCACCACCTATCGTCT	

Table 3. Continued

Virus	Target gene	Amplicon size (bp)	Oligonucleotide function	Oligonucleotide sequence	Reference
Human Coronavirus (HCoV) group 1 (229E and NL63)	Polymerase 1b	≈85	Forward primer F2	TTTATGGTGGTTGGAAATAATATGTTG	[30]
			Reverse primer R2	GGCAAAGCTCTATCACATTTGG	
			Probe P1	ATAATCCCAACCCATRAG	
		≈85	Forward primer F3	TGGCGGGTGGGATAAATATGT	[30]
			Reverse primer R3	GAGGGCATAGCTCTATCACACTTAGG	
Human Coronavirus (HCoV) group 2 (OC43 and HKU1)	Polymerase 1b	≈85	Probe P2	ATAGTCCCATCCCATCAA	[30]
			Forward primer F1	TGGTGGCTGGGACGATATGT	
			Reverse primer R1	GGCATAGCACGATCACACTTAGG	
		≈85	Probe P1	ATAATCCCAACCCATRAG	[30]
			Forward primer F-OC	CCTTATTAAAGATGTTGACAAATCCTGTAC	
Human Herpesviruses		≈85	Reverse primer R-OC	AATACGTAGTAGGTTTGGCATAGCAC	[30]
			Probe P-OC	CACACTTAGGATAGTCCCA	
		76	Forward primer	AACTCAGCCTTCCCTAAGACCA	[28]
			Reverse primer	GGGAGCACTGAGGCAAGTTC	
		74	Probe	FAM-CAATGGCTGCAGTCAGGCCATGG-TAMRA	[28]
Epstein-Barr virus (EBV)	BNT p143	74	Forward primer	GGAACCTGGTCATCCTTTGC	[28]
			Reverse primer	ACGTGCATGGACCGGTTAAT	
			Probe	FAM-CGCAGGCACTCGTACTGCTCGCT(AS)-TAMRA	
		166	Forward primer	TTCTCGTTCCCTCACCTGCCCTCCC	[28]
			Reverse primer	GCAGGCACACGTAAACGCACGCT	
Herpes simplex virus type 1 (HSV-1)	US 4 gene	71	Probe	FAM-CGTCTGGACCAACCGCCACACAGGT(AS)-TAMRA	[28]
			Forward primer	CGCCAAAATACGCCTTAGCA	
			Reverse primer	GAAGGTTCTTCCCGGGAAT	
		71	Probe	FAM-CTCGCTTAAGATGGCCGATCCCAATC-TAMRA	[28]

Table 3. Continued

Virus	Target gene	Amplicon size (bp)	Oligonucleotide function	Oligonucleotide sequence	Reference
Varicella zoster virus (VZV)	ORF 38	82	Forward primer	AAGTTCCCCCGTTTCGC	[28]
			Reverse primer	TGGACTTGAAGATGAACCTTAATGAAGC	
			Probe	FAM-CCGCAACAACACTGCAGTATATATCGTCTCA-TAMRA	
Influenza (IFV) A virus (generic)	AMsw	99	Forward primer M+25	AGATGAGTCTTCTAACCGAGGTCG	[31, 32]
			Reverse primer M-124BB	CCWGC AAARACATCYTCAAGTYTCTG	
			Reverse primer M-124sw	CTGCAAAGACACTTTCAGTCTCTG	
			Probe M+64 MGB	FAM-TCAGGCCCCCTCAA-MGBNFQ	
Seasonal influenza (IFV) A/H1 virus	HA1	127	Forward primer FluA H1 F832	GGATCAGGAATCATCAMYTCAAATGC	[31]
			Reverse primer FluA H1 R959	GGACACTCTCCTATTGTGACTGGGTG	
			Probe FluA H1 MGB914	FAM-CTGCTGTTTATAGCTCC-MGBNFQ	
			Forward primer H3F-162	TCCTCATCAGATCCTTGATG	
Seasonal influenza (IFV) A/H3 virus	HA3	129	Reverse primer H3R-291	ACAGTTGCTGTAGGCTTTGC	[31]
			Probe H3S-284	FAM-CTCTATTGGGRGACCC-MGBNFQ	
			Forward primer FluSw H1 F236	TGGGAATCCAGAGTGTGAATCACT	
Pandemic influenza (IFV) A/H1v virus	HA1v	82	Reverse primer FluSw H1 R318	CGTTCCATTGCTCTGAAC TAGTGTT	[31]
			Probe FluSw H1 TM292	FAM-CCACAATGTAGGACCATGAGCTTGCTGT-BHQ1	
			Forward primer N1P-1078 AN	AYGGYAATGGTGTGTGGATMGGRAG	
Seasonal influenza (IFV) A/N1 virus	NA1	160	Reverse primer N1P-160 bp	ARCTYCCRCCTRTAYCCHGACCARTCRGT	[31]
			Probe N1S MGB	FAM-TCCAYCCRTTRGGRTCCCAA-MGBNFQ	
			Forward primer N2P-P-769 AN	GATACTAAAAATACTATTTCATTGAGGAGG	
Seasonal influenza (IFV) A/N2 virus	NA2	165	Reverse primer N2P 934 AN	ATATCTACDATGGGCCTATTGGAGC	[31]
			Probe N2-S-840 MGB	FAM-CAYTCCTCGACATGCTG-MGBNFQ	
			Forward primer N1 F1255	AGACCTTGCTTGTGGGTTGAAC	
Pandemic influenza (IFV) A/N1v virus	NA1	79	Reverse primer N1 R1334	AAGGATATGCTGCTCCRCCTAGT	[31]
			Probe N1 TM1310	FAM-CAGATTGTGTTCTCTTTGGGTGCGCCCT-BHQ1	

Table 3. Continued

Virus	Target gene	Amplicon size (bp)	Oligonucleotide function	Oligonucleotide sequence	Reference
Avian influenza (IFV) A/H5 virus	HA5	229	Forward primer H5+1456	ACGTATGACTATCCACAATACTCAG	[32, 33]
			Reverse primer H5-1685	AGACCAGCTACCATGATTGC	
			Probe H5+1637	FAM-TCAAACAGTGGCGAGTTCCTAGCA-TAMRA	
Avian influenza (IFV) A/H7 virus	HA5	98	Forward primer H7+1244	ATTGGACACGAGACGCAATG	[32, 33]
			Reverse primer H7-1342	TTCTGAGTCCGCAAGATCTATTG	
			Probe H7+1281	FAM-TAATGCTGAGCTGTGTGGCA-TAMRA	
Influenza (IFV) B virus (generic)	BM	89	Forward primer BMP-13	GAGACACAATTGCCTACCTGC	[31]
			Reverse primer BMP-102AN	TTCCCACCRAACCARCARTGTAAT	
			Probe BMP-72 MGB	FAM-CTGCTTTGGCCTTCTC-MGBNFQ	
Human metapneumo-virus (HMPV) subtype A	Fusion protein	69	Forward primer A	GCCGTTAGCTTCAGTCAATTCAA	[34]
			Reverse primer A	TCCAGCATTTGTCTGAAAAATTGC	
			Probe A	FAM-CAACATTTAGAAAACCTTCT-MGBNFQ	
Human metapneumo-virus (HMPV) subtype B	Fusion protein	74	Forward primer B	GCTGTCAGCTTCAGTCAATTCAA	[34]
			Reverse primer B	GTTATCCCTGCATTGCTGTGAAAACT	
			Probe B	FAM-CGCACAAACATTTAGGAATCTTCT-MGBNFQ	
Human parainfluenza virus type 1 (HPIV-1)	Polymerase	84	Forward primer	ACAGATGAAATTTTCAAAGTGCTACTTTAGT	[27]
			Reverse primer	GCCTCTTTTAAATGCCATATTATCATTAGA	
			Probe	ATGGTAATAAATCGACTCGCT	
Human parainfluenza virus type 2 (HPIV-2)	Polymerase	78	Forward primer	TGCATGTTTATACTACTGATCTTGCTAA	[27]
			Reverse primer	GTTTCGAGCAAAATGGATTATGGT	
			Probe	ACTGTCTTCAATGGAGATAT	
Human parainfluenza virus type 3 (HPIV-3)	Matrix	66	Forward primer	TGCTGTTTCGATGCCAACAA	[27]
			Reverse primer	ATTTTATGCTCCTATCTAGTGGAAAGACA	
			Probe	TTGCTCTTGCTCCTCA	
Human parainfluenza virus type 4 (HPIV-4)	Phosphoprotein	246	Forward primer LPW 1778	AAAGAATTAGGTGCAACCAAGTC	[35]
			Reverse primer LPW 1779	GTGTCTGATCCCAATAAGCAGC	

Table 3. Continued

Virus	Target gene	Amplicon size (bp)	Oligonucleotide function	Oligonucleotide sequence	Reference
Picorna-/Enterovirus (EV)1	5'-noncoding region	392	Forward primer	GCACTTCTGTTCCTCC	[36]
			Reverse primer	CGGACACCCCAAAGTAG	
			Probe	AAGTAGTCGGTTCCGC	
Respiratory syncytial virus (RSV) consensus types A + B	Matrix	80 (RSV-A) 81 (RSV-B)	Forward primer RSV	GGAAACATACGTGAACAAGCTTCA	[37]
			Reverse primer A	CATCGTCTTTTCTAAAGACATTGTATTGA	
			Reverse primer B	TCATCATCTTTTCTAGAAACATTGTACTGA	
			RSV probe	FAM-TGTTATGTGGAGCCTT-MGBNFQ	
			EXO forward primer	GCCTGGTGCAAAAATTGCTT	
Respiratory syncytial virus subtype A (RSV-A)	External control (jellyfish)	130	EXO reverse primer	TCGTTCAATTTGTTCTTTTGTGGAA	[38]
			EXO probe	VIC-CAGCTATTGCAACGCCAT-MGBNFQ	
			Forward primer	AATACAGCCAAATCTAACCAACTTTACA	
Respiratory syncytial virus subtype B (RSV-B)	Polymerase	94	Reverse primer	GCCAAAGGAAGCATGCAATAAA	[38]
			Probe A	FAM-TGCTATTGTGCACTAAAG-MGBNFQ	
			Forward primer	AATACAGCCAAATCTAACCAACTTTACA	
			Reverse primer	GCCAAAGGAAGCATGCAATAAA	
			Probe B	VIC-CACTATTCTCTTACTAAAGATGTC-MGBNFQ	
Human Rhinovirus (HRV)	5'-noncoding region	207	Forward primer	CPXGCCZGCGTGCG	[39]
			Reverse primer	GAAACACGGACACCCCAAAGTA	
			Probe	TCCTCCGGCCCTGAATGYGGC	

¹ Further subtyping of the several members of the genus Enterovirus can be archived using the protocol of W.A. Nix [40].

Abbreviations: FAM: 6-carboxyfluorescein; TAMRA: 6-carboxytetramethylrhodamine; MGB: Molecular-Groove Binding Non-fluorescence Quencher; BHQ1: 3'-terminaler BlackHole1™ Dark Quencher

infants with CF and frequently causes hospitalization for acute pulmonary exacerbations in these patients [51]. Nearly 50% of CF patients with RSV infection during the respiratory virus season (October to March) require hospitalization. Reduced post-season maximal flow and functional residual capacity of CF patients is associated with LRTIs induced by RSV especially in male individuals. LRTIs due to RSV that are treated by day care can lead to an increased gas trapping [50]. Hospitalization is prolonged and characterized by significant morbidity. Mechanical ventilation and home oxygen therapy for persistent hypoxemia at discharge can become necessary. CF patients with a history of RSV infections show chronic respiratory signs and lower chest radiograph scores more frequently than other young CF patients [51]. Prophylaxis with palivizumab lowers the hospitalization rate for RSV infection in adult populations at risk of severe infection. There are studies giving hint that CF infants may benefit from RSV prophylaxis with palivizumab as well [52, 53], whereas other authors reported no clinically meaningful differences in outcomes between the palivizumab and placebo groups [54]. It seems that the severity of pre-existing medical disorders makes the patients generally more susceptible to respiratory infections and RSV prophylaxis with palivizumab may modulate the degree of illness [55].

The antiviral drug ribavirin is approved for treatment of severe RSV disease, for example for treatment of RSV infections in stem cell or bone marrow transplant recipients in combination with parenteral immunoglobulins [56]. However, only a marginal clinical benefit has been shown, and it is therefore not routinely indicated for the treatment of RSV disease [57, 58].

The immunosuppressive agent leflunomide was shown to reduce pulmonary viral loads in an animal model using RSV-inoculated cotton rats [59]. These experimental data promise that orally administered leflunomide could be an alternative antiviral therapeutic for RSV infections. While specific antiviral mechanisms have not been elucidated so far, the favorable effects of leflunomide are obviously due to its well-documented anti-inflammatory activity besides its potential to reduce viral load [59]. Further studies investigating the outcome of RSV infections under leflunomide therapy are needed.

Purified fusion protein (PFP) vaccines against RSV were developed as therapeutic approaches for RSV-seropositive children with CF. An efficient use of the PFP-2 vaccine against lower respiratory tract illness during the RSV season was demonstrated in 1996 [60]. Sequential annual PFP-2 vaccination appeared to be safe and not associated with exaggerated respiratory disease [61]. A third generation, purified fusion protein (PFP-3) vaccine was developed to prevent severe respiratory syncytial virus disease in high-risk groups like CF patients. The PFP-3 vaccine induced a robust immune response that lasted throughout the RSV season. RSV-specific, neutralizing antibody and binding antibody to the fusion protein were significantly increased in PFP-3 vaccinees. However, the titres declined slowly after 28 days [62].

The infection rates, epidemiology and clinical impact observed for human metapneumovirus are comparable to that for RSV in CF children 7–18 years of age. Infection intervals are typically associated with LRTI while hospitalization rates are not affected [63]. Incidence of human metapneumovirus infections among respiratory infections is 5–12% [64].

Infections with influenza A virus in CF patients are associated with acute respiratory deterioration and are often preventable by the annual flu vaccination [65, 66]. Influenza significantly increases the incidence of hospitalization and of less serious respiratory illness in children with CF [67]. However, influenza can also lead to severe exacerbations in CF patients [66, 68]. Influenza virus infections cause worsening of lung function, disease progression, and increase the propensity to bacterial infections in CF. CF patients with influenza have lower Shwachman scores and are more likely to present elevated C-reactive protein levels than CF patients suffering from other non-bacterial infections. They also have higher mean decreases in forced expiratory volume per second and in forced expiratory flow in first 25% of vital capacity [15].

Immunization against influenza A virus is relatively safe and induces an adequate antibody response [69]. Patients with CF should be offered immunization at the beginning of each influenza season. The successful use of both split-virion influenza vaccines and sub-unit vaccines in CF patients was already described in 1987 with satisfying serological responses [70]. Influenza A virus cold-adapted vaccines appeared to be safe, immunogenic alternatives to influenza A inactivated intramuscular trivalent vaccines for CF patients and their families [71]. Both virosome and subunit vaccines induced an efficient immune response in CF patients and were well tolerated by children and adolescents with CF [72].

In summary, influenza vaccinations induce a satisfactory serological antibody response. The incidence of adverse effects are associated with the respective type of influenza vaccine with relatively low rates of adverse effects for the intranasal live vaccine and higher rates for split virus vaccines. However, these events are not severe [73]. Influenza vaccination is generally recommended to patients with CF although the currently available evidence to support routine influenza vaccination is limited in CF. There are hints that the current vaccination practice may play a role in the prevention of subsequent acquisition of influenza (*Table 4*) [69].

Rapid diagnostic tests and the use of antiviral drugs play an additional prophylactic role in minimizing lung damage. In the 1970s, amantadine-HCl — an antiviral drug clinically effective against most strains of influenza A virus with a lot of side effects — was applied to patients suffering from CF [74]. Nowadays, the neuraminidase inhibitors zanamivir and oseltamivir are the antiviral agents of choice in the management and prophylaxis of influenza in CF patients [75]. Pharmacokinetical studies suggest that a unitary dose of oseltamivir higher than the presently recommended dose of 75 mg for patients without CF is necessary for CF

Table 4. Viral infectious agents affecting the lung function in patients with cystic fibrosis

Viral infectious agents of the upper respiratory tract	Viral infectious agents of the lower respiratory tract	Further viral agents affecting the lung function in cystic fibrosis patients
Human Adenovirus (HAdV) 1, 2, 5 (C); 3, 7 (B)	Adenovirus (HAdV) 4 (E); 7, 14, 21 (B)	Cytomegalovirus (CMV)
Human Coronavirus (hCoV) 1/2	Human Bocavirus (HBoV) 1	Epstein–Barr virus (EBV)
Enterovirus (EV)	Influenza A/B virus (IFV-A/B)	Herpes-simplex-Virus (HSV) 1/2
Parainfluenza virus (HPIV) 1–4	Human metapneumovirus (HMPV)	Varicella zoster virus (VZV)
Human Rhinovirus (HRV)	Respiratory syncytial virus (RSV)	

patients most probably because CF is associated with an increase in the glomerular filtration rate and in the lean body weight/total body weight ratio [76]. An appropriate dosing regimen has still to be determined in future studies.

The newly described human bocavirus-1 (HBoV) was associated with a variety of signs and symptoms like rhinitis, pharyngitis, cough, dyspnoea, wheezing, pneumonia, otitis media, and fever [77]. Since many of these potential manifestations have not been systematically explored and high co-infection rates with other pathogens were recorded, it is unclear to date whether this virus is a pure bystander or a pathogenic agent. Up to 15.5% of nasopharyngeal swab samples of children with respiratory symptoms were tested positive for HBoV-1 [78], and life-threatening cases were reported [79]. Thus, it should be considered as a potential cause of respiratory tract illness. However, its role for deterioration of lung function of CF patients has still to be examined.

Non-respiratory viruses affecting the lung function in CF patients: The human herpes virus group

Primary Epstein–Barr virus (EBV) infection can be associated with severe pulmonary exacerbations and subsequent deterioration in the clinical course of CF [80]. EBV in adolescent CF patients regularly leads to weight loss, lower pulmonary function tests and lower clinical scores compared with controls. These differences are still detectable six months after the diagnosis of the infection. Frequency of exacerbations requiring hospitalization increases after EBV infections [80].

Varicella zoster virus (VZV) infections can result in infective pulmonary exacerbations of CF even in adult patients requiring intravenous acyclovir and additional antibiotic treatment. Early treatment with acyclovir in combination with appropriate antibiotics is likely to prevent pulmonary deterioration in such cases [81]. VZV vaccination is recommended for seronegative adolescents as prophylaxis procedure [82].

Viral infections with agents of the herpes virus group are described to be associated with severe intra-uterine and

peri-partum pathologies in fetuses and newborns with CF. Congenital cytomegalovirus (CMV) infections can be associated with malformations like bile duct obstruction in newborn infants with a pre-dominantly pancreatic CF manifestation [83]. CF with pulmonary and meconial syndrome combined with generalized herpes including medulla oblongata involvement can cause the death of newborns due to an obturation of the respiratory routes with pathologic bronchial secretion and parasitic exudative mass (Table 4) [84].

Aspects of the inflammatory and immune response to viral infections in cystic fibrosis patients

The lower airway inflammation that characterizes CF is not primarily initiated by the genetic defect. Infection initiates and sustains airway inflammation in CF patients [85]. Viral infection is the primary cause of respiratory morbidity in infants suffering from CF [86]. Already within the first 3 months of life lower respiratory infections are present in almost 40% of infants with CF. More than one third is symptom free [25]. Increased virus replication, impaired specific anti-bacterial defence and increased adherence of bacteria to the mucous membrane play a role in the pathogenesis of viral respiratory infections in CF [17].

Host factors allow increased virus replication and cytokine production (interleukin-6 and -8) [87] supporting the severity of virus disease in CF. Increased virus load appears as a consequence of lacking nitric oxide synthase 2 (NOS2) and 2', 5' oligoadenylate synthetase (OAS) 1 induction in response to virus or IFN-gamma. This can be attributed to impaired activation of signal transducer and activator of transcription 1 (STAT 1), a fundamental component of antiviral defence. NO donor or NOS2 overexpression provide protection from virus infection in CF, suggesting that NO supports the antiviral host defence in the human airway [86].

CF cells, epithelial cells expressing a mutant dysfunctional CFTR that have an exaggerated activation of the transcriptional regulatory complex nuclear factor (NF)-κB, and in consequence an increased production

of proinflammatory cytokines [88], are more susceptible to viral agents [13]. A lesser antiviral and greater early inflammatory response is likely to contribute to severe respiratory illnesses of CF patients with viral infections. There are multiple alterations in the antiviral defence of CF epithelium compared with normal cells. Gene expression is significantly modified by influenza virus in both normal cells and CF cells, with a similar pattern of gene response but with overall less numbers of responsive genes in CF. CF cells show less IFN-gamma-related antiviral gene induction but increased inflammatory cytokine gene induction after infection [13].

Antigen-antibody complex mediated activation of complement may occur in CF patients with viral lower respiratory tract infections (LRTI). CF patients without clinical evidence of immune complex disease suffering from LRTI express depressed levels of the third and fourth complement components, which return to normal values after recovery. CF patients with LRTI without viral infection, CF patients in a stable clinical state and non-CF patients with LRTI do not show complement depression [89]. Compared to non-infected CF patients and controls, virus-infected CF patients have elevated BAL inflammatory indices (neutrophils, elastase activity, interleukin-1, -6, -8, -10 and IFN-alpha) [22, 85]. The presence of pathogens in the lower airways correlates with levels of inflammation, compliance of the respiratory system and degree of air trapping [90]. Airway inflammation follows respiratory infection and improves when pathogens are eradicated from the airways, especially in young children. Newly diagnosed infants who suffer from CF without infection show BAL profiles comparable with control subjects. In older children, the development and persistence of infection is accompanied by increased inflammatory markers, whereas these markers are decreased in the absence or with the clearance of infection (*Table 5*) [85].

Mimicry-like effects in cystic fibrosis

CF is able to mimic viral infections of the liver. There are reports about children with non-diagnosed CF followed up

for many years with an original diagnosis of non-A/non-B hepatitis. The development of serious cirrhosis with portal hypertension due to CF is possible. CF — though rarely presenting with initial hepatic signs — can manifest with long-term hepatic symptoms. Analysis of sweat chloride concentration in cases of hepatic disease of unknown origin is strongly recommended [91].

Adenovirus — both harmful agent and tool for viral somatic gene therapy in cystic fibrosis patients

Adenovirus infections occur regularly in CF patients. IgM antibodies against adenovirus can be found in more than 50% and IgG antibodies even in more than 95% of CF patients [92]. They are significantly higher in CF patients older than seven years than in younger patients and in age-matched controls. High increases of antibody titers can be associated with chronic decreases of FVC and FEV-1. Wild-type adenovirus infections are prevalent in CF patients and seem to be associated with deteriorations of lung function [92].

Clinical trials were performed using replication-deficient adenoviruses as vectors for gene transfer into the airways of CF patients [92]. The aim of somatic gene therapy is the delivery of the normal allele directly to the respiratory epithelium using Ela-adenovirus type 2- or 5-based vectors. For safety reasons, the adenovirus vectors are rendered replication-deficient by deletion of the Ela region. However, CF patients may have detectable Ela sequences in the respiratory epithelium with the theoretical potential of such sequences to support the replication of Ela-depleted adenovirus vectors so Ela-screenings are unavoidable prior to gene therapy (*Table 6*) [93]. The host immune response and low vector efficiency are impediments to effective CF transmembrane regulator (CFTR) gene transfer in patients with CF [94]. CF children show a normal antibody response after adenovirus infection. Pre-existing antibodies may protect against reinfection with negative effects to somatic gene therapy [95]. Some serous

Table 5. Immunological and inflammatory factors contributing to viral infections in patients with cystic fibrosis

– Increased virus replication
– Impaired specific anti-bacterial defence
– Increased adherence of bacteria
– Lack of nitric oxide synthase 2 and 2', 5' oligoadenylate synthetase 1
– Impairment of activation of signal transducer and activator of transcription 1
– Less IFN-gamma related antiviral gene induction after viral infection
– Increased inflammatory cytokine gene induction (IL-6 and -8) after viral infection
– Depressed levels of the third and fourth complement components during viral infections
– Elevation of BAL inflammatory indices in broncho-alveolar lavage (BAL)

Table 6. Limitations and risks of viral somatic gene therapy with adenoviruses in patients with cystic fibrosis

- Pre-existing antibodies after previous adenovirus infections
- Low vector efficiency due to poorly susceptible submucosal tracheal gland cells
- E1a sequences in the respiratory epithelium with the potential to support the replication of E1a-depleted adenovirus vectors

cell lines from submucosal tracheal glands are poorly susceptible to adenovirus infection and adenovirus-mediated gene transduction (Table 6). The major limiting steps apparently include the primary events of interaction like cell attachment and entry due to missing cellular receptors. Re-targeting of the adenovirus vectors to other receptors is necessary under such conditions to achieve a higher transducing efficiency [96].

Although first generation recombinant adenoviruses with deleted E1a and E1b genes, have been useful for *in vivo* applications of gene therapy, expression of the recombinant gene has been transient and often associated with the development of inflammation. First-generation adenovirus-mediated gene transfer to mouse lungs induced an expression of viral proteins leading to destructive cellular immune responses and repopulation of the lung with non-transgenic cells [97]. Second-generation E1-deleted viruses that were further crippled by a temperature-sensitive mutation in the E2a gene were associated with a substantially longer expression of the recombinant allele and less inflammation. Stable expression of human CF transmembrane conductance regulator was achieved in lungs of CF mice instilled with second-generation vectors [97]. Recombinant adeno-associated serotype 2-based vectors (rAAV2) were useful for CF gene therapy because they elicited little or no inflammatory response and resulted generally in a stable expression of the transferred allele [98]. Adeno-associated virus vector (AAV-CFTR) administration to the maxillary sinus led to a successful, dose-dependent allele transfer to the maxillary sinus and alterations in the transepithelial potential difference. These changes were regarded as suggestive for a functional effect. Little or no cytopathic host immune response was observed [98].

Adenovirus-based somatic gene therapy is a fast developing technology for a causal therapy of CF. Further research is still necessary to ensure the safe and efficient future use of this new therapeutic option.

Summary

A wide spectrum of viruses was demonstrated to a role in the deterioration of lung function of CF patients play to varying degrees (see Table 4). This deterioration of lung function is mostly due to a virus-triggered exacerbation of bacterial lower respiratory tract infections. The traditional approach is focused on the treatment of acute pulmonary exacerbations of CF patients with intravenous antimicrobial agents. However, prophylactic strategies to prevent initial infection or to delay chronic infection with *P. aeruginosa* or else chronic maintenance therapy to

slow deterioration of lung function may also improve the clinical status [1]. Recognition of the role of inflammation, even early in life and in the absence of clinical symptoms, encouraged the treatment with anti-inflammatory agents. Novel strategies include the disruption of biofilm formation, the stimulation of chloride conductance, and the replacing of the abnormal CFTR [1]. More efficient approaches to avoid respiratory viral infections should become more important in future therapeutic strategies. At present, influenza vaccination and chemoprophylaxis with neuraminidase inhibitors as well as RSV prophylaxis with palivizumab are the only practicable procedures in the management of respiratory virus infections in CF patients, apart from mere — mostly PCR-based — diagnostics.

Only few data illustrate the relationship of respiratory viruses and CF yet. By gaining further knowledge of this relationship, future clinical practice could be changed to boost the survival of these patients [2, 19]. Further knowledge about the interaction of virus infections with bacteria in CF lung disease might result in new therapeutic strategies [17].

References

1. Rajan S, Saiman L: Pulmonary infections in patients with cystic fibrosis. *Semin Respir Infect* 17(1), 47–56 (2002)
2. Wat D: Impact of respiratory viral infections on cystic fibrosis. *Postgrad Med J* 79(930), 201–203 (2003)
3. Abman SH et al.: Early bacteriologic, immunologic, and clinical courses of young infants with cystic fibrosis identified by neonatal screening. *J Pediatr* 119(2), 211–217 (1991)
4. Tunney MM et al.: Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. *Am J Respir Crit Care Med* 177, 995–1001 (2008)
5. Jørgensen IM et al.: Epidemic spread of *Pandoraea apista*, a new pathogen causing severe lung disease in cystic fibrosis patients. *Pediatr Pulmonol* 36(5), 439–446 (2003)
6. Moore JE et al.: *Pandoraea apista* isolated from a patient with cystic fibrosis: problems associated with laboratory identification. *Br J Biomed* 59(3), 164–166 (2002)
7. Pitulle C et al.: Novel bacterium isolated from a lung transplant patient with cystic fibrosis. *J Clin Microbiol* 37(12), 3851–3855 (1999)
8. Chiron R et al.: Clinical and microbiological features of *Inquilinus* sp. isolates from five patients with cystic fibrosis. *J Clin Microbiol* 43(8), 3938–3943 (2005)
9. Wellinghausen N et al.: *Inquilinus limosus* in patients with cystic fibrosis, Germany. *Emerg Infect Dis* 11(3), 457–459 (2005)
10. Hordvik NL et al.: Effects of acute viral respiratory tract infections in patients with cystic fibrosis. *Pediatr Pulmonol* 7(4), 217–222 (1989)

11. Stroobant J: Viral infection in cystic fibrosis. *J R Soc Med* 79(Suppl 12), 19–22 (1986)
12. Shale DJ: Viral infections: a role in the lung disease of cystic fibrosis? *Thorax* 47(2), 69 (1992)
13. Xu W et al.: Cystic fibrosis and normal human airway epithelial cell response to influenza a viral infection. *J Interferon Cytokine Res* 26(9), 609–627 (2006)
14. Wang EE et al.: Association of respiratory viral infections with pulmonary deterioration in patients with cystic fibrosis. *N Engl J Med* 311(26), 1653–1658 (1984)
15. Wat D et al.: The role of respiratory viruses in cystic fibrosis. *J Cyst Fibros* 7(4), 320–328 (2008)
16. Wat D, Doull I: Respiratory virus infections in cystic fibrosis. *Paediatr Respir Rev* 4(3), 172–177 (2003)
17. van Ewijk BE et al.: Viral respiratory infections in cystic fibrosis. *J Cyst Fibros* 4 (Suppl 2), 31–36 (2005)
18. Ramsey BW et al.: The effect of respiratory viral infections on patients with cystic fibrosis. *Am J Dis Child* 143(6), 662–668 (1989)
19. Prober CG: The impact of respiratory viral infections in patients with cystic fibrosis. *Clin Rev Allergy* 9(1–2), 87–102 (1991)
20. Clifton IJ et al.: Ten years of viral and non-bacterial serology in adults with cystic fibrosis. *Epidemiol Infect* 136(1), 128–134 (2008)
21. Ong EL et al.: Infective respiratory exacerbations in young adults with cystic fibrosis: role of viruses and atypical microorganisms. *Thorax* 44(9), 739–742 (1989)
22. Armstrong D et al.: Severe viral respiratory infections in infants with cystic fibrosis. *Pediatr Pulmonol* 26(6), 371–379 (1998)
23. Freymouth F et al.: Presence of the new human metapneumovirus in French children with bronchiolitis. *Pediatr Infect Dis J* 22(1), 92–94 (2003)
24. Olesen HV et al.: Viral and atypical bacterial infections in the outpatient pediatric cystic fibrosis clinic. *Pediatr Pulmonol* 41(12), 1197–1204 (2006)
25. Armstrong DS et al.: Lower respiratory infection and inflammation in infants with newly diagnosed cystic fibrosis. *BMJ* 310(6994), 1571–1572 (1995)
26. Punch G et al.: Method for detection of respiratory viruses in the sputa of patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 24(1), 54–57 (2005)
27. Kuypers J et al.: Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children. *J Clin Microbiol* 44(7), 2382–2388 (2006)
28. Watzinger F et al.: Real-time quantitative PCR assays for detection and monitoring of pathogenic human viruses in immunosuppressed pediatric patients. *J Clin Microbiol* 42(11), 5189–5198 (2004)
29. Lu X et al.: Real-time PCR assays for detection of bocavirus in human specimens. *J Clin Microbiol* 44(9), 3231–3235 (2006)
30. Kuypers J et al.: Clinical disease in children associated with newly described coronavirus subtypes. *Pediatrics* 119(1), e70–76 (2007)
31. Schulze M et al.: Diagnostic approach for the differentiation of the pandemic influenza A(H1N1)v virus from recent human influenza viruses by real-time PCR. *PLoS One* 5(4), e9966 (2010)
32. Spackman E et al.: Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *J Clin Microbiol* 40(9), 3256–3260 (2002)
33. Lee CW, Suarez DL: Application of real-time RT-PCR for the quantitation and competitive replication study of H5 and H7 subtype avian influenza virus. *J Virol Methods* 119(2), 151–158 (2004)
34. Kuypers J et al.: Detection and quantification of human metapneumovirus in pediatric specimens by real-time RT-PCR. *J Clin Virol* 33(4), 299–305 (2005)
35. Lau SK et al.: Human parainfluenza virus 4 outbreak and the role of diagnostic tests. *J Clin Microbiol* 43(9), 4515–4521 (2005)
36. Pitkäranta A et al.: Detection of rhinovirus in sinus brushings of patients with acute community-acquired sinusitis by reverse transcription-PCR. *J Clin Microbiol* 35(7), 1791–1793 (1997)
37. Nokso-Koivisto J et al.: Human picornavirus and coronavirus RNA in nasopharynx of children without concurrent respiratory symptoms. *J Med Virol* 66(3), 417–420 (2002)
38. Kuypers J et al.: Evaluation of quantitative and type-specific real-time RT-PCR assays for detection of respiratory syncytial virus in respiratory specimens from children. *J Clin Virol* 31(2), 123–129 (2004)
39. Lu X et al.: Real-time reverse transcription-PCR assay for comprehensive detection of human rhinoviruses. *J Clin Microbiol* 46(2), 533–539 (2008)
40. Nix WA et al.: Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. *J Clin Microbiol* 44(8), 2698–2704 (2006)
41. Henrickson KJ, Hall CB: Diagnostic assays for respiratory syncytial virus disease. *Pediatr Infect Dis J* 26(11 Suppl), S36–40 (2007)
42. Principi N, Esposito S: Antigen-based assays for the identification of influenza virus and respiratory syncytial virus: why and how to use them in pediatric practice. *Clin Lab Med* 29(4), 649–660 (2009)
43. Popow-Kraupp T, Aberle JH: Diagnosis of respiratory syncytial virus infection. *Open Microbiol J* 5, 128–134 (2011)
44. Landry ML: Diagnostic tests for influenza infection. *Curr Opin Pediatr* 23(1), 91–97 (2011)
45. Collinson J et al.: Effects of upper respiratory tract infections in patients with cystic fibrosis. *Thorax* 51(11), 1115–1122 (1996)
46. van Ewijk BE et al.: Prevalence and impact of respiratory viral infections in young children with cystic fibrosis: prospective cohort study. *Pediatrics* 122(6), 1171–1176 (2008)
47. Burns JL et al.: Respiratory viruses in children with cystic fibrosis: viral detection and clinical findings. *Influenza Other Respi Viruses*. doi: 10.1111/j.1750–2659.2011.00292.x. [Epub ahead of print] (2011)
48. Gerna G et al.: Correlation of rhinovirus load in the respiratory tract and clinical symptoms in hospitalized immunocompetent and immunocompromised patients. *J Med Virol* 81(8), 1498–1507 (2009)
49. Smyth AR et al.: Effect of respiratory virus infections including rhinovirus on clinical status in cystic fibrosis. *Arch Dis Child* 73(2), 117–120 (1995)
50. Hiatt PW et al.: Effects of viral lower respiratory tract infection on lung function in infants with cystic fibrosis. *Pediatrics* 103(3), 619–626 (1999)
51. Abman SH et al.: Role of respiratory syncytial virus in early hospitalizations for respiratory distress of young infants with cystic fibrosis. *J Pediatr* 113(5), 826–830 (1988)
52. Giebels K et al.: Prophylaxis against respiratory syncytial virus in young children with cystic fibrosis. *Pediatr Pulmonol* 43(2), 169–174 (2008)

53. Shadman KA, Wald ER: A review of palivizumab and emerging therapies for respiratory syncytial virus. *Expert Opin Biol Ther* 11(11), 1455–1467 (2011)
54. Robinson KA et al.: Palivizumab for prophylaxis against respiratory syncytial virus infection in children with cystic fibrosis. *Cochrane Database Syst Rev* 2, CD007743 (2012)
55. Paes B et al.: Respiratory hospitalizations and respiratory syncytial virus prophylaxis in special populations. *Eur J Pediatr*. [Epub ahead of print] (2011)
56. Ghosh S et al.: Respiratory syncytial virus infections in autologous blood and marrow transplant recipients with breast cancer: combination therapy with aerosolized ribavirin and parenteral immunoglobulins. *Bone Marrow Transplant* 28(3), 271–275 (2001)
57. Empey KM et al.: Pharmacologic advances in the treatment and prevention of respiratory syncytial virus. *Clin Infect Dis* 50(9), 1258–1267 (2010)
58. Krilov LR: Respiratory syncytial virus disease: update on treatment and prevention. *Expert Rev Anti Infect Ther* 9(1), 27–32 (2011)
59. Dunn MC et al.: Inhibition of respiratory syncytial virus in vitro and in vivo by the immunosuppressive agent leflunomide. *Antivir Ther* 16(3), 309–317 (2011)
60. Piedra PA et al.: Purified fusion protein vaccine protects against lower respiratory tract illness during respiratory syncytial virus season in children with cystic fibrosis. *Pediatr Infect Dis J* 15(1), 23–31 (1996)
61. Piedra PA et al.: Sequential annual administration of purified fusion protein vaccine against respiratory syncytial virus in children with cystic fibrosis. *Pediatr Infect Dis J* 17(3), 217–224 (1998)
62. Piedra PA et al.: Immunogenicity of a new purified fusion protein vaccine to respiratory syncytial virus: a multi-center trial in children with cystic fibrosis. *Vaccine* 21(19–20), 2448–2460 (2003)
63. Garcia DF et al.: Human metapneumovirus and respiratory syncytial virus infections in older children with cystic fibrosis. *Pediatr Pulmonol* 42(1), 66–74 (2007)
64. van den Hoogen BG et al.: A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 7(6), 719–724 (2001)
65. Conway SP et al.: Acute severe deterioration in cystic fibrosis associated with influenza A virus infection. *Thorax* 47(2), 112–114 (1992)
66. Viviani L et al.: Impact of the A (H1N1) pandemic influenza (season 2009–2010) on patients with cystic fibrosis. *J Cyst Fibros* 10(5) 370–376 (2011)
67. Ferson MJ et al.: Impact of influenza on morbidity in children with cystic fibrosis. *J Paediatr Child Health* 27(5), 308–311 (1991)
68. Pribble CG et al.: Clinical manifestations of exacerbations of cystic fibrosis associated with nonbacterial infections. *J Pediatr* 117(2 Pt 1), 200–204 (1990)
69. Wat D et al.: Is there a role for influenza vaccination in cystic fibrosis? *J Cyst Fibros* 7(1), 85–88 (2008)
70. Adlard P, Bryett K: Influenza immunization in children with cystic fibrosis. *J Int Med Res* 15(6), 344–351 (1987)
71. Gruber WC et al.: Comparison of live attenuated and inactivated influenza vaccines in cystic fibrosis patients and their families: results of a 3-year study. *J Infect Dis* 169(2), 241–247 (1994)
72. Schaad UB et al.: Comparison of immunogenicity and safety of a virosome influenza vaccine with those of a subunit influenza vaccine in pediatric patients with cystic fibrosis. *Antimicrob Agents Chemother* 44(5), 1163–1167 (2000)
73. Tan A et al.: Vaccines for preventing influenza in people with cystic fibrosis. *Cochrane Database Syst Rev* (2), CD001753 (2000)
74. Wright PF et al.: Evaluation of the safety of amantadine-HCl and the role of respiratory viral infections in children with cystic fibrosis. *J Infect Dis* 134(2), 144–149 (1976)
75. Jagannath VA et al.: Neuraminidase inhibitors for the treatment of influenza infection in people with cystic fibrosis. *Cochrane Database Syst Rev* 17(3), CD008139 (2010)
76. Jullien V et al.: Pharmacokinetics and diffusion into sputum of oseltamivir and oseltamivir carboxylate in adults with cystic fibrosis. *Antimicrob Agents Chemother* 55(9), 4183–4187 (2011)
77. Jartti T et al.: Human bocavirus-the first 5 years. *Rev Med Virol* 22(1), 46–64 (2012)
78. Koseki N et al.: Detection of human bocavirus 1-4 from nasopharyngeal swab samples collected from patients with respiratory tract infections. *J Clin Microbiol* [Epub ahead of print] (2012)
79. Edner N et al.: Life-threatening respiratory tract disease with human bocavirus-1 infection in a 4-year-old child. *J Clin Microbiol* 50(2), 531–532 (2012)
80. Winnie GB, Cowan RG: Association of Epstein–Barr virus infection and pulmonary exacerbations in patients with cystic fibrosis. *Pediatr Infect Dis J* 11(9), 722–726 (1992)
81. Ong EL et al.: Varicella-zoster infection in adults with cystic fibrosis: role of acyclovir. *Scand J Infect Dis* 23(3), 283–285 (1991)
82. Malfroot A et al.: Immunisation in the current management of cystic fibrosis patients. *J Cyst Fibros* 4(2), 77–87 (2005)
83. Oppenheimer EH, Esterly JR: Letter: Congenital cytomegalovirus infection and bile duct obstruction in newborn infant with cystic fibrosis of pancreas. *Lancet* 2(7836), 1031–1032 (1973)
84. Kakabadze SA, Tumanov VP: Generalized herpes with mucoviscidosis, pneumocystosis and meconial disease-rare causes of death in newborn infants. *Sud Med Ekspert* 36(2), 8–12 (1993)
85. Armstrong DS et al.: Lower airway inflammation in infants with cystic fibrosis detected by newborn screening. *Pediatr Pulmonol* 40(6), 500–510 (2005)
86. Zheng S et al.: Impaired innate host defense causes susceptibility to respiratory virus infections in cystic fibrosis. *Immunity* 18(5), 619–630 (2003)
87. Stecenko AA et al.: Dysregulated cytokine production in human cystic fibrosis bronchial epithelial cells. *Inflammation* 25(3), 145–155 (2001)
88. Blackwell TS et al.: Dysregulated NF-kappaB activation in cystic fibrosis: evidence for a primary inflammatory disorder. *Am J Physiol Lung Cell Mol Physiol* 281(1), L69–70 (2001)
89. Strunk RC et al.: Serum complement depression during viral lower respiratory tract illness in cystic fibrosis. *Arch Dis Child* 52(9), 687–690 (1977)
90. Dakin CJ et al.: Inflammation, infection, and pulmonary function in infants and young children with cystic fibrosis. *Am J Respir Crit Care Med* 165(7), 904–910 (2002)
91. Resti M et al.: False diagnosis of non-A/non-B hepatitis hiding two cases of cystic fibrosis. *Eur J Pediatr* 150(2), 97–99 (1990)
92. Rosenacker J et al.: Adenovirus infection in cystic fibrosis patients: implications for the use of adenoviral vectors for gene transfer. *Infection* 24(1), 5–8 (1996)

93. Eissa NT et al.: Evaluation of the respiratory epithelium of normals and individuals with cystic fibrosis for the presence of adenovirus E1a sequences relevant to the use of E1a-adenovirus vectors for gene therapy for the respiratory manifestations of cystic fibrosis. *Hum Gene Ther* 5(9), 1105–1114 (1994)
94. Wagner JA et al.: Safety and biological efficacy of an adeno-associated virus vector-cystic fibrosis transmembrane regulator (AAV-CFTR) in the cystic fibrosis maxillary sinus. *Laryngoscope* 109(2 Pt 1), 266–274 (1999)
95. Piedra PA et al.: Incidence and prevalence of neutralizing antibodies to the common adenoviruses in children with cystic fibrosis: implication for gene therapy with adenovirus vectors. *Pediatrics* 101(6), 1013–1019 (1998)
96. Gaden F et al.: Mechanism of restriction of normal and cystic fibrosis transmembrane conductance regulator-deficient human tracheal gland cells to adenovirus infection and ad-mediated gene transfer. *Am J Respir Cell Mol Biol* 27(5), 628–640 (2002)
97. Yang Y et al.: Inactivation of E2a in recombinant adenoviruses improves the prospect for gene therapy in cystic fibrosis. *Nat Genet* 7(3), 362–369 (1994)
98. Flotte TR et al.: Phase I trial of intranasal and endobronchial administration of a recombinant adeno-associated virus serotype 2 (rAAV2)-CFTR vector in adult cystic fibrosis patients: a two-part clinical study. *Hum Gene Ther* 14(11), 1079–1088 (2003)