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COMMUNITY-ACQUIRED PNEUMONIA IN CHILDREN

Aetiology, clinical features, and complications

Maria Hartiala

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1714 | MEDICA – ODONTOLOGICA | TURKU 2023

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University of Turku

Faculty of Medicine
Department of Clinical Medicine
Paediatrics
Doctoral Programme in Clinical Research

Supervised by

Professor Ville Peltola, MD, PhD
Department of Clinical Medicine
Paediatrics, University of Turku
Turku University Hospital
Turku, Finland

Elina Lahti, MD, PhD
Outpatient Clinic for Children and Adolescents
City of Turku Welfare Division
Turku, Finland

Reviewed by

Docent Eliisa Kekäläinen, MD, PhD
Department of Bacteriology and Immunology
University of Helsinki
Helsinki, Finland

Professor Matti Uhari, MD, PhD
Department of Paediatrics
University of Oulu
Oulu, Finland

Opponent

Professor Matti Korppi, MD, PhD
Department of Paediatrics
University of Tampere
Tampere, Finland

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To my family

UNIVERSITY OF TURKU
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ABSTRACT

Pneumonia is an important cause of morbidity and hospitalization in children worldwide. Since the development of nucleic acid amplification techniques, rhinovirus (RV) is frequently detected in community-acquired pneumonia (CAP), but the causative role of RV in pneumonia is still questioned. Empyema is a severe complication of pneumonia, and the evaluation of its long-term consequences is necessary.

We studied the viral aetiology of childhood CAP by searching for 18 respiratory viruses and six bacteria in sputum specimens (n = 76). The clinical characteristics and prevalence of RV pneumonia and its risk factors were evaluated by retrospectively comparing the medical record data of RV-positive (n = 82) and RV-negative (n = 231) children hospitalized for CAP. We also prospectively investigated viral and bacterial biomarker levels in children hospitalized for CAP (n = 24), focusing on RV pneumonia. Finally, we investigated the long-term outcome of childhood parapneumonic empyema (n = 26) at 3–19 years' follow-up by a detailed interview, physical examination, lung imaging and lung function tests.

Viruses were detected in 72%, bacteria in 91%, and both in 66% of children hospitalized for CAP. RV, human bocavirus, and human metapneumovirus were the most commonly found viruses. Treatment failures were documented in viral-bacterial co-infections. Young age and a history of preterm birth were associated with RV-positive pneumonia, but the clinical features of pneumonia were similar in RV-positive and RV-negative children. RV-positive children had elevated levels of bacterial biomarkers, but a viral biomarker myxovirus resistance protein A remained low. Lung magnetic resonance imaging showed abnormal findings in 92% and significant pleural scarring in 25% of the children recovered from empyema, but most patients had normal lung function, chest radiograph and clinical recovery.

Viral-bacterial co-detections are common in childhood CAP and potentially associated with treatment failure. RV is commonly detected in young children with pneumonia and it is often associated with bacterial co-infection. Making the decision to withdraw antibiotics in children with pneumonia is challenging. Further studies and strategies are needed to differentiate viral from bacterial or mixed viral-bacterial pneumonia. The long-term recovery from parapneumonic empyema seems to be good with current treatment strategies.

KEYWORDS: children, empyema, pneumonia, rhinovirus

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TIIVISTELMÄ

Keuhkokuume on maailmanlaajuisesti merkittävä lasten sairastavuuden ja sairaalahoidon aiheuttaja. Rinovirus (RV) on yleinen löydös lasten keuhkokuumeessa, mutta sen rooli keuhkokuumeen aiheuttajana on edelleen epäselvä. Empyeema on keuhkokuumeen vakava komplikaatio, jonka pitkäaikaisvaikutusten selvittäminen on tärkeää.

Selvitimme lasten avosyntyisen keuhkokuumeen virusetiologiaa tutkimalla 18 viruksen ja kuuden bakteerin esiintyvyyttä yskösnäytteistä (n = 76). RV-keuhkokuumeen taudinkuvaa, esiintyvyyttä ja riskitekijöitä tutkittiin vertailemalla retrospektiivisesti keuhkokuumeen vuoksi sairaalahoitoon joutuneiden RV-positiivisten (n = 82) ja RV-negatiivisten (n = 231) lasten potilaskertomustietoja. Tutkimme myös virus- ja bakteerimerkkiaineiden tasoja lasten sairaalahoitoa vaativassa keuhkokuumeessa (n = 24), erityisesti RV-keuhkokuumeessa. Lasten empyeeman pitkäaikaisvaikutuksia selvitettiin 3–19 vuoden kuluttua sairastamisesta kliinisellä tutkimuksella sekä keuhkojen toimintakokeilla ja kuvantamisella (n = 26).

Viruksia löydettiin 72 %:lta, bakteereja 91 %:lta ja molempia 66 %:lta lapsista, jotka tarvitsivat sairaalahoitoa keuhkokuumeen vuoksi. RV, bokavirus ja metapneumovirus olivat yleisimmin löydetyt virukset. Hoidon epäonnistumista havaittiin virus-bakteerisekainfektioissa. Lapsen nuori ikä ja keskosuustausta olivat yhteydessä RV-positiiviseen keuhkokuumeeseen, mutta keuhkokuumeen taudinkuva oli samankaltainen RV-positiivisilla ja RV-negatiivisilla lapsilla. Bakteeri-infektion merkkiaineiden tasot olivat koholla mutta virusmerkkiaineen, myksovirusresistenssiproteiini A:n, pitoisuus veressä oli matala RV-positiivisilla lapsilla. Keuhkojen magneettikuvauksella havaittiin poikkeavia löydöksiä 92 %:lla ja merkittävää arpea keuhkopussissa 25 %:lla empyeeman sairastaneista lapsista, mutta kliininen paraneminen, keuhkojen toiminta ja keuhkokuva olivat valtaosalla normaalit.

Virus-bakteerisekainfektiot ovat yleisiä lasten avosyntyisessä keuhkokuumeessa ja ovat mahdollisesti yhteydessä hoidon epäonnistumiseen. RV löytyy usein nuorilta keuhkokuumetta sairastavilta lapsilta ja usein yhdessä bakteeri-infektion kanssa. Päätös antibioottihoidon aloittamatta jättämisestä on haastava. Lisää tutkimuksia ja toimintasuunnitelmia tarvitaan virusinfektion erottamiseksi bakteeri- tai virus-bakteerisekainfektioista. Pitkäaikaisseurannassa empyeemasta paraneminen vaikuttaa hyvältä nykyisillä hoitokäytännöillä.

AVAINSANAT: empyeema, keuhkokuume, lapset, rinovirus

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Abbreviations

AdV	Adenovirus
CAP	Community-acquired pneumonia
CI	Confidence interval
CoV	Coronavirus
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
Ct	Cycle threshold
EV	Enterovirus
FEV1	Forced expiration volume in 1 sec
FVC	Forced vital capacity
HBoV	Human bocavirus
ICAM-1	Intercellular adhesion molecule 1
Ig	Immunoglobulin
IFN	Interferon
IL	Interleukin
IP-10	Interferon gamma-induced protein 10
IQR	Interquartile range
HMPV	Human metapneumovirus
LRTI	Lower respiratory tract infection
MRI	Magnetic resonance imaging
MBL	Mannose-binding lectin
MxA	Myxovirus resistance protein A
NAAT	Nucleic acid amplification test
OR	Odds ratio
PIV	Parainfluenza virus
PCR	Polymerase chain reaction
PCT	Procalcitonin
PCV	Pneumococcal conjugate vaccine
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcriptase polymerase chain reaction
RV	Rhinovirus

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
TLR	Toll-like receptor
TNF	Tumor necrosis factor
URTI	Upper respiratory tract infection
WBC	White blood cell

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Honkinen M, Lahti E, Österback R, Ruuskanen O, Waris M. Viruses and bacteria in sputum samples of children with community-acquired pneumonia. *Clin Microbiol Infect*, 2012; 18: 300-7.

II Hartiala M, Lahti E, Forsström V, Vuorinen T, Ruuskanen O, Peltola V. Characteristics of Hospitalized Rhinovirus-Associated Community-Acquired Pneumonia in Children, Finland, 2003-2014. *Front Med*, 2019; 6: 235.

III Hartiala M, Lahti E, Toivonen L, Waris M, Ruuskanen O, Peltola V. Biomarkers of viral and bacterial infection in rhinovirus pneumonia. *Front Pediatr*, 2023; 11: 1137777.

IV Honkinen M, Lahti E, Svedström E, Jartti T, Virkki R, Peltola V, Ruuskanen O. Long-term recovery after parapneumonic empyema in children. *Pediatr Pulmonol*, 2014; 49: 1020-7.

1 Introduction

Pneumonia is a common cause of hospitalization in children. According to global estimates, 120 million episodes of pneumonia in children younger than 5 years of age are recorded annually (Rudan et al. 2013, Walker et al. 2013). The introduction of penicillin in the 1940s resulted in a significant reduction in pneumonia mortality and the implementation of pneumococcal conjugate vaccines (PCVs) since 2000 resulted in a substantial decrease in the incidence of childhood pneumonia and the predominance of respiratory viruses as pneumonia pathogens (Griffin et al. 2013, Rudan et al. 2013, Jain et al. 2015, Rhedin et al. 2015, Nascimento-Carvalho et al. 2018a, Wahl et al. 2018, Eklundh et al. 2021).

Determining the aetiology of pneumonia is challenging, as obtaining specimens from the lungs is not feasible in routine practice. With modern molecular diagnostic techniques, especially by the development of nucleic acid amplification tests (NAATs), an increasing number of micro-organisms can be detected. Recent aetiological studies, most of them using nasopharyngeal samples, have reported increasing detection rates for mixed viral–bacterial infections in children with community-acquired pneumonia (CAP) (Wang et al. 2015). Rhinovirus (RV) is the most common cause of respiratory tract infections in children worldwide, and it is frequently detected in CAP (Juvén et al. 2000, Peltola et al. 2009, Don et al. 2010, Ruuskanen et al. 2011, Jain et al. 2015, Rhedin et al. 2015, Wang et al. 2015, Toivonen et al. 2016, Nascimento-Carvalho et al. 2018a). However, there is a lack of published clinical data of RV-associated pneumonia in children. There are even controversies whether RV is a causative agent or a bystander in pneumonia.

C-reactive protein (CRP) and procalcitonin (PCT) are the most widely used biomarkers to predict bacterial infection but the usefulness of them in differentiating viral and bacterial pneumonia is not explicit (Toikka et al. 2000). Myxovirus resistance protein A (MxA) is a new promising biomarker for viral infections (Haller et al. 2011, Toivonen et al. 2015). To avoid the overuse of antibiotics, further studies are needed to differentiate viral from bacterial or mixed viral-bacterial pneumonia.

Empyema is a severe complication of childhood CAP, and despite the implementation of pneumococcal vaccines, it still causes a significant proportion of paediatric intensive care unit admissions (Subhi et al. 2022). During the acute phase

of empyema, lung function is disturbed. It is important to investigate the long-term anatomical and functional consequences in the lungs after empyema to optimize treatment and follow-up strategies.

2 Review of the Literature

2.1 Definition of pneumonia

Pneumonia is an acute lower respiratory tract infection (LRTI), an inflammation of lung tissue caused by an infectious agent (Ruuskanen and Mertsola 1999). Pneumonia and bronchiolitis are the most important LRTIs (GBD 2016 Lower Respiratory Infections Collaborators 2018). For the exact diagnosis of pneumonia, radiological verification is needed along with symptoms of acute LRTI, most commonly fever, cough, and tachypnoea or dyspnoea (British Thoracic Society, 2002). Radiological facilities are not always available in developing countries. Moreover, the utility of chest radiogragh is recently questioned due to its diagnostic and technical limitations (Kazi et al. 2022). In 1990, the World Health Organization (WHO) produced a guideline for clinical detection of pneumonia including cough or breathing difficulties, and tachypnea (>50 breaths/min in children <12 months and >40 breaths/min in children ≥12 months of age) or lower chest wall indrawing (WHO 1990). This broad definition of pneumonia ensures that all severe cases will be detected, but it probably leads to overtreatment with antibiotics. Interestingly, in a prospective study from US, only 34% of children with radiologically confirmed pneumonia met the WHO case definition of pneumonia (Wingerter et al. 2012). In the Pneumonia Etiology Research for Child Health (PERCH) project and in the Trial of Respiratory infections in children for ENhanced Diagnostics (TREND) study, which classificated paediatric pneumonia with an algorithm along with WHO's clinical classification of pneumonia, children whose chest indrawing resolved after bronchodilator therapy were excluded (Scott et al. 2012, Rhedin et al. 2019).

CAP is acquired outside the hospital (British Thoracic Society, 2002). Hospital-acquired pneumonia, ventilator-associated pneumonia, aspirate pneumonia, and neonate pneumonia are distinct entities which are not considered in this review.

2.2 Epidemiology

Pneumonia is an important cause of morbidity and mortality in children worldwide, accounting for 14–19% of all deaths of children aged <5 years (McIntosh 2002, Wardlaw 2006, Rudan et al. 2008, WHO 2022). In 2000, the estimated global

incidence of clinical pneumonia in young children (<5 years) was 155.8 million cases, and ten years later, it was 122.4 million cases, 95% of which in developing countries (Rudan et al. 2004, Rudan et al. 2013). The number of LRTI episodes among children of <5 years of age is highest in South Asia (18.76 million cases in 2016 with the incidence of 122.1 episodes per 1,000 children), and the highest incidence is reported in Oceania (171.5 per 1,000 children) (GBD 2016 Lower Respiratory Infections Collaborators 2018). In Finland, 3.5–4% of children of <5 years of age and 1.5% of older children used to have radiologically confirmed pneumonia yearly and half of the first group needed hospitalization (Jokinen et al. 1993, Heiskanen-Kosma 1998), but the incidences have declined substantially after the introduction of PCV. Along with the substantial decrease in the incidence of childhood pneumonia, the predominance of respiratory viruses as pneumonia pathogens is emerging (le Roux and Zar 2017, Eklundh et al. 2021).

Approximately 1.6 million children younger than 5 years died from pneumonia (18% of all deaths in this age group) worldwide in 2008, and 0.92 million (15.5%) in 2015, respectively (Black et al. 2010, Liu et al. 2016). The LRTI mortality rates of children have reduced substantially between 2007 and 2017 because of the implementation of vaccines, appropriate antibiotic therapy, and improvements concerning hygienic, nutritional, and educational issues (GBD 2017 Causes of Death Collaborators 2018). Regional differences in mortality are still significant, and the highest LRTI mortality rates are reported in the Central African Republic with 460 deaths per 100,000 children of <5 years of age (GBD 2016 Lower Respiratory Infections Collaborators 2018), while in developed countries, mortality for CAP with no comorbid conditions is rare (Prayle et al. 2011, Lantto et al. 2013).

Due to improvements in molecular diagnostic methods, antimicrobial therapies, and prevention strategies, as well as due to new epidemics and emerging viruses, the epidemiology of pneumonia keeps changing.

2.3 Diagnostics

Diagnosis of pneumonia is commonly based on the clinical presentation of acute symptoms of LRTI and radiological findings. In outpatient care with non-severe cases, the clinical diagnosis of pneumonia based on acute symptoms and signs of infection, such as crackles in the lung auscultation, is possible (Bradley et al. 2011, Harris et al. 2011). The most common symptoms of pneumonia are fever and cough (Juvén et al. 2003, Jain et al. 2015). Digital auscultation with automated analysis to diagnose pneumonia still requires further evaluation (Ahmed et al. 2022). Biomarkers studied from blood sample can assist in the clinical diagnosis of pneumonia, and microbial diagnostic tests can aid in predicting the course of illness

and guide the treatment. New biomarkers derived from host transcriptional profile analysis are promising but not yet validated (Zar et al. 2017).

2.3.1 White blood cells

High white blood cell (WBC) count (commonly used threshold >15 × 10^9/L) is thought to be associated with bacterial infection, as well as an absolute neutrophil count >10 × 10^9/L, but the accuracy of them is insufficient (Korppi et al. 1993, De et al. 2014).

2.3.2 C-reactive protein and procalcitonin

CRP is the most widely used biomarker to predict bacterial infection. CRP is an acute phase protein peaking 36-50 h from the onset of infection, and in a meta-analysis of eight studies a CRP value over the cutoff varying from 40 to 60 mg/L was suggestive of bacterial infection with a positive predictive value of 64% (Flood et al. 2008). Serum CRP level is increased by proinflammatory cytokines (IL-6, IL-1β, tumor necrosis factor (TNF) α), and it can be increased also by any inflammation or tissue damage. In a Finnish study of common paediatric infections, along with bacterial infections, 67% of adenovirus cases presented with CRP >80 mg/L and/or WBC >15 × 10^9/L (Peltola et al. 2006). Moreover, in septicaemia, 93% of patients with *S. pneumoniae* but only 52% of patients with *S. aureus* had CRP level over 80 mg/L and/or WBC >15 × 10^9/L (Peltola et al. 2006). Otherwise, IL-6 receptor deficiency or IL-6 antibodies, for instance, can extremely rarely impair the elevation of CRP (Puel et al. 2008, Bloomfield et al. 2019, Spencer et al. 2019). Combining elevated CRP level (a suggested threshold of 72 mg/L) with clinical symptoms, such as the presence of fever or the absence of rhinorrhoea, can improve the differentiation of bacterial from presumed viral pneumonia in children (Bhuiyan et al. 2019b).

PCT rises more rapidly (peak in 12-24 h) than CRP and it is induced by the same cytokines, but also its usefulness in differentiating viral and bacterial pneumonia is not explicit (Gilbert 2010, Kamat et al. 2020). Serum PCT level >0.5 μg/L suggests bacterial cause in adults with CAP (Kamat et al. 2020). PCT-guided (cutoff point 0.25 μg/L) initiation of antibiotic treatment decreased the use of antibiotics, the length of antibiotic treatment, and the adverse effects of antibiotics without increasing treatment failures in otherwise healthy children with clinically and radiologically diagnosed uncomplicated pneumonia (Esposito et al. 2011). In young febrile infants, PCT has been shown to be more accurate than CRP to predict invasive bacterial infection (Milcent et al. 2016). Similarly, in a recent study by Bashir et al., PCT was associated with disease severity in children with CAP more strongly than CRP (Bashir et al. 2022). In children hospitalized with CAP, the measurement of these non-specific inflammatory parameters can be recommended for the estimation of disease severity

and response to treatment. In a recent systematic review and meta-analysis, CRP and PCT were considered the best diagnostic biomarkers for bacterial pneumonia in children, with sensitivity and specificity from 64 to 70%, and the suggested statistically optimal cutoff levels being 53 mg/L for CRP and 0.59 μg/L for PCT based on the Youden index, with areas under the receiver operating characteristic curve of 0.71 and 0.70 for CRP and PCT, respectively (Gunaratnam et al. 2021).

2.3.3 Pro-inflammatory cytokines

The pro-inflammatory cytokine IL-6, the concentration of which rises within minutes with a half-life of about an hour, is a potential biomarker of pneumococcal infection among children with CAP, and is also elevated among cases with pleural effusion (Vasconcellos et al. 2020). A cutoff point of 12.5 ng/L for serum IL-6 concentration was suggested to predict pneumococcal CAP among children under 5 years (Vasconcellos et al. 2018). Regarding acute asthma, serum IFN-ɣ-induced protein 10 (IP-10) level was shown to increase in rhinovirus-induced asthma exacerbations (Wark et al. 2007). Viperin, an interferon-regulated antiviral protein, is a potential marker of symptomatic viral infection (Proud et al. 2008, Helbig and Beard 2014, Yahya et al. 2017). Gene expression patterns in blood leukocytes might assist in the discrimination of different viral and bacterial infections (Ramilo et al. 2007). In febrile children, a 2-transcript host RNA signature can discriminate bacterial from viral infection (Herberg et al. 2016).

2.3.4 Myxovirus resistance protein A

MxA is an intracellular, cytoplasmic GTPase that is induced exclusively by type I (α, β) and type III (λ) interferons, highly expressed during viral infections but not in bacterial infections (Halminen et al. 1997, Haller and Kochs 2011, Toivonen et al. 2015). As an antiviral protein, MxA adheres to the important parts of the virus to disable its function and replication. Blood MxA level rises rapidly in hours (peak in about 16 h), and with a half-life of 2–4 days it remains elevated during the acute course of infection (Ronni et al. 1993, Haller and Kochs 2011, Toivonen et al. 2015). Several different viruses induce MxA expression. In a study by Toivonen et al., the median MxA protein level was 695 μg/L in virus-positive children with respiratory symptoms, 110 μg/L in asymptomatic virus-negative children, and 145 μg/L in asymptomatic virus-positive children (Toivonen et al. 2015). MxA level has been shown to be elevated in symptomatic viral infections regardless of the concomitant bacterial infection, and one suggested cutoff level of MxA was 256 μg/L to differentiate viral infections from bacterial or asymptomatic viral infections (Piri et al. 2022). In a recent study of Rhedin et al., a cutoff of MxA 430 μg/L discriminated between viral and

bacterial pneumonia with 100% specificity and 93% sensitivity, though there were only four bacterial pneumonia cases (Rhedin et al. 2022). In that study, the median MxA level in LRTI cases caused by RV was about 200 μg/L, whereas the highest median levels were seen according to AdV (1961 μg/L), RSV (1226 μg/L), PIV (985 μg/L), influenza virus (783 μg/L), and HMPV (759 μg/L) (Rhedin et al. 2022).

2.3.5 Radiology

Chest radiograph is the mainstay of diagnosing pneumonia, as the symptoms can be variable and non-specific. Even though a chest radiograph is the standard imaging modality in pneumonia diagnostics, it has limitations concerning its diagnostic accuracy, challenges in obtaining high-quality images, and notable interobserver variability (Kazi et al. 2022). Chest radiograph is indicated at least in cases of locally decreased or absent breath sounds, slow or missing response to antibiotic treatment, or hypoxaemia (Bradley et al. 2011, Harris et al. 2011). Lobar alveolar infiltrates on the chest radiograph are strongly suggestive of bacterial pneumonia, whereas interstitial infiltrates are seen in both viral and bacterial pneumonias (Virkki et al. 2002). The size of consolidation and the left-sided location of pneumonia are considered to predict a more severe course of pneumonia (Grafakou et al. 2004). Multilobar or nonlobar infiltrates are also shown to predict a more severe outcome of CAP in children (Williams et al. 2016). Follow-up chest radiography is not routinely recommended in non-complicated CAP (Virkki et al. 2005).

Lung ultrasonography is commonly used to evaluate the pleural space and to guide catheter placement for drainage, but its introduction into clinical practice in the diagnostics of pneumonia has been slow, despite its high sensitivity for identification of lung consolidation (Stadler et al. 2017, Kazi et al. 2022). Its absolute credits are its bedside availability and the absence of radiation exposure. Moreover, doppler ultrasound enables to detect early necrotic changes. In the follow-up, the absence of a pleural gliding sign on ultrasound indicates the presence of a pleurodesis. However, there is notable interobserver variation in reporting ultrasound characteristics (Haggie et al. 2022).

Computed tomography scans are recommended in complex cases or selected patients in case of non-specific radiographic findings, complications, suspicion of an underlying lesion, or recurrent or non-resolving pneumonia (Franquet 2018).

Lung magnetic resonance imaging (MRI), a radiation free modality, has been shown to be an accurate and effective technique for evaluating pneumonia and its complications in children (Peltola et al. 2008, Yucel et al. 2021). Dynamic MRI has the potential for evaluating chest motion and lung volumetry during the breathing cycle (Plathow et al. 2004, Swift et al. 2007). In children with complicated pneumonia, lung MRI detected pulmonary abscess/necrosis formations and

empyema significantly better than chest radiograph (with a prevalence of 72.7% and 74.1% versus 27.3% and 11.1%, respectively) and slightly better than chest radiograph and lung ultrasound together (55.6% and 63.1%, respectively) (Konietzke et al. 2020). The admission of contrast media is not mandatory in the detection of abscesses or empyema by MRI (Konietzke et al. 2020).

2.4 Microbial diagnostics

The development of nucleic acid amplification techniques and the availability of rapid molecular diagnostic tests has revolutionised the microbial diagnostics of respiratory viral infections. NAAT detects microbial nucleic acids. Comparably, culture reflects the replication of a microorganism. Antigen tests are immunoassays that can rapidly detect the presence of a specific microbial antigen. NAAT is more sensitive than culture, antigen detection, or serologic assays (Jartti et al. 2013). The properties of the most common microbiological diagnostic methods in pneumonia are compared in Table 1. NAATs include polymerase chain reaction (PCR) and reverse transcriptase (RT-) PCR, which amplifies genetic material by thermal cycling, but also other amplification techniques, such as transcription mediated amplification or loop-mediated isothermal amplification. Qualitative (end-point) NAAT only detects microbial nucleic acids and quantitative NAAT, such as qPCR or qRT-PCR, also determines the amount of nucleic acid at real time by the use of fluorescent probes. In quantitative NAAT, a known amount of a standard molecule is added to every sample, and the sample nucleic acid amplification signal is compared to the standard curve.

Nasopharygeal carriage of some potential viral and bacterial pneumonia pathogens in healthy children is commonly known and poses a diagnostic challenge (Jansen et al. 2011a, Dunne et al. 2018, Bhuiyan et al. 2019a). Serology distinguishes acute from chronic infection and thus can be used to confirm the pathogenic role of microbes, though it has a limited impact on clinical decision making because of requiring paired serum samples (Murdoch et al. 2012, Sawatwong et al. 2012, Feikin et al. 2013, Zhang et al. 2016). A high viral load (a low cycle threshold (Ct) value) in a NAAT can be considered to indicate the real pathogenicity of a virus (Mardian et al. 2021). The Ct value is defined as the amplification cycle number at which the fluorescence of a NAAT product exceeds the background signal, and it can be used as a semiquantitative measure of viral load in the sample. Simultaneous detection of multiple viruses or bacteria from one sample has been enabled by the development of multiplex NAAT assays. Multiplex NAAT assays are cost-effective in determining the aetiology of respiratory infections in children, as clinical signs and symptoms are usually overlapping and co-infections are common, and compared to single-target NAATs, specificity and sensitivity are similar with slight virus-specific

differences (Jansen et al. 2011b, Jartti et al. 2013, Parker et al. 2015). So far, NAAT assays are used mainly in the inpatient settings. Real-time metagenomic sequencing to determine all microbiota present is a promising new technique to improve the microbial diagnosis of pneumonia (Pendleton et al. 2017, Chiu and Miller 2019, Deng et al. 2022, Yang et al. 2022). As a target-independent approach, metagenomic sequencing can detect novel, rare and unexpected pathogens as well as co-infections and can provide comprehensive information about pathogens, but interpreting the results may be challenging (Chen et al. 2022).

Table 1. The most common microbiological diagnostic methods in pneumonia and their pros (+) and cons (-).

Method	Target	Capacity
Nucleic acid amplification test	Genetic material of microbes	+ High sensitivity and specificity + Rapid + Can be quantitative + Automated technology + Product can be genotyped or sequenced + Multiplex possibility - Detection of colonization (false positives) - Expensive
Serologic test	Antibodies produced in response to an infection	+ Can distinguish acute from chronic infection + Can detect systemic infection - Slow
Antigen detection	Proteins of microbes	+ Good specificity, variable sensitivity + Rapid + Point of care - No assays for routine detection of e.g. RV
Culture	Live microbes	- Some viruses do not grow in routine cultures (e.g. RV-C) - Time-consuming - Virus culture is laborious and requires expertise

Numerous different types of specimens are used for the direct detection of LRTI pathogens. Blood cultures, pleural fluid samples from thoracentesis, and transthoracic needle aspirates are sterile and bronchoalveolar lavages nonsterile samples with invasive techniques (Loens et al. 2009). Noninvasive techniques include oropharyngeal swab, nasal swab, nasopharyngeal swab or aspirate, and sputum samples (Loens et al. 2009). Noninvasive upper respiratory tract specimens are commonly used for the identification of respiratory viruses and bacteria, as they are easily obtained. Nasal swabs have comparable sensitivity to nasopharyngeal aspirates

for the detection of all major respiratory viruses except respiratory syncytial virus (RSV) (Heikkinen et al. 2002). In LRTIs, most of the pathogenic microbes detected from sputum samples are often identified also in upper respiratory tract specimens, though sputum samples often carrying higher amounts of viruses and bacteria in these cases (Lahti et al. 2009). The association is not so consistent the other way around, and upper airway microbe detection does not necessarily reflect the cause of LRTI. However, nasopharyngeal colonization may have proceeded to an invasive infection, if the same pathogen is detected simultaneously in sputum and nasopharyngeal samples. The induced sputum procedure has been shown to be safe, even among severely ill children aged <5 years in low-income-country settings (Grant et al. 2012, DeLuca et al. 2017). Discomfort is a common side effect of the sputum procedure, but more severe adverse effects, such as a drop in oxygen saturation or a new requirement of bronchodilator, are rare (0.3%) (Lahti et al. 2009, DeLuca et al. 2017). Microbial contamination from the upper respiratory tract may occur and should be considered when analysing induced sputum samples, lessening its superiority over the more easily obtained swab samples (Hammitt et al. 2012). Cleaning the nasopharynx of the mucus before sputum collection is a way to minimise nasopharyngeal contamination (Lahti et al. 2009). The safety and utility of the lung aspirate procedure has been established in young children hospitalized with severe pneumonia, but, even though it is the gold standard specimen for pneumonia diagnostics, lung aspirates are not widely used in clinical practice (Vuori-Holopainen et al. 2002, Ebruke et al. 2021). Adverse events of the lung aspirate procedure, including minor bleeding and pneumothorax, are reported to be rare (<3%) and transient (Ideh et al. 2011). Nevertheless, even by lung tap, the focus of infected lung tissue may be missed.

2.4.1 Viral aetiology

Respiratory viruses are commonly detected in children with CAP (Table 2). In both developing and developed countries, RSV, RV, human metapneumovirus (HMPV), human bocavirus (HBoV), and parainfluenza viruses (PIVs) are the most frequently identified respiratory viruses in children with CAP (Ruuskanen et al. 2011, Pratt et al. 2022). Viral-viral co-detections are also common and increasingly identified in CAP (Table 2). The classifications and structural details of the most common respiratory viruses are listed in Table 3.

RSV continues to be the major viral pathogen in pneumonia globally (Juvén et al. 2000, Berkley et al. 2010, Garcia-Garcia et al. 2012, Jain et al. 2015, Rhedin et al. 2015, Pneumonia Etiology Research for Child Health (PERCH) Study Group 2019, Gareca Perales et al. 2021, Rueda et al. 2022). Bronchiolitis and acute otitis media are even more common clinical findings in children infected by RSV (Peltola et al. 2009). Young children are at a higher risk of having severe pneumonia caused

by RSV (Ruuskanen and Ogra 1993, Jonnalagadda et al. 2017, Bunthi et al. 2021). The role of RV in the aetiology of CAP is increasingly discussed, and RV was recently detected in up to 46% of children with CAP (Nascimento-Carvalho 2018). RV is more profoundly elaborated in the next chapter.

At a tertiary center in Finland, 14% of children with influenza infection had radiologically verified pneumonia (Lahti et al. 2006). Similarly, in a more recent Finnish study, 16% of hospitalized children with influenza infection had pneumonia, regardless of the type (A or B) of influenza virus (Mattila et al. 2020).

Newly described respiratory viruses, HBoV and HMPV, are also notable viruses in the aetiology of childhood CAP (Fry et al. 2007, Cilla et a. 2008, Rhedin et al 2015, Nascimento-Carvalho et al. 2018a). HBoVs have been associated with a variety of symptoms from rhinitis to gastroenteritis, and species 1 of HBoVs, isolated in 2005, is predominantly a respiratory pathogen (Allander et al. 2005, Jartti et al. 2012, Christensen et al. 2019). HBoV is frequently detected in viral co-infections (71%) (Garcia-Garcia et al. 2012). Unlike most respiratory viruses, HBoV is known to have prolonged shedding for months after an acute infection (Martin et al. 2010, Christensen et al. 2019). Therefore, quantitative NAAT with serology is a recommended diagnostic approach for diagnosing acute HBoV infection, and HBoV mRNA and antigen detections are showing promising results (Christensen et al. 2019). Recently in Brazil, HBoV was detected by PCR in 21% of children with non-severe CAP, and acute HBoV infection was confirmed serologically in 24% of them (Nascimento-Carvalho et al. 2018b). HMPV, discovered in 2001, has clinical similarities with RSV, and can cause severe pneumonia especially in infants younger than 1 year of age (van den Hoogen et al. 2001, Don et al. 2008, Rhedin et al. 2015, Wang et al. 2021b).

PIVs, of which types 1 and 2 are most often associated with croup and type 3 with pneumonia, were recently detected in 21% of children with pneumonia in Brazil, and globally, approximately 13% of acute LRTIs in children younger than 5 years are estimated to be attributable to PIVs (Nascimento-Carvalho et al. 2018a, Howard et al. 2021, Wang et al. 2021c). The detection rate of the four endemic human coronaviruses (CoVs) 229E, NL63, OC43, and HKU1, which can cause various respiratory symptoms, is highest in children aged 1–5 years (Varghese et al. 2018). In childhood CAP, CoVs have been detected in up to 7–8% of studied children (Cilla et a. 2008, Cevey-Macherel et al. 2009, Nascimento-Carvalho et al. 2018a). Adenoviruses (AdVs) cause a wide variety of illnesses, including pneumonia, and were recently detected in 38% of children with non-severe CAP in Brazil and in 11% of children hospitalized for CAP in United states (Nascimento-Carvalho et al. 2018a, Jain et al. 2015). Among enteroviruses (EVs), which are structurally closely related to RVs, EV-D68 usually causes respiratory illness and is an emerging pathogen associated with a severe acute respiratory illness (Bosis and Esposito 2017, Savage et al. 2018). Similar proportions of common respiratory viruses are seen in both inpatient and outpatient

settings in children with CAP (Yun et al. 2022). More uncommon viruses as causative agents for childhood CAP include varicella-zoster virus, hantaviruses, parechoviruses, Epstein-Barr virus, human herpesviruses 6 and 7, herpes simplex virus, mimivirus, cytomegalovirus, and measles virus (Ruuskanen et al. 2011).

Pneumonia-related hospitalizations and viral detections are most common among children younger than 5 years of age (Jain et al. 2015, Pratt et al. 2022, Rueda et al. 2022). In a Spanish study of children hospitalized with radiologically confirmed pneumonia, viral detection rate was significantly higher in children aged <18 months than in older children (83% vs. 67%) (Garcia-Garcia et al. 2012). In addition to an age of <5 years, ongoing viral epidemic, slow onset of symptoms, rhinitis, wheezing, sole interstitial infiltrates or bilateral chest radiograph findings, and slow or missing response to antibiotic treatment are features suggesting a viral aetiology of pneumonia (Ruuskanen et al. 2011). Seasonal patterns of activity are typical for many respiratory viruses, as RV peaks are seen in autumn and spring, RSV in every other year in late autumn but recently with fluctuating seasonality, and influenza and HCoV in winter (Monto et al. 2002, Varghese et al. 2018, Li et al. 2019, Moriyama et al. 2020, Renko and Tapiainen 2020). Pathogen transmission can occur via direct or indirect contact, droplets, or aerosols (Leung et al. 2021, Wang et al. 2021a).

The importance of respiratory viruses in causing severe pneumonia and new epidemics is yet re-emphasised by the Coronavirus disease 2019 (COVID-19) pandemic. COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), presents often as interstitial pneumonia with common findings in chest radiograph being peribronchial thickening, ground-glass opacities, consolidation, and pleural effusion (Oterino Serrano et al. 2020). The clinical picture of COVID-19 in children varies from asymptomatic or mild to severe or critical infection, and a multisystem inflammatory syndrome may occur after an acute infection by immunological mechanisms (Miao et al. 2020, Parisi et al. 2020). In a recent study of severe viral pneumonia in children, SARS-CoV-2 was shown to cause a milder clinical course than other respiratory viruses, mostly RV/EV or RSV (Ng et al. 2022). Due to some uncertain factors, SARS-CoV-2 affects adults more severely than children (Lu et al. 2020, Ludvigsson 2020, Parisi et al. 2020, Zimmermann et al. 2020). The proposed explanations include the greater potential of the innate immune system of children, a cross-reactivity between immune response to early childhood vaccines and SARS-CoV-2, and the possible lower expression levels and functionality of angiotensin-converting enzyme 2 (ACE2) receptor, by which the virus enters respiratory epithelial cells, in children (Parisi et al. 2020). As seen in respiratory infections with seasonal CoV, viral and bacterial co-infections may complicate the clinical presentation of COVID-19 (Zhu et al. 2020, de Koff et al. 2021). In addition, empyema has been identified as a rare complication of COVID-19 (Abbasi et al. 2021).

Table 2. Viral aetiology of community-acquired pneumonia in children. Modified from original publication I.

	Rueda et al. 2022	**Nascimento-Carvalho et al. 2018a**	**Jain et al. 2015**	**Rhedin et al. 2015**	**García-García et al. 2012**	**Honkinen et al. 2012**	**O' Callaghan-Gordo et al. 2011**	**Wolf et al. 2010**	**Cevey-Macherel et al. 2009**	**Cilla et al. 2008**	**Samransamruajkit et al. 2008**	**Nascimento-Carvalho et al. 2008**	**Hamano-Hasegawa et al. 2008**	**Tsolia et al. 2004**	**Juvén et al. 2000**
Country	Colombia	Brazil	United States	Sweden	Spain	Finland	Mozambique	Israel	Switzerland	Spain	Thailand	Brazil	Japan	Greece	Finland
No. of cases	525	774	2222	121	884	76	807	1296	99	338	257	184	1700	75	254
Hospitalized (%)	100	NG	100	76	100	100	100	77	100	53	100	100	NG	100	100
Age (years)	Median 2.7	Median 2.1	Median 2	Range 0–5	Median 1.5	Mean 4.7	Range 0–5	Range 0–4	Mean 2.4	Median <3	Median 1.3	Median 1.6	Range 0–19	Median 7.2	Mean 3.8
Viral detection methods	PCR, Ag, Serol	PCR	PCR, Serol	PCR	PCR	PCR, Cul, Ag	PCR	PCR, Cul, Ag	PCR, Cul, Ag, Serol	PCR, Cul, Ag	PCR	PCR, Ag, Serol	PCR	PCR	PCR, Cul, Ag, Serol
No. of viruses studied	15	16	13	15	16	18	11	8	13	14	8	9	12	9	12
Aetiology detected (%)	84	NS	81	NS	NS	97	NS	NS	86	NG	NS	78	77	77	85
Blood-culture positive (%)	4	NS	NG	NS	2	NS	11	NS	1	1	NS	5	NG	3	1
Bacteria (%)	46	NS	15	NS	NS	91	NS	NS	53	NG	NS	42	50	40	53
Viruses (%)	72	91	73	81	73	72	49	47	67	67	49	60	43	65	62

	Rueda et al. 2022	Nascimento-Carvalho et al. 2018a	Jain et al. 2015	Rhedin et al. 2015	García-García et al. 2012	Honkinen et al. 2012	O' Callaghan-Gordo et al. 2011	Wolf et al. 2010	Cevey-Macherel et al. 2009	Cilla et al. 2008	Samransamruajkit et al. 2008	Nascimento-Carvalho et al. 2008	Hamano-Hasegawa et al. 2008	Tsolia et al. 2004	Juvén et al. 2000
≥2 viruses (%)	15	63	NG	31	30	30	20	5	12	18	NG	NG	2	9	14
Viruses and bacteria (%)	34	NS	7	NS	NS	66	NS	NS	33	NG	NS	23	15	28	30
RV (%)	21	46	27	23	19	30	41	NS	20	14	NS	21	15	45	24
RSV (%)	31	25	28	32	31	7	11	23	13	20	16	15	9	3*	29
HBoV (%)	1	22	NS	12	13	18	NS	NS	NS	14	NS	NS	3	NS	NS
HMPV (%)	7	13	13	23	5	14	8	8	13	12	9	NS	7	1	NS
AdV (%)	13	38	11	15	13	11	21	3	7	3	7	3	2	12	7
PIV types 1–3 (%)	23**	21	7	2	5**	13**	7**	3	13	11**	5	17	7	8	10
Influenza virus (%)	18	9	7	7	5	5	8	3	14	7	13	9	<2***	7	4
EV (%)	2	26	NS	2	NG	3	4	NS	13	NS	NS	5	<2***	NS	NS
CoV (%)	3	8	5	5	1	7	NS	NS	7	7	NS	NS	<2***	NS	3

Ag = antigen detection, CuI = viral culture, NG = not given, NS = not studied, Serol = viral antibody enzyme immunoassay from paired serum samples
* Studied children aged 5–14 years.
** PCR included types 1–4.
*** Influenza A and B viruses, coronavirus OC43, enterovirus.

Table 3. Characteristics of the most common respiratory viruses.

Virus	Classification	Genome type*	Diameter (nm)	Viral family
RV	Species A, B, and C with over 150 types	+ssRNA	15–30**	Picornaviridae
RSV	Groups A and B	-ssRNA	150–250	Pneumoviridae
HBoV	Species 1–4, HBoV2 divided into strains A and B	+ssDNA	18–26**	Parvoviridae
HMPV	Groups A and B	-ssRNA	150–600	Pneumoviridae
AdV	51 serotypes	dsDNA	90–100**	Adenoviridae
PIV	Types 1, 2, 3, and 4	-ssRNA	150–250	Paramyxoviridae
Influenza virus	Types A, B, and C	-ssRNA	80–120	Orthomyxoviridae
EV	Species A–D with up to 70 types in each species	+ssRNA	25–30**	Picornaviridae
CoV	Types OC43, 229E, NL63, HKU1	+ssRNA	80–220	Coronaviridae

* ss=single-stranded; ds=double-stranded; +ss=positive-sense single-stranded; -ss=negative-sense single-stranded
** non-enveloped viruses

2.4.1.1 Rhinovirus

RV, discovered in the 1950s, is a small non-enveloped RNA virus belonging to the family of picornaviruses (Jacobs et al. 2013). RV is classified into three species (A, B, and C) with approximately 160 different types (Daleno et al. 2013, Jacobs et al. 2013). RV-C was discovered in 2003. The genome of RV is a positive single-stranded RNA consisting of a single gene encoding 11 proteins, including viral capsid proteins. Genotyping of RVs is usually performed by RT-PCR amplification and sequence analysis of gene regions for structural viral protein (VP) 1 or VP4/VP2 (Jacobs et al. 2013).

RV replication occurs in the nasal mucosa and posterior nasopharynx, as well as in the epithelial cells of lower respiratory tract (Kennedy et al. 2012). RVs have been detected even directly from lung tissue in children with LRTI (Imakita et al. 2000). It has been shown in vitro that RV can replicate well at lower airway temperatures (Papadopoulos et al. 1999, Hayden et al. 2004). For binding and endocytosis, RV-A uses intercellular adhesion molecule 1 (ICAM-1) receptor or less frequently low-density lipoprotein receptors (LDL-R), RV-B uses ICAM-1, and RV-C uses cadherin-related family 3 (CDHR3) (Basnet et al. 2019). Toll-like receptors (TLRs) are essential in the recognition of viral RNA in the endosome. Inflammatory

response for RV is mediated by the nuclear factor (NF) kappa B pathway and production of interferon (IFN)-beta, IFN-gamma, interleukin (IL)-8, and IL-1b, leading to the increased production of T cells and neutrophil cytokines (Bizot et al. 2021). Some immunodeficiencies, such as non-functional melanoma differentiation-associated protein 5 (MDA-5), have been reported to lead to recurrent and severe RV infections, because of poor IFN production and a lack of immune responses (Lamborn et al. 2017).

Since the 2000s and the development of new molecular methods, RV is increasingly detected by PCR. Antibodies are serotype specific. Thus, simultaneous circulation of several RV types in populations year-round explains the high frequency of RV infections. Recently, despite the high phylogenetic diversity of RV, the development of RV species-specific antibody test has been successful (Megremis et al. 2018).

RV is the most common cause of upper and lower respiratory tract infections in children worldwide (Peltola et al. 2009, Vandini et al. 2019). RV infections cause a remarkable clinical and socioeconomic burden in the community (Halabi et al. 2022). High RV transmission rates were documented even during the social restrictions of COVID-19 pandemic (Kuitunen et al. 2021, Varela et al. 2022). RV-A is the most common RV species appearing year-round, RV-C is often associated with more severe symptoms and mostly detected in the winter, while RV-B plays a minor role concerning the frequency and severity of RV infections (Esposito et al. 2012, Lee et al. 2012, Ahn et al. 2018, Ai et al. 2022, Esneau et al. 2022). Even fatal outcome of RV-C infection has been reported (Lupo et al. 2015). Severe RV (A45) infection with central nervous system involvement and multiorgan dysfunction was recently reported in a previously healthy 10-year-old girl (Liu et al. 2022).

In infants hospitalized with viral bronchiolitis, RV has been associated with a higher T helper type-2 (Th2) immune response than RSV (Fedele et al. 2018). Th2-like immune responses are typical in atopic children, and allergic sensitization can lead to more severe RV-induced LRTIs (Jackson et al. 2012). RV-induced wheezing in the first three years of life involves a higher risk of childhood asthma (Jackson et al. 2008, Liu et al. 2017). RV is known to be the major pathogen in wheezing illnesses, such as bronchiolitis, recurrent wheezing, or asthma exacerbation (Peltola et al. 2008, Peltola et al. 2009, Jacobs et al. 2013, Kennedy et al. 2014, Jartti and Gern 2017, Ahn et al. 2018), but the clinical characteristics of RV-associated pneumonia in children have not been thoroughly investigated. RV is detected in 14–46% of children with CAP (Table 2). However, the role of RV as the causative agent of pneumonia is unclear, as a substantial proportion of asymptomatic children are positive for RV by PCR of upper respiratory tract specimens (Jartti et al. 2008a, van der Zalm et al. 2009, Jansen et al. 2011a, Jain et al. 2015, Rhedin et al. 2015, Spichak et al. 2016, Zar et al. 2016, Baillie et al. 2018). In adults, the pathogenic role of RV

was demonstrated by comparing asymptomatic controls with CAP patients (Self et al. 2016). The possibility of prolonged carriage of RV for months and even years after the onset of infection is worth noticing in immunosuppressed children, but otherwise RV clearance occur in 1–3 weeks after symptomatic infection (Peltola et al. 2008, Peltola et al. 2013, Bizot et al. 2021). A feasible cutoff value of viral load ($\geq 10^{4.5}$ copies/ml) for clinical relevance of RV detection has been reported, but high RV loads were seen also in some asymptomatic controls (Jansen et al. 2011a).

Despite active research, no specific antiviral therapy or vaccine for RV exists. Among children with allergic asthma, an anti-immunoglobulin (Ig) E therapy omalizumab has been studied for the prevention of RV-associated asthma exacerbations with promising results (Esquivel et al. 2017). Recently, TLR3 blockage has been studied as one of the possible options for treatment (Silkoff et al. 2018). Consideration of an intranasal application of interferons and antibodies for ICAM-1 as a RV-specific treatment is worth further investigations (Padayachee et al. 2021).

2.4.2 Bacterial aetiology

Streptococcus pneumoniae is the most common bacterial pathogen in CAP. It has been detected in 37–46% of hospitalized and 18% of non-hospitalized children with CAP (Juvén et al. 2000, Michelow et al. 2004, Don et al. 2005, Cevey-Macherel et al. 2009). The incidence of CAP along with other invasive pneumococcal infections has declined substantially after the introduction of PCVs (Korppi et al. 2013, Palmu et al. 2013), which in Finland occurred in autumn 2010. Comparably, *Mycoplasma pneumoniae* is detected in 7–14% of hospitalized and 27% of non-hospitalized children with CAP, most commonly in school-age children (Juvén et al. 2000, Korppi et al. 2003, Michelow et al. 2004, Don et al. 2005, Don et al. 2010, Jain et al. 2015, Bhuiyan et al. 2019a, Kutty et al. 2019, Rueda et al. 2022). Tsolia et al. have detected *M. pneumoniae* in 35% of school-age children hospitalized with CAP (Tsolia et al. 2004). Seasonal variation and epidemics are common among *M. pneumoniae* infections (Lv et al. 2022). *Moraxella catarrhalis* and *Haemophilus influenzae* are often detected in respiratory infections including pneumonia but these pathogenic bacteria are also often detected from the nasopharynx of healthy children; similarly *S. pneumoniae* is often carried in the nasopharynx of healthy children, in as many as 87% of children at least once during their first 2 years of life (Syrjänen et al. 2001, (Bhuiyan et al. 2019a). In previously healthy children, *M. catarrhalis* is a rare cause of pneumonia (Sy et al. 2010). *H. influenzae* type b used to be a common cause of LRTIs and other severe infections before the implementation of the *H. influenzae* type b conjugate vaccine (included in the national vaccination programme of Finland from 1993). *Staphylococcus aureus* and *Streptococcus pyogenes* can

rarely cause a severe pneumonia. *Chlamydia pneumoniae* is rarely found in school-age children and *Chlamydia trachomatis* in neonates with pneumonia.

Bacteraemia is uncommonly (2.5%) associated with CAP in children (Neuman et al. 2017). The urine antigen assay for *S. pneumoniae* is not useful for diagnosing bacterial pneumonia in children, as it is non-specific because of the frequency of pneumococcal colonisation (Dowell et al. 2001, Bradley et al. 2011). Pneumococcal antigen detection from pleural fluid is useful in diagnosing pneumococcal empyema (Martinón-Torres et al. 2012). Several multiplex NAAT platforms for bacteria are available for clinicians, in addition to traditional methods including gram staining, culture, and serology.

A rapid onset of symptoms, high fever, tachypnoea, lobar alveolar infiltrates in chest radiograph, and a rapid response to antibiotic treatment suggest bacterial aetiology of pneumonia (Ruuskanen et al. 2011).

2.5 Risk factors

Pneumonia occurs most commonly in vulnerable individuals. Children of <5 years of age have an increased risk of CAP (McAllister et al. 2019). Other main risk factors for CAP in children include prematurity, lack of exclusive breastfeeding, malnutrition, indoor and outdoor air pollution, and household crowding (Bradley et al. 2011, Jackson et al. 2013). Immunocompromised children are at a higher risk of developing pneumonia. Genetic variants of innate immune factors such as mannose-binding lectin (MBL), TLRs, IL-6, and TNF-α have been associated with a higher risk of respiratory infections (Koch et al. 2001, Revai et al. 2009, Toivonen et al. 2017). In early infancy, the association between RV infection and increased nasopharyngeal colonization by *S. pneumoniae* has been linked to genetic variations of MBL (Karppinen et al. 2013). In the first year of life, maternal HIV, maternal smoking, male sex, and malnutrition were associated with an increased incidence of pneumonia in a South African birth cohort (le Roux et al. 2015). Incomplete vaccination, concerning particularly PCV and *H. influenzae* type b conjugate vaccination but also measles vaccination, is an obvious risk factor for childhood pneumonia. An association of parental asthma, earlier wheezing, and exposure to tobacco smoke with a more severe clinical course of pneumonia has been reported (Erdem et al. 2018). Many host and disease characteristics, such as age below two months, diagnosis of *Pneumocystis jirovecii*, chronic underlying disease, HIV/AIDS, and severe malnutrition, as well as socio-economic and environmental factors, such as young maternal age, low maternal education, low socioeconomic status, second-hand smoke exposure, and indoor air pollution, are risk factors for mortality of CAP (Sonego et al. 2015). Dysbiosis or imbalance in the normal lung microbiome is also essential in the pathogenesis of pneumonia (Dickson et al. 2014, Zar et al. 2017).

2.6 Treatment and prevention

Empirical antibiotic therapy is directed at the most likely aetiological pathogens, most importantly *S. pneumoniae*. In general, oral amoxicillin is used for milder cases, especially for those who do not require hospitalization (Atkinson et al. 2007a, Bradley et al. 2011, Harris et al. 2011), whilst intravenous therapy, usually benzyl penicillin or ampicillin, is administered in more severe cases needing hospitalization (Nascimento-Carvalho and Nascimento-Carvalho 2019). In a randomised controlled equivalence trial by Atkinson et al., oral amoxicillin was shown to be as effective as intravenous benzyl penicillin in most children admitted to hospital with pneumonia, but most severe cases were excluded in this study (Atkinson et al. 2007b). Macrolides are used as monotherapy or combined with beta-lactam antibiotics if *M. pneumoniae* infection is likely (Bradley et al. 2011). Current recommendations for the optimal duration (7–10 days) of antibiotic treatment for CAP are based on sparse evidence (Bradley et al. 2011, Le Saux and Robinson 2015). In a recent double-blinded clinical trial, a short-course (5 days) antibiotic therapy with high-dose amoxicillin appeared to be comparable to the standard care (10 days) for the treatment of previously healthy children with CAP not requiring hospitalization (Pernica et al. 2021). Other studies with similar results stand for a shorter (3–5 days) antibiotic course, but there are many limitations concerning the definition of pneumonia, the lack of chest radiographs, variations in disease severity or the high proportion of probable viral pneumonias (Greenberg et al. 2014, Bielicki et al. 2021, Pernica et al. 2021, Kuitunen et al. 2022, Li et al. 2022, Williams et al. 2022). Withholding antibiotic treatment in LRTIs is increasingly considered, but severe pneumonia and empyema remain therapeutic emergencies (Cohen et al. 2017). In other LRTIs than pneumonia, antibiotic treatment has not been shown to enhance recovery (Little et al. 2021).

Supporting care includes bed rest, maintenance of cleared upper airways, and adequate intake of fluids, electrolytes, and calories. In case of hypoxia, simple oxygen administration via nasal cannulae or mask, or ventilatory support with high-flow nasal oxygen or non-invasive ventilator, such as continuous positive airway pressure (CPAP), is necessary. In severe cases, initiation of invasive mechanical ventilation should be considered.

Anti-influenza agents such as oseltamivir are used for influenza pneumonia, but otherwise antiviral therapies are not available, even though CAP has a viral aetiology in many patients. Adjunctive corticosteroid therapy is not recommended routinely, not even in complicated CAP (Tagarro et al. 2017), neither are intravenous immunoglobulins for immunocompetent children or monoclonal or polyclonal antibodies.

Vaccines against *S. pneumoniae* and *H. influenzae* type b have played an important role in pneumonia prevention. Moreover, an annual influenza vaccination

reduces risk of pneumonia as a complication of influenza. Palivizumab for certain infants and children with increased risk of severe RSV infection is justifiable. Overall, non-pharmaceutical preventive methods, including hand hygiene, social distancing, and use of respiratory masks, are effective methods in avoiding respiratory infections (Jacobs et al. 2013).

2.7 Outcome and complications

The great majority of children with uncomplicated pneumonia have no long-term sequelae and make a complete recovery (Virkki et al. 2005, Harris et al. 2011). However, childhood pneumonia may be associated with asthma, impaired airway function and chronic respiratory symptoms and could be a marker for a group of children at risk of chronic respiratory disease, both obstructive and restrictive lung disease (Gold et al. 1989, Johnston et al 1998, Castro-Rodríguez et al. 1999, Virkki et al. 2005, Eastham et al. 2008, Edmond et al. 2012, Chan et al. 2015, Rantala et al. 2015, Collaro et al. 2021). Severe respiratory infection may impair lung growth when occurring at the time of rapid lung development (Johnston et al. 1998). In a study by Castro-Rodríguez, 26% of children with radiologically confirmed uncomplicated pneumonia during the first three years of life had physician-diagnosed asthma at the age of 11 years (Castro-Rodríguez et al. 1999). Similarly, in a recent study by Collaro et al., pneumonia before five years of age was associated with an increased risk of childhood asthma (Collaro et al. 2021).

In a recent study creating risk models for severe pneumonia in children (<18 years), the strongest predictors of severe outcome were extremes of age, vital signs, chest indrawing, and radiological infiltration pattern (multilobar or nonlobar infiltrates) (Williams et al. 2016). Delayed or inadequate antimicrobial therapy can lead to poor outcomes. About 3% of CAP cases in children become complicated (Legg and Rampton 2020). Complications of pneumonia should be suspected if there is no response to appropriate antibiotic treatment within 48–72 hours (Balfour-Lynn et al. 2005, Lahti et al. 2007). Early detection of treatment failure is crucial. Complications of CAP can be local/pulmonary, such as parapneumonic effusion, empyema, necrotising pneumonia, or lung abscess, or systemic, such as bacteraemia, multiorgan failure, acute respiratory distress syndrome, or disseminated intravascular coagulation (Pabary and Balfour-Lynn 2013, de Benedictis et al. 2020). Death due to complicated pneumonia in children is fortunately rare. *S. pneumoniae* and *S. aureus* are the most common causative organisms of complicated CAP (Azzari et al. 2019, Liese et al. 2019). Extensive destruction and liquefaction of lung tissue are characteristics for necrotising pneumonia, the incidence of which is up to 7% of CAP cases in children (Masters et al. 2017). Despite its severe nature, necrotising pneumonia usually has a good long-term recovery (Masters et al. 2017).

2.7.1 Empyema

Pleural effusion can be exudative, fibrinopurulent, or organized with fibroblastic activity and peel formation. Empyema, simply described as pus in the pleural space, is an advanced stage of parapneumonic effusion. The dysregulation of hydrostatic and oncotic pressure balance between the systemic and pulmonary circulations and the pleural space, and obstruction of lymphatic drainage contribute to the pleural fluid accumulation (Feller-Kopman and Light 2018). Inflammatory markers, primarily high systemic immune-inflammation index (SII = peripheral blood platelet x neutrophil/lymphocyte counts (cells/L)), low lymphocyte-monocytes ratio, high CRP, and high absolute neutrophil count, can be useful in differentiating empyema from parapneumonic effusion (Güneylioğlu et a. 2022).

In the past few decades, the incidence of empyema in children has increased worldwide (Byington et al. 2002, Lahti et al. 2007, Byington et al. 2010, Grijalva et al. 2011). Moreover, the introduction of the 7-valent PCV (PCV7, which covers serotypes 4, 6B, 9V, 14, 18C, 19F, 23F) was still associated with an increased incidence of empyema, obviously due to the non-vaccine serotypes, while the incidence of CAP declined (de Benedictis et al. 2020). In Utah, after the licensure of PCV7 in 2000, empyema was detected in even 36% of children with pneumonia treated in a hospital in 2007 (Byington et al. 2010). When the PCV7 was globally replaced with the 13-valent PCV (PCV13, which also covers serotypes 1, 3, 5, 6A, 7F, 19A), the incidence of empyema declined substantially (Wiese et al. 2016, Diaz-Conradi et al. 2019, Liese et al. 2019). After the 13-valent PCV implementation, group A streptococcus became the leading cause of empyema (45%), while pneumococcal infections decreased significantly (from 79% to 36%, $p<0.001$) (Madhi et al. 2019). However, as a proportion of all paediatric intensive care unit admissions, empyema admissions were noticed to increase in the post-PCV13 era (Subhi et al. 2022). In Germany, the incidence of parapneumonic pleural effusion or empyema decreased from 2009 to 2013 (general immunization with PCV13 since 2009) but then subsequently increased until 2018 (Sorg et al. 2021).

Prolonged fever, tachypnoea, pain on abdominal palpation, and high serum CRP levels are clinical predictors for parapneumonic empyema in children (Lahti et al. 2007). Bacterial pneumonia and preadmission ibuprofen usage have been identified as risk factors for the development of parapneumonic empyema in children (Elemraid et al. 2015).

Systemic antimicrobials directed primarily at *S. pneumoniae* and *S. aureus* (e.g. intravenous penicillin or cefuroxime combined with clindamycin) are the basis of the treatment, combined with interventional procedures if needed. In symptomatic effusions, pleural drainage should be performed (Balfour-Lynn et al. 2005, Sahn 2007), and the use of intrapleural fibrinolytics may enhance recovery (Islam et al. 2012). If these non-operative interventions are insufficient, video-assisted

thoracoscopic surgery (VATS) might be necessary to advance the resolution of empyema (Pacilli and Nataraja 2019), and thoracotomy, or a mini-thoracotomy from a smaller incision, is rarely indicated. The need of supportive therapy with oxygen, mechanical ventilator, or nutrition must be actively assessed.

Only few studies have investigated the long-term anatomical and functional consequences in the lungs after empyema (McLaughlin et al. 1984, Redding et al. 1990, Kohn et al. 2002, Satish et al. 2003, Cohen et al 2012, de Benedictis et al. 2019, Maffey et al. 2019). Previously reported complications of empyema include chest wall shape abnormality, pleural thickening, and abnormalities in lung function (McLaughlin et al. 1984, Redding et al. 1990, Kohn et al. 2002, Bradley et al. 2011, Harris et al. 2011). Long-term clinical outcome of paediatric pleural empyema is good in most cases, although pleural thickening was found in chest radiograph in 85% of patients 1–6 months after discharge from hospital (Cohen et al 2012). Pleural thickening detected by chest radiograph has been observed to resolve over a period of 2–16 months (Satish et al. 2003). British Thoracic Society guidelines recommend that children with empyema should be followed-up after discharge until they have recovered completely and their chest radiograph has returned to near normal (Balfour-Lynn et al. 2005, Harris et al. 2011).

3 Aims

The main purpose of this thesis was to evaluate the microbial diagnostics of pneumonia, to assess the role of RV in CAP and biomarker levels in RV-pneumonia, and to study the recovery of children after complicated CAP.

The specific aims of the study were:

1. to evaluate thoroughly the roles of all of the most common respiratory micro-organisms in the aetiology of CAP, and to determine the prevalence of mixed viral-bacterial infections. (I)
2. to assess the risk factors for, clinical characteristics and prevalence of RV-associated pneumonia in children. (II)
3. to establish the levels of viral and bacterial biomarkers in RV-associated pneumonia in children. (III)
4. to investigate the recovery of children hospitalized with severe CAP complicated by empyema. (IV)

4 Materials and Methods

4.1 Subjects and study design

The thesis consists of four studies carried out at the Department of Paediatrics and Adolescent Medicine, Turku University Hospital, Turku, Finland. The number of patients in each study and the study periods are listed in Table 4. In all studies, pneumonia was defined as the presence of alveolar or interstitial pneumonic infiltrates on the chest radiograph with simultaneous signs and/or symptoms of an acute infection. In study IV, the criteria for parapneumonic empyema were the signs and symptoms of acute infection, radiological evidence of pneumonia and pleural fluid, and the detection of purulent pleural fluid by pleural puncture. Tachypnoea was defined (in study II) as respiratory rate >60/min in infants younger than 2 months of age, >50/min in infants from 2 to 12 months, >40/min in children from 1 to 5 years and >30/min in children 6 years of age or older.

Table 4. Study populations.

	Population	**Age criteria**	**Time of admission**
Study I	76 children with CAP	6 months – 15 years	Jan 2006 – Apr 2007*
Study II	313 children with CAP	<18 years	2003–2014
Study III	24 children with CAP**	<16 years	May 2014 – Dec 2015
Study IV	26 children with parapneumonic empyema***	<16 years	Jan 1991 – Feb 2007

* The Paediatric Infectious Disease Ward of Turku University Hospital

** Patients with any severe underlying condition were excluded.

*** Of the 37 empyema patients eligible for the study, 2 had died due to causes unrelated to empyema, 2 were too young to undertake spirometry and MRI, 2 had a chronic neurological illness and 5 were not reached, resulting in a study population of 26 persons.

In study II, we searched the Electronic Registry of the Turku University Hospital for International Classification of Diseases (ICD-10) codes related to pneumonia (J12–18, J10.0, J11.0, J85, J86, J90) to identify children who were hospitalized with

CAP. Of this patient population, we identified those with a diagnostic PCR test for RV performed during the hospitalization for CAP.

In study III, we added a comparison group of children with a RV-positive upper respiratory tract infection (URTI) from the study by Toivonen et al. to evaluate the role of MxA (Toivonen et al. 2015). From that study population, we included all RV-positive children who had respiratory symptoms, no other viral findings, and no signs of bacterial co-infection (CRP <20 mg/L and normal WBC count [5.0–15.0 × 10^9/L]), resulting in a comparison group of 13 children. Children with RV-positive pneumonia were separately compared to children with pneumonia positive for another respiratory virus, excluding a child positive for RV and another virus, and to the comparison group of children with RV-positive URTI.

4.2 Data collection (I–IV)

The medical records of the study children were reviewed to collect the clinical and background data. In addition, in study III, enrolled children with their caregivers were interviewed with the use of a structured questionnaire, and in study IV, patients underwent a detailed interview with a standardized respiratory questionnaire and a complete physical examination at the control visit.

4.3 Sputum samples (I)

Sputum production was induced by inhalation of 5.0% hypertonic saline solution. The sputum sample was obtained by aspirating through the nostrils in 69 (91%) children and by expectoration in 7 (9%) children who were old enough to produce an adequate sputum sample. A good-quality sputum sample with 25 leukocytes per low-power field was obtained from 76 of 133 children otherwise eligible for the study. The details of the sputum collection method have been described previously by Lahti et al. (Lahti et al. 2009).

4.4 Viral detection (I–III)

Different viral detection methods were conducted as listed in Table 5, using sputum samples in study I, nasal swabs in study III, and variable samples in study II. In study II, viral detection methods were used in accordance with the clinical practices at the time of admission. In study III, nasal swab (Copan, Brescia, Italy) samples for viral detection were collected on admittance, and RV-positive patients were followed weekly by nasal swabs until negative for RV. RV PCR Ct values, which are inversely proportional to the viral loads, were determined in study III and compared between children with pneumonia and URTI.

Table 5. Viral detection methods.

Method	Viruses studied	Performed as described previously	Study
In-house end-point RT-PCR	RV, EV	Lönnrot et al. 1999	II
Multiplex RT-PCR for 12 viruses*	AdV, influenza A and B viruses, RSV A and B, HMPV, PIV types 1, 2 and 3, RV A and B, CoV 229E/NL63 and OC43/HKU1		I, II
Multiplex RT-PCR for 16 viruses**	Those listed in multiplex RT-PCR for 12 viruses, and PIV type 4, RV C, EV, HBoV		II, III
Real-time PCR	AdV	Supporting Information of study I	I
Real-time PCR	HBoV	Lahti et al. 2009	I
Real-time RT-PCR	EV, RV, RSV	Peltola et al. 2008, Österback et al. 2013	I–III
Real-time RT-PCR	HMPV	Lahti et al. 2009	I
Real-time RT-PCR	Human parechoviruses	Benschop K et al. 2008	I
Real-time RT-PCR	PIV4, influenza C virus	Supporting Information of study I	I
Sequence analysis	RV species determination	Peltola et al. 2008	I, III
Viral antigen detection by time-resolved fluoroimmunoassay	Influenza A and B viruses, PIV types 1, 2 and 3, RSV, AdV	Lahti et al. 2009	I

* Seeplex RV12 ACE Detection; Seegene, Seoul, Korea
** Anyplex RV16 Detection; Seegene, Seoul, Korea

4.5 Bacterial detection (I, III)

In study I, gram staining (standard method), semiquantitative bacterial culture (standard method), quantitative *S. pneumoniae* PCR on *S. pneumoniae* culture positive samples, and *M. pneumoniae* PCR on sputum specimens and the IgM immunoassay test for *M. pneumoniae* were previously performed as described by Lahti et al. (Lahti et al., 2009). We completed these bacterial studies by multiplex PCR (Prove-It Sepsis) searching for respiratory bacteria (*S. pneumoniae*, *H. influenzae*, *S. aureus* and *S. pyogenes*), performed by the manufacturer at Mobidiag (Helsinki, Finland) for 14 samples with negative results or only normal/mixed flora found in previous bacterial studies. All of the patients were previously immunized against *H. influenzae* type b, and therefore only non-typeable serogroups of *H. influenzae* were studied.

In study III, *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. aureus* were cultured from nasopharyngeal swab (Copan) samples. Bacterial blood culture, *S. pneumoniae* antigen test from urine, and serum *M. pneumoniae* antibodies were also studied.

4.6 Other laboratory tests (III, IV)

In study III, the concentration of MxA was determined in a whole blood sample collected on the first morning after admittance. Blood was diluted 1:20 in hypotonic buffer and stored at -70 °C until measurement of the MxA level by an enzyme immunoassay as described earlier (Toivonen et al. 2015). WBC, plasma CRP levels, and plasma PCT levels were determined by routine laboratory methods on admittance, on the following morning, and again if clinically needed.

In study IV, complete blood count, serum IgG, IgA, and IgM levels, IgG subclass levels, and IgG antibody responses to tetanus immunization were studied at the Laboratory of Turku University Hospital, and the results were compared to standard age-related values. The complement function was measured using standard protocols for the classical pathway, the alternative pathway, and the lectin pathway (COMPL 300 Wielisa kit, Wieslab, Lund, Sweden). The cutoff levels for the decreased activity of the complement were 60% for the classical pathway, 40% for the alternative pathway, and 10% for the lectin pathway. Serum MBL concentration and MBL genotyping were studied at the Department of Medical Microbiology, University of Turku. The wild type of *MBL2*, the gene for human MBL, was referred to as A, and the MBL2-coding region variants were referred to as B, C, or D, based on the location of the gene variation. Serum MBL levels less than 0.8 mg/L were considered abnormally low.

4.7 Radiology (IV)

Contrast medium-enhanced lung MRIs were obtained with a Siemens 1.5-T MR imager (Magnetom Avanto, Siemens, Erlangen, Germany) without general anaesthesia as described earlier (Peltola et al. 2008). All MRIs were reviewed by one paediatric radiologist (E.S.) using Kodak Carestream 5.0 software (Eastman Kodak, Rochester, NY) and standard soft-copy reading techniques. Images were evaluated particularly for pleural scarring/thickening, nodules, micronodules (<5 mm), peribronchovascular changes, linear consolidation, mediastinal shift, and mediastinal organs. According to their importance and extent, MRI findings were classified into three groups: (0) no findings, (1) moderate findings, and (2) significant findings. Pleural scarring was considered significant if it extended over

10 mm. Contraindications for MRI were implanted devices or foreign bodies, the possibility of being pregnant, an age of less than 5 years and the lack of co-operation.

Chest radiographs and computed tomography scans of the study patients during empyema were re-reviewed by one paediatric radiologist (R.V.) to see how many patients had necrotizing pneumonia or lung abscess in addition to empyema. Radiographic findings of necrotizing pneumonia included the destruction of lung parenchyma, loss of contrast enhancement (indicating devitalized tissue), and the development of multiple thin-walled (<2 mm) fluid- or air-filled cavities lacking an enhancing border (Donnelly and Klosterman 1998). Lung abscess was defined as a thick-walled (>2 cm) cavity with an enhancing border and loss of normal architecture in the surrounding parenchyma (Donnelly and Klosterman 1998).

The follow-up chest radiographs included in this study were obtained using Canon DR imaging systems (Canon CXDI-40C and CXDI-31; Canon, Medical Equipment Group, Tochigi, Japan), and each follow-up chest radiograph was reviewed by a paediatric radiologist at the Department of Paediatric Radiology, Turku University Hospital.

4.8 Lung function tests (IV)

Forced expiration volume in 1 sec (FEV1) and forced vital capacity (FVC) were obtained with a Jaeger Masterlab spirometre (Jaeger AG, Würzburg, Germany) using the best three efforts. A bronchodilator challenge was carried out after determination of the baseline. Three doses of salbutamol (0.2 mg/dose) were administered by an inhaler (Buventol Easyhaler; Orion Corporation, Espoo, Finland). After 15 min, a re-measurement of pulmonary function was carried out in the same manner as above. After the bronchodilator challenge, any changes in FEV1 and FVC of >12% or >200 ml were considered clinically significant. The results were compared to the age-, height-, and weight-related standard values of the Finnish people (Koillinen et al. 1998).

4.9 Statistical analysis

Descriptive statistics are given as proportions, medians, or means with ranges, interquartile ranges (IQR), or standard deviations (SD). The statistical analyses were performed with SAS version 9.2 and 9.4 (SAS Institute Inc., Cary, NC, USA) or SPSS version 23.0 (IBM SPSS Statistics, IBM Corp., Armonk, NY, USA).

In study I, univariate associations between age and the frequency distribution of viral and bacterial pathogens were analysed with logistic regression. Univariate associations between the distributions of viral, bacterial, and mixed viral–bacterial detections and the clinical picture of illness were studied with multinomial logistic

regression analysis. A multiple multinomial logistic regression analysis was performed with all of the predictor variables (age, a WBC count and a serum CRP level on admission, and the highest fever during stay at hospital) simultaneously. Associations between viral, bacterial, and mixed viral–bacterial detections and the chest radiograph were studied with Fisher's exact tests. For children with at least one virus, the univariate association between predictor variables and number of viruses was studied with logistic regression analysis. Thereafter, a multiple logistic regression analysis was performed with all of the predictor variables simultaneously. The clinical pictures of the most commonly found combinations of viruses and bacteria (the three most common viruses (RV, HBoV and HMPV) with one or more bacteria, and the three most common bacteria (*S. pneumoniae*, *H. influenzae* and *M. catarrhalis*) with one or more viruses) were compared by using one-way analysis of variance for continuous variables and Fisher's exact tests for chest radiographs. *M. pneumoniae* serology results were excluded from our statistics, as they were available in only 47% of the study population.

In study II, the χ^2 test or Fisher's exact test were performed to compare proportions between the groups, and the Wilcoxon rank sum test was used to compare continuous data. A multivariate logistic regression analysis was conducted to examine the independent risk factors (age, sex, asthma or reactive airway disease, premature birth, neurological condition, cardiovascular disease, and atopic eczema or sensitization to aeroallergen) for RV-positive CAP.

In study III, for continuous data, comparisons between two groups were performed by use of the *t* test. For categorical data, comparisons were performed by use of Fisher's exact test.

In study IV, basic descriptive values were calculated but no statistical tests were performed.

4.10 Ethics

The prospective studies (I, III, IV) were approved by the Ethics Committee of the Hospital District of South-West Finland. Signed, informed consent was obtained from the participant (in study IV, if ≥18 years of age) or the parent or guardian of each participating child before enrolment.

5 Results

5.1 Viruses and bacteria in sputum samples of children with community-acquired pneumonia

Respiratory microbes were detected in the sputum samples of 74 (97%) of the 76 children studied. Viruses were identified by antigen detection and PCR, and bacteria by culture and PCR. Viruses were found in 55 (72%) and bacteria in 69 (91%). Both viral and bacterial pathogens were found in 50 (66%), and altogether 64 children (84%) had any co-detections (viral-viral, viral-bacterial, or bacterial-bacterial). Proportions of microbiological findings are shown in Figure 1. Two viruses were detected in 17 (22%) and three viruses in six (8%). One child had six microorganisms (*H. influenzae, M. catarrhalis, S. pneumoniae*, EV, HBoV, and RV) simultaneously detected, and altogether, seven children (9%) had two or three bacteria with two or three viruses co-detected. The detailed microbiological findings are listed in Table 2 of original publication I. The mean age of the 76 study children was 4.7 years (SD ±3.9 years).

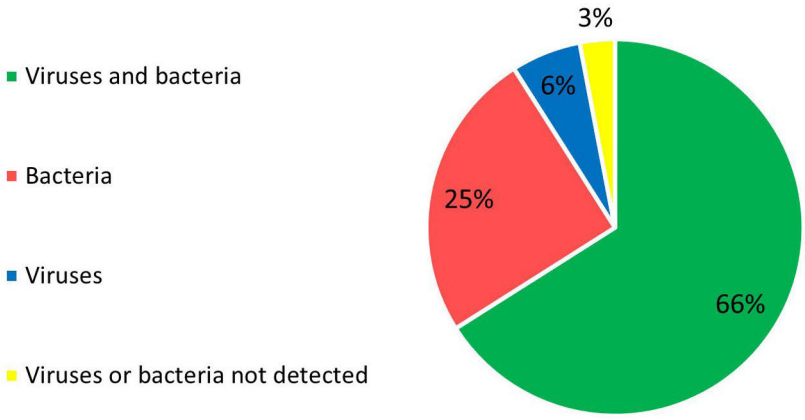

Figure 1. Proportions of microbiological findings in induced sputum in children with community-acquired pneumonia.

The most commonly detected viruses were RV (in 30% of the children), HBoV (in 18%), HMPV (in 14%), AdV (in 11%), PIV3 (in 8%), CoV (in 7%), and RSV (in 7%) (Figure 2). Sequence analysis of 14 RV-positive samples revealed nine belonging to RV A species and five to RV C species. A few cases of influenza A virus (n = 3), EV (n = 2), human parechovirus (n = 2), PIV1 (n = 2), influenza B virus (n = 1), PIV2 (n = 1), and PIV4 (n = 1) were detected. The most common combination of viruses was HBoV–RV (n = 6), and as much as 79% of HBoV-positive samples (vs. 57% of RV-positive samples) were also positive for other viruses, whereas HMPV and RSV were most commonly found as sole viral agents (64% and 60%, respectively). Viruses were detected in 80% of children <5 years of age.

Bacterial detections were common, even though sputum samples were collected after the onset of intravenous antibiotic treatment in 34 (45%) of the studied children. The most commonly detected pathogen was *S. pneumoniae* (in 50% of the children), and 66% of *S. pneumoniae* -positive children had a concomitant viral detection. RV–*S. pneumoniae* was the most commonly found combination of viruses and bacteria (n = 12). *H. influenzae* (in 38%), *M. catarrhalis* (in 28%) and *S. aureus* (in 13%) were other frequently detected bacteria (Figure 2). The most common combination of co-detected bacteria was *S. pneumoniae* with *M. catarrhalis* (n = 12). The multiplex PCR detected bacteria in 71% (10/14) of patients with negative bacterial culture results.

5.1.1 Clinical, laboratory and radiological findings

Significant differences in the clinical picture of pneumonia were not found between different aetiological groups (Table 6). Furthermore, the presence of a single versus multiple viruses as the aetiology was not associated with the clinical presentation. Interstitial infiltration (14%) on the chest radiograph indicated a sole viral or mixed viral–bacterial infection, whereas those with a sole bacterial infection had only alveolar infiltrations on the chest radiograph (Table 6). Of the 19 children with a sole bacterial detection, 89% had a CRP level ≥60 mg/L and 68% had a WBC count ≥15.0 $\times 10^9$/L on admission, whereas the same values of children with evidence of both viruses and bacteria were 64% and 50%, respectively. The only significant clinical distinction between different concomitantly detected micro-organisms was the higher WBC count of those with *S. pneumoniae* and one or more viruses compared to those with *H. influenzae* and one or more viruses (p=0.001) (Table 6). Treatment failure (fever ≥38ºC lasting for ≥48 h regardless of antibiotic treatment) occurred in six children (8%), and all of them had evidence of mixed viral-bacterial infection.

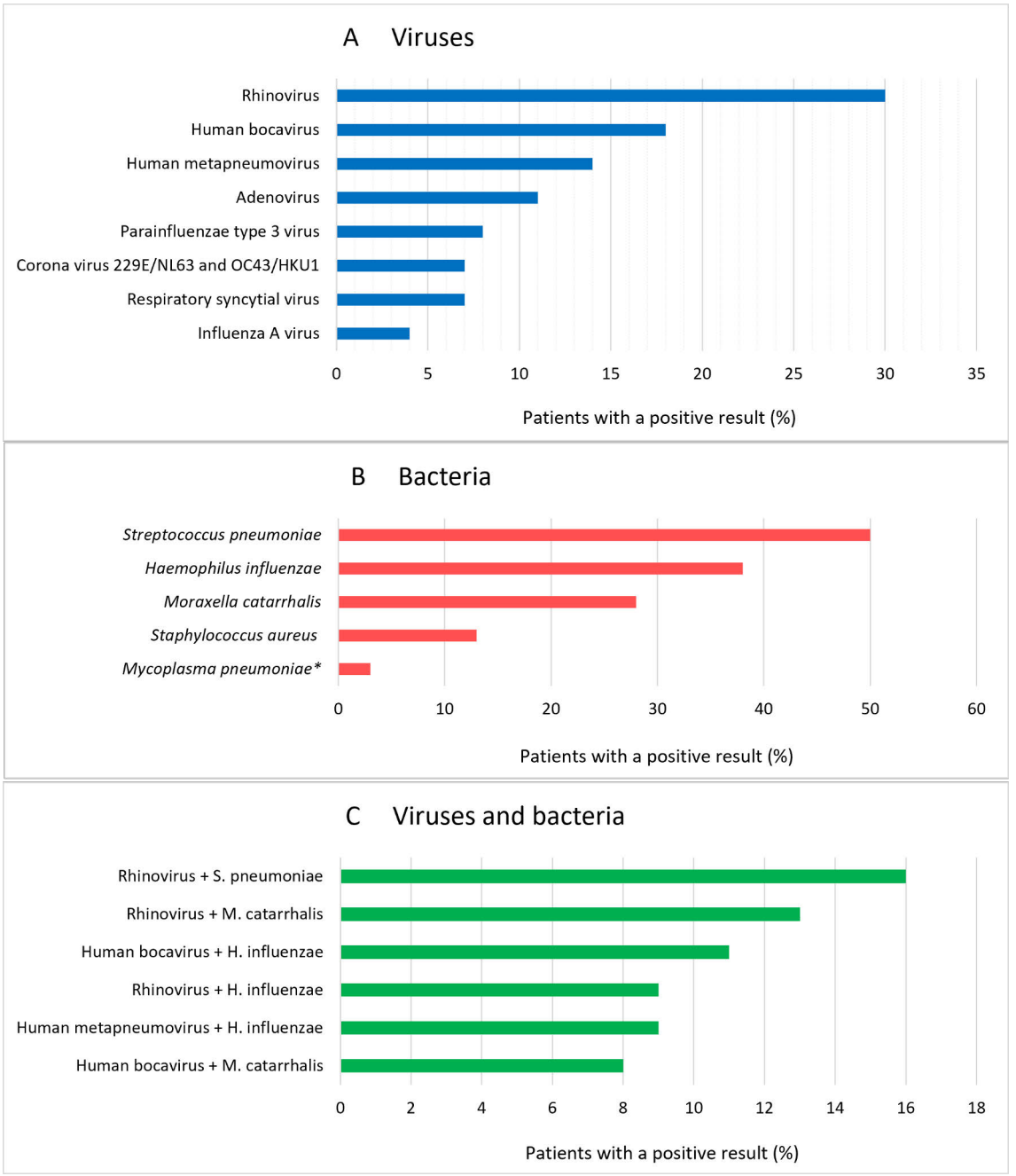

Figure 2. Frequencies of the most common viral (A), bacterial (B), and viral-bacterial (C) detections in children with community-acquired pneumonia. Total 76 patients. **M. pneumoniae* was found altogether in 22% of those 36 patients who were studied both serologically and by PCR.

Table 6. Clinical, laboratory and radiological findings of children with community-acquired pneumonia associated with viruses, bacteria, both viruses and bacteria, or the most common combinations of viruses and bacteria. Modified from original publication I.

Characteristic	Viruses	Bacteria	Both viruses and bacteria	RV with ≥1 bacterium	HPMV with ≥1 bacterium	*S. pneumoniae* with ≥1 virus	*H. influenzae* with ≥1 virus
N (%)	4 (5)	19 (25)	50 (66)	10 (13)	7 (9)	14 (18)	14 (18)
Age (years)*	2.0 (0.6)	6.0 (4.0)	4.4 (3.9)	5.4 (4.9)	4.1 (1.8)	6.4 (5.4)	3.5 (2.1)
On admission							
WBC count ($\times 10^9$/L)*	18.6 (6.1)	20.1 (8.0)	17.0 (8.8)	21.0 (8.4)	11.3 (9.3)	21.4*** (6.5)	11.3*** (6.2)
Serum CRP level (mg/L)*	82.5 (146.4)	166.4 (78.2)	121.8**[1] (103.1)	129.9 (95.0)	50.3 (33.3)	132.6 (99.1)	99.3 (78.9)
Fever (ºC)*	40.1**[2] (0.4)	39.7**[1] (0.4)	39.7**[7] (0.4)	39.6**[1] (0.6)	39.6 (0.3)	39.9**[1] (0.4)	39.7**[2] (0.6)
Chest radiograph findings							
Alveolar infiltrates, *n* (%)	3 (75)	19 (100)	43 (86)	9 (90)	7 (100)	12 (86)	12 (86)
Interstitial infiltrates, *n* (%)	1 (25)	0 (0)	9 (18)	1 (10)	1 (14)	2 (14)	3 (21)

* Values are mean (SD); ** Number of missing data; *** Significant difference (p=0.001) between the groups of *S. pneumoniae* with viruses and *H. influenzae* with viruses. Otherwise, there were no significant differences (p<0.05) in the studied variables between different aetiological groups.

5.2 Characteristics of hospitalized rhinovirus-associated community-acquired pneumonia in children

During the years 2003–2014, 81–143 children per year needed hospitalization for CAP in Turku University Hospital (Turku, Finland) (Figure 3).

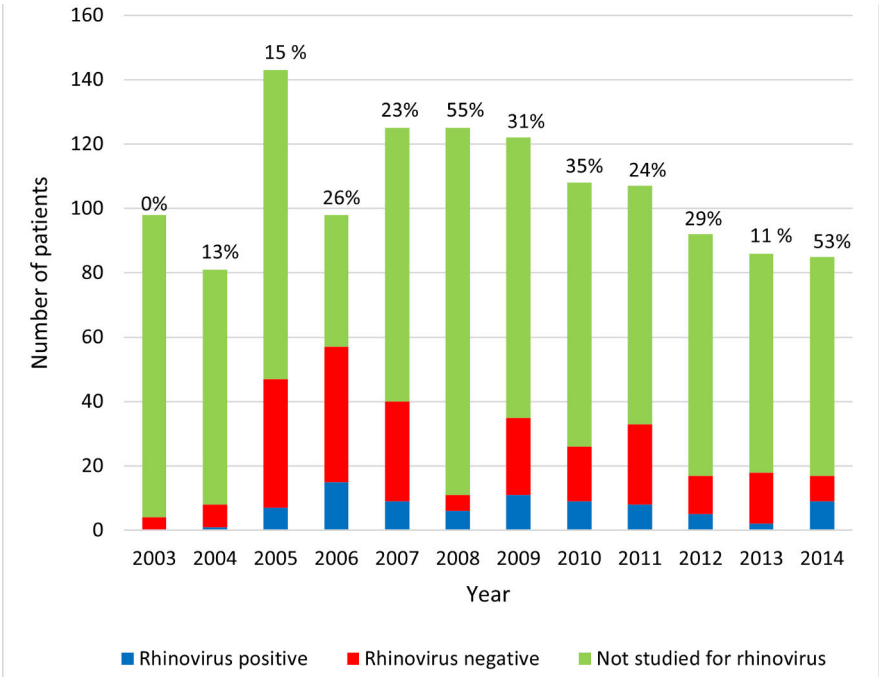

Figure 3. The number of pneumonia inpatients stratified by RV status during the years 2003–2014. The percentage over the bar indicates the proportion of RV-positive patients among those who where studied for RV. Modified from original publication II.

The collection of the final study population is shown in Figure 4. Of 1270 inpatients, 25% had PCR diagnostics for RV done during the hospitalization, and in 26% of them, RV was detected. RV pneumonia was shown to have a peak of occurrence in October (Figure 5). Other viruses were detected by PCR in 13 (16%) of 82 children positive for RV and in 45 (19%) of 231 children negative for RV. Pathogenic bacteria were detected in the blood culture of five children: 1 RV-positive and 3 RV-negative children had *S. pneumoniae* and 1 RV-negative child had *S. pyogenes* bacteraemia.

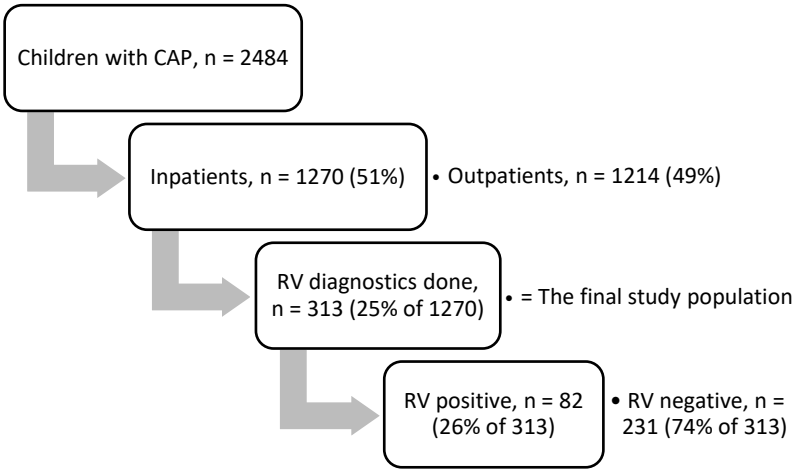

Figure 4. The enrolment of children with RV-associated community-acquired pneumonia.

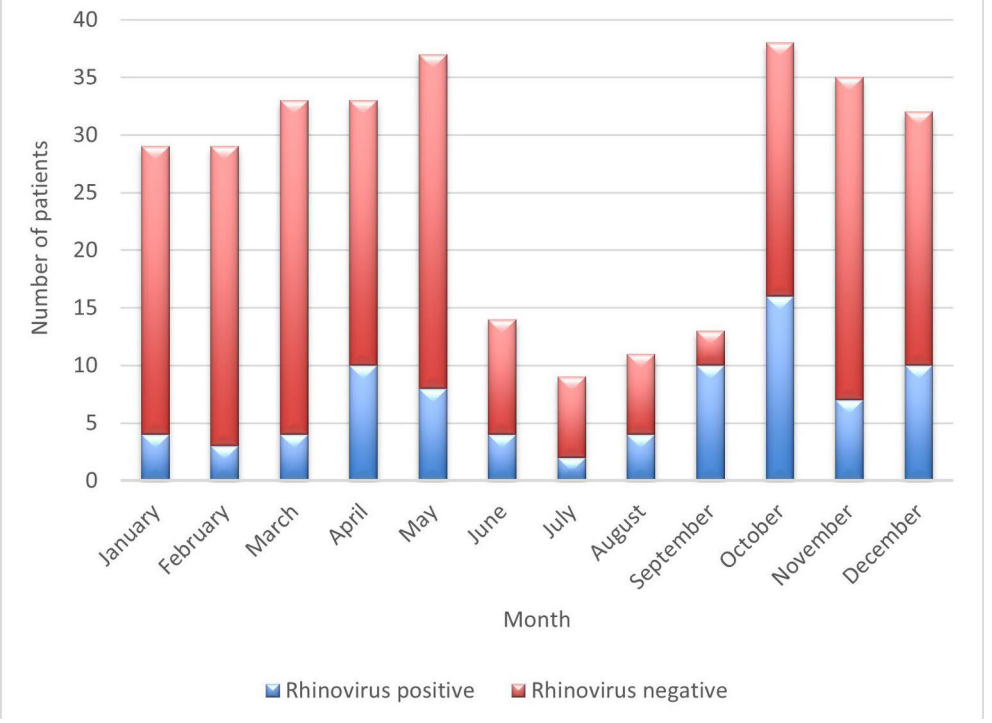

Figure 5. The total number of pneumonia inpatients studied for RV per month from 2003 to 2014, and numbers of RV-positive and RV-negative patients.

The median age of the final study population (n = 313) was 3.1 (IQR 1.5–7.4) years (Table 7). RV-positive children were younger than RV-negative children

(median age 2.6 [IQR 1.1–4.6] years vs. 3.5 [IQR 1.7–8.3] years, p = 0.002). A total of 171 (55%) were males and 142 (45%) females.

RV-positive children had a history of preterm birth more frequently than RV-negative children (16% vs. 5%, p = 0.002 in the univariate analysis; odds ratio (OR) 2.89, 95% confidence interval (CI) 1.21–6.92, p = 0.017 in the multivariate logistic regression analysis) (Table 7). At the time of admission, asthma was diagnosed in 15% and atopic eczema or sensitization to aeroallergen in 27% of RV-positive children, while the occurrence in RV-negative children was 12% and 19%, respectively (no statistically significant differences, Table 7).

The clinical features and laboratory findings of pneumonia in RV-positive and RV-negative children are compared in Table 8. Although fever and cough were certainly the most common symptoms in all children, fever was documented less frequently in RV-positive than in RV-negative children (84% vs. 97%, p < 0.001). RV-positive children had a shorter duration of symptoms before referral than RV-negative children (median 3 [IQR 1–7] days vs. 5 [IQR 2–8] days, p = 0.006). The WBC count determined on admission was higher in RV-positive than in RV-negative children (median 16.4 [IQR 12.2–25.0] × 10^9/L vs. 14.1 [8.5–20.4] × 10^9/L, p = 0.002), whereas CRP concentrations did not differ significantly (Table 8). The above-mentioned findings (significant differences associated with age, a history of preterm birth, the duration of symptoms before referral, the frequency of fever, and the median WBC count) remained similar when compared sole RV-positive children (no other viruses detected) (n = 69) with virus-negative children (n = 186).

The outcomes of pneumonia were similar in children with or without RV (Table 8). The median duration of hospitalization was 2 days. Compared to RV-negative children, RV-positive children more often needed intensive care unit admission (20% vs. 15%), oxygen supplementation (34% vs. 27%), and invasive mechanical ventilation (10% vs. 5%), but the differences were not statistically significant. All patients were treated with antibiotics during hospitalization, and 13% of RV-positive and 21% of RV-negative children had antimicrobial therapy initiated before referral to the hospital. Complicated pneumonia was documented in 6% of RV-positive and in 9% of RV-negative patients. The total mortality rate was 0.3% with one death in the RV-negative group; a 1-year-old boy with no underlying conditions, positive for influenza B and adenovirus, died of pneumonia after 5 days of hospital treatment.

Table 7. Demographic characteristics and underlying conditions of children with community-acquired pneumonia requiring hospitalization; RV-positive patients compared to RV-negative patients. Modified from original publication II.

Demographic characteristics and underlying conditions	RV-positive, N = 82	RV-negative, N = 231	Univariate analysis *P*-value	Logistic regression analysis *P*-value	Logistic regression analysis OR (95% CI)
Age, yr – median (IQR)	2.59 (1.08–4.59)	3.51 (1.68–8.26)	0.002*	0.010	0.91 (0.86–0.98)
Age group – no. (%)					
<2 years	33 (40)	77 (33)			
2–4 years	32 (39)	55 (24)			
5–9 years	10 (12)	53 (23)			
10–17 years	7 (9)	46 (20)			
Males – no. (%)	49 (60)	122 (53)	0.278**	0.241	1.38 (0.81–2.35)
Underlying condition – no. (%)	39 (48)	94 (41)	0.280**		
Atopic eczema or sensitization to aeroallergen	22 (27)	43 (19)	0.115**	0.154	1.59 (0.84–3.01)
Preterm birth	13 (16)	12 (5)	0.002**	0.017	2.89 (1.21–6.92)
Asthma or reactive airway disease	12 (15)	28 (12)	0.558**	0.363	1.45 (0.65–3.20)
Cardiovascular disease	6 (7)	8 (3)	0.209***	0.366	1.76 (0.52–5.97)
Neurological condition	3 (4)	11 (5)	1.000***	0.526	0.61 (0.14–2.78)
Malignancy or immunosuppression	1 (1)	6 (3)	0.681***		

* Wilcoxon rank-sum test; ** χ^2 test; *** Fisher's exact test

Table 8. Clinical features, laboratory results and outcomes of children with community-acquired pneumonia requiring hospitalization; RV-positive patients compared to RV-negative patients. Modified from original publication II.

Clinical features, laboratory results and outcomes	**RV-positive, N = 82**	**RV-negative, N = 231**	***P*-value**
Clinical features – no. (%)			
Cough	58 (71)	185 (80)	0.081[a]
Fever	69 (84)	223 (97)	<0.001[a]
Tachypnoea	25 (30)	67 (29)	0.800[a]
Dyspnea or shortness of breath	29 (35)	65 (28)	0.220[a]
Rhinitis or nasal congestion	36 (44)	83 (36)	0.201[a]
Sore throat or hoarse voice	7 (9)	13 (6)	0.355[a]
Headache	7 (9)	23 (10)	0.708[a]
Muscle pain	5 (6)	10 (4)	0.550[b]
Chest pain or abdominal pain	12 (15)	42 (18)	0.465[a]
Vomiting	24 (29)	69 (30)	0.918[a]
Otitis media	17 (21)	67 (29)	0.146[a]
Abnormal breath sounds	52 (63)	151 (65)	0.750[a]
Crackles	37 (45)	99 (43)	0.722[a]
Decreased breath sounds	17 (21)	58 (25)	0.425[a]
Wheezing	10 (12)	26 (11)	0.819[a]
Duration of symptoms before referral, days – median (IQR)	3.0 (1.0–7.0)	5.0 (2.0–8.0)	0.006[c]
Antibiotic treatment received before referral – no. (%)	11 (13)	49 (21)	0.123[a]
Laboratory results			
WBC count on admission, $\times 10^9$/L – median (IQR)[d]	16.4 (12.2–25.0)	14.1 (8.5–20.4)	0.002[c]
Highest WBC count, $\times 10^9$/L – median (IQR)[e]	17.4 (13.4–25.1)	14.8 (9.5–20.6)	0.003[c]
0–3.9 $\times 10^9$/L – no. (%)	1 (1)	4 (2)	
4–14.9 $\times 10^9$/L – no. (%)	28 (35)	112 (49)	
≥15 $\times 10^9$/L – no. (%)	52 (64)	113 (49)	
CRP on admission, mg/L – median (IQR)[f]	79 (20–192)	87 (25–181)	0.998[c]
Highest CRP, mg/L – median (IQR)[g]	86 (28–192)	100 (30–214)	0.582[c]
<20 mg/L – no. (%)	18 (22)	44 (19)	
20–39 mg/L – no. (%)	8 (10)	25 (11)	
40–79 mg/L – no. (%)	13 (16)	30 (13)	
≥80 mg/L – no. (%)	42 (52)	131 (57)	

Outcomes			
Length of stay, days – median (IQR)	2.0 (1.0–4.0)	2.0 (1.0–3.0)	0.802[c]
Intensive care unit admission – no. (%)	16 (20)	35 (15)	0.358[a]
Oxygen supplementation – no. (%)	28 (34)	63 (27)	0.239[a]
Invasive mechanical ventilation – no. (%)	8 (10)	11 (5)	0.112[b]
Lung abscess, necrotising pneumonia or empyema – no. (%)	5 (6)	20 (9)	0.473[a]
Death in the hospital – no. (%)	0 (0)	1 (0.4)	

[a] χ^2 test.
[b] Fisher's exact test.
[c] Wilcoxon rank-sum test.
[d] Data available on 80 RV-positive and 228 RV-negative children.
[e] Data available on 81 RV-positive and 229 RV-negative children.
[f] Data available on 79 RV-positive and 228 RV-negative children.
[g] Data available on 81 RV-positive and 230 RV-negative children.

5.3 Biomarkers of viral and bacterial infection in rhinovirus pneumonia

RV was detected in 29% and any viruses in 71% of the 24 study children (12 females and 12 males) hospitalized for CAP. The median age was 3.3 years (IQR 2.3–6.6 years). One patient had two concomitant viruses (RV and AdV). The rapid virus antigen detection test showed only one viral finding (RSV), which was also detected by viral multiplex-PCR and RT-PCR for EV, RV, and RSV. Of 7 RV-positive cases, 4 had RV-A and 2 had RV-C (Table 9). In the follow-up of RV-positive patients, 4 of 7 were negative for RV one week after the first sample, and after two weeks all were negative for RV.

Blood MxA concentration was measured from 6 children with RV pneumonia and no other viral findings (median age 1.6 years). It was detectable in all cases but concentrations were low varying from 70 ug/L to 450 ug/L (median 100 ug/L). The levels were lower than in those 10 with another virus (median 495 µg/L, range 70–1620 µg/L, p = 0.034) (Table 10). The median MxA level in children without any viral detections was 120 ug/L (range 110–230 ug/L). Children with RV-positive pneumonia had also lower MxA levels than children with RV-positive URTI (median 620 µg/L, range 200–3120 µg/L, p = 0.011) (Table 11). The median Ct value for RV was lower (23.2) in children with RV pneumonia compared to children with RV URTI (28.7), suggesting higher viral load in children with pneumonia, but the difference was not statistically significant. The median age of the RV-positive URTI comparison group was 0.8 years.

Biomarkers for bacterial infection were markedly increased in all 7 children with RV pneumonia: the median (range) of highest values for WBC count was 18.0 (10.3–25.7) × 10^9/L, for CRP 290 (190–345) mg/L, and for PCT 6.61 (1.99–25.73) µg/L.

One RV-positive child had a blood culture positive for *S. pneumoniae* (Figure 6). In addition, the *S. pneumoniae* antigen test from urine was positive in 4, and potentially pathogenic bacteria (*S. pneumoniae* and/or *M. catarrhalis*) were detected from nasopharyngeal samples in 4 of 7 RV-positive children. Chest radiographs revealed alveolar changes (as shown in Figure 6) in 6 of 7 children with RV pneumonia. All RV-positive children were treated with antibiotics with rapid recovery (Table 9).

The clinical characteristics of pneumonia were similar in RV-positive and other virus-positive children (Table 12). The most common symptoms were cough (100%), fever (96%), rhinitis (75%), and vomiting (71%). The median duration of symptoms before hospitalization and diagnosis of RV pneumonia was 2.5 days (IQR 1.0–4.8 days) vs. 4.0 days (IQR 3.0–10.0) in children with other virus-positive pneumonia ($p = 0.150$). The median length of hospitalization was 2.0 days (IQR 1.8–2.0 days) among RV-positive children and 1.0 day (IQR 1.0–2.0 days) among other virus-positive children ($p = 0.041$).

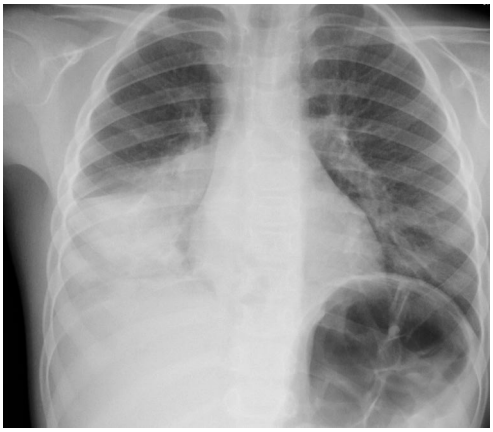

Figure 6. A chest radiograph of a boy aged 5 years and 9 months with CAP. Preceding symptoms were fever for 3 days, fatigue, poor appetite, abdominal pain, right-sided chest pain, and tachypnoea. On admission, CRP was 345 mg/L, PCT 13.67 µg/L, WBC count 10.3×10^9/L, and MxA 70 µg/L. RV was positive from nasal swab, and *S. pneumoniae* was detected in blood and nasopharyngeal sample. The initial chest radiograph shows alveolar consolidation in the right lung and a small amount of pleural fluid. The boy was treated with intravenous penicillin G. After 2 days of treatment, the boy was discharged from hospital with a course of oral amoxycillin for 7 days. After hospitalization, the first follow-up nasal swab sample was negative for RV. Cough, hoarse voice, rhinitis, and nasal congestion lasted for 8 days.

Table 9. Clinical and laboratory features of 7 children with community-acquired pneumonia positive for RV. Modified from original publication III.

	1	2	3	4	5	6	7
Gender	Female	Female	Male	Male	Male	Male	Male
Age, yr	6.1	1.8	5.8	3.1	1.0	1.5	1.1
RV type	C18	A63	C23	Not done	A22	A56	A58
Cycle threshold (Ct) value of RV	19.8	22.7	21.1	36.9	26.9	23.6	21.1
Other viral findings	Adenovirus						
MxA, µg/L	Not done	450	70	100	100	100	140
Highest WBC count, $\times 10^9$/L (normal range 5-15 $\times 10^9$/L)	24.5	14.4	10.3	18.9	17.8	18.0	25.7
Band forms, % of total neutrophils (normal range <5%)	Not done	2	9	40	4	26	Not done
Highest CRP, mg/L (normal range <10 mg/L)	190	332	345	223	290	244	298
Highest PCT, µg/L (normal range <0.5 µg/L)	Not done	7.19	13.67	6.03	1.99	25.73	5.65
Bacterial findings							
bacterial blood culture	Negative	Negative	*S. pneumoniae*	Negative	Negative	Negative	Negative
bacterial culture from nasopharyngeal swab	*M. catarrhalis*	*S. pneumoniae* *M. catarrhalis*	*S. pneumoniae*	Negative	Negative	*S. pneumoniae* *M. catarrhalis*	Not done
S.pneumoniae antigen test from urine	Negative	Positive	Positive	Not done	Positive	Positive	Not done
Chest radiograph findings	Alveolar consolidation, pleural effusion	Alveolar consolidation	Alveolar consolidation	Interstitial infiltrates	Alveolar consolidation, dense	Alveolar consolidation, dense	Alveolar consolidation
Duration of symptoms before referral, days	3	3	4	1	7	1	2
Length of fever in hospital, days	1.5	< 1	< 1	1.5	< 1	< 1	< 1
Length of hospitalization, days	2	2	2	2	2	1	2
Antibiotic treatment received during hospitalization	Penicillin + azithromycin	Penicillin	Penicillin	Cefuroxime - ceftriaxone	Penicillin	Penicillin	Penicillin - cefuroxime

Table 10. The median (IQR) of the highest values for MxA, WCB, CRP, and PCT in RV-positive and other virus-positive children hospitalized for pneumonia (viral co-infections were not included).

	RV positive (n = 6)	**Another virus positive (n = 10)***	***P*-value****
MxA (µg/L)	100 (93–218)	495 (108–855)	0.034
WBC (×10^9/L)	17.9 (13.4–20.6)	13.7 (10.0–27.0)	0.724
CRP (mg/L)	294 (239–335)	223 (112–284)	0.084
PCT (µg/L)	6.61 (4.74–16.69)	2.29 (0.61–6.85)	0.058

* RSV n = 3, HMPV n = 2, AdV n = 1, CoV n = 1, EV n = 1, HBoV n = 1, influenza A virus n = 1
** *t* test

Table 11. Comparison of children with RV-positive pneumonia and children with RV-positive upper respiratory tract infection (URTI). Modified from original publication III.

	RV-positive pneumonia, n = 6	**RV-positive URTI, n = 13**	***P*-value***
Age, yr – median (IQR)	1.64 (1.06–3.77)	0.79 (0.64–1.09)	0.095
Cycle threshold (Ct) value – median (IQR)	23.2 (21.5–26.1)	28.7 (25.8–31.7)	0.135
MxA, µg/L – median (IQR)	100 (93–218)	620 (310–1120)	0.011

* *t* test

Table 12. Symptoms of RV-positive and other virus-positive children with community-acquired pneumonia (viral co-infections were not included).

Symptoms – no. (%)	**RV positive, n = 6**	**Another virus positive, n = 10**	***P*-value***
Cough	6 (100)	10 (100)	
Fever	6 (100)	9 (90)	1.000
Rhinitis	6 (100)	8 (80)	0.500
Vomiting	5 (83)	6 (60)	0.588
Nasal congestion	5 (83)	6 (60)	0.588
Dyspnea	3 (50)	3 (30)	0.607
Hoarse voice	3 (50)	8 (80)	0.299

* Fisher's exact test

5.4 Long-term recovery after parapneumonic empyema in children

The mean age of the 26 study patients (15 females and 11 males) was 4.4 years (SD ±3.5 years) at the time of empyema, and 12.6 years (SD ±5.9 years) at the time of the follow-up visit. One child had empyema in both lungs, 14 only in the right lung, and 11 only in the left lung. In addition to pleural empyema, a lung abscess was found in one and necrotising pneumonia in six children. The mean duration of fever before hospitalization was 5.6 days (SD ±2.3 days), and the mean duration of hospitalization was 15 days (SD ±6.0 days). In addition to antibiotic treatment, all 26 children were initially treated with at least one pleural puncture (n = 3) or pleural drainage (n = 23). Nine children (35%) were treated with fibrinolytics, ten (38%) underwent a thoracotomy, and ten (38%) required ventilator treatment. Detailed information on the treatments and microbial aetiology (most commonly *S. pneumoniae* [in 69%]) of our empyema study population is listed in Table 13. All children recovered uneventfully after hospitalization.

All the 26 participants had normal findings in the physical examination at follow-up 3–19 years (mean 8.2 years) later. In the interim, four of them had suffered from a second pneumonia but without empyema, and none had suffered from other severe infections or recurrent sinusitis. Six participants had the common cold at least five times per year. Three participants had atopic eczema at the time of follow-up visit.

Subtle defects in immunity were detected in eight participants (31%) (Table 14). A mutation in the MBL gene was detected in seven, a low serum MBL level in four, and a decreased activity of the lectin pathway in five participants. One participant had a low level of IgG2 subclass (0.66 g/L). Six participants (23%) had elevated blood eosinophils (0.68–1.21 × 10^9/L), and two had mild neutropenia with otherwise normal laboratory test results. All the participants had serum IgG antibodies against tetanus.

The chest radiograph showed abnormal findings in 36% (9/25) of study population, and all of them had findings also in the lung MRI (Table 14). The MRI revealed pleural scarring in 92% (22/24) of the children, six of which had significant pleural scarring (extension longer than 1 cm) and 16 had moderate pleural scarring. Four of the six subjects with significant findings in the MRI 4–11 years after empyema were treated with a thoracotomy during the acute phase of empyema. In addition to pleural scarring, abnormal findings in the lung tissue including micronodules <5 mm (n = 4), nodules (n = 1), linear consolidation (n = 2), and minor mediastinal shift (n = 1) were revealed by the MRI. Bronchiectasis was not seen in any participant. Of the six participants with a history of necrotising pneumonia and empyema, one had a subpleural micronodule and significant pleural scarring in the follow-up MRI, four had moderate pleural scarring, and one had no findings.

Table 13. Aetiological findings and treatment of empyema patients, and lung MRI findings 3–19 years (mean 8 years) after empyema. Modified from original publication IV.

Age at admission (years)	Gender (male / female)	Duration of fever before hospitalization (days)	Antibiotic treatment[a]	Aetiology[b]	Pleural drainage (at day 0– in hospital) (days)	Thoraco-tomy (N)	Fibrinolytic treatment[c]	Ventilator treatment (days)	Duration of hospitalization (days)	MRI finding[d]
0,6	M	4	cefur, azi, met, cli, cefta, amo-cla	Pnc, EV	4		str	14	29	0
0,8	F	5	cefur, gen, ery, ceftr		4				14	0
1,0	F	8	pen, cefur, cla, cli		5				13	1
1,1	F	3	pen, cefur, met, azi	StrV	3	1	str	1	18	1
1,1	F	7	cefur, azi, cli	Pnc	1				8	1
1,4	M	11	pen, cefur, met, cli, mer, ceftr, cip	Pnc, EV	7	1		2	12	2
1,4	F	5	pen, cefur, cli, azi	Pnc	2		str	5	13	1
1,7	M	3	pen, cefur, cli, azi	Pnc					8	1
1,7	F	4	pen, met, cli, azi, cefur, cefta	Pnc	3				15	1
1,8	F	4	pen, ery, cefur, met	EV	4		uro		18	2
2,4	M	9	cefur, cli, azi	Pnc	10	1		0,5	16	2
2,6	M	5	cefur, azi,	Pnc, EV, RV					7	1
3,0	M	4	cefur, azi, cli	Pnc	3	1	alt	2	23	1
3,1	F	5	cefur, ery, met	Mpn	1				11	1
3,3	M	10	pen	Pnc	1				6	2

Age at admission (years)	Gender (male / female)	Duration of fever before hospitalization (days)	Antibiotic treatment[a]	Aetiology[b]	Pleural drainage (at day 0– in hospital) (days)	Thoraco-tomy (N)	Fibrinolytic treatment[c]	Ventilator treatment (days)	Duration of hospitalization (days)	MRI finding[d]
5,1	M	8	cefur, ery, gen, cla, met	PIV3	4	1			12	2
5,3	F	3	pen, cefur, cli, azi, mer	Pnc, EV, Mpn	2		str	9	18	1
5,4	F	5	cefur, cli, met, azi, cla	Pnc	1			3	10	1
5,9	M	5	pen, cefur, azi, met	Pnc, Mor	5	1	alt		16	1
6,3	F	4	cefur, azi, pen, cli	Pnc, RV	2		str		13	1
7,3	M	10	pen, azi, cefur, cli	Pnc, Cpn	3	1	alt	1	14	2
8,1	M	10	pen, cefur, cli	Pnc	2	1			12	1
9,5	F	5	cefur, azi, ceftr, van, amo-cla	Pnc	16	1		1	22	1
10,0	F	5	cefur, met, cefad, cli, gen	InA	2				27	
10,8	F	6	pen, cefur, cla, cli						13	1
12,6	F	3	cefur, ery	Pnc	4	1			24	

[a] Abbreviations of antibiotics: cefur = cefuroxime (n = 25), cli = clindamycin (n = 16), azi = azithromycin (n = 15), pen = penicillin G (n = 14), met = metronidazole (n = 10), ery = erythromycin (n = 5), cla = clarithromycin (n = 4), ceftr = ceftriaxone (n = 3), gen = gentamicin (n = 3), amo-cla = amoxicillin-clavulanate (n = 2), cefta = ceftazidime (n = 2), mer = meropenem (n = 2), cefad = cefadroxil (n = 1), cip = ciprofloxacin (n = 1), van = vancomycin (n = 1).

[b] Abbreviations of microbes: Pnc = *Streptococcus pneumoniae* (n = 18), EV = enterovirus (n = 5), RV = rhinovirus (n = 2), Mpn = *Mycoplasma pneumoniae* (n = 2), Cpn = *Chlamydia pneumoniae* (n = 1), InA = influenza A virus (n = 1), Mor = *Moraxella catarrhalis* (n = 1), PIV3 = parainfluenza virus type 3 (n = 1), StrV = *Streptococcus viridans* (n = 1).

[c] Abbreviations of fibrinolytics: str = streptokinase (n = 5), alt = alteplase (n = 3), uro = urokinase (n = 1).

[d] MRI findings: 0 = no findings, 1 = moderate findings, 2 = significant findings.

At the follow-up visit, spirometry was normal in 80% (20/25) of the study population; two participants had previously diagnosed asthma, two more participants had lung function values referring to asthma, and one had decreased lung volume as a result of lung resection. The mean FVC was 96% (range 78–121%) of the standard values before the bronchodilator challenge, and 96% (range 79–125%) after the bronchodilator challenge. The mean FEV1 before and after the bronchodilator challenge was 93% (range 80–117%), and 96% (range 84–112%), respectively. Of the six participants with significant pleural scarring, only one presented with abnormal spirometry test results. Continuous or recurrent respiratory symptoms were reported by 13 participants (50%); 11 of them (12 had MRI obtained) had moderate or significant scarring in the pleura, and 3 of them had an abnormal spirometry. Sixty-seven percent of those with significant scarring in the pleura reported persistent respiratory symptoms.

Table 14. The follow-up results of 26 empyema patients.

Age at follow-up (years)	Time from empyema (years)	Lung MRI finding (0=normal, 1=moderate, 2=significant)	Chest radiograph finding (0=normal, 1=abnormal)	Lung function in spirometry (0=normal, 1=abnormal)	Persistent respiratory symptoms[a] (0=no, 1=yes)	Abnormality in immunology tests[b] (0=normal)
10,7	10,1	0	0	1	0	MBL-A/B, LP↓
8,8	8,1	0	0	0	1[c]	0
6,3	5,4	1	0	0	1 (atopic eczema)	0
6,7	5,6	1	0	0	0	0
8,2	7,1	1	1	0	0[c]	0
9,0	7,6	2	1	0	0	0 (neut↓)
10,7	9,3	1	0	0	1	0 (eos↑)
7,1	5,4	1	0	0	0	0
12,3	10,6	1	1	0	1	MBL-A/B, MBL↓, LP↓
12,7	10,9	2	1	0	0	0 (eos↑)
8,9	6,5	2	1	0	1	MBL-A/B, MBL↓, LP↓
8,7	6,1	1	0	0	1	0
6,2	3,2	1	0		0	IgG2↓
20,1	17,0	1	0	0	1 (atopic eczema)	0

Age at follow-up (years)	Time from empyema (years)	Lung MRI finding (0=normal, 1=moderate, 2=significant)	Chest radiograph finding (0=normal, 1=abnormal)	Lung function in spirometry (0=normal, 1=abnormal)	Persistent respiratory symptoms[a] (0=no, 1=yes)	Abnormality in immunology tests[b] (0=normal)
7,0	3,7	2	1	0	1	0 (eos↑)
12,1	6,9	2	1	0	1 (atopic eczema)	0 (eos↑)
13,5	8,2	1	0	0	0	MBL-A/B, MBL↓, LP↓
11,5	6,1	1	0	0	0	MBL-A/B
10,5	4,6	1	0	0	0	0
15,2	9,0	1	0	0	1	0
13,0	5,7	2	0	1	1 (asthma)[c]	0 (neut↓, eos↑)
15,8	7,8	1	1	1	0	MBL-A/D
18,7	9,1	1	0	1	1 (asthma)[c]	0 (eos↑)
29,2	19,2		1	1[d]	1	MBL-B/D, MBL↓, LP↓
17,2	6,4	1	0	0	0	0
27,0	14,5			0	0	0

[a] such as impaired tolerance of physical distress or prolonged cough after a common cold

[b] eos = eosinophils; IgG2 = immunoglobulin G2 subclass; LP = lectin pathway; MBL = mannose-binding lectin, variants A/B/D; neut = neutrophils; ↑ = increased; ↓ = decreased

[c] had suffered from a second pneumonia

[d] decreased lung volume as a result of lung resection, other abnormalities of lung function were obstructive

6 Discussion

6.1 Microbial aetiology of childhood community-acquired pneumonia

We showed that a potential pneumonia pathogen was detected from induced sputum in almost all (97%) children hospitalized for CAP, when comprehensively tested by PCR. Moreover, 84% of the cases were found to be associated with viral–viral, viral–bacterial, or bacterial–bacterial co-detections. Similar results were reported in a South African study with both viral and bacterial pathogens detected in 87% of children with pneumonia, tested by PCR from nasopharyngeal swabs (Zar et al. 2016).

Quantitative PCR is the most sensitive method for detecting respiratory pathogens, and it has displaced the conventional methods in pneumonia aetiology studies (Jartti et al. 2013). However, the interpretation of viral and bacterial detections is not straightforward and establishing causality is difficult. Viruses may be detected due to a concomitant URTI. Furthermore, RV and other respiratory viruses have often been detected in asymptomatic children (up to 60% in children <1 year of age) (van Gageldonk-Lafeber et al. 2005, Jartti et al. 2008b). However, prolonged shedding of RV does not occur in immunocompetent children, contrary to some other respiratory viruses, most typically, HBoV, which may show prolonged shedding for months after acute infection (Fry et al. 2007). The detection of some respiratory DNA viruses such as AdV, may not be indicative of a symptomatic infection as they can be found by PCR due to latency. Moreover, the virulence of certain bacteria, such as *M. catarrhalis*, is low and they are therefore less likely causes of pneumonia (Sy et al. 2010).

Half of all children evaluated at the emergency department for CAP required hospitalization in our study (II), similarly as earlier reported in Finland (Jokinen et al. 1993, Heiskanen-Kosma et al. 1998). However, a decreasing trend was observed from 2008 to 2014 in the yearly number of children hospitalized for CAP. Worldwide, children´s hospitalizations for all-cause pneumonia as well as viral pneumonia has declined markedly after the introduction of PCVs (Fathima et al. 2018). A 10-valent PCV was included in the Finnish national immunization program for all children in 2010. Viruses will probably become even more substantial causes of pneumonia in the future as pneumococcal pneumonia becomes less common due to vaccinations.

In recent years, increased attention has focused on viral pneumonia, and SARS-CoV-2 and influenza virus pandemics have re-emphasised the importance of respiratory viruses as causes of severe pneumonia. In our study (I), viruses were identified in 72% of children hospitalized for CAP, which was the highest detection rate of viral aetiology reported theretofore (Juvén et al. 2000, Tsolia et al. 2004, Cilla et al. 2008, Nascimento-Carvalho et al. 2008, Cevey-Macherel et al. 2009). In subsequent studies, the viral detection rate has varied from 72 to 91% (Jain et al. 2015, Rhedin et al. 2015, Nascimento-Carvalho et al. 2018a, Rueda et al. 2022). We detected two viruses in 22% of the cases and three viruses in 8%, and the mean age of children with multiple viral findings was 2.6 years. Previously, Cilla et al. have reported two viruses in 15% of studied children with pneumonia and three viruses in 3%, and recently, Nascimento-Carvalho et al. reported two or more viruses simultaneously identified in 63% of 774 pneumonia patients, with the median age being 2.4 years (Cilla et al. 2008, Nascimento-Carvalho et al. 2018a). The most common viral-viral co-detection included HBoV and RV in our study. Of those with HBoV in the sputum, in nearly 80% of cases other viruses were also detected, which is in concordance with previous studies (Allander et al. 2007, Fry et al. 2007, Esposito et al. 2008, Garcia-Garcia et al. 2012).

The most frequently detected viruses in our study (I) were RV, HBoV and HMPV, accounting for up to 57% of all viral detections. RV was detected in 26–30% of childhood CAP cases in our three studies (I, II, III), which is in line with other studies of childhood CAP reporting the detection rate of RV from 14 to 45% (Juvén et al. 2000, Tsolia et al. 2004, Cilla et al. 2008, Hamano-Hasegawa et al. 2008, Nascimento-Carvalho et al. 2008, Cevey-Macherel et al. 2009, O'Callaghan-Gordo et al. 2011, Jain et al. 2015, Rhedin et al. 2015, Mancino et al. 2020, Pratt et al. 2022). Recently, RV has been identified in up to 46% of children with non-severe CAP (Nascimento-Carvalho et al. 2018a). The predominance of RV A species followed by RV C in our studies (I, III) is in concordance with other pneumonia studies in children (Esposito et al. 2012, Ahn et al. 2018, Ai et al. 2022). Our detection rates for HBoV (18%) and HMPV (14%) were also in line with earlier studies (3–14% and 7–13%, respectively), but RSV was detected at lower rates (7%) than reported by other studies (9–29%), as our study period was between two RSV epidemics (Juvén et al. 2000, Cilla et al. 2008, Hamano-Hasegawa et al. 2008, Nascimento-Carvalho et al. 2008, Samransamruajkit et al. 2008, Cevey-Macherel et al. 2009, Wolf et al. 2010, O'Callaghan-Gordo et al. 2011). In more recent studies, RSV, HMPV, and HBoV have been detected in up to 32%, 23%, and 22% of children with pneumonia, respectively (Rhedin et al. 2015, Nascimento-Carvalho et al. 2018a).

Thorough diagnosing of specific viral aetiology of pneumonia in the absence of effective antiviral drugs and vaccines against most respiratory viruses may seem

unnecessary. However, potential benefits of virus detection include at least: 1) the surveillance of local epidemiology of seasonal and emerging viruses, 2) better prediction of the clinical course of illness, 3) prevention of transmission by isolation or other methods, and finally, 4) identification of crucial targets for future investigations. Viral testing has been shown to have only a minimal impact on reducing antibiotic use in CAP (Schulert et al. 2014, Blatt et al. 2017, Rao et al. 2021, Mattila et al. 2022).

In addition to viral findings, PCR substantially broadened the detection rate of bacteria in sputum samples from children with CAP, resulting in the bacterial detection rate of 91%, and 41% of them included more than one bacterium. Negative results in the bacterial culture in the presence of a positive bacterial PCR result is most probably explained by the fact that nearly half of our study population had intravenous antibiotic treatment started before the collection of sputum samples.

6.2 Mixed viral-bacterial pneumonia

Two-thirds (66%) of our patients had viral–bacterial co-detections. Previous aetiological studies of childhood CAP have reported viral–bacterial co-detection rates between 15% and 33% (Juvén et al. 2000, Michelow et al. 2004, Tsolia et al. 2004, Hamano-Hasegawa et al. 2008, Nascimento-Carvalho et al. 2008, Cevey-Macherel et al. 2009, Lahti et al. 2009). Viral-bacterial co-infections are increasingly recognized and possibly associated with a more severe course of pneumonia or treatment failure, as in our study, particularly the combination of RV and *S. pneumoniae* (Juvén et al. 2000, Juvén et al. 2004, Jennings et al. 2008, Costa et al. 2014, Brealey et al. 2015, Jain et al. 2015, Cawcutt and Kalil 2017, Nolan et al. 2018, Nathan et al. 2020, Yan et al. 2022). Concomitant viral and bacterial detections complicate the investigation of the role of each microorganism in the pathogenesis and clinical features of illness.

A preceding viral infection can facilitate bacterial colonization to proceed to secondary bacterial infection. Several mechanisms as to how RV increases bacterial susceptibility have been presented, such as an increased permeability of the airway epithelium, and the release of inflammatory cytokines (Jacobs et al. 2013). In vitro, Ishizuka et al. found that the adhesion of *S. pneumoniae* to airway epithelial cells is stimulated by RV infection via increases in the platelet-activating factor receptors (Ishizuka et al. 2003). In a study by Passariello et al., over-expression of ICAM-1 was one suggested mechanism by which RV infection increases the ability of *S. aureus* to internalize into pneumocytes (Passariello et al. 2006). It has been shown that RV exposure leads to the impairment of immune response to bacterial products and phagocytosis of bacteria in human macrophages (Oliver et al. 2008, Finney et al. 2019). In a recent ex vivo study by Wronski et al., RV was shown to replicate in

airway epithelial cells and genomic analyses revealed the induction of a type I IFN-driven antiviral immune response and epithelial cell-associated pathways in response to RV infection (Wronski et al. 2021). A temporal association between RV circulation in the community and invasive *S. pneumoniae* infections has been reported in children (Peltola et al. 2011), and a seasonal peak in pneumonia incidence in winter coinciding with viral LRTIs has been reported (Ben-Shimol et al. 2015). Karppinen et al. have demonstrated that a symptomatic RV infection facilitates the acquisition of *S. pneumoniae* from the community and within-family pneumococcal transmission (Karppinen et al. 2017). In febrile infants, bacterial infection has been found more commonly in those with RV than those with other viruses detected (Blaschke et al. 2018). Viral co-infections are common in children with invasive pneumococcal disease and are possibly associated with higher mortality (Techasaensiri et al. 2010). Mixed influenza virus–*S. aureus* infection can cause severe, fatal pneumonia in children (Connor and Powell 1985, Thomas et al. 2003, Finelli et al. 2008, Reed et al. 2009). In a recent Malaysian study by Nathan et al., viral-bacterial co-detection in induced sputum of children hospitalized with pneumonia was associated with a higher CRP but no other markers of clinical severity (Nathan et al. 2020). Noteworthy in our study is the finding that all children with treatment failure (8% of the study population) had evidence of mixed viral-bacterial infection.

More severe clinical illness has been associated with viral-viral co-infections in some studies. Children with viral co-infections were hospitalized more frequently compared with those with a single virus in a Spanish study of more than 300 children with CAP (Cilla et al. 2008). Esposito et al. also found that HBoV co-infections with other viruses were associated with greater disease burden leading to more hospitalizations and absence from school in children with respiratory tract infection than in those with HBoV infection alone (Esposito et al. 2008). This fits with the fact that prolonged shedding of HBoV is common and the detection of HBoV does not necessarily signify HBoV infection. On the contrary, in a systemic review by Goka et al., multiple respiratory virus detection was not associated with disease severity or risk of pneumonia (Goka et al. 2014). Interestingly, a primary RV infection may even have a protective effect against other respiratory viruses, including SARS-CoV-2, via stimulation of innate anti-viral immunity, potentially depending on RV subtypes (Esneau et al. 2022).

6.3 Rhinovirus pneumonia

We found that RV-positive pneumonia patients were younger than RV-negative patients. A recent multicenter case-control study from developing countries also showed the high frequency of RV detections in children <5 years of age hospitalized

with CAP (Bénet et al. 2017). In our study, RV-positive patients were considerably more often prematurely born than others. This is in agreement with the findings of Miller et al. (Miller et al. 2012). Prematurely born children may also be tested more actively for RV which may affect the generalizability of the results in study II. Several pathophysiological mechanisms have been proposed to explain the observed increased risk of respiratory disorders, including RV infections, following preterm birth. These include diminished immune responses, impaired lung fuction as well as virally activated inflammatory and airway re-modeling pathways (Townsi et al. 2018). Interestingly, among preterm infants, gut microbiota modification with specific prebiotics and probiotics during the first months of life has been noticed to reduce the risk of RV infections (Luoto et al. 2014). The symptom profile is determined largely by the nature and extent of the immune response to the virus (Kennedy et al. 2012).

In our study, RV pneumonias occurred year-round with peaks in autumn and spring, similar to earlier reports (Monto et al. 2002). Clinical features of pneumonia were similar in RV-positive and RV-negative children in our study. The most common symptoms were fever and cough in both groups. Interestingly, wheezing was not more prevalent among RV-positive children, though they had asthma slightly more often than RV-negative children. Most cases had high CRP and WBC levels and all were treated with antibiotics because of suspected bacterial pneumonia. The median WBC counts were even higher in the RV-positive than in the RV-negative group (study II). Similarly, increased WBC levels have been earlier observed in RV-related LRTIs (Berce et al. 2015). RV-positive children in study III with one exception had alveolar changes in chest radiograph strongly supporting bacterial infections, and the clinical response to antibiotic treatment was rapid in all of them. One RV-positive child with interstitial infiltrates and remarkable respiratory symptoms might have had viral pneumonia with a systemic secondary bacterial infection like sepsis, though the bacterial blood culture of this child was negative. In our retrospective data (study II), the severity of pneumonia and the response to treatment were largely similar between RV-positive and RV-negative children. RV-infected children slightly more often needed oxygen supplementation, treatment at the intensive care unit, and invasive mechanical ventilation, but the differences were not significant. Similar to our findings, Louie et al. found RV to be associated with a severe course of pneumonia in children (Louie et al. 2009). However, only few studies have investigated the clinical characteristics of RV pneumonia in children. In a study of Annamalay et al., no significant between-group differences were found considering the clinical, laboratory and microbial findings of RV-positive and RV-negative children hospitalized with pneumonia (Annamalay et al. 2016). In a study of Ahn et al., younger age, shorter duration of fever, and higher frequencies of chest retraction and wheezing were associated with RV detections in children hospitalized

for acute LRTIs (Ahn et al. 2018). Baillie et al. have also reported wheezing to be more prevalent among RV-positive versus RV-negative children with pneumonia, and moreover, those with RV-C were more likely to present with wheezing compared to those with RV-A (Baillie et al. 2019, Baillie et al. 2021). Otherwise, only marginal differences have been reported in studies comparing children with LRTI (CAP or an unspecified LRTI) caused by different RV types (Xiang et al. 2008, Iwane et al. 2011, Esposito et al. 2012, Annamalay et al. 2016, Ahn et al. 2018, Baillie et al. 2019).

The role of RV as a true pneumonia pathogen is debatable and sometimes considered to be overestimated (Hayden et al. 2004, Fry et al. 2011, Iwane et al. 2011, Ruuskanen and Järvinen 2014, Shi et al. 2015, Spichak et al. 2016, Drysdale et al. 2017, Korppi 2017, Baillie et al. 2018). In common clinical settings, the identification of RV directly from the lungs is not feasible, and upper respiratory tract specimens are mainly used in pneumonia aetiology studies in children. However, a life-threatening case of pneumonia with RV as the only detected pathogen from specimens including bronchoalveolar lavage has been reported (Broberg et al. 2011), and, in post-mortem studies from children with pneumonia, RV has been isolated directly from the lung tissue (Las Heras and Swanson 1983, Imakita et al. 2000). In a recent case report of a 20-month-old child with parapneumonic empyema, RV was the only detected pathogen (Mann et al. 2020). RV has been detected from bronchoalveolar lavage from adults with severe CAP (Karhu et al. 2014, Wang et al. 2017, Ishiguro et al. 2019, Zhang et al. 2020, Morimoto et al. 2021). RV viremia has been observed particularly among CAP patients with RV-C (Fuji et al. 2011, Lu et al. 2017, Baillie et al. 2020). In a Swedish study comprising 121 CAP cases and 240 healthy controls, there were no significant differences in the RV findings between the cases and controls (23% vs 27%) (Rhedin et al. 2015). Of note, controls with respiratory symptoms reported within the last 7 days were not excluded, and moreover, CAP cases had lower Ct values than controls indicating a higher viral load. In a recent Colombian study, 21% of 525 CAP cases and 10% of 61 healthy controls had RV detections (Rueda et al. 2022). Still, RV detection in an asymptomatic subject is not proof of its nonpathogenicity. Asymptomatic infections are recognised for many respiratory viruses. This is well documented recently in children with SARS-CoV-2 infections. Asymptomatic RV detection may reflect previous infection or precede the onset of symptoms, but persistent shedding of RV after an acute infection is not known to occur in otherwise healthy subjects. In immunocompetent children, the mean virus shedding time after RV infection is as short as 11 days (Peltola et al. 2013). In our prospective pneumonia study (III), all children with RV-induced CAP were negative for RV two weeks after it was first detected, suggesting an acute RV-associated pneumonia in these children.

Controversy remains regarding the correlation of viral loads in upper respiratory tract specimens to the clinical course of the infection. In some studies, high RV loads have been associated with more severe respiratory symptoms and LRTIs (Utokaparch et al. 2011, Bruning et al. 2015, Ng et al. 2018). On the contrary, Kennedy et al. found that children with wheezing had similar RV loads compared to those with only rhinitis (Kennedy et al. 2014). In a recent study by Baillie et al., a similar distribution of RV species and comparable RV loads were observed among all subjects but RV-viremia was significantly more prevalent among children hospitalized for pneumonia compared to controls (Baillie et al. 2020). Serologic assays that could confirm acute RV infection are not in routine use. The development of effective vaccines and/or drug treatments for RV could reveal its true impact on childhood pneumonia. More pathophysiological studies and knowledge of possible biomarkers of RV infection are needed.

6.4 Viral and bacterial biomarkers and antibiotic treatment of community-acquired pneumonia

Several different viruses induce MxA expression. Surprisingly, RV-positive children of our pneumonia study (III) had significantly lower levels of blood MxA protein (median 100 μg/l) than those with other virus pneumonias (495 μg/L) and those with RV-positive URTI (620 μg/L). Except for one RV-positive case with high MxA, the levels were similar to those reported in asymptomatic virus-negative children (Toivonen et al. 2015). It is previously shown that in children with a symptomatic non-pneumonic RV infection the mean MxA level is 500–600 μg/L (Toivonen et al. 2015). Young age might contribute to an elevated MxA level, but not as much as the difference seen in MxA levels between our RV-positive pneumonia patients and the comparison group of RV-positive URTI. In pneumonia, the bacterial co-infection may dominate the inflammation cascade and downregulate MxA expression. However, it is tempting to speculate that in our cases, a relative interferon system deficiency could have resulted in uncontrolled RV replication and more severe clinical illness, as seen in COVID-19 (Stertz and Hale 2021), though opposite findings have also been presented concerning the association of MxA with severity of COVID-19 (Lehtinen et al. 2022). The hypothesis is supported by our observation that in 6 of 7 children Ct values of RV were < 27 indicating high viral loads and furthermore supporting the pathogenic role of RV. Contrary to this suggestion, low MxA could suggest that RV infection occurred before the onset of pneumonia and the interferon response to virus infection had diminished before patient enrolment. Interestingly, in a recent study by Rubió et al., higher abundance of Picornaviruses, mostly RVs, in the respiratory virome profile was associated with lower innate immune responses (Rubió et al. 2022).

The biomarkers for bacterial infections (WBC, CRP, and PCT) were increased in children with bacterial and mixed viral-bacterial pneumonia, but also in children with viral pneumonia in our study (I). They were also markedly increased in children with RV-positive CAP (study III). More specific biomarkers are needed to exclude or confirm bacterial co-infection in RV-associated and other viral pneumonias as the specificity of CRP and WBC is insufficient in differentiation between viral and bacterial infections.

The necessity of antibiotic treatment is the major question in pneumonia management. Along with the concern of antibiotic resistance, antibiotics have several adverse effects and long-term consequences in individuals (Duong et al. 2022). Clinicians need accurate and rapid tools to guide the use of antibiotics and to help decide in which children antibiotics could be withheld safely. The British Thoracic Society guidelines recommended already two decades ago that antiobiotic therapy can be withheld in young, mildly ill children in whom viral infection is likely (British Thoracic Society 2002). Mixed viral-bacterial detections are very common, but there is a need to recognize which of these cases are self-limiting and do not require antibiotic treatment. The detection of a virus by PCR can not distinguish between an asymptomatic viral carriage and a symptomatic viral infection, and also detected bacteria can be contaminants from the nasopharynx. In our studies, identification of RV did not result in the withholding of antibiotic treatment, but the matter could be different in milder pneumonia cases not needing hospitalization. Commonly used bacterial biomarkers CRP and PCT have notable limitations in differentiating causes of pneumonia (Rhedin et al. 2021). The capacity of a single biomarker is limited. The use of both viral and bacterial biomarkers is supported by the high prevalence of viral-bacterial co-infections. The blood MxA/CRP ratio (with a proposed cutoff level of 18.6) is suggested to be more comprehensive in differentiating between viral and bacterial infections than MxA measurement alone (Piri et al. 2022). The combination of CRP and/or PCT, MxA, and multiplex PCR could be sufficient to diagnose a viral pneumonia. New approaches and emerging technologies are still needed. One promising novel diagnostic assay integrates the concentrations of three host proteins: CRP, IP-10, and TNF-related apoptosis-inducing ligand (TRAIL) (Oved et al. 2015, van Houten et al. 2017, Papan et al. 2022). TRAIL concentrations increase in viral infection and decrease in bacterial infection. Integrating studies of host response, microbe detection, and airway microbiome is a modern approach in pneumonia diagnostics (Langelier et al. 2018). Antigen detection and PCR point-of-care tests for respiratory pathogens are already widely used, and a novel recombinase polymerase amplification-based point-of-care test is a promising innovation with a high sensitivity but still a short turnaround time as it does not require thermal cycling (Rhedin et al. 2019). Metabolomic analysis, which means characterizing of metabolites in biofluids, is a potential tool to improve

pneumonia diagnosis (Laiakis et al. 2010). A new promising method is the detection of virus-specific inflammatory reaction by a host gene expression analysis, which is able to discriminate between symptomatic viral infection and asymptomatic viral detection by transcriptional profiling (Ramilo et al. 2007, Popper et al. 2009, Hu et al. 2013, Heinonen et al. 2016, Herberg et al. 2016, Lydon et al. 2019). Metagenomic new-generation sequencing from bronchoalveolar lavage fluid has recently been shown to be highly efficient in the pathogen diagnosis of childhood pneumonia, and it is suggested to be used in combination with convential microbiological tests (Deng et al. 2022, Yang et al. 2022). Novel methods, especially the use of transcriptomic methods will probably revolutionize the diagnostics of viral pneumonia.

6.5 Recovery from parapneumonic empyema

Even though pleural scarring can be seen in lung MRI in the majority of children many years after empyema, our study shows that the long-term functional outcome of paediatric parapneumonic empyema is good. In addition, patients with necrotizing pneumonia and empyema recovered well. Other studies have reported similar findings, also after the decortication of empyema (Satish et al. 2003, Grotenhuis et al. 2008, Casali et al. 2009, Cohen et al. 2012, de Benedictis et al. 2019, Maffey et al. 2019). Persistent respiratory symptoms were nevertheless reported by many subjects, but the possible association of persistent respiratory symptoms and empyema remains undefined.

Lung MRI was expectedly much more sensitive than a chest radiograph in assessing the anatomical sequelae of empyema. The MRI revealed pleural scarring in 92%, while only one third of the patients had findings also in the follow-up chest radiograph. Even with MRI, abnormalities of lung parenchyma were infrequently found, and radiological signs of chronic lung disease, such as bronchiectasis or pulmonary fibrosis, were not detected. Significant (>1 cm) pleural scarring was seen in 27% in the follow-up MRI. Most of those with significant pleural scarring had undergone thoracotomy or had pleural drainage for at least 3 days. Significant pleural scars could be hypothesized to have an influence on lung function, for example, during heavy exercise, infection, or anaesthesia. Cohen et al. described an excellent 1-year outcome of childhood empyema, though most patients showed minor pleural thickening (mean 1.5 mm) in the follow-up chest radiograph (Cohen et al. 2012). The clinical significance of extensive pleural scarring and parenchymal abnormalities is not known.

Normal pulmonary function was a common finding in our study, as only 16% of subjects had evidence of reversible airway obstruction and only one had a restrictive defect. Some longitudinal studies have looked into the pulmonary function in children with empyema, but the outcome results in these studies have been

inconsistent due to small sample sizes, different subgroups of empyema patients, and the improved quality of care in the course of time (McLaughlin et al. 1984, Redding et al. 1990, Kohn et al. 2002, Satish et al. 2003, Cohen et al. 2012). In a study by McLaughlin et al. almost four decades ago, five of eight children had evidence of a mild restrictive defect and one patient had airway obstruction consistent with asthma (McLaughlin et al. 1984). Similarly, in a study by Redding et al., 47% of 15 children had evidence of mild airway obstruction (Redding et al. 1990). Kohn et al. reported 19% of 36 children having a mild restrictive pattern and 16% having mild obstructive changes (Kohn et al. 2002). In contrast, in a study by Satish et al., a full recovery of pulmonary function was reported among all 13 patients 3–24 months after empyema (Satish et al. 2003). Comparably, Cohen et al. reported only 2 of 34 patients having abnormal spirometry results 12 months after empyema (Cohen et al. 2012). According to most recent studies, pulmonary function returns to normal within a few months after empyema (de Benedictis et al. 2019, Maffey et al. 2019). It might be that children with pre-existing small airways are over-represented among children developing pneumonia or empyema, rather than pneumonia or empyema leading to the development of obstructive airway disease. Despite a normal spirometry in most subjects, continuous or recurrent respiratory symptoms, such as a reduced ability to perform vigorous exercise and a prolonged cough after a common cold, were commonly reported in our study. This finding is in line with earlier studies reporting that pneumonia is an independent risk factor for chronic respiratory symptoms and persistent cough (Gold et al. 1989, Eastham et al. 2008).

Minor immunological abnormalities, mostly MBL deficiencies, were found in one-third of our study subjects, and four subjects without MBL deficiencies suffered from a second pneumonia, which is considered as a warning sign for immunodeficiency. Increased susceptibility to other severe infections was not recorded. The clinical significance of the subtle immunological abnormalities has been questioned (Pan and Hammarström 2000, Verdu et al. 2006). An increased risk of death among MBL-deficient patients with severe pneumococcal infection has been reported, as well as severe or recurrent respiratory infections in children with a low level of serum MBL (Summerfield et al. 1997, Eisen et al. 2008). However, MBL deficiency has not been reported to be associated to the susceptibility to thoracic empyema (Chapman et al. 2010). The MBL B variant was detected in 23% and the D variant in 8% of our study subjects, as compared to previously reported percentages of European populations with the B variant occurring in 22–28% and the D variant in 14% (Turner 2003). Thus, even though empyema is a severe complication of pneumonia, immunodeficiency does not seem to be overrepresented among children with empyema and should not be routinely searched for.

6.6 Limitations

The modest number of patients is an obvious limitation of our study, especially in studies III and IV. The diversity of findings in relation to the size of our study population limits the conclusions that can be drawn. As well, the lack of a control group of healthy children (I, II, IV) is recognized.

Our study population was from a single center which may affect the generalizability of the results. Our study included only hospitalized CAP patients. In the empyema study, we had a severe cohort given the 40% ventilated rate.

In study I, the duration of the study period was short and did not comprise RSV epidemics at all. On the contrary, in study II, during the 12-year study period the diagnostic methods and clinical practices have varied, as in study IV, in which a cohort of empyema patients was collected over many years and treatment patterns as well as the natural history of the underlying disease may have varied.

The retrospective setting in study II causes some limitations. The clinical data was collected from the medical records, which might be incomplete. The study population was somewhat selected as RV tests were not routinely performed for all CAP patients. However, our study population can be considered to be a representative sample of overall CAP inpatients as key figures in patients undergoing RV detection in this study and in CAP inpatients in our prospective aetiology study (I) are comparable.

Our study was mostly PCR-defined. The use of serology could have established infections caused by some viruses; also RVs can induce serologic response but it is virus type-specific (Jacobs et al. 2013, Saarinen et al. 2020). Serology has been shown to increase the diagnostic yield over NAAT for some virus infections, e.g. PIV by up to 49% and by 12% for RSV (Zhang et al. 2016). Combining NAAT and serologic assays can provide a more complete picture of respiratory viral infections and can separate co-infection from co-detection (Jartti et al. 2013, Zhang et al. 2016). Detection of viruses by culture, which reflects virus replication, would have strengthened our conclusions by detecting live, contagious viruses, though the sensitivity is low. Moreover, the roles of bacterial culture from nasopharyngeal swab and *S. pneumoniae* antigen test from urine are debatable, since both methodologies can detect bacterial colonization in children. The microbial results need cautious interpretation.

In study II, other viruses than RV and bacteria were not comprehensively analysed, and data on the types of RV was not available. However, the patient population of this study was overlapping with our aetiology study, in which 64% of RV findings belonged to RV A species and 36% to RV C species, and viral-bacterial co-infections were frequent (study I). Viral loads were available only in study III.

The challenge of good specimen collection is worth noticing. For the viral detection, sputum samples were used in study I, nasal swabs were used in study III,

and variable samples were used in study II. In study I, only the sputum samples with a high leukocyte count were included, which may have influenced the results especially in terms of viruses which may not induce leukocytes to the sputum. Sputum samples may also include contaminants from the nasopharynx. On the other hand, respiratory viruses can be detected also in lower airway samples of asymptomatic children (Thavagnanam et al. 2010). Nasopharyngeal swabs are more feasible in clinical practice. However, especially in study III, a minimally invasive procedure like bronchoalveolar lavage, which is not routinely done, would have supported the role of RV or another virus as a cause of pneumonia.

In study IV, there are limitations related to lung function tests, including the inexperience of especially young patients in the spirometry, the lack of data on lung function before the episode of empyema, and the lack of plethysmography data. Moreover, exercise testing with dynamic values could have revealed more information on the respiratory function (Tsubota et al. 1994).

6.7 Prospects for the future

A better understanding of the pathogenesis of pneumonia is still needed. The establishment of causal factors in the microbiome and integration of multi-omics data are future steps. Moreover, in future pneumonia aetiology studies, explicit case definitions based on radiological evidence, formal evaluation and potentially wider use of lung aspirates, and emphasizing of comprehensive post-mortem evaluations are suggested (Feikin et al. 2017).

More studies would be needed to further elucidate the role of RV in pneumonia. For instance, interesting targets of studies related to RV pneumonia might be serum cytokine profiles, gene expression patterns in WBCs, neutrophil surface protein markers, and serum metabolomics. Further studies are needed to establish the clinical characteristics of RV pneumonia in outpatients. The development of diagnostic biomarkers able to confirm RV as a true cause of the disease would be highly beneficial because of the high global burden of pneumonia and high prevalence of RV in children with pneumonia. Furthermore, drugs or vaccines against RV could possibly have a significant effect on the treatment and prevention of childhood pneumonia.

An important issue is the necessity of antibiotic treatment in CAP, especially in outpatients with milder disease. Would viral detections, particularly when combined with near-normal CRP level and potentially increased MxA level, or the use of other novel diagnostic assays, result in the withholding of antibiotic treatment? Overall, advanced viral diagnostics is important for surveillance of local epidemiology and detection of new viruses.

7 Summary

In study I, using modern molecular diagnostic techniques, high rates of both viruses and bacteria could be identified in childhood CAP. The clinical significance of mixed viral-bacterial infections remains unclear, although we found a potential association between mixed viral-bacterial infections and treatment failure. These findings support the view that all children with severe pneumonia should be treated with antibiotics, as the detection of a virus does not allow a concomitant bacterial infection to be ruled out. Still, in the post-pneumococcal conjugate vaccine era, viral pneumonia should receive greater attention in future treatment and prevention studies.

In study II, RV was frequently detected in young children hospitalized for CAP, and a history of premature birth was identified as a factor associated with RV-positive pneumonia. RV-associated pneumonia seemed to be a rather severe disease with high levels of inflammatory biomarkers and a clinical course often requiring intensive care. The clinical features of pneumonia did not clearly differ between RV-positive and RV-negative children. Further studies are needed to clarify the clinical significance of RV detection in children with pneumonia.

In study III, a high RV load followed by rapid clearance of RV suggested a pathogenic role of RV in CAP. Increased levels of bacterial biomarkers and the predominance of alveolar changes in chest radiograph supported the conception of a viral-bacterial co-infection in RV-positive pneumonia. Low MxA levels in children with RV-positive CAP suggest an aberrant interferon response to RV in children with viral-bacterial pneumonia but more studies on this topic are needed.

In study IV, the long-term recovery of children with parapneumonic empyema was good. Though many children had pleural scarring in the MRI several years after empyema, parenchymal abnormalities were rarely found, and radiological findings of chronic lung disease were not detected. Considering the normal lung function, chest radiograph and clinical recovery of most patients, more intensive follow-up of all empyema patients does not seem necessary.

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References

Abbasi, R., Javanmardi, F. S., Mokhtari, A., Hosseinpour, P., Shahriarirad, R. & Ebrahimi, K., 2021. Management of pleural empyema in a 12-year-old obese patient with COVID-19: a pediatric case report. *BMC Pediatr*, 21(1), 531.

Ahmed, S., Sultana, S., Khan, A. M., Islam, M. S., Habib, G. M., McLane, I. M., et al., 2022. Digital auscultation as a diagnostic aid to detect childhood pneumonia: A systematic review. *J Glob Health*, 12, 04033.

Ahn, J. G., Kim, D. S. & Kim, K. H., 2018. Clinical characteristics and cytokine profiles of children with acute lower respiratory tract infections caused by human rhinovirus. *PLoS One*, 13(7), e0198624.

Ai, J., Zhu, Y., Duan, Y., Zhang, M., Lu, G., Sun, Y., et al., 2022. A multicenter study on molecular epidemiology of rhinovirus isolated from children with community acquired pneumonia in China during 2017-2019. *Infect Genet Evol*, 106, 105384.

Allander, T., Tammi, M. T., Eriksson, M., Bjerkner, A., Tiveljung-Lindell, A. & Andersson, B., 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci U S A*, 102(36), 12891–6.

Allander, T., Jartti, T., Gupta, S., Niesters, H. G., Lehtinen, P., Österback, R., et al., 2007. Human bocavirus and acute wheezing in children. *Clin Infect Dis*, 44(7), 904–10.

Annamalay, A. A., Lanaspa, M., Khoo, S. K., Madrid, L., Acácio, S., Zhang, G., et al., 2016. Rhinovirus species and clinical features in children hospitalised with pneumonia from Mozambique. *Trop Med Int Health*, 21(9), 1171–80.

Atkinson, M., Yanney, M., Stephenson, T. & Smyth, A., 2007a. Effective treatment strategies for paediatric community-acquired pneumonia. *Expert Opin Pharmacother*, 8(8), 1091–101.

Atkinson, M., Lakhanpaul, M., Smyth, A., Vyas, H., Weston, V., Sithole, J., et al., 2007b. Comparison of oral amoxicillin and intravenous benzyl penicillin for community acquired pneumonia in children (PIVOT trial): a multicentre pragmatic randomised controlled equivalence trial. *Thorax*, 62(12), 1102–6.

Azzari, C., Serranti, D., Nieddu, F., Moriondo, M., Casini, A., Lodi, L., et al., 2019. Significant impact of pneumococcal conjugate vaccination on pediatric parapneumonic effusion: Italy 2006-2018. *Vaccine*, 37(20), 2704–2711.

Baillie, V. L., Olwagen, C. P. & Madhi, S. A., 2018. Review on Clinical and Molecular Epidemiology of Human Rhinovirus-Associated Lower Respiratory Tract Infections in African and Southeast Asian Children. *Pediatr Infect Dis J*, 37(7), e185–194.

Baillie, V. L., Moore, D. P., Mathunjwa, A., Morailane, P., Simões, E. A. F. & Madhi, S. A., 2019. Molecular Subtyping of Human Rhinovirus in Children from Three Sub-Saharan African Countries. *J Clin Microbiol*, 57(9), e00723-19.

Baillie, V. L., Moore, D. P., Mathunjwa, A., Morailane, P., Simões, E. A. F. & Madhi, S. A., 2020. A prospective case-control study on the association of Rhinovirus nasopharyngeal viral load and viremia in South African children hospitalized with severe pneumonia. *J Clin Virol*, 125, 104288.

Baillie, V. L., Moore, D. P., Mathunjwa, A., Baggett, H. C., Brooks, A., Feikin, D. R., et al., 2021. Epidemiology of the Rhinovirus (RV) in African and Southeast Asian Children: A Case-Control Pneumonia Etiology Study. *Viruses*, 13(7), 1249.

Balfour-Lynn, I. M., Abrahamson, E., Cohen, G., Hartley, J., King, S., Parikh, D., et al., 2005. BTS guidelines for the management of pleural infection in children. *Thorax*, 60(Suppl 1), i1–21.

Bashir, A., Khan, R., Thompson, S. & Caceres, M., 2022. A retrospective observational study of biomarker levels and severity assessment in pediatric community-acquired pneumonia. *Medicine (Baltimore)*, 101(32), e30010.

Basnet, S., Palmenberg, A. C. & Gern, J. E., 2019. Rhinoviruses and Their Receptors. *Chest*, 155(5), 1018–1025.

Ben-Shimol, S., Greenberg, D., Hazan, G., Shemer-Avni, Y., Givon-Lavi, N. & Dagan, R., 2015. Seasonality of both bacteremic and nonbacteremic pneumonia coincides with viral lower respiratory tract infections in early childhood, in contrast to nonpneumonia invasive pneumococcal disease, in the pre-pneumococcal conjugate vaccine era. *Clin Infect Dis*, 60(9), 1384–7.

Bénet, T., Sánchez Picot, V., Messaoudi, M., Chou, M., Eap, T., Wang, J., et al., 2017. Microorganisms Associated With Pneumonia in Children <5 Years of Age in Developing and Emerging Countries: The GABRIEL Pneumonia Multicenter, Prospective, Case-Control Study. *Clin Infect Dis*, 65(4), 604–612.

Benschop, K., Molenkamp, R., van der Ham, A., Wolthers, K. & Beld, M., 2008. Rapid detection of human parechoviruses in clinical samples by real-time PCR. *J Clin Virol*, 41(2), 69–74.

Berce, V., Unuk, S., Duh, D., Homšak, M. & Vičič, M., 2015. Clinical and laboratory characteristics of viral lower respiratory tract infections in preschool children. *Wien Klin Wochenschr*, 127(Suppl 5), S255–62.

Berkley, J. A., Munywoki, P., Ngama, M., Kazungu, S., Abwao, J., Bett, A., et al., 2010. Viral etiology of severe pneumonia among Kenyan infants and children. *JAMA*, 303(20), 2051–7.

Bhuiyan, M. U., Snelling, T. L., West, R., Lang, J., Rahman, T., Granland, C., et al., 2019a. The contribution of viruses and bacteria to community-acquired pneumonia in vaccinated children: a case-control study. *Thorax*, 74(3), 261–269.

Bhuiyan, M. U., Blyth, C. C., West, R., Lang, J., Rahman, T., Granland, C., et al., 2019b. Combination of clinical symptoms and blood biomarkers can improve discrimination between bacterial or viral community-acquired pneumonia in children. *BMC Pulm Med*, 19(1), 71.

Bielicki, J. A., Stöhr, W., Barratt, S., Dunn, D., Naufal, N., Roland, D., et al., 2021. Effect of Amoxicillin Dose and Treatment Duration on the Need for Antibiotic Re-treatment in Children With Community-Acquired Pneumonia: The CAP-IT Randomized Clinical Trial. *JAMA*, 326(17), 1713–1724.

Bizot, E., Bousquet, A., Charpié, M., Coquelin, F., Lefevre, S., Le Lorier, J., et al., 2021. Rhinovirus: A Narrative Review on Its Genetic Characteristics, Pediatric Clinical Presentations, and Pathogenesis. *Front Pediatr*, 9, 643219.

Black, R. E., Cousens, S., Johnson, H. L., Lawn, J. E., Rudan, I., Bassani, D. G., et al., 2010. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet*, 375(9730), 1969–87.

Blaschke, A. J., Korgenski, E. K., Wilkes, J., Presson, A. P., Thorell, E. A., Pavia, A. T., et al., 2018. Rhinovirus in Febrile Infants and Risk of Bacterial Infection. *Pediatrics*, 141(2), e20172384.

Blatt, S. P., Yultyev, A., Huang, M., Friedstrom, S. & Steinbrunner, J., 2017. Impact of respiratory virus molecular testing on antibiotic utilization in community-acquired pneumonia. *Am J Infect Control*, 45(12), 1396–1398.

Bloomfield, M., Parackova, Z., Cabelova, T., Pospisilova, I., Kabicek, P., Houstkova, H., et al., 2019. Anti-IL6 Autoantibodies in an Infant With CRP-Less Septic Shock. *Front Immunol*, 10, 2629.

Bosis, S. & Esposito, S., 2017. Enterovirus D68-Associated Community-Acquired Pneumonia in the Pediatric Age Group. *Curr Infect Dis Rep*, 19(3), 12.

Bradley, J. S., Byington, C. L., Shah, S. S., Alverson, B., Carter, E. R., Harrison, C., et al., 2011. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clin Infect Dis*, 53(7), e25–76.

Brealey, J. C., Sly, P. D., Young, P. R. & Chappell, K. J., 2015. Viral bacterial co-infection of the respiratory tract during early childhood. *FEMS Microbiol Lett*, 362(10), fnv062.

British Thoracic Society Standards of Care Committee, 2002. British Thoracic Society Guidelines for the Management of Community Acquired Pneumonia in Childhood. *Thorax*, 57(Suppl 1), i1–24.

Broberg, E., Niemelä, J., Lahti, E., Hyypiä, T., Ruuskanen, O. & Waris, M., 2011. Human rhinovirus C--associated severe pneumonia in a neonate. *J Clin Virol*, 51(1), 79–82.

Bruning, A. H. L., Thomas, X. V., van der Linden, L., Wildenbeest, J. G., Minnaar, R. P., Jansen, R. R., et al., 2015. Clinical, virological and epidemiological characteristics of rhinovirus infections in early childhood: A comparison between non-hospitalised and hospitalised children. *J Clin Virol*, 73, 120-126.

Bunthi, C., Rhodes, J., Thamthitiwat, S., Higdon, M. M., Chuananon, S., Amorninthapichet, T., et al., 2021. Etiology and Clinical Characteristics of Severe Pneumonia Among Young Children in Thailand: Pneumonia Etiology Research for Child Health (PERCH) Case-Control Study Findings, 2012-2013. *Pediatr Infect Dis J*, 40(9S), S91–100.

Byington, C. L., Spencer, L. Y., Johnson, T. A., Pavia, A. T., Allen, D., Mason, E. O., et al., 2002. An epidemiological investigation of a sustained high rate of pediatric parapneumonic empyema: risk factors and microbiological associations. *Clin Infect Dis*, 34(4), 434–40.

Byington, C. L., Hulten, K. G., Ampofo, K., Sheng, X., Pavia, A. T., Blaschke, A. J., et al., 2010. Molecular epidemiology of pediatric pneumococcal empyema from 2001 to 2007 in Utah. *J Clin Microbiol*, 48(2), 520–5.

Casali, C., Storelli, E. S., Di Prima, E. & Morandi, U., 2009. Long-term functional results after surgical treatment of parapneumonic thoracic empyema. *Interact Cardiovasc Thorac Surg*, 9(1), 74–8.

Castro-Rodríguez, J. A., Holberg, C. J., Wright, A. L., Halonen, M., Taussig, L. M., Morgan, W. J., et al., 1999. Association of radiologically ascertained pneumonia before age 3 yr with asthmalike symptoms and pulmonary function during childhood: a prospective study. *Am J Respir Crit Care Med*, 159(6), 1891–7.

Cawcutt, K. & Kalil, A. C., 2017. Pneumonia with bacterial and viral coinfection. *Curr Opin Crit Care*, 23(5), 385–390.

Cevey-Macherel, M., Galetto-Lacour, A., Gervaix, A., Siegrist, C. A., Bille, J., Bescher-Ninet, B., et al., 2009. Etiology of community-acquired pneumonia in hospitalized children based on WHO clinical guidelines. *Eur J Pediatr*, 168(12), 1429–36.

Chan, J. Y., Stern, D. A., Guerra, S., Wright, A. L., Morgan, W. J. & Martinez, F. D., 2015. Pneumonia in childhood and impaired lung function in adults: a longitudinal study. *Pediatrics*, 135(4), 607–16.

Chapman, S. J., Vannberg, F. O., Khor, C. C., Rautanen, A., Maskell, N. A., Davies, C. W., et al., 2010. Mannose-binding lectin genotypes: lack of association with susceptibility to thoracic empyema. *BMC Med Genet*, 11, 5.

Chen, Y., Fan, L. C., Chai, Y. H. & Xu, J. F., 2022. Advantages and challenges of metagenomic sequencing for the diagnosis of pulmonary infectious diseases. *Clin Respir J*., 16(10), 646–656.

Chiu, C. Y. & Miller, S. A., 2019. Clinical metagenomics. *Nat Rev Genet*, 20(6), 341–355.

Christensen, A., Kesti, O., Elenius, V., Eskola, A. L., Døllner, H., Altunbulakli, C., et al., 2019. Human bocaviruses and paediatric infections. *Lancet Child Adolesc Health*, 3(6), 418–426.

Cilla, G., Oñate, E., Perez-Yarza, E. G., Montes, M., Vicente, D. & Perez-Trallero, E., 2008. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. *J Med Virol*, 80(10), 1843–9.

Cohen, E., Mahant, S., Dell, S. D., Traubici, J., Ragone, A., Wadhwa, A., et al., 2012. The long-term outcomes of pediatric pleural empyema: a prospective study. *Arch Pediatr Adolesc Med*, 166(11), 999–1004.

Cohen, R., Angoulvant, F., Biscardi, S., Madhi, F., Dubos, F. & Gillet, Y., 2017. Antibiotic treatment of lower respiratory tract infections. *Arch Pediatr*, 24(12S), S17–21.

Collaro, A. J., Chang, A. B., Marchant, J. M., Chatfield, M. D., Vicendese, D., Blake, T. L., et al., 2021. Early Childhood Pneumonia Is Associated with Reduced Lung Function and Asthma in First Nations Australian Children and Young Adults. *J Clin Med*, 10(24), 5727.

Connor, E. & Powell, K., 1985. Fulminant pneumonia caused by concomitant infection with influenza B virus and Staphylococcus aureus. *J Pediatr*, 106(3), 447–50.

Costa, L. F., Queiróz, D. A., Lopes da Silveira, H., Bernardino Neto, M., de Paula, N. T., Oliveira, T. F., et al., 2014. Human rhinovirus and disease severity in children. *Pediatrics*, 133(2), e312–21.

Daleno, C., Piralla, A., Scala, A., Senatore, L., Principi, N. & Esposito, S., 2013. Phylogenetic analysis of human rhinovirus isolates collected from otherwise healthy children with community-acquired pneumonia during five successive years. *PLoS One*, 8(11), e80614.

De, S., Williams, G. J., Hayen, A., Macaskill, P., McCaskill, M., Isaacs, D., et al., 2014. Value of white cell count in predicting serious bacterial infection in febrile children under 5 years of age. *Arch Dis Child*, 99(6), 493–9.

de Benedictis, F. M., Carloni, I., Osimani, P., Cobellis, G., Martino, A., Lanza, C., et al., 2019. Prospective evaluation of lung function in children with parapneumonic empyema. *Pediatr Pulmonol*, 54(4), 421–427.

de Benedictis, F. M., Kerem, E., Chang, A. B., Colin, A. A., Zar, H. J. & Bush, A., 2020. Complicated pneumonia in children. *Lancet*, 396(10253), 786–798.

de Koff, E. M., van Houten, M. A., Sanders, E. A. M. & Bogaert, D., 2021. Severity of Respiratory Infections With Seasonal Coronavirus Is Associated With Viral and Bacterial Coinfections. *Pediatr Infect Dis J*, 40(1), e36–39.

DeLuca, A. N., Hammitt, L. L., Kim, J., Higdon, M. M., Baggett, H. C., Brooks, W. A., et al., 2017. Safety of Induced Sputum Collection in Children Hospitalized With Severe or Very Severe Pneumonia. *Clin Infect Dis*, 64(suppl_3), S301–308.

Deng, W., Xu, H., Wu, Y. & Li, J., 2022. Diagnostic value of bronchoalveolar lavage fluid metagenomic next-generation sequencing in pediatric pneumonia. *Front Cell Infect Microbiol*, 12, 950531.

Díaz-Conradi, A., Hernández, S., García-García, J. J., Muñoz-Almagro, C., Moraga-Llop, F., Ciruela, P., et al., 2019. Complicated pneumococcal pneumonia with pleural effusion or empyema in the 13-valent pneumococcal conjugate vaccine era. *Pediatr Pulmonol*, 54(5), 517–524.

Dickson, R. P., Erb-Downward, J. R. & Huffnagle, G. B., 2014. Towards an ecology of the lung: new conceptual models of pulmonary microbiology and pneumonia pathogenesis. *Lancet Respir Med*, 2(3), 238–46.

Don, M., Fasoli, L., Paldanius, M., Vainionpää, R., Kleemola, M., Räty, R., et al., 2005. Aetiology of community-acquired pneumonia: serological results of a paediatric survey. *Scand J Infect Dis*, 37(11-12), 806–12.

Don, M., Korppi, M., Valent, F., Vainionpää, R. & Canciani, M., 2008. Human metapneumovirus pneumonia in children: results of an Italian study and mini-review. *Scand J Infect Dis*, 40(10), 821–6.

Don, M., Canciani, M. & Korppi, M., 2010. Community-acquired pneumonia in children: what's old? What's new? *Acta Paediatr*, 99(11), 1602–8.

Donnelly, L. F. & Klosterman, L. A., 1998. Cavitary necrosis complicating pneumonia in children: sequential findings on chest radiography. *AJR Am J Roentgenol*, 171(1), 253–6.

Dowell, S. F., Garman, R. L., Liu, G., Levine, O. S. & Yang, Y. H., 2001. Evaluation of Binax NOW, an assay for the detection of pneumococcal antigen in urine samples, performed among pediatric patients. *Clin Infect Dis*, 32(5), 824–5.

Drysdale, S. B., Mejias, A. & Ramilo, O., 2017. Rhinovirus - not just the common cold. *J Infect*, 74(Suppl 1), S41–46.

Dunne, E. M., Murad, C., Sudigdoadi, S., Fadlyana, E., Tarigan, R., Indriyani, S. A. K., et al., 2018. Carriage of Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, and Staphylococcus aureus in Indonesian children: A cross-sectional study. *PLoS One*, 13(4), e0195098.

Duong, Q. A., Pittet, L. F., Curtis, N. & Zimmermann, P., 2022. Antibiotic exposure and adverse long-term health outcomes in children: A systematic review and meta-analysis. *J Infect*, 85(3), 213–300.

Eastham, K. M., Hammal, D. M., Parker, L. & Spencer, D. A., 2008. A follow-up study of children hospitalised with community-acquired pneumonia. *Arch Dis Child*, 93(9), 755–9.

Ebruke, B. E., Deloria Knoll, M., Haddix, M., Zaman, S. M. A., Prosperi, C., Feikin, D. R., et al., 2021. The Etiology of Pneumonia From Analysis of Lung Aspirate and Pleural Fluid Samples: Findings From the Pneumonia Etiology Research for Child Health (PERCH) Study. *Clin Infect Dis*, 73(11), e3788–3796.

Edmond, K., Scott, S., Korczak, V., Ward, C., Sanderson, C., Theodoratou, E., et al., 2012. Long term sequelae from childhood pneumonia; systematic review and meta-analysis. *PLoS One*, 7(2), e31239.

Eisen, D. P., Dean, M. M., Boermeester, M. A., Fidler, K. J., Gordon, A. C., Kronborg, G., et al., 2008. Low serum mannose-binding lectin level increases the risk of death due to pneumococcal infection. *Clin Infect Dis*, 47(4), 510–6.

Eklundh, A., Rhedin, S., Ryd-Rinder, M., Andersson, M., Gantelius, J., Gaudenzi, G., et al., 2021. Etiology of Clinical Community-Acquired Pneumonia in Swedish Children Aged 1-59 Months with High Pneumococcal Vaccine Coverage-The TREND Study. *Vaccines (Basel)*, 9(4), 384.

Elemraid, M. A., Thomas, M. F., Blain, A. P., Rushton, S. P., Spencer, D. A., Gennery, A. R., et al., 2015. Risk factors for the development of pleural empyema in children. *Pediatr Pulmonol*, 50(7), 721-6.

Erdem, S. B., Can, D., Girit, S., Çatal, F., Şen, V., Pekcan, S., et al., 2018. Does atopy affect the course of viral pneumonia? *Allergol Immunopathol (Madr)*, 46(2), 119–126.

Esneau, C., Duff, A. C. & Bartlett, N. W., 2022. Understanding Rhinovirus Circulation and Impact on Illness. *Viruses*, 14(1), 141.

Esposito, S., Bosis, S., Niesters, H. G., Tremolati, E., Sabatini, C., Porta, A., et al., 2008. Impact of human bocavirus on children and their families. *J Clin Microbiol*, 46(4), 1337–42.

Esposito, S., Tagliabue, C., Picciolli, I., Semino, M., Sabatini, C., Consolo, S., et al., 2011. Procalcitonin measurements for guiding antibiotic treatment in pediatric pneumonia. *Respir Med*, 105(12), 1939–45.

Esposito, S., Daleno, C., Tagliabue, C., Scala, A., Tenconi, R., Borzani, I., et al., 2012. Impact of rhinoviruses on pediatric community-acquired pneumonia. *Eur J Clin Microbiol Infect Dis*, 31(7), 1637–45.

Esquivel, A., Busse, W. W., Calatroni, A., Togias, A. G., Grindle, K. G., Bochkov, Y. A., et al., 2017. Effects of Omalizumab on Rhinovirus Infections, Illnesses, and Exacerbations of Asthma. *Am J Respir Crit Care Med*, 196(8), 985–992.

Fathima, P., Blyth, C. C., Lehmann, D., Lim, F. J., Abdalla, T., de Klerk, N., et al., 2018. The Impact of Pneumococcal Vaccination on Bacterial and Viral Pneumonia in Western Australian Children: Record Linkage Cohort Study of 469589 Births, 1996-2012. *Clin Infect Dis*, 66(7), 1075–1085.

Fedele, G., Schiavoni, I., Nenna, R., Pierangeli, A., Frassanito, A., Leone, P., et al., 2018. Analysis of the immune response in infants hospitalized with viral bronchiolitis shows different Th1/Th2 profiles associated with respiratory syncytial virus and human rhinovirus. *Pediatr Allergy Immunol*, 29(5), 555–557.

Feikin, D. R., Njenga, M. K., Bigogo, G., Aura, B., Gikunju, S., Balish, A., et al., 2013. Additional diagnostic yield of adding serology to PCR in diagnosing viral acute respiratory infections in Kenyan patients 5 years of age and older. *Clin Vaccine Immunol*, 20(1), 113–4.

Feikin, D. R., Hammitt, L. L., Murdoch, D. R., O'Brien, K. L. & Scott, J. A. G., 2017. The Enduring Challenge of Determining Pneumonia Etiology in Children: Considerations for Future Research Priorities. *Clin Infect Dis*, 64(suppl_3), S188–196.

Feller-Kopman, D. & Light, R., 2018. Pleural Disease. *N Engl J Med*, 378(8), 740–751.

Finelli, L., Fiore, A., Dhara, R., Brammer, L., Shay, D. K., Kamimoto, L., et al., 2008. Influenza-associated pediatric mortality in the United States: increase of Staphylococcus aureus coinfection. *Pediatrics*, 122(4), 805–11.

Finney, L. J., Belchamber, K. B. R., Fenwick, P. S., Kemp, S. V., Edwards, M. R., Mallia, P., et al., 2019. Human Rhinovirus Impairs the Innate Immune Response to Bacteria in Alveolar Macrophages in Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*, 199(12), 1496–1507.

Flood, R. G., Badik, J. & Aronoff, S. C., 2008. The utility of serum C-reactive protein in differentiating bacterial from nonbacterial pneumonia in children: a meta-analysis of 1230 children. *Pediatr Infect Dis J*, 27(2), 95–9.

Fuji, N., Suzuki, A., Lupisan, S., Sombrero, L., Galang, H., Kamigaki, T., et al., 2011. Detection of human rhinovirus C viral genome in blood among children with severe respiratory infections in the Philippines. *PLoS One*, 6(11), e27247.

Franquet T., 2018. Imaging of Community-acquired Pneumonia. *J Thorac Imaging*, 33(5), 282–294.

Fry, A. M., Lu, X., Chittaganpitch, M., Peret, T., Fischer, J., Dowell, S. F., et al., 2007. Human bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. *J Infect Dis*, 195(7), 1038–45.

Fry, A. M., Lu, X., Olsen, S. J., Chittaganpitch, M., Sawatwong, P., Chantra, S., et al., 2011. Human rhinovirus infections in rural Thailand: epidemiological evidence for rhinovirus as both pathogen and bystander. *PLoS One*, 6(3), e17780.

García-García, M. L., Calvo, C., Pozo, F., Villadangos, P. A., Pérez-Breña, P. & Casas, I., 2012. Spectrum of respiratory viruses in children with community-acquired pneumonia. *Pediatr Infect Dis J*, 31(8), 808–13.

Gareca Perales, J., Soleto Ortiz, L., Loayza Mafayle, R., Machuca Soto, B., Hidalgo Flores, L., López Montaño, J., et al., 2021. Diagnosis of Community-acquired Pneumonia in Hospitalized Children: A Multicenter Experience in Bolivia. *Pediatr Infect Dis J*, 40(1), 32–38.

GBD 2016 Lower Respiratory Infections Collaborators, 2018. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect Dis*, 18(11), 1191–1210.

GBD 2017 Causes of Death Collaborators, 2018. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*, 392(10159), 1736–1788.

Gilbert, D. N., 2010. Use of plasma procalcitonin levels as an adjunct to clinical microbiology. *J Clin Microbiol*, 48(7), 2325–9.

Gold, D. R., Tager, I. B., Weiss, S. T., Tosteson, T. D. & Speizer, F. E., 1989. Acute lower respiratory illness in childhood as a predictor of lung function and chronic respiratory symptoms. *Am Rev Respir Dis*, 140(4), 877–84.

Goka, E. A., Vallely, P. J., Mutton, K. J. & Klapper, P. E., 2014. Single and multiple respiratory virus infections and severity of respiratory disease: a systematic review. *Paediatr Respir Rev*, 15(4), 363–70.

Grafakou, O., Moustaki, M., Tsolia, M., Kavazarakis, E., Mathioudakis, J., Fretzayas, A., et al., 2004. Can chest X-ray predict pneumonia severity? *Pediatr Pulmonol*, 38(6), 465–9.

Grant, L. R., Hammitt, L. L., Murdoch, D. R., O'Brien, K. L. & Scott, J. A., 2012. Procedures for collection of induced sputum specimens from children. *Clin Infect Dis*, 54(Suppl 2), S140–5.

Greenberg, D., Givon-Lavi, N., Sadaka, Y., Ben-Shimol, S., Bar-Ziv, J. & Dagan, R., 2014. Short-course antibiotic treatment for community-acquired alveolar pneumonia in ambulatory children: a double-blind, randomized, placebo-controlled trial. *Pediatr Infect Dis J*, 33(2), 136–42.

Griffin, M. R., Zhu, Y., Moore, M. R., Whitney, C. G. & Grijalva, C. G., 2013. U.S. hospitalizations for pneumonia after a decade of pneumococcal vaccination. *N Engl J Med*, 369(2), 155-63.

Grijalva, C. G., Zhu, Y., Nuorti, J. P. & Griffin, M. R., 2011. Emergence of parapneumonic empyema in the USA. *Thorax*, 66(8), 663–8.

Grotenhuis, B. A., Janssen, P. J. & Eerenberg, J. P., 2008. The surgical treatment of stage III empyema: the effect on lung function. *Minerva Chir*, 63(1), 23–7.

Gunaratnam, L. C., Robinson, J. L. & Hawkes, M. T., 2021. Systematic Review and Meta-Analysis of Diagnostic Biomarkers for Pediatric Pneumonia. *J Pediatric Infect Dis Soc*, 10(9), 891–900.

Güneylioğlu, M. M., Güngör, A., Göktuğ, A., Üner, Ç., Bodur, İ., Yaradılmış, R. M., et al., 2022. Evaluation of the efficiency of the systemic immune-inflammation index in differentiating parapneumonic effusion from empyema. *Pediatr Pulmonol*, 57(7), 1625–1630.

Haggie, S., Selvadurai, H., Gunasekera, H., Fitzgerald, D. A., Lord, D. & Chennapragada, M. S., 2022. Pediatric empyema: Are ultrasound characteristics at the time of intervention predictive of reintervention? *Pediatr Pulmonol*, 57(7), 1643–1650.

Halabi, K. C., Stockwell, M. S., Alba, L., Vargas, C., Reed, C., Saiman L, et al., 2022. Clinical and socioeconomic burden of rhinoviruses/enteroviruses in the community. *Influenza Other Respir Viruses*, 16(5), 891–896.

Haller, O. & Kochs, G., 2011. Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity. *J Interferon Cytokine Res*, 31(1), 79–87.

Halminen, M., Ilonen, J., Julkunen, I., Ruuskanen, O., Simell, O. & Mäkelä M. J., 1997. Expression of MxA protein in blood lymphocytes discriminates between viral and bacterial infections in febrile children. *Pediatr Res*, 41(5), 647–50.

Hamano-Hasegawa, K., Morozumi, M., Nakayama, E., Chiba, N., Murayama, S. Y., Takayanagi, R., et al., 2008. Comprehensive detection of causative pathogens using real-time PCR to diagnose pediatric community-acquired pneumonia. *J Infect Chemother*, 14(6), 424–32.

Hammitt, L. L., Murdoch, D. R., Scott, J. A., Driscoll, A., Karron, R. A., Levine, O. S., et al., 2012. Specimen collection for the diagnosis of pediatric pneumonia. *Clin Infect Dis*, 54(Suppl 2), S132–9.

Harris, M., Clark, J., Coote, N., Fletcher, P., Harnden, A., McKean, M., et al., 2011. British Thoracic Society guidelines for the management of community acquired pneumonia in children: update 2011. *Thorax*, 66(Suppl 2), ii1–23.

Hayden, F. G., 2004. Rhinovirus and the lower respiratory tract. *Rev Med Virol*, 14(1), 17–31.

Heikkinen, T., Marttila, J., Salmi, A. A. & Ruuskanen, O., 2002. Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. *J Clin Microbiol*, 40(11), 4337–9.

Heinonen, S., Jartti, T., Garcia, C., Oliva, S., Smitherman, C., Anguiano, E., et al., 2016. Rhinovirus Detection in Symptomatic and Asymptomatic Children: Value of Host Transcriptome Analysis. *Am J Respir Crit Care Med*, 193(7), 772–82.

Heiskanen-Kosma, T., Korppi, M., Jokinen, C., Kurki, S., Heiskanen, L., Juvonen, H., et al., 1998. Etiology of childhood pneumonia: serologic results of a prospective, population-based study. *Pediatr Infect Dis J*, 17(11), 986–91.

Helbig, K. J. & Beard, M. R., 2014. The role of viperin in the innate antiviral response. *J Mol Biol*, 426(6), 1210–9.

Herberg, J. A., Kaforou, M., Wright, V. J., Shailes, H., Eleftherohorinou, H., Hoggart, C. J., et al., 2016. Diagnostic Test Accuracy of a 2-Transcript Host RNA Signature for Discriminating Bacterial vs Viral Infection in Febrile Children. *JAMA*, 316(8), 835–45.

Howard, L. M., Rankin, D. A., Spieker, A. J., Gu, W., Haddadin, Z., Probst. V., et al., 2021. Clinical features of parainfluenza infections among young children hospitalized for acute respiratory illness in Amman, Jordan. *BMC Infect Dis*, 21(1), 323.

Hu, X., Yu, J., Crosby, S. D. & Storch, G. A., 2013. Gene expression profiles in febrile children with defined viral and bacterial infection. *Proc Natl Acad Sci U S A*, 110(31), 12792–7.

Ideh, R. C., Howie, S. R., Ebruke, B., Secka, O., Greenwood, B. M., Adegbola, R. A., et al., 2011. Transthoracic lung aspiration for the aetiological diagnosis of pneumonia: 25 years of experience from The Gambia. *Int J Tuberc Lung Dis*, 15(6), 729–35.

Imakita, M., Shiraki, K., Yutani, C. & Ishibashi-Ueda, H., 2000. Pneumonia caused by rhinovirus. *Clin Infect Dis*, 30(3), 611–2.

Ishiguro, T., Yoshida, Y., Kobayashi, Y., Shimizu, Y. & Takayanagi, N., 2019. Primary rhinovirus pneumonia in which bronchoalveolar lavage fluid yielded human rhinovirus. *Respir Med Case Rep*, 28, 100910.

Ishizuka, S., Yamaya, M., Suzuki, T., Takahashi, H., Ida, S., Sasaki, T., et al., 2003. Effects of rhinovirus infection on the adherence of Streptococcus pneumoniae to cultured human airway epithelial cells. *J Infect Dis*, 188(12), 1928–39.

Islam, S., Calkins, C. M., Goldin, A. B., Chen, C., Downard, C. D., Huang, E. Y., et al., 2012. The diagnosis and management of empyema in children: a comprehensive review from the APSA Outcomes and Clinical Trials Committee. *J Pediatr Surg*, 47(11), 2101–10.

Iwane, M. K., Prill, M. M., Lu, X., Miller, E. K., Edwards, K. M., Hall, C. B., et al., 2011. Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. *J Infect Dis*, 204(11), 1702–10.

Jackson, D. J., Gangnon, R. E., Evans, M. D., Roberg, K. A., Anderson, E. L., Pappas, T. E., et al., 2008. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med*, 178(7), 667–72.

Jackson, D. J., Evans, M. D., Gangnon, R. E., Tisler, C. J., Pappas, T. E., Lee, W. M., et al., 2012. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Respir Crit Care Med*, 185(3), 281–5.

Jackson, S., Mathews, K. H., Pulanic, D., Falconer, R., Rudan, I., Campbell, H., et al., 2013. Risk factors for severe acute lower respiratory infections in children: a systematic review and meta-analysis. *Croat Med J*, 54(2), 110–21.

Jacobs, S. E., Lamson, D. M., St George, K. & Walsh, T. J., 2013. Human rhinoviruses. *Clin Microbiol Rev*, 26(1), 135–62.

Jain, S., Williams, D. J., Arnold, S. R., Ampofo, K., Bramley, A. M., Reed, C., et al., 2015. Community-acquired pneumonia requiring hospitalization among U.S. children. *N Engl J Med*, 372(9), 835–45.

Jansen, R. R., Wieringa, J., Koekkoek, S. M., Visser, C. E., Pajkrt, D., Molenkamp, R., et al., 2011a. Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. *J Clin Microbiol*, 49(7), 2631–6.

Jansen, R. R., Schinkel, J., Koekkoek, S., Pajkrt, D., Beld, M., de Jong, M. D., et al., 2011b. Development and evaluation of a four-tube real time multiplex PCR assay covering fourteen respiratory viruses, and comparison to its corresponding single target counterparts. *J Clin Virol*, 51(3), 179–85.

Jartti, T., Jartti, L., Peltola, V., Waris, M. & Ruuskanen, O., 2008a. Identification of respiratory viruses in asymptomatic subjects: asymptomatic respiratory viral infections. *Pediatr Infect Dis J*, 27(12), 1103–7.

Jartti, T., Lee, W. M., Pappas, T., Evans, M., Lemanske, R. F. Jr. & Gern, J. E., 2008b. Serial viral infections in infants with recurrent respiratory illnesses. *Eur Respir J*, 32(2), 314–20.

Jartti, T., Hedman, K., Jartti, L., Ruuskanen, O., Allander, T. & Söderlund-Venermo, M., 2012. Human bocavirus-the first 5 years. *Rev Med Virol*, 22(1), 46–64.

Jartti, T., Söderlund-Venermo, M., Hedman, K., Ruuskanen, O. & Mäkelä, M. J., 2013. New molecular virus detection methods and their clinical value in lower respiratory tract infections in children. *Paediatr Respir Rev*, 14(1), 38–45.

Jartti, T. & Gern, J. E., 2017. Role of viral infections in the development and exacerbation of asthma in children. *J Allergy Clin Immunol*, 140(4), 895–906.

Jennings, L. C., Anderson, T. P., Beynon, K. A., Chua, A., Laing, R. T., Werno, A. M., et al., 2008. Incidence and characteristics of viral community-acquired pneumonia in adults. *Thorax*, 63(1), 42–8.

Johnston, I. D., Strachan, D. P. & Anderson, H. R., 1998. Effect of pneumonia and whooping cough in childhood on adult lung function. *N Engl J Med*, 338(9), 581–7.

Jokinen, C., Heiskanen, L., Juvonen, H., Kallinen, S., Karkola, K., Korppi, M., et al., 1993. Incidence of community-acquired pneumonia in the population of four municipalities in eastern Finland. *Am J Epidemiol*, 137(9), 977–88.

Jonnalagadda, S., Rodríguez, O., Estrella, B., Sabin, L. L., Sempértegui, F. & Hamer, D. H., 2017. Etiology of severe pneumonia in Ecuadorian children. *PLoS One*, 12(2), e0171687.

Juvén, T., Mertsola, J., Waris, M., Leinonen, M., Meurman, O., Roivainen, M., et al., 2000. Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr Infect Dis J*, 19(4), 293–8.

Juvén, T., Ruuskanen, O. & Mertsola, J., 2003. Symptoms and signs of community-acquired pneumonia in children. *Scand J Prim Health Care*, 21(1), 52–6.

Juvén, T., Mertsola, J., Waris, M., Leinonen, M. & Ruuskanen O., 2004. Clinical response to antibiotic therapy for community-acquired pneumonia. *Eur J Pediatr*, 163(3), 140–4.

Kamat, I. S., Ramachandran, V., Eswaran, H., Guffey, D. & Musher, D. M., 2020. Procalcitonin to Distinguish Viral From Bacterial Pneumonia: A Systematic Review and Meta-analysis. *Clin Infect Dis*, 70(3), 538–542.

Karhu, J., Ala-Kokko, T. I., Vuorinen, T., Ohtonen, P. & Syrjälä, H., 2014. Lower respiratory tract virus findings in mechanically ventilated patients with severe community-acquired pneumonia. *Clin Infect Dis*, 59(1), 62–70.

Karppinen, S., Vuononvirta, J., He, Q., Waris, M. & Peltola V., 2013. Effects of Rhinovirus Infection on Nasopharyngeal Bacterial Colonization in Infants With Wild or Variant Types of Mannose-Binding Lectin and Toll-Like Receptors 3 and 4. *J Pediatric Infect Dis Soc*, 2(3), 240–7.

Karppinen, S., Teräsjärvi, J., Auranen, K., Schuez-Havupalo, L., Siira, L., He, Q., et al., 2017. Acquisition and Transmission of Streptococcus pneumoniae Are Facilitated during Rhinovirus Infection in Families with Children. *Am J Respir Crit Care Med*, 196(9), 1172-1180.

Kazi, S., Hernstadt, H., Abo, Y. N., Graham, H., Palmer, M., Graham, S. M., et al., 2022. The utility of chest x-ray and lung ultrasound in the management of infants and children presenting with severe pneumonia in low-and middle-income countries: A pragmatic scoping review. *J Glob Health*, 12, 10013.

Kennedy, J. L., Turner, R. B., Braciale, T., Heymann, P. W. & Borish, L., 2012. Pathogenesis of rhinovirus infection. *Curr Opin Virol*, 2(3), 287–93.

Kennedy, J. L., Shaker, M., McMeen, V., Gern, J., Carper, H., Murphy, D., et al., 2014. Comparison of viral load in individuals with and without asthma during infections with rhinovirus. *Am J Respir Crit Care Med*, 189(5), 532–9.

Koch, A., Melbye, M., Sørensen, P., Homøe, P., Madsen, H. O., Mølbak, K., et al., 2001. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. *JAMA*, 285(10), 1316–21.

Kohn, G. L., Walston, C., Feldstein, J., Warner, B. W., Succop, P. & Hardie, W. D., 2002. Persistent abnormal lung function after childhood empyema. *Am J Respir Med*, 1(6), 441–5.

Koillinen, H., Wanne, O., Niemi, V. & Laakkonen, E., 1998. Terveiden suomalaisten lasten spirometrian ja uloshengityksen huippuvirtauksen viitearvot. *Suom Lääkärilehti*, 53, 395–402.

Konietzke, P., Mueller, J., Wuennemann, F., Wagner, W. L., Schenk, J. P., Alrajab, A., et al., 2020. The value of chest magnetic resonance imaging compared to chest radiographs with and without additional lung ultrasound in children with complicated pneumonia. *PLoS One*, 15(3), e0230252.

Korppi, M., Kröger, L. & Laitinen, M., 1993. White blood cell and differential counts in acute respiratory viral and bacterial infections in children. *Scand J Infect Dis*, 25(4), 435–40.

Korppi, M., Heiskanen-Kosma, T. & Kleemola, M., 2003. Mycoplasma pneumoniae causes over 50% of community-acquired pneumonia in school-aged children. *Scand J Infect Dis*, 35(4), 294.

Korppi, M., Karppa, H. & Vuento, R., 2013. Universal pneumococcal vaccination of <2-year-olds decreased pneumococcal bacteremia and antibiotic resistance in <5-year-olds. *Acta Paediatr*, 102(6), e239–40.

Korppi, M., 2017. The role of rhinoviruses is overestimated in the aetiology of community-acquired pneumonia in children. *Acta Paediatr*, 106(3), 363–365.

Kuitunen, I., Artama, M., Haapanen, M. & Renko, M., 2021. Rhinovirus spread in children during the COVID-19 pandemic despite social restrictions-A nationwide register study in Finland. *J Med Virol*, 93(10), 6063–6067.

Kuitunen, I., Jääskeläinen, J., Korppi, M. & Renko, M., 2022. Antibiotic treatment duration for community acquired pneumonia in outpatient children in high-income countries - a systematic review and meta-analysis. *Clin Infect Dis*, ciac374.

Kutty, P. K., Jain, S., Taylor, T. H., Bramley, A. M., Diaz, M. H., Ampofo, K., et al., 2019. Mycoplasma pneumoniae Among Children Hospitalized With Community-acquired Pneumonia. *Clin Infect Dis*, 68(1), 5–12.

Lahti, E., Peltola, V., Virkki, R. & Ruuskanen, O., 2006. Influenza pneumonia. *Paediatr Infect Dis J*, 25(2), 160–4.

Lahti, E., Peltola, V., Virkki, R., Alanen, M. & Ruuskanen, O., 2007. Development of parapneumonic empyema in children. *Acta Paediatr*, 96(11), 1686–92.

Lahti, E., Peltola, V., Waris, M., Virkki, R., Rantakokko-Jalava, K., Jalava, J., et al., 2009. Induced sputum in the diagnosis of childhood community-acquired pneumonia. *Thorax*, 64(3), 252–7.

Laiakis, E. C., Morris, G. A., Fornace, A. J. & Howie, S. R., 2010. Metabolomic analysis in severe childhood pneumonia in the Gambia, West Africa: findings from a pilot study. *PLoS One*, 5(9), e12655.

Lamborn, I. T., Jing, H., Zhang, Y., Drutman, S. B., Abbott, J. K., Munir, S., et al., 2017. Recurrent rhinovirus infections in a child with inherited MDA5 deficiency. *J Exp Med*, 214(7), 1949–1972.

Langelier, C., Kalantar, K. L., Moazed, F., Wilson, M. R., Crawford, E. D., Deiss, T., et al., 2018. Integrating host response and unbiased microbe detection for lower respiratory tract infection diagnosis in critically ill adults. *Proc Natl Acad Sci U S A*, 115(52), E12353–12362.

Lantto, M., Renko, M. & Uhari, M., 2013. Changes in infectious disease mortality in children during the past three decades. *Pediatr Infect Dis J*, 32(9), e355–9.

Las Heras, J. & Swanson, V. L., 1983. Sudden death of an infant with rhinovirus infection complicating bronchial asthma: case report. *Pediatr Pathol*, 1(3), 319-23.

le Roux, D. M., Myer, L., Nicol, M. P. & Zar, H. J., 2015. Incidence and severity of childhood pneumonia in the first year of life in a South African birth cohort: the Drakenstein Child Health Study. *Lancet Glob Health*, 3(2), e95–103.

le Roux, D. M. & Zar, H. J., 2017. Community-acquired pneumonia in children - a changing spectrum of disease. *Pediatr Radiol*, 47(11), 1392–1398.

Le Saux, N., Robinson, J. L. & Canadian Paediatric Society, Infectious Diseases and Immunization Committee, 2015. Uncomplicated pneumonia in healthy Canadian children and youth: Practice points for management. *Paediatr Child Health*, 20(8), 441–50.

Lee, W. M., Lemanske, R. F. Jr., Evans, M. D., Vang, F., Pappas, T., Gangnon, R., et al., 2012. Human rhinovirus species and season of infection determine illness severity. *Am J Respir Crit Care Med*, 186(9), 886–91.

Legg, J. & Rampton, C., 2018. British Thoracic Society paediatric pneumonia audit. National audit period: 1 November 2016–31 January 2017. From ⟨https://www.brit-thoracic.org.uk/quality-improvement/clinical-audit/bts-national-audit-reports/⟩.

Lehtinen, O., Broman, N., Waris, M., Vuorinen, T., Peltola, V., Löyttyniemi, E., et al., 2022. Association of human myxovirus resistance protein A with severity of COVID-19. *BMC Infect Dis*, 22(1), 755.

Leung, N. H. L., 2021. Transmissibility and transmission of respiratory viruses. *Nat Rev Microbiol*, 19(8), 528–545.

Li, Y., Reeves, R. M., Wang, X., Bassat, Q., Brooks, W. A., Cohen, C., et al., 2019. Global patterns in monthly activity of influenza virus, respiratory syncytial virus, parainfluenza virus, and metapneumovirus: a systematic analysis. *Lancet Glob Health*, 7(8), e1031–1045.

Li, Q., Zhou, Q., Florez, I. D., Mathew, J. L., Shang, L., Zhang, G., et al., 2022. Short-Course vs Long-Course Antibiotic Therapy for Children With Nonsevere Community-Acquired Pneumonia: A Systematic Review and Meta-analysis. *JAMA Pediatr*, 176(12), 1199-1207.

Liese, J. G., Schoen, C., van der Linden, M., Lehmann, L., Goettler, D., Keller, S., et al., 2019. Changes in the incidence and bacterial aetiology of paediatric parapneumonic pleural effusions/empyema in Germany, 2010-2017: a nationwide surveillance study. *Clin Microbiol Infect*, 25(7), 857–864.

Little, P., Francis, N. A., Stuart, B., O'Reilly, G., Thompson, N., Becque, T., et al., 2021. Antibiotics for lower respiratory tract infection in children presenting in primary care in England (ARTIC PC): a double-blind, randomised, placebo-controlled trial. *Lancet*, 398(10309), 1417–1426.

Liu, L., Oza, S., Hogan, D., Chu, Y., Perin, J., Zhu, J., et al., 2016. Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet*, 388(10063), 3027–3035.

Liu, L., Pan, Y., Zhu, Y., Song, Y., Su, X., Yang, L., et al., 2017. Association between rhinovirus wheezing illness and the development of childhood asthma: a meta-analysis. *BMJ Open*, 7(4), e013034.

Liu, J., Zhao, H., Feng, Z., Liu, Y., Feng, Q., Qian, S., et al., 2022. A severe case of human rhinovirus A45 with central nervous system involvement and viral sepsis. *Virol J*, 19(1), 72.

Loens, K., Van Heirstraeten, L., Malhotra-Kumar, S., Goossens, H. & Ieven, M., 2009. Optimal sampling sites and methods for detection of pathogens possibly causing community-acquired lower respiratory tract infections. *J Clin Microbiol*, 47(1), 21–31.

Louie, J. K., Roy-Burman, A., Guardia-Labar, L., Boston, E. J., Kiang, D., Padilla, T., et al., 2009. Rhinovirus associated with severe lower respiratory tract infections in children. *Pediatr Infect Dis J*, 2(4), 337–9.

Lu, X., Schneider, E., Jain, S., Bramley, A. M., Hymas, W., Stockmann, C., et al., 2017. Rhinovirus Viremia in Patients Hospitalized With Community-Acquired Pneumonia. *J Infect Dis*, 216(9), 1104–1111.

Lu, X., Zhang, L., Du, H., Zhang, J., Li, Y. Y., Qu, J., et al., 2020. SARS-CoV-2 Infection in Children. *N Engl J Med*, 382(17), 1663–1665.

Ludvigsson, J. F., 2020. Systematic review of COVID-19 in children shows milder cases and a better prognosis than adults. *Acta Paediatr*, 109(6), 1088–1095.

Luoto, R., Ruuskanen, O., Waris, M., Kalliomäki, M., Salminen, S. & Isolauri E., 2014. Prebiotic and probiotic supplementation prevents rhinovirus infections in preterm infants: a randomized, placebo-controlled trial. *J Allergy Clin Immunol*, 133(2), 405–13.

Lupo, J., Schuffenecker, I., Morel-Baccard, C., Bardet, J., Payen, V., Kaiser, L., et al., 2015. Disseminated rhinovirus C8 infection with infectious virus in blood and fatal outcome in a child with repeated episodes of bronchiolitis. *J Clin Microbiol*, 53(5), 1775–7.

Lv, Y. T., Sun, X. J., Chen, Y., Ruan, T., Xu, G. P. & Huang, J. A., 2022. Epidemic characteristics of *Mycoplasma pneumoniae* infection: a retrospective analysis of a single center in Suzhou from 2014 to 2020. *Ann Transl Med*, 10(20), 1123.

Lydon, E. C., Henao, R., Burke, T. W., Aydin, M., Nicholson, B. P., Glickman, S. W., et al., 2019. Validation of a host response test to distinguish bacterial and viral respiratory infection. *EBioMedicine*, 48, 453–461.

Lönnrot, M., Sjöroos, M., Salminen, K., Maaronen, M., Hyypiä, T. & Hyöty, H., 1999. Diagnosis of enterovirus and rhinovirus infections by RT-PCR and time-resolved fluorometry with lanthanide chelate labeled probes. *J Med Virol*, 59(3), 378–84.

Madhi, F., Levy, C., Morin, L., Minodier, P., Dubos, F., Zenkhri, F., et al., 2019. Change in Bacterial Causes of Community-Acquired Parapneumonic Effusion and Pleural Empyema in Children 6 Years After 13-Valent Pneumococcal Conjugate Vaccine Implementation. *J Pediatric Infect Dis Soc*, 8(5), 474–477.

Maffey, A., Colom, A., Venialgo, C., Acastello, E., Garrido, P., Cozzani, H., et al., 2019. Clinical, functional, and radiological outcome in children with pleural empyema. *Pediatr Pulmonol*, 54(5), 525–530.

Mancino, E., Cristiani, L., Pierangeli, A., Scagnolari, C., Nenna, R., Petrarca, L., et al., 2020. A single centre study of viral community-acquired pneumonia in children: No evidence of SARS-CoV-2 from October 2019 to March 2020. *J Clin Virol*, 128, 104385.

Mann, Y., Zeller, P., Carrillo-Kappus, K., Victor, M. & Moore M., 2020. Severe Pulmonary Infection in a 20-Month-Old Female. *Case Rep Infect Dis*, 2020, 7301617.

Mardian, Y., Menur Naysilla, A., Lokida, D., Farida, H., Aman, A. T., Karyana, M., et al., 2021. Approach to Identifying Causative Pathogens of Community-Acquired Pneumonia in Children Using Culture, Molecular, and Serology Tests. *Front Pediatr*, 9, 629318.

Martin, E. T., Fairchok, M. P., Kuypers, J., Magaret, A., Zerr, D. M., Wald, A., et al., 2010. Frequent and prolonged shedding of bocavirus in young children attending daycare. *J Infect Dis*, 201(11), 1625–32.

Martinón-Torres, F., Dosil-Gallardo, S., Perez del Molino-Bernal, M. L. , Sánchez, F. P., Tarrago, D., Alvez, F., et al., 2012. Pleural antigen assay in the diagnosis of pediatric pneumococcal empyema. *J Crit Care*, 27(3), 321.e1–4.

Masters, I. B., Isles, A. F. & Grimwood, K., 2017. Necrotizing pneumonia: an emerging problem in children? *Pneumonia (Nathan)*, 9, 11.

Mattila, J. M., Vuorinen, T. & Heikkinen, T., 2020. Comparative Severity of Influenza A and B Infections in Hospitalized Children. *Pediatr Infect Dis J*, 39(6), 489–493.

Mattila, S., Paalanne, N., Honkila, M., Pokka, T. & Tapiainen, T., 2022. Effect of Point-of-Care Testing for Respiratory Pathogens on Antibiotic Use in Children: A Randomized Clinical Trial. *JAMA Netw Open*, 5(6), e2216162.

McAllister, D. A., Liu, L., Shi, T., Chu, Y., Reed, C., Burrows, J., et al., 2019. Global, regional, and national estimates of pneumonia morbidity and mortality in children younger than 5 years between 2000 and 2015: a systematic analysis. *Lancet Glob Health*, 7(1), e47–57.

McIntosh K., 2002. Community-acquired pneumonia in children. *N Engl J Med*, 346(6), 429–37.

McLaughlin, F. J., Goldmann, D. A., Rosenbaum, D. M., Harris, G. B., Schuster, S. R. & Strieder, D. J., 1984. Empyema in children: clinical course and long-term follow-up. *Pediatrics*, 73(5), 587–93.

Megremis, S., Niespodziana, K., Cabauatan, C., Xepapadaki, P., Kowalski, M. L., Jartti, T., et al., 2018. Rhinovirus Species-Specific Antibodies Differentially Reflect Clinical Outcomes in Health and Asthma. *Am J Respir Crit Care Med*, 198(12), 1490–1499.

Miao, H., Li, H., Yao, Y., Wu, M., Lu, C., Wang, J., et al., 2020. Update on recommendations for the diagnosis and treatment of SARS-CoV-2 infection in children. *Eur J Clin Microbiol Infect Dis*, 39(12), 2211–2223.

Michelow, I. C., Olsen, K., Lozano, J., Rollins, N. K., Duffy, L. B., Ziegler, T., et al., 2004. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. *Pediatrics*, 113(4), 701–7.

Milcent, K., Faesch, S., Gras-Le Guen, C., Dubos, F., Poulalhon, C., Badier, I., et al., 2016. Use of Procalcitonin Assays to Predict Serious Bacterial Infection in Young Febrile Infants. *JAMA Pediatr*, 170(1), 62–9.

Miller, E. K., Bugna, J., Libster, R., Shepherd, B. E., Scalzo, P. M., Acosta, P. L., et al., 2012. Human rhinoviruses in severe respiratory disease in very low birth weight infants. *Pediatrics*, 129(1), e60–7.

Monto, A. S., 2002. The seasonality of rhinovirus infections and its implications for clinical recognition. *Clin Ther*, 24(12), 1987–97.

Morimoto, Y., Ishiguro, T., Kasuga, K., Takano, K., Ozawa, R., Isono, T., et al., 2021. Two Cases of Primary Rhinovirus Pneumonia with Multiple Pulmonary Nodules. *Intern Med*, 60(3), 435-439.

Moriyama, M., Hugentobler, W. J. & Iwasaki, A., 2020. Seasonality of Respiratory Viral Infections. *Annu Rev Virol*, 7(1), 83–101.

Murdoch, D. R., O'Brien, K. L., Driscoll, A. J., Karron, R. A., Bhat, N., Pneumonia Methods Working Group, et al., 2012. Laboratory methods for determining pneumonia etiology in children. *Clin Infect Dis*, 54(Suppl 2), S146–52.

Nascimento-Carvalho, C. M., Ribeiro, C. T., Cardoso, M. R., Barral, A., Araújo-Neto, C. A., Oliveira, J. R., et al., 2008. The role of respiratory viral infections among children hospitalized for community-acquired pneumonia in a developing country. *Pediatr Infect Dis J*, 27(10), 939–41.

Nascimento-Carvalho, A. C., Vilas-Boas, A. L., Fontoura, M. H., Vuorinen, T., Nascimento-Carvalho, C. M. & PNEUMOPAC-Efficacy Study Group, 2018a. Respiratory viruses among children with non-severe community-acquired pneumonia: A prospective cohort study. *J Clin Virol*, 105, 77–83.

Nascimento-Carvalho, A. C., Vilas-Boas, A. L., Fontoura, M. H., Xu, M., Vuorinen, T., Söderlund-Venermo, M., et al., 2018b. Serologically diagnosed acute human bocavirus 1 infection in childhood community-acquired pneumonia. *Pediatr Pulmonol*, 53(1), 88–94.

Nascimento-Carvalho, A. C. & Nascimento-Carvalho, C. M., 2019 Clinical management of community-acquired pneumonia in young children. *Expert Opin Pharmacother*, 20(4), 435–442.

Nathan, A. M., The, C. S. J., Jabar, K. A., Teoh, B. T., Tangaperumal, A., Westerhout, C., et al., 2020. Bacterial pneumonia and its associated factors in children from a developing country: A prospective cohort study. *PLoS One*, 15(2), e0228056.

Neuman, M. I., Hall, M., Lipsett, S. C., Hersh, A. L., Williams, D. J., Gerber, J. S., et al., 2017. Utility of Blood Culture Among Children Hospitalized With Community-Acquired Pneumonia. *Pediatrics*, 140(3), e20171013.

Ng, K. T., Oong, X. Y., Lim, S. H., Chook, J. B., Takebe, Y., Chan, Y. F., et al., 2018. Viral Load and Sequence Analysis Reveal the Symptom Severity, Diversity, and Transmission Clusters of Rhinovirus Infections. *Clin Infect Dis*, 67(2), 261–268.

Ng, D. C., Tan, K. K., Ting, G. S. S., Ling, C., Fadzilah, N. F. B., Tan, S. F., et al., 2022. Comparison of Severe Viral Pneumonia Caused by SARS-CoV-2 and Other Respiratory Viruses Among Malaysian Children During the COVID-19 Pandemic. *Front Pediatr*, 10, 865099.

Nolan, V. G., Arnold, S. R., Bramley, A. M., Ampofo, K., Williams, D. J., Grijalva, C. G., et al., 2018. Etiology and Impact of Coinfections in Children Hospitalized With Community-Acquired Pneumonia. *J Infect Dis*, 218(2), 179-188.

O'Callaghan-Gordo, C., Bassat, Q., Morais, L., Díez-Padrisa, N., Machevo, S., Nhampossa, T., et al., 2011. Etiology and epidemiology of viral pneumonia among hospitalized children in rural Mozambique: a malaria endemic area with high prevalence of human immunodeficiency virus. *Pediatr Infect Dis J*, 30(1), 39–44.

Oliver, B. G., Lim, S., Wark, P., Laza-Stanca, V., King, N., Black, J. L., et al., 2008. Rhinovirus exposure impairs immune responses to bacterial products in human alveolar macrophages. *Thorax*, 63(6), 519–25.

Oterino Serrano, C., Alonso, E., Andrés, M., Buitrago, N. M., Pérez Vigara, A., Parrón Pajares, M., et al., 2020. Pediatric chest x-ray in covid-19 infection. *Eur J Radiol*, 131, 109236.

Oved, K., Cohen, A., Boico, O., Navon, R., Friedman, T., Etshtein, L., et al., 2015. A novel host-proteome signature for distinguishing between acute bacterial and viral infections. *PLoS One*, 10(3), e0120012.

Pabary, R. & Balfour-Lynn, I. M., 2013. Complicated pneumonia in children. *Breathe*, 9(3), 210–222.

Pacilli, M. & Nataraja, R. M., 2019. Management of paediatric empyema by video-assisted thoracoscopic surgery (VATS) versus chest drain with fibrinolysis: Systematic review and meta-analysis. *Paediatr Respir Rev*, 30, 42–48.

Padayachee, Y., Flicker, S., Linton, S., Cafferkey, J., Kon, O. M., Johnston, S. L., et al., 2021. Review: The Nose as a Route for Therapy. Part 2 Immunotherapy. *Front Allergy*, 2, 668781.

Palmu, A. A., Jokinen, J., Borys, D., Nieminen, H., Ruokokoski, E., Siira, L., et al., 2013. Effectiveness of the ten-valent pneumococcal Haemophilus influenzae protein D conjugate vaccine (PHiD-CV10) against invasive pneumococcal disease: a cluster randomised trial. *Lancet*, 381(9862), 214–22.

Pan, Q. & Hammarström, L., 2000. Molecular basis of IgG subclass deficiency. *Immunol Rev*, 178, 99–110.

Papadopoulos, N. G., Sanderson, G., Hunter, J. & Johnston, S. L., 1999. Rhinoviruses replicate effectively at lower airway temperatures. *J Med Virol*, 58(1), 100–4.

Papan, C., Argentiero, A., Porwoll, M., Hakim, U., Farinelli, E., Testa, I., et al., 2022. A host signature based on TRAIL, IP-10, and CRP for reducing antibiotic overuse in children by differentiating bacterial from viral infections: a prospective, multicentre cohort study. *Clin Microbiol Infect*, 28(5), 723-730.

Parisi, G. F., Indolfi, C., Decimo, F., Leonardi, S. & Miraglia Del Giudice, M., 2020. COVID-19 Pneumonia in Children: From Etiology to Management. *Front Pediatr*, 8, 616622.

Parker, J., Fowler, N., Walmsley, M. L., Schmidt, T., Scharrer, J., Kowaleski, J.,et al., 2015. Analytical Sensitivity Comparison between Singleplex Real-Time PCR and a Multiplex PCR Platform for Detecting Respiratory Viruses. *PLoS One*, 10(11), e0143164.

Passariello, C., Schippa, S., Conti, C., Russo, P., Poggiali, F., Garaci, E., et al., 2006. Rhinoviruses promote internalisation of Staphylococcus aureus into non-fully permissive cultured pneumocytes. *Microbes Infect*, 8(3), 758-66.

Peltola, V., Mertsola, J. & Ruuskanen, O., 2006. Comparison of total white blood cell count and serum C-reactive protein levels in confirmed bacterial and viral infections. *J Pediatr*, 149(5), 721–4.

Peltola, V., Ruuskanen, O. & Svedström, E., 2008. Magnetic resonance imaging of lung infections in children. *Pediatr Radiol*, 38(11), 1225–31.

Peltola, V., Waris, M., Österback, R., Susi, P., Ruuskanen, O. & Hyypiä, T., 2008. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. *J Infect Dis*, 197(3), 382–9.

Peltola, V., Jartti, T., Putto-Laurila, A., Mertsola, J., Vainionpää, R., Waris, M., et al., 2009. Rhinovirus infections in children: a retrospective and prospective hospital-based study. *J Med Virol*, 81(10), 1831–8.

Peltola, V., Heikkinen, T., Ruuskanen, O., Jartti, T., Hovi, T., Kilpi, T., et al., 2011. Temporal association between rhinovirus circulation in the community and invasive pneumococcal disease in children. *Pediatr Infect Dis J*, 30(6), 456–61.

Peltola, V., Waris, M., Kainulainen, L., Kero, J. & Ruuskanen, O., 2013. Virus shedding after human rhinovirus infection in children, adults and patients with hypogammaglobulinaemia. *Clin Microbiol Infect*, 19(7), E322–7.

Pendleton, K. M., Erb-Downward, J. R., Bao, Y., Branton, W. R., Falkowski, N. R., Newton, D. W., et al., 2017. Rapid Pathogen Identification in Bacterial Pneumonia Using Real-Time Metagenomics. *Am J Respir Crit Care Med*, 196(12), 1610–1612.

Pernica, J. M., Harman, S., Kam, A. J., Carciumaru, R., Vanniyasingam, T., Crawford, T., et al., 2021. Short-Course Antimicrobial Therapy for Pediatric Community-Acquired Pneumonia: The SAFER Randomized Clinical Trial. *JAMA Pediatr*, 175(5), 475–482.

Piri, R., Yahya, M., Ivaska, L., Toivonen, L., Lempainen, J., Nuolivirta, K., et al., 2022. Myxovirus Resistance Protein A as a Marker of Viral Cause of Illness in Children Hospitalized with an Acute Infection. *Microbiol Spectr*, 10(1), e0203121.

Plathow, C., Ley, S., Fink, C., Puderbach, M., Heilmann, M., Zuna, I., et al., 2004. Evaluation of chest motion and volumetry during the breathing cycle by dynamic MRI in healthy subjects: comparison with pulmonary function tests. *Invest Radiol*, 39(4), 202–9.

Pneumonia Etiology Research for Child Health (PERCH) Study Group, 2019. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. *Lancet*, 394(10200), 757–779.

Popper, S. J., Watson, V. E., Shimizu, C., Kanegaye, J. T., Burns, J. C. & Relman, D. A., 2009. Gene transcript abundance profiles distinguish Kawasaki disease from adenovirus infection. *J Infect Dis*, 200(4), 657–66.

Pratt, M. T. G., Abdalla, T., Richmond, P. C., Moore, H. C., Snelling, T. L., Blyth, C. C., et al., 2022. Prevalence of respiratory viruses in community-acquired pneumonia in children: a systematic review and meta-analysis. *Lancet Child Adolesc Health*, 6(8), 555-570.

Prayle, A., Atkinson, M. & Smyth A., 2011. Pneumonia in the developed world. *Paediatr Respir Rev*, 12(1), 60–9.

Proud, D., Turner, R. B., Winther, B., Wiehler, S., Tiesman, J. P., Reichling, T. D., et al., 2008. Gene expression profiles during in vivo human rhinovirus infection: insights into the host response. *Am J Respir Crit Care Med*, 178(9), 962–8.

Puel, A., Picard, C., Lorrot, M., Pons, C., Chrabieh, M., Lorenzo, L., et al., 2008. Recurrent staphylococcal cellulitis and subcutaneous abscesses in a child with autoantibodies against IL-6. *J Immunol*, 180(1), 647–54.

Ramilo, O., Allman, W., Chung, W., Mejias, A., Ardura, M., Glaser, C., et al., 2007. Gene expression patterns in blood leukocytes discriminate patients with acute infections. *Blood*, 109(5), 2066–77.

Rantala, A. K., Jaakkola, M. S., Mäkikyrö, E. M., Hugg, T. T. & Jaakkola, J. J., 2015. Early Respiratory Infections and the Development of Asthma in the First 27 Years of Life. *Am J Epidemiol*, 182(7), 615–23.

Rao, S., Lamb, M. M., Moss, A., Mistry, R. D., Grice, K., Ahmed, W., et al., 2021. Effect of Rapid Respiratory Virus Testing on Antibiotic Prescribing Among Children Presenting to the Emergency Department With Acute Respiratory Illness: A Randomized Clinical Trial. *JAMA Netw Open*, 4(6), e2111836.

Redding, G. J., Walund, L., Walund, D., Jones, J. W., Stamey, D. C. & Gibson, R. L., 1990. Lung function in children following empyema. *Am J Dis Child*, 144(12), 1337–42.

Reed, C., Kallen, A. J., Patton, M., Arnold, K. E., Farley, M. M., Hageman, J., et al., 2009. Infection with community-onset Staphylococcus aureus and influenza virus in hospitalized children. *Pediatr Infect Dis J*, 28(7), 572–6.

Renko, M. & Tapiainen, T., 2020. Change in respiratory syncytial virus seasonality in Finland. *Acta Paediatr*, 109(1), 202–203.

Revai, K., Patel, J. A., Grady, J. J., Nair, S., Matalon, R. & Chonmaitree T., 2009. Association between cytokine gene polymorphisms and risk for upper respiratory tract infection and acute otitis media. *Clin Infect Dis*, 49(2), 257–61.

Rhedin, S., Lindstrand, A., Hjelmgren, A., Ryd-Rinder, M., Öhrmalm, L., Tolfvenstam, T., et al., 2015. Respiratory viruses associated with community-acquired pneumonia in children: matched case-control study. *Thorax*, 70(9), 847–53.

Rhedin, S., Eklundh, A., Ryd-Rinder, M., Naucler, P., Mårtensson, A., Gantelius, J., et al., 2019. Introducing a New Algorithm for Classification of Etiology in Studies on Pediatric Pneumonia: Protocol for the Trial of Respiratory Infections in Children for Enhanced Diagnostics Study. *JMIR Res Protoc*, 8(4), e12705.

Rhedin, S., Elfving, K. & Berggren, A., 2021. Novel Biomarkers Differentiating Viral from Bacterial Infection in Febrile Children: Future Perspectives for Management in Clinical Praxis. *Children (Basel)*, 8(11), 1070.

Rhedin, S., Eklundh, A., Ryd-Rinder, M., Peltola, V., Waris, M., Gantelius, J., et al., 2022. Myxovirus resistance protein A for discriminating between viral and bacterial lower respiratory tract infections in children - The TREND study. *Clin Microbiol Infect*, S1198-743X(22)00264-6.

Ronni, T., Melén, K., Malygin, A. & Julkunen I., 1993. Control of IFN-inducible MxA gene expression in human cells. *J Immunol*, 150(5), 1715–26.

Rubió, J. R., Megremis, S., Pasioti, M., Lakoumentas, J., Constantinides, B., Xepapadaki, P., et al. Respiratory virome profiles reflect antiviral immune responses. *Authorea*, preprint August 2022.

Rudan, I., Tomaskovic, L., Boschi-Pinto, C., Campbell, H. & WHO Child Health Epidemiology Reference Group, 2004. Global estimate of the incidence of clinical pneumonia among children under five years of age. *Bull World Health Organ*, 82(12), 895–903.

Rudan, I., Boschi-Pinto, C., Biloglav, Z., Mulholland, K. & Campbell, H., 2008. Epidemiology and etiology of childhood pneumonia. *Bull World Health Organ*, 86(5), 408–16.

Rudan, I., O'Brien, K. L., Nair, H., Liu, L., Theodoratou, E., Qazi, S., et al., 2013. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. *J Glob Health*, 3(1), 010401.

Rueda, Z. V., Aguilar, Y., Maya, M. A., López, L., Restrepo, A., Garcés, C., et al., 2022. Etiology and the challenge of diagnostic testing of community-acquired pneumonia in children and adolescents. *BMC Pediatr*, 22(1), 169.

Ruuskanen, O. & Ogra, P. L., 1993. Respiratory syncytial virus. *Curr Probl Pediatr*, 23(2), 50–79.

Ruuskanen, O. & Mertsola, J., 1999. Childhood community-acquired pneumonia. *Semin Respir Infect*, 14(2), 163–72.

Ruuskanen, O., Lahti, E., Jennings, L. C. & Murdoch, D. R., 2011. Viral pneumonia. *Lancet*, 377(9773), 1264–75.

Ruuskanen, O. & Järvinen, A., 2014. What is the real role of respiratory viruses in severe community-acquired pneumonia? *Clin Infect Dis*, 59(1), 71–3.

Saarinen, N., Lehtonen, J., Veijola, R., Lempainen, J., Knip, M., Hyöty, H., et al., 2020. Multiplexed High-Throughput Serological Assay for Human Enteroviruses. *Microorganisms*, 8(6), 963.

Sahn, S. A., 2007. Diagnosis and management of parapneumonic effusions and empyema. *Clin Infect Dis*, 45(11), 1480–6.

Samransamruajkit, R., Hiranrat, T., Chieochansin, T., Sritippayawan, S., Deerojanawong, J., Prapphal, N., et al., 2008. Prevalence, clinical presentations and complications among hospitalized children with influenza pneumonia. *Jpn J Infect Dis*, 61(6), 446–9.

Satish, B., Bunker, M. & Seddon, P., 2003. Management of thoracic empyema in childhood: does the pleural thickening matter? *Arch Dis Child*, 88(10), 918–21.

Savage, T. J., Kuypers, J., Chu, H. Y., Bradford, M. C., Buccat, A. M., Qin, X., et al., 2018. Enterovirus D-68 in children presenting for acute care in the hospital setting. *Influenza Other Respir Viruses*, 12(4), 522–528.

Sawatwong, P., Chittaganpitch, M., Hall, H., Peruski, L. F., Xu, X., Baggett, H. C., et al., 2012. Serology as an adjunct to polymerase chain reaction assays for surveillance of acute respiratory virus infections. *Clin Infect Dis*, 54(3), 445–6.

Schulert, G. S., Hain, P. D. & Williams, D. J., 2014. Utilization of viral molecular diagnostics among children hospitalized with community acquired pneumonia. *Hosp Pediatr*, 4(6), 372–6.

Scott, J. A., Wonodi, C., Moïsi, J. C., Deloria-Knoll, M., DeLuca, A. N., Karron, R. A., et al., 2012. The definition of pneumonia, the assessment of severity, and clinical standardization in the Pneumonia Etiology Research for Child Health study. *Clin Infect Dis*, 54(Suppl 2), S109–16.

Self, W. H., Williams, D. J., Zhu, Y., Ampofo, K., Pavia, A. T., Chappell, J. D., et al. 2016. Respiratory Viral Detection in Children and Adults: Comparing Asymptomatic Controls and Patients With Community-Acquired Pneumonia. *J Infect Dis*, 213(4), 584–91.

Shi, T., McLean, K., Campbell, H. & Nair, H., 2015. Aetiological role of common respiratory viruses in acute lower respiratory infections in children under five years: A systematic review and meta-analysis. *J Glob Health*, 5(1), 010408.

Silkoff, P. E., Flavin, S., Gordon, R., Loza, M. J., Sterk, P. J., Lutter, R., et al, 2018. Toll-like receptor 3 blockade in rhinovirus-induced experimental asthma exacerbations: A randomized controlled study. *J Allergy Clin Immunol*, 141(4), 1220–1230.

Sonego, M., Pellegrin, M. C., Becker, G. & Lazzerini, M., 2015. Risk factors for mortality from acute lower respiratory infections (ALRI) in children under five years of age in low and middle-income countries: a systematic review and meta-analysis of observational studies. *PLoS One*, 10(1), e0116380.

Sorg, A. L., Obermeier, V., Liese, J. G. & von Kries, R., 2021. Incidence trends of parapneumonic pleural effusions/empyema in children 2009 to 2018 from health insurance data: Only temporal reduction after the introduction of PCV13. *Vaccine*, 39(26), 3516–3519.

Spencer, S., Köstel Bal, S., Egner, W., Lango Allen, H., Raza, S. I., Ma, C. A., 2019. Loss of the interleukin-6 receptor causes immunodeficiency, atopy, and abnormal inflammatory responses. *J Exp Med*, 216(9), 1986–1998.

Spichak, T. V., Yatsyshina, S. B., Katosova, L. K., Kim, S. S. & Korppi, M. O., 2016. Is the role of rhinoviruses as causative agents of pediatric community-acquired pneumonia over-estimated? *Eur J Pediatr*, 175(12), 1951–1958.

Stadler, J. A. M., Andronikou, S. & Zar, H. J., 2017. Lung ultrasound for the diagnosis of community-acquired pneumonia in children. *Pediatr Radiol*, 47(11), 1412–1419.

Stertz, S. & Hale, B. G., 2021. Interferon system deficiencies exacerbating severe pandemic virus infections. *Trends Microbiol*, 29(11), 973–982.

Subhi, R., Gelbart, B., Ching, N., Thompson, J., Osowicki, J., Rozen, T. H., et al., 2022. Characteristics, management and changing incidence of children with empyema in a paediatric intensive care unit. *J Paediatr Child Health*, 58(6), 1046–1052.

Summerfield, J. A., Sumiya, M., Levin, M. & Turner, M. W., 1997. Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. *BMJ*, 314(7089), 1229–32.

Swift, A. J., Woodhouse, N., Fichele, S., Siedel, J., Mills, G. H., van Beek, E. J., et al., 2007. Rapid lung volumetry using ultrafast dynamic magnetic resonance imaging during forced vital capacity maneuver: correlation with spirometry. *Invest Radiol*, 42(1), 37–41.

Sy, M. G. & Robinson, J. L., 2010. Community-acquired Moraxella catarrhalis pneumonia in previously healthy children. *Pediatr Pulmonol*, 45(7), 674–8.

Syrjänen, R. K., Kilpi, T. M., Kaijalainen, T. H., Herva, E. E. & Takala, A. K., 2001. Nasopharyngeal carriage of Streptococcus pneumoniae in Finnish children younger than 2 years old. *J Infect Dis*, 184(4), 451–9.

Tagarro, A., Otheo, E., Baquero-Artigao, F., Navarro, M. L., Velasco, R., Ruiz, M., et al., 2017. Dexamethasone for Parapneumonic Pleural Effusion: A Randomized, Double-Blind, Clinical Trial. *J Pediatr*, 185, 117–123.e6.

Techasaensiri, B., Techasaensiri, C., Mejías, A., McCracken, G. H. Jr. & Ramilo O., 2010. Viral coinfections in children with invasive pneumococcal disease. *Pediatr Infect Dis J*, 29(6), 519–23.

Thavagnanam, S., Christie, S. N., Doherty, G. M., Coyle, P. V., Shields, M. D. & Heaney, L. G., 2010. Respiratory viral infection in lower airways of asymptomatic children. *Acta Paediatr*, 99(3), 394–8.

Thomas, P., Riffelmann, M., Schweiger, B., Dominik, S. & von König, C. H., 2003. Fatal influenza A virus infection in a child vaccinated against influenza. *Pediatr Infect Dis J*, 22(2), 201–2.

Toikka, P., Irjala, K., Juvén, T., Virkki, R., Mertsola, J., Leinonen, M., et al., 2000. Serum procalcitonin, C-reactive protein and interleukin-6 for distinguishing bacterial and viral pneumonia in children. *Pediatr Infect Dis J*, 19(7), 598–602.

Toivonen, L., Schuez-Havupalo, L., Rulli, M., Ilonen, J., Pelkonen, J., Melen, K., et al., 2015. Blood MxA protein as a marker for respiratory virus infections in young children. *J Clin Virol*, 62, 8–13.

Toivonen, L., Schuez-Havupalo, L., Karppinen, S., Teros-Jaakkola, T., Rulli, M., Mertsola, J., et al., 2016. Rhinovirus Infections in the First 2 Years of Life. *Pediatrics*, 138(3), e20161309.

Toivonen, L., Vuononvirta, J., Mertsola, J., Waris, M., He, Q. & Peltola, V., 2017. Polymorphisms of Mannose-binding Lectin and Toll-like Receptors 2, 3, 4, 7 and 8 and the Risk of Respiratory Infections and Acute Otitis Media in Children. *Pediatr Infect Dis J*, 36(5), e114-e122.

Townsi, N., Laing, I. A., Hall, G. L. & Simpson, S. J., 2018. The impact of respiratory viruses on lung health after preterm birth. *Eur Clin Respir J*, 5(1), 1487214.

Tsolia, M. N., Psarras, S., Bossios, A., Audi, H., Paldanius, M., Gourgiotis, D., et al., 2004. Etiology of community-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. *Clin Infect Dis*, 39(5), 681–6.

Tsubota, N., Yanagawa, M., Yoshimura, M., Murotani, A. & Hatta, T., 1994. The superiority of exercise testing over spirometry in the evaluation of postoperative lung function for patients with pulmonary disease. *Surg Today*, 24(2), 103–5.

Turner, M. W., 2003. The role of mannose-binding lectin in health and disease. *Mol Immunol*, 40(7), 423–9.

Utokaparch, S., Marchant, D., Gosselink, J. V., McDonough, J. E., Thomas, E. E., Hogg, J. C., et al., 2011. The relationship between respiratory viral loads and diagnosis in children presenting to a pediatric hospital emergency department. *Pediatr Infect Dis J*, 30(2), e18-23.

van den Hoogen, B. G., de Jong, J. C., Groen, J., Kuiken, T., de Groot, R., Fouchier, R. A., et al., 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med*, 7(6), 719–24.

van der Zalm, M. M., van Ewijk, B. E., Wilbrink, B., Uiterwaal, C. S., Wolfs, T. F. & van der Ent, C. K., 2009. Respiratory pathogens in children with and without respiratory symptoms. *J Pediatr*, 154(3), 396–400, 400.e1.

van Gageldonk-Lafeber, A. B., Heijnen, M. L., Bartelds, A. I., Peters, M. F., van der Plas, S. M. & Wilbrink, B., 2005. A case-control study of acute respiratory tract infection in general practice patients in The Netherlands. *Clin Infect Dis*, 41(4), 490–7.

van Houten, C. B., de Groot, J. A. H., Klein, A., Srugo, I., Chistyakov, I., de Waal, W., et al., 2017. A host-protein based assay to differentiate between bacterial and viral infections in preschool children (OPPORTUNITY): a double-blind, multicentre, validation study. *Lancet Infect Dis*, 17(4), 431–440.

Vandini, S., Biagi, C., Fischer, M. & Lanari, M., 2019. Impact of Rhinovirus Infections in Children. *Viruses*, 11(6), 521.

Varela, F. H., Sartor, I. T. S., Polese-Bonatto, M., Azevedo, T. R., Kern, L. B., Fazolo, T., et al., 2022. Rhinovirus as the main co-circulating virus during the COVID-19 pandemic in children. *J Pediatr (Rio J)*, S0021-7557(22)00044-4.

Varghese, L., Zachariah, P., Vargas, C., LaRussa, P., Demmer, R. T., Furuya, Y. E., et al., 2018. Epidemiology and Clinical Features of Human Coronaviruses in the Pediatric Population. *J Pediatric Infect Dis Soc*, 7(2), 151–158.

Vasconcellos, Â. G., Clarêncio, J., Andrade, D., Cardoso, M. A., Barral, A. & Nascimento-Carvalho, C. M., 2018. Systemic cytokines and chemokines on admission of children hospitalized with community-acquired pneumonia. *Cytokine*, 107, 1–8.

Vasconcellos, Â. G., Clarêncio, J., Andrade, D., Araújo-Neto, C. A., Barral, A. & Nascimento-Carvalho, C. M., 2020. Systemic cytokines/chemokines associated to radiographic abnormalities in pneumonia in children. *Cytokine*, 135, 155191.

Verdu, P., Barreiro, L. B., Patin, E., Gessain, A., Cassar, O., Kidd, J. R., et al., 2006. Evolutionary insights into the high worldwide prevalence of MBL2 deficiency alleles. *Hum Mol Genet*, 15(17), 2650–8.

Virkki, R., Juvén, T., Rikalainen, H., Svedström, E., Mertsola, J. & Ruuskanen, O., 2002. Differentiation of bacterial and viral pneumonia in children. *Thorax*, 57(5), 438–41.

Virkki, R., Juvén, T., Mertsola, J. & Ruuskanen, O., 2005. Radiographic follow-up of pneumonia in children. *Pediatr Pulmonol*, 40(3), 223–7.

Vuori-Holopainen, E., Salo, E., Saxén, H., Hedman, K., Hyypiä, T., Lahdenperä, R., et al., 2002. Etiological diagnosis of childhood pneumonia by use of transthoracic needle aspiration and modern microbiological methods. *Clin Infect Dis*, 34(5), 583–90.

Wahl, B., O'Brien, K. L., Greenbaum, A., Majumder, A., Liu, L., Chu, Y., et al., 2018. Burden of Streptococcus pneumoniae and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000-15. *Lancet Glob Health*, 6(7), e744–757.

Walker, C. L. F., Rudan, I., Liu, L., Nair, H., Theodoratou, E., Bhutta, Z. A., et al., 2013. Global burden of childhood pneumonia and diarrhoea. *Lancet*, 381(9875), 1405–1416.

Wang, M., Cai, F., Wu, X., Wu, T., Su, X. & Shi, Y., 2015. Incidence of viral infection detected by PCR and real-time PCR in childhood community-acquired pneumonia: a meta-analysis. *Respirology*, 20(3), 405–12.

Wang, K., Xi, W., Yang, D., Zheng, Y., Zhang, Y., Chen, Y., et al., 2017. Rhinovirus is associated with severe adult community-acquired pneumonia in China. *J Thorac Dis*, 9(11), 4502-4511.

Wang, C. C., Prather, K. A., Sznitman, J., Jimenez, J. L., Lakdawala, S. S., Tufekci, Z., et al., 2021a. Airborne transmission of respiratory viruses. *Science*, 373(6558), eabd9149.

Wang, X., Li, Y., Deloria-Knoll, M., Madhi, S. A., Cohen, C., Ali, A., et al., 2021b. Global burden of acute lower respiratory infection associated with human metapneumovirus in children under 5 years in 2018: a systematic review and modelling study. *Lancet Glob Health*, 9(1), e33–43.

Wang, X., Li, Y., Deloria-Knoll, M., Madhi, S. A., Cohen, C., Arguelles, V. L., et al., 2021c. Global burden of acute lower respiratory infection associated with human parainfluenza virus in children younger than 5 years for 2018: a systematic review and meta-analysis. *Lancet Glob Health*, 9(8), e1077–1087.

Wardlaw, T., Salama, P., Johansson, E. W. & Mason, E., 2006. Pneumonia: the leading killer of children. *Lancet*, 368(9541), 1048–50.

Wark, P. A., Bucchieri, F., Johnston, S. L., Gibson, P. G., Hamilton, L., Mimica, J., et al., 2007. IFN-gamma-induced protein 10 is a novel biomarker of rhinovirus-induced asthma exacerbations. *J Allergy Clin Immunol*, 120(3), 586–93.

World Health Organization, 1990. Acute respiratory infections in children: case management in small hospitals in developing countries, a manual for doctors and other senior health workers. No. WHO/ARI/90.5. From ⟨WHO_ARI_90.5.pdf⟩.

World Health Organization, 2022. Pneumonia in children. From ⟨https://www.who.int/news-room/fact-sheets/detail/pneumonia⟩.

Wiese, A. D., Griffin, M. R., Zhu, Y., Mitchel, E. F. Jr. & Grijalva, C. G., 2016. Changes in empyema among U.S. children in the pneumococcal conjugate vaccine era. *Vaccine*, 34(50), 6243–6249.

Williams, D. J., Zhu, Y., Grijalva, C. G., Self, W. H., Harrell, F. E. Jr., Reed, C., et al., 2016. Predicting Severe Pneumonia Outcomes in Children. *Pediatrics*, 138(4), e20161019.

Williams, D. J., Creech, C. B., Walter, E. B., Martin, J. M., Gerber, J. S., Newland, J. G., et al., 2022. Short- vs Standard-Course Outpatient Antibiotic Therapy for Community-Acquired Pneumonia in Children: The SCOUT-CAP Randomized Clinical Trial. *JAMA Pediatr*, 176(3), 253–261.

Wingerter, S. L., Bachur, R. G., Monuteaux, M. C. & Neuman, M. I., 2012. Application of the world health organization criteria to predict radiographic pneumonia in a US-based pediatric emergency department. *Pediatr Infect Dis J*, 31(6), 561–4.

Wolf, D. G., Greenberg, D., Shemer-Avni, Y., Givon-Lavi, N., Bar-Ziv, J. & Dagan, R., 2010. Association of human metapneumovirus with radiologically diagnosed community-acquired alveolar pneumonia in young children. *J Pediatr*, 156(1), 115–20.

Xiang, Z., Gonzalez, R., Xie, Z., Xiao, Y., Chen, L., Li, Y., et al., 2008. Human rhinovirus group C infection in children with lower respiratory tract infection. *Emerg Infect Dis*, 14(10), 1665–7.

Yahya, M., Rulli, M., Toivonen, L., Waris, M. & Peltola, V., 2017. Detection of Host Response to Viral Respiratory Infection by Measurement of Messenger RNA for MxA, TRIM21, and Viperin in Nasal Swabs. *J Infect Dis*, 216(9), 1099–1103.

Yan, Y., Sun, J., Ji, K., Guo, J., Han, L., Li, F., et al., 2022. High incidence of virus among respiratory pathogens in children with lower respiratory tract infection in northwestern China. *J Med Virol*, Epub ahead of print.

Yang, A., Chen, C., Hu, Y., Zheng, G., Chen, P., Xie, Z., et al., 2022. Application of Metagenomic Next-Generation Sequencing (mNGS) Using Bronchoalveolar Lavage Fluid (BALF) in Diagnosing Pneumonia of Children. *Microbiol Spectr*, 10(5), e0148822.

Yucel, S., Aycicek, T., Ceyhan Bilgici, M., Dincer, O. S. & Tomak, L., 2021. 3 Tesla MRI in diagnosis and follow up of children with pneumonia. *Clin Imaging*, 79, 213–218.

Yun, K. W., Wallihan, R., Desai, A., Alter, S., Ambroggio, L., Cohen, D. M., et al., 2022. Clinical Characteristics and Etiology of Community-acquired Pneumonia in US Children, 2015-2018. *Pediatr Infect Dis J*, 41(5), 381–387.

Zar, H. J., Barnett, W., Stadler, A., Gardner-Lubbe, S., Myer, L. & Nicol, M. P., 2016. Aetiology of childhood pneumonia in a well vaccinated South African birth cohort: a nested case-control study of the Drakenstein Child Health Study. *Lancet Respir Med*, 4(6), 463–72.

Zar, H. J., Andronikou, S. & Nicol, M. P., 2017. Advances in the diagnosis of pneumonia in children. *BMJ*, 358, j2739.

Zhang, Y., Sakthivel, S. K., Bramley, A., Jain, S., Haynes, A., Chappell, J. D., et al., 2016. Serology Enhances Molecular Diagnosis of Respiratory Virus Infections Other than Influenza in Children and Adults Hospitalized with Community-Acquired Pneumonia. *J Clin Microbiol*, 55(1), 79–89.

Zhang, H., Han, Y., Jin, Z., Ying, Y., Lan, F., Huang, H., et al., 2020. Detection of Viruses by Multiplex Real-Time Polymerase Chain Reaction in Bronchoalveolar Lavage Fluid of Patients with Nonresponding Community-Acquired Pneumonia. *Can Respir J*, 2020, 8715756.

Zhu, X., Ge, Y., Wu, T., Zhao, K., Chen, Y., Wu, B., et al., 2020. Co-infection with respiratory pathogens among COVID-2019 cases. *Virus Res*, 285, 198005.

Zimmermann, P. & Curtis, N., 2020. Coronavirus Infections in Children Including COVID-19: An Overview of the Epidemiology, Clinical Features, Diagnosis, Treatment and Prevention Options in Children. *Pediatr Infect Dis J*, 39(5), 355–368.

Österback, R., Tevaluoto, T., Ylinen, T., Peltola, V., Susi, P., Hyypiä, T., et al., 2013. Simultaneous detection and differentiation of human rhino- and enteroviruses in clinical specimens by real-time PCR with locked nucleic acid probes. *J Clin Microbiol*, 51(12), 3960–7.

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