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CHILDHOOD COMMUNITY-ACQUIRED PNEUMONIA

by

Elina Lahti

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From the Department of Pediatrics, Turku University Hospital, and the Department of Medical Microbiology and Immunology, Turku University, Turku, Finland

Supervised by

Professor Olli Ruuskanen, MD
Department of Pediatrics
University of Turku
Turku, Finland

Reviewed by

Docent Eeva Salo, MD
Hospital for Children and Adolescents
University of Helsinki
Helsinki, Finland

and

Docent Harri Saxén, MD
Hospital for Children and Adolescents
University of Helsinki
Helsinki, Finland

Opponent

Docent Marjo Renko, MD
Department of Pediatrics
University of Oulu
Oulu, Finland

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To my family

ABSTRACT

Elina Lahti
Childhood community-acquired pneumonia
Department of Pediatrics, Department of Medical Microbiology and Immunology, Turku University, Turku, Finland

Background: Community-acquired pneumonia is a leading cause of morbidity and mortality in children worldwide. New, rapid methods are needed to improve the microbiologic diagnosis of pneumonia in clinical practice. The increasing incidence of parapneumonic empyema in children accentuates the importance of the identification of the causative agent and clinical predictors of empyema.
Aims and methods: Two prospective studies were conducted to find feasible diagnostic methods for the detection of causative agents of pneumonia. The usefulness of pneumolysin-targeted real-time PCR in the diagnosis of pneumococcal disease was studied in children with pneumonia and empyema, and the clinical utility of induced sputum analysis in the microbiologic diagnosis of pneumonia was investigated in children with pneumonia. In addition, two retrospective clinical studies were performed to describe the frequency and clinical profile of influenza pneumonia in children and the frequency, clinical profile, and clinical predictors of empyema in children.
Results: Pneumolysin-PCR in pleural fluid significantly improved the microbiologic diagnosis of empyema by increasing the detection rate of pneumococcus almost tenfold to that of pleural fluid culture (75 % vs. 8 %). In whole blood samples, PCR detected pneumococcus in only one child with pneumonia and one child with pneumococcal empyema. Sputum induction provided good-quality sputum specimens with high microbiologic yield. *Streptococcus pneumoniae* (46 %) and rhinovirus (29 %) were the most common microbes detected. The quantification results of the paired sputum and nasopharyngeal aspirate specimens provided support that the majority of the bacteria (79 %) and viruses (55 %) found in sputum originated from the lower airways. Pneumonia was detected in 14 % of children with influenza infection. A history of prolonged duration of fever, tachypnea, and pain on abdominal palpation were found to be independently significant predictors of empyema.
Conclusions: Pneumolysin-targeted real-time PCR is a useful and rapid method for the diagnosis of pneumococcal empyema in children. Induced sputum analysis with paired nasopharyngeal aspirate analysis can be of clinical value in the microbiologic diagnosis of pneumonia. Influenza pneumonia is an infrequent and generally benign disease in children with rare fatalities. Repeat chest radiograph and ultrasound imaging are recommended in children with pneumonia presenting with clinical predictors of empyema and in children with persistent fever and high CRP levels during hospitalization.

Key words: children, empyema, induced sputum, influenza virus, pneumonia, polymerase chain reaction, *Streptococcus pneumoniae*, viral pneumonia

TIIVISTELMÄ

Elina Lahti
Lasten avosyntyinen keuhkokuume
Lastenklinikka, Lääketieteellinen mikrobiologia ja immunologia, Turun Yliopisto, Turku

Tausta: Lasten keuhkokuume aiheuttaa maailmanlaajuisesti merkittävää sairastavuutta ja kuolleisuutta. Käytännön työhön kaivataan uusia diagnostisia menetelmiä, joiden avulla keuhkokuumeen aiheuttaja voitaisiin löytää aiempaa useammin ja nopeammin. Keuhkokuumeen komplikaation, empyeeman yleistyminen korostaa empyeeman mikrobiologisen diagnostiikan ja ennustekijöiden tuntemisen tärkeyttä.
Tavoitteet ja menetelmät: Kahdessa prospektiivisessa tutkimuksessa etsittiin käytännön työhön soveltuvia diagnostisia menetelmiä keuhkokuumeen aiheuttajan selvittämiseksi. Pneumolysiini-PCR –menetelmän hyödyllisyyttä pneumokokki-sairauksien diagnostiikassa tutkittiin keuhkokuumetta ja empyeemaa sairastavilla lapsilla ja indusoidun yskösnäytteen soveltuvuutta keuhkokuumeen mikrobiologiseen diagnostiikkaan selvitettiin keuhkokuumetta sairastavilla lapsilla. Lisäksi suoritettiin kaksi retrospektiivista tutkimusta lasten influenssakeuhkokuumeen yleisyyden ja taudinkuvan määrittämiseksi ja empyeeman yleisyyden, taudinkuvan ja ennustekijöiden selvittämiseksi.
Tulokset: Pleuranesteen pneumolysiini-PCR paransi merkittävästi empyeeman mikrobiologista diagnostiikkaa lisäämällä pneumokokin osoittamista melkein kymmenkertaisesti viljelyyn verrattaessa (75 % vs 8 %). Kokoverinäytteissä pneumolysiini-PCR identifioi pneumokokin vain yhdellä keuhkokuumetta sairastavalla lapsella ja yhdellä empyeemaa sairastavalla lapsella. Yskösnäytteen indusointi tarjosi edustavia yskösnäytteitä. *Streptococcus pneumoniae* (46 %) ja rinovirus (29 %) olivat yleisimmin todetut mikrobit. Yskösnäytteiden ja nenänielun imulimanäytteiden parittainen, kvantitatiivinen vertailu osoitti, että suurin osa yskösnäytteestä osoitetuista bakteereista (79 %) ja viruksista (55 %) oli peräisin alahengitysteistä. Influenssa-keuhkokuume todettiin 14 %:lla influenssaa sairastavista lapsista. Empyeeman itsenäisiä ennustekijöitä todettiin olevan pitkittynyt kuume ennen sairaalaan tuloa, ja tihentynyt hengitys ja vatsan palpaatioarkuus sairaalaan tullessa.
Päätelmät: Pleuranesteen pneumolysiini-PCR on nopea ja hyödyllinen menetelmä lasten pneumokokin aiheuttaman empyeeman diagnostiikassa. Indusoidun yskösnäytteen mikrobiologinen analysointi ja parittainen vertailu nenänielunäytteeseen voi tarjota kliinisesti tärkeää tietoa keuhkokuumeen aiheuttajasta. Influenssakeuhkokuume on lapsilla harvinainen ja useimmiten lievä sairaus, ja kuolleisuus influenssa-keuhkokuumeeseen on hyvin harvinaista. Keuhkojen kontrollikuvausta ja ultraäänitutkimusta suositellaan keuhkokuumetta sairastaville lapsille, joilla on sairaalaan tullessa tai sairaalahoidon aikana todettavissa empyeeman ennustekijöitä.

Avainsanat: Lapset, empyeema, indusoitu yskös, influenssavirus, keuhkokuume, polymeraasiketjureaktio, *Streptococcus pneumoniae*, viruskeuhkokuume

TABLE OF CONTENTS

ABBREVIATIONS

ALRI	Acute lower respiratory tract infection
CAP	Community-acquired pneumonia
CFU	Colony-forming unit
CI	Confidence interval
CRP	C-reactive protein
CT	Computed tomography
ESR	Erythrocyte sedimentation rate
HBoV	Human bocavirus
hMPV	Human metapneumovirus
MRI	Magnetic resonance imaging
OR	Odds ratio
PCR	Polymerase chain reaction
PCT	Procalcitonin
RSV	Respiratory syncytial virus
SD	Standard deviation
WBC	White blood cell
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I – IV, and on some supplementary unpublished data.

I Lahti E, Mertsola J, Kontiokari T, Eerola E, Ruuskanen O, Jalava J. Pneumolysin polymerase chain reaction for diagnosis of pneumococcal pneumonia and empyema in children.
Eur J Clin Microbiol Infect Dis 2006; 25:783-9.

II Lahti E, Peltola V, Waris M, Virkki R, Rantakokko-Jalava K, Jalava J, Eerola E, Ruuskanen O. Induced sputum in the diagnosis of childhood community-acquired pneumonia.
Submitted.

III Lahti E, Peltola V, Virkki R, Ruuskanen O. Influenza pneumonia.
Pediatr Infect Dis J 2006; 25:160-4.

IV Lahti E, Peltola V, Virkki R, Alanen M, Ruuskanen O. Development of parapneumonic empyema in children.
Acta Paediatr 2007; 96:1686-92.

1. INTRODUCTION

Pneumonia is the leading cause of childhood morbidity and mortality worldwide. According to the estimates of the World Health Organization (WHO), pneumonia accounts for almost one-fifth of overall childhood mortality (Williams et al., 2002; Bryce et al., 2005). Although the introduction of penicillin in the 1940s resulted in a significant reduction in pneumonia mortality in developed countries, the morbidity caused by childhood pneumonia has remained substantial in the developed world (Murphy et al., 1981; Jokinen et al., 1993; Ruuskanen and Mertsola 1999; Yorita et al., 2008).

The establishment of microbiologic diagnosis of childhood pneumonia using conventional methods is difficult. A definitive diagnosis requires the isolation of the microbe from the site of infection, demanding invasive procedures such as transthoracic needle aspiration or transtracheal aspiration. These procedures are potentially harmful and, therefore, can not be employed in routine diagnostics. Isolation of a microbe from a blood culture is accepted as indirect evidence of the etiology of pneumonia. However, blood cultures are positive only in 1 % to 8 % of children with pneumonia (Claesson et al., 1989; Gendrel et al., 1997; Juven et al., 2000; Tsolia et al., 2004; Don et al., 2005). The advent of polymerase chain reaction (PCR) technology has provided new perspectives into the diagnostics of infectious diseases (Chan and Morris, 2007) but only a limited number of studies have investigated the usefulness of PCR in the detection of pneumococcal pneumonia in children (Dagan et al., 1998; Toikka et al., 1999a; Michelow et al., 2002). Sputum gram staining and culture are often performed to define the causative agent of pneumonia in adults. In children, the potential benefit of sputum analysis has not been properly studied as children can not easily produce adequate sputum samples.

In an era of increasing antibiotic resistance and complicated pneumonias, the capability to define the causative agent of pneumonia is of great importance. The main objective of the present thesis was to find feasible diagnostic methods for the detection of the etiologic agent of pneumonia in clinical practice. The study investigated the accuracy of pneumolysin-targeted real-time PCR in whole blood and pleural fluid samples in the diagnosis of pneumococcal pneumonia and empyema in children and the clinical utility of induced sputum analysis in the microbiologic diagnosis of childhood pneumonia. In addition, the frequency and clinical profile of influenza pneumonia and parapneumonic empyema was described, and clinical predictors for the development of empyema were determined.

2. REVIEW OF THE LITERATURE

2.1 Definition of pneumonia

Pneumonia is defined as inflammation of lung tissue due to an infectious agent (Ruuskanen and Mertsola, 1999; Mizgerd and Skerret, 2008). Radiologic verification (Graham, 1990; Redd et al., 1994; Jadavji et al., 1997; Mandell et al., 2007) and presence of acute symptoms or signs of infection are required for the clinical diagnosis of pneumonia (McIntosh, 2002; British Thoracic Society, 2002). Radiologic verification of pneumonia is essential in the developed world where it is important to differentiate pneumonia from other acute lower respiratory tract infections (ALRI) such as bronchiolitis and wheezy bronchitis, which are solely viral infections and do not need to be treated with antibiotics. The shortcoming of radiologic verification is that the development of pneumonic infiltrates is a dynamic process (Figure 1). The chest radiographic finding is therefore dependent on the timing, and may be misleadingly normal if the chest radiograph is obtained in the early phase of the illness (British Thoracic Society, 2002; Mandell et al., 2007). There is also high intra- and inter-observer variation in the interpretation of chest radiograph findings (Davies et al., 1996; Kiekara et al., 1996). In addition, the sensitivity of chest radiography in diagnosing pneumonia is not optimal, as shown in studies with computed tomography (CT) as a reference standard (Syrjälä et al., 1998; Lähde et al., 2002). In the developing countries, radiographic facilities are rarely available, and the diagnosis is based solely on clinical signs and symptoms. The WHO recommends the use of tachypnea and chest wall retractions to diagnose pneumonia in clinical settings (Mulholland et al., 1992; Shann et al., 1984a; WHO, 1990; Pio 2003). This broad definition ensures that all severe cases of pneumonia will be detected, but also leads to overdiagnosis and overtreatment of pneumonia among children with uncomplicated viral respiratory tract infection.

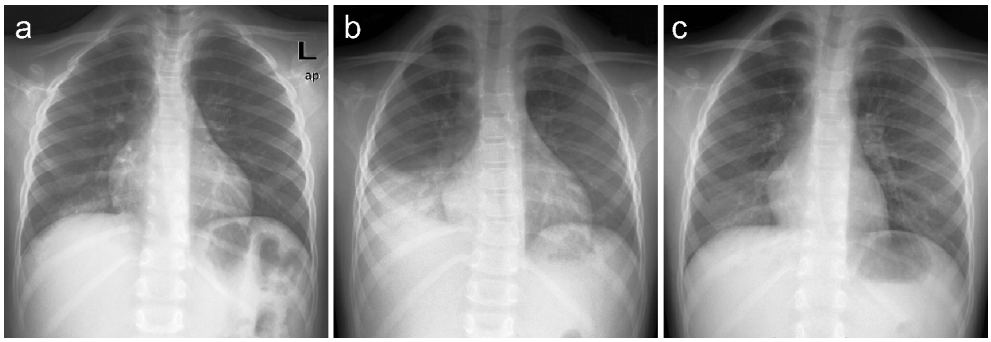

Figure 1. Development of pneumonic infiltrates in a five-year old boy with pneumonia. Chest radiograph on admission (**a**), one day after admission (**b**), and five days after admission (**c**).

Community-acquired pneumonia (CAP) is defined as pneumonia acquired outside hospital (British Thoracic Society, 2002) and nosocomial pneumonia as pneumonia occurring 48 hours or more after hospital admission (Flanders et al., 2006). Pneumonia that has been acquired within a 4 week period after birth is classified as neonatal pneumonia (Duke, 2005). The focus of this review is on CAP.

2.2 Epidemiology

Childhood pneumonia is an important cause of morbidity and mortality worldwide. More than two million children younger than five years of age die from pneumonia every year, accounting for almost one-fifth of overall childhood mortality (Williams et al., 2002; Bryce et al., 2005) (Figure 2). The estimated incidence of clinical pneumonia in developing countries is 0.29 episodes per child-year, equating to an annual incidence of more than 150 million cases (Rudan et al., 2004). This is a close approximation of the global incidence of childhood pneumonia since 95 % of pneumonias occur in the developing world (Rudan et al., 2004).

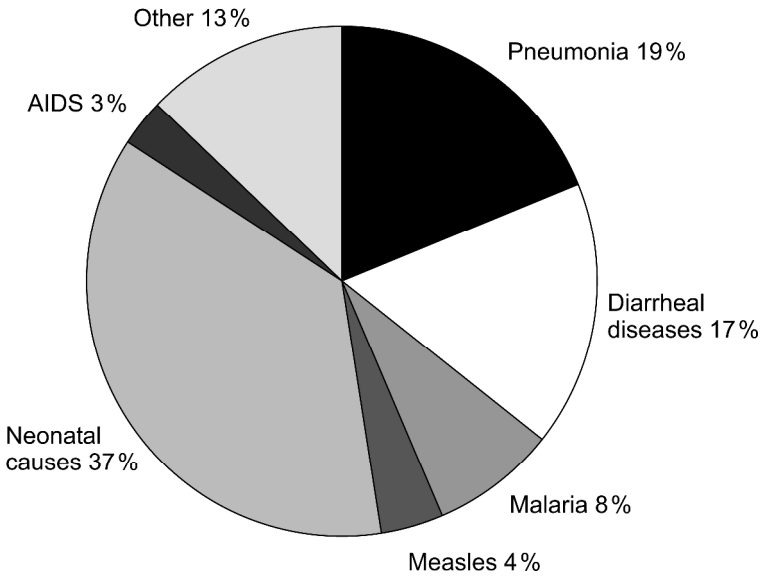

Figure 2. WHO estimates of global causes of death in children younger than five years of age (figure adapted from an article by Bryce et al., 2005).

Although the greatest disease burden occurs in the developing countries, childhood pneumonia also remains a substantial cause of morbidity in the developed world. It is estimated that up to 25 % of all pediatric admissions are due to lower respiratory tract infections (Yorita et al., 2008). Only few community-based studies have investigated the incidence of pneumonia in children. In a cornerstone Finnish study all radiologically confirmed cases of pneumonia in four municipalities in Eastern Finland

between 1981 and 1982 were prospectively reported to a pneumonia register (Jokinen et al., 1993). In this study, the incidence of pneumonia for children younger than five years of age was 36.0/1000/year and 16.2/1000/year for children aged 5–14 years. In a study in the USA, the age-specific incidence of clinically diagnosed pneumonia per 1000 inhabitants per year was 40 in children aged 6 months to 5 years, 22 in those aged 5–9 years, 11 in those aged 9–12 years, and 7 in those aged 12–15 years (Murphy et al., 1981). A male predominance in young children was found in both studies (Jokinen et al., 1993; Murphy et al., 1981). The incidence of clinically diagnosed CAP in other community-based studies has ranged from 7.6/1000/year to 55.9/1000/year (MacIntyre et al., 2003; Black et al., 2002; Bos et al., 2003; Weigl et al., 2003). Half of the children less than five years of age are treated in the hospital whereas most of the older children are treated at home (Foy et al., 1973; Jokinen et al., 1993; MacIntyre et al., 2003; Clark et al., 2007a). Mortality without underlying illness is very low in the developed world (Foy et al., 1973; Jokinen et al., 1993; Ruuskanen and Mertsola, 1999; Dowell et al., 2000).

2.3 Pathogenesis

Lung tissue is normally sterile. Numerous anatomical, biochemical and immunological defense mechanisms such as the upper airway filter, mucociliary clearance, cough reflex, and innate and adaptive immunity protect the lungs against infectious agents. Pneumonia develops as a consequence of a deficiency in these pulmonary defence mechanisms or after exposure to a highly virulent or numerous microbes. Pneumonia can occur after inhalation or aspiration of pathogens or infrequently after hematogenous spread (Nelson et al., 1995; Schidlow and Callahan, 1996; Mizgerd, 2008). The short distance between upper respiratory tract and alveoli, the small diameter of the airways, profuse mucus production, and the immaturity of the immune defence predisposes children to pneumonia. In addition, the nasopharyngeal colonization of pneumonia causing bacteria is common in young children (Syrjänen et al., 2001; Bogaert et al., 2004; Zemlickova et al., 2006).

The appearance of a microbe into the lung tissue triggers a complex inflammatory response in the lungs. Innate immunity is responsible for the acute, unspecific response and adaptive immunity for the specific, memory-based response. Neutrophils, alveolar macrophages, lung epithelial cells, and plasma proteins are important factors in the innate immunity, whereas T- and B –lymphocytes are the main factors in adaptive immunity. Numerous inflammatory mediators such as interleukins and tumor necrosis factor-alpha, and pattern-recognition receptors such as Toll-like receptors are essential in transmitting information between these different players in immune response (Mizgerd, 2008). During infection, neutrophils migrate out of the pulmonary capillaries into the alveoles via a cascade of consecutive rolling, adhesion, transmigration, and chemotaxis events (Mizgerd, 2002, Burns et al., 2003; Salmi and Jalkanen, 2005). After phagocytosis, neutrophils kill microbes with bacteriocidal proteins, proteolytic enzymes, and oxidizing agents (Nathan, 2006). Alveolar macrophages and dendritic cells expressing pattern-recognition receptors act as antigen-presenting cells and activate T-lymphocytes (Moore et al., 2001; Akira et al., 2006). Macrophages also

reinforce the inflammatory response by secreting proteases, oxidants, and inflammatory mediators such as tumor necrosis factor-alpha, interleukin-1, and interleukin-23 (Mizgerd, 2008). Plasma proteins including natural antibodies, complement proteins, and C-reactive protein (CRP), contribute by serving opsonic, bacteriostatic, and microbicidal functions during infection (Mizgerd, 2008). Activated T-lymphocytes activate B-lymphocytes to produce antigen specific antibodies. Antibodies are responsible for the specific immune response via opsonization, complement activation, antibody-dependent cytotoxicity, agglutination, and neutralization (Moore et al., 2001).

The outcome of a pulmonary infection depends on the virulence of the causative organism and the inflammatory response in the lung. Insufficient inflammatory response can result in life-threatening infection, but an excessive response can lead to life-threatening inflammatory lung injury (Mitzgerd, 2008). In severe infection, loss of infected alveolar epithelial cells, accumulation of degradation products, cell edema, and loss of surfactant causes lungs to become stiff and inelastic. Infected, unventilated areas of the lung remain perfused leading to hypoxemia (Schidlow and Callahan, 1996).

Increasing evidence exists on the ability of one respiratory tract infection to alter the immunity and pathology of another respiratory pathogen. These mechanisms can be either protective or exacerbating depending on the particular microbes involved (Page et al., 2006; Didierlaurent et al., 2007). The classic view that viruses pave the way for bacterial infections has gained reinforcement in epidemiologic and animal studies. O'Brien and co-workers showed that children with severe pneumococcal pneumonia were more likely to have experienced an influenza-like illness within 1-4 weeks before admission and were more likely than control subjects to have a positive influenza A serology (O'Brien et al., 2000). Similarly, Stensballe et al. found that recent hospitalization for respiratory syncytial virus (RSV) infection or non-RSV infection increased the risk of invasive pneumococcal disease during early childhood (Stensballe et al., 2008). In a study by Peltola et al., a high level of influenza virus neuramidase activity supported the adherence of *Streptococcus pneumoniae* and the development of secondary bacterial pneumonia in a mouse model (Peltola et al., 2005). Furthermore, Didierlaurent and colleaques have reported that sustained desensitization of lung sentinel cells to Toll-like receptor ligands after influenza or RSV infection reduces chemokine production and nuclear factor κB action in alveolar macrophages. This leads to reduced neutrophil recruitment which may cause a predisposition to the development of bacterial pneumonia (Didierlaurent et al., 2008).

2.4 Risk factors

The most important risk factor for childhood pneumonia in developing countries is malnutrition, being an underlying cause of pneumonia in 52 % of the fatal cases (Caulfield et al., 2004). Other risk factors include indoor air pollution, poor socioeconomic status, overcrowding, low birth weight, young maternal age, lack of breast feeding, attendance at day-care center, and a history of asthma, pneumonia or

wheezing (Victora et al., 1994; Mahalanabis et al., 2002). In a Finnish study, risk factors for pneumonia for children under 5 years of age were recurrent respiratory tract infections during the past year (odds ratio (OR) 5.5; 95 % confidence interval (CI) 2.0–14.2), a history of wheezing episodes (OR 5.3; 95 % CI 2.3–12.2), and a history of acute otitis media treated by tympanocentesis before the age of 2 years (OR 3.6; 95 % CI 1.5–8.9). For older children, significant risk factors were a history of recurrent respiratory tract infections in the past year (OR 3.0; 95 % CI 1.1–7.9) and a history of wheezing episodes (OR 2.1; 95 % CI 1.0–4.3) (Heiskanen-Kosma et al., 1997). Thus, susceptibility to respiratory infections predisposes children to pneumonia.

The majority of pneumonias develop in previously healthy children but the probability for the development of pneumonia is increased if child has an underlying disease impairing the normal pulmonary defence mechanisms such as pulmonary anomaly, congenital or acquired immunodeficiency or immunosupression, lung disease such as cystic fibrosis, or neurologic syndrome (Schidlow and Callahan, 1996).

2.5 Microbiologic etiology

Prospective studies evaluating the etiology of CAP in children have identified the causative agent of pneumonia in 43 % to 88 % of the cases (Table 1). The variations in the detection rate of different causative agents in different studies are mostly explained by the extensiviness and sensitiveness of the microbiologic methods used, and by variations in inclusion criteria, epidemiologic situation and geographical location of the studies (Ruuskanen and Mertsola, 1999; British Thoracic Society, 2002). A definite etiologic diagnosis of pneumonia requires the isolation of a microbe from blood, lung tissue or pleural fluid. In the developed countries, however, blood cultures are seldom positive, and samples collected directly from the lung tissue or pleural space are seldom available (Table 1). Therefore, current knowledge of the etiology of childhood pneumonia in developed countries is based mostly on studies using secondary diagnostics methods such as bacterial serology.

Table 1. Etiology (%) of community-acquired childhood pneumonia in the developed countries.

	Turner et al. 1987	Claesson et al. 1989	Ruuska-nen et al. 1992	Korppi et al. 1993a	Gendrel et al. 1997	Heiskanen-Kosma et al. 1998	Wubbel et al. 1999	Clements et al. 2000	Juven et al. 2000	Miche-low et al 2004a	Tsolia et al. 2004	Don et al. 2005
Country	USA	Sweden	Finland	Finland	France	Finland	USA	UK	Finland	USA	Greece	Italy
Number of patients	98	336	50	195	104	201	168	89	254	154	75	101
Hospitalized patients, (%)	20	50	100	100	50	32	0	100	100	100	100	27
Duration, months	16	14	8	12	30	12	23	12	36	15	12	15
Age, years	59 <2 yr 39 ≥2 yr	medians 1 and 5[a]	mean 4.4	117<2yr 78 ≥2yr	mean 5.6	105<5 yr 96≥5yr	63 % < 5 yr	2 months–15.2 yr	mean 3.8	median 2.75	median 7.2	mean 4.7
Etiology detected	48	48	88	51	84	66	43	54	85	79	77	65
Blood culture positive	3	1	0	NS	8	NS	0	2	1/125	NR	3	1/35
Streptococcus pneumoniae	17	13	38	32[b]	14	28	27	8	37	44	7	18
Mycoplasma pneumoniae	0	10	20	2	41	22	7	16	7	14	35	27
Haemophilus influenzae	2	NS	12	9	NS	6	NS	NS	9	NS	0	3
Moraxella catarrhalis	NS	NS	10	2	NS	3	NS	NS	4	NS	0	1
Bacteria, total	19	23	62	32	55	51	28	32	53	60	40	44
RSV	28	20	30	27	10	21	8	14	29	13	3	17
Rhinovirus	2	NS	10	NS	NS	NS	NS	NR	24	3	45	NS
Adenovirus	1	5	10	5	4	2	2	NR	7	7	12	3
Influenza virus A or B	2	3	2	0	4	0	3	NR	4	22	7	9
Parainfluenza virus type 1,2,3	5	3	8	3	6	3	3	NR	10	13	8	12
Viruses, total	39	29	60	35	29	25	20	26	62	45	65	42
Mixed viral-bacterial, total	10	6	34	16	8	10	3	3	30	23	28	20

[a]Medians of age given for hospitalized patients and outpatients, respectively.
[b]In the original study, *Streptococcus pneumoniae* was detected in 21 % of the patients. The detection rate of pneumococcus rose to 32 %, when antibodies to immune complexes containing pneumococcal capsular polysaccharides, C-polysaccharide, and pneumolysin were measured in a later study (Korppi and Leinonen 1997).
NR, not reported. NS, not studied.

2.5.1 Bacteria

Bacteria account for up to 62 % of CAP cases in children in the developed countries (Table 1). Lung-puncture studies that have mainly been conducted in the developing world, have confirmed the importance of *S. pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*, as causes of severe pneumonia in children (Shann et al., 1984b; Forgie et al., 1991; Vuori-Holopainen and Peltola, 2001). Figure 3 shows the bacterial etiology of childhood pneumonia in Finland.

S. pneumoniae is the major bacterial cause of pneumonia in children throughout the world, in both outpatients and hospitalized patients, and in children of all ages (Adegbola et al., 1994; Heiskanen-Kosma et al., 1998; Juven et al., 2000; Michelow et al., 2004a). The yield of *S. pneumoniae* in studies employing blood culture and serologic methods has ranged from 7 % to 38 % (Claesson et al., 1989; Ruuskanen et al., 1992; Wubbel et al., 1999; Juven et al., 2000; Tsolia et al., 2004; Don et al., 2005). When the PCR method has also been used, the detection rate of pneumococcus has been up to 44 % (Michelow et al., 2004a). Only one study has investigated the microbiologic etiology of childhood CAP using transthoracic needle aspiration and modern microbiologic methods in developed countries. In this Finnish study, *S. pneumoniae* was clearly the most common causative agent detected, found in 18 of 34 children (53 %) with alveolar pneumonia (Vuori-Holopainen et al., 2002). In a study by Ruiz-Conzález et al., which compared routine diagnostics methods and transthoracic needle aspiration, *S. pneumoniae* was found to be the leading cause of pneumonia in adult patients in whom the causative agent of pneumonia could not have been established with routine diagnostics methods (Ruiz-González et al., 1999). Thus, the current incidence figures of pneumococcal pneumonia may probably underestimate the true incidence of the disease. The documented reduction of over 30 % in the incidence of childhood CAP after the introduction of heptavalent pneumococcal conjugate vaccine also provides evidence of the importance of *S. pneumoniae* in the etiology of pneumonia in children (Shinefield and Black, 2000; Black et al., 2002; Hansen et al., 2006).

Mycoplasma pneumoniae is an important cause of pneumonia in children especially over 5 years of age and in nonhospitalized children (Foy et al., 1970; Gendrel et al., 1997; Heiskanen-Kosma et al., 1998; Tsolia et al., 2004). In a study by Korppi et al., *M. pneumoniae* was identified in over 50 % of the children aged 5 to 14 years, and 90 % of these children were treated at home (Korppi et al., 2004). Evidence has also accumulated of the frequency of *M. pneumoniae* infection among young children and hospitalized children (Block et al., 1995; Hammerschlag, 1995; Principi et al., 2001). In a study by Othman et al., 40 % of the children with M. pneumoniae infection were under 5 years of age (Othman et al., 2005), and in a study by Tsolia et al. *M. pneumoniae* infection was detected in 35 % of the hospitalized children with pneumonia (Tsolia et al., 2004). *Chlamydia pneumoniae* is a less frequent causative agent of pneumonia in children, identified in 3–10 % of the cases, and like *M. pneumoniae* is most commonly detected in school-aged children (Heiskanen-Kosma et al., 1998; Wubbel et al., 1999; Juven et al., 2000; Michelow et al., 2004).

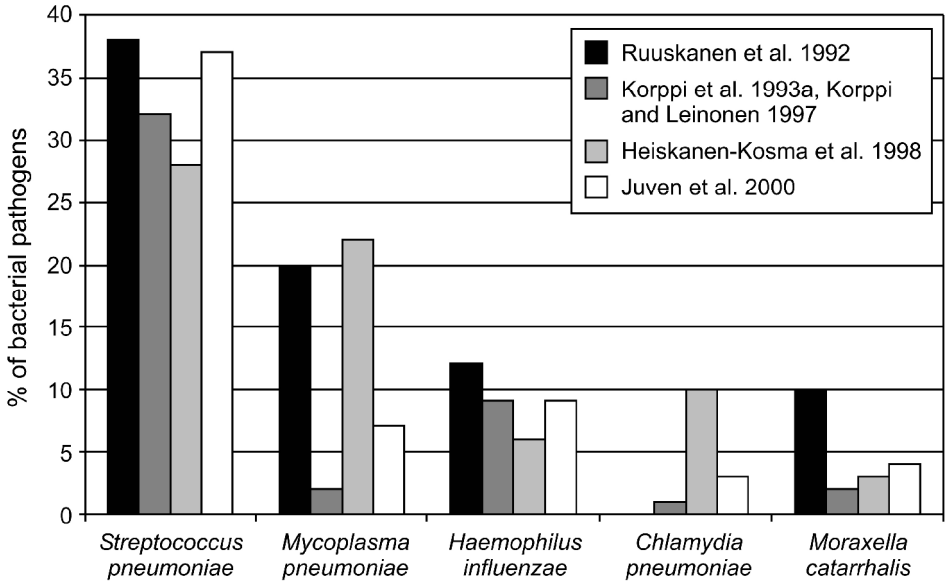

Figure 3. Bacterial etiology of childhood pneumonia in Finland.

Noncapsulated *H. inluenzae* infection has been documented serologically in 2 % to 12 % of children with radiologically verified pneumonia (Table 1). Most of these infections have been reported to occur as mixed infections with a virus or another bacterium (Heiskanen-Kosma et al., 1998; Juven et al., 2000). In a study by Wang et al., noncapsulated *H. influenzae* was cultured from 12 of 25 bronchoalveolar lavage samples in children with pneumonia, providing evidence of its true role in the etiology of childhood pneumonia (Wang et al., 1994). Furthermore, *H. influenzae* has frequently been detected from lung aspirate samples in children with pneumonia in the developing world (Shann et al., 1984b). However, it must be stressed that no infection due to *H. influenzae* was diagnosed in the Finnish lung aspiration study (Vuori-Holopainen et al., 2002).

Moraxella catarrhalis is an uncommon cause of childhood pneumonia and has mostly been detected in mixed infections, suggesting that it is rarely a primary cause of pneumonia (Claesson and Leinonen, 1994; Heiskanen-Kosma et al., 1998; Juven et al., 2000). *S. aureus* has been detected only in 1 % of children with pneumonia in the developed countries (Gendrel et al., 1997; Juven et al., 2000; Michelow et al., 2004a; Don et al., 2005). Other infrequent causes of pneumonia in children in the developed countries are *Streptococcus pyogenes*, *Escherichia coli*, *Bordetella pertussis*, and *Legionella pneumophila* (Clements et al., 2000; McIntosh, 2002; Lichenstein et al., 2003; Michelow et al., 2004a).

2.5.2 Viruses

A wide spectrum of viruses, such as RSV, rhinovirus, parainfluenza types 1, 2, and 3 viruses, influenza A and B viruses, adenovirus, enteroviruses, coronaviruses, human

metapneumovirus (hMPV), human bocavirus (HBoV), varicella-zoster virus, human herpes virus 6, and Epstein-Barr virus, have been identified in children with pneumonia (Ruuskanen and Mertsola, 1999; McIntosh, 2002; Sinaniotis, 2004; Van den Hoogen et al., 2001; Allander, 2008). Overall, viruses account for 20 % to 65 % of cases of childhood pneumonia, and are more frequently detected in infants and young children (Table 1). In a study by Juven et al., that involved 254 children hospitalized with pneumonia, the viral etiology of pneumonia was investigated by a wide range of microbiologic methods including virus culture, antigen detection, serologic tests, and PCR (Juven et al., 2000). The overall etiology of pneumonia was found in 85 % of the children, and viruses were identified in 62 %. The percentages of viral finding in children < 2 years of age, 2 to 5 years of age, and > 5 years of age were 80 %, 58 %, and 37 %, respectively.

RSV is clearly the most common viral cause of pneumonia in children. It has been detected approximately in one-third of the children with pneumonia (Table 1). RSV infection is most often found in children younger than 2 years of age (Ruuskanen and Ogra, 1993) and its occurrence follows an epidemic pattern, occurring predominantly during the winter months in temperate climates (Stensballe et al., 2003). In Finland, RSV epidemics appear biannually in the spring and late fall in odd-numbered years (Waris, 1991). It is of note that RSV is among the few viruses that have been identified in the lung tissue in children with pneumonia (Adegbola et al., 1994; Shann et al., 1984b).

The role of rhinovirus in childhood pneumonia has long been underestimated due to the difficulties in isolating rhinovirus in cell cultures. Since the advent of sensitive PCR methods, increasing evidence has accumulated of the importance of rhinovirus in lower respiratory tract infections (Papadopoulos, 2004). Rhinovirus has been shown to be able to replicate in the lower respiratory tract tissue and to be able to infect the human bronchial epithelium (Subauste et al., 1995; Papadopoulos et al., 1999; Papadopoulos et al., 2000). In addition, rhinoviruses have been detected from bronchial samples (Schmidt and Fink, 1991) and directly from lung tissue (Imakita et al., 2000) from children with lower respiratory tract infection. Recent studies employing sensitive PCR methods have detected rhinovirus in nasal samples in 24 % to 45 % of children with pneumonia (Juven et al., 2000; Tsolia et al., 2004). The corresponding detection rate by culture has been approximately 10 % (Ruuskanen et al., 1992; Isaacs, 1989).

Newly discovered viruses, hMPV and HBoV, have been associated with lower respiratory tract infections in children (Van den Hoogen et al., 2001; Allander, 2008). Recent studies have identified hMPV in 1 % to 5 % of children with pneumonia (Tsolia et al., 2004; Werno et al., 2004; Don et al., 2005; Lin et al., 2005) and pneumonia has been diagnosed in up to 38 % of the children with hMPV associated acute respiratory tract infection (Peiris et al., 2003; Principi et al., 2004; Williams et al., 2004; Caracciolo et al., 2008). The role of HBoV as a causative agent of acute lower respiratory tract infection is more difficult to assess, since HBoV seems to persist after primary infection for a longer time than other respiratory viruses (Schildgen et al., 2008), and it is most frequently detected in co-infections (Allander et al., 2007; Fry et al., 2007; Esposito et al., 2008). In a prospective study by Fry et al.,

HBoV infection was detected in 4.5 % of hospitalized patients with pneumonia (Fry et al., 2007). Among children under 5 years of age, it was associated with 12 % of the pneumonia cases. It is of note that 91 % of these HBoV infections had co-infection with other viruses. Esposito and colleagues studied 1332 children attending the Emergency Room of the Institute of Pediatrics, University of Milan (Milan, Italy) for any acute medical reason excluding surgical diseases and trauma (Esposito et al., 2008). HBoV was identified in 7.4 % of all the children and in 11.4 % of children with respiratory tract disease. Fifty-one percent of these HBoV findings were detected together with at least one other respiratory virus. Interestingly, the prevalence of lower respiratory tract infections was higher in these co-infections.

Influenza virus pneumonia is probably the best example of primary viral pneumonia (Louria et al., 1959; Yeldandi and Colby, 1994). According to recent studies, 4 % to 22 % of childhood pneumonias are caused by influenza viruses (Juven et al., 2000; Michelow et al., 2004a; Tsolia et al., 2004; Don et al., 2005).

The exact role of viruses in the etiology of pneumonia is not clear. Viruses can either be the primary pathogenic event in the development of pneumonia or they can pave the way for bacterial pneumonia by making the host more susceptible to bacterial infection. When a wide spectrum of sensitive detection methods is used, viruses are detected in upper respiratory tract samples in up to 80 % of pneumonias in infants and young children (Juven et al., 2000) and in up to 65 % of pneumonias in school-aged children (Tsolia et al., 2004). These findings may indicate that viruses are rather a rule than an exception in the development of pneumonia in children (Tsolia et al., 2004). However, the mere presence of a virus in nasopharynx offers no direct evidence of the etiologic association with pneumonia. Only few studies have looked for viruses from samples aspirated directly from the lungs of children with pneumonia. Vuori-Holopainen et al. detected viruses in lung aspirates only from two children with alveolar pneumonia (Vuori-Holopainen et al., 2002). Adegbola et al. detected viruses (adenovirus, parainfluenza, herpes simplex, RSV or influenza) in 30 % of lung aspirates from Gambian children with pneumonia (Adegbola et al., 1994).

2.5.3 Mixed infections

Mixed infections are frequent in children with pneumonia. Therefore, the detection of a virus or a bacterium in a child with pneumonia does not rule out the co-involvement of other etiologic agents (Ruuskanen and Mertsola, 1999; McIntosh, 2002).

2.5.3.1 Viral-bacterial infections

Mixed viral-bacterial infection is the most frequent form of mixed infection, detected in up to 34 % of the cases (Table 1). The most commonly found viral-bacterial combinations in children with pneumonia are concomitant finding of *S. pneumoniae* and RSV or *S. pneumoniae/M. pneumoniae* and rhinovirus (Korppi et al., 1993a; Heiskanen-Kosma et al., 1998; Juven et al., 2000; Tsolia et al., 2004). Bacterial coinfection has been detected in 44 % of children with RSV pneumonia (Korppi et al., 1993a; Heiskanen-Kosma et al., 1998; Juven et al., 2000), and in one-half of the children with rhinovirus

associated pneumonia (Juven et al., 2000; Tsolia et al., 2004). High frequency of concomitant bacterial infection has also been detected in influenza, parainfluenza, and hMPV infections (Juven et al., 2000; Don et al., 2005; Lin et al., 2005). The proportion of concomitant viral infection in children with *S. pneumoniae* varies between 44 % and 55 % (Korppi et al., 1993a; Juven et al., 2000; Don et al., 2005).

The true substance of mixed viral-bacterial findings is not fully understood. It has been proposed that virus induced aspiration of bacteria into the lungs or escape of bacteria into the bloodstream could result in the production of bacterial antibodies or immune complexes even though the bacteria would not be the cause of pneumonia (McIntosh, 2002). On the other hand, mixed viral-bacterial infections have been detected in lung aspirate samples (Adegbola et al., 1994) supporting their true concomitant role in the development of pneumonia. Evidence has also accumulated of the ability of mixed infections to induce a more severe inflammation and clinical disease than individual bacterial or viral infections (Juven et al., 2004; Jennings et al., 2008). A concomitant influenza virus and *S. aureus* infection has been shown to be able to cause severe, fatal pneumonia in children (Connor and Powell, 1985; Thomas et al., 2003; Centers for Disease Control and Prevention, 2008). In a pneumonia study by Juven et al., one-half of the children with treatment failure had evidence of mixed viral-bacterial infection (Juven et al., 2004). Corresponding association with mixed infections and insufficient response to therapy has also been detected in children with acute otitis media (Chonmaitree et al., 1990). Furthermore, mixed rhinovirus-pneumococcal infection has been shown to be associated with severe pneumonia in adults (Jennings et al., 2008).

Viral-bacterial infection can be either concomitant viral-bacterial pneumonia or secondary bacterial pneumonia, in which the virus no longer can be recovered, but the preceding viral infection can be demonstrated by serological methods (Louria et al., 1959). Mechanisms that have been proposed to be responsible for the enhanced development of bacterial pneumonia during or after viral infection include distruption of epithelial integrity, changes in airway function such as ciliary dysfunction, up-regulation of bacterial adhesion receptors, and desensitization of lung sentinel cells to Toll-like receptor ligands (McCullers, 2006; Didierlaurent et al., 2008). The most studied interaction between virus and bacteria is probably the interaction between the influenza virus and pneumococcus in lung infection, a combination probably responsible for the great part of the mortality associated with the influenza pandemics of 1918 and 1957 (Louria et al., 1959; McAuley et al., 2007). One potential mechanism for the development of concomitant influenza virus and pneumococcal pneumonia or secondary pneumococcal pneumonia is that the influenza virus enhances the adherence of pneumococcus to epithelial cells by directly damaging the epithelium or by increasing the adherence of pneumococcus with its neuraminidase activity (McCullers, 2006). Another mechanism could be the combined effect of the influenza virus and pneumococcus on the inflammatory response. Influenza and pneumococcus are both recognized by Toll-like receptors, generating a similar cytokine response. In a mouse model by Smith et al., elevated levels of cytokines, including tumor necrosis factor-alpha, interleukin-1, interleukin-2, and interleukin-10, in secondary bacterial pneumonia, resulted in a massive influx of neutrophils and macrophages into the lung,

amplifying the immune response, leading to inflammatory damage, but not an effective clearance of bacteria (Smith et al., 2007). On the other hand, Didierlaurent and colleagues have shown evidence suggesting that influenza infection can lead to prolonged desensitization of lung sentinel cells to Toll-like receptor ligands, which is associated with reduced chemokine production and reduced neutrophil and macrophage recruitment (Didierlauerent et al., 2008). The authors hypothesize that this kind of inability of innate immunity to return to the preinfection state after influenza infection may contribute to the increased susceptibility to secondary pneumococcal pneumonia. Interestingly, the synergistic effect of viral-bacterial infection may not be unidirectional. McCullers and Rehg found that the viral lung load of influenza was increased after pneumococcal challenge in a mouse model of secondary bacterial pneumonia (McCullers and Rehg, 2002).

2.5.3.2. Dual bacterial and viral infections

Two bacteria or two viruses may also be associated with childhood pneumonia (Shann et al., 1984b; Heiskanen-Kosma et al., 1998; Juven et al., 2000; Tsolia et al., 2004; Don et al., 2005). *S. pneumoniae* and *M. pneumoniae* co-infection is the most frequently found dual bacterial infection in children with pneumonia (Heiskanen-Kosma et al., 1998; Toikka et al., 2000a; Korppi et al., 2004; Don et al., 2005). Like viral-bacterial co-infections, dual viral infections have been shown to be linked with greater disease severity than single infections (Semple et al., 2005; Richard et al., 2008; Esposito et al., 2008). In a recent study by Esposito et al., HBoV co-infections were associated with greater disease burden in children with respiratory tract infection as it involved more examinations, hospitalizations, loss of school days, and antibiotic prescriptions than HBoV infection alone (Esposito et al., 2008).

2.6 Diagnosis

2.6.1 Signs and symptoms

Fever, cough, and dyspnea are the most frequently reported symptoms in children with pneumonia in the developed countries (Tan et al., 1998; Toikka et al., 1999b; Esposito et al., 2002; Juven et al., 2003; Clark et al., 2007b). However, one-fourth of the children with pneumonia may not have cough (Tan et al., 1998; Esposito et al., 2002; Juven et al., 2003), and some of them may present without any respiratory symptoms (Juven et al., 2003). The general appearance may also be misleadingly good (Ruuskanen and Mertsola, 1999; Juven et al., 2003). Chest pain or abdominal pain as a sign of pleural irritation, are uncommon symptoms in children (Tan et al., 1998; Ruuskanen and Mertsola, 1999; Toikka et al., 1999b; Juven et al., 2003). Pneumonia is a relatively infrequent illness among children presenting for emergency care with symptoms of lower respiratory tract infection (Leventhal, 1982; Taylor et al., 1995). In a recent study, radiologically verified pneumonia was diagnosed in only 9 % of children presenting with cough and fever, respiratory distress or chest pain (Mahabee-Gittens et al., 2005). The identification of children with pneumonia among the children with other respiratory tract infection on the basis of symptoms alone is, therefore, difficult.

Tachypnea is considered to be the most reliable clinical sign that differentiates upper and lower respiratory tract infections in febrile children (Leventhal, 1982; Zukin et al., 1986; Berman et al., 1991; Harari et al., 1991; Taylor et al., 1995; Ruuskanen and Mertsola, 1999). Palafox and colleagues found that the presence of tachypnea was the single most sensitive and specific clinical indicator of pneumonia, with 74 % sensitivity and 67 % specificity among children less than 5 years of age (Palafox et al., 2000). In a study by Taylor et al., tachypnea as a sign of pneumonia had a sensitivity of 74 % and specificity of 77 % (Taylor et al., 1995). On the other hand, WHO defined tachypnea was present only in one-half of the pneumonia cases in children in studies by Tan et al. in the United States and Juven et al. in Finland (Tan et al., 1998; Juven et al., 2003). The respiratory rate thresholds for acute lower respiratory tract infection according to the WHO are a respiratory rate of > 60/minute for children less than 2 months of age, > 50/minute for children aged 2 to 12 months, and >40 /minute for children aged 1 to 5 years (WHO, 1990). This easily detected diagnostic sign of pneumonia is helpful in the developing world where radiographic facilities are rarely available, trained staff are lacking, and pneumonia mortality is high. In the developed countries, however, tachypnea as a sign of pneumonia should be used with caution, since most children presenting with tachypnea may have bronchiolitis, wheezy bronchitis, or asthma instead of pneumonia (Ruuskanen and Mertsola, 1999). In addition to tachypnea, chest wall indrawing is found to be a useful indicator of childhood pneumonia in the developing world (Cherian et al., 1988; WHO, 1990; Harari et al., 1991).

Crackles on lung auscultation are considered to indicate pulmonary parenchymal disease. Crackles are defined as short, explosive, non-musical sounds that occur when atelectatic or fluid filled airways and alveoli open at the end of inspiration (Schidlow and Callahan, 1996). Crackles on lung auscultation is a typical finding suggesting pneumonia, but the sensitivity of crackles as an indicator of pneumonia is relatively poor. The sensitivity has ranged from 43 % to 57 % and specificity from 75 % to 80 % (Leventhal, 1982; Zukin et al., 1986; Grossman and Caplan, 1988; Palafox et al., 2000). Decreased breath sounds and dullness on percussion of the chest wall are signs of dense consolidation or pleural effusion, and are more frequently found in children with bacterial pneumonia (Schidlow and Callahan, 1996; Tan et al., 1998; Juven et al., 2001; Juven et al., 2003). It is of note that normal breathing sounds on lung auscultation does not rule out the possibility of pneumonia in children (Juven et al., 2003, Toikka et al., 1999b). Although the inter-observer agreement of lung auscultation findings has only been moderate (Margolis and Gadomski, 1998; Elphick et al., 2004) lung auscultation is an important part of clinical examination as it can provide valuable information on the level of respiratory distress.

If all clinical signs (respiratory distress, tachypnea, crackles, and decreased breath sounds) are negative, pneumonia is considered to be unlikely (Jadavji et al., 1997; British Thoracic Society, 2002). However, in a study by Rothrock et al., these criteria were only 45 % sensitive and 66 % specific for diagnosing pneumonia in urban settings (Rothrock et al., 2001). In a multivariate model of Lynch and colleagues, a combination of fever, decreased breath sounds, crackles, and tachypnea had high sensitivity of 98 % but specificity of only 8 % (Lynch et al., 2004a).

The clinical presentation of pneumonia varies according to the etiology and patient's age (British Thoracic Society, 2002; Juven et al., 2003; Lichenstein et al., 2003). According to the British Thoracic Society fever > 38.5 °C, respiratory rate > 50 breaths/minute, and chest recession refers to bacterial pneumonia, and young age, wheezing, fever < 38.5 °C, and marked chest recession refers to viral etiology (British Thoracic Society, 2002). In a study by Juven et al., thoracic pain, headache, and decreased breathing sounds were more frequently observed in children with bacterial pneumonia whereas dyspnea and rhonchi on lung auscultation were more frequent in children with viral etiology (Juven et al., 2003).

The clinical presentation of pneumonia with a defined etiology is presented in Table 2. Classical pneumococcal pneumonia is associated with rapid onset of high fever, breathlessness, tachypnea, chest wall indrawing and unwell appearance (British Thoracic Society, 2002). Children with bacteremic pneumococcal pneumonia are generally more ill than children with serologically diagnosed pneumococcal pneumonia (Tan et al., 1998; Toikka et al., 1999b; Juven et al., 2001). Interestingly, in a study by Toikka et al., cough was reported only in 55 % of children with bacteremic pneumococcal pneumonia (Toikka et al., 1999b). Cough may be absent in alveolar pneumonia since alveoli are poorly endowed with cough receptors, and the cough occurs only when lysis is present and debris is swept into the airways (British Thoracic Society, 2002). Gradual onset of symptoms, non-productive cough, malaise, and headache are symptoms most commonly associated with *M. pneumoniae* pneumonia (Hammerschlag, 1995; British Thoracic Society, 2002). However, the clinical findings of pneumonia of different causative agents are largely overlapping, and therefore the etiology can not be accurately identified on the basis of the signs and symptoms alone (Jadavji et al., 1997; Juven et al., 2003; Korppi et al., 2008a).

Table 2. Clinical presentation of pneumonia in children with defined etiology in the developed countries.

Symptom or sign	All children with pneumonia (n = 254) Juven et al. 2000	Children with pneumococcal pneumonia (n= 254) Tan et al. 1998	Children with *Mycoplasma pneumoniae* pneumonia (n=68) Esposito et al. 2001	Children with RSV pneumonia (n=26) Juven et al. 2001
Fever >37.5 °C	96 %	91 %	85 %	92 %
Cough	76 %	72 %	65 %	89 %
Rhinorrhea	48 %	42 %	NR	58 %
Dyspnea	37 %	28 %	NR	62 %
Thoracic pain	10 %	10 %	NR	0 %
Tachypnea	51 %	49 %	12 %	95 %
Auscultation				
Rales/crackles	24 %	42 %	88 %[a]	39 %
Diminished breathing sounds	15 %	54 %	NR	0 %
Wheezing	20 %	20 %	15 %	35 %

[a] Reported as rales

2.6.2 Non-specific inflammatory parameters

Serum C-reactive protein (CRP), white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and serum procalcitonin (PCT) are measured with the intention to differentiate bacterial from viral pneumonia and to assess the severity of the disease. These non-specific inflammatory parameters have proved valuable in identifying septic or bacteremic infections in children (Jaye and Waites, 1997; Gendrel et al., 1999) but their utility in the screening of focal, non-invasive bacterial infections such as pneumonia is not fully defined (Jaye and Waites, 1997; Gendrel et al., 1999; Korppi, 2004).

Table 3 presents the results of selected studies on the sensitivities and specificities of CRP for distinguishing bacterial and viral pneumonia. Recently published meta-analysis evaluated the value of serum CRP in differentiating bacterial from nonbacterial pneumonia in 1230 children (Flood et al., 2008). In this study, CRP concentration exceeding 40 to 60 mg/l was found to weakly predict bacterial etiology, with a positive predictive value of 64 %. In a study by Prat et al., a cut-off point of 65 mg/l for CRP and 2 ng/ml for PCT differentiated bacterial etiology from viral etiology with sensitivities and specificities of 79 % and 67 % for CRP, and 69 % and 79 % for PCT (Prat et al., 2003). No significant differences were found for leukocyte count. In a recent study by Korppi et al., in which the microbial etiology of pneumonia was assessed by extensive serologic methods, the combination of CRP >80 mg/l, WBC > 17.0 x 10^9/l, PCT > 0.84 µg/l, or ESR > 63 mm/h had a sensitivity of 61 % and specificity of 65 % in differentiating between pneumococcal and viral pneumonia (Korppi, 2004). In agreement with the findings of numerous previous studies, the authors concluded that although the mean values of inflammatory parameters are higher in children with bacterial than viral pneumonia the findings are too widely distributed to allow accurate differential diagnosis in clinical pediatric practice (Nohynek et al., 1995a; Korppi et al., 1997; Heiskanen-Kosma and Korppi, 2000; Toikka et al., 2000b; Korppi et al., 2004; Michelow et al., 2004a; Don et al., 2007). Accordingly, British Thoracic Society guidelines do not recommend routine measurement of inflammatory markers in children with pneumonia in outpatient care (British Thoracic Society, 2002). However, it must be stressed that all studies evaluating the utility of non-specific inflammatory parameters in screening of bacterial pneumonia are hampered by the limitations of the available microbiologic tests and by the high frequency of mixed viral-bacterial infections.

Recent data suggest that serum CRP and PCT values are useful in estimating the disease severity and prognosis in adult patients with CAP (Boussekey et al., 2005; Chalmers et al., 2008; Menendez et al., 2008). High serum PCT value is also found to be a valuable predictor for disease severity in children with pneumonia (Moulin et al., 2001; Don et al., 2007). In addition, PCT could be potentially useful in evaluating the response to treatment in bacterial infections since it decreases quickly after appropriate treatment (Moulin et al., 2001; Korppi, 2003). In a recent study by Clark et al., serum CRP did not correlate with the overall disease severity in children with pneumonia but was high in children with pleural effusion (Clark et al., 2007b). Correspondingly, Hsieh et al. found that CRP > 120 mg/l at presentation was an independent predictor

for parapneumonic empyema and necrotizing pneumonia in children (Hsieh et al., 2004). Thus, the measurement of non-specific inflammatory parameters can be recommended in children with pneumonia in hospitalized children.

Table 3. Sensitivity and specificity of CRP in differentiating bacterial from viral pneumonia.

	CRP cutoff value (mg/l)	Sensitivity	Specificity
Toikka et al. 2000b	80	0.59	0.68
	150	0.31	0.88
Moulin et al. 2001	60	0.70	0.52
Virkki et al. 2002	40	0.66	0.53
	80	0.52	0.72
Prat et al. 2003	65	0.79	0.67

2.6.3. Radiologic investigations

Chest radiography is considered to be the gold standard for diagnosing pneumonia in the developed countries since the symptoms and clinical findings of pneumonia are variable and nonspecific (Graham, 1990; Redd et al., 1994; Jadavji et al., 1997; Ruuskanen and Mertsola, 1999; McIntosh, 2002; Mandell et al., 2007). The clinical indications for radiologic investigations in children are, however, not fully defined. A recent Cochrane review found no evidence indicating that performing a chest radiograph would affect the clinical outcome of ALRI in children aged 2 months to 5 years treated as outpatients (Swingler and Zwarenstein, 2008). In addition, antibiotic prescription was more frequent among children who underwent radiography (Swingler et al., 1998). The authors concluded that routine use of chest radiography in outpatient care is therefore unnecessary. In line with this recommendation, British Thoracic Society guidelines suggest that in children with mild uncomplicated ALRI, pneumonia diagnosis could be done on the basis of clinical presentation alone (British Thoracic Society, 2002). However, it seems reasonable that in countries like Finland, where children attend the doctor in the early phase of illness, the diagnosis of pneumonia should be based on chest radiography to avoid overprescription of antibiotics.

Like non-specific inflammatory markers, chest radiography is considered to be too insensitive to be useful in differentiating bacterial from viral pneumonia accurately (McCarthy et al., 1981; Turner et al., 1987; Courtoy et al., 1989; British Thoracic Society, 2002). The presence of an alveolar infiltrate, especially of a lobar pattern increases the likelihood of bacterial pneumonia but interstitial infiltrates are found in both viral and bacterial pneumonias (Korppi et al., 1993b; Virkki et al., 2002). Perihilar, peribronchial infiltrates are common in young children with viral respiratory tract infections, and as a sole finding should not be interpreted as pneumonia (Wildin et al., 1988; Ruuskanen and Mertsola, 1999).

All studies evaluating the utility of chest radiograph in screening of bacterial pneumonia are hampered by the limitations of the microbiologic tests used and by the

high intra- and inter-observer variation in the interpretation of chest radiograph findings (Davies et al., 1996; Kiekara et al., 1996). In addition, there have been no validated definitions for chest radiograph interpretation. In 2005, the WHO radiology working group developed standardized definitions for radiologic pneumonia to facilitate comparison of the results of vaccine trials and epidemiologic studies of pneumonia (Cherian et al., 2005). This definition classifies the radiograph findings as normal appearance, significant pathology (consolidation, infiltration or effusion), end-point consolidation (a dense or fluffy consolidation that occupies a portion or whole of a lobe that may or may not contain air-bronchograms), other (non-end-point) infiltrate including interstitial infiltrate, and pleural effusion. The inter-observer agreement of these findings was high but the clinical value of these definitions needs to be determined in clinical trials.

Follow-up radiographs are not recommended in childhood CAP if the child has recovered uneventfully (Grossman et al., 1979; Gibson et al., 1993; British Thoracic Society, 2002; Virkki et al., 2005; Suren et al., 2008). However, the resolution of lobar collapse, circular infiltrates, pleural effusion, pneumatoceles, and pulmonary abscess should be ensured by follow-up radiography (Jadavji et al., 1997; British Thoracic Society, 2002; Kim and Donnelly, 2007). In addition, a repeat chest radiograph is recommended in children with treatment failure since the incidence of complicated pneumonia is increasing (British Thoracic Society, 2002; Virkki et al., 2005). In complicated cases, CT has been shown to be beneficial as it often reveals clinically significant findings not apparent on chest radiograph (Donnelly and Klosterman, 1998; Jaffe et al., 2008).

CT is also more sensitive than conventional chest radiography in overall pneumonia diagnostics but the clinical significance of CT findings when findings of radiography are negative is unclear (Syrjälä et al., 1998; Lähde et al., 2002). The use of CT is also limited by the high radiation load. The radiation dose of a chest CT is nearly 70 times the dose of a chest radiograph (Frush et al., 2003). The addition of the lateral radiograph to the frontal view is considered to rarely provide additional information for clinical decision making, and is not considered necessary in children with pneumonia (Kiekara et al., 1996; British Thoracic Society, 2002; Lynch et al., 2004b). However, it must be mentioned that in a recent, large study by Rigsby et al., the sensitivity of the frontal view alone for the radiographic diagnosis of childhood pneumonia was only 85 % (Rigsby et al., 2004). Low-field projection magnetic resonance imaging (MRI) is a promising alternative to conventional pediatric chest radiography (Figure 4). In a study by Rupprecht et al., a steady state free precession projection MRI was shown to overcome the limitations (long examination time and motion artefacts caused by heart activity and breathing) present in conventional MRI methods, and was especially beneficial in detecting pleural effusions and small pneumonic infiltrates in pediatric patients with suspected pneumonia (Rupprecht et al., 2002).

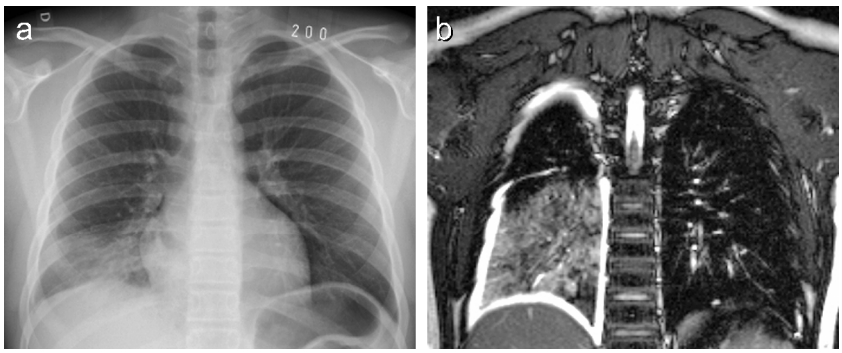

Figure 4. Pneumonia caused by *M. pneumoniae* in a 14-year-old boy. (**a**) Initial chest radiograph shows an infiltrate in the right lower lobe. (**b**) MRI done 2 days later. The T2-weighted coronal image shows parenchymal edema in the entire right lower lobe and pleural fluid collections (Figure adapted from an article by Peltola et al., in press).

2.6.4. Microbiologic investigations

The establishment of definite microbiologic diagnosis of CAP is essential in children with severe or complicated pneumonia, and in children with treatment failure. In addition, the establishment of etiologic diagnosis provides valuable information on the overall epidemiologic situation and the antibiotic sensitivity patterns in society. Routine microbiologic investigations are not indicated in children with pneumonia in outpatient care (British Thoracic Society, 2002). In children with severe pneumonia, however, it is important to pursue a microbiologic diagnosis, although the diagnostic tests often lack adequate sensitivity or specificity (British Thoracic Society, 2002). In clinical practice, blood culture, serologic tests for *M. pneumoniae* and *C. pneumoniae,* and viral antigen detection and viral PCR in nasopharyngeal aspirates, are the methods available for use.

2.6.4.1 Culture

Culture remains the standard method for determining bacterial and viral pathogens in both developing and developed countries although the microbiologic yield by culture is low. In clinical practice, blood culture is recommended to be obtained from all children with suspected bacterial pneumonia and from hospitalized patients (Jadavji et al., 1997; British Thoracic Society, 2002). The value of a positive blood culture in the microbiologic diagnosis of pneumonia is high but its sensitivity is low. In the developed countries, blood culture rarely has an effect on treatment as only 1 % to 8 % of pediatric blood cultures are positive, almost always yielding *S. pneumoniae* (Table 1). In the developing countries, the proportion of blood culture-positive cases is somewhat higher (Shann et al., 1984b; Forgie et al., 1991; Adegbola et al., 1994). The low positive yield of blood cultures is partly explained by prior antibiotic treatment and by a low and intermittent level of bacteremia in children with pneumonia (Sullivan et al., 1982; Bell et al., 1985). In a study by Sullivan et al., children with *S. pneumoniae* or *H. influenzae* pneumonia had a low concentration of bacteria in the blood, usually < 10 colony forming units (CFU)/ml, whereas children with meningitis often had >100

CFU/ml (Sullivan et al., 1982). The detection of bacteremia can thus be improved by increasing the blood culture volume and by obtaining two separate samples of blood (Isaacman et al., 1996; Cockerill et al., 2004). However, the main reason for the low bacterial yield in blood cultures is that most childhood pneumonias are probably non-bacteremic (Turner et al., 1987; Claesson et al., 1989; Ruuskanen et al., 1992; Gendrel et al., 1997; Wubbel et al., 1999; Clements et al., 2000; Juven et al., 2000; Tsolia et al., 2004; Don et al., 2005).

Isolation of a microbe from the infected lung tissue is the gold standard for the microbiologic diagnosis of pneumonia (Brtish Thoracic Society, 2002). In an extensive review of 3560 transthoracic needle aspirations, the total microbiologic yield from lung tap specimen by basic diagnostics methods was found to be high, up to 66 % in Europe and Africa (Vuori-Holopainen et al., 2001). In a Finnish study, transthoracic needle aspiration and modern microbiologic methods disclosed the etiology of alveolar pneumonia in 69 % of the cases in which a representative specimen was obtained (Vuori-Holopainen et al., 2002). However, lung tap and other invasive procedures such as bronchoalveolar lavage are potentially harmful and can be considered only for the most critically ill patients who have failed to respond to standard therapy (Ruuskanen and Mertsola, 1999, British Thoracic Society, 2002). Isolation of a microbe from pleural fluid is also considered indicative of a proven microbiologic etiology. When a significant amount of pleural fluid is present, it should be aspirated for diagnostic purposes (British Thoracic Society, 2002).

Positive bacterial culture from nasopharyngeal aspirate does not indicate proven bacterial pneumonia since the nasopharyngeal carriage of pneumonia causing bacteria is high in children (Korppi et al., 1992; Syrjänen et al., 2001; British Thoracic Society, 2002; Bogaert et al., 2004; Zemlickova et al., 2006). Sputum gram staining and culture is considered potentially useful in preadolescents and adolescents but adequate sputum specimens are difficult to obtain in younger children (Jadavji et al., 1997; Lichenstein et al., 2003). In adults, routine use of sputum gram stain and culture is recommended for patients needing intensive care unit admission, for patients with failure of outpatient antibiotic therapy, and for patients with pleural effusion (Mandell et al., 2007).

2.6.4.2 Antigen detection

Antigen detection techniques can provide a rapid diagnosis and are particularly useful in patients with preceding antibiotic treatment. The recently introduced immunochromatographic test detecting the C polysaccharide cell wall antigen of *S. pneumoniae* in serum and urine has proven to be fairly sensitive and specific in detecting pneumococcal pneumonia in adults (Werno and Murdoch, 2008). In children, the diagnostic value is diminished by the high rate of false-positive results, which results from nasopharyngeal colonization with *S. pneumoniae* (Dominguez et al., 2003; Neuman and Harper, 2003; Esposito et al., 2004; Charkaluk et al., 2006; Anjay and Anoop, 2008). When compared with conventional diagnostic methods, the test has a sensitivity of 87 %–100 % and a specificity of 56 %–81 % in detecting pneumococcal pneumonia in children (Dominguez et al., 2003; Esposito et al., 2004; Charkaluk et al.,

2006). Recently, pneumococcal antigen detection has also been applied to pleural fluid specimens obtained from children with empyema, and the preliminary results have been promising (Le Monnier et al., 2006; Ploton et al., 2006).

Unlike bacteria, viruses, except rhinovirus and HBoV, rarely colonize the respiratory tract in the absence of disease (Ruuskanen and Mertsola, 1999; Van Gageldonk-Lafeber et al., 2005). Therefore, the detection of antigens of common respiratory viruses (RSV, influenza A and B viruses, parainfluenza virus types 1, 2, and 3, adenovirus) from the nasopharyngeal mucus is routinely used for diagnosis of viral respiratory tract infection (Arstila and Halonen, 1988; Ruuskanen and Mertsola, 1999; British Thoracic Society, 2002). During epidemics, the use of rapid, point-of-care diagnostic tests for RSV and influenza is beneficial as it can facilitate appropriate clinical management such as antiviral treatment, reasonable antibiotic usage, and patient isolation (Rodriguez et al., 2002; Zheng et al., 2004; Cruz et al., 2007). However, although the sensitivity and specificity of these methods are fairly good, the mere presence of a virus in the nasopharynx offers no direct evidence of the etiology of pneumonia.

2.6.4.3 Serology

Serologic testing in paired sera is the standard method for the diagnosis of *M. pneumoniae* and *C. pneumoniae* infection since isolation of these organisms is time-consuming and insensitive, and is therefore not suitable for routine diagnosis (Waris et al., 1998; Daxboeck et al., 2003). Measurement of IgM antibodies in acute-phase serum by enzyme immunoassay has proven valuable in the diagnosis of *M. pneumoniae* pneumonia in children when symptoms have lasted over 10 days (Waris et al., 1998). The cold agglutinin test that can be performed at the bedside is of limited clinical value as serum cold agglutinins are positive in only 33 % to 70 % of children with mycoplasma infection (Broughton, 1986).

Serologic methods have been employed to determine the pneumococcal etiology of childhood CAP in research settings (Claesson et al., 1989; Ruuskanen et al., 1992; Korppi et al., 1993a; Heiskanen-Kosma et al., 1998; Wubbel et al., 1999; Juven et al., 2000; Michelow et al., 2004a; Don et al., 2005). The pneumococcal antigens used have been capsular C-polysaccharide, capsular type-specific polysaccharides, and pneumolysin. Studies using both antibody assays and immune complex assays have found serological evidence of pneumococcal infection in over 30 % of pneumonia cases in children (Korppi and Leinonen, 1997; Juven et al., 2000; Michelow et al., 2004a). The sensitivity of these assays, when compared with culture-positive cases, has ranged from 47 % to 92 % (Forgie et al., 1991; Michelow et al., 2002). Although the detection rate of significant antibody responses in healthy children has been low, only 1 % to 3 % (Nohynek et al., 1995b; Korppi and Leinonen, 1998), pneumococcal acute otitis media and acquisition of a new pneumococcal serotype in nasopharynx have been found to be able to induce a significant pneumococcal antibody response (Soininen et al., 2002; Korppi et al., 2008b). Due to the lack of a gold standard for the detection of non-bacteremic pneumococcal pneumonia, serologic methods are insufficiently validated and are therefore not recommended for routine diagnostics. Serologic tests

for *H. influenzae* and *M. catarrhalis* are also available in research settings (Nohynek et al., 1995b; Heiskanen-Kosma et al., 1998; Salo and Leinonen, 1999; Juven et al., 2000).

Serologic tests for common respiratory viruses are seldom needed in clinical practise. However, antibody testing is useful as a complementary tool to confirm the diagnosis retrospectively in undefined cases and in research studies (Sullivan and Jordan, 1988; Ruuskanen and Mertsola, 1999).

2.6.4.4 Polymerase chain reaction

Nucleic acid amplification tests have provided a new perspective into the diagnostics of infectious diseases (Chan and Morris, 2007). PCR technology allows very small amounts of target DNA to be detected in a timely manner. It is also an advantage that non-viable organisms can be amplified. However, despite the great potential of PCR, several weaknesses such as high cost and contamination susceptibility, have limited the widespread use of this technology in clinical practice.

Since the advent of nucleic acid amplification methods, great expectations have been placed on PCR to improve the microbiologic diagnosis of bacterial pneumonia. Studies investigating the applicability of PCR from blood samples to the diagnosis of pneumococcal pneumonia in children and adults have shown variable clinical sensitivity and specificity ranges of 29 % to 100 % and 83 % to 100 %, respectively compared with blood culture results (Rudolph et al., 1993; Salo et al., 1995; Dagan et al., 1998; Toikka et al., 1999a; Dominguez et al., 2001; Michelow et al., 2002; Murdoch et al., 2003). The highest sensitivity (100 %) in children was reported by Michelow et al., who tested several blood fractions (whole blood, buffy coat and plasma), which is laborious and expensive, and therefore not suitable for routine diagnostics (Michelow et al., 2002). The possible explanations for the suboptimal clinical sensitivity of PCR could be the rapid clearance of *S. pneumoniae* from the blood stream and the small sample volume used in PCR reactions resulting in false-negative results in specimens with a very low number of pathogens in the original sample (Werno and Murdoch, 2008).

PCR is used for the detection of *M. pneumoniae* in nasopharyngeal aspirates in children with pneumonia since *M. pneumoniae* rarely colonize the respiratory tract of healthy children (Waris et al., 1998; Michelow et al., 2004b; Morozumi et al., 2006). As with pneumococcal PCR, the sensitivities and specificities have ranged widely depending on the primers and PCR technology used. In a recent study by Morozumi et al., the sensitivity and specificity of real-time PCR in detecting *M. pneumoniae* pneumonia in children were 99.2 % and 97.5 %, respectively, when compared with culture results (Morozumi et al., 2006). In a Finnish study by Waris et al., however, PCR was positive in only 50 % of the *M. pneumoniae* cases detected by serology and/or by culture (Waris et al., 1998).

In pleural fluid samples, the use of broad range 16S PCR has significantly improved the bacterial yield in children with empyema (Eastham et al., 2004; Saglani et al.,

2005; Le Monnier et al., 2006). In a recent study by Saglani et al., the addition of PCR increased organism detection in pleural fluid from 19 % to 69 % (Saglani et al., 2005).

The development of PCR methodologies has also been crucial in understanding the magnitude of rhinovirus-related infections since virus isolation by conventional methods is difficult. In children with pneumonia, the detection rate of rhinovirus by reverse-transcription PCR has been up to fourfold greater when compared with the virus culture method (Isaacs, 1989; Ruuskanen et al., 1992; Juven et al., 2000; Tsolia et al., 2004). However, the high frequency (15 %─35 %) of positive PCR findings in asymptomatic children makes it difficult to fully define the quantitative role of rhinovirus in childhood pneumonia (Rakes et al., 1999; Van Benten et al., 2003; Jartti et al., 2004; Van Gageldonk-Lafeber et al., 2005; Winther et al., 2006). PCR is also the current method of choice for the clinical diagnosis of hMPV and HBoV infections. hMPV is rarely detected in healthy children (Van den Hoogen et al., 2003; Van Gageldonk-Lafeber et al., 2005) but HBoV infection can be followed by a asymptomatic virus shedding in the respiratory tract leading to false-positive clinical diagnosis of HBoV infection (Schildgen et al., 2008). In a recent study by Kantola et al-, 59 % of children with acute wheezing who were positive for HBoV according to PCR, elicited acute serologic response. The majority of these children had a high load of HBoV DNA in the nasopharynx, supporting the hypothesis that a high HBoV DNA load indicates acute primary infection, whereas a low load could be of less clinical significance (Kantola et al., 2008). PCR has also been found to be significantly more sensitive than antigen detection in identification of adenovirus in nasopharyngeal mucus (Jennings et al., 2004; Arnold et al., 2008).

2.7 Treatment

The principal question in pneumonia management is whether all children with CAP need to be treated with antibiotics. To date, there is no clear consensus on this issue. Some expert opinions recommend that all children with pneumonia should receive antibiotic treatment since it is impossible to exclude the presence of bacterial infection (Ruuskanen and Mertsola, 1999; McIntosh, 2002; Korppi, 2003). Others, such as the British Thoracic Society guidelines, recommend that antibiotic therapy can be withheld in young, mildly ill children in whom viral infection is likely (McCracken, 2000; British Thoracic Society, 2002; Stein and Marostica, 2007). Only one randomised prospective study in the developed world has investigated the necessity of antibiotic treatment in childhood bronchiolitis and CAP (Friis et al., 1984). In this study, 136 children diagnosed as having pneumonia on the basis of fine crepitating rales on lung auscultation or pulmonary consolidation on chest radiograph (positive chest radiography in 80%) were allocated to receive either antibiotic treatment or no antibiotic treatment. No clinically significant differences were found between these two groups in the course of the acute illness, or with the development of pulmonary complications. The authors concluded that routine antibiotic treatment of ALRI in infants and small children admitted to the hospital is not indicated, even if pulmonary consolidation is present. The generality of these findings is, however, limited by the fact that most of the study children had relatively mild signs and symptoms as all

children with severe disease were excluded. In addition, the study was performed during an RSV epidemic so the majority of the study children probably had bronchiolitis instead of pneumonia. Furthermore, one-fourth of the children with no initial antibiotic treatment were eventually treated with antibiotics.

The clear advantage of antibiotic therapy on pneumonia mortality in children has been shown in studies conducted at the beginning of the era of antibiotics in the late 1930s and early 1940s (Shann, 1986). In adults, the only pneumonia study that included a no-treatment arm was published by Evans and Gainsford in 1938 (Evans and Gainsford, 1938). In this study, mortality rate among untreated, hospitalized patients with acute-onset lobar pneumonia was found to be 27 % compared with 8 % detected among patients treated with sulphonamide. At present, placebo-controlled studies on pneumonia treatment are not ethically possible.

2.7.1 Management in outpatient care

Children presenting with mild to moderate symptoms can be treated safely at home (British Thoracic Society, 2002). The treatment should be re-evaluated if the child's condition deteriorates, or there is no improvement after 48 hours of treatment (British Thoracic Society, 2002).

In clinical practice, the selection of initial antibiotic treatment is empiric as the causative agent of pneumonia is almost never known. The first-line antibiotic therapy in both outpatient care and hospital care should always be active against *S. pneumoniae* as it is the most common and significant pathogen of pneumonia. Most pneumonia guidelines recommend amoxicillin for the first-line therapy in children less than 5 years of age and macrolides in children older than 5 years of age (Jadavji et al., 1997; Bradley, 2002; British Thoracic Society, 2002; McIntosh, 2002; Korppi, 2003; Lichenstein et al., 2003) (Table 4). The rationale behind these age-based recommendations is that beetalactams are not active against *M. pneumoniae* and *C. pneumoniae* which are frequent causative agents of pneumonia in older children. The simple use of macrolides in children of all ages, on the other hand, can no longer be recommended as the worldwide resistance to macrolides among *S. pneumoniae* is high, approximately 35 % (Felmingham et al., 2007). The most recently published expert opinion on pneumonia management recommends amoxicillin for all children with CAP without co-morbidity (Atkinson et al., 2007a). Addition of macrolide therapy is suggested only if no clinical improvement is observed after 48 hours treatment with amoxicillin. This recommendation is based on results of a closed loop audit from England which investigated a new treatment protocol promoting the use of beetalactams (Clements et al., 2000). The new management protocol was found to be effective, and no association with increased rate of treatment failure was observed. This new management recommendation favoring amoxicillin use appears rational as the rate of pneumococcal macrolide resistance is increasing and macrolide resistance is capable of causing clinical treatment failures (Lonks et al., 2002; Jacobs and Johnson, 2003; Iannini et al., 2007). In addition, macrolide resistance for *M. pneumoniae* has unexpectedly emerged as reported in a recent study in Japan (Morozumi et al., 2008). Pneumococcal penicillin resistance, in contrast, has shown to be of less clinical importance. Available data

strongly suggest that intermediate pneumococcal penicillin nonsusceptibility does not increase the risk of treatment failure in children with CAP (Friedland, 1995; Tan et al., 1998; Deeks et al., 1999). The worldwide prevalence of penicillin nonsusceptible isolates of *S. pneumoniae* in 2005 was 37 %, of which 24 % were fully resistant (Felmingham et al., 2007). The lowest rates of penicillin resistance are observed in Northern Europe and North America, and the highest in Southern Europe, Southern Africa and the Far East (Felmingham et al., 2007). The antibiotic resistance profile of *S. pneumoniae* isolates in children collected between 2006 and 2007 in South-West Finland is presented in Figure 5 (personal communication, Olli Meurman).

Table 4. Some treatment recommendations for antimicrobial therapy in children with pneumonia treated at outpatient care.

	British Thoracic Society guidelines 2002	McIntosh 2002	Korppi 2003	Lichenstein et al. 2003	Atkinson et al. 2007a
Age < 5 years	Amoxicillin or amoxicillin/ clavulanate, cefaclor, erythromycin, clarithromycin or azithromycin	Amoxicillin	Amoxicillin	Amoxicillin or amoxicillin/ clavulanate or cefuroxime or macrolide	Amoxicillin Addition of macrolide if no clinical improvement in 48 hours
Age > 5 years	Macrolide (erythromycin, clarithromycin, azithromycin)	Erythromycin, clarithromycin, azithromycin, or doxycycline for children > 8 years of age	Macrolide (erythromycin, azithromycin, clarithromycin) or doxycycline for children > 8 years of age	Macrolide or tetracycline for children > 8 years of age	Amoxicillin Addition of macrolide if no clinical improvement in 48 hours

Management guidelines are only directional and the choice of empiric antibiotic therapy should be based on the patient's age, clinical presentation, local resistance pattern of predominant bacteria, and local epidemiologic factors (McIntosh, 2002; Korppi, 2003; Pelton and Hammerschlag, 2005). For example, amoxicillin is always the first-line therapy if *S. pneumoniae* is thought to be the likely pathogen, and macrolides should be used if atypical bacteria are suspected (British Thoracic Society, 2002). Secondary antibiotic choices in outpatient care include phenoxymethylpenicillin (penicillin V), cefaclor, amoxicillin/clavulanate, co-trimoxazole, and doxycycline in children older than 8 years of age.

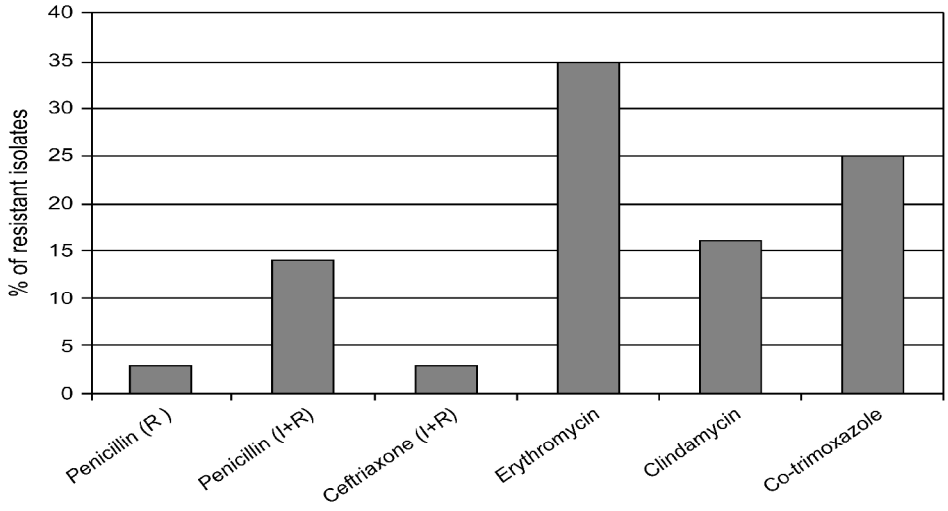

Figure 5. Antibiotic resistance rates among *S. pneumoniae* isolates in children collected between 2006 and 2007 in South-West Finland.

2.7.2 Management in hospital

Children with pneumonia presenting with impaired general condition, respiratory distress, oxygen requirement, a significant amount of pleural fluid, dehydration, frequent vomiting, and treatment failure on appropriate oral antibiotic therapy need to be admitted to hospital (Jadavji et al., 1997; British Thoracic Society, 2002; Korppi, 2003). In addition, age < 6 months and noncompliant parents are indicators for hospital management.

Intravenous benzylpenicillin (penicillin G) is usually recommended as the first-line therapy for hospitalized children with pneumonia in areas with low level penicillin resistance (Ruuskanen and Mertsola, 1999; Korppi, 2003; Atkinson et al., 2007a). In severely ill patients with pneumonia, the antibiotic therapy should also cover *H. influenzae* and *S. aureus*, and therefore cefuroxime is recommended. Macrolides should be added when *M. pneumoniae* infection is suspected. In adults, the addition of a macrolide to beetalactam therapy has been found to be associated with reduced mortality in the most severely ill patients (Waterer et al., 2001; Martinez et al., 2003; Baddour et al., 2004). However, it is not yet known whether this benefit is associated with the extended spectrum of pathogens covered or with the potential ability of macrolides to modulate the host inflammatory response (Culic et al., 2001; Laterre, 2008). Other possible antibiotic choices in hospital care are ampicillin, ceftriaxone, cefotaxime, clindamycin, and meropenem. In a recent study by Bradley et al., levofloxacin was found to be as well tolerated and effective as standard-of-care antibiotics for the treatment of pneumonia in children (Bradley et al., 2007). However, fluorokinolones are not yet licensed for children because of their potential to induce arthropathy in juvenile animals (Schaad, 2007).

A recent multicentre, randomized, controlled, non-blinded equivalence trial comparing oral amoxicillin and intravenous benzylpenicillin has provided new insight into the management of pneumonia in hospitalized children (Atkinson et al., 2007b). This study randomized 246 children to receive either oral amoxicillin for 7 days or intravenous benzylpenicillin followed by oral amoxicillin after discharge. All study children had radiologically verified pneumonia severe enough to require hospital admission. Exclusion criteria were wheeze, oxygen saturations < 85 %, shock requiring > 20 ml/kg fluid resuscitation, immunodeficiency, pleural effusion requiring drainage, chronic lung condition, penicillin allergy, and age < 6 months. In this study, oral amoxicillin and intravenous penicillin were found to have equivalent efficacy for the treatment of pneumonia in previously healthy children as assessed by the duration of fever, oxygen requirement, and number of complications. In addition, oral treatment allowed children to be discharged home sooner and to avoid pain and distress for cannulation. The authors concluded that all but the sickest children with pneumonia should be treated with oral amoxicillin. Corresponding results were found in a study by Addo-Yobo et al., comparing parenteral penicillin and oral amoxicillin in treatment for severe pneumonia in developing countries (Addo-Yobo et al., 2004). Whether most children with pneumonia could be treated entirely at outpatient care with oral antibiotics is, however, not yet known. In a recent study by Hazir et al., home treatment with high-dose oral amoxicillin was found to be equivalent to hospital treatment with parenteral ampicillin for treatment of WHO defined severe pneumonia (Hazir et al., 2008). However, these results can not be directly applied to general pneumonia management as the pneumonia diagnosis was not based on chest radiography and a high proportion of children had wheeze, suggesting a high prevalence of viral infections.

There is currently little data on the most appropriate duration of antibiotic treatment. Intravenous therapy can be changed to oral treatment when clear evidence of improvement is observed (British Thoracic Society, 2002). Azithromycin therapy for 3 days has been shown to be effective for treatment of pneumonia in children (Kogan et al., 2003), but the recommended duration of other oral antibiotics is 5 to 7 days, and 10 days in cases of severe infection (Dagan, 1993; Korppi, 2003; Atkinson et al., 2007a). In non-severe, WHO defined pneumonia, treatment with oral amoxicillin for 3 days has been found to be equally effective as treatment for 5 days (Pakistan Multicentre Amoxicillin Short Course Therapy pneumonia study group, 2002; Agarwal et al., 2004).

2.7.3 General management

General management of pneumonia includes oxygen therapy if oxygen saturation is 92 % or less and fluid therapy at 80 % basal level (after hypovolemia has been corrected) if the patient is vomiting or severely ill. In addition, antipyretics and analgetics can be used to keep the child comfortable and to help coughing. Chest physical therapy is not found to be beneficial, and is not recommended for children with pneumonia (British Thoracic Society, 2002). In fact, in a recent study by Paludo et al., chest physiotherapy was associated with prolonged duration of coughing and rhonchi in lung auscultation (Paludo et al., 2008).

2.8 Outcome and complications

In developed countries, the great majority of children with pneumonia recover rapidly and uneventfully (Ruuskanen et al., 1992; Gendrel et al., 1997; Harris et al., 1998; Juven et al., 2004; Bradley et al., 2007; Clark et al., 2007b). Long term follow-up is therefore considered unnecessary (Ruuskanen and Mertsola, 1999; British Thoracic Society, 2002; Virkki et al., 2005). In a study by Juven et al., the median duration of fever after the onset of antibiotic therapy in hospitalized children was 14 hours and treatment failure (fever lasting for ≥ 48 hours) was reported in only 9 % of the cases (Juven et al., 2004). RSV (46 %) and *H. influenzae* (39 %) were the pathogens most commonly found in children with treatment failure (Juven et al., 2004). The most frequently reported reasons for treatment failure in developed countries include *M. pneumoniae* infections, viral infections, mixed viral-bacterial infections, and antibiotic resistance (Ruuskanen et al., 1992; Gendrel et al., 1997; Juven et al., 2004).

Complications of pneumonia include parapneumonic pleural effusion, empyema, lung abscess, necrotizing pneumonia, and pneumotocele (British Thoracic Society, 2002; Tan et al., 1995). Although these complications are infrequent especially in the developed countries, the possibility of complicated disease should be evaluated if a child remains pyrexial or unwell 48 hours after administration of antibiotics (British Thoracic Society, 2002).

2.8.1 Parapneumonic effusion and empyema

The frequency of parapneumonic empyema in children in the past decades has increased in the United States as well as in Europe and Asia (Rees et al., 1997; Byington et al., 2002; Tan et al., 2002; Eastham et al., 2004; Hsieh et al., 2004; Spencer et al., 2006; Roxburgh et al., 2008). *S. pneumoniae* has been found to be the principal pathogen, with a predominance of serotype 1 (Byington et al., 2002; Eastham et al., 2004). Other important causative agents of empyema are *S. aureus* and *S. pyogenes* (Chonmaitree and Powell, 1983; Rees et al., 1997; Byington et al., 2002; Eastham et al., 2004; Nyambat et al., 2008). It is unclear whether the increasing incidence of empyema is related to changes in the virulence of the causative organisms, to changes in antibiotic prescription polices, or to other factors. Interestingly, the introduction of the heptavalent pneumococcal conjugate vaccine did not lead to a reduction in the empyema incidence in the United States although the overall rate of invasive pneumococcal disease declined. In fact, the incidence of parapneumonic empyema is still increasing, and in addition to serotype 1, serotypes 3 and 19A have become prevalent, all of which are nonvaccine serotypes (Byington et al., 2006).

The development of parapneumonic effusion is a continuum that can be divided into three stages: exudative, fibrinopurulent and organisational (Hamm and Light, 1997). Empyema is defined as presence of pus in the pleural space (Balfour-Lynn et al., 2005; Sahn, 2007). Time is of essence in the diagnosis and treatment of parapneumonic effusion as appropriate treatment can prevent the development of empyema and its progression. In clinical practice, diagnostic and therapeutic thoracocentesis should be performed as soon as possible when pleural effusion is detected (Sahn and Light, 1989;

Balfour-Lynn et al., 2005). Pleural fluid analysis allows the staging of pleural effusion and guides the initial management. Uncomplicated parapneumonic effusion is characterized by clear appearance, pH > 7.30, a glucose level > 6 mg/L, lactate dehydrogenase level < 700 IU/L, and negative microbiologic results whereas complicated effusion is associated with purulent appearance, a pleural fluid pH < 7.20, a glucose level < 4 mg/L, lactate hydrogenase level > 1000 IU/L, and positive gram staining and culture results (Sahn, 2007). Loculated pleural fluid also indicates complicated effusion.

As the disease process is dynamic, a step-wise treatment approach is usually considered beneficial (Sonnappa and Jaffe, 2007). Treatment options include intravenous antibiotics alone or in combination with thoracocentesis, chest tube drainage, intrapleural fibrinolytics, video-assisted thoracoscopy, minithoracotomy, and open decortication. In cases of small uncomplicated effusions antibiotic treatment alone is likely to be effective whereas complicated effusions require drainage (Balfour-Lynn et al., 2005; Sonnappa and Jaffe, 2007). Intrapleural fibrinolytics are recommended for any complicated effusion and empyema in children as they have been found to increase pleural drainage and to significantly shorten the length of hospitalization compared to intrapleural saline installation (Thomson et al., 2002; Balfour-Lynn et al., 2005). In a study by Sonnappa et al., intrapleural urokinase was even shown to be equivalent to video-assisted thoracoscopic surgery in the treatment of empyema (Sonnappa et al., 2006). Chest tube drainage with intrapleural fibrinolytics was also found to be the most cost-effective strategy for treating childhood empyema in a recent cost-effectiveness analysis (Cohen et al., 2008). In a controlled, double-blinded, randomized trial in adults, however, intrapleural streptokinase did not improve mortality, the rate of surgery, or the length of hospital stay among patients with pleural infection (Maskell et al., 2005).

Currently, surgical intervention is recommended in cases with failure of antibiotics, chest tube drainage, and fibrinolytic treatment (Balfour-Lynn et al., 2005). The benefits of early surgery have been discussed but consensus on this issue has not yet been reached. A meta-analysis comparing primary operative therapy with non-operative therapy concluded that primary operative therapy is associated with a lower mortality rate, a lower reintervention rate, a shorter length of hospitalization, a shorter time with thoracostomy tube, and a shorter course of antibiotic treatment, compared with nonoperative treatment (Avansino et al., 2005). However, this meta-analysis was hampered by the scarcity of prospective, controlled trials and by a lack of proper staging of the empyema.

2.9 Prevention

Introduction of the heptavalent pneumococcal conjugate vaccine has led to a significant reduction in the prevalence of childhood pneumonia in the United States (Grijalva et al., 2007). The Kaiser Permanente effectiveness trial showed 20.5 % vaccine effectiveness against radiologically verified pneumonia episodes (Black et al., 2002). When more specific WHO criteria for radiologic diagnosis were used, the

vaccine efficacy increased to 30 % (Hansen et al., 2006). In a randomized, double-blind, placebo-controlled trial in Gambia, the nine-valent pneumococcal conjugate vaccine showed high efficacy (37 %) against radiologic pneumonia and substantially reduced hospital admissions and improved child survival (Cutts et al., 2005). The 16 % reduction in infant mortality documented in this study provides strong evidence of the significant benefits that the pneumococcal vaccine could have on overall childhood mortality. The Finnish expert committee on childhood pneumococcal vaccination recommends that pneumococcal conjugate vaccine should be included into the Finnish National Vaccination Programme (Saxén et al., 2008).

After the introduction of the heptavalent pneumococcal conjugate vaccine, increasing evidence has emerged of invasive pneumococcal infections, including pneumonia and empyema caused by non-vaccine pneumococcal serotypes (Byington et al., 2006; Singleton et al., 2007; Munoz-Almagro et al., 2008). The most prevalent serotypes responsible for this replacement phenomenon have been serotype 1 and serotype 19A both of which have high invasive potential (Byington et al., 2006; Singleton et al., 2007; Munoz-Almagro et al., 2008). In a study by De Schutter et al., the nine-valent vaccine containing serotype 1 significantly increased the theoretical coverage of the conjugate vaccine compared with the seven-valent vaccine, especially in invasive pneumonia (De Schutter et al., 2006). However, the repertoire of over 90 different pneumococcal serotypes poses great challenge to the development of extensive conjugate vaccine. In the future, vaccines based on highly conserved protein antigens of *S. pneumoniae* could have potential for prevention of pneumococcal infections caused by all serotypes of pneumococcus in both children and the elderly (Giefing et al., 2008).

The trivalent inactivated influenza vaccine and the trivalent live attenuated intranasal influenza vaccine have been shown to provide significant protection against pneumonia and influenza events in children (Centers for Disease Control and Prevention, 2004a, Piedra et al., 2008). Annual influenza vaccination for children aged 6–35 months with trivalent inactivated influenza vaccine was included into the Finnish National Vaccination Programme in 2007.

3. AIMS OF THE STUDY

The general aim of the present thesis was to improve the current understanding of childhood community-acquired pneumonia in the fields of microbiologic diagnosis, etiology, viral disease, and complicated disease.

The specific aims of the study were:

- To evaluate the clinical value of pneumolysin-targeted real-time PCR in the diagnosis of pneumococcal pneumonia and empyema in children.
- To investigate the usefulness and clinical value of microbiologic analysis of induced sputum specimens in the etiologic diagnosis of childhood pneumonia.
- To describe the frequency and characteristics of influenza virus-related pneumonia in children.
- To find clinical predictors for parapneumonic empyema in children.

4. MATERIALS AND METHODS

The thesis consists of two prospective and two retrospective studies carried out at the Department of Pediatrics, Turku University Hospital. In all studies, pneumonia was defined as presence of pneumonic infiltrates on the chest radiograph with a simultaneous finding of signs and/or symptoms of acute infection. The criteria for parapneumonic empyema were purulent pleural fluid, positive pleural fluid bacteriology, radiologic evidence of empyema, and/or a need for surgical intervention, i.e. thoracoscopic surgery or thoracotomy.

4.1 Patients and study design

Table 5. Study patients

Study I	30 children with CAP 12 children with parapneumonic empyema 19 children with a suspected pneumococcal bacteremia 19 children with a nonpneumococcal infection as controls
Study II	76 children with CAP
Study III	134 children with influenza virus-related CAP
Study IV	37 children with parapneumonic empyema 74 children with alveolar uncomplicated CAP as controls

Study I

Pneumolysin-targeted real-time PCR in whole blood and pleural fluid samples was compared to blood and pleural fluid culture in the diagnosis of pneumococcal pneumonia and empyema in children. The prospective study enrolled children aged 6 months to 15 years with CAP admitted to the Department of Pediatrics, Turku University Hospital between April 2004 and January 2005, and children with parapneumonic empyema admitted to the Department of Pediatrics, Turku University Hospital or to the Department of Pediatrics, Oulu University Hospital between November 2003 and October 2005. In addition, children with a clinical picture suggestive of pneumococcal bacteremia without pneumonia were included in the study. Children with a clinically nonpneumococcal infection were used as controls. The study was performed in collaboration with the Department of Medical Microbiology and Immunology, Turku University, and the National Public Health Institute, Turku.

Study II

A prospective study was carried out to investigate the virologic and bacteriologic yield from induced sputum specimens from children with CAP, and to study whether analysis of paired nasopharyngeal aspirate and induced sputum specimens could be useful in the microbiologic diagnosis of childhood CAP. Children aged 6 months to 15 years treated for radiologically verified CAP in the Pediatric Infectious Disease Ward

of Turku University Hospital between January 2006 and April 2007, were eligible for the study. The study was done in collaboration with the Department of Medical Microbiology and Immunology, and the Department of Virology, Turku University, and the Department of Clinical Microbiology, Turku University Hospital.

Study III

A retrospective study was carried out to describe the frequency and characteristics of influenza virus-related pneumonia in children. The study involved children less than 16 years of age treated as inpatients or outpatients at the Department of Pediatrics, Turku University Hospital, during a 24-year period from 1980 trough 2003. Children with influenza A or B antigen detected in nasopharyngeal aspirate were identified from the files of the Department of Virology, Turku University. Children with laboratory-documented influenza and simultaneous finding of infiltrates compatible with pneumonia on the chest radiograph were included in the study.

Study IV

The study involved children less than 16 years of age discharged from the Department of Pediatrics, Turku University Hospital with a diagnosis of CAP or empyema over a 15.5-year period from January 1991 to August 2006. Study children were retrospectively identified from the database of Turku University Hospital and the medical records of children with a discharge diagnosis of empyema were reviewed for the presence of parapneumonic empyema. In addition, to ensure that no empyema case was missed as a result of an inadequate discharge diagnosis, the medical records of all children with a discharge diagnosis of CAP treated with thoracocentesis or chest tube drainage were reviewed for the presence of empyema. Patients with postoperative or posttraumatic empyema, and patients with non-infectious causes of pleural fluid, such as malignant disease or rheumatoid disease, were excluded from the study.

Two distinct groups of children with uncomplicated CAP with alveolar consolidation on the chest radiograph were included as controls to allow the determination of the clinical predictors for empyema in children. The first comparison group consisted of children discharged with the diagnosis of CAP with dense alveolar consolidation on the chest radiograph. Thirty-seven consecutive children fulfilling these criteria during a 3-year period from January 2003 through November 2005, when the incidence of parapneumonic empyema was highest, were included. Another comparison group was included in the study to allow comparison of the kinetics of inflammatory parameters between empyema patients and uncomplicated pneumonia patients. This comparison group consisted of children included in an etiologic study of childhood CAP conducted from 1993 through 1995 in Turku, Finland (Juven et al., 2000). Inflammatory parameters were prospectively followed-up in the study. Patients were included in the second comparison group on the basis of alveolar consolidation on the chest radiograph and CRP values on admission comparable with those in children with parapneumonic

empyema. Thirty-seven children with CRP values closest to those with empyema were included.

4.2 Data collection

The following clinical and laboratory data on all study children were collected through a systematic review of medical records: age; gender; underlying illness; preceding antibiotic treatment before presentation; duration of fever and symptoms before admission; signs and symptoms at presentation (tachypnea was definite with a respiratory rate >60/min in infants less 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 over 5 years of age); duration of fever (≥37.5 ºC in study III, ≥38.0 ºC in studies II and IV) in the hospital; duration of hospitalization; the level of inflammatory parameters; and the available microbiology results. In addition, in study II, the parent or guardian was requested to complete a symptom questionnaire in order to obtain more accurate data on the symptoms of pneumonia before hospitalization.

4.3 Pneumolysin-targeted real-time PCR

The pneumolysin-targeted real-time PCR method was adapted from the study of Saukkoriipi et al. with the author's approval (Saukkoriipi et al., 2002). PCR was performed using the LightCycler Instrument (Roche Diagnostics GmbH, Mannheim, Germany) in which PCR amplification and analysis take place simultaneously in a closed system allowing specific and rapid analysis. The PCR was optimized for the purposes of the study by titrating the template DNA, primer, probe, and MgCl concentrations in the reaction mixture using whole blood specimens spiked with *S. pneumoniae* as samples. The *in vitro* sensitivity of the PCR assay was determined using 10-fold dilutions of genomic DNA from *Streptococcus pneumoniae* ATCC® 6314D, and DNA extracted from 33 clinical isolates of *S. pneumoniae*. The *in vitro* specificity was determined using DNA extracted from 12 clinical isolates of viridans group streptococci and with 27 different bacterial organisms representing common bacterial pathogens and normal bacterial flora in children. The clinical isolates were obtained from National Public Health Institute, Turku Finland.

Whole blood samples for PCR analysis from children with pneumonia or suggestive pneumococcal bacteremia without pneumonia, and from control patients were collected at presentation prior to the initiation of antibiotic therapy. The whole blood samples were collected in tubes containing EDTA in context with the collection of peripheral blood samples for routine bacterial culture. Pleural fluid samples for PCR analysis and routine bacterial culture were collected from children with empyema in context with diagnostic and therapeutic pleurocentesis within a median of 70 hours after the initiation of antibiotic treatment.

DNA from clinical samples and DNA from clinical isolates of *S. pneumoniae* and other bacterial organisms was extracted using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) in accordance with the

manufacturer's instructions. Whole blood samples and pleural fluid samples were processed using the protocol for whole blood (Study I), and nasopharyngeal aspirate samples and sputum samples using the protocol for bacteria (Study II). Before DNA extraction, purulent nasopharyngeal aspirate samples and sputum samples were homogenized by incubation with 0.1% dithiothreitol in equal amounts of the sample volume (Study II). Then nasopharyngeal samples, and sputum samples as well as pleural fluid samples in study I were concentrated by centrifugation (13000xg, 5 min), and 200 µl of the concentrate was used for DNA extraction. An additional incubation at 37ºC for 15 minutes with 15 µl of lysozyme (10mg/ml) was performed for all samples before DNA extraction to ensure prober lysis of the pneumococcal cell wall.

A 206bp fragment of the pneumolysin encoding gene of *S. pneumoniae* was used as a target in the PCR assay. The oligonucleotide sequences for primers and fluorescent-labeled hybridization probes used in this study have been previously described (Saukkoriipi et al., 2002). PCR was performed using the LightCycler Instrument (Roche Diagnostics GmbH, Mannheim, Germany), and assays were carried out in LightCycler capillaries in a 20 µl reaction volume using the LightCycler FastStart DNA Master Hybridization kit (Roche Diagnostics GmbH, Mannheim, Germany) as described earlier (Saukkoriipi et al., 2002). In brief, the reaction mixture contained 2 µl of LightCycler FastStart DNA Master Hybridization probes -reaction mixture, 4 mM $MgCl_2$, 1 µM of each primer, 0.2 µM of each probe, and 2 µl of extracted DNA template. The PCR run was performed as follows: initial denaturation at 95ºC for 10 min followed by 50 cycles of amplification, each consisting of 10 s of denaturation at 95 ºC, 15 s of annealing at 57ºC and 9 s of elongation at 72 ºC. Melting curve analysis was performed at 95ºC for 20 s, 40ºC for 20 s and 85ºC for 0 s. The standard curve for *S. pneumoniae* quantification was done using genomic DNA from *Streptococcus pneumoniae* ATCC 6314D (American Type Culture Collection, Manassas, VA, USA) in tenfold dilutions ranging from 0.4 pg of pneumococcal DNA to 40 000 pg of pneumococcal DNA per reaction (Study II). Standard precautions were taken to avoid contamination, and pneumococcal DNA from clinical isolate and sterile distilled water were used as positive and negative controls in each run.

Blood culture and pleural fluid culture were performed using standard methods.

4.4 Induced sputum analysis

A nasopharyngeal sample was aspirated with a disposable catheter connected to a mucus extractor prior to sputum induction. Nasopharyngeal aspirate was taken from both nostrils by inserting the catheter into the back wall of the nasopharynx and drawing back while applying suction with an electronic suction device to obtain a nasopharyngeal sample and to clean the nasopharynx of nasopharyngeal mucus. The procedure of sputum induction is presented in Figure 6. Sputum samples were collected on admission previous to administration of intravenous antibiotic treatment. However, in case of poor general condition in a study child, or during the night-time, sputum induction was performed after administration of one or two antibiotic dosages.

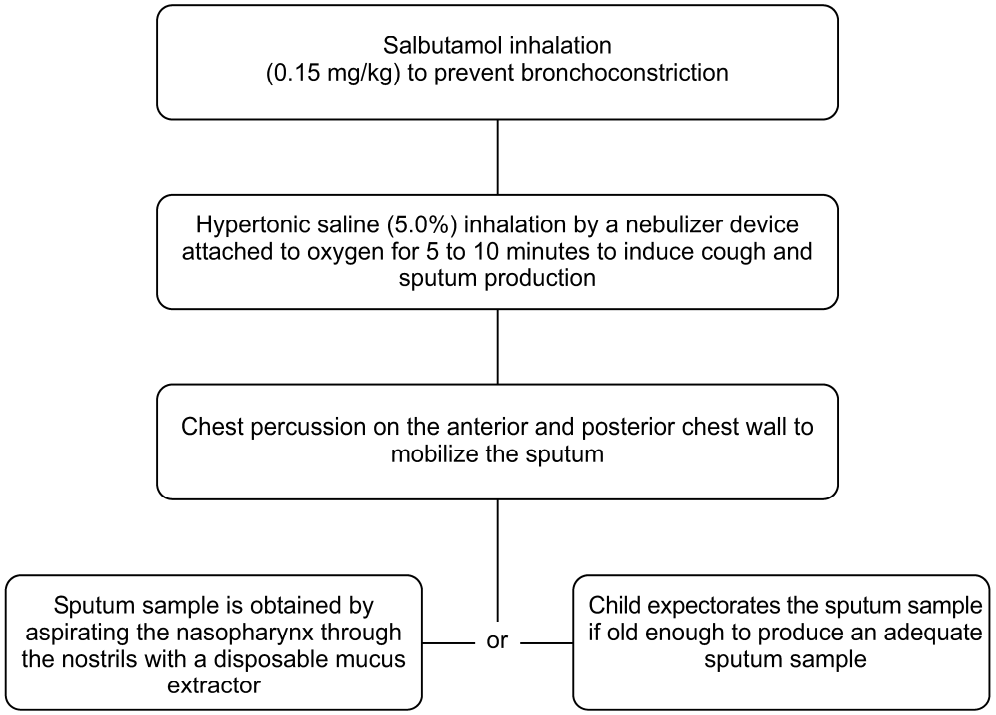

Figure 6. Procedure of sputum induction.

After sample collection, nasopharyngeal aspirates and sputum samples were immediately prepared for microbiologic studies by dipping one sterile Coban swab and three sterile cotton swabs into each sputum and nasopharyngeal aspirate sample. The Coban swab was placed in a Coban Amies gel agar transport system with charcoal (Coban diagnostics, Murrieta, CA, USA) for bacterial culture; one cotton swab was applied over a microscope glass slide for bacterial gram staining; two cotton swabs were placed in sterile tubes for viral antigen detection and for viral PCR analyses; and the remainder of the samples were used for *M. pneumoniae* and *S. pneumoniae* PCR. Specimens were stored at +4ºC until analyzed the following working day.

The quality of the sputum specimen was assessed by evaluating the gram staining by light microscopy under low power (10 x lens objective). Sputum samples were considered of good quality if they had <25 squamous epithelial cells and >25 leukocytes per low-powered field (Geckler et al., 1977). Good-quality specimens were analyzed further. Figure 7 shows the microbiologic studies performed on induced sputum specimens. All microbiologic studies, except the quality analysis, were done on both sputum and nasopharyngeal aspirate specimens. Blood cultures and an IgM enzyme immunoassay test for *M. pneumoniae* were done according at the discretion of the duty pediatrician using standard methods.

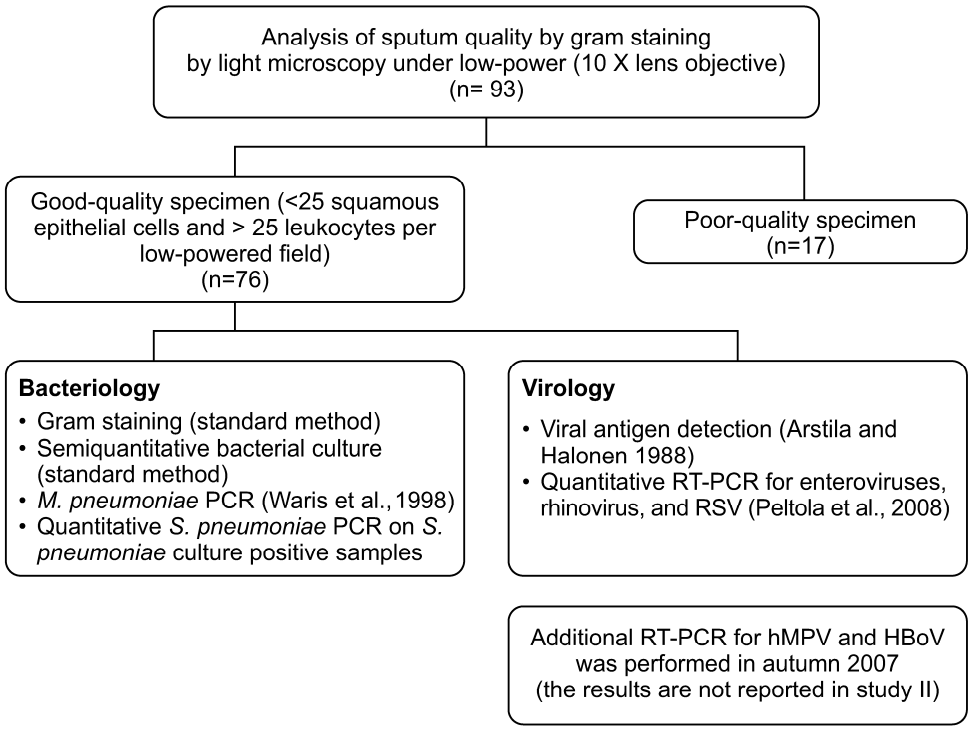

Figure 7. Microbiologic analyses performed on sputum specimens.

Additional PCR analyses for hMPV and HBoV were carried out in autumn 2007. hMPV PCR was performed using the Amplitect Quantification Kit for Metapneumovirus (hMPV) Genomes and Ambigen qPCR Mastermix (both from AME Biosciences, Toroed, Norway) as recommended by the manufacturer. Bocavirus PCR was done as previously described (Koskenvuo et al., 2008).

4.5 Radiologic investigations

The chest radiographs of all study children in studies II, III, and IV were reviewed by a pediatric radiologist (Raimo Virkki, MD) according to a systematic formula. The findings were classified as alveolar and/or interstitial infiltrate, atelectasis, hilar enlargement, hyperaeration, and pleural fluid. Local, loculate pleural infiltrate was considered a radiologic finding suggestive of empyema. The location of radiologic findings in the pulmonary lobes was recorded. The pediatric radiologist was unaware of the clinical characteristics of the study children. In study I, the chest radiographs were evaluated by the radiologist on duty.

4.6 Statistical analysis

The χ^2 test, or Fisher's exact test (II, III, IV) were performed to compare categoric data, and the Kruskal-Wallis test (II, III) and the Student's t-test or the Mann-Whitney U test

(IV) were used to compare continuous data. A p-value < 0.05 was considered significant. In study II, a simple kappa coefficient was calculated to assess the agreement between the induced sputum and nasopharyngeal samples. In study IV, independent clinical predictors for empyema were identified using multivariate logistic regression analysis, and the 95% confidence interval for the incidence of empyema among hospitalized children with community-acquired pneumonia was calculated using binominal distribution.

4.7 Ethics

The prospective studies I and II were approved by the Ethics Committee of the Hospital District of South-West Finland. Signed informed consent was obtained from the parent or guardian of each participating child before enrolment.

5. RESULTS

5.1. Pneumolysin-targeted real-time PCR in the diagnosis of pneumococcal pneumonia and empyema

The analytical sensitivity of the PCR assay was 4 fg of pneumococcal DNA, corresponding to 2 genome equivalents of pneumococcal DNA/reaction. The PCR assay correctly detected all the clinical isolates of *S. pneumoniae* tested, resulting in an *in vitro* sensitivity of 100 %, whereas all nonpneumococcal bacterial organisms, including *Streptococcus mitis*, *Streptococcus oralis* and *Streptococcus sanguis*, were negative, resulting in an *in vitro* specificity of 100 %.

In a clinical trial, *S. pneumoniae* was detected in the pleural fluid by PCR in 9 of 12 (75 %) children with empyema (Table 6). PCR increased the detection rate of pneumococcus to almost tenfold when compared to pleural fluid culture (75 % vs. 8 %). In whole blood samples, PCR detected *S. pneumoniae* in only 1 child of 30 children with pneumonia and in one of the three children with pneumococcal empyema who had whole blood samples analyzed by PCR. In three children with blood cultures positive for *S. pneumoniae*, PCR failed to detect *S. pneumoniae* in whole blood samples. The frozen pneumococcal bacterial isolates obtained from these three children were positive by PCR. All whole blood samples from children with a nonpneumococcal infection were negative by PCR.

Table 6. Clinical diagnosis and laboratory findings in 13 patients with positive finding in blood or pleural fluid PCR, or blood or pleural fluid culture.

Clinical diagnosis	Leukocyte count (10^9/l)	CRP value (mg/l)	Pleural fluid PCR	Pleural fluid culture	Blood PCR	Blood culture
Empyema	10.1	155	pos.	neg.	ND	pos.
Empyema	52	274	pos.	pos.	pos.	neg.
Empyema	8.3	281	pos.	neg.	ND	neg.
Empyema	19.6	158	pos.	neg.	ND	neg.
Empyema	20.6	452	pos.	neg.	ND	neg.
Empyema	17.9	253	pos.	neg.	neg.	neg.
Empyema	20.1	176	pos.	neg.	neg.	neg.
Empyema	12.7	317	pos.	neg.	ND	ND
Empyema	17.2	251	pos.	neg.	ND	neg.
Pneumonia	22.3	79	ND	ND	neg.	pos.
Pneumonia	34.5	311	ND	ND	neg.	pos.
Pneumonia	15.4	64	ND	ND	pos.	neg.
Pneumococcemia	22.5	5	ND	ND	neg.	pos.

5.2 Induced sputum in the microbiologic diagnosis of CAP

A good-quality sputum sample was obtained from 76 (82 %) of the 93 children in whom the sputum quality was analyzed. The sputum sample was obtained by expectoration in seven (9 %) children and by aspirating through the nostrils in 69 (91 %) children. No serious adverse events were recorded during the sputum induction but children found hypertonic saline inhalation and repeated nasopharyngeal aspirations unpleasant.

A possible microbial etiology was identified in 64 (84 %) of the 76 children with a good-quality sputum sample (Table 7). The proportions of bacterial, viral and mixed bacterial-viral findings are presented in Figure 8. *S. pneumoniae* was the most frequently isolated bacterium, detected in almost one-half of the study children, followed by *M. catarrhalis*, noncapsulated *H. influenzae*, and *S. aureus*. The great majority (94 %) of the *S. pneumoniae* isolates were penicillin-susceptible whereas the detection rate of macrolide resistant pneumococci was high (54 %). Evidence of *M. pneumoniae* infection was detected in induced sputum samples by PCR in two children, and by IgM serology in 7 of the 36 children tested. Altogether, evidence of *M. pneumoniae* infection was identified in 8 (11 %) children. Previously given antibiotic treatment had a significant influence on the detection rate of *S. pneumoniae* and *H. influenzae* in sputum samples (Table 7).

Rhinovirus was the most common virus detected, identified in 29 % of the children by PCR (Table 7). RSV was found rarely, which is explained by the fact that this study was conducted during a time period when there was no on-going RSV epidemic; the winter epidemic 2006 had just ended and the spring epidemic 2007 had not yet started. The most frequently detected viral-bacterial combinations, found in 15 % and in 13 % of the children, were those of rhinovirus with *S. pneumoniae* and rhinovirus with *M. catarrhalis*, respectively. The most common bacterial combination was that of *S. pneumoniae* and *M. catarrhalis*, found in 16 % of the study children.

One-fourth of all bacterial findings were only detected in sputum, and the amount of bacteria in the remainder of the sputum specimens compared to nasopharyngeal aspirate was higher in 14 % and equal in 70 % of all the comparisons. The amount of bacteria in *S. pneumoniae* culture positive samples was also quantified by quantitative pneumolysin PCR and the amount of pneumococci in sputum was higher in 45 % of the cases ($p = 0.891$). All viruses (rhinovirus, RSV, enteroviruses, parainfluenza type 3 virus, influenza A and B viruses) detected in sputum were also detected in nasopharyngeal aspirates. The amount of rhinovirus in sputum by quantitative PCR was higher in 82 % of the comparisons ($p = 0.020$).

Table 7. Bacteria and viruses in induced sputum in children with community-acquired pneumonia.

Microbe	All children (n = 76) No. (%)	Children without preceding antibiotic treatment (n = 37) No. (%)	Children with preceding antibiotic treatment (n = 39)[a] No. (%)
Bacterium			
Streptococcus pneumoniae[b]	35 (46 %)	23 (62 %)	12 (31 %)[c]
Haemophilus influenzae	22 (29 %)	5 (14 %)	17 (44 %)[d]
Moraxella catarrhalis	21 (28 %)	11 (30 %)	10 (26 %)
Staphylococcus aureus	9 (12 %)	5 (14 %)	4 (10 %)
Mycoplasma pneumoniae[e]	2 (3 %)	1 (3 %)	1 (3 %)
Other bacterium[f]	3 (4 %)	2 (5 %)	1 (3 %)
Normal/mixed flora	11 (14 %)	5 (14 %)	6 (15 %)
Neg	2 (3 %)	0 (0 %)	2 (5 %)
Total	60 (79 %)	30 (81 %)	30 (77 %)
Virus			
Rhinovirus	22 (29 %)	13 (35 %)	9 (23 %)
Human bocavirus[g]	14 (18%)	4 (11 %)	10 (26 %)
Human metapneumovirus[g]	10 (13 %)	3 (8 %)	7 (18 %)
Respiratory syncytial virus	3 (4 %)	0 (0 %)	3 (8 %)
Enteroviruses	2 (3 %)	1 (3 %)	1 (3 %)
Parainfluenzae type 3 virus	1 (1 %)	1 (3 %)	0 (0 %)
Influenza A virus	1 (1 %)	0 (0 %)	1 (3 %)
Influenza B virus	1 (1 %)	1 (3 %)	0 (0 %)
Adenovirus	0 (0 %)	0 (0 %)	0 (0 %)
Parainfluenzae type 1 virus	0 (0 %)	0 (0 %)	0 (0 %)
Parainfluenzae type 2 virus	0 (0 %)	0 (0 %)	0 (0 %)
Total[g]	42 (55 %)	17 (46 %)	25 (64 %)

[a]Antibiotic treatment was as follows: penicillin G 68 %; penicillin G and macrolide or clindamycin 13 %; intravenous cephalosporin 9 %; intravenous cephalosporin and macrolide or clindamycin 3 %; and oral amoxicillin or macrolide 7 %. Sputum induction was performed previous to administration of one intravenous antibiotic dosage in 9 children, after two intravenous antibiotic dosages in 21 children, after three intravenous antibiotic dosages in 3 children, and after oral antibiotic treatment in 6 children.
[b]In one child, *Streptococcus pneumoniae* was also isolated from blood culture.
[c]$p = 0.006$ by χ^2 test, comparison between children with preceding antibiotic treatment and those without.
[d]$p = 0.004$ by χ^2 test, comparison between children with preceding antibiotic treatment and those without.
[e]*Mycoplasma pneumoniae* was detected by PCR.
[f]*Streptococcus agalactie, Streptococcus pyogenes*, *Pseudomonas* like gram-negative rod.
[g] PCR for human bocavirus and human metapneumovirus was performed retrospectively and the results are not included in study II.

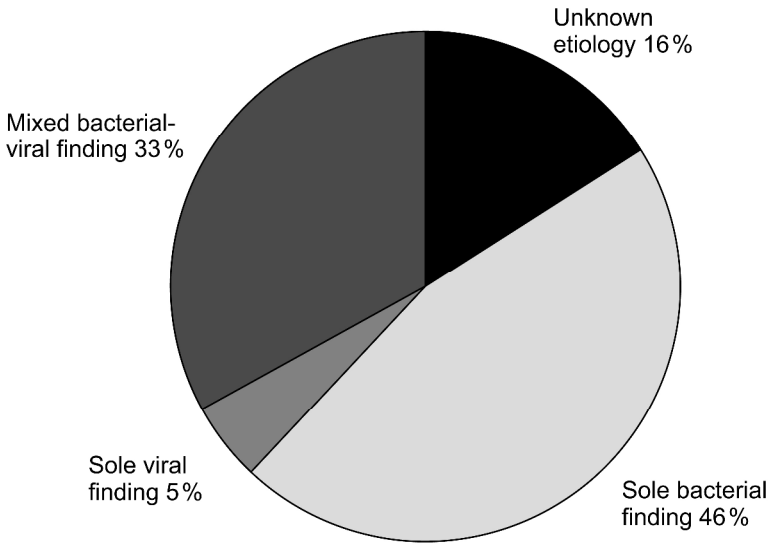

Figure 8. The proportion of bacterial, viral, and mixed bacterial-viral findings in 76 children with community-acquired pneumonia.

Children with *S. pneumoniae* in sputum had higher WBC counts on admission than those without *S. pneumoniae* in sputum (means ± SD, 21.7 ± 8.2 vs. 15.3 ± 8.1; $p < 0.001$). They also had higher CRP levels on admission (means ± SD, 161 ± 105 vs. 115 ± 96) but this difference was not statistically significant (Table 8). Typical findings for pneumococcal pneumonia i.e. high body temperature (≥39.0 °C), leukocytosis (WBC count ≥ 15.0 x 10^9/L), and high CRP level (CRP ≥ 60 mg/L) were found in 66 % of the study children with *S. pneumoniae*. Children with concomitant rhinovirus/ pneumococcal infection had the highest mean WBC counts and mean CRP levels on admission (Table 8).

Treatment failure (fever ≥ 38.0°C lasting for ≥ 48 hours) was recorded in 6 (8 %) of the 76 children. A potential causative agent/s of pneumonia was identified in four children: *S. aureus* and *M. pneumoniae* in one; *S. aureus* and RSV in one; *H. influenzae* in one; and *H. influenzae* and *M. catarrhalis* in one child.

PCR for HBoV and hMPV in induced sputum was performed retrospectively in autumn 2007. HBoV was found by PCR in 14 (18 %) children, and hMPV in 10 (13 %) children. When these findings were taken into account, a possible microbiologic etiology was found in 91 % of the 76 children with pneumonia and evidence of viral infection was detected in 55 % of the patients.

Table 8. Clinical findings in 76 children with pneumonia according to the etiologic agent in sputum.

Finding	Any etiology (n = 76)	*Streptococcus pneumoniae* (n = 35)[a]	*Haemophilus influenzae* (n = 22)[b]	*Moraxella catarrhalis* (n = 21)[c]	*Staphylococcus aureus* (n = 9)[d]	Rhinovirus (n = 22)[e]	Rhinovirus and *Streptococcus pneumoniae* (n=11)	Rhinovirus without *Streptococcus pneumoniae* (n=11)
Age (year), mean ± SD	4.7 ± 3.9	5.1 ± 3.8	3.7 ± 2.5	3.2 ± 2.0	7.1 ± 4.1	3.8 ± 3.6	4.8 ± 4.8	2.8 ± 1.7
WBC (10^9/L), mean ± SD	18.2 ± 8.7	21.7 ± 8.2[f]	14.1 ± 8.6[f]	21.1 ± 8.8	16.5 ± 8.6	21.3 ± 8.1[g]	24.2 ± 7.9[g]	18.3 ± 7.4[h]
CRP (mg/L), mean ± SD	137 ± 102	161 ± 105	109 ± 65	138 ± 116	130 ± 71	143 ± 128	181 ± 141	106 ± 107
Alveolar infiltrates, (%)	86	94	86	86	100	77	91	64
Interstitial infiltrates, (%)	12	9	23	14	0	27	9	45[h]
Pleural fluid, (%)	11	14	9	10	22	9	9	9
Dense infiltration, (%)	38	46	18[g]	33	33	32	45	18
Duration of fever after beginning of treatment (hour), mean ± SD	15 ± 16	10 ± 7	17 ± 16	13 ± 13	19 ± 22	13 ± 9	11 ± 7	14 ± 12

[a] *Streptococcus pneumoniae* as sole bacterium in 17 children.
[b] *Haemophilus influenzae* as sole bacterium in 9 children.
[c] *Moraxella catarrhalis* as sole bacterium in 4 children.
[d] *Staphylococcus aureus* as sole bacterium in 1 child.
[e] Rhinovirus alone in 4 children.
[f] $p < 0.001$, [g] $p < 0.05$ comparisons between children with the particular aetiological agent in question and children without the particular aetiological agent in question by χ^2 test or by Kruskal-Wallis test.
[h] $p < 0.05$, the Cochran-Mantel-Haenszel test was performed to study the influence of rhinovirus when the influence of *Streptococcus pneumoniae* was adjusted.

5.3 Frequency and characteristics of influenza pneumonia

Pneumonia was detected in 134 (14 %) of 936 children with influenza infection. The median age of the children with influenza pneumonia was 2.2 years (interquartile range, 1.0-3.6 years). Fever (98 %), cough (84 %), and rhinorrhea (65 %) were the most frequently reported symptoms (Figure 9). The fever was high (≥ 39.0 ºC) in 90 % of the children. Classic symptoms of influenza, i.e. headache and myalgia, were rare, also among those old enough (> 3 years of age) to describe their symptoms. Typical pneumonic crackles on lung auscultation were heard in 22 % of the children, and decreased breath sounds in 10 %. Normal auscultation findings were obtained in 32 % of the children, and 47 % had no symptoms or signs suggestive of pneumonia, i.e. dyspnea, tachypnea, decreased breath sounds or crackles on lung auscultation.

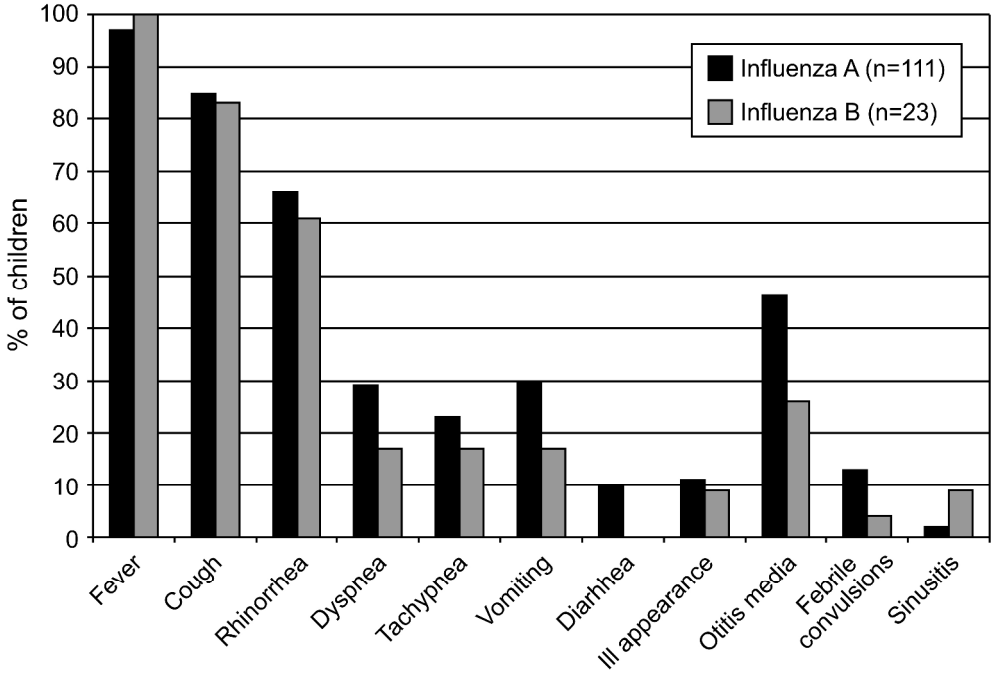

Figure 9. Clinical findings at presentation in 134 children with influenza pneumonia.

At presentation, the WBC count was <15 x 10^9/l in 89 % and serum CRP concentration <80 mg/l in 85 % of the children. One-half of the children had both WBC counts <15 x 10^9/l and CRP concentrations <20 mg/l at presentation. Leukopenia was detected in 10 % of the children. On the chest radiograph, one-half of the children had solely interstitial infiltrates, one-quarter solely alveolar, and one-quarter both alveolar and interstitial infiltrates. Clear consolidation was seen in 42 % of the children with alveolar infiltrates on the chest radiograph.

Three children had a laboratory-documented concomitant bacterial infection (*Pseudomonas Maltophilia* in one and *S. aureus* in two). Because bacterial pneumonia

often remains microbiologically undetected, the incidence of surrogate markers of bacterial complications was analyzed. The following findings were considered suggestive of bacterial infection: CRP >80 mg/l, WBC count >15 x 10^9/l and alveolar consolidation on the chest radiograph. All three criteria were met in four children and two or three of these criteria were met in 19 % of the children. Laboratory-documented double viral infection was documented in nine children.

In total, 84 % of the study children received antibiotic treatment during their influenza pneumonia episode. The hospitalization rate among children with influenza pneumonia was 68 % and the median duration of fever (temperature, ≥ 37.5 ºC) in hospitalized children was 30 h (range, 6-198 h). No difference was seen in the duration of fever between children who received antibiotic treatment and those who did not. Complicated pneumonias were rare, and mortality was low (0.7 %).

5.4 Clinical predictors of parapneumonic empyema

The incidence of empyema among hospitalized children with community-acquired pneumonia increased significantly during the study period, from the rate of 0.5% detected between 1991 and 1998 to 3.3% detected between 1999 and 2006 (Figure 10).

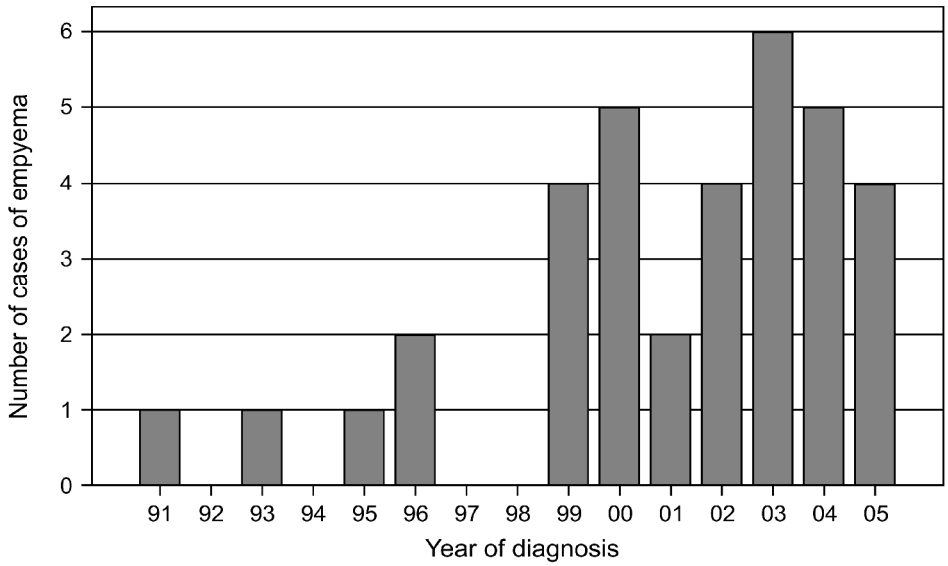

Figure 10. Annual numbers of cases of empyema during the study period (Figure from study IV).

Variables that were significantly associated with empyema were prolonged duration of fever, antibiotic treatment and breathing difficulty before admission, and tachypnea, decreased breath sounds on lung auscultation, pain on abdominal palpation, and low blood oxygen saturation levels on admission (Table 9). In the multivariate logistic regression analysis of clinical variables of empyema, tachypnea (p = 0.010), pain on

abdominal palpation (p = 0.019), and history of prolonged fever (p = 0.017) were found to be independently significant variables. CRP level of ≥ 120 mg/L on admission was also associated with empyema (89 % vs. 68 %; p = 0.024). At the initial evaluation, 35 % of the children with empyema were considered to have uncomplicated pneumonia on admission.

Table 9. Patient characteristics and clinical findings in children with empyema and in children with pneumonia without empyema on admission (Table from study IV).

Characteristic	Empyema (n = 37)	Pneumonia without empyema (n = 37)	p-value
Age (year); median (range)	5.3 (0.1–16.3)	4.3 (1.0–14.8)	0.893
Boys/girls (%)	41/59	41/59	1.000
Underlying illness (%)	16	32	0.104
History before admission			
Duration of fever (day); median (range)	5.0 (1–13)	3.0 (1–7)	<0.001
Antibiotic treatment received (%)	43	14	0.005
Macrolide treatment received (%)	30	8	0.018
Betalactam treatment received (%)	22	5	0.041
Clinical findings (%)			
Fever	100	100	1.000
Cough	89	89	1.000
Rhinorrhea	57	70	0.227
Chest pain	30	27	0.797
Breathing difficulty (according to parental report)	49	19	0.007
Dyspnea	54	32	0.060
Tachypnea	78[a]	36[b]	0.002
Decreased breath sounds on auscultation	60	27	0.005
Pneumonic crackles on auscultation	16	19	0.760
Pain on abdominal palpation	22	3	0.013
Oxygen saturation (%); median (range)	94 (85–99)[c]	96 (91–99)[d]	0.036

[a]Data available on 27 children
[b]Data available on 28 children
[c]Data available on 29 children
[d]Data available on 28 children

During hospitalization, prolonged fever (Figure 11) and persistence of high serum CRP levels were associated with empyema. The duration of fever (temperature, ≥38.0 ºC) in hospital in children with empyema was 7.0 days compared with 0.7 days in those with uncomplicated pneumonia (P <0.001). Sensitivity and specificity of the CRP level of ≥ 120 mg/L 48 hours after admission for detecting empyema was 86 % and 63 %, respectively.

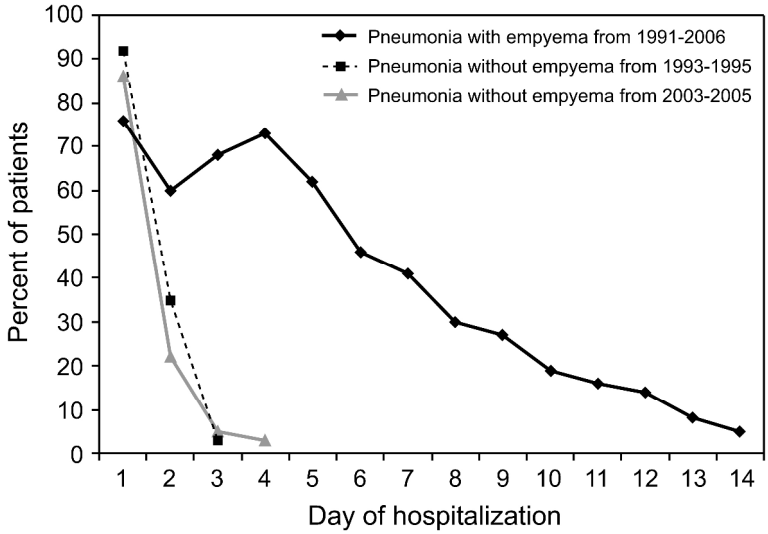

Figure 11. Fever kinetics in children with empyema (n=37) and in those with dense alveolar pneumonia without empyema (n=37 in both groups) during hospitalization. Percentage of febrile patients (≥38.0ºC) after onset of antibiotics (Figure from study IV).

6. DISCUSSION

6.1 Microbiologic diagnosis of pneumonia

Although the clinical entity of pneumonia (fever, cough, thoracic pain, and sputum production) was described by Hippocrates already in 400 B.C., and the first bacteria (*S. pneumoniae, Klebsiella pneumoniae*) as causative agents of pneumonia were identified in the 1880s, much is yet to be discovered in the field of pneumonia in the 21st century. Establishment of microbiologic diagnosis of pneumonia in clinical practice remains one of the major challenges. A concern about the decline in microbial studies and the quality and yield of microbiology done in patients with pulmonary infection has been expressed among clinicians treating adult patients (Bartlett, 2004; Musher, 2005). The increasing incidence of bacterial antibiotic resistance and the increasing number of complicated diseases accentuates the importance of pathogen identification also in the child population, although most children recover uneventfully with empiric antibiotic therapy.

At present, the available microbiologic methods rarely identify the causative agent of pneumonia in children in clinical practise as blood cultures are positive only in < 10 % of the cases (Turner et al., 1987; Claesson et al., 1989; Ruuskanen et al., 1992; Gendrel et al., 1997; Wubbel et al., 1999; Clements et al., 2000; Juven et al., 2000; Tsolia et al., 2004; Don et al., 2005), pleural fluid and lung aspirate samples are rarely available, and sputum samples can not be obtained from small children. Serologic methods, except for the *M. pneumoniae* IgM test, are not suitable for routine diagnostics and pneumococcal antigen detection in serum and urine is hampered by the false-positive findings caused by nasopharyngeal carriage (Dominguez et al., 2003; Esposito et al., 2004; Korppi et al., 2008b). In addition, although viral infections can be successfully detected by antigen detection or PCR in nasopharyngeal aspirate samples, the identification of a viral agent in the nasopharynx does not exclude the presence of concomitant bacterial infection nor offer direct evidence of the etiology of pneumonia.

The basis of the microbiologic studies performed in the present thesis was strictly clinical. As the causative agent of pneumonia remains frequently undetected, we aimed to improve the microbiologic diagnosis of childhood pneumonia in every day clinical practise by investigating the accuracy and usefulness of pneumolysin-targeted real-time PCR in whole blood and pleural fluid samples, and the clinical utility of induced sputum analysis in the etiologic diagnosis of pneumonia.

6.1.1 Pneumolysin-targeted real-time PCR

The introduction of PCR in the mid 1980s revolutionized the field of molecular science (Saiki et al., 1985; Mullis and Faloona, 1987). Kary Mullis was awarded the Nobel Prize in Chemistry in 1993 for this invention. In PCR, the target DNA is amplified exponentially by a thermostable DNA polymerase enzyme through multiple cycles of DNA denaturation, primer hybridisation, and extension (Yang and Rothman, 2004).

Consequently, the PCR method has the theoretical potential to generate billions of copies of target DNA from a single copy in less than one hour (Yang and Rothman, 2004). The clinical applications of PCR have rapidly expanded, and currently the PCR technique is employed in cancer diagnostics as well as in the detection of genetic diseases, and even in criminal investigation.

In the field of infectious diseases, PCR was greeted with great enthusiasm. The greatest benefits have been achieved in viral diagnostics, in which the quantitative PCR has also allowed the monitoring of treatment response (Yang and Rothman, 2004; Espy et al., 2006; Sloots et al., 2008). In the diagnostics of bacterial infections, however, PCR has not been able to fully fulfil the expectations placed on its great sensitivity. Moreover, the susceptibility to contamination and high costs have limited the widespread use of PCR (Chan and Morris, 2007). Currently, the PCR assay for *Chlamydia trachomatis* is practically the only bacterial PCR method that has replaced traditional methods in clinical practice (Puolakkainen et al., 1998). Another PCR-based diagnostic assay which has gained widespread acceptance is that for *Mycobacterium tuberculosis* (D'Amato et al., 1995, Piersimoni et al., 1997). However, although it significantly shortens the time to diagnosis compared with culture, it is officially approved to be used only in adjunct to the conventional smear and culture (Yang and Rothman, 2004; Chan and Morris, 2007). Apart from the detection of *C. trachomatis* and *M. tuberculosis* by PCR only a limited number of PCR assays for the diagnosis of bacterial infections have been approved by the US Food and Drug Administration (Yang and Rothman, 2004). Other PCR methods, including widely used PCR assays for *Bordetella pertussis*, *M. pneumoniae*, and *C. pneumoniae*, are in-house systems developed in research laboratories.

The first PCR method for the detection of *S. pneumoniae* was published by Rudolph et al. in 1993 (Rudolph et al., 1993). Since then, several methods based on the amplification of different targets, including pneumolysin and autolysin-encoding genes have been described (Salo et al., 1995; Dagan et al., 1998; Toikka et al., 1999a; Dominguez et al., 2001; Michelow et al., 2002; Murdoch et al., 2003; Van Haeften et al., 2003). The most important PCR study in the field of microbiologic diagnosis of pneumonia in children has been the study by Michelow et al. published in 2002 (Michelow et al., 2002). This study showed a 100 % sensitivity of pneumolysin-based PCR in blood samples (whole blood, buffy coat or plasma) compared with culture in the detection of pneumococcal infection in children with a lower respiratory tract infection. The specificity among control subjects was 95 %. Overall, 44 % of the study children had a positive PCR result in either whole blood, buffy coat, or plasma samples. In other studies investigating the usefulness of PCR in blood samples in the diagnosis of invasive pneumococcal infections in children, the sensitivity has ranged from 50 % to 100%, and specificity from 83 % to 100 % (Zhang et al., 1995; Dagan et al., 1998; Toikka et al., 1999a).

In our study, the results of pneumolysin-targeted PCR in blood samples were disappointing. The PCR failed to detect *S. pneumoniae* in whole blood samples from three children with positive blood culture results, and, in total, *S. pneumoniae* was detected by PCR in whole blood only in two of the 52 children with pneumonia,

empyema or suspected pneumococcemia. The reason for the low clinical sensitivity of pneumolysin PCR from whole blood samples in our study is not clear. The *in vitro* sensitivity of the 2 genome equivalents of pneumococcal DNA/reaction detected in our study was similar or superior to earlier reported sensitivities (Rudolph et al., 1993; Toikka et al., 1999a; Dominguez et al., 2001; Michelow et al., 2002), and the *in vitro* sensitivity and specificity of 100 % when tested with isolated strains could not be any better. The pneumolysin primers, DNA extraction method, and LightCycler technology employed in this study have been successfully used in studies of PCR used for the diagnosis of other pneumococcal infections (Saukkoriipi et al., 2002; Van Haeften et al., 2003). Furthermore, the DNA extracted from whole blood samples can be considered good quality because no significant delay occurred in DNA extraction and no inhibitory agents were detected in the samples.

The probable explanation for the low clinical sensitivity of PCR from whole blood samples simply seems to be the low bacterial concentrations in the blood in our patients. Sullivan et al. (Sullivan et al., 1982) as well Bell et al. (Bell et al., 1985) found that the magnitude of *S. pneumoniae* bacteremia correlates with the severity of the infection. Children with pneumococcemia or pneumococcal pneumonia have low levels of bacteremia, usually <10 CFU/ml, whereas children with meningitis often have >100 CFU/ml. As only a small part of the original sample can be exploited in the PCR analysis, it is possible that with so few bacteria/ml present, no bacteria are present in the final PCR reaction. In our study, children with pneumonia or suspected pneumococcemia were referred to the hospital in a very early phase of the illness, were generally in good condition and recovered rapidly and uneventfully. The severity of the infection and the amount of bacteremia have, therefore, probably been very low in our study children. In the study by Michelow et al. (Michelow et al., 2002), in contrast, all study children were high-risk hospitalized children, indicating that the magnitude of bacteremia in these children was probably higher. The use of different blood fractions, i.e. buffy coat, serum, and plasma, instead of whole blood as samples for PCR would probably have improved the sensitivity of our PCR, as Toikka et al. (Toikka et al., 1999a) and Michelow et al. (Michelow et al., 2002) have previously shown in their PCR studies. However, the use of several blood fractions is laborious and expensive, and therefore is not feasible for clinical diagnostics. The small number of blood samples studied in our study is also a limitation. With more extensive patient material, more bacteremic diseases could have been detected, which would have allowed for more reliable sensitivity analysis.

In children with parapneumonic empyema, the PCR assay in pleural fluid significantly improved the diagnostic yield of pneumococcus compared with traditional culture. *S. pneumoniae* was detected by PCR in the pleural fluid of 75 % of children with empyema, increasing the detection rate of pneumococcus almost tenfold to that of pleural fluid culture. This finding is in agreement with the results of Eastham et al. (Eastham et al., 2004) who found evidence of *S. pneumoniae* infection by pneumolysin PCR in over 70 % of culture-negative children with empyema. Whether all positive PCR findings represent true positive findings is difficult to determine, because collection of pleural fluid samples from healthy children for specificity studies is not

possible. However, considering that all 39 nonpneumococcal bacterial organisms as well as all 19 whole blood samples from children with clinically nonpneumococcal infection tested were negative by PCR, specificity was clearly no problem in our study. In addition, we used melting curve analysis to confirm the positive pneumococcal findings. This method has proven to be useful in differentiating *S. pneumoniae* from other α-hemolytic streptococci that may also contain the pneumolysin gene and cause false-positive findings in pneumolysin-based PCR (Kaijalainen et al., 2005). The most probable reason for the failure of culture to detect *S. pneumoniae* in PCR positive samples was the prior antibiotic treatment that all study children with empyema had received before collection of pleural fluid samples.

The better clinical sensitivity of PCR in pleural fluid compared with whole blood samples is explained by two factors. First, the amount of bacteria in pleural fluid is most likely to be higher than the amount of bacteria in blood. Secondly, bacteria present in the original pleural fluid sample were concentrated by centrifugation before DNA extraction and PCR analysis, whereas blood samples were not. Consequently, the DNA extracted from pleural fluid included all bacteria present in the original sample (volume 0.5–3 ml), whereas DNA extracted from blood included only bacteria present in the 0.2 ml volume that were used for the DNA extraction. To improve the clinical sensitivity of PCR in whole blood samples, the bacteria present in the original sample should be concentrated without concentrating the excessive human DNA present in blood. One way to avoid the concentration of human DNA is to lyse erythrocytes and white blood cells before the centrifugation. In a recent study by Kee et al., the PCR-based assay for the detection of *S. pneumoniae* in whole blood was successfully improved by increasing the original blood volume used in DNA extraction by centrifuging the sample after the lysis of erythrocytes (Kee et al., 2008). However, the level of bacteremia in children with pneumonia may be too low and transient for blood samples to be considered optimal diagnostic samples in pneumonia at all.

In conclusion, our data indicate that pneumolysin-targeted real-time PCR in pleural fluid is a valuable method for the etiologic diagnosis of pneumococcal empyema in children. As pleural fluid samples are often collected after administration of antibiotic treatment, PCR is more sensitive method than culture which can detect only viable organisms. The ease and rapidity of the LightCycler technology make real-time PCR an applicable tool for routine diagnostics. In the evaluation of blood samples, however, blood culture is the superior method for the diagnosis of bacteremic pneumococcal disease.

6.1.2 Induced sputum in the diagnosis of CAP in children

In the developed countries, the majority of pneumonia cases in children are probably nonbacteremic (Turner et al., 1987; Claesson et al., 1989; Ruuskanen et al., 1992; Gendrel et al., 1997; Wubbel et al., 1999; Clements et al., 2000; Juven et al., 2000; Tsolia et al., 2004; Don et al., 2005). Thus, in most cases the causative agent of pneumonia can not be isolated from blood, not even with the most sensitive method. Sputum, bronchoalveolar lavage fluid, pleural fluid, transthoracic needle aspiration fluid, and lung biopsy are alternative diagnostic samples in pneumonia. Sputum is the

only noninvasive sample, and therefore the only sample suitable for routine diagnostics.

In adults with pneumonia, the routine use of sputum gram staining and culture is recommended for patients needing intensive care unit admission, for patients with failure of outpatient antibiotic therapy, and for patients with pleural effusion (Mandell et al., 2007). Factors that favour routine use of sputum gram staining and culture are that these methods are inexpensive, noninvasive, and easy to perform. In a recent study by Musher et al., a good-quality sputum specimen was available in 55 % of adult patients (Musher et al., 2004). The sensitivities of sputum gram staining and culture in detecting pneumococci in patients with bacteremic pneumococcal pneumonia with a good-quality sputum specimen without previous antibiotic treatment were good; 80 % and 93 %, respectively.

In children with pneumonia, routine sputum analysis has not been recommended because young children cannot produce adequate sputum samples, and in contrast to adults, the nasopharyngeal colonization of *S. pneumoniae* (19–38 %), *H. influenzae* (13–25 %), *M. catarrhalis* (22–39 %), and *S. aureus* (16–36 %) is common in healthy children (Korppi et al., 1992; Syrjänen et al., 2001; British Thoracic Society, 2002; Bogaert et al., 2004; Zemlickova et al., 2006). Sputum induction with hypertonic saline solution has proven useful in children in the detection of microbes normally not found in the nasopharynx such as *M. tuberculosis* and *Pneumocystis jirovecii* (Zar et al., 2000; Zar et al., 2005). Sputum induction has also been shown to be effective in obtaining sputum in adult patients who fail to expectorate a sample (Bandyopadhyay et al., 2000). The applicability of this procedure in children with pneumonia has not been properly studied. We performed a prospective study to investigate the usefulness of paired nasopharyngeal aspirate and induced sputum specimen analysis in the microbiologic diagnosis of CAP in children. The quality of the sputum samples was carefully analyzed and only samples rich in leukocytes and short of squamous epithelial cells were included into the final analysis. In addition, the nasopharyngeal contamination of sputum samples was minimized by cleaning the nasopharynx of the mucus prior to sputum collection.

Our study had three major findings. First, sputum induction in children with CAP was found to be safe and efficient, as it provided a good-quality sputum specimen in 82 % of the study children. In adults with pneumonia, the proportion of good-quality samples has ranged from 40 % to 70 % (Gleckmann et al., 1988; Bandyopadhyay et al., 2000; Roson et al., 2000; Ewig et al., 2002; Musher et al., 2004). Second, the bacteriologic and virologic yield of sputum specimens was good as a possible microbial etiology of pneumonia was identified in 91 % of the children with a valid sputum sample when HBoV and hMPV results were also included. Third, the quantification results of the paired sputum and nasopharyngeal aspirate specimens provided support for the notion that the majority of the bacteria and viruses found in sputum most probably originated from the lower airways.

The limitations of this study must also be noted. We can not provide unquestionable evidence that sputum specimens were truly representative samples from the lower

respiratory tract as lung aspirates were not collected. In addition, although only good-quality sputum samples were included in the final analysis, the possibility that sputum samples may have been contaminated with nasopharyngeal bacteria or viruses can not be excluded. However, in one-fourth of the cases, the bacterium was only found in sputum suggesting that in these cases induced sputum most probably identified the causative agent of pneumonia. Considering that blood culture provided the etiology only in 1 % of the children, induced sputum offered a huge improvement in the microbiologic diagnosis of pneumonia.

In conclusion, our observations provide evidence that induced sputum analysis could be useful in the microbiologic diagnosis of childhood pneumonia. In accordance with the recommendation for adults, we think that induced sputum analysis could be beneficial in children needing intensive care unit admission, in children with failure of first-line antibiotic therapy, and in children with pleural effusion (Mandell et al., 2007). However, our observations do not support routine use of induced sputum analysis for all children with CAP since most children were successfully treated with empirical antibiotic therapy, and children found the hypertonic saline inhalation and repeated nasopharyngeal aspirations unpleasant.

6.2 Etiology of pneumonia

Numerous studies have investigated the etiology of childhood CAP (Turner et al., 1987; Claesson et al., 1989; Ruuskanen et al., 1992; Gendrel et al., 1997; Heiskanen-Kosma et al., 1998; Wubbel et al., 1999; Clements et al., 2000; Juven et al., 2000; Tsolia et al., 2004; Don et al., 2005). However, to our knowledge, no study has reported the virologic or the bacteriologic yield of good-quality sputum specimens in children with pneumonia. Although our sputum findings can not be considered as definite evidence of the etiology of pneumonia due to the potential risk of nasopharyngeal contamination, the results provide interesting information on the causative agents of pneumonia.

S. pneumoniae was the most frequently isolated pathogen, detected in one-half of all study children, and in two-thirds of children without previous antibiotic treatment. In previous studies, the detection rate of pneumococcus has ranged from 7 % to 44 % (Claesson et al., 1989; Gendrel et al., 1997; Heiskanen-Kosma et al., 1998; Wubbel et al., 1999; Juven et al., 2000; Michelow et al., 2004a; Tsolia et al., 2004). Although the higher yield of *S. pneumoniae* in our study may partly be explained by nasopharyngeal bacterial contamination, it may also reflect the true incidence of pneumococcal pneumonia in children. In fact, the 50 % detection rate identified in our study is in agreement with the reported 53 % detection rate in lung aspirates in children with alveolar pneumonia (Vuori-Holopainen et al., 2002). It is of note that almost 90 % of our study children had alveolar pneumonia on the chest radiograph. The identification of pneumococcal etiology in children with pneumonia is unlikely to have influence on patient management in clinical practice as first-line antibiotic therapy usually covers pneumococcus. However, the information on the antibiotic resistance pattern of pneumococcus provided by the sputum culture can be of clinical importance.

An unexpected finding was that *S. aureus* was detected in good-quality sputum specimens in 12 % of the children, suggesting that the incidence of *S. aureus* pneumonia in developed countries may have previously been underestimated (Gendrel et al., 1997; Juven et al., 2000; Michelow et al., 2004a; Don et al., 2005). All but one of these *S. aureus* cases were found in induced sputum only. In addition, two children with *S. aureus* in sputum failed to respond to first line antibiotic therapy (penicillin G), suggesting that these findings may have had true clinical implications. Early detection of *S. aureus* is important since *S. aureus* is known to be able to cause severe and complicated pneumonias such as empyema and lung abscesses (Connor and Powell, 1985; Tan et al., 1995; Byington et al., 2002; Patradoon-Ho and Fitzgerald, 2007). Thus the timely detection of *S. aureus* by sputum gram staining would be of true clinical importance.

Rhinovirus was clearly the most common viral finding, detected by PCR in the sputum of 29 % of the children. This finding is in agreement with previous studies which have identified rhinovirus by PCR in nasal samples in 24 %– 45 % of children with pneumonia (Juven et al., 2000; Tsolia et al., 2004). The mere presence of rhinovirus in sputum, however, offers no direct evidence of the etiology of pneumonia as 82 % of the children with rhinovirus had evidence of concomitant bacterial infection. An interesting finding was that mixed rhinovirus/pneumococcal infection seemed to induce a more severe inflammation response than sole rhinovirus or sole pneumococcal infection which is in agreement with the recent results by Jennings et al. that mixed rhinovirus/pneumococcal infection is associated with severe disease in adults (Jennings et al., 2008).

The role of newly discovered viruses, HBoV and hMPV, in the etiology of pneumonia is not fully established. In our study, these new viruses accounted for up to 42 % of the all viral cases detected. HBoV was detected in sputum samples in 18 % of the children, and hMPV in 13 %. In previous studies, HBoV infection has been found in nasopharyngeal aspirate samples in 4.5 % of hospitalized patients with pneumonia (Fry et al., 2007) and the incidence of hMPV among children with pneumonia has ranged from 1 % to 5 % (Tsolia et al., 2004; Werno et al., 2004; Don et al., 2005; Lin et al., 2005). In accordance with previous studies, we found a high viral co-infection rate (64 %) in children with HBoV (Allander et al., 2007; Fry et al., 2007; Esposito et al., 2008). The viral co-infection rate in children with hMPV was 40 %. Whether the detection of these new respiratory viruses in children with pneumonia reflects a primary infection or persistence of the virus in the nasopharynx after a previous infection can not be determined. However, 4 of 10 hMPV cases, and 3 of 14 HBoV cases were only detected in sputum specimens suggesting that in these cases HBoV and hMPV clearly originated from the lower respiratory tract.

6.3 Viral pneumonia with special emphasis on influenza pneumonia

After the advent of improved diagnostic methods the proportion of viral etiology in children with pneumonia has increased substantially (Juven et al., 2000; Tsolia et al., 2004; Nascimento-Carvalho, in press). In recent studies, viruses have been identified in

up to 80 % of infants and children with pneumonia (Juven et al., 2000; Tsolia et al., 2004; Nascimento-Carvalho, in press). In our sputum study, evidence of viral infection was identified in 55 % of the children with pneumonia when HBoV and hMPV results were also included. The precise role of viruses in the development of pneumonia remains, however, undetermined. We aimed to investigate the frequency and clinical profile of influenza pneumonia in children, and to estimate the magnitude of bacterial co-infections in viral pneumonia. Influenza pneumonia was chosen as a research subject as it was considered to be the best example of classic viral pneumonia.

Influenza is associated with high morbidity in children. The reported attack rates during annual epidemics vary greatly, with an annual average of 20 % (Fox et al., 1982; Neuzil et al., 2002; Heikkinen et al., 2004). Among children with pneumonia, the detection rate of the influenza virus has ranged from 4 % to 22 % (Juven et al., 2000; Michelow et al., 2004a; Tsolia et al., 2004; Don et al., 2005). Although overall mortality due to influenza in the child population is low, life-threatening diseases and deaths from influenza pneumonia in children have been reported (Connor and Powell, 1985; O'Brien et al., 2000; Centers for Disease Control and Prevention, 2004b). In our study, influenza pneumonia was found to be a relatively infrequent and a benign illness in children. Unlike adults, most children with influenza pneumonia recovered uneventfully, and mortality was low. The most common disease features of influenza pneumonia recorded in our study were fever, cough, and rhinorrhea. Classic symptoms of influenza, i.e. headache and myalgia, were rare. In addition, half of the study children presented with no clinical findings suggesting pneumonia. Differentiation of influenza pneumonia from uncomplicated influenza infection and from bacterial pneumonia on the basis clinical findings alone is, therefore, difficult.

Three types of pneumonia associated with influenza virus infection are well recognized in adults: primary influenza virus pneumonia, coexisting influenza virus and bacterial pneumonia, and secondary bacterial pneumonia (Louria et al., 1959; Cox and Subbarao, 1999). The greatest disease severity and mortality has been associated with the synergism between the influenza virus and bacteria (Connor and Powell, 1985; O'Brien et al., 2000; McCullers and Rehg, 2002; McAuley et al., 2007; Seki et al., 2007). The ability of mixed infections to induce a more severe inflammation and clinical disease than sole viral or sole bacterial infections has also been detected among other viral infections (Juven et al., 2004; Jennings et al., 2008). In our study, the majority of children with influenza pneumonia were considered to have primary viral pneumonia as one-half of the study children had both a WBC count of $<15 \times 10^9$/l and a CRP concentration of <20 mg/l at presentation. The exact role of coexisting or secondary bacterial infection in our study was difficult to establish as only 2 % of the children had a laboratory-documented concomitant bacterial infection. Because bacterial pneumonia often remains microbiologically undetected, we analyzed the incidence of surrogate markers of bacterial complications. A high CRP level (>80 mg/l), a high WBC count ($>15 \times 10^9$/l), and the presence of alveolar infiltrates on the chest radiograph were considered suggestive of bacterial infection. Because none of the findings are specific for bacterial infection, at least two findings were required for the diagnosis of a probable bacterial infection. According to these criteria, one-fifth of the

study children were considered to have concomitant bacterial pneumonia. This is likely to be an underestimation of the true incidence of concomitant bacterial pneumonia as preceding antibiotic treatment may have influenced the laboratory and radiologic findings in some children, and secondary bacterial complications occurring when the influenza virus is no longer secreted remained undetected in our study. Previous studies that have employed serologic diagnostic methods for viral and bacterial pneumonia, have found evidence of concomitant bacterial infection in more than half of the children with influenza infection (Juven et al., 2000; Don et al., 2005).

A clinically relevant question is whether all children with pneumonia, including children with viral pneumonia, should receive antibiotic therapy. In our study, 84 % of the children with laboratory-documented influenza infection received antibiotic treatment during the influenza pneumonia episode, although the majority of the pneumonias seemed to be of sole viral origin. No difference was observed in the duration of fever between children who received antibiotic treatment and those who did not.

In study II, however, only a minority of children with pneumonia were found to have a sole viral finding in a good-quality sputum sample. The high bacterial yield in sputum may be partly explained by the nasopharyngeal bacterial contamination as already previously discussed, but it may also reflect the true high proportion of bacterial etiology in hospitalized children with pneumonia. The high rate of undetected bacterial etiology could help explain the unexpected finding of Juven et al. that children with sole viral pneumonia showed a similar response to antibiotic treatment to those with sole bacterial pneumonia (Juven et al., 2004).

Our results do not argue for or against the necessity of antimicrobial therapy in children with viral pneumonia. However, in the light of the high prevalence of mixed viral-bacterial infections in children with pneumonia (Juven et al., 2000; Tsolia et al., 2004; Don et al., 2005), it is obvious that identification of viral infection does not exclude the possibility of bacterial infection. While the greatest antibiotic consumption is reported among children with upper respiratory tract infections and not among children with pneumonia, there is no need to withhold antibiotic therapy in children with viral pneumonia by appealing to increasing antibiotic resistance. Thus, as far as there is no reliable method to exclude the bacterial infection, it seems rational to treat all children with radiologically verified pneumonia with antibiotics.

A specific antiviral treatment is available for influenza pneumonia. Oseltamivir treatment has been shown to be associated with reduced risk for pneumonia in children with clinically diagnosed influenza infection (Barr et al., 2007). In a recent study by Sugaya et al., zanamivir was found to be equivalent to oseltamivir in reducing febrile period of influenza infection in children over 4 years of age (Sugaya et al., 2008), but the effectiveness of zanamivir in preventing pneumonia in children with influenza has not yeat been studied. The greatest benefit from anti-influenza drugs is gained if therapy is started early, within 48 hours after onset of symptoms (Harper et al., 2004). In our study, however, only one-quarter of the children presented at hospital within 48 hours after the onset of symptoms.

6.4 Recovery and complications

In agreement with previous reports (Ruuskanen et al., 1992; Gendrel et al., 1997; Harris et al., 1998; Juven et al., 2004; Bradley et al., 2007; Clark et al., 2007b) the majority of our study children with CAP improved well. Only one child of the total 314 children with pneumonia reviewed died from severe pneumonia. The rapid recovery rate of uncomplicated pediatric pneumonia reported by several previous studies (Ruuskanen et al., 1992; Dagan, 1993, Toikka et al., 1999b; Juven et al., 2004) was also confirmed by our study. It took on average of 14 (study IV) to 15 hours (study II) for children with uncomplicated pneumonia to become afebrile. The treatment failure rate detected in study II was 8 %. The most frequently found organisms in children with treatment failure were *S. aureus* (33 %), *H. influenzae* (33 %), and HBoV (67 %). All but one of these cases were mixed infections, supporting the previous findings that mixed infections are associated with a more severe disease than sole viral or sole bacterial infection (Connor and Powell, 1985; Thomas et al., 2003; Juven et al., 2004; Jennings et al., 2008).

Although the overall incidence of complicated disease remained low, a significant increase in the incidence of parapneumonic empyema was observed during the study period. The incidence increased from the rate of 0.5 % detected between 1991 and 1998 to the rate of 3.3 % detected between 1999 and 2006. A corresponding increase in the incidence of parapneumonic empyema has been reported in Europe, Asia and USA (Rees et al., 1997; Byington et al., 2002; Tan et al., 2002; Eastham et al., 2004; Hsieh et al., 2004; Spencer et al., 2006; Roxburgh et al., 2008). As only a few studies have been conducted on the prediction of parapneumonic empyema in children with pneumonia (Byington et al., 2002; Tan et al., 2002; Hsieh et al., 2004) we performed a retrospective study to describe the clinical characteristics and clinical predictors for parapneumonic empyema in children.

Our data demonstrated that early recognition of developing empyema in young children is a challenging task. At the initial evaluation, one-third of study children with empyema were considered to have uncomplicated pneumonia. The pleural fluid and radiologic findings suggestive of empyema that were already present on the chest radiograph on admission remained frequently undetected, thus delaying the diagnosis of empyema. The increasing awareness of empyema among radiologists and pediatricians will probably improve the early detection of empyema in children. Clinical predictors for empyema are, however, still needed.

A history of prolonged fever, tachypnea, and pain on abdominal palpation on admission were found to be independently significant predictors for empyema in children. In addition, antibiotic treatment and breathing difficulty before admission and high serum CRP level, decreased breath sounds on lung auscultation and low blood oxygen saturation levels on admission were found to be associated with empyema. As all these clinical features can also be detected in uncomplicated pneumonias, these findings can not unequivocally be used to either diagnose or exclude empyema. However, empyema should always be borne in mind when children with pneumonia present with these clinical predictors for empyema.

In hospital, prolonged duration of fever and persistence of high CRP levels were associated with empyema. CRP levels ≥ 120 mg/L 48 hours after admission were found to be significantly more frequent among children with empyema than among those with uncomplicated pneumonia. This observation suggests that CRP can be used as an indicator of the response to treatment of pneumonia and empyema. Our observations are also in agreement with the previous recommendations that the treatment should be re-evaluated, if there is no improvement after 48 hours on treatment (British Thoracic Society, 2002).

In conclusion, children with pneumonia presenting with a history of prolonged fever, high CRP level, tachypnea, and pain on abdominal palpation are at risk for having or developing parapneumonic empyema. Consequently, repeat chest radiograph and ultrasound imaging in addition to thorough interpretation of the initial chest radiograph are recommended in children presenting with these clinical predictors for empyema and in children with persistent fever and high CRP levels during hospitalization.

SUMMARY AND CONCLUSIONS

In study I, pneumolysin-targeted real-time PCR in pleural fluid was found to be superior to culture in the microbiologic diagnosis of pneumococcal empyema. The ease and rapidity of the real-time technology used make this PCR method an applicable tool for routine diagnostics. In the evaluation of blood samples, however, blood culture remained superior to PCR for the diagnosis of bacteremic pneumococcal pneumonia.

In study II, sputum induction provided good-quality sputum specimens with high microbiologic yield in children with pneumonia. The possible causative agent of pneumonia was identified in 91 % of the cases. In clinical practice, induced sputum analysis can be recommended for children with pneumonia with treatment failure of first-line antibiotic therapy and for children with complicated disease. In these children, microbiologic diagnosis of pneumonia is necessary and sputum analysis can provide valuable information on the causative agent/s of pneumonia. However, our observations do not support the routine use of induced sputum analysis for all children with pneumonia since children found the sputum induction procedure unpleasant and the possibility of nasopharyngeal bacterial contamination of sputum specimens can not be excluded.

Study III showed that influenza pneumonia in children is a relatively infrequent and benign illness. Unlike adults, most children with influenza pneumonia recovered uneventfully, and mortality was low. The majority of the children with influenza pneumonia had normal WBC counts and low serum CRP concentrations at presentation suggesting that most cases were of sole viral origin. The high proportion of mixed viral-bacterial infections in children with pneumonia detected in study II, however, suggests that it would be rational to treat all hospitalized children with radiologically verified pneumonia with antibiotics.

Study IV demonstrated that early recognition of developing empyema is a challenging task in clinical settings. Timely detection and treatment of parapneumonic pleural effusion is essential as appropriate treatment can prevent the development of empyema and its progression. In our study, children presenting with a history of prolonged duration of fever, high CRP level, tachypnea, and pain on abdominal palpation were found to be at risk for having or developing empyema. During hospitalization, persistent high fever and high CRP levels were found to be associated with empyema. Consequently, repeat chest radiograph and ultrasound imaging are recommended in children presenting with clinical predictors for empyema.

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