TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 868

MEDICA - ODONTOLOGICA

COMMUNITY-ACQUIRED PNEUMONIA IN ADULT PATIENTS WITH SPECIAL REFERENCE TO RAPID METHODS FOR ETIOLOGICAL DIAGNOSIS

by

Ulla Hohenthal

TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2009

From the Department of Medicine
University of Turku, Finland

Supervised by

Professor Pirkko Kotilainen, MD, PhD
Department of Medicine, Division of Infectious Diseases
University of Turku
Turku, Finland

and

Professor (emeritus) Jukka Nikoskelainen, MD, PhD
Department of Medicine, Division of Infectious Diseases
University of Turku
Turku, Finland

Reviewed by

Docent Asko Järvinen, MD, PhD
Department of Medicine, Division of Infectious Diseases
University of Helsinki
Helsinki, Finland

and

Docent Markku Koskela, MD, PhD
Department of Microbiology
University of Oulu
Oulu, Finland

Dissertation opponent

Professor Ville Valtonen, MD, PhD
Department of Medicine, Division of Infectious Diseases
University of Helsinki
Helsinki, Finland

ISBN 978-951-29-4045-5 (PRINT)
ISBN 978-951-29-4046-2 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2009

to Ilona

ABSTRACT

Ulla Hohenthal

Community-acquired pneumonia in adult patients with special reference to rapid methods for etiological diagnosis

From the Department of Medicine, University of Turku, Finland
Annales Universitatis Turkuensis
Painosalama Oy – Turku, Finland 2009

Background. Community-acquired pneumonia (CAP) is a serious cause of morbidity. It affects 60 000 adults per year in Finland. Despite advances in the management of CAP, mortality ranges from 6% to 15%. Identifying the cause of a lower respiratory tract infection remains a challenge.

Aims. The aim was to investigate the etiology of CAP in adult patients treated at the Turku University Hospital, Finland. The purpose was to evaluate the therapeutic implications of various rapid diagnostic techniques.

Methods. In Studies I and III there were 384 consecutive adult patients admitted between December 1999 and December 2004 for CAP in the Department of Infectious Diseases, Turku University Hospital. In Study I, PCR and antigen detection techniques were used to identify pathogens in addition to standard microbiological methods. Study II included a subset of 231 patients who had their throat swab specimens examined also for the presence of rhinoviruses and enteroviruses. In Study III, plasma C-reactive protein (CRP) concentrations were measured during the first five days of hospitalization in 384 adult patients with CAP. The data were evaluated using comprehensive statistical analyses to assess the value of CRP in estimating the severity and complications of CAP. In Study IV, leukocyte receptor expression was examined on admission from 68 patients with CAP. In Study V, the results of 71 bronchoalveolar lavage (BAL) samples of patients treated for CAP in the Department of Medicine from 1996 throughout 2000 were retrospectively analyzed to assess the value of BAL in the etiological diagnosis of the disease.

Results. 230 microbial agents in 209 patients were identified, with 135 (58.7%) identified by antigen detection or PCR tests. Of these 135 microbial agents, 95 (70.4%) were identified only by these rapid methods. Respiratory viral antigen detection was positive in 11.1% with the highest diagnostic yield (20.3%) in patients with severe pneumonia. In the subset of 231 patients, the total proportion of viruses causing CAP was 20%. Among the 47 patients with viruses, a concomitant bacterial infection was detected in 17 (36%) patients. Of the 231 patients, 19 (8.2%) were positive for respiratory picornaviruses by RT-PCR. Significantly higher CRP values on admission were detected in Pneumonia Severity Index class III-V compared to class I-II ($p < 0.001$). A CRP level of >100 mg/l on day 4 after admission was suggestive of treatment failure or development of complications. The mean expression of complement receptor (CR1) on neutrophils was significantly higher in patients with pneumococcal pneumonia than influenza A pneumonia. Among the 71 BAL samples, only one (1.3%) quantitative bacterial culture was diagnostic for CAP. New diagnostic methods provided the etiological diagnosis infrequently, increasing the total diagnostic yield for CAP to 9.8% in BAL samples.

Conclusions. Besides yielding the etiological diagnosis rapidly, new methods add to the total diagnostic yield in CAP. The diagnostic yield of rapid methods differs by severity of CAP. Respiratory viruses are frequently detected among patients with CAP. Patients with virus-associated CAP are often severely ill. CRP may be used as an additional tool to assess disease severity in patients with CAP. In particular, CRP is valuable as a predictor of the development of complications during the treatment of CAP. The determination of the expression of CR1 may be of value as an additional rapid tool differentiating between bacterial or viral CAP. The value of BAL in patients with CAP on antibiotics was very small, and its therapeutic implications were minimal.

Keywords: Community-acquired pneumonia, etiology, PCR, CRP, BAL

TIIVISTELMÄ

Ulla Hohenthal

Aikuispotilaan kotisyntyisen keuhkokuumeen etiologinen diagnostiikka mikrobiologisilla pikamenetelmillä

Sisätautien klinikka, Turun yliopistollinen keskussairaala, Turun yliopisto, Suomi
Turun yliopiston julkaisuja
Painosalama Oy – Turku, Suomi 2009

Tausta. Keuhkokuume on vakava sairaus, johon sairastuu Suomessa vuosittain n. 60 000 aikuista. Huolimatta siitä, että taudin hoito on kehittynyt, siihen liittyy yhä merkittävä, 6-15%:n kuolleisuus. Alahengitystieinfektion aiheuttajamikrobien tunnistaminen on myös edelleen haasteellista.

Tavoitteet. Tämän työn tavoitteena oli tutkia Turun yliopistollisessa keskussairaalassa hoidettujen aikuispotilaiden keuhkokuumeen etiologiaa sekä selvittää uusien mikrobiologisten pikamenetelmien hyödyllisyyttä taudinaiheuttajan toteamisessa.

Aineisto. Osatöiden I ja III aineisto koostui 384 Turun yliopistollisen keskussairaalaan infektio-osastolla hoidetusta keuhkokuumepotilaasta. Osatyössä I tutkittiin keuhkokuumeen aiheuttajamikrobeja käyttämällä perinteisten menetelmien lisäksi antigeeniosoitukseen ja PCR-tekniikkaan perustuvia pikamenetelmiä. Osatyö II käsitti 231 potilaasta koostuvan alaryhmän, jossa tutkittiin potilaiden nielun limanäytteestä rinovirusten ja enterovirusten esiintyvyyttä. Osatyössä III potilailta tutkittiin plasman C-reaktiivisen proteiinin (CRP) pitoisuus ensimmäisten viiden sairaalahoitopäivän aikana. Laajoja tilastotieteellisiä analyysejä käyttämällä selvitettiin CRP:n käyttökelpoisuutta sairauden vaikeusasteen arvioinnissa ja komplikaatioiden kehittymisen ennustamisessa. Osatyössä IV 68 keuhkokuumepotilaan sairaalaan tulovaiheessa otetuista näytteistä määritettiin neutrofiilien pintareseptorien ekspressio. Osatyössä V analysoitiin sisätautien vuodeosastoilla vuosina 1996-2000 keuhkokuumepotilaille tehtyjen keuhkohuuhtelunäytteiden laboratoriotutkimustulokset.

Tulokset. Keuhkokuumeen aiheuttaja löytyi 209 potilaalta, aiheuttajamikrobeja löydettiin kaikkiaan 230. Näistä aiheuttajista 135 (58.7%) löydettiin antigeenin osoituksella tai PCR-menetelmillä. Suurin osa, 95 (70.4%), todettiin pelkästään kyseisillä pikamenetelmillä. Respiratorinen virus todettiin antigeeniosoituksella 11.1% keuhkokuumepotilaalla. Eniten respiratorisia viruksia löytyi vakavaa keuhkokuumetta sairastavilta potilailta (20.3%). 231 keuhkokuumepotilaan alaryhmässä todettiin PCR-menetelmällä picornavirus 19 (8.2%) potilaalla. Respiratorinen virus löytyi tässä potilasryhmässä kaiken kaikkiaan 47 (20%) potilaalta. Näistä 17:llä (36%) löytyi samanaikaisesti bakteerin aiheuttama infektio. CRP-tasot olivat sairaalaan tulovaiheessa merkitsevästi korkeammat vakavaa keuhkokuumetta (PSI-luokat III-V) sairastavilla potilailla kuin lievää keuhkokuumetta (PSI-luokat I-II) sairastavilla potilailla ($p < 0.001$). Yli 100 mg/l oleva CRP-taso neljän päivän kuluttua sairaalaan tulosta ennusti keuhkokuumeen komplikaatiota tai huonoa hoitovastetta. Neutrofiilien komplementtireseptorin ekspressio oli pneumokokin aiheuttamaa keuhkokuumetta sairastavilla merkitsevästi korkeampi kuin influenssan aiheuttamaa keuhkokuumetta sairastavilla. BAL-näytteistä vain yhdessä 71:stä (1.3%) todettiin diagnostinen bakteerikasvu kvantitatiivisessa viljelyssä. Uusilla menetelmilläkin keuhkokuumeen aiheuttaja löytyi vain 9.8% BAL-näytteistä.

Päätelmät. Uusilla antigeeniosoitus- ja PCR-menetelmillä keuhkokuumeen etiologia voidaan saada selvitettyä nopeasti. Lisäksi näitä menetelmiä käyttämällä taudin aiheuttajamikrobi löytyi huomattavasti suuremmalta osalta potilaista kuin pelkästään tavanomaisia menetelmiä käyttämällä. Pikamenetelmien hyödyllisyys vaihteli taudin vaikeusasteen mukaan. Respiratorinen virus löytyi huomattavan usein keuhkokuumetta sairastavilta potilailta, ja näiden potilaiden taudinkuva oli usein vaikea. Tulovaiheen korkeaa CRP-tasoa voidaan käyttää lisäkeinona arvioitaessa keuhkokuumeen vaikeutta. CRP on erityisen hyödyllinen arvioitaessa hoitovastetta ja riskiä komplikaatioiden kehittymiseen. Neutrofiilien komplementtireseptorin ekspression tutkiminen näyttää lupaavalta pikamenetelmältä erottamaan bakteerien ja virusten aiheuttamat taudit toisistaan. Antimikrobihoitoa saavilla potilailla BAL-tutkimuksen löydökset olivat vähäiset ja vaikuttivat hoitoon vain harvoin.

Avainsanat: Kotisyntyinen keuhkokuume, etiologia, PCR, CRP, BAL

TABLE OF CONTENTS

ABBREVIATIONS **8**
LIST OF ORIGINAL PUBLICATIONS **9**
1. INTRODUCTION **10**
2. REVIEW OF THE LITERATURE **12**
2.1. Epidemiology of community-acquired pneumonia (CAP) 12
2.2. Etiology 13
2.3. Diagnosis 19
2.3.1. Symptoms and signs 19
2.3.2. C-reactive protein (CRP) and procalcitonin 20
2.3.3. Phagocyte complement receptors (CR) 21
2.3.4. Radiography 21
2.4. Microbiological methods for the etiological diagnosis of CAP 22
2.4.1. Blood culture 22
2.4.2. Sputum gram stain and sputum culture 23
2.4.3. Antigen Detection 24
2.4.4. Polymerase chain reaction (PCR) –based methods 25
2.4.5. Serology 26
2.4.6. Bronchoscopy and bronchoalveolar lavage (BAL) 27
2.5. Etiological agents 27
2.5.1. *Streptococcus pneumoniae* 27
2.5.1.1. Bacteremic pneumococcal pneumonia 30
2.5.1.2. Laboratory diagnosis of *S. pneumoniae* 31
2.5.2. *Mycoplasma pneumoniae* 32
2.5.2.1. Laboratory diagnosis of *M. pneumoniae* 35
2.5.3. *Chlamydophila pneumoniae* 37
2.5.3.1. Laboratory diagnosis of *C. pneumoniae* 38
2.5.4. *Legionella* spp. 38
2.5.4.1. Laboratory diagnosis of *Legionella* spp. 39
2.5.5. *Staphylococcus aureus* 40
2.5.6. Gram negative bacteria 41
2.5.7. Other bacteria and yeasts as causative agents of CAP 42
2.5.8. Viral pneumonia 42
2.5.8.1. Influenza viruses 42
2.5.8.2. Respiratory syncytial virus (RSV) 45
2.5.8.3. Parainfluenza virus (PIV) 46
2.5.8.4. Adenovirus 46
2.5.8.5. Rhinovirus and enterovirus 46
2.5.8.6. Other viruses 47
2.5.8.7. Detection of respiratory viruses 47
2.6. Guideline recommendations for microbiological investigations in CAP patients 49
2.7. Severity of CAP and site of care 49
2.7.1. Pneumonia severity index (PSI) 52
2.7.2. CURB-65 (confusion–urea–respiratory rate–blood pressure–age ≥65 years) 52
2.7.3. American Thoracic Society (ATS) score 53
2.7.4. SMART-COP 54
2.7.5. CRP and procalcitonin 54
2.8. Antimicrobial treatment 56
2.9. Clinical and radiographic response to treatment and hospital discharge 58
2.10. Treatment failure 59
2.10.1. CRP and procalcitonin as markers of treatment failure 60
2.10.2. Management of treatment failure 61

2.11. Outcome 62
3. AIMS OF THE STUDY 64
4. PATIENTS, SUBJECTS AND METHODS 65
4.1. Patients, study designs and data collection (I-V) 65
4.2. Diagnostic criteria (I-V) 66
4.3. Microbiological examinations (I-IV) 67
4.4. Bronchoscopy and BAL (V) 67
4.5. Definitions for etiological diagnosis (I-V) 68
4.6. Determination of CRP (III, IV) 68
4.7. Measurement of leukocyte receptor expression (IV) 68
4.8. Statistical analyses (I, III, IV) 69
4.9. Ethics 69
5. RESULTS 70
5.1. Etiological diagnosis of CAP (I, II, V) 70
5.1.1. Rapid diagnostic methods for etiological diagnosis of CAP (I) 70
5.1.2. Rhinoviruses and enteroviruses as causative agents of CAP (II) 71
5.1.3. Diagnostic yield of various microbiological methods (I) 71
5.1.3.1. Antigen detection 71
5.1.3.2. PCR 72
5.1.3.3. Other methods 72
5.1.4. Diagnostic yield according to severity of pneumonia and preceding antimicrobial therapy (I) 73
5.2. Usefulness of CRP in assessing CAP severity, complications and etiology (III) ... 75
5.2.1. CRP on admission 75
5.2.2. CRP and clinical stabilization 76
5.2.3. CRP during the follow-up 76
5.3. Usefulness of complement receptor 1, complement receptor 3, and FcγR1 on neutrophils in differentiating between bacterial and viral pneumonia (IV) ... 76
5.4. Value of BAL for etiological diagnosis of CAP in a routine setting (V) 78
5.4.1. BAL cultures 79
5.4.2. BAL PCR assays 79
5.4.3. Total diagnostic yield and impact of BAL on therapy 79
6. DISCUSSION 81
6.1. Etiological diagnosis of CAP (I, II, V) 81
6.1.1. Rapid diagnostic methods for etiological diagnosis of CAP (I, II) 81
6.1.1.1. CAP due to bacterial pathogens (I) 81
6.1.1.2. Viral agents associated with CAP (I, II) 82
6.1.1.3. Limitations of epidemiological information (I) 83
6.1.1.4. Usefulness of rapid microbiological methods with respect to disease severity (I) 84
6.2. Usefulness of CRP to assess disease severity, complications and etiology (III) . 84
6.3. Usefulness of CR1, CR3, and FcγR1 on neutrophils for differentiation between bacterial and viral pneumonia (IV) 86
6.4. Value of BAL in etiological diagnosis of CAP in a routine setting (V) 87
6.4.1. Quantitative bacterial culture of the BAL fluid 87
6.4.2. Viral isolations from BAL fluid 88
6.4.3. Specific PCR assays on BAL fluid 88
6.4.4. Diagnostic yield of BAL and etiology of CAP 89
6.4.5. Slowly responding pneumonia and BAL 89
7. SUMMARY AND CONCLUSIONS 90
8. ACKNOWLEDGEMENTS 92
9. REFERENCES 94
ORIGINAL PUBLICATIONS I – V 109

ABBREVIATIONS

BAL	bronchoalveolar lavage
CAP	community-acquired pneumonia
COPD	chronic obstructive pulmonary disease
CR	complement receptor
CRP	C-reactive protein
CRP1	C-reactive protein on admission
CRP2	C-reactive protein at the time of clinical stability
CURB-65	confusion–urea–respiratory rate–blood pressure–age ≥65 years
ICU	intensive care unit
PCR	polymerase chain reaction
PIV	parainfluenza virus
PSI	pneumonia severity index
RSV	respiratory syncytial virus
RT-PCR	reverse transcriptase polymerase chain reaction
spp.	species

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by Roman numerals I-V.

I. Hohenthal U, Vainionpää R, Meurman O, Vahtera A, Katiskalahti T, Nikoskelainen J, Kotilainen P. Aetiological diagnosis of community-acquired pneumonia: utility of rapid microbiological methods with respect to disease severity. *Scand J Infect Dis* 2007; 40: 131-138.

II. Hohenthal U, Vainionpää R, Nikoskelainen J, Kotilainen P. The role of rhinoviruses and enteroviruses in community acquired pneumonia in adults. *Thorax* 2008; 63: 658-659.

III. Hohenthal U, Hurme S, Helenius H, Heiro M, Meurman O, Nikoskelainen J, Kotilainen P. Utility of C-reactive protein in assessing the disease severity and complications of community-acquired pneumonia. *Clin Microbiol Infect* 2009 Jun 22. (Epub ahead of print)

IV. Hohenthal U, Nuutila J, Lilius EM, Laitinen I, Nikoskelainen J, Kotilainen P. Measurement of complement receptor 1 on neutrophils in bacterial and viral pneumonia. *BMC Infect Dis* 2006; 6:11.

V. Hohenthal U, Sipilä J, Vainionpää R, Meurman O, Rantakokko-Jalava K, Nikoskelainen J, Kotilainen P. Diagnostic value of bronchoalveolar lavage in community-acquired pneumonia in a routine setting: a study on patients treated in a Finnish University Hospital. *Scand J Infect Dis* 2004; 36:198-203.

1. INTRODUCTION

Rapid etiological diagnosis of community-acquired pneumonia (CAP) is important since it is usually impossible to judge the type of the causative agent on a clinical basis. The changes on a chest radiograph (Boersma et al. 2006) and the laboratory tests that measure inflammation are not specific enough to allow an etiological diagnosis of CAP (Almirall et al. 2004, García-Vázquez et al. 2003). Of the traditional diagnostic methods, blood cultures are positive in only 5-16% of CAP patients (Bohte et al. 1995, Ishida et al. 1998, Roson et al. 2001, Socan et al. 1999, van der Eerden et al. 2005, Örtqvist et al. 1990). Moreover, valid samples for sputum culture are difficult to obtain and serological tests provide the etiological diagnosis too slowly. In clinical practise, an etiological agent of CAP is identified in less than 30% of the hospitalized patients (Fine et al 1999, Lidman et al. 2002). Consequently, also antimicrobial treatment is most often empirical. However, during the last decade, new microbiological techniques, e.g., antigen detection and gene amplification (Cheng et al. 2004, Rantakokko-Jalava and Jalava 2001, Roson et al. 2004[b], Strålin et al. 2006, Tondella et al. 2002, Waris et al. 1998), have made rapid etiological diagnosis of CAP possible.

In adult patients, the most common etiological agents of CAP are bacteria, the number one of which is *Streptococcus pneumoniae*. Other common etiological agents are *Mycoplasma pneumoniae, Chlamydophila pneumoniae, Haemophilus influenzae* and influenza A virus (Bochud et al. 2001, Bohte et al. 1995, Gutierrez et al. 2005, Jokinen et al. 2001, Lim et al. 2001, Roson et al. 2001). As the diagnostic methods of viral infection have improved, the proportion of viral pneumonias identified in adult patients has increased during the years. Of the viral agents, influenza viruses are the most common ones (Bohte et al. 1995, Gutierrez et al. 2005, Lim et al. 2001, Ruiz et al. 1999[a]). Currently, little is known about the role of respiratory picornaviruses as causative agents of CAP in adults and about their contribution to disease severity (Angeles Marcos et al. 2006, Jennings et al. 2008).

Many studies have shown the clinical utility of the plasma C-reactive protein (CRP) concentration as an acute-phase reactant in the management of patients with various infections, including septicemia, meningitis and infective endocarditis (Hansson et al. 1993, Heiro et al. 2005, Povoa 2002). Previous studies have also shown that the CRP value may contribute to establishing the diagnosis of CAP (Almirall et al. 2004, Castro-Guardiola et al. 2000, Flanders et al. 2004, Holm et al. 2007, Smith et al. 1995[a]). In addition, the use of CRP as a tool in the etiological workup of CAP has been investigated in a number of studies but the results have been discordant (Almirall et al. 2004, García-Vázquez et al. 2003, Hedlund and Hansson 2000, Kragsbjerg et al. 1995, Örtqvist et al. 1995). Similarly, the results of the studies evaluating the use of CRP as a prognostic factor have been somewhat inconsistent (Brunkhorst et al. 2002, Chalmers et al. 2008[a], García-Vázquez et al. 2003, Hedlund and Hansson 2000), although the CRP levels have been higher in patients with bacteremic pneumonia than nonbacteremic pneumonia (Hedlund and Hansson 2000, Kragsbjerg et al. 1995, Örtqvist et al. 1995). In addition to CRP, there is a need for new specific markers of inflammation which might help to distinguish between bacterial and viral infection. One such candidate is the neutrophil

complement receptor. There are preliminary data showing that quantitative assessment of complement receptors on neutrophils may improve the distinction between bacterial and viral infection in adult febrile patients (Nuutila et al. 2006).

The number of studies exploiting bronchoscopy and bronchoalveolar lavage (BAL) in the diagnosis of CAP has been small in comparison to the number focusing on e.g. nosocomial pneumonia or pneumonia in immunocompromized patients (Dalhoff et al. 1993, Ewig et al. 1996, Feinsilver et al. 1990, Hohenadel et al. 2001, Jimenez et al. 1993, Korsgaard et al. 2002, Rasmussen et al. 2001, Sörensen et al. 1989, Thorpe et al. 1987, Torres and El-Ebiary 2000). Most patients with CAP can be successfully treated without invasive diagnostic procedures. Thus, in the routine clinical setting, it is not usually reasonable to perform BAL on newly admitted CAP patients before starting empiric antimicrobial treatment. The situation is different if the patient has a very severe clinical presentation, or when unusual pathogens are suspected.

This study was initiated to evaluate the usefulness of novel rapid microbiological methods in the etiological diagnostics of CAP. In addition, the role and contribution to disease severity of rhinoviruses and enteroviruses in adult CAP patients was evaluated. Furthermore, the value of CRP in assessing the severity, complications and etiology of CAP was analyzed, and whether the expression of leukocyte receptors on admission is a useful preliminary test to differentiate between bacterial and viral infection was examined. In addition, the diagnostic value of bronchoscopy and BAL was assessed in a routine setting in patients with CAP requiring BAL for clinical reasons.

2. REVIEW OF THE LITERATURE

2.1. Epidemiology of community-acquired pneumonia (CAP)

According to WHO data, 3–4 million people die due to pneumonia, a large proportion of whom are children or elderly. Pneumonia is the third most common cause of death in the world accounting for 7% of the total mortality of 56 million people (Lopez et al. 2006). Pneumonia is a common health problem in industrialized countries, as well, and may still be life threatening despite the availability of effective antibiotic therapy and supportive care in intensive care units (ICU). Pneumonia ranks among the ten leading causes of death in the USA and the European countries. In the USA, pneumonia causes approximately 60 000 deaths annually, which equals 20 deaths per 100 000 population (Mokdad et al. 2004).

According to population-based studies the annual incidence of CAP among the adult population varies between 1.6 to 9 cases per 1000 per year (Almirall et al. 2000, Woodhead 2002, Woodhead et al. 1987). The incidence of CAP is higher in males than females (Almirall et al. 2000, Gutierrez et al. 2006, Jokinen et al. 1993). There is a seasonal variation in the incidence of CAP: the incidence is highest in winter (Almirall et al. 2000, Woodhead et al. 1987). Age is strongly associated with the the incidence of CAP. Among patients aged ≥ 65 years the incidence of CAP is two to five times higher than among adult patients aged < 65 years (Almirall et al. 2000, Gutierrez et al. 2006, Jokinen et al. 1993, Woodhead 2002). According to various studies 22-42% of the patients with CAP require hospitalization (Guest and Morris 1997, Jokinen et al. 2001, Woodhead et al. 1987). Patients aged 65 years or older account for about half of all patients hospitalized for CAP (Guest and Morris 1997, Niederman 1998). At least partly due to the aging of the population, hospital admissions for pneumonia have been rising during the recent years (Thomsen et al. 2006, Trotter et al. 2008). Between 5% and 10% of the hospitalized patients require management in an ICU (Thomsen et al. 2006, Trotter et al. 2008, Wilkinson and Woodhead 2004, Woodhead et al. 2006). The mortality rate among patients in open care is less than 1% and among hospitalized patients 4% to 15% (Colice et al. 2004, Mandell 2004, Woodhead 2002). Mortality rates are highest among elderly patients (18 %), and patients with severe disease who require treatment in an ICU (22% to more than 50%) (Fine et al. 1996, Jokinen et al. 1993, Woodhead et al. 2006).

Regarding Finland, Jokinen *et al.* provided detailed epidemiological data in a population-based study run in Eastern Finland in 1981-1982 (Jokinen et al. 1993). The incidence of CAP was 11.6 per 1000 habitants per year. The incidence was highest in children and elderly. The age-specific incidence per 1000 habitants per year was as follows: age < 5 years, 36.0; age 5-14 years, 16.2; age 15-59 years, 6.0; age 60-74 years, 15.4; and age ≥ 75 years, 34.2. The incidence was higher in males (13.9/1000) than females (9.4/1000). The rate of hospitalization was 42% and mortality 4%. Säynäjäkangas *et al.* studied the incidence of hospitalization for pneumonia in Finland between 1972 and 1993. Among working-aged people, the annual rate of pneumonia related hospitalization was 2.67/1000 persons among men and 1.10/1000 persons among women (Säynäjäkangas et al. 1997[b]). In the population aged ≥ 65 years, the annual rate of hospital admissions for pneumonia

increased during the study period from 15.5 per 1000 persons in 1972 to 23.9 per 1000 persons in 1993 (Säynäjäkangas et al. 1997[a]).

Managing CAP has also a substantial economic impact. In the 1990s the annual cost to treat CAP was estimated to be 8.4 to 12.2 billion dollars in the USA (Colice et al. 2004, Niederman et al. 1998). Most of this cost was due to treatment of hospitalized patients with CAP; the mean treatment cost for an outpatient episode of CAP was approximately 500 dollars and for an inpatient episode 6000 to 10 000 dollars (Colice et al. 2004, Niederman et al. 1998). In the United Kingdom, the annual cost to treat CAP in the 1990s was £441 million. Hospital treatment for CAP accounted for 96% of the total costs. The average cost for outpatient treatment of CAP was £100 per episode and for hospitalized patients £1700-£5100 per episode (Guest and Morris 1997). In these studies the cost of sick leave has not been accounted which would increase the economic burden of CAP. More than half of employed patients with CAP require sick leave for more than two weeks (Almirall et al. 2000, Woodhead et al. 1987).

2.2. Etiology

Studies on the microbial etiology of CAP have shown that the portion of causative pathogens varies by geographical areas, study populations used, concurrent epidemics, microbiological techniques and definitions of the etiological diagnosis of CAP. Despite these numerous differences in study settings, a regular finding in all studies on the etiology of CAP is that *Streptococcus pneumoniae* is the most common pathogen causing CAP. Even in different geographical areas with different climates and socioeconomic status of the population *S. pneumoniae* is the most common pathogen of CAP. *Mycoplasma pneumoniae, Chlamydophila pneumoniae* and influenza A virus are typically agents for which the occurrence is dependent on the current epidemiological situation whereas the occurrence of *Legionella* spp. varies in particular according to the geographical area studied (Beigel 2008, Karvonen et al. 1993, Pönkä 1980, Ricketts and Joseph 2007). It has also been found that a small range of key pathogens cause most cases of CAP all around the world. *S. pneumoniae* is followed by *Haemophilus influenzae, M. pneumoniae, C. pneumoniae* and influenza A virus (Almirall et al. 2000, Bohte et al. 1995, Charles et al. 2008[a], Diaz et al. 2007, Gutierrez et al. 2006, Jokinen et al. 2001, Lieberman et al. 1996, Lim et al. 2001, Miyashita et al. 2005, Roson et al. 2001, Ruiz et al. 1999[a], Scott et al. 2000[b], Wattanathum et al. 2003, Woodhead et al. 1987, Örtqvist et al. 1990). Especially during the last years, the importance of viruses as causative agents of CAP has been recognized. Of the viruses, influenza A is the most important etiological agent (Jennings et al. 2008, Templeton et al. 2005).

There are only few population-based studies on the etiology of CAP in adults including both hospitalized patients and patients treated at home (**Table 1**). *S. pneumoniae* has been the most common pathogen in all of these studies with frequences of 11-41%. *C. pneumoniae* and *M. pneumoniae* are the next common agents of CAP with frequences of 3-10% and 1-10% (Almirall et al. 2000, Gutierrez et al. 2006, Jokinen et al. 2001, Woodhead et al. 1987). In Spanish studies *Legionella pneumophila* is among the most common pathogens (2-4%) (Almirall et al. 2000, Gutierrez et al.

2006) According to a Finnish study from the early 1980s, *C. pneumoniae* and *M. pneumoniae* were identified each in 10% of the patients with CAP and were the most common pathogens after *S. pneumoniae*, which was identified in 41% of the patients (Jokinen et al. 2001). The total portion of CAP caused by viruses in these studies varied from 4% to 13% (Almirall et al. 2000, Gutierrez et al. 2006, Jokinen et al. 2001, Woodhead et al. 1987). In a population-based study of hospitalized patients in the USA in 1991, *S. pneumoniae* (7%), *M. pneumoniae* (5%), *C. pneumoniae* (2%) and *Legionella* spp. (2%) were the most common etiological agents of CAP (Marston et al. 1997).

Table 1. Etiological agents of community-acquired pneumonia in population-based studies. Figures are n (%)

	Almirall Spain 2000	Gutierrez Spain 2006	Jokinen Finland 2001	Woodhead UK 1987	Marston[a] USA 1997
Study population	>14 years n=241	≥15 years n=493	≥15 years n=345	16-79 years n=236	≥18 years n=2776
Diagnostic methods	bc, spag, hiag, ser	bc, sc, spag, lpag, ser	spag ser	bc, sc, vc, spag ser	bc, sc, vc, ser
S. pneumoniae	27 (11%)	83 (17%)	125 (41%)	85 (36%)	187 (7%)[d]
C. pneumoniae	22 (9%)	15 (3%)	30 (10%)	NS	29 (2%)[d]
M. pneumoniae	9 (4%)	38 (8%)	30 (10%)	3 (1%)	67 (5%)[d]
Legionella spp.	5 (2%)	21 (4%)	NS	1 (<1%)	47 (2%)[d]
H. influenzae	1 (<1%)	9 (2%)	12 (4%)	24 (10%)	41 (1%)[d]
Gram negative bacteria[b]	2 (<1%)	16 (3%)	NS	3 (1%)	54 (2%)[d]
S. aureus	0	2 (<1%)	NS	2 (1%)	16 (<1%)[d]
Respiratory virus	32 (13%)	20 (4%)	27 (9%)	30 (13%)	92 (3%)[d]
Mixed[c]	10 (4%)	28 (6%)	49 (16%)	26 (11%)	50 (2%)[d]
Unknown	137 (57%)	243 (49%)	121 (40%)	107 (45%)	1777 (64%)[d]

bc=blood culture, sc=sputum culture, vc=viral culture, spag=*Streptococcus pneumoniae* antigen detection, hiag=*Haemophilus influenzae* antigen detection, lpag=*Legionella* spp. antigen detection, ser=serological detection, NS=not studied

[a]Hospitalized patients. Possible cases are not included in these numbers.

[b]Gram negative enteric bacteria and *P. aeruginosa*

[c]Two or more etiological agents

[d]Possible cases are not included

Only a limited number of studies have been published on the etiology of CAP in primary care in adult patients treated at home. *S. pneumoniae* has been the most frequent agent detected in 20% - 32% of patients with CAP, followed by *M. pneumoniae* (14-18%), *H. influenzae* (2-28%), influenza A virus (7-10%) and *C. pneumoniae* (5%) (Bochud et al. 2001, Lagerström et al. 2003) (**Table 2**). The only exception to the leading position of *S. pneumoniae* as the primary causative agent of CAP has been among patients with CAP admitted to hospital emergency unit and treated as outpatients (**Table 2**). In these studies

M. pneumoniae and *C. pneumoniae* have been equally or even more common causative agents compared to *S. pneumoniae* (Gutierrez et al. 2005, Marrie et al. 1996, Miyashita et al. 2005, Wattanathum et al. 2003). Respiratory viruses were identified in up to 21% of these patients. Also in one study focusing on the etiology of nonsevere CAP in patients treated either as outpatients or inpatients, *M. pneumoniae* was the most common pathogen (20%) followed by *C. pneumoniae* (17%) and *S. pneumoniae* (11%) (Beovic et al. 2003). In another study of patients with nonsevere pneumonia, *S. pneumoniae* was the most frequent causative agent (28%) followed by *M. pneumoniae,* (16%) and *C. pneumoniae* (11%) (Falguera et al. 2001). Neither of these two studies included any virological tests.

Table 2. Etiological agents of community-acquired pneumonia in outpatients. Figures are n (%)

	Bochud[a] Switzerland 2001	Lagerström[a] Sweden 2002	Gutierrez[b] Spain 2005	Marrie[b] Canada 1996	Miyashita[b] Japan 2005	Wattanathum[b] Thailand 2003
Study population	>15 years n=170	>10 years n=82	≥15 years n=132	≥18 years n=149	adult n=106	adult n=98
Diagnostic methods	bc, sc, vc, spag, ser	sc, ser	bc, sc, spag, lpag, ser	ser	bc, sc, spag, lpag, ser	bc, sc, spag, lpag, ser
S. pneumoniae	34 (20%)	26 (32%)	19 (14%)	NS	13 (12%)	13 (13%)
C. pneumoniae	9 (5%)	4 (5%)	NR	21 (14%)	12 (11%)	36 (37%)
M. pneumoniae	23 (14%)	15 (18%)	17 (13%)	39 (26%)	29 (27%)	29 (30%)
Legionella spp.	1 (<1%)	0	4 (3%)	1 (<1%)	0	8 (8%)
H. influenzae	3 (2%)	23 (28%)	3 (2%)	NS	5 (5%)	0
Gram negative bacteria[c]	0	1 (1%)	1 (<1%)	NS	0	0
S. aureus	0	6 (7%)	0	NS	1 (<1%)	0
Respiratory virus	18 (11%)	13 (16%)	5 (4%)	12 (8%)	2 (2%)	NS
Mixed[d]	15 (9%)	21 (26%)	5 (4%)	7 (5%)	8 (8%)	13 (13%)
Unknown	78 (46%)	20 (24%)	62 (47%)	75 (50%)	50 (47%)	24 (25%)

bc=blood culture, sc=sputum culture, vc=viral culture, spag=*Streptococcus pneumoniae* antigen detection, hiag=*Haemophilus influenzae* antigen detection, lpag=*Legionella* spp. antigen detection, ser=serological detection, NS=not studied, NR= not reported

[a]Primary care based studies

[b]Hospital emergency based studies

[c]Gram negative enteric bacteria and *P. aeruginosa*

[d]Two or more etiological agents

Most of the etiological studies of CAP involve hospitalized patients (**Table 3**). *S. pneumoniae* is universally the most common (20% - 40%) etiological agent of CAP among them. Usually *M. pneumoniae, H. influenzae, C. pneumoniae* and influenza A virus follow *S. pneumoniae* with varying frequencies and ranking (Bohte et al. 1995, Charles et al. 2008[a], Diaz et al. 2007, Gutierrez et al. 2005, Lauderdale et al. 2005, Lieberman et al. 1996, Lim et al. 2001, Miyashita et al. 2005, Ruiz et al. 1999[a], Wattanathum et al. 2003, Örtqvist et al. 1990). There is some variation by geographical

Table 3. Etiological agents of community-acquired pneumonia in hospitalized patients. Figures are n (%)

Study	Population	Diagnostic methods	*S.pneumoniae*	*C.pneumoniae*	*M.pneumoniae*	*Legionella spp.*	*H. influenzae*	Gram negative bacteria[a]	*S. aureus*	Respiratory virus	Mixed	Unknown
Charles, 2008 Australia	>18 years n=885	bc,sc,spag, lpag,vPCR, ser	123 (14%)	11 (1%)	78 (9%)	30 (3%)	45 (5%)	27 (3%)	11 (1%)	131 (15%)	75 (9%)	481 (54%)
Díaz, 2007 Chile	≥16 years n=176	bc,sc,spag, lpag,vag,ser	43 (24%)	3 (2%)	3 (2%)	4 (2%)	5 (3%)	1 (<1%)	1 (<1%)	23 (13%)	13 (7%)	78 (44%)
Gutierrez, 2005 Spain	≥15 years n=361	bc,sc,spag, lpag, ser	64 (18%)	NR	21 (6%)	17 (5%)	6 (2%)	15 (4%)	2 (<1%)	14 (4%)	23 (6%)	175 (49%)
Jennings, 2008 New Zealand	≥18 years n=304	bc,sc,vc, ser spag,lpag, vag,vPCR	93 (31%)	NR	8 (3%)	11 (4%)	34 (11%)	NR	7 (2%)	88 (29%)	49 (16%)	127 (42%)
Johnstone, 2008 Canada	≥18 years n=193	bc,sc,vPCR, mPCR, lpPCR,ser	14 (7%)	4 (2%)	4 (2%)	1 (<1%)	2 (1%)	6 (3%)	2 (1%)	29 (15%)	8 (11%)	118 (61%)
Lauderdale, 2004 Taiwan	>16 years n=168	bc,sc,spag, lpag, ser	40 (24%)	12 (7%)	24 (14%)	2 (1%)	8 (5%)	11 (7%)	3 (2%)	17 (9%)	21 (13%)	69 (41%)
Lim, 2001 UK	≥16 years n=267	bc,sc,spag, lpag, ser	129 (48%)	35 (13%)	9 (3%)	9 (3%)	20 (7 %)	4 (1%)	4 (1%)	62 (23%)	75 (28%)	68 (25%)
Miyashita, 2005 Japan	adult n=400	bc,sc,spag, lpag, ser	105 (26%)	27 (7%)	37 (9%)	6 (2%)	52 (13%)	16 (4%)	13 (3%)	12 (3%)	56 (14%)	138 (35%)
Rosón, 2001 Spain	adult n=533	bc,sc,lpag, TNA, ser	135 (25%)	11 (2%)	12 (2%)	35 (7%)	34 (6%)	10 (2%)	0	6 (1%)	19 (7%)	250 (47%)
Ruiz, 1999 Spain	adult n=395	bc, sc, ser	39 (21%)	9 (5%)	9 (5%)	14 (8%)	11 (6%)	16 (9%)	5 (3%)	26 (14%)	41 (23%)	213 (54%)
Scott, 2000 Kenya	≥15 years n=281	bc,sc,spag, TNA, ser	129 (46%)	0	7 (3%)	0	10 (4%)	4 (1%)	4 (1%)	16 (6%)	30 (11%)	99 (35%)
van der Eerden, 2005 Netherlands	≥18 years n=262	bc,sc,spag, lpag, BAL, ser	97 (37%)	1 (<1%)	23 (9%)	14 (5%)	19 (7%)	13 (5%)	10 (4%)	10 (4%)	36 (14%)	104 (40%)
Wattanathum, 2003 Thailand	adult n=147	bc,sc,spag, lpag, ser	33 (22 %)	24 (16%)	10 (7%)	8 (5%)	4 (3%)	20(14%)	5 (3%)	NS	9 (6%)	42 (29%)

bc=blood culture, sc=sputum culture, vc=viral culture, spag=*Streptococcus pneumoniae* antigen detection, lpag=*Legionella* spp. antigen detection, vag=viral antigen detection, mpPCR=*Mycoplasma pneumoniae* PCR test, lpPCR=*Legionella* spp. PCR test, vPCR=viral PCR test, ser=serological detection, TNA=transthoracic needle aspiration, NS= not studied, NR=not reported.

[a] Gram negative enteric bacteria and *P. aeruginosa*

area. In Mediterranian countries *L. pneumophila* is among the four most common agents (4% - 16%) of the hospitalized patients with CAP whereas in northern countries *L. pneumophila* is rare (Gutierrez et al. 2005, Lieberman et al. 1996, Lim et al. 2001, Roson et al. 2001, Ruiz et al. 1999[a], Woodhead 2002, Örtqvist et al. 1990). In studies from Asian countries, members of *Enterobacteriaceae* and *Pseudomonas* spp. are more common (4% - 13%) than in other parts of the world where these pathogens are usually found at low frequencies (1% - 3%) (Bohte et al. 1995, Diaz et al. 2007, Lauderdale et al. 2005, Lim et al. 2001, Miyashita et al. 2005, Roson et al. 2001, Scott et al. 2000[b], Wattanathum et al. 2003). In a study from Kenya, *Mycobacterium* spp. was identified in 13% of the patients with CAP and *Salmonella* spp. in 2%. However, in this study unlike in most other studies, HIV-positive patients were included and consisted half of the study group (Scott et al. 2000[b]). The portion of respiratory viruses among hospitalized patients has most commonly been around 10%, with the frequencies varying from 1% to 29% (Bohte et al. 1995, Charles et al. 2008[a], Diaz et al. 2007, Gutierrez et al. 2005, Jennings et al. 2008, Lauderdale et al. 2005, Lieberman et al. 1996, Lim et al. 2001, Roson et al. 2001, Ruiz et al. 1999[a], Scott et al. 2000[b], Örtqvist et al. 1990).

Except for the predominance of *S. pneumoniae*, the etiological agents of CAP in patients treated in ICUs for severe CAP differ in some respects from the etiological agents in the rest of hospital-treated patients (**Table 4)**. On the other hand, Valencia *et al*. reported that among patients with severe CAP, defined as PSI class V, the etiological agents occurred in similar frequencies whether the patients were treated in an ICU or ward (Valencia et al. 2007). *M. pneumoniae* and *C. pneumoniae* are rare causative agents in patients with severe CAP, while *L. pneumophila* is at least as common among ICU patients as among other hospitalized patients with CAP. Although the frequencies of gram negative enteric bacteria and *P. aeruginosa* are quite low also among patients admitted to an ICU, these pathogens are more common among ICU patients than other hospitalized patients (Leroy et al. 1995, Paganin et al. 2004, Rello et al. 2003, Ruiz et al. 1999[a], Ruiz et al. 1999[b], Valencia et al. 2007). Some studies have reported also that *Staphylococcus aureus* is among the most common causative agents of CAP among ICU-treated patients (Leroy et al. 1995, Valencia et al. 2007). In some Asian countries *Burkholderia pseudomallei* may cause severe pneumonia (Tan et al. 1998). Viral diagnostics has rarely been included in the studies on the etiological diagnosis of CAP in patients admitted to an ICU, but when this has been done, viruses have been detected in 5-6% of the patients (Ruiz et al. 1999[b], Valencia et al. 2007).

Table 4. Etiological agents of community-acquired pneumonia in patients admitted to an intensive care unit. Figures are n (%)

	Leroy et al. France 1995	Paganin et al. France 2004	Rello et al. Spain 2003	Ruiz et al. Spain 1999	Valencia et al. Spain 2007
Study population	63.9 years[a] n=299	54.7 years[a] n=112	61.1 years[a] n=204	65 years[a] n=89	70 years[a] n= 92
Diagnostic methods	bc, sc, ta, ser, PF bronchoscopic samples[b]	bc, ser, PF, BAL[c]	bc, ser, PF, lpag protected-brush specimen[d]	bc, sc, ta, ser, PF, bronchoscopic samples[b]	bc, sc, spag, lpag, ser, PF bronchoscopic samples[b]
S. pneumoniae	80 (31%)	48 (43%)	41 (20%)	21 (24%)	30 (47%)
C. pneumoniae	NR	0	1 (<1%)	6 (7%)	1 (2%)
M. pneumoniae	2 (<1%)	0	2 (<1%)	3 (3%)	
Legionella spp.	0	2 (2%)	23 (11%)	2 (2%)	3 (5%)
H. influenzae	26 (10%)	1 (<1%)	11 (5%)	5 (6%)	6 (9%)
Gram negative bacteria[e]	55 (18%)	30 (27%)	12 (6%)	14 (16%)	13 (14%)
S. aureus	57 (22%)[f]	2 (2%)	5 (2%)	2 (2%)	4 (6%)
Respiratory virus	NS	NS	0	5 (6%)	3 (5%)
Mixed	52 (17%)	3 (3%)	0	16 (18%)	12 (19%)
Unknown	102 (34%)	24 (21%)	87 (43%)	42 (47%)	37 (40%)

bc=blood culture, sc=sputum culture, ta=culture of tracheobronchial aspirate, PF=pleural fluid culture, spag=*Streptococcus pneumoniae* antigen detection, lpag=*Legionella* spp. antigen detection, ser=serological detection

[a]Mean age

[b]According to clinical judgement

[c]In most patients as a routine procedure

[d]In patients who required mechanical ventilation

[e] Gram negative enteric bacteria and *P. aeruginosa*

[f]*Staphylococcus* spp.

S. pneumoniae is usually the predominant etiological agent throughout all age groups (Jokinen et al. 2001, Lim et al. 2001, Örtqvist et al. 1990). However, in some studies *M. pneumoniae* has been detected with similar to or even higher frequencies compared to *S. pneumoniae* in the youngest age group (15-44 years) (Gutierrez et al. 2005, Lauderdale et al. 2005, Lieberman et al. 1996). In general, *M. pneumoniae* is a rare cause of CAP in elderly patients (Jokinen et al. 2001, Lim et al. 2001, Loeb 2003, Ruiz et al. 1999[a], Örtqvist et al. 1990). Gram negative enteric bacteria and *P. aeruginosa* may cause CAP in elderly patients, especially among patients with comorbidities or those who live in nursing homes or long-term care facilities (Charles et al. 2008[a], Loeb 2003, Marrie 2000, Vila-Corcoles et al. 2009). Apart from age, comorbidities may also have some impact on the etiology of CAP. Pulmonary comorbidity predisposes to CAP caused by gram negative enteric bacteria and *P. aeruginosa* (Charles et al. 2008[a], Gutierrez et al. 2005, Ruiz et al. 1999[a]*)*. Hepatic comorbidity and alcoholism are associated with pneumococcal pneumonia (Ruiz et al. 1999[a]). Instead, seldom has any association been reported between a viral etiology of CAP and age or comorbidities (Gutierrez et al. 2005, Jokinen et al. 2001, Lauderdale et al. 2005, Lieberman et al. 1996, Lim et al. 2001).

The proportion of patients with a mixed etiology of CAP varies from 4% to 16% in population- based studies (Almirall et al. 2000, Gutierrez et al. 2006, Jokinen et al. 2001, Woodhead et al. 1987), from 6% to 38% in the studies of hospitalized patients. The most common combinations are pneumococci with viruses, with *C. pneumoniae,* or with *M. pneumoniae.* (Bohte et al. 1995, Charles et al. 2008[a], Diaz et al. 2007, Gutierrez et al. 2005, Lauderdale et al. 2005, Lieberman et al. 1996, Lim et al. 2001, Miyashita et al. 2005, Ruiz et al. 1999[a], Wattanathum et al. 2003, Örtqvist et al. 1990).

In observational or retrospective studies of CAP, an etiological diagnosis has been established in less than 30% of the hospitalized patients (Fine et al. 1999, Lidman et al. 2002). Even with extensive use of microbiological methods 40% to 50% of all cases remain with an unknown etiology (Almirall et al. 2000, Bohte et al. 1995, Charles et al. 2008[a], Diaz et al. 2007, Gutierrez et al. 2005, Jokinen et al. 2001, Lauderdale et al. 2005, Miyashita et al. 2005, Roson et al. 2001, Ruiz et al. 1999[a], Scott et al. 2000[b], Valencia et al. 2007, Wattanathum et al. 2003). To examine the etiology of CAP defined as unknown by conventional methods, Ruiz-González *et al.* performed cultures and PCR and antigen tests for common respiratory pathogens in lung aspirates obtained by transthoracic needle aspiration. The study of lung aspirates provided evidence of a microbial etiology in 36 (65%) of the 55 patients with pneumonia of unknown etiology by conventional methods. *S. pneumoniae* was identified in one-third of these patients (Ruiz-Gonzalez et al. 1999). On the other hand, in recent PCR-studies, testing of specimens obtained from the upper respiratory tract has yielded an etiological diagnosis in up to 76% of the patients. Above all what these studies have shown, is the high incidence of viruses associated with CAP. A virus was identified in 15% - 56% of the patients with CAP. Rhinoviruses undetected by conventional methods were the most common viral pathogens (4% - 17%). Up to 50% of the patients with a viral diagnosis had a concomitant bacterial diagnosis (Charles et al. 2008[a], Jennings et al. 2008, Johnstone et al. 2008[b], Templeton et al. 2005).

2.3. Diagnosis

2.3.1. Symptoms and signs

Patients with CAP present with a large number of symptoms. Fever and cough are the most common ones. Fever is present in 65% to 90% and cough in 75% to 96% of patients with CAP. Other typical respiratory complaints include sputum production, dyspnea and chest pain. Patients with CAP may also have nonrespiratory symptoms like fatigue, chills, headache and myalgia (Bochud et al. 2001, Castro-Guardiola et al. 2000, Flanders et al. 2004, Gennis et al. 1989, Metlay et al. 1997[b], Ruiz et al. 1999[a]). Elderly patients complain of fewer symptoms than patients aged less than 65 years. Altered mental status is a typical symptom for elderly patients with CAP (Marrie 2000, Metlay et al. 1997[b]). Symptoms are not specific for CAP and distinguish poorly between CAP and other causes of respiratory illnesses. The likelihood ratio for a single symptom in distinguishing pneumonia from other respiratory diseases is close to 1.0 (Metlay and Fine 2003, Metlay et al. 1997[a]). The prevalence of pneumonia is less than 15% among adult patients presenting at the primary care with symptoms of acute respiratory tract infection (Flanders et al. 2004, Macfarlane et al. 2001).

Apart from fever, physical findings include tachypnea, tachycardia and dullness to percussion. Auscultation of the lungs may reveal rales or bronchial or decreased breath sounds or, sometimes, wheezing (Flanders et al. 2004, Gennis et al. 1989, Metlay and Fine 2003, Metlay et al. 1997[a], Wipf et al. 1999). Physical findings are not specific for pneumonia and thus no single physical finding is particularly helpful in making the diagnosis of pneumonia. The positive likelihood ratios for various findings in chest examination or vital signs are in the range of 1.4 to 8.6 (Metlay and Fine 2003). In addition, intraobserver variation in recognizing these findings is obvious (Metlay and Fine 2003, Metlay et al. 1997[a], Wipf et al. 1999).

Because the accuracy of individual symptoms and signs for predicting pneumonia is low, studies have attempted to build prediction rules that incorporate several symptoms and signs. However, maximal probabilities of these prediction rules are only less than 50%. For example with the baseline prevalence of pneumonia of 5%, a patient with cough, fever, tachycardia and crackles has a probability of pneumonia from 18% to 42% (Metlay and Fine 2003, Metlay et al. 1997[a]). On the other hand, in the absence of tachycardia, tachypnea, fever and abnormal chest examination, the probability of pneumonia is small.

Historically, the clinical features of CAP have been characterized as being either typical or atypical. The typical pneumonia syndrome caused by *S. pneumoniae* or other pyogenic bacteria is characterized by sudden onset of high fever, chills, pleuritic chest pain and purulent sputum. The atypical pneumonia caused by *M. pneumoniae, C. pneumoniae, Legionella* spp*,* or viruses is characterized by subacute illness, nonproductive cough and nonrespiratory complaints like headache and diarrhea. However, studies have shown that it is not possible to predict the microbial etiology by the presenting signs and symptoms (Beovic et al. 2003, Bochud et al. 2001, Ruiz et al. 1999[a]).

2.3.2. C-reactive protein (CRP) and procalcitonin

CRP is a protein of the acute phase, synthesized by the hepatocytes. Its production is stimulated mainly by interleukin 6, interleukin 1 and tumor necrosis factor α in response to infection or tissue inflammation (Clyne and Olshaker 1999). Some studies have evaluated the use of CRP to diagnose radiologically proved pneumonia (Almirall et al. 2004, Flanders et al. 2004, Holm et al. 2007, Müller et al. 2007, van der Meer et al. 2005). Among the patients with lower respiratory infection, median CRP levels were significantly higher in patients with CAP than in those not having CAP (Almirall et al. 2004, Flanders et al. 2004). However, CRP is neither sufficiently sensitive to rule out nor sufficiently specific to rule in pneumonia (van der Meer et al. 2005). The reported sensitivity for CRP levels of 20-40 mg/l to diagnose pneumonia was 70-89% and the specificity was 44-90% (Almirall et al. 2004, Flanders et al. 2004, Holm et al. 2007, Müller et al. 2007). Accuracy was improved when the clinical signs are combined with the CRP value (Müller et al. 2007).

A number of studies have investigated the use of CRP as a tool for providing an etiological diagnosis to CAP. Some studies have reported no difference in the CRP levels between different etiologies (Hedlund and Hansson 2000, Kosmas et al. 1997), while others have found significantly higher CRP levels only in bacteremic pneumococcal pneumonia

(Kragsbjerg et al. 1995, Örtqvist et al. 1995). On the other hand, one study found that the CRP values were significantly higher in patients with *L. pneumophila* pneumonia than in any other group (García-Vázquez et al. 2003). Another study reported significantly higher CRP levels in pneumococcal pneumonia or *L. pneumophila* pneumonia compared to other etiological groups (Almirall et al. 2004).

Procalcitonin, the precursor of calcitonin, is normally produced by neuroendocrine cells, e.g., the thyroid gland. During systemic infection procalcitonin is produced by a variety of parenchymal cells, including liver, kidney, and monocytes (Christ-Crain and Muller 2007). Procalcitonin is comparable to CRP in identifying patients with pneumonia (Holm et al. 2007, Müller et al. 2007). In the study by Holm *et al.* a cut-off point of >0.06 ng/ml of procalcitonin identified the patients with CAP with a sensitivity of 70% and specificity of 66% (Holm et al. 2007). Respectively in the study by Müller *et al.* a cut-off point of >0.5 μg/l of procalcitonin identified patients with CAP with a sensitivity of 57% and specificity of 83% (Müller et al. 2007). Christ-Crain *et al*. showed that the use of procalcitonin-guided antibiotic therapy in CAP resulted in a substantial reduction of antibiotic use. Antibiotics were withheld for 15% of the patients compared to 1% of those receiving standard therapy. In addition, the use of procalcitonin-guided therapy resulted in 55% shorter duration of antibiotic treatment with no adverse effect on outcome (Christ-Crain et al. 2006).

2.3.3. Phagocyte complement receptors (CR)

Activation of the complement system is one of the major effector pathways in bacterial infection. The receptors for the complement molecules, CR1 and CR3 are only weekly expressed on the surface of resting neutrophils being mostly stored in vesicles and granules. In infection, degranulation and fusion of the vesicles and granules with plasma membrane leads to an upregulation of CR1 and CR3. Flow cytometry can be used to quantify the receptor expression on neutrophils (Nuutila and Lilius 2007). In the study by Nuutila *et al.* the expression levels of CR1 and CR3 on neutrophils in bacterial infections were over 3-fold and 2-fold higher, respecticely, compared to viral infections and controls. The sensitivities of CR1 and CR3 in differentiating between bacterial and viral infections were 92% and 81% and specifities 85% and 63%. Respectively the sensitivity of CRP was 88% and specificity 85% (Nuutila et al. 2006).

2.3.4. Radiography

Chest radiograph is considered as the gold standard for the diagnosis of pneumonia. American Thoracic Society (ATS) guidelines recommend a chest radiograph to establish the diagnosis of pneumonia in all patients with suspected CAP to assess the extent of disease and to identify any pleural effusion (Mandell et al. 2007). The Finnish National Guidelines (Käypä hoito -suositus) recommend chest radiograph for patients with symptoms of lower respiratory tract infection (fever, cough, purulent sputum production, dyspnea, wheezing, chest pain) and in case the symptoms have a sudden onset, the disease affects the patient's general condition, the patient has risk factors (age, underlying diseases) or if the symptoms of lower respiratory tract have worsened after initial recovery (Honkanen et al. 2008).

Apart from infection, an opacity on the chest radiograph may be due to a variety of clinical conditions, e.g., edema fluid, malignancy, vasculitis and pulmonary manifestations of adverse drug reactions (Mandell et al. 2000, Marrie 1994). Moreover, coexisting pulmonary disease can make interpretation of radiographs difficult (Tarver et al. 2005). Chest radiography performed early in the course of pneumonia may not show any infiltration (Bartlett and Mundy 1995, Franquet 2001). Thus, the radiographic findings must be interpreted in the context of the medical history and physical examination findings (Marrie 1994). Studies have shown considerable interobserver variability in the radiographic diagnosis of pneumonia and interobserver reliability for identifying the pattern of infiltrates is poor, even among radiologists (Albaum et al. 1996, Boersma et al. 2006, Young and Marrie 1994). Compared to chest radiograph, computed tomography is more sensitive for diagnosing CAP. In a Finnish study of 47 patients with clinical symptoms and signs suspicious for CAP, HRCT identified 26 CAP cases while chest radiography identified only 18 cases (Syrjälä et al. 1998). HRCT improves also the accuracy of typing of CAP (Syrjälä et al. 1998). However, the clinical significance of these findings is unclear and the method is also associated with radiation exposure. Rather, repeat chest radiography is recommended for patients hospitalized for suspected pneumonia but whose radiographic diagnosis is uncertain (Mandell et al. 2007).

The patterns of infection in the lung parenchyma can be divided into three radiologic patterns: lobar pneumonia, bronchopneumonia and interstitial pneumonia. Classically, these patterns are associated with different causative agents. Lobar pneumonia involving single or multible lobes is a typical presentation of pneumococcal pneumonia. Bronchopneumonia infections are centered on large inflamed airways with patchy involvement appearing as poorly defined areas of airspace consolidation and a patchy pattern. Bronchopneumonia is typically caused by *S. aureus* and gram negative bacteria. In interstitial pneumonia, the infection is situated predominantly in the interstitial tissue of the alveolar septa and surrounding small airways and vessels. Interstitial pneumonia is typically caused by *M. pneumoniae* and viruses (Franquet 2001, Tarver et al. 2005). However, often the radiologic appearance of pneumonia cannot be categorized into one of these patterns. There is also variation in the radiologic manifestations of pneumonia caused by a specific organism. Consequently, radiographic changes cannot be used to distinguish the etiology of CAP (Boersma et al. 2006, Macfarlane et al. 1984).

2.4. Microbiological methods for the etiological diagnosis of CAP

2.4.1. Blood culture

Detection of bacteremia and fungemia by blood culture has profound diagnostic and therapeutic importance. Positive blood cultures can establish the etiological agent for pneumonia and provide susceptibility testing of this agent and thus optimization of antimicrobial therapy. Yet, a single blood culture specimen may not be sufficient to detect all bacteremias and fungemias (Lee et al. 2007). Investigators have reported on the relationship between the volume of blood cultured and the yield of microorganisms (Cockerill et al. 2004, Lee et al. 2007, Li et al. 1994). Obtaining serial blood cultures makes it easier to interpret the clinical significance of organisms (Richter et al. 2002).

The conventional practice has been to obtain blood specimens at or around the time of temperature elevation. This practice is based on the principle that the presence of organism in the intravascular space leads to elaboration of cytokines which, in turn, raises the body temperature. However, there are studies showing that the rates of bacteremia detection are not enhanced by collecting blood cultures at the time when patients have temperature spikes (Riedel et al. 2008). Generally, an interval of 30 to 60 minutes between blood culture sets is recommended for optimal results. However Li *et al*. found no significant difference in the yield of blood culture sets drawn simultaneously and those drawn with an interval period between the sets (Li et al. 1994). The use of antibiotics either before presentation or in the healthcare facility reduces the diagnostic yield of blood culture (Glerant et al. 1999, Metersky et al. 2004, van der Eerden et al. 2005).

Blood cultures are usually incubated for five to seven days. A number of commercial blood culture systems are available for the detection of bacteremia. These automated systems allow continuous monitoring i.e., a growth reading is automatically taken every 10 to 20 minutes throughout the day to detect positive cultures as quickly as possible. The mean time to detection is less than 24 hours and most positive blood cultures are reported to the clinician as gram stains within 24 hours of sample collection (Bengtsson et al. 1998, Cockerill et al. 1997, Huang et al. 1998). The time to a positive reading varies by microorganism and magnitude of bacteremia; the median time is 11-14 hours for *S. pneumoniae* and 12-24 hours for *S. aureus* (Cockerill et al. 1997, Martinez et al. 2007, Peralta et al. 2006).

In CAP, the diagnostic yield of blood culture varies from 3 to 16% (Benenson et al. 2007, Bohte et al. 1995, Ishida et al. 1998, Metersky et al. 2004, Roson et al. 2001, van der Eerden et al. 2005, Örtqvist et al. 1990).

2.4.2. Sputum gram stain and sputum culture

Microscopic examination and culture of expectorated sputum have been the mainstay of laboratory evaluation of pneumonia. A section of the purulent portion of the sputum sample is spread thinly over a glass slide and stained by Gram's method. To maximize the diagnostic yield and to minimize the possibility of oropharyngeal contamination, all gram stains are first screened under a microscope at low power (x100) to assess the appropriateness of the sample. Sputum samples containing more than 25 neutrophils and fewer than 10 squamous epithelial cells in a microscopic field are considered of good quality (Brown and Lerner 1998, Saubolle and McKellar 2001). The good quality samples are then screened for predominant bacterial morphology under oil immersion microscopy. The presence of a predominant morphotype is considered when the gram stain shows at least eight to ten bacterial organisms in each high-power field (Brown and Lerner 1998). Sputum samples of good quality are plated onto culture media, usually blood agar and chocolate agar. Semi-quantitative culture methods are used to facilitate the interpretation of the result of the sputum culture and the results are usually analyzed in combination with the findings of the gram stain (Ewig et al. 2002).

Gram staining allows fast, preliminary identification of the etiological agent. On the other hand, sputum is often contaminated by upper airway flora and the yield of the gram stain of a sputum sample is highly dependent on the investigator, who must be stringend and

apply the relevant strict criteria (Fine et al. 1991). One of the main problems of the sputum gram stain and culture is that 40% or more of the patients with CAP cannot produce a sputum sample. In addition, less than 40% of the patients can produce a sputum sample of an adequate quality (Ewig et al. 2002, Garcia-Vazquez et al. 2004, Miyashita et al. 2008, Roson et al. 2000). The sensitivity of the sputum gram stain and culture is low. In patients with pneumococcal pneumonia the sensitivity of the gram stain of a valid sputum sample is 35% to 68% and the sensitivity of sputum culture is 33% to 79%. (Garcia-Vazquez et al. 2004, Miyashita et al. 2008, Musher et al. 2004, Roson et al. 2000).

The diagnostic yields of sputum gram stain and culture are lower in patients with preceding antimicrobial treatment (Miyashita et al. 2008, Musher et al. 2004, Roson et al. 2000), while no relationship has been reported between the severity of CAP and the diagnostic yield of sputum examination (Garcia-Vazquez et al. 2004, van der Eerden et al. 2005).

2.4.3. Antigen Detection

Detection of specific microbial antigens is a rapid method for detecting the presence of an organism directly in a clinical sample. Depending on the pathogen, respiratory samples from the upper and lower respiratory tract, pleural fluid and urinary samples are the specimens that are used for antigen detection in patients with CAP.

The simplest methods for antigen detection are based on agglutination. The presence of a specific antigen in a clinical sample is detected by agglutination of antibody-coated latex particles or red cells. Latex agglutination tests were previously used for detection of *S. pneumoniae.* The main limitation of the test was the low sensitivity, especially in urine samples. As the latex agglutination test of *H. influenzae* detects only capsular types, the use of this test is of limited value in CAP (Ajello et al. 1987, Boersma et al. 1991).

The immunofluorescence (IF) based antigen detection methods employ a histochemical technique that utilizes a detector antibody labelled with a fluorescent compound. These fluorescent-labelled antibodies (direct IF) or specific antibodies and fluorescent-labelled anti-immunoglobulin (indirect IF) are overlaid on a slide where the respiratory specimen is applied. In case an antigen is present in the specimen, there is emission of fluorescent light, which is detected with fluorescent microscopy. Interpretation of the result requires trained and experienced personnel (Leland and Ginocchio 2007). Studies on the IF tests to detect *Legionella* spp., *M. pneumoniae* and *C. pneumoniae* in respiratory samples have yielded low sensitivities (Garnett et al. 1998, Murdoch 2003[a], Waites and Talkington 2004). The sensitivity of IF to detect respiratory viruses is, on the other hand, considerably better (Leland and Ginocchio 2007). IF tests are most commonly used methods to detect *Pneumocystis jirovecii* (Nato et al. 1992)

Immunological antigen detection methods also include enzyme immunoassay (EIA/ ELISA), fluorescent immunoassay (FIA), and optical immunoassay (OIA). These immunological assays are most often used for virus detection (Grandien 1996, Leland and Ginocchio 2007, Mäkelä et al. 1998). Studies of these methods to detect various bacterial agents have been done, but these methods are seldom used clinically (Sjögren et al. 1987, Waterer et al. 2001, Venkatesan and Macfarlane 1992). The basic principle

of EIA/ELISA is the use of a monoclonal antibody conjugated with an enzyme which, upon reacting with its substrate, forms a measurable reaction product (Carpenter 2007). A color reaction product is produced and the color change is monitored visually or spectrophotometrically. The FIA systems use fluorescent-labelled antibodies. When these fluorescent-labelled antibodies bind to the antigen from the respiratory tract sample fluorescence emerges (Carpenter 2007). OIA test uses a mirrorlike surface of a silicon wafer coated with optical molecular thin film and a capture antibody specific for microorganism studied. After incubation with a substrate, the test surface is examined under a bright light source. A positive result is visualized as a change of colour (Herrmann et al. 2001).

In clinical practice, commercially available immunochromatographic antigen detection methods are the most common. This method is available for *S. pneumoniae* and *Legionella* spp. The commonly used Binax Now *S. pneumoniae* immunochromatographic test uses a rabbit anti-*S. pneumoniae* antibody, conjugated to visualizing particles. The antibody binds to the pneumococcal C polysaccharide antigen present in a urine sample. The resulting complex is immobilized by a band of rabbit anti-*S. pneumoniae* antibodies absorbed onto a nitrocellulose membrane. A control line which contains goat antibody against rabbit antibodies captures the excess visualizing conjugate. The result is read by the naked eye after 15 min. A color line on the sample and control lines indicates a positive antigen test (Smith et al. 2003). The urinary antigen detection test for *Legionella* spp. is performed similarly with *Legionella* specific antibodies (Murdoch 2003[a]).

2.4.4. Polymerase chain reaction (PCR) –based methods

Current molecular techniques are most often based on the PCR technology, which is a nucleic acid amplification test that detects microbial nucleic acid in clinical samples (Ieven 2007). The basic steps of PCR include extraction of DNA from a patient sample and amplification of a target gene. A partial DNA sequence must be known for designing primers that hybridize to specific regions of target DNA. Enzymes are used to copy this DNA via multiple rounds of extension and denaturation; this results in exponential amplification of the DNA fragment of interest (Yang and Rothman 2004). Detection of the PCR products can be achieved by hybridization, gel electrophoresis or by an EIA-based method (Ieven 2007). Primers of a broad range PCR test are shared by a given taxonomic group such as the 16S rRNA gene that is found in all bacteria. Products of broad range PCR are identified by hybridization with a specific probe or by DNA sequencing. Since 16S rRNA can detect almost all bacteria, this method can be applied only in an otherwise sterile specimen e.g. pleural fluid in CAP patients (Yang and Rothman 2004).

There are several variations of the PCR technique. Since many viruses are RNA-based, reverse transcriptase (RT)-PCR was developed. In RT-PCR, RNA is converted to a complementary DNA copy before PCR is performed. RT-PCR can be used to detect the viability of microbial cells through examination of microbial mRNA. Because RNA is rapidly degraded after cell death, this technique detects only viable organisms, unlike the conventional PCR method, which cannot distinguish between living and dead organisms (Yang and Rothman 2004). In nested PCR, two rounds of PCR are performed, increasing the sensitivity of the methods for detection of very small amounts of target DNA. Multiplex

PCR uses multiple sets of primers to test for several targets simultaneously (Ieven 2007, Yang and Rothman 2004). Multiplex PCR assays are generally less sensitive and the specificity may also be reduced compared to conventional PCR assays (Murdoch 2003[b]).

In quantitative real-time PCR amplification and detection of the products occur in a single tube. This system reduces the detection time and due to the closed system also the risk of contamination (Ieven 2007, Murdoch 2003[b]). The reaction is performed using fluorescent DNA intercalating dyes or fluorescent-labelled DNA probes. The signal intensity increases with increasing DNA amplification. Consequently, this method allows also quantification of the PCR products and the gene copies of the original sample. The sensitivity and specificity are comparable to the conventional PCR method. Multiplex PCR can also be performed with this technique (Ieven 2007).

PCR can provide a diagnosis earlier than cultures and in cases where standard techniques are too laborious, or slow, or remain negative. The technique is probably less affected by previous antimicrobial therapy. However, the lack of routine antimicrobial susceptibility testing is one of the disadvantages of the method (Ieven 2007, Murdoch 2003[b]). PCR is extremely sensitive and may detect pathogens at concentrations below those of standard methods. As a consequence of the high sensitivity, contamination is possible and will yield false positive results (Ieven 2007, Murdoch 2003[b], Yang and Rothman 2004). Sometimes it may be impossible to differentiate between colonization and disease on the basis of the positive PCR test. Results of real-time PCR can help to interpret positive tests by quantifying organisms and establishing cut-offs that distinguish colonization from infection (Murdoch 2003[b]). PCR inhibitors in samples can lead to false negative results. There may also be too little sample to allow the detection of DNA or an ineffective release of DNA from the cells during processing. Moreover, the PCR equipment and reagents are often expensive and PCR requires trained personnel (Ieven 2007, Murdoch 2003[b], Yang and Rothman 2004). On the other balance, replacement of traditional methods by PCR may reduce personnel time (Espy et al. 2006).

In situ PCR can be used to direct detection RNA or DNA of a microorganism on tissue or cytological samples (Hayden et al. 2001, Nuovo 2007). Fluorescence in situ hybridization is another method to directly visualize micro-organisms. This method is based on ribosomal ribonucleic acid-targeted oligonucleotide probes labelled with fluorescent dye (Declerck and Ollevier 2006, Buchbinder et al. 2004)

2.4.5. Serology

The major disadvantage of a diagnosis based on the detection of antibodies in a patient's serum is that antibodies cannot be detected in the acute phase of the disease. The immunoglobulin M (IgM) response does not appear during the first week of illness and IgG response appears even more slowly (Daxboeck et al. 2003, Dowell et al. 2001). In addition, in most respiratory diseases, the IgM response appears only in primary infection (Dowell et al. 2001, Vikerfors et al. 1988). Thus, usually two samples (paired sera) are required, the first sample taken in the acute phase of the disease and the second 2 to 3 weeks later. Detection of a significant rise in the antibody titer is considered as the most reliable demonstration of a causative agent (Beersma et al. 2005, Dowell et al. 2001, Talkington et al. 2004). Among the most common etiological agents, *M. pneumoniae* and *C. pneumoniae*

are mainly diagnosed by serology (Daxboeck et al. 2003, Kumar and Hammerschlag 2007). Above all, serological tests are well suited for epidemiological studies (Jokinen et al. 2001). These tests are also the only ones available to detect some of the more rare causative agents of CAP, e.g., *Coxiella burnetii* and *Francisella tularensis* (Mandell et al. 2007). Antibodies to microbial agents can be measured by precipitation, hemagglutination, neutralization, IF, complement fixation, EIA/ELISA and radioimmunoassay. Today, the most commonly used method is EIA/ELISA. In this method, specific antibodies in the serum sample bind to the solid phase antigen and are detected with an enzyme-labelled anti-immunoglobulin secondary antibody. Isotype-specific enzyme-labelled anti Ig-antibodies can be used to determine the specific Ig class present. The product of the enzymatic reaction is measured by automated analyzers (Carpenter 2007).

2.4.6. Bronchoscopy and bronchoalveolar lavage (BAL)

The introduction of the flexible fiberoptic bronchoscope in the late 1960s gave physicians direct access to the lower airway. With the development of protected specimen brush in 1979, the problem of contamination of the samples by upper airway flora could be diminished (Wimberley et al. 1982). Quantitative bacterial culture is used to distinguish colonization from infection. A bacterial growth of greater or equal to 10^3 cfu/ml in protected brush-sample specimens is significant**.** BAL is performed by advancing the bronchoscope distally into a subsegmental bronchus until the airway is occluded proximally. After that, 20 to 50 ml aliquots of sterile saline are instilled into the lung periphery followed by gentle aspiration (Torres and el-Ebiary 1998). Usually volumes of at least 100 ml and up to 240 ml are used in the diagnostic evaluation of pneumonia. The retrieved volume ranges from 5% to 70% of the total instilled volume. The sampling area of BAL is selected on the basis of the location of the infiltrate on chest radiography or by direct visualization of purulent secretions (Torres and el-Ebiary 1998). BAL is easily contaminated by oropharyngeal flora especially when performed in nonintubated patients. However, higher specificity can be obtained by using quantitative culture with a threshold value of 10^4 cfu/ml (Baughman and Conrado 1998, Rasmussen 2001). The recovery of bacteria from the BAL fluid by culture declines after commencement of antimicrobial treatment (Rasmussen et al. 2001). PCR tests applied to the BAL samples may increase the diagnostic yield of BAL especially in the pretreated patients (Strålin et al. 2006). BAL has proved especially useful for diagnosing pneumonia caused by *Pneumocystis jirovecii* and *Mycobacterium tuberculosis* (Baugham and Conrado 1998). Complications of BAL are generally infrequent and minor but sometimes may be harmful for the patient. Complications include postbronchoscopy fever, reduction in oxygenation, and rarely hemoptysis, pneumothorax or exacerbation of respiratory failure (Torres and el-Ebiary 1998).

2.5. Etiological agents

2.5.1. *Streptococcus pneumoniae*

S. pneumoniae was identified in 1881 concurrently by Louis Pasteur and George Stenberg. In the 1880s the causative role of this organism in human lobar pneumonia was established by Carl Friedlander and Albert Fankel and the organism was named *Pneumococcus* (Watson et al. 1993). The present name *Streptococcus pneumoniae* was

given in 1974. In addition to being a cause for pneumonia, *S. pneumoniae* causes also otitis, sinusitis, meningitis, and, less frequently, endocarditis, arthritis and a variety of other infectious diseases. In addition to being the most common pathogen causing CAP, *S. pneumoniae* may also cause nosocomial pneumonia (Bouza et al. 2005, Sopena and Sabria 2005).

S. pneumoniae are gram positive coccoid bacteria with an external polysaccharide capsule. The capsule protects the bacteria from phagocytosis and is consequently the most important factor for the virulence of *S. pneumoniae* (Bogaert et al. 2004, Tuomanen et al. 1995). On the basis of antigenic differences in capsular polysaccharides, 90 serotypes of *S. pneumoniae* have been identified**.** These serotypes are grouped into 46 serogroups, based on immunological similarities (Henrichsen 1995). There are geographical, temporal and age-related differences in the distribution of the different serotypes (Feikin and Klugman 2002). Moreover, different pneumococcal serotypes vary greatly in the prevalence at which they are recovered from invasive disease, like bacteremic pneumonia. Approximately 10 serogroups account for most of the invasive diseases (Hausdorff et al. 2000).

By activating complement and stimulating the production of cytokines, the cell wall of *S. pneumoniae* is a potent inducer of inflammation. Components of the cell wall are also important for the ability of bacteria to attach to endothelial cells during the course of invasive disease. Pneumolysin, autolysin and pneumococcal surface protein A are other noncapsular factors that contribute to the virulence of *S. pneumoniae* (Bogaert et al. 2004, Tuomanen et al. 1995).

The initial step in the pathogenesis of pneumococcal infection is attachment of the organism to the mucosal epithelium of nasopharynx. In general, this is followed by asymptomatic carriage. Nasopharyngeal colonization is common among children. The peak incidence of pneumococcal colonization occurs during the first three years of life, when up to 69% of the children are colonized. After that, the colonization rate gradually declines and reaches a stable level of less than 10% after the age 10 years (Bogaert et al. 2004, Regev-Yochay et al. 2004). In one of the few studies focusing on pneumococcal carriage in an adult population, the carriage rate was 4% and the same in the elderly population as among young adults (Regev-Yochay et al. 2004). However, in family settings, the carriage rates among adults have usually been higher, between 11% and 15% (Greenberg et al. 2006, Watt et al. 2004). The median duration of carriage is two to four weeks, longest in children younger than 1 year old and shortest in adults. Those with an acute infection are carriers for a mean of 10 days longer than asymptomatic contacts (Ekdahl et al. 1997). Pneumococcal carriage is an important source of horizontal spread of this pathogen in the community. Outbreaks of pneumococcal pneumonia have occurred in institutes like nursing homes and military camps (Nuorti et al. 1998, Vainio et al. 2009). However, as there are numerous steps intervening between transmission of the organism and development of the disease, pneumococcal pneumonia is not generally regarded as contagious (Musher 2003).

The incidence of bacteremic pneumococcal pneumonia, as well as other invasive pneumococcal infections, is highest among children under two years of age and in adults older than 65 years (Breiman et al. 1990, Klemets et al. 2008[a], Pastor et al. 1998, Plouffe

et al. 1996). In the study by Jokinen *et al*. pneumococcal pneumonia was significantly more frequent among patients aged ≥60 years than among patients aged 15-59 years (Jokinen et al. 2001). The incidence of pneumococcal infections is related to seasons; it is the highest during winter. The association between season and pneumococcal infection is probably mediated through the seasonal incidence of viral illness. The seasonal fluctuation of pneumococcal infections and association with the occurrence of respiratory viruses is more pronounced in adults than in children (Kim et al. 1996, Talbot et al. 2005). Racial and sosioeconomic factors have also an impact on the incidence of pneumococcal disease (Breiman et al. 1990, Pastor et al. 1998, Plouffe et al. 1996). In Finland, Klemets *et al.* have shown an increase in the incidence of invasive pneumococcal disease from 8.2 to 11.5/100 000 during the years 1995-2002 (Klemets et al. 2008[a]).

A majority of the hospitalized patients with pneumococcal pneumonia have at least one underlying condition (Jover et al. 2008, Miyashita et al. 2002, Musher et al. 2000, Shariatzadeh et al. 2005, Sopena et al. 2004). In the study of Jokinen *et al*. half of the patients with pneumococcal pneumonia were treated as outpatients, and only one-third of them had one or more predisposing chronic condition; smoking was not, however, included in this analysis (Jokinen et al. 2001). Underlying conditions that are frequently recognized in patients with pneumococcal pneumonia are smoking, alcoholism, chronic lung disease, cardiac failure, diabetes mellitus and malignancy (Jover et al. 2008, Miyashita et al. 2002, Musher et al. 2000, Shariatzadeh et al. 2005, Sopena et al. 2004). In a multivariate analysis of another case-control study, male sex, black race, chronic illness, low level of education, living in a household with children attending a day care center and active or passive smoking were significantly associated with pneumococcal disease (Nuorti et al. 2000). Although highly active antiretroviral treatment has reduced the incidence of pneumococcal disease in HIV-infected patients, the incidence is still significantly higher compared to HIV-negative patients (Grau et al. 2005, Jordano et al. 2004). HIV is also the most common risk factor for recurrent invasive pneumococcal disease. Another common risk factor is some malignant disorder. The documented rate of recurrence of invasive pneumococcal infection is 2.3% to 5.3% (Einarsdottir et al. 2005, King et al. 2003, Turett et al. 2001).

The classic description of the clinical features of pneumococcal pneumonia includes sudden onset, chills, fever, pleuritic chest pain and productive cough (Marrie 1999, Örtqvist et al. 2005). However, the clinical picture may vary greatly, and a lack of fever and respiratory symptoms may occur even in patients with bacteremia (Torres et al. 1998, Örtqvist et al. 1988). When patients with pneumococcal pneumonia are compared to those with *C. pneumoniae* or *M. pneumoniae* pneumonia, shortness of breath, chest pain, sputum production and hypotension are clinical features that are more frequent in pneumococcal pneumonia. A high white blood cell count and a high concentration of CRP in the serum are more common laboratory findings in patients with pneumococcal pneumonia than mycoplasma or chlamydophila pneumonia. Also, patients with pneumococcal pneumonia have less frequently received antimicrobial treatment before admission (Farr et al. 1989, Kauppinen et al. 1996, Miyashita et al. 2002, Örtqvist et al. 1988).

2.5.1.1. Bacteremic pneumococcal pneumonia

In adults, pneumonia is the most common focal disease associated with pneumococcal bacteremia –approximately 80% of the patients with pneumococcal bacteremia have pneumonia (Breiman et al. 1990, Pastor et al. 1998). In hospitalized patients with CAP, 8% to 37% of the patients with pneumococcal pneumonia are bacteremic. However, the microbial methods used besides blood culture to detect *S. pneumoniae,* vary in these studies (Lieberman et al. 1996, Lim et al. 2001, Roson et al. 2001, Sopena et al. 1999, Örtqvist et al. 1990). In studies of mild CAP with most of the patients treated as outpatients, 1% to 8% of the patients with pneumococcal pneumonia were bacteremic (Beovic et al. 2003, Ortega et al. 2005, Woodhead et al. 1987). In contrast, in studies of severe CAP, 48% of patients with pneumococcal pneumonia were bacteremic (Paganin et al. 2004). Moreover, *S. pneumoniae* is the etiological agent in most (62%-93%) bacteremic pneumonias (Almirall et al. 2000, Falguera et al. 2001, Ortega et al. 2005, Paganin et al. 2004, Roson et al. 2001, Sopena et al. 1999, Örtqvist et al. 1990).

In all except one of the studies that have compared patients with bacteremic pneumococcal pneumonia with nonbacteremic pneumococcal pneumonia, there was no significant difference in the mean age or sex distribution of the patients (Brandenburg et al. 2000, Jover et al. 2008, Musher et al. 2000, Örtqvist et al. 1988). Of the underlying conditions, alcoholism is significantly more common in patients with bacteremic pneumococcal pneumonia than nonbacteremic pneumococcal pneumonia (Jover et al. 2008, Musher et al. 2000, Örtqvist et al. 1988). Generally, the clinical features of the disease in these two patient groups have been reportedly very similar; in one study, however, respiratory symptoms were present in only half of the patients with bacteremic pneumococcal pneumonia compared to in nearly 90% of the patients with nonbacteremic disease (Brandenburg et al. 2000, Jover et al. 2008, Musher et al. 2000, Örtqvist et al. 1988). In the study by Musher *et al*., bilateral infiltrates and pleural effusions were significantly more common in bacteremic patients. However, in a multivariate analysis only air bronchogram turned out to be a significant differentiator between bacteremic and nonbacteremic patients (Musher et al. 2000). Also according to other studies chest radiography is similar in these two patient groups (Brandenburg et al. 2000, Jover et al. 2008, Musher et al. 2000, Örtqvist et al. 1988).

A complicated course is more common for patients with bacteremic pneumococcal pneumonia than nonbacteremic pneumococcal pneumonia. (Brandenburg et al. 2000, Musher et al. 2000, Shariatzadeh et al. 2005). Empyema and extrapulmonary (metastatic) infections, e.g., meningitis and endocarditis, are mainly seen in bacteremic pneumococcal pneumonia (Brandenburg et al. 2000, Musher et al. 2000, Örtqvist et al. 1988). There are studies showing equal or higher need for ICU admission in patients with bacteremic pneumococcal pneumonia compared to patients with either nonbacteremic pneumococcal pneumonia or to patients with any nonbacteremic pneumonia. The frequency of ICU admission among the patients with bacteremic pneumococcal pneumonia varies from 15% to 44% and in patients with nonbacteremic pneumonia from 10% to 25% (Brandenburg et al. 2000, Musher et al. 2000, Shariatzadeh et al. 2005). No significant differences have been found in mortality rates between patients with bacteremic pneumococcal pneumonia and nonbacteremic cases (Brandenburg et al. 2000, Jover et al. 2008, Musher et al. 2000, Shariatzadeh et al. 2005). In nonbacteremic pneumococcal pneumonia

mortality has varied from 3% to 15% whereas it has been 8% to 27% in bacteremic disease (Brandenburg et al. 2000, Jover et al. 2008, Musher et al. 2000, Shariatzadeh et al. 2005). Higher mortality in bacteremic pneumonia occurs during the first week of hospitalization (Musher et al. 2000). It has also been reported that more than half of the patients with bacteremic pneumococcal pneumonia with a fatal outcome die within three days after admission (Shariatzadeh et al. 2005, Örtqvist et al. 1988). Patients with either anatomical or functional asplenia are a special risk group for fulminant infection; in this mortality may reach 55% (Bisharat et al. 2001, Marrie 1999). In the study by Klemets *et al*. mortality of invasive pneumococcal disease among nonelderly adult patients was highest in patients with alcohol-related diseases, non-hematological malignancies, chronic liver disease, cardiac failure and HIV (Klemets et al 2008[b]).

2.5.1.2. Laboratory diagnosis of *S. pneumoniae*

A definitive pneumococcal etiology can be established by the recovery of the organism by culture from uncontaminated specimens like blood or pleural fluid. The sensitivity of blood culture is low, as only a minority of pneumococcal pneumonias are bacteremic. The use of PCR on blood, serum or plasma samples does not give a better diagnostic yield than blood culture (Dominguez et al. 2001, Murdoch et al. 2003). Pleural effusions are present in 13-30% of patients with pneumococcal pneumonia, only a fraction of whom have a sufficient amount of fluid for aspiration (Jover et al. 2008, Musher et al. 2000, Shariatzadeh et al. 2005, Örtqvist et al. 1988). Also, bacterial culture of pleural fluid is an insensitive method, since pleural fluid cultures are positive in only 11% to 32% of the cases (Boersma et al. 1993, Falguera et al. 2002, Porcel et al. 2007). Pneumococcal antigen detection on a pleural fluid sample has a significantly higher diagnostic yield: 71 - 89% of the pleural fluid samples of patients with proven pneumococcal pneumonia are positive by pneumococcal antigen detection. False positive results are possible but the specificity of the test on pleural fluid sample is high, 92-93% (Boersma et al. 1993, Porcel et al. 2007). In children, the use of PCR for the detection of *S. pneumoniae* in pleural fluid samples has increased the diagnostic yield of pleural fluid examination on pneumococcal pneumonia considerably (Lahti et al. 2006, Le Monnier et al. 2006). The only study evaluating the use of PCR on pleural fluid in adult patients with pneumococcal pneumonia yielded a sensitivity of 78% and specificity of 93% of the test (Falguera et al. 2002).

Microscopic demonstration of numerous gram positive diplococci in a sputum sample of good quality from a patient with CAP is strongly suggestive of pneumococcal pneumonia. This may be supported by the finding of *S. pneumoniae* as the predominant isolate in cultures of sputum specimens. Since *S. pneumoniae* in a sputum sample may represent nasopharyngeal colonization of the patient, the etiological diagnosis obtained by sputum culture is considered only as probable (Werno and Murdoch 2008). Although detection of *S. pneumoniae* by PCR from a sputum sample is highly sensitive, PCR poses an even more difficult challenge to distinguish infection from colonization (Murdoch 2003[b], Murdoch et al. 2003). However, with a real-time quantitative PCR method it is possible to quantify pneumococcal DNA and assign diagnostic cut-offs to correspond to quantitative culture. Real-time quantitative PCR has increased the diagnostic yield of sputum sample almost by the double compared to sputum culture. An additional benefit

of this method is that, compared to culture, it yields positive results more frequently also in patients on antibiotic treatment before sputum sampling (Johansson et al. 2008).

Previous pneumococcal antigen tests using latex agglutination, ELISA and countercurrent immunoelectrophoresis had cross reactions with non-pneumococcal organisms, especially when the tests were applied to upper respiratory tract samples. Tests based on latex agglutination were poor at detecting antigen in urine, and ELISA-based methods were time consuming (Boersma et al. 1991, Sjögren et al. 1987, Venkatesan and Macfarlane 1992). A newer commercial immunochromatographic membrane assay (Binax NOW *Streptococcus pneumoniae* Urinary Antigen test, Binax, Portland, ME, USA) detects the pneumococcal C polysaccharide and is specific for the pneumococcal cell wall which is common to all serotypes. False positive results are rare in pneumonia and also in other infections caused by organisms other than *S. pneumoniae*, and thus the specificity of the test is 90-100% (Genne et al. 2006[a], Gutierrez et al. 2003, Roson et al. 2004[b], Smith et al. 2003). Pneumococcal vaccine may cause a false positive result for few days after vaccination (Priner et al. 2008). After pneumococcal pneumonia, urinary antigen detection may remain positive for several weeks (Andreo et al. 2008). When compared to conventional methods, the sensitivity of the pneumococcal urinary antigen test has been 64-94%, and it has been especially high in patients with bacteremic pneumococcal pneumonia (77-92%) and in patients with severe disease (94%) (Genne et al. 2006[a], Gutierrez et al. 2003, Roson et al. 2004[b], Smith et al. 2003). Also, a small increase in sensitivity can be achieved by using a concentrated urine sample (Murdoch et al. 2001). The diagnostic yield of pneumococcal antigen detection is lower among patients who have received antibiotics to treat the current infection (Gutierrez et al. 2003, van der Eerden et al. 2005).

Measurement of the antibody responses to capsular C polysaccharide, to pneumolysin, to pneumococcal surface adhesion A and to capsular type-specific polysaccharides has been used in antibody assays based on EIA. Etiological diagnosis is based on a significant increase in antibodies in paired sera. Immune complex assays measure capsular C polysaccharide, pneumolysin and type-specific polysaccharides and the corresponding antibodies. High titers of the immune complex in acute or convalescent serum samples are considered diagnostic (Korppi et al. 2008). The sensitivity, and especially the specificity, of these serological tests have been suboptimal in adult patients (Korppi et al. 2008, Musher et al. 2001, Scott et al. 2000[a], Werno and Murdoch 2008). Serological responses are detected also in patients who are asymptomatic carriers (Musher et al. 1997). Serological tests have been used almost exclusively in research and as a tool to study vaccine efficacy (Korppi et al. 2008, Leinonen and Mäkelä 2001, Scott et al. 2000[a], Werno and Murdoch 2008).

2.5.2. *Mycoplasma pneumoniae*

Soon after the sulfonamides and penicillins were introduced in the 1940s, some cases of pneumonia did not respond to these antibiotics. Neither gram staining nor bacterial culture could detect any etiological agent in these cases. The etiological organism of this atypical pneumonia was first isolated from the sputum of a patient with pneumonia in 1944 by Eaton *et al.* (Eaton et al. 1944). This small organism, named the Eaton agent,

was capable to pass through viral filters and it was first considered to be a virus (Waites and Talkington 2004). It was not until the 1960s that this organism was successfully cultured. It was identified as a mycoplasma of the class *Mollicutes* and in 1963 it was designated *M. pneumoniae* (Chanock 1963).

Mycoplasmas are the smallest self-replicating organisms capable of cell-free existence. The cell volume of *M. pneumoniae* is less than 5% of a typical bacillus and it cannot be detected by light microscopy. The small genome of *M. pneumoniae*, about one sixth of the size of *Escherichia coli*, has limited biosynthetic potential. Thus, mycoplasmas depend on host cells to supply the necessary nutrients. Although *M. pneumoniae* depends on close host-cell contact for survival, it is primarily an extracellular pathogen (Waites and Talkington 2004). However, in vitro studies have shown that it can penetrate cell membranes and invade cells (Dallo and Baseman 2000, Yavlovich et al. 2004).

M. pneumoniae lacks a cell wall. Instead, it is bound by a cell membrane which consists of a protein network and sterols. The organism may also elaborate capsular material external to the cell membrane. *M. pneumoniae* has at the one end an attachment organelle to facilitate attachment to cell membranes. The P1 protein of the attachment organelle is the major element responsible for the interaction of *M. pneumoniae* with host cells. P1 is also the target of many of the antibodies produced by the host in response to the *M. pneumoniae* infection.

Attachment of *M. pneumoniae* to the respiratory epithelium is necessary for the initiation of infection. After adherence to the epithelial cells, *M. pneumoniae* causes local damage to ciliar function and structure and even loss of cilia. Metabolism of the epithelial cells may be affected and epithelial cells may become vacuolated or exfoliated. These mechanisms are mediated by release of enzymatic and cytolytic metabolites, e.g., hydrogen peroxide, and through the immunological properties of *M. pneumoniae* (Waites and Talkington 2004). Current evidence suggests that cytokine production and lymphocyte activation may play a role in the pathogenesis of pneumonia as well as in the pathogenesis of the extrapulmonary manifestations of *M. pneumoniae* (Tanaka et al. 1996, Tanaka et al. 2002, Waites and Talkington 2004). The more vigorous the cytokine and cell-mediated immune response, the more severe is the pulmonary injury (Tanaka et al. 2002). There is also some evidence that host-cell mediated immunity may influence the pattern of pulmonary lesions (Tanaka et al. 1996).

M. pneumoniae is transmitted from person to person by respiratory droplets generated by coughing. Infected persons carry the organism in their nose, throat, trachea and sputum. The incubation period varies from two to three weeks (Foy et al. 1966). *M. pneumoniae* can be isolated from infected persons for several weeks after the onset of illness and even after antibiotic therapy (Foy et al. 1966, Grayston et al. 1965, Nilsson et al. 2008). Instead, asymptomatic carriage is uncommon, although it may become more common during epidemics (Foy et al. 1966, Nilsson et al. 2008). Unlike many other respiratory pathogens, *M. pneumoniae* lacks an obvious seasonal pattern of occurrence (Foy et al. 1979). However, there are also epidemiological studies showing a higher rate of *M. pneumoniae* infections during fall and winter and lower rates during summer (Grayston et al. 1965, Pönkä 1980).

M. pneumoniae infections commonly spread among family members (Dorigo-Zetsma et al. 2001[b], Foy et al. 1966, Nilsson et al. 2008). In a follow-up study of families with an index case of *M. pneumoniae* disease, at least one secondary case was detected in 64% of the families. Within these families, 84% of the children and 41% of the adults became infected (Foy et al. 1966). Outbreaks have been documented in semiclosed or closed settings, like military units and religious communities (Klement et al. 2006, Waring et al. 2001). Epidemiological surveys have shown periodicity in the incidence of *M. pneumoniae* infections: the rate of infections peaks often at 3- to 7-year intervals (Foy et al. 1979, Hauksdottir et al. 1999, Jansson et al. 1971, Pönkä 1980). Communities in large geographical areas, such as the Nordic countries, tend to face *M. pneumoniae* epidemics concurrently (Hauksdottir et al. 1999, Pönkä 1980). Due to the long incubation period of *M. pneumoniae* and the prolonged persistence of the organism in the respiratory tract, the epidemics usually last for several months (Foy et al. 1979, Jansson et al. 1971, Pönkä 1980). The occurence of different P1 adhesin subtypes might be the reason for the cyclic occurrence of *M. pneumoniae* epidemics in the community. The incidence of *M. pneumoniae* pneumonia varies greatly by age. The incidence is highest during the first two decades of life and peaks in children aged 5 to 14 years (Foy et al. 1979). There is an associaton between smoking and *M. pneumoniae* infection according to a study conducted during an outbreak of *M. pneumoniae* among soldiers (Klement et al. 2006). Compared to adult patients with pneumonia caused by other bacterial pathogens, patients with mycoplasma pneumonia are younger and more often have no underlying diseases (Miyashita et al. 2002, von Baum et al. 2009).

M. pneumoniae infections may be manifested in the upper respiratory tract, the lower respiratory tract or both. According to an epidemiological survey the frequency of clinical pneumonia among persons infected with *M. pneumoniae* is 10% in children and 2% in adults (Foy et al. 1979). Higher frequencies, 11% in adults, have been reported during outbreaks of *M. pneumoniae* (Klement et al. 2006). Mycoplasma pneumonia is usually a mild disease with a low frequency of hospitalization and a minimal mortality (Foy 1993, von Baum et al. 2009).

The onset of mycoplasma pneumonia is gradual. Patients have usually been symptomatic for several days before admission (Pönkä 1978). Fever and typically dry, occasionally productive cough is present in almost all patients with mycoplasma infections (Clyde 1993, Mansel et al. 1989, Miyashita et al. 2002). Sore throat and headache are also common and occur in more than half of the patients with mycoplasma pneumonia. Chills, shortness of breath and chest pain are rare (Clyde 1993, Mansel et al. 1989, Miyashita et al. 2002). In patients with mycoplasma pneumonia, cough may continue for three to four weeks. The duration of cough is shorter when the patient has been treated with antibiotics (Pönkä 1978).

M. pneumoniae infections are associated with several extrapulmonary manifestations. Most common of these complications are skin and mucosal involvement and central nervous system manifestations. A wide range of dermatologic conditions have been reported in conjunction with mycoplasma pneumonia e.g. erythematous maculopapular and vesicular rashes and erythema multiforme. In addition, *M. pneumoniae* is the most common microbial agent associated with the Stevens-Johnson syndrome (Sanchez-

Vargas and Gomez-Duarte 2008, Waites and Talkington 2004). Meningoencephalitis and encephalitis are the most common of the central nervous system manifestations, followed by polyradiculitis and aseptic meningitis (Sanchez-Vargas and Gomez-Duarte 2008, Waites and Talkington 2004). Less common manifestations are myocarditis, pericarditis, hemolytic anemia and renal or other organ involvemet. Autoimmune reactions may be responsible for many of these complications. Yet, as *M. pneumoniae* has been detected by PCR in cerebrospinal fluid, synovial fluid, pericardial fluid and skin lesions, the role of direct invasion of the organism in the pathogenesis of extrapulmonary manifestations cannot be excluded. Extrapulmonary manifestations may occur either before, during or after pulmonary disease, as well as in the absence of any respiratory symptoms (Waites and Talkington 2004). In studies with both children and adult patients with mycoplasma infection, extrapulmonary manifestations have been observed in 5% to 12% of the patients. Not surprising, the highest frequency occurs in hospitalized patients (Grayston et al. 1965, Mansel et al. 1989, Pönkä 1978).

2.5.2.1. Laboratory diagnosis of *M. pneumoniae*

Culture of *M. pneumoniae* is laborious, expensive and extremely slow. It requires incubation of up to several weeks. Even in experienced laboratories the sensitivity of the culture is only 60%. Also, the utility of antigen detection methods is low due to poor sensitivity as a consequence of cross- reactivity with other mycoplasmas (Waites and Talkington 2004).

PCR can be used for detection of *M. pneumoniae* in respiratory samples as well as in samples of body fluids and tissue samples. Nasopharyngeal swabs, throat swabs, sputum and BAL are the respiratory samples suitable for the PCR testing. Compared to nasopharyngeal and throat swab samples, the yield of PCR is higher when sputum samples are analyzed (Dorigo-Zetsma et al. 2001[a], Räty et al. 2005). However, only about half of the patients with *M. pneumoniae* pneumonia are able to produce a sputum sample (Mansel et al. 1989).

Numerous in-house PCR assays to detect *M. pneumoniae* have been developed. The described methods have different extraction methods, target regions and amplification methods. The P1 gene and 16S rRNA are the most common targets (Loens et al. 2003). Regardless of various patient populations, sample types and PCR methods, the findings of the studies of the use of *M. pneumoniae* PCR have consistently shown that PCR is highly specific and more sensitive than culture (Loens et al. 2003, Morozumi et al. 2004, Waring et al. 2001). Some of the studies on *M. pneumoniae* PCR have reported a poor correlation between antibody response and positive PCR result, which may be due to asymptomatic carriage (Dorigo-Zetsma et al. 2001[a], Loens et al. 2003, Templeton et al. 2003). Also, *M. pneumoniae* can be detected by PCR before an antibody response is diagnostic. Conversely, the PCR test is more probably positive during the first weeks of the disease, while the antibody response remains positive for a longer period (Daxboeck et al. 2003, Nilsson et al. 2008, Waites and Talkington 2004). Moreover, a diagnostic antibody response is not observed in all patients with *M. pneumoniae* infection (Daxboeck et al. 2003). In a study population of adult patients with CAP, Dorigo-Zetsma *et al*. found that the patients with positive PCR and negative serology were significantly older than

those who were positive by both PCR and serology (Dorigo-Zetsma et al. 2001[a]).There are also studies showing a good agreement between PCR and serology (Morozumi et al. 2004, Nilsson et al. 2008).

Even before the bacteriological characterization of *M. pneumoniae*, the presence of cold agglutinins was used in the diagnostics of atypical pneumonia. Cold agglutinins are IgM antibodies that are considered to develop either as a result of antigenic alteration of the erythrocytes caused by *M. pneumoniae* or due to development of cross-reactive autoantibodies directed to antigen 1 on erythrocytes during *M. pneumoniae* infection. Cold agglutinins appear in about 50% of patients with *M. pneumoniae* infection one to two weeks after the onset of infection. Cold agglutinins are induced also in patients with other bacterial and viral infections. Although the test for cold agglutinins is fast and easy to perform, the popularity of testing cold agglutinins has declined with the development of more specific and sensitive serological methods (Daxboeck et al. 2003, Waites and Talkington 2004).

Serological diagnosis has been the cornerstone of *M. pneumoniae* diagnosis. IgM antibodies appear during the first week of illness, peak during the third week and decline to low levels within a few months (Beersma et al. 2005, Daxboeck et al. 2003). In the first week after the onset of illness, IgM is positive in only a minority, about 20%, of the patients. Although a positive IgM finding in the acute phase sera is diagnostic for *M. pneumoniae*, a convalescent serum sample is often needed to confirm the diagnosis (Beersma et al. 2005, Talkington et al. 2004). IgM antibodies are not always produced in a reinfection of adult patients (Vikerfors et al. 1988). IgG antibodies appear approximately 2 weeks later than IgM antibodies. Adults may produce IgG more quickly as an anamnestic response to reinfection (Talkington et al. 2004). In Finland, during a nonepidemic period, the seroprevalence to *M. pneumoniae* in an adult population was 40%. In subjects aged over 65 years, the seroprevalence was 60% (Tuuminen et al. 2000). Thus, on IgG-based diagnosis, a defined rise in antibody titer in paired sera is considered diagnostic.

The complement fixation test was the first method developed for serological testing for *M. pneumoniae*. Until recently it has also been the most popular serological test. Complement fixation measures mainly the early IgM response and, only to a minor extent, IgG antibodies. The diagnostic value of complement fixation test is limited in the elderly. The antigen used in this test may undergo cross-reactions with other bacteria and human tissues. A four-fold increase in titer in paired serum samples or a highly elevated single titer is considered diagnostic (Daxboeck et al. 2003, Waites and Talkington 2004).

Today, EIAs are the most widely used mycoplasma serology tests. Most of the EIA tests detect IgM and IgG antibodies separately, while some of the tests detect them simultaneously, or detect only IgM antibodies. Although some of the EIA assays show modest specificity, usually specificity is good, >95%. Instead, the assays show wide variation in sensitivity, which ranges from 32% to 84% (Beersma et al. 2005, Petitjean et al. 2002). The sensitivity and specificity of EIA IgM assays are usually similar to the complement fixation test. However, testing for IgG in addition to IgM in paired serum samples generates better diagnostic yields than those obtained by complement fixation tests (Beersma et al. 2005).

2.5.3. *Chlamydophila pneumoniae*

C. pneumoniae was isolated in 1965 from a conjunctival swab specimen during a trachoma vaccination trial in Taiwan. The association of *C. pneumoniae* with CAP was demonstrated by Saikku *et al.* in 1985, when they described an epidemic of mild pneumonia affecting adolescents and young adults in northern Finland in 1978 (Saikku et al. 1985). *C. pneumoniae* was initially thought to be an exclusively human pathogen. Now, several studies have demonstrated that *C. pneumoniae* may be a causative agent of infections in a variety of animal species, although zoonotic transmission has not thus far been reported (Kutlin et al. 2007). Pneumonia caused by *C. pneumoniae* may be sporadic or epidemic. Outbreaks of *C. pneumoniae* have been observed in families, schools, nursing homes and among military recruits (Kleemola et al. 1988, Mordhorst et al. 1992, Nakashima et al. 2006, Pether et al. 1989). Transmission is apparently rather inefficient, but does occur from person to person via respiratory secretions (Kuo et al. 1995). The prolonged case-to-case intervals in outbreaks indicate that *C. pneumoniae* has a long incubation time, nearly four weeks (Kauppinen and Saikku 1995, Kuo et al. 1995). Initial reports of *C. pneumoniae* pneumonia emphasized that the pneumonia is rather mild and affects mostly adolescents and young adults. However, *C. pneumoniae* may be associated with severe pneumonia (Balis et al. 2003, Kauppinen and Saikku 1995), also in the elderly (Kauppinen et al. 1996, Miyashita et al. 2002, Nakashima et al. 2006). Among Finnish adult patients with CAP, there was no difference by age in the frequency of *C. pneumoniae* (Jokinen et al. 2001).

C. pneumoniae is a common cause of a wide variety of respiratory infections, usually only mildly symptomatic or even asymptomatic (Kuo et al. 1995). During epidemics among military recruits in Finland, about 10% of *C. pneumoniae* infections manifested themselves as pneumonia (Kleemola et al. 1988). *C. pneumoniae* infections are often acquired early in life. Thus, seroprevalence of *C. pneumoniae* is high: about 50% of all adults have detectable levels of antibodies to the organism. The seroprevalence continues to increase into older age groups and reaches approximately 75% in the elderly (Karvonen et al. 1993, Kuo et al. 1995). Accordingly, among adults *C. pneumoniae* pneumonia is more often a reinfection than a primary infection (Kauppinen et al. 1996, Miyashita et al. 2002). In the community, *C. pneumoniae* infections appear in epidemic cycles of up to ten years with intervening periods of higher and lower prevalence (Gnarpe et al. 1999, Karvonen et al. 1993).

There is no association between *C. pneumoniae* pneumonia and specific underlying conditions (Kauppinen et al. 1996, Miyashita et al. 2002). A gradual onset of symptoms is typical. Upper respiratory symptoms, pharyngitis and hoarseness may precede pneumonia. Cough is very common and is often prolonged; sputum production is often scant and nonpurulent. Fever may be of low grade or even absent (Kauppinen and Saikku 1995, Kuo et al. 1995). Miyashita *et al*. reported that the average body temperature on admission is lower among patients with *C. pneumoniae* pneumonia than pneumococcal pneumonia (Miyashita et al. 2002). Kauppinen *et al*. reported that symptoms of central nervous system, headache and confusion are more common among patients with *C. pneumoniae* pneumonia than pneumococcal pneumonia. Also, in that study patients with *C. pneumoniae* had a longer duration of symptoms and they had more often received antibiotic treatment before admission (Kauppinen et al. 1996).

2.5.3.1. Laboratory diagnosis of *C. pneumoniae*

C. pneumoniae, being an obligate intracellular bacterium, is difficult to isolate from clinical samples. Also, as *C. pneumoniae* may invade deeper layers of the epithelium, it can be difficult to obtain a proper sample for isolation (Kauppinen et al. 1996). Technical complexity, limited availability and low yield do not make the use of culture as a routine diagnostic tool attractive (Kumar and Hammerschlag 2007).

The most common way to diagnose *C. pneumoniae* infection is serology. Assays available for detection of *C. pneumoniae*-specific antibodies include MIF tests, and EIAs/ELISAs (Kumar and Hammerschlag 2007). Owing to the high seroprevalence among adults, paired serum samples are usually required for a serological diagnosis of *C. pneumoniae* pneumonia. The serological response may also take a considerable time to develop. In primary infections, IgM antibodies appear up to 2 to 3 weeks after the onset of illness and remain positive for 2 to 6 months. IgG antibodies appear 6 to 8 weeks after infection. Usually, IgM antibodies do not appear in reinfections. Instead, the IgG antibody titer increases quickly, within two weeks, in reinfections (Dowell et al. 2001).

MIF is the most widely used method and the gold standard for the diagnosis of *C. pneumoniae.* The method is, however, technically demanding and its interpretation is subjective (Dowell et al. 2001). With MIF, single IgM titers of ≥1:16 or 4-fold increases of IgG titers are recommended as criteria for acute infection (Dowell et al. 2001). A number of EIA/ELISA methods have been commercially developed. These methods are relatively simple to perform and not very time consuming. Reading of the result is photometrical and thus more objective (Hoymans et al. 2003). In addition, antibodies to lipopolysaccharide, the antigen used in EIA/ELISA, develop early in infection which allows the detection of seropositivity earlier than with MIF (Ekman et al. 1993). The reported sensitivities and specificities of these methods range from 62% to 97% and from 71% to 87%, respectively (Hoymans et al. 2003).

PCR has been used for detection of *C. pneumoniae* in nasopharyngeal swab specimens, throat swab specimens, sputum and BAL specimens (Dowell et al. 2001, Kumar and Hammerschlag 2007). At least 31 in-house PCR assays for detection of *C. pneumoniae* have been published (Kumar and Hammerschlag 2007). Among 18 different PCR assays using various *C. pneumoniae*-specific primers, only 4 assays met the validation criteria set by a group of experts in the field. The sensitivity and specificity of most of the PCR methods are unknown (Dowell et al. 2001). PCR assays cannot differentiate between acute *C. pneumoniae* infections and nasopharyngeal carriage (Miyashita et al. 2001). In addition, significant intercenter discordance of detection rates has been reported, even when the same tests have been used (Kumar and Hammerschlag 2007). An additional open question is what is the optimal sample for PCR testing for *C. pneumoniae*. The question will relate to the fact that the epithelium of the upper respiratory tract contains *C. pneumoniae* only in extremely small numbers (Kauppinen and Saikku 1995).

2.5.4. *Legionella* spp.

An outbreak of pneumonia at the American Legion Convention in Philadelpia in 1976 led to the identification of a new pathogen, *Legionella pneumophila* (McDade et al.

1977). The disease was called Legionnaires' disease. Since then more than 50 *Legionella* spp. have been identified and *L. pneumophila* has been found to comprise at least 16 different serogroups (Diederen 2008). *L. pneumophila* accounts for 90% of the cases of legionellosis and serogroup 1 for more than 70% of them (Ricketts and Joseph 2007, von Baum et al. 2008). *Legionella* is an etiological agent for both community-acquired and nosocomial pneumonia and occurs both sporadically and in outbreaks. The incidence varies by geographic area and season. In Europe, the highest incidence (25-28 per million persons) has been reported in Spain and Croatia (Ricketts and Joseph 2007), whereas the incidence of legionellosis in Finland has been 3-9 per million persons (Hulkko et al. 2008). In countries with a moderate climate, like Finland, legionellosis is often travel-associated (Diederen 2008, Hulkko et al. 2008). The source of *Legionella* spp. is often a water supply system or air conditioning system. *Legionellae* are usually acquired by inhalation of an infectious aerosol, but also microaspiration of water contaminated with legionella may cause transmission. The incubation time of legionellosis is 2 to 10 days (Diederen 2008).

Typically, *L. pneumophila* causes severe pneumonia with high mortality rates. However, advances in the etiological diagnostics of legionella have substantially increased the rate of diagnosis of *L. pneumophila* and it has become evident that *L. pneumophila* is a potential pathogen of pneumonia also in ambulatory patients (von Baum et al. 2008). Mortality rates have decreased since 1990, when mortality was around 26% to current rates of around 10% in community-acquired *L. pneumophila* pneumonia (Benin et al. 2002). In addition to respiratory signs and findings legionella pneumonia may be associated with diarrhea, headache, elevated creatine kinase and hyponatremia (Sopena et al. 1998). It has been shown that it is not possible to distinguish patients with legionella pneumonia from patients with other etiological agents on a purely clinical basis (Diederen 2008). Pneumonia due to non-*pneumophila Legionella* spp. resembles pneumonia due to *L. pneumophila* (Muder and Yu 2002). Recognized risk factors for nosocomial and community-acquired Legionnaires' disease are older age, current smoking, diabetes, end-stage renal failure, and cancer, especially hematological malignancies (Marston et al. 1994). In a recent study focusing on sporadic community-acquired Legionnaires' disease, diabetes and smoking were the only independent risk factors for the disease (Den Boer et al. 2006).

2.5.4.1. Laboratory diagnosis of *Legionella* spp.

Legionellae are slow-growing fastidious bacteria, and successful culture requires selective media and prolonged incubation periods. Culture on buffered-charcoal yeast extract (BCYE) plates is the gold standard for the laboratory diagnosis of legionellosis (Diederen 2008). *Legionella* spp. can be isolated from blood cultures, but the yield is poor, only 0% to 6% (Waterer et al. 2001). Sputum and bronchoscopy samples are the samples of choice. A major limitation of sputum culture is that fewer than 50% of the patients with legionella pneumonia produce sputum (Sopena et al. 1998). Some patients with legionella pneumonia produce sputum that has little purulence. However the rejection criteria applied to conventional sputum culture should not be applied to sputum samples taken for legionella isolation (Ingram and Plouffe 1994, Murdoch 2003[a]). The sensitivity of culturing sputum for *Legionella* spp. is usually less than 50%, if serology is used as

the standard (Murdoch 2003[a]). Direct fluorescent antibody staining can also be used for detection of *Legionella* spp. from respiratory samples. The test is quick but technically demanding. The sensitivity of the test does not exceed that of culture (Murdoch 2003[a]).

Detection of *Legionella* antigen in urine sample permits early diagnosis of legionella pneumonia. *Legionella* antigenuria can be detected one day after the onset of the symptoms and persists for days to weeks. Methods based on radioimmunoassay and EIA have been available soon after the first recognized outbreak of *L. pneumophila* in Philadelphia. Today, a commercial immunochromatographic assay is most commonly used. This test is easy to perform and provides a result within 15 min (Murdoch 2003[a]). Urinary antigen detection tests are most sensitive for the detection of *L. pneumophila* serogroup 1, less sensitive for other serogroups and poorly sensitive for other *Legionella* spp. The average sensitivity of the test is 70% to 80%, and the specificity 99% (Diederen 2008). Today, urinary antigen detection is the most common method to identify *Legionella* infection (Ricketts and Joseph 2007).

Besides providing the test result rapidly, *Legionella* PCR testing allows concomitant detection of all *Legionella* spp. (Diederen et al. 2008, Murdoch 2003[a]). In routine clinical practice the sensitivity of the *Legionella* PCR test on respiratory samples is 86% to 92%, and the specificity 95% to 98% (Diederen et al. 2008). The PCR test has also been applied on urine and serum samples, but the sensitivity of the test on these samples is lower than on respiratory samples (Diederen et al. 2008, Murdoch 2003[a]).

Serological testing for *Legionella* spp. is a valuable epidemiological tool but has little impact on clinical decision making because of the time delay before the result is available. Indirect immunofluorescence is the most popular method. Other methods include ELISA, counterimmunophoresis and microagglutination techniques (Waterer et al. 2001). The measurement of specific IgM is an unreliable marker of an acute infection and a convalescent serum sample is required to confirm the diagnosis of legionella. A 4-fold or greater increase in reciprocal antibody titer to ≥128 is considered diagnostic (Murdoch 2003[a]). The reported sensitivities of serological assays vary from 60% to 80% (Diederen 2008, Waterer et al. 2001). Infection with *Legionella* spp. other than *L. pneumophila* leads to seroconversion in fewer than half of the cases and the identification of different species and serogroups by serological testing may not be possible (Waterer et al. 2001).

2.5.5. *Staphylococcus aureus*

Blood culture and sputum culture are available to detect *S. aureus* in patients with CAP. Serological diagnosis of *S. aureus* may be useful in bacteremia and endocarditis (Larinkari and Valtonen 1984) but less is known about the usefulness of staphylococcal serology in pneumonia. *S. aureus* is an infrequent agent of CAP; it is more common in patients with severe pneumonia and in elderly patients with CAP than young and less severely ill patients (Leroy et al. 1995, Tan et al. 1998, Vila-Corcoles et al. 2009, Zalacain et al. 2003). During the influenza season, especially during pandemics, *S. aureus* can lead to secondary bacterial infection in previously healthy persons (Kallen et al. 2009). In recent years several cases of severe necrotizing pneumonia caused by methicillin-resistant *S. aureus* (MRSA) have been reported in the USA and to a lesser extent in Europe.

The organism responsible for these cases has a specific virulence factor, the Panton-Valentine leukocidin. Patients with this type of necrotizing pneumonia are often young and previously healthy. Concomitant or preceding influenza or another viral infection is common (Kallen et al. 2009).

2.5.6. Gram negative bacteria

Gram negative bacteria causing CAP are usually detected by sputum culture. Some of the gram negative bacteria, like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae,* occasionally cause invasive, bacteremic disease which can be detected by blood culture (Paganin et al. 2004, Tan et al. 1998)**.** During the recent years, the use of multiplex PCR for detection of the main CAP agents including *Haemophilus influenzae* has been developed. Considering *H. influenzae,* the results of PCR tests have been comparable to sputum culture, although a positive result of *H. influenzae* by PCR may represent colonization (Morozumi et al. 2006, Strålin et al. 2006). Epidemiological studies have utilized serological testing for *H. influenzae* and *Moraxella catarrhalis* (Almirall et al. 2000, Jokinen et al. 2001).

The most common gram negative bacterial species causing CAP is *H. influenzae*. In adult patients, CAP is usually caused by some nontypeable strain of *H. influenzae* (Mandell 2004). Up to 80% of healthy persons are carriers of these nontypeable *H. influenzae* strains. In patients with chronic pulmonary disease, the lower respiratory tract may be colonized by *H. influenzae* (Murphy et al. 1999). Consequently, the prevalence of *H. influenzae* pneumonia is higher among patients with COPD (Mandell et al. 2007). *M. catarrhalis* is a less common colonizer of the oropharynx (1-5% of healthy adults). Similar to *H. influenzae,* adults with chronic lung disease have a higher rate of respiratory tract colonization with *M. catarrhalis* than healthy adults. In general, M. *catarrhalis* is an infrequent cause of CAP. Yet, patients with COPD and elderly patients may develop this disease (Murphy 1998). Neither *H. influenzae* nor *M. catarrhalis* tend to cause fulminant pneumonia (Mandell 2004, Murphy 1998).

Unlike *H. influenzae,* other gram negative rods, such as *K. pneumoniae* and *P. aeruginosa,* are uncommon causes of CAP. However, they are potential causative agents of severe pneumonia, especially among patients admitted to an ICU and elderly patients. Gram negative rods other than *H. influenzae* are detected from 6 % to 27% of CAP patients admitted to an ICU and from 12% to 24% of elderly patients (Leroy et al. 1995, Paganin et al. 2004, Rello et al. 2003, Ruiz et al. 1999[b], Valencia et al. 2007, Vila-Corcoles et al. 2009, Zalacain et al. 2003). Arancibia *et al.* compared the clinical features of patients with CAP due to gram negative bacteria and patients with CAP due to other etiological agents, including *H. influenzae.* They found that patients with CAP due to gram negative bacteria had a lower body temperature (37.2°C vs. 37.6°C), were less likely to report chills (19% vs. 41%) or preceding symptoms of upper airway infections (11% vs. 34%) and were more likely to present with dyspnea (85% vs. 72%). Acute respiratory failure (77% vs. 57%) and severe sepsis or septic shock (42% vs. 22%) were present significantly more often (Arancibia et al. 2002). Typically, gram negative rods are encountered in patients with comorbidities, particularly in patients with a pulmonary comorbidity. COPD and bronchiectasis are the main pulmonary comorbidities predisposing to these

pathogens (Arancibia et al. 2002, Mandell 2004, Ruiz et al. 1999[a]). The mortality rates in pneumonia caused by gram negative rods are higher compared to other etiological agents, up to 18% among hospitalized patients and 44% to 75% among patients admitted to an ICU (Arancibia et al. 2002, Kang et al. 2008, Paganin et al. 2004, Rello et al. 2003, Ruiz et al. 1999[a], Tan et al. 1998).

2.5.7. Other bacteria and yeasts as causative agents of CAP

Other less common causes of CAP include *Streptococcus pyogenes*, *Francisella tularensis, Chlamydia psittaci, Coxiella burnetii,* anaerobes, and yeasts. These pathogens have to be considered if the patient has a known risk factor, e.g., exposure to particular animals or travel to endemic areas (Mandell et al. 2007). In Finland, *F. tularensis* is a possible etiological agent that has to be considered depending on the geographical area and epidemiological situation (Hulkko et al. 2008, Syrjälä et al. 1985). Farming activities such as making and handling dry hay are common sources of infection in tularemia cases with respiratory symptoms (Syrjälä et al. 1985).

2.5.8. Viral pneumonia

In previous studies, the percentage of CAP of viral etiology has ranged from 2% to 23%. Influenza viruses have been the most common viral agents (Jokinen et al. 2001, Lim et al. 2001, Roson et al. 2001, Woodhead et al. 1987) **(Table 5)**. In recent studies, with the use of PCR tests, viral agents have been identified in 15% to 56% of the patients with CAP. Besides influenza viruses, rhinovirus and cononavirus have been the most common viral agents in these studies (Angeles Marcos et al. 2006, Charles et al. 2008[a], Jennings et al. 2008, Johnstone et al. 2008[b], Templeton et al. 2005). More than half of the patients with CAP with a viral etiology have some comorbidity (de Roux et al. 2004, Jennings et al. 2008, Johnstone et al. 2008[b]). Patients with pure viral pneumonia are significantly more likely to have a history of cardiac disease or chronic heart failure than patients with mixed or pure bacterial pneumonia (de Roux et al. 2004, Johnstone et al. 2008[b]). Moreover, in more than half of the patients with CAP associated with a respiratory virus, the illness has been severe (de Roux et al. 2004, Jennings et al. 2008, Johnstone et al. 2008[b]). CAP of viral etiology cannot be differentiated from CAP of other etiologies by clinical picture, radiologic presentation or laboratory values (de Roux et al. 2004). Bacterial agents co-occur often with viral agents. Also, during previous influenza pandemics, bacterial pneumonia probably had a predominat role as a cause of death (Morens et al. 2008). The mechanisms by which viral infection predisposes to bacterial infections include destruction of respiratory epithelium, virus-induced immunosuppression and an inflammatory response to viral infection that may up-regulate expression of molecules that bacteria utilize as receptors (Kirchberger et al. 2007, Peltola and McCullers 2004).

2.5.8.1. Influenza viruses

Influenza viruses are members of the family *Orthomyxoviridae* and are classified into three distinct types, influenza A, influenza B and influenza C viruses. Influenza A viruses are further subtyped based on differences in the surface glycoproteins hemagglutinin and neuraminidase. Antigenic variation due to point mutations in the hemagglutinin and neuraminidase genes occurs in influenza A and B viruses. As a consequence of this

Table 5. Percentage of various respiratory viruses in hospitalized patients with community-acquired pneumonia (CAP)

	Study population	Diagnostic methods	Influenza A	Influenza B	RSV	PIV	Adeno-virus	Rhino-virus	Corona-virus	Metapneumo-virus
Bohte, 1995 Netherlands	≥18 years n=334	ser	4.2%[a]	4.2%[a]	0.9%	2.4%	0.6%	NS	NS	NS
Charles, 2008 Australia	>18 years n=885	vPCR, ser[b]	7.7%[a]	7.7%[a]	1.9%	0.5%	0.3%	5.2%[c]	NS	NS
de Roux, 2005 Spain	≥18 years n=338	ser	8.0%	3.0%	1.5%	3.3%	1.5%	NS	NS	NS
Díaz, 2007 Chile	≥ 16 years n=176	vag,	4.5%[a]	4.5%[a]	0	7.4%	1.1%	NS	NS	NS
Gutierrez, 2005 Spain	≥ 15 years n=361	ser	2.8%[a]	2.8%[a]	1.1%	NS	0	NS	NS	NS
Jennings, 2008 New Zealand	≥ 18 years n=304	vc, vag, vPCR, ser	7.6%	2.0%	3.9%	2.0%	3.6%	10.2%	2.0%	0
Johnstone, 2008 Canada	≥ 18 years n=193	vPCR	1.6%	2.0%	2.6%	1.6%	1.0%	2.0%	2.0%	3.6%
Lauderdale, 2004 Taiwan	>16years n=168	ser	6.5%	0	1.2%	1.2%	1.2%	NS	NS	NS
Lieberman, 1996 Israel	adult n=346	ser	2.3%	2.9%	1.4%	2.6%	1.4%	NS	NS	NS
Lim, 2001 UK	≥ 16 years n=267	ser	18.7%	0.7%	4.1%	NS	0.4%	0.7%	NS	NS
Rosón, 2001 Spain	adult n=533	ser	0	NS	0.8%	0.4%	NS	NS	NS	NS
Ruiz, 1999 Spain	adult n=395	ser	2.5%	1.5%	0.3%	1.8%	0.5%	NS	NS	NS
Scott, 2000 Kenya	≥ 15 years n=281	ser	4.3%	0.7%	0	NS	0.7%	NS	NS	NS
Templeton, 2005 Netherlands	≥ 18 years n=105	vc, vPCR, ser	10.5%	2.9%	2.9%	7.6%	3.8%	17.1%	13.3%	0
Örtqvist,1990 Sweden	≥ 18 years n=277	vc, ser	2.5%[a]	2.5%[a]	2.9%	4.7%	5.4%	NS	NS	NS

vc=viral culture, vag=viral antigen detection, vPCR=viral PCR test, ser=serology, NS=not studied
[a]Numbers include both influenza A and influenza B viruses
[b]influenza A and influenza B serology
[c]PCR test for picornaviruses including both rhinoviruses and enteroviruses

antigenic drift, influenza A and B viruses cause epidemic disease in humans annually and are more common than influenza C which causes only sporadic and usually mild disease. Unlike influenza B, influenza A has a natural reservoir in animals, especially domestic and wild birds, and swine. This reservoir allows more profound antigenic variation. This antigenic shift is the basis of the emergence of pandemic strains of influenza A (Beigel 2008, Cox and Subbarao 1999, Trifonov et al. 2009). Although 16 subtypes of hemagglutinin and 9 types of neuraminidase of influenza A have been identified, only subtypes 1-3 of the hemagglutinin proteins and subtypes 1-2 of the neuraminidase proteins have caused epidemic and pandemic activity in man since 1900. Human cases and outbreaks of avian influenza have also occurred. Avian influenza virus acquiring the capacity for effective human-to-human transmission is another mechanism for the development of a new pandemic influenza virus (Beigel 2008, Cox and Subbarao 1999, Gambotto et al. 2008). The ongoing pandemia is caused by a new strain of human H1N1 influenza A virus which was first identified in Mexico in April 2009. Genomic analysis of the virus indicates that it is closely related to common reassortant swine influenza A viruses isolated in Europe, North America, and Asia (Trifonov 2009).

In the northern climates influenza epidemics occur during the winter, whereas in the tropics influenza appears throughout the year (Beigel 2008). Influenza epidemics are associated with excess morbidity and mortality. There is some year-to-year variation in influenza-associated hospitalization and mortality; excess hospitalization and mortality is higher in the years when influenza viruses H3N2 predominate compared to the years when influenza viruses H1N1 or influenza B predominate (Thompson et al. 2004, Thompson et al. 2003). An annual estimate of hospitalization for pneumonia and influenza in the USA is 95 000 for primary pneumonia and 130 000 for any listed pneumonia. Considering that influenza virus activity is associated with an increase in hospitalizations also for a broad range of cardiopulmonary causes, influenza is estimated to be associated annually with 200 000 to 300 000 hospitalizations. Hospitalization rates are highest among patients aged 65 years or older, above all in patients 85 years or older (Thompson et al. 2004). Annual estimates of underlying pneumonia and influenza deaths in the USA were 8000 and all-cause of deaths associated with influenza 50 000. Most of the underlying pneumonia and influenza deaths occurred among persons aged 65 years or older (Thompson et al. 2003).

Small-particle aerosols, droplets and direct contact are the transmission pathways for influenza (Weber and Stilianakis 2008). The influenza virus attaches and invades the epithelial cells of the respiratory tract. Viral replication in these epithelial cells leads to release of proinflammatory cytokines and necrosis of ciliated cells. The incubation period is from 1 to 4 days. Viral shedding begins before the onset of illness and continues 1 to 3 days after the onset of illness. In children and immunocompromised patients shedding of the virus can continue even for weeks (Beigel 2008).

The classic clinical symptoms of influenza are fever with an abrupt onset, accompanied by myalgia, sore throat and non-productive cough. The proportion of patients complaining of these and other symptoms like headache and malaise varies by age. The duration of fever is typically 2 to 3 days (Beigel 2008, Cox and Subbarao 1999). Pneumonia associated with influenza may be primary viral pneumonia, mixed viral and bacterial

pneumonia or secondary bacterial pneumonia. In the study by Oliveira *et al.* nearly half of the adult patients hospitalized for influenza A had pneumonia. The patients who developed pneumonia were older and they had more often asthma or COPD compared to patients with only upper respiratory disease of influenza A. Symptoms of the patients with pneumonia and upper respiratory tract infection were similar except that the patients with pneumonia had significantly more often shortness of breath. Admission to an ICU and mortality rates were high among the patients with influenza A and pneumonia, 59% and 29%, respectively (Oliveira et al. 2001). Although the majority of the patients with influenza pneumonia are old and have comorbid conditions, such as heart disease, COPD, renal disease, diabetes or immunosuppression, serious influenza pneumonia may also occur in previously healthy patients (Oliveira et al. 2001).

2.5.8.2. Respiratory syncytial virus (RSV)

RSV belongs to the *Paramyxoviridae* family and was first isolated in 1956 (Blount et al. 1956). RSV is divided into two major groups, A and B. During outbreaks these strains circulate simultaneously with alternating predominance (Waris 1991). In Finland, RSV follows a regular 2-year pattern: during the odd-numbered years there is a limited spring outbreak, followed by a more forceful winter outbreak around the next New Year (Hulkko et al. 2008, Waris 1991).

Transmission of RSV occurs by large droplets and fomites and requires thus close person-to-person contact or contact with contaminated environmental surfaces and autoinoculation (Falsey and Walsh 2000, Hall 2001). After an incubation period, RSV replicates in the nasopharyngeal epithelium. With spread to the lower respiratory tract, necrosis of the epithelium and increased mucus secretion obstruct the small airways. Complete restoration of the airways may take weeks (Falsey and Walsh 2000, Hall 2001). The mean duration of viral shedding is four days (Hall et al. 2001).

Virtually all children have experienced RSV in the first few years of their life and reinfections occur throughout life (Falsey and Walsh 2000, Hall 2001). RSV is one of the most important viral agents causing severe lower respiratory tract infection in infants and young children (Jartti et al. 2004, Juven et al. 2000). Reinfections of RSV in adults present often as mild upper respiratory tract infections, but lower respiratory tract symptoms do occur in 22% of previously healthy adult patients with RSV infection (Hall et al. 2001). Compared to influenza, RSV is less often associated with fever and headache, but more often with nasal congestion, ear and sinus involvement and productive cough. The mean duration of illness is 9.5 days, which is significantly longer than in influenza (Hall et al. 2001). In a study on adult hospitalized patients with pneumonia, wheezing by history or wheezing or rhonchi on physical examination were the clinical features that most clearly separated the RSV-infected patients from patients with pneumonia caused by influenza, bacteria or an atypical agent. Of 57 RSV-infected patients 21% required treatment in an ICU, and 7% required respiratory support (Dowell et al. 1996). RSV is especially among the elderly an important cause of acute lower respiratory tract disease. The estimated yearly attack rate of RSV in an institution for elderly people is 5% to 10% of all residents; pneumonia rates vary from 10% to 20% among those infected with RSV and death rates from 2% to 5% (Falsey and Walsh 2000). In the USA, the annual estimate

of pneumonia and RSV deaths is 2700, 90% of which occurs in persons aged 65 years or older (Thompson et al. 2003).

2.5.8.3. Parainfluenza virus (PIV)

PIVs were isolated in the late 1950s. They belong to the *Paramyxoviridae* family and are genetically and antigenically divided into types 1 to 4 (Henrickson 2003). Close contact or surface contamination and autoinoculation by large droplets are apparent modes of transmission, while airborne transmission is considered to be unlikely. The respiratory epithelium is the major site of virus binding and infection. Focal tissue destruction caused by PIV is usually mild and rapidly repaired (Henrickson 2003).

Most children are infected by PIV 1-3 during the first three years of life. According to seroprevalence studies, PIV4 infections occur mainly later in childhood. Reinfections occur throughout life. PIV infections are usually mild infections of the upper respiratory tract. In children, in immunocompromised adults and in the elderly PIV1 and PIV3 are also important causes of lower respiratory tract infections. PIVs vary by seasonal epidemiology and by type. Peak seasons for PIV1 and PIV2 occur biennially in the fall or early winter and annually in the spring for PIV3 (Hall 2001, Henrickson 2003). In a study among adults hospitalized for lower respiratory tract infection, PIV 1 was detected in 2.5%, PIV 3 in 3.1%, and PIV2 in 0.2% of the patients. Of the patients with PIV3 infection, 59% had an infiltrate on chest radiograph, 23% required treatment in an ICU, and 15% required respiratory support (Marx et al. 1999).

2.5.8.4. Adenovirus

Adenoviruses are a common cause of many infections in childhood. Since the first isolation of the adenovirus in 1953, 51 different serotypes have been recognized. Less than 10 of the serotypes cause most of the human infections (Schmitz et al. 1983). Transmission of adenovirus infections occurs by direct contact, by small-droplet aerosols and by the orofecal route. The incubation period of the adenovirus is 4 to 5 days (Baum 2005). Epidemics of adenovirus infections have been reported in military recruits. In adults, adenoviruses rarely cause CAP, but when this is the case adenovirus pneumonia may be severe and even fatal even among immunocompetent patients (Hakim and Tleyjeh 2008, Retalis et al. 1996).

2.5.8.5. Rhinovirus and enterovirus

Rhinoviruses and enteroviruses belong to the *Picornaviridae* family and share basic properties, e.g., 40% to 60% homology between their genomes. More than 100 rhinovirus serotypes and more than 60 enterovirus serotypes have been identified (Kirchberger et al. 2007, Modlin 2005). Rhinoviruses are the most common causes of the common cold appearing throughout the year with seasonal peaks in fall and spring (Kirchberger et al. 2007, Mäkelä et al. 1998, Peltola et al. 2008). Upper respiratory tract infection is also the most common manifestation of enterovirus infection. The enteroviruses are causative agents of a wide range of other illnesses, e.g., meningitis, myopericarditis and poliomyelitis (Modlin 2005). Infections caused by rhinoviruses and enteroviruses may be asymptomatic. On the other hand, asymptomatic shedding of these viruses may occur

during the incubation period of an infection or after a symptomatic infection (Modlin 2005, Peltola et al. 2008).

Both rhinoviruses and enteroviruses are common causes of lower respiratory tract infection in children (Jartti et al. 2004, Juven et al. 2000). In adult patients with CAP, rhinovirus has been detected in 10-18% (Jennings et al. 2008, Templeton et al. 2005). In contrast, Angeles Marcos *et al.* report that enterovirus is only seldom detected from adult patients with CAP. Among the 340 patients they studied, enterovirus was detected in only one (Angeles Marcos et al. 2006). Rhinovirus infection increases the adherence of the pneumococcus to epithelial cells lining the respiratory tract (Ishizuka et al. 2003). Rhinovirus infection may also modulate the immune system and this may play a role in the development of secondary bacterial pneumonia (Kirchberger et al. 2007, Oliver et al. 2008). In the study by Jenning *et al.*, *S. pneumoniae* was detected in 11 of the 31 CAP patients with rhinovirus detected from the upper respiratory tract (Jennings et al. 2008). Outbreaks of rhinovirus with severe respiratory disease have been documented in nursing homes and long-term care facilities (Hicks et al. 2006, Wald et al. 1995). Rhinoviruses and enteroviruses have been identified in immunocompromised patients with severe lower respiratory tract infection (Ison et al. 2003, Parody et al. 2007).

2.5.8.6. Other viruses

Coronaviruses, members of the *Coronaviridae* family, are frequent causative agents of the common cold. These viruses have been identified in 1% to 13% of adult patients with CAP (Angeles Marcos et al. 2006, Jennings et al. 2008, Templeton et al. 2005). The severe acute respiratory syndrome (SARS) epidemic, which began in China in 2002 was due to a coronavirus (Peiris et al. 2003). The human metapneumovirus, a member of the *Metapneumoviridae* family, was discovered in 2001 and the bocavirus, a member of the family *Parvoviridae*, in 2005. These viruses seem to be rare causes of respiratory tract infection among adults (Kahn 2006, Schildgen et al. 2008), although at least one outbreak of lower respiratory tract infections caused by human metapneumovirus has been documented in a long-term care facility (Boivin et al. 2007).

2.5.8.7. Detection of respiratory viruses

Until the 1990s, virus isolation and serology have been the mainstays of the clinical laboratory for diagnosing respiratory virus infections. During the past two decades, antigen detection and molecular methods have become more widely used for viral diagnosis. Compared to viral culture and serology, these new methods provide a test result more rapidly, usually within hours. In addition, virus isolation in cell cultures involves technical expertise whereas antigen detection and molecular tests are less cumbersome. Nasopharyngeal washes, aspirates and swabs are the most often used respiratory samples for viral culture, antigen detection and molecular methods (Ginocchio 2007, Leland and Ginocchio 2007, Mahony 2008).

Virus isolation in cell cultures requires viable, infective viruses. Thus, appropriate selection, collection, transport and processing of clinical samples are important for successful virus isolation. Consequently, viral culture is less sensitive for labile viruses such as RSV. Also, some viruses, like the rhinovirus, require special cell lines or

culture environments. On the other hand, because infectivity is required, culture is the only method that can differentiate an infectious virus from a noninfectious virus. Shell vial culture, cocultivated cell lines and transgenic cell lines are new methods of viral culture that have reduced the time needed for virus detection from the previous 5-10 days required by the traditional tube culture to 24-48 hours (Leland and Ginocchio 2007).

Antigen detection of respiratory viruses includes membrane based EIAs, OIAs, FIA and IF based tests. All these methods are performed directly on clinical samples. EIAs and OIAs are commercially available rapid tests that are used as point-of-care testing. These tests are available for influenza A, influenza B and RSV. Up to $10^5 – 10^6$ viral particles are needed to obtain a positive result. Accordingly, the sensitivities of EIAs and OIAs are low compared to the other methods. During an epidemic season, the assay specificity is high, up to 100% but is markedly reduced outside the season (Ginocchio 2007, Leland and Ginocchio 2007). The sensitivity of time-resolved FIA is no less than 95%, which compared to viral culture denotes marked improvement compared to EIA. In addition to influenza A, influenza B and RSV, TR-FIA detects also PIV types 1-3 and adenovirus (Mäkelä et al. 1998, Waris et al. 1988).

The IF methods are most common one-step direct methods in which the monoclonal antibody of the specific virus is labelled with fluorescence dye. IF reagents containing monoclonal antibodies for multiple viruses allow simultaneous detection of several viruses. IF screening for the seven respiratory viruses (adenovirus, influenza A virus, influenza B virus, PIV types 1-3 and RSV) is now common. The sensitivities and specificities of IF vary depending on the virus. Compared to viral culture IF is more sensitive for RSV whereas it is less sensitive for adenovirus. Overall, IF is somewhat less sensitive and less specific than viral culture. Sensitivity of pooled respiratory viral antigen screening has been reported as 81%. Test results of IF are available within less than 2 hours. However, IF reagents are not available for all respiratory viruses like rhinovirus and coronavirus (Ginocchio 2007, Leland and Ginocchio 2007).

Viruses can be detected directly from clinical specimens by using highly specific nucleic acid probes that are complementary to the target viral RNA or DNA sequences. The amount of viruses needed for detection by PCR is drastically less than required for antigen detection. Moreover, PCR allows detection of respiratory viruses that are not detected or are not easily detected by cell culture or antigen detection such as rhinovirus, coronavirus, human metapneumovirus and bocavirus. Consequently, PCR demonstrates superior sensitivity over viral culture and antigen detection methods. In addition, nucleic acid methods allow laboratories to rapidly detect and identify previously unknown viruses. These methods have played a crucial role in identifying the SARS virus and avian influenza H5N1 virus. The use of multiplex PCR allows detection of several, up to 19, respiratory viruses simultaneously. The drawback of PCR that also limits a wider use of the method is the expense of this testing (Mahony 2008).

2.6. Guideline recommendations for microbiological investigations in CAP patients

The Finnish, British, European, and American guidelines do not recommend microbiological investigations in primary care or for outpatients (Honkanen et al. 2008, Mandell et al. 2007, Woodhead et al. 2005). The American and the British guidelines consider though that for epidemiologic reasons or management decisions, exceptions may be applied to some pathogens like influenza. Also the Finnish guidelines regard the use of a rapid influenza detection method as useful during the early phase of an epidemic. In addition, the Finnish guidelines recommend the use of *Legionella* spp. urinary antigen detection in case legionella pneumonia is suspected. As a whole, the Finnish guidelines consider that microbiological tests are rarely useful in the treatment of CAP patients and do not give any specific recommendations for the use of various diagnostic tests. The British guidelines regard that the investigations performed should be guided by the severity of pneumonia, epidemiological risk factors, and respond to treatment whereas the American guidelines give a general recommendation to strongly encourage diagnostic testing whenever the result is likely to change individual antibiotic management. The recommendations of the European, American, British and Swedish guidelines regarding the microbiological investigations of hospitalized CAP patients are given in **Table 6**.

2.7. Severity of CAP and site of care

In the assessment and management of CAP, disease severity assessment is crucial. It guides which therapeutic options to take to, e.g., suitability for outpatient treatment or need for hospitalization, ICU admission, the extent of examinations and choice of antimicrobial agent (Mandell et al. 2007). Optimal management of patients with CAP requires recognition of seriously ill patients requiring hospitalization or admission to the ICU. On the other hand, a major impact of costs related to CAP is determined whether or not a patient is admitted to hospital (Colice et al. 2004, Guest and Morris 1997, Niederman et al. 1998). Clinical judgement alone tends to overestimate a patient's risk of death (Fine et al. 1997[b]). Therefore various prognostic scoring systems predicting the mortality rate have been developed. These prognostic scoring systems have been used to guide the decision of hospitalization (Fine et al. 1997[a], Lim et al. 2003). However, in addition to disease severity, hospital admission requirements depend on many variables, e.g., comorbidity, adequate home facilities and patient compliance. Prediction rules do not consider the patients' preferences for site of care. Accordingly, prediction rules are meant to contribute rather than supersede the physician's judgment (Mandell et al. 2007, Woodhead 2004). The decision to admit patients with CAP to an ICU is usually dictated by the need for mechanical ventilation or hemodynamic support and closer monitoring of the patient. Until recently, the use of different scoring systems to predict the need of ICU admission has not been much studied (Angus et al. 2002, Ewig et al. 2004, Liapikou et al. 2009, Valencia et al. 2007). The use of the systemic inflammatory response syndrome as a predictor of severe sepsis or mortality in CAP patients has been inferior compared to pneumonia-specific scoring systems (Barlow et al. 2007, Dremsizov et al. 2006).

Table 6. Guideline recommendations for the use of microbiological investigation in hospitalized patients with community-acquired pneumonia

	European Respiratory Society (Woodhead et al. 2005)	**Infectious Diseases Society of America and American Thoracic Society** (Mandell et al. 2007)	**British Guidelines** (BTS guidelines, 2001)	**Swedish Guidelines** (Hedlund et al. 2005)
Blood culture	All patients	ICU admission, cavitary infiltrates, leukopenia, alcohol abuse, chronic severe liver disease, asplenia, pleural effusion, positive pneumococcal urinary antigen detection (Optional for patients without these conditions)	All patients	All patients
Sputum culture	When a purulent sputum sample can be obtained	ICU, cavitary infiltrates, alcohol abuse, positive pneumococcal urinary antigen detection, pleural effusion, failure of outpatient antibiotic therapy, severe obsctructive/ structural lung disease if a good-quality specimen can be obtained *Legionella* culture in patients with positive *Legionella* urinary antigen detection or with suspected Legionnaires' disease	Patients with nonsevere CAP who are able to expectorate purulent sputum sample and patients with severe CAP and those who fail to improve[a] *Legionella* culture in patients with severe pneumonia and when *Legionella* is suspected on epidemiological grounds	All patients *Legionella* culture in patients with severe pneumonia and when *Legionella* is suspected
Pleural fluid analysis	A significant pleural effusion	Pleural effusion >5cm	If present	

S. pneumoniae urinary antigen test	Patients with severe CAP	ICU admission, leukopenia, alcohol abuse, chronic severe liver disease, asplenia, pleural effusion, failure of outpatient treatment	Patients with severe CAP	Patients with severe CAP
Legionella spp. urinary antigen test	Patients with severe CAP, and when clinically or epidemiologically suspected	ICU admission, alcohol abuse, pleural effusion, failure of outpatient treatment, recent travel	Patients with severe CAP or specific risk factors and during outbreaks	Patients with severe CAP and when *Legionella* is suspected
Molecular tests	May be considered for the detection of influenza and RSV during the winter season, and for atypical pathogens provided that the tests are validated and results obtained rapidly	No recommendations	(Research tool)	*Mycoplasma* and *Legionella* PCR in patients with severe CAP
Serology	Not recommended	No recommendations	During outbreaks, patients with severe CAP or who are unresponsive to β-lactam or with epidemiological risk factor	Patients with severe CAP
Bronchoalveolar lavage	Can be considered in intubated patients and in nonresolving pneumonia	Can be considered in intubated patients. Endotracheal aspirate is recommended for all intubated patients	Can be considered in intubated patients	Should be considered in critically ill patients and in patients who fail to respond to antibiotic therapy

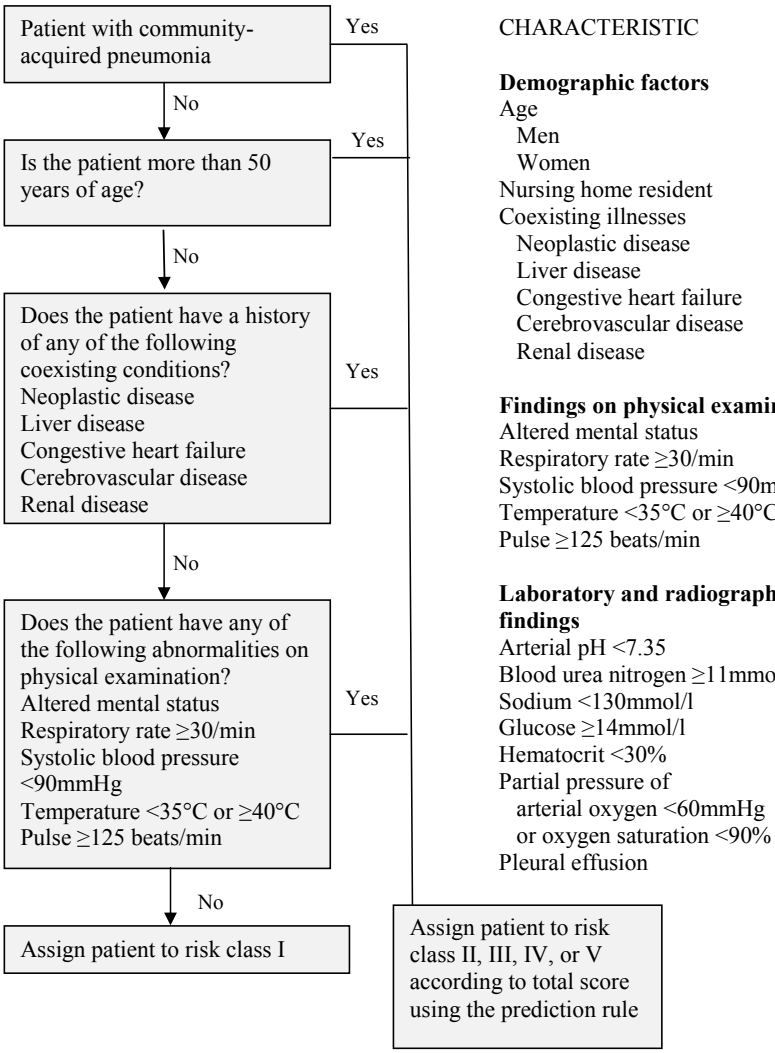
[a]In addition in patients with severe pneumonia, a respiratory sample for antigen detection of respiratory viruses, *Chlamydophila* spp. and in patients with risk factors also antigen detection of *Pneumocystis jirovecii*

2.7.1. Pneumonia severity index (PSI)

Fine *et al.* developed a prediction tool to identify accurately patients who have CAP at low risk of death within 30 days of presentation and who thus might be managed as outpatients. The PSI was derived from data on 14 199 inpatients with CAP, it was independently validated with data from 38 039 inpatients with CAP and 2287 inpatients and outpatients prospectively enrolled in the Pneumonia PORT cohort study. The PSI is based on age, comorbid disease, clinical findings and laboratory investigations, totally 20 variables (**Figure 1**). The PSI stratifies patients into five mortality risk classes: class I with a mortality of 0.1%, class II 0.6%, class III 0.9-2.8%, class IV 8.2-9.3% and class V 27.0-29.2%. A similar progression of risk was evident when PSI classes were analyzed against the duration of hospital treatment, ICU admission and hospital admission for those initially managed as outpatients (Fine et al. 1997[a]). On the basis of associated mortality rates, it has been suggested that risk class I and II patients can be treated as outpatients provided that the patient does not exhibit any serious abnormalities in his vital signs. Risk class III patients should be treated with a brief observation period or a short hospitalization, and risk classes IV and V patients should be hospitalized (Mandell et al. 2007). Application of this score system has reduced avoidable hospital admission without increasing mortality (Marrie et al. 2000[b], Renaud et al. 2007[b]). A number of limitations of this scoring system have been recognized. Age and comorbidity affects overall score very heavily, potentially underestimating the need for hospitalization of younger patients. The PSI system with many variables has also been considered as cumbersome especially for the needs of primary care (Niederman 2007). The PSI is neither good in discriminating the most severely ill patients, nor can it be used to predict which patients require intensive care (Niederman 2007, Valencia et al. 2007).

2.7.2. CURB-65 (confusion–urea–respiratory rate–blood pressure–age ≥65 years)

The CURB-65 score was derived from the British Thoracic Society score which was developed primarily to identify patients with severe CAP at high risk mortality. The CURB-65 scoring system uses 5 variables: confusion, raised urea (>7 mmol/l), respiratory rate (≥ 30/min), blood pressure (systolic < 90 mmHg or diastolic ≤ 60 mmHg) and age ≥ 65 years. For each aberration from the ideal, 1 point is scored, and the maximum score may thus be 6. This scoring system allows patients to be stratified by risk of death: score 0 carries a mortality risk of 0.7%, score 1 of 2.1%, score 2 of 9.2%, score 3 of 14.5%, score 4 of 40%, and score 5 of 57% (Lim et al. 2003). Patients with scores 0 and 1 may be considered for outpatient management, whereas patients with scores 2-5 should be hospitalized (Lim et al. 2003, Woodhead 2004). Treatment in an ICU may be considered for patients with a score of ≥3. However, also the CURB-65 is too sensitive in predicting the need for admission to an ICU (Angus et al. 2002, Ewig et al. 2004). The CURB-65 scoring system is simpler than the PSI. In addition, if urea is omitted as a CRB-65 score, this allows scoring system to be used also in primary care where laboratory investigations are not always available (Lim et al. 2003). The main limitation of the CURB-65 scoring system is that it does not account for comorbidities and may underestimate the mortality risk of elderly patients (Niederman 2007).



CHARACTERISTIC	NO OF POINTS ASSIGNED
Demographic factors	
Age	
Men	Age (in yr)
Women	Age (in yr)-10
Nursing home resident	+10
Coexisting illnesses	
Neoplastic disease	+30
Liver disease	+20
Congestive heart failure	+10
Cerebrovascular disease	+10
Renal disease	+10
Findings on physical examination	
Altered mental status	+20
Respiratory rate ≥30/min	+20
Systolic blood pressure <90mmHg	+20
Temperature <35°C or ≥40°C	+15
Pulse ≥125 beats/min	+10
Laboratory and radiographic findings	
Arterial pH <7.35	+30
Blood urea nitrogen ≥11mmol/l	+20
Sodium <130mmol/l	+20
Glucose ≥14mmol/l	+10
Hematocrit <30%	+10
Partial pressure of arterial oxygen <60mmHg or oxygen saturation <90%	+10
Pleural effusion	+10

Stratification of Risk Score

RISK	RISK CLASS	SCORE	MORTALITY
Low	I	Based on algorithm	0.1%
Low	II	≤70	0.6%
Low	III	71-90	0.9%
Moderate	IV	91-130	9.3%
High	V	>130	27.0%

Figure 1. PSI risk classes.
Figure modified from Fine et al. 1997[a]

2.7.3. American Thoracic Society (ATS) score

The ATS score was designed to recognize patients who require care in an ICU. The original ATS guidelines identified 9 criteria for severe CAP: a need for mechanical ventilation, an increase in the size of infiltrates on radiography by > 50% within 48

hours, septic shock or need for pressor drugs for > 4h, acute renal failure, respiratory rate ≥ 30/min, Pa_{O2}/Ft_{O2} < 250, bilateral pneumonia or multilobular pneumonia, systolic blood pressure ≤ 90 mmHg and diastolic blood pressure ≤ 60 mmHg. The presence of any one of these criteria was used to define severe CAP (Niederman et al. 1993). The original guideline was highly sensitive (98%), but not very specific (32%) (Ewig et al. 1998).

In the modified ATS score, the criteria were divided into minor criteria that could be present on admission and major criteria that could be present on admission or later in the hospital stay. The minor criteria included Pa_{O2}/Ft_{O2} < 250, multilobar pneumonia and systolic blood pressure ≤ 90 mmHg. The major criteria included a need for mechanical ventilation and septic shock. The presence of either 2 minor criteria or one major criterion predicted the need for ICU admission with a sensitivity of 69-78% and a specificity of 94-97% (Ewig et al. 2004, Ewig et al. 1998). Compared to the PSI and CURB-65 scores, the modified ATS score has turned out to have better discriminatory capacity for ICU admission, although also the modified ATS score is too sensitive in predicting the need for ICU admission (Angus et al. 2002, Ewig et al. 2004).

The most recent ATS guidelines for the treatment of adults with CAP retained the same two major severity criteria and developed a new set of minor severity criteria: respiratory rate ≥30 breaths/min, Pao2/Fio2 ≤250, multilobar infiltrates, confusion and/or disorientation, uremia (BUN level ≥20 mg/dl), leukopenia (WBC count < 4 x 10^9 cells/l), thrombocytopenia (platelet count <100 x 10^9 platelets/l), hypothermia (core temperature <36°C) and hypotension (SBP <90 mmHg; requiring aggressive fluid resuscitation). The guidelines recommend consideration of ICU admission if ≥3 minor severity criteria were present (Mandell et al. 2007). This scoring system has been recently validated in a study of 2102 patients with CAP, 235 of whom were admitted to the ICU. This scoring system of severe CAP predicted ICU admission with a sensitivity of 71% and a specificity of 88%. (Liapikou et al. 2009).

2.7.4. SMART-COP

Charles *et al.* described a new score for predicting the need for intensive respiratory or vasopressor support in patients with CAP. The scoring system is composed of 8 clinical and laboratory factors (**Figure 2**). A SMART-COP score of ≥3 points identified 92% of the patients who needed intensive respiratory or vasopressor support during the hospitalization. Specificity of this scoring system was 62%. An increasing score was also associated with higher mortality. In this study by Charles *et al.* the sensitivities of PSI and CURB-65 to predict the need for intensive respiratory or vasopressor support were 74% and 39% and specificities 49% and 74% (Charles et al. 2008[b]).

2.7.5. CRP and procalcitonin

Previous studies have reported no correlation between CRP and PSI classes, CRB-65 score or mortality (García-Vázquez et al. 2003, Hedlund and Hansson 2000, Krüger et al. 2008, Müller et al. 2007, Örtqvist et al. 1995). There is, however, a correlation between CRP values and duration of hospital stay (Hedlund and Hansson 2000, Örtqvist et al.

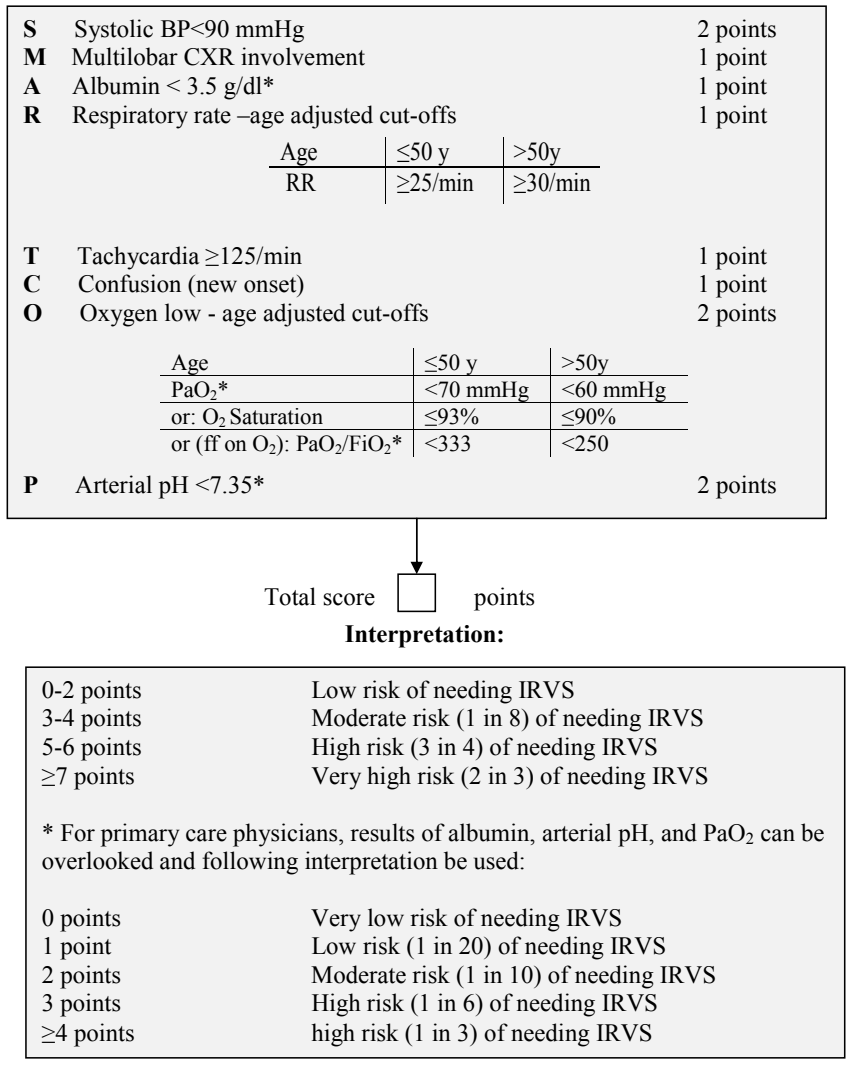


Figure 2. SMART-COP. IRVS, intensive respiratory or vasopressor support
Figure modified from Charles et al. 2008[b]

1995). Contrary to previous results, Seppä *et al.* showed that in elderly patients with CAP, a CRP value ≥ 100 mg/l on admission was independently associated with death (Seppä et al. 2001) and the study by Chalmers *et al.* showed that elevated CRP ≥ 100 mg/l on admission was associated with increased 30-day mortality, need for respiratory support and complicated pneumonia in adult patients with CAP. The specificity of CRP ≥ 100 mg/l was low (<36%) but it was highly sensitive (95-98%) in predicting an adverse outcome. Subsequently, the authors suggest that a CRP value < 100 mg/l could be used to predict patients who may be safely treated as outpatients on clinical grounds (Chalmers et al. 2008[a]). Consistently, Almirall *et al.* found that a cut-off value of CRP 106-110 mg/l differentiates the need of inpatient care from outpatient care (Almirall et al. 2004). There are only few studies that show that CRP levels in patients with bacteremic pneumonia

are higher compared to patients with nonbacteremic pneumonia (Hedlund and Hansson 2000, Örtqvist et al. 1995).

In contrast to CRP, a correlation between procalcitonin levels on admission and PSI score, CRB-65 score and mortality has been established (Masiá et al. 2005, Müller et al. 2007). Procalcitonin levels rise as the severity of the pneumonia increases as assessed by the CRB-65 sore (Krüger et al. 2008). In the study by Masiá *et al.* significantly higher procalcitonin levels on admission were measured in patients with complications or death (Masiá et al. 2005). Compared to CRP, procalcitonin seems to be a better predictor of bacteremia (Müller et al. 2007). Serial measurements have also been used to define prognosis in patients with severe CAP. Among 100 ICU patients with CAP, increased procalcitonin concentration from day 1 to day 3 was a poor prognostic factor (Boussekey et al. 2006).

2.8. Antimicrobial treatment

At the time of initial diagnosis, antibiotics are given empirically. Since in half of the cases the causative agent remains unrecognized, the antimicrobial treatment is often also continued empirically. Therapy must be focused on the pathogens most likely to be present. Accordingly, *S. pneumoniae* is agent number one that uniquely must be covered according to the guidelines of empiric treatment of CAP (BTS Guidelines 2001, Hedlund et al. 2005, Honkanen et al. 2008, Mandell et al. 2007, Woodhead et al. 2005). Initial empiric treatment is selected by categorizing patients on the basis of site of therapy (outpatient, inpatient, ICU), severity of illness and the presence or absence of comorbidities. In addition, treatment recommendations must be modified on the basis of local conditions of microbial resistance patterns (Honkanen et al. 2008, Mandell et al. 2007, Woodhead et al. 2005). The evidence for an adverse effect on resistance is best documented for macrolides (Daneman et al. 2006). Instead, in patients with bacteremic pneumococcal pneumonia no relationship between mortality and penicillin MIC $\geq$ 2 μg/ml has been found and high-dose β-lactams are still considered to be an appropriate choice in cases with penicillin MIC levels of $\leq$ 2 μg/ml (Weinstein et al. 2009). Recommendations for empirical antibiotics for community-acquired pneumonia according to the Finnish, European, and American guidelines are given in **Table 7.** The Swedish guidelines still consider penicillin as the preferred agent for most outpatients and also for inpatients with non-severe CAP. Amoxicillin is recommended in these cases when infection by *H. influenzae* or *S. pneumoniae* with reduced susceptibility is suspected. Macrolide or alternatively doxycycline is recommended in case atypical pathogens are suspected. According to the Swedish guidelines, penicillin is the first choice of treatment also in patients with moderately severe CAP, cefuroxime or cefotaxime are alternatives especially in patients with comorbidities. In patients with critical illness the Swedish guidelines recommend cefuroxime/cefotaxime with addition of erythromycin or alternatively penicillin with addition of moxifloxacin/levofloxacin (Hedlund et al. 2005). The British guidelines are in line with the European guidelines. However, in the British guidelines a combination of β-lactam and macrolide is recommended for almost all patients hospitalized for clinical reasons wheather the disease is severe or not (BTS Guidelines 2001). If a specific pathogen is subsequently identified by diagnostic testing,

Table 7. Recommendations for empirical antibiotics for community-acquired pneumonia

	Finnish Guidelines (Honkanen et al. 2008)	**European Respiratory Society** (Woodhead et al. 2005)	**Infectious Diseases Society of America and American Thoracic Society** (Mandell et al. 2007)
Outpatient previously healthy	Amoxicillin[a] or telithromycin / doxicycline In case travelling history or preceding antimicrobial treatment during the previous month, a respiratory fluoroquinolone	Amoxicillin or tetracycline	Macrolide (Doxycycline) In regions with high rate of macrolide-resistant *S. pneumoniae* consider alternative agents as below[b]
Outpatient with comorbidities	as above or a respiratory fluoroquinolone	as above	A respiratory fluoroquinolone or β-lactam plus macrolide[b]
Inpatient: non-ICU treatment	Cefuroxime or penicillin G ± macrolide or doxicycline Alternatively a respiratory fluoroquinolone	Penicillin G, aminopenicillin, or 2nd/3rd cephalosporin ± macrolide Alternatively a respiratory fluoroquinolone	A respiratory fluoroquinolone or β-lactam plus macrolide
Inpatient: ICU treatment / severe pneumonia	Cefuroxime or ceftriaxone plus a respiratory fluoroquinolone or macrolide	3rd Cephalosporin plus macrolide or a respiratory fluoroquinolone	Cefotaxime, ceftriaxone or ampicillin-sulbactam plus azithromycin/a respiratory fluoroquinolone
Risk factors for *P. aeruginosa*		Antipseudomonal cephalosporin, acylureidopenicillin/plus β-lactamase inhibitor, or carbapenem plus ciprofloxacin	Antipseudomonal β-lactam plus ciprofloxacin/levofloxacin or antipseudomonal β-lactam plus aminoglycoside and azithromycin/ antipneumococcal fluoroquinolone
Special concerns			If MRSA is a consideration, add vancomycin or linezolid

[a]Amoxicillin plus macrolide or doxicyclin is optional
[b]In case use of antimicrobials within previous 3 months, an alternative from a different class should be selected

therapy may be focused (BTS Guidelines 2001, Hedlund et al. 2005, Honkanen et al. 2008, Mandell et al. 2007, Woodhead et al. 2005).

Whether the empirical treatment should include antibiotics that are active against atypical agents is controversial. *M. pneumoniae* and *C. pneumoniae* are common causes of pneumonia especially among outpatients. On the other hand, the disease caused by these agents is often mild and carries a low mortality. No benefit of empirical atypical coverage was shown in hospitalized patients in a meta-analysis of randomized controlled trials comparing treatment regimens with and without coverage of atypical agents (Shefet et al. 2005). Similarly, in a meta-analysis of trials of patients with mild to moderate CAP, antibiotics active against atypical agents were not shown to improve the outcome (Mills et al. 2005). A recent report reviewed studies involving outpatients with CAP and concluded that there was no advantage in using specific antibacterials for treatment of mild CAP in otherwise healthy outpatients (Maimon et al. 2008). However, observational studies have shown a reduction in mortality among hospitalized patients given treatment with β-lactam plus a macrolide or monotherapy with a fluoroquinolone, compared to patients on a cephalosporin alone (Bratzler et al. 2008, Brown et al. 2003, Gleason et al. 1999). Still, it remains to be clarified whether the benefit is associated with the spectrum of pathogens covered or some anti-inflammatory properties of these antibiotics (Bartlett 2008, Laterre 2008). Pneumonia caused by *L. pneumophila* may be a severe disease and thus antimicrobial treatment covering atypical agents is included in the guidelines of empirical treatment of severe CAP. Also the meta-analyses have shown the benefit of the empiric coverage of atypical agents in cases with legionella pneumonia (Mills et al. 2005, Shefet et al. 2005).

Early treatment of influenza, within 48 hours of the onset of symptoms in ambulatory adults with inhaled zanamivir or oral oseltamivir, appears to reduce the likelihood of lower respiratory tract complications (Kaiser et al. 2000, Treanor et al. 2000). The impact of such treatment on patients who are hospitalized with influenza pneumonia is unclear. Empirical antiviral treatment in patients suspected of having influenza is usually not recommended. According to the European guidelines empirical antiviral treatment can be considered during influenza epidemics in high risk patients who have had typical influenza symptoms for less than two days (Woodhead et al. 2005). In the American guidelines, early treatment, within 48 hours of the onset of symptoms, with zanamivir or oseltamivir is recommended for an identified influenza case. From an infection control standpoint the American guidelines consider it reasonable to treat hospitalized patients even with symptoms for longer than 48 hours (Mandell et al. 2007).

2.9. Clinical and radiographic response to treatment and hospital discharge

Most patients will respond rapidly to empirical therapy, with clinical response usually occurring within 3-4 days (Halm et al. 1998, Menendez et al. 2004[a]). Objective parameters of treatment response include resolution of respiratory symptoms (cough, sputum production, dyspnea), defervescence, normalization of oxygen saturation and respiratory rate and resolution of radiographic findings. The rate of response to treatment is dependent on age, comorbid illness, severity of CAP, the pathogen and the chest radiographic findings (Bartlett et al. 2000, Halm et al. 1998, Menendez et al. 2004[a]).

Halm *et al*. established a definition of stability for CAP that includes 5 components: a temperature ≤37.8ºC, a heart rate ≤100 beats/min, a respiratory rate ≤ 24 breaths/min, systolic blood pressure ≥90 mmHg and oxygen saturation ≥90%. They demonstrated that the initial severity of pneumonia correlated with the number of days until clinical stability (Halm et al. 1998). Menendez *et al.* reported that dyspnea, multilobar involvement, pleural effusion and confusion are the initial factors that were associated with later clinical stability in patients with severe CAP, whereas adherence to antibiotic guidelines was associated to earlier clinical stability (Menendez et al. 2004[a]). Once a patient's condition becomes stable, the risk of serious clinical deterioration is 1% or less (Halm et al. 1998). Conversely, patients who are discharged before their condition has stabilized have a higher risk-adjusted rate of death, readmission and failure to return to their usual activities compared to those discharged with stabilized disease (Halm et al. 2002). A switch from intravenous to oral treatment and discharge of the patient is appropriate when the patient is clinically stable, with a normal mental status and able to maintain oral intake (Mandell et al. 2007).

Although the majority of the patients with CAP become clinically stable within a few days, the total recovery time may be several weeks. At six weeks after cessation of antibiotic therapy, 64% of patients still report ≥1 CAP-related symptoms, most often fatigue (45%), followed by cough (35%) and shortness of breath (33%) (Marrie et al. 2000[a]).

Radiographic findings commonly lag behind clinical findings and do not correlate well with clinical improvement. The radiograph often worsens initially after the therapy is started. Radiographic deterioration is a particular feature of Legionnaires' disease and of bacteremic pneumococcal pneumonia (Macfarlane et al. 1984). Also the rate of resolution of pneumonia is related to the causative agent of pneumonia and is faster for mycoplasma pneumonia compared to CAP caused by *L. pneumophila* or *S. pneumoniae* (Macfarlane et al. 1984). In another study, radiographic resolution of pneumonia occurred more rapidly in younger patients and in those with only a single lobe involved compared to those with multiple lobes involved. Complete radiographic clearing of pneumonia occurred for 50% of patients at 2 weeks and for 67% at 4 weeks (Mittl et al. 1994). In elderly patients the resolution of pneumonia is even slower: 35% at 3 weeks and 60% at 6 weeks (El Solh et al. 2004).

2.10. Treatment failure

Although not clear-cut and validly defined, treatment failure is considered to be present when an adequate clinical response is not obtained despite antibiotic treatment. The severity of CAP, the baseline characteristics of the patients and the site of treatment are factors that are related to different treatment responses (Mandell et al. 2007, Menendez and Torres 2007). The time of evaluation is also essential for defining treatment failure. The most frequently used period is 72 hours which is in line with the median time to reach clinical stability (Halm et al. 1998). While most authors consider treatment failure only after 72 hours of antibiotic treatment (Arancibia et al. 2000), others consider earlier evaluation (Menendez et al. 2004[b], Roson et al. 2004[a]).

Early failure is usually defined as progressive pneumonia or clinical deterioration with acute respiratory failure requiring respiratory support with or without septic shock

within 72 hours of hospital admission (Mandell et al. 2007, Menendez and Torres 2007). Progressive pneumonia is the most common cause of early failure. Other causes of early failure are empyema, uncovered pathogen, inaccurate diagnosis or metastatic infection, e.g., endocarditis or meningitis (Mandell et al. 2007, Roson et al. 2004[a]).

Treatment failure occurring after 72 hours of hospitalization is defined as deterioration and development of respiratory failure or hypotension. The absence of or delay in achieving clinical stability after more than 72 hours of treatment is also considered treatment failure (Mandell et al. 2007, Menendez and Torres 2007, Menendez et al. 2004[b]). In addition to the same causes as in early failure, nosocomial pneumonia, exacerbation of comorbid illness, intercurrent non-infectious disease and adverse drug reactions are possible causes of treatment failure after 72 hours of treatment (Arancibia et al. 2000, Mandell et al. 2007, Menendez et al. 2004[b]). Although antimicrobial resistance is increasing, resistance is an uncommon cause for treatment failure (Arancibia et al. 2000, Genne et al. 2003, Roson et al. 2004[a]).

Severity of illness at presentation and multilobar pneumonia are common risk factors for both early and late treatment failure (Menendez et al. 2004[b], Roson et al. 2004[a]). Other factors for either early or late treatment failure are pleural effusion, cavitation, leukopenia, hyponatremia, discordant therapy, and CAP caused by *Legionella* spp. or gram negative bacteria (Menendez et al. 2004[b], Roson et al. 2004[a]). Also host-related factors, e.g., comorbidites and possibly also genetic differences in inflammatory response, are additional factors that contribute to treatment failure (Genne et al. 2006[b], Menendez and Torres 2007, Menendez et al. 2004[b]).

Treatment failure occurs in 6% to 16% of all hospitalized patients with CAP. The incidence of early treatment failure varies from 6% to 9% and the incidence of late treatment failure from 6% to 11% of hospitalized patients (Arancibia et al. 2000, Genne et al. 2003, Genne et al. 2006[b], Menendez et al. 2004[b], Roson et al. 2004[a]). Mortality is understandably significantly higher among patients with treatment failure compared to treatment responders (Menendez et al. 2004[b], Roson et al. 2004[a]). Mortality rates of 25% to 43% have been recorded in patients with treatment failure (Arancibia et al. 2000, Menendez et al. 2004[b], Roson et al. 2004[a]). In addition, treatment failure results in prolonged hospital stay (Menendez et al. 2004[b]).

2.10.1. CRP and procalcitonin as markers of treatment failure

One of the major advantages of CRP and procalcitonin assessments is that serial measurements can be used as markers of treatment response. With appropriate treatment, both CRP and procalcitonin levels fall rapidly (Bruns et al. 2008, Chalmers et al. 2008[a], Kosmas et al. 1997, Menendez et al. 2008, Smith et al. 1995[b]).

Menéndez *et al.* demonstrated that CRP and procalcitonin levels on day 1 were significantly higher in patients with early or late treatment failure (232 mg/l vs. 136 mg/l and 1.5 ng/ml vs. 0.5 ng/ml) and an elevated level of CRP on day 3 was a predictor of a late treatment failure (Menendez et al. 2008). The value of CRP as a marker of treatment failure has been confirmed by Chalmers *et al.* who established that a CRP level that fails to fall by 50% or more within 4 days is associated with increased total rates of mechanical ventilation or

need for inotropic support or both, a higher total incidence of complicated pneumonia and increased 30-day mortality. Patients who were discharged before day 4 and had a level of CRP ≥100 mg/l at discharge were readmitted significantly more often (8.7%) compared to patients with level of CRP < 100 mg/l at discharge (0.6%) (Chalmers et al. 2008[a]). The study by Bruns *et al.* showed that a delayed decline of CRP level is associated with a higher risk of having received inappropriate antibiotic treatment (Bruns et al. 2008).

2.10.2. Management of treatment failure

Treatment failure will generally result in at least 1 of 3 clinical responses: transfer of the patients to a higher level of care, further diagnostic testing or escalation or change in antibiotic treatment. Antibiotic changes before 72 hours of therapy are recommended only for patients with deterioration or in whom new data of etiological diagnosis or epidemiologic clues suggest alternative etiologies (Honkanen et al. 2008, Mandell et al. 2007). Also, if the patient is not clinically stable after 72 hours of treatment, and if the microorganism or host factors associated with delayed response are present, continued therapy without antibiotic change is considered appropriate. On the other hand, if there is no explanation for a slow response, if there is no response after 7 days of therapy or when there is clinical deterioration, careful re-evaluation is necessary (Menendez and Torres 2007, Niederman et al. 2001).

A further history of risk factors, e.g., animal exposure and travel, may give a clue to an unusual pathogen and to a need for special microbiological tests and change of treatment. One should consider risk factors for *P. aeruginosa*, *S. aureus* or other pathogens in patients who fail to respond to appropriate empirical therapy. Alternatively, CAP could be caused by a virus not responding to any antimicrobial treatment. With a history of tuberculosis exposure or other risk factors for tuberculosis, the skin test for tuberculosis and sputum sample for acid-fast staining and mycobacterial culture should be obtained. In addition, unrecognized conditions affecting immunity, above all HIV, should be taken to account. Complete re-evaluation of the clinical history may also suggest other alternative non-infectious diagnoses (Mandell et al. 2007, Menendez and Torres 2007, Niederman et al. 2001).

A chest radiograph or a CT scan may reveal pleural effusion, and if this is the case, an ultrasound-guided diagnostic thoracocentesis should be performed as soon as possible. Aspiration of pus, a putrid odor associated with anaerobic infection, a positive gram stain or culture result, pH < 7.20, a glucose level < 40 mg/dl and a LDH level > 1000 IU/l support the diagnosis of empyema and the need to drain the pleural space (Sahn 2007). Patients with empyema, particularly those with an altered mental status, esophageal disease or a history of alcoholism should be treated with antibiotics that cover the anaerobes (Sahn 2007). Chest radiograph and especially CT may also show the presence of a lung abscess or the acute respiratory distress syndrome complicating pneumonia, or alternatively, a non-infectious illness only mimicking CAP. Other diagnostic procedures may be needed to detect or exclude metastatic infections or exacerbation of comorbid illness (Mandell et al. 2007, Menendez and Torres 2007, Niederman et al. 2001).

Bronchoscopy and BAL allow the direct observation of the airways and obtain samples directly within the infected lobe. Samples obtained by bronchoscopy and bronchial brush or BAL can lead to identification of an unusual organism or a drug-resistant

pathogen even in the presence of antibiotics. In addition, mechanical factors that delay resolution, e.g., an endobronchial tumor can be detected by bronchoscopy. Running a cell count of BAL samples may reveal eosinophilia or lymphocytosis and this orients the physician to think of non-infectious causes of pulmonary infiltrates (Menendez and Torres 2007, Niederman et al. 2001). In the IDSA/ATS guidelines, bronchoscopy with BAL is recommended if the differential diagnosis of nonresponse includes non-infectious pneumonia (Mandell et al. 2007).

Blood cultures should be repeated if the patient's condition deteriorates or if he has progressive pneumonia. Positive blood culture results should increase the suspicion of antibiotic resistant isolates or a metastatic infection, e.g., endocarditis. Particularly in ICU patients, nosocomial pneumonia or extrapulmonary infections, e.g., intravascular catheter infections, should be considered (Mandell et al. 2007). Drug fever and antibiotic-associated diarrhea are adverse reactions to antibiotic treatment that might explain why treatment fails. Stopping the ß-lactam component of therapy may be helpful, if there is a suspicion of drug fever (Mandell et al. 2007).

2.11. Outcome

Less than 10% of the patients with CAP treated as outpatients are subsequently hospitalized within 30 days. The rate of hospitalization of outpatients is higher in patients categorized to higher PSI classes (Fine et al. 1997[a], Minogue et al. 1998, Renaud et al. 2007[a]). Accordingly, outpatients who are subsequently hospitalized are older and more often have comorbid illnesses (Minogue et al. 1998). The reason for hospitalization is most commonly CAP-related but in up to one third of the cases it may be comorbidity-related (Minogue et al. 1998). Mortality among outpatients is low, usually less than 1% (Fine et al. 1999, Minogue et al. 1998, Renaud et al. 2007[a]). However, one study comparing the outpatients with subsequent hospitalization and those with no hospitalization found a significant difference in the mortality between these two groups (4.2% vs. 0.3%) (Minogue et al. 1998).

According to a meta-analysis including 25 629 hospitalized CAP patients and a recent German study including 388 406 hospitalized CAP patients, mortality rate among these patients is nearly 14% (Ewig et al. 2009, Fine et al. 1996). The leading immediate cause of death is respiratory failure (Fine et al. 1999, Garcia-Vidal et al. 2008). Among hospitalized patients, mortality rates are highest in the patients with severe pneumonia, especially in those requiring treatment in an ICU. In the meta-analysis by Fine *et al.* mortality of the CAP patients treated in an ICU was 37% (Fine et al. 1996). Mortality is also higher among elderly patients (14-18%), particularly among elderly patients admitted from nursing homes (25%-31%) (Ewig et al. 2009, Fine et al. 1996). Other factors that are related to a higher incidence of mortality are bacteremic pneumonia and multilobar pneumonia (Fine et al. 1996, Garau et al. 2008). Malignancy and neurologic disease were the comorbidities that were found to be associated with high mortality in both of the two large CAP studies (Ewig et al. 2009, Fine et al. 1996). In a study of 577 elderly hospitalized patients with CAP, 12% were rehospitalized within 30 days after the initial discharge. Most (74%) of the hospitalizations were comorbidity-related (Jasti et al. 2008).

Acute cardiac events are relatively common among elderly patients with CAP. Musher *et al.* studied the occurrance of myocardial infarction, congestive heart failure and arrhythmia at the time of hospital admission in 170 patients with pneumococcal pneumonia. Acute cardiac event was present in 19% of the patients, 7% of them had acute myocardial infarction (Musher et al. 2007). Similarly, in the study by Ramirez *et al.* acute myocardial infarction was present on admission in 15% of the 86 patients with severe CAP. In this study including a total of 500 CAP patients, acute myocardial infarction was identified in 5.8% during hospitalization (Ramirez et al. 2008). These findings are in line with a growing amount of epidemiologic, experimental, and clinical evidence that has linked infection as a risk factor to various atherosclerotic diseases including acute myocardial infarction (Valtonen 1999). It is of note that in both of these CAP studies mortality was high, 28-40%, among the patients with CAP and acute myocardial infarction (Musher et al. 2007, Ramirez et al. 2008). Activation of the coagulation system, increased cytokine expression and inflammation, endothelial dysfunction, hypoxemia and hypotension are possible explanations for the pathogenesis of AMI in CAP patients (Musher et al. 2007, Ramirez et al. 2008, Valtonen 1999). The study by Chalmers *et al.* gives support for a possible connection between systemic inflammation caused by CAP and mortality. They found that statin use was associated with reduced markers of systemic inflammation and with significantly lower 30-day mortality (Chalmers et al. 2008[b]). In addition, Yende *et al.* found that among CAP patients elevated level of inflammatory markers at hospital discharge was associated with an increased risk of death during one year follow-up (Yende et al. 2008).

Follow-up studies have shown that patients with CAP have a significantly higher long-term mortality than age-matched control subjects. In the study by Koivula *et al.* 122 elderly patients (both outpatients and hospitalized patients) who survived an episode of CAP were followed up for mortality for a median of 9.2 years. Subsequent risk of total mortality, pneumonia-related mortality, and cardiovascular mortality were increased in persons who survived CAP. Of these patients, 89% were alive after one year, 80% after two years, 60% after five years and 39% after ten years. The respective figures for the control group were 96% after one year, 92% after two years, 81% after five years and 61% after ten years (Koivula et al. 1999). In a study of 3284 hospitalized CAP patients with a mean age of 69 years, outcomes were registered for a median of 3.8 years. With respect to all-cause mortality, 12% of the patients died within 30 days, 28% within one year, and 53% by the study end. Cardiovascular disease was the most common cause of death (31%) followed by respiratory disease (26%), and cancer (18%). During the follow-up period 16% of the patients were readmitted for a repeat episode of pneumonia (Johnstone et al. 2008[a]). Mortensen *et al*. analyzed mortality in CAP patients who survived 90 days after presentation to the hospital. Besides a significantly higher long-term mortality among patients with CAP compared to age-matched controls, this study showed that mortality was higher across all age groups (Mortensen et al. 2003). Age, male sex, and comorbidities are the most commonly found factors associated with long-term mortality in CAP patients (Johnstone et al. 2008[a], Koivula et al. 1999, Mortensen et al. 2003).

3. AIMS OF THE STUDY

The purpose of this study was to evaluate the utility of rapid methods in the etiological diagnostics and assessment of disease severity and complications in patients treated for CAP in a Finnish university hospital.

The specific aims were:

1. to evaluate to what extent it is possible to diagnose the etiological agent in the acute phase of CAP using novel, rapid microbiological methods, such as antigen detection and gene amplification (PCR),

2. to evaluate the role of rhinoviruses and enteroviruses as causative agents of CAP in adults and their contribution to disease severity,

3. to examine the value of CRP in assessing the disease severity and complications as well as etiology of CAP,

4. to assess whether the expression of leukocyte receptors on admission could be used as a preliminary test to differentiate between bacterial and viral etiology of CAP and

5. to assess the diagnostic value of bronchoscopy and BAL in patients with CAP in a routine clinical setting

4. PATIENTS, SUBJECTS AND METHODS

4.1. Patients, study designs and data collection (I-V)

We included in this study all adult patients (aged ≥16 years) admitted between December 1999 and December 2004 for CAP to the Department of Infectious Diseases, Turku University Hospital, Turku, Finland. The hospital is a 1000-bed teaching facility serving as a tertiary-care center for the southwestern part of Finland, and as a primary care facility for infectious diseases in a catchment area of about 200,000 inhabitants. The exclusion criteria included patients with immunosuppression (e.g. HIV infection, chemotherapy or systemic corticosteroid treatment with a dosage of >20 mg prednisolone-equivalent per day), an emerging alternative diagnosis during the follow-up (e.g., pulmonary emboli or malignancy), pneumonia caused by tuberculosis or aspiration and hospitalization within the previous 10 days. In addition, 71 patients with CAP who had undergone BAL for serious or slowly responding pneumonia during 1996-2000 were included (**Table 8**). Clinical and microbiological data of the patients were collected from the hospital records. The assessment of the severity of CAP was performed using the Pneumonia Severity Index (PSI) (Fine et al. 1997[a]). The clinical stability of the patients was defined as described by Halm et al. (Halm et al. 1998).

Table 8. Patients and study designs (I – V)

Study	Study design	Time period	Patient population	Number of patients	Purpose of the study
I	Prospective	1999 – 2004	Patients admitted for CAP	384	Evaluation of the etiology of CAP
II	Prospective	1999 – 2004	Patients admitted for CAP and examined for picornaviruses	231	Evaluation of the role of picornaviruses in CAP
III	Prospective	1999 – 2004	Patients admitted for CAP	384	Evaluation of the utility of CRP in the assessment of disease severity, etiology and complications in CAP
IV	Prospective	1999 – 2004	Patients admitted for CAP and examined for leukocyte receptors	68	To assess the utility of the expression of leukocyte receptors in differentiating between bacterial and viral pneumonia
V	Retrospective	1996 – 2000	Patients with CAP undergoing BAL in a routine clinical setting	71	To assess the value of BAL in the etiological diagnosis of CAP

Studies I and III. These studies were prospective. There were 384 adults, who had a mean (SD) age of 49.8 (19.2) years. The demographic and clinical characteristics of these patients are described in **Table 9**. The mean duration (SD) from the onset of symptoms to the hospitalization was 3.8 (3.0) days (range 1 - 21 days). In accordance with the PSI prognostic score, 124 (32.3%) patients were in class I, 113 (29.4%) in class II, 59 (15.4%) in class III, and 88 (22.9%) in classes IV and V combined.

Table 9. Characteristics of 384 patients with community-acquired pneumonia (Study I)

Characteristic	Total	Patients with preceding antimicrobial therapy[a] (*n* = 110)	Patients without preceding antimicrobial therapy (*n* = 274)	*P*
Mean age ± SD (years)	49.8 ± 19.2	44.7 ± 17.4	51.9 ± 19.5	<0.001
Male	201 (52.3)	45 (40.9)	154 (56.2)	0.007
Smokers	125 (32.6)	30 (27.3)	95 (34.7)	0.186
Underlying disease	167 (43.5)	34 (30.9)	133 (48.5)	0.002
Cardiovascular disease	70 (18.2)	10 (9.1)	60 (21.9)	0.003
Asthma	35 (9.1)	7 (6.4)	28 (10.2)	0.326
Diabetes mellitus	28 (7.3)	8 (7.3)	20 (7.3)	1.000
Chronic obstructive pulmonary disease	17 (4.4)	4 (3.6)	13 (4.7)	0.787
Alcoholism	15 (3.9)	0 (0)	15 (5.5)	0.008
Admitted from nursing home	7 (1.8)	2 (1.8)	5 (1.8)	1.000
PSI I-II	237 (61.7)	87 (79.1)	150 (54.7)	<0.001
PSI III-V	147 (38.3)	23 (20.9)	124 (45.3)	<0.001

*Antibiotic treatment before admission, regardless of the amount of the doses taken.
Data are presented as number of subjects (%) unless otherwise indicated

Study II. This study formed part of a larger prospective study (**I**). Included was a subset of 231 patients who had their throat swab specimens examined also for the presence of rhinoviruses and enteroviruses.

Study IV. This study was a prospective study. Included were 68 patients with CAP who had their leukocyte receptor expression examined on admission.

Study V. This retrospective study included 71 patients with CAP who had undergone BAL in 1996-2000. The demographic and clinical characteristics of these patients are described in **Table 10**. All BALs were requested by the attending physicians on clinical grounds, either due to diagnostic or to therapeutic difficulties associated with the patient's disease.

Table 10. Patient characteristics (n=71) (Study V)

Characteristic	
Age, years	59±13.5 (range 29-82)
Sex	48 (68) male, 23 (32) female
Smokers	24 (34)
Cardiovascular disease	10 (14)
Chronic obstructive pulmonary disease	9 (13)
Alcoholism	9 (13)
Other underlying diseases	17 (24)

Data are presented as number of subjects (%) unless otherwise indicated

4.2. Diagnostic criteria (I-V)

The diagnostic criteria for CAP included a new infiltrate on chest radiograph in a patient with either fever or clinical signs/symptoms of lower respiratory tract infection (cough, sputum production, dyspnea, pleuritic chest pain, crackles on auscultation), or both.

4.3. Microbiological examinations (I-IV)

The clinical specimens for microbiology were obtained as described (**I-IV**). Standard methods were used to culture aerobic and anaerobic bacteria and viruses. Sputum culture was performed in a semiquantitative manner only when sputum samples had more than 25 polymorphonuclear leukocytes and less than 10 epithelial cells per low-power microscopy field. For the detection of *Legionella* spp., specimens were cultured on buffered charcoal-yeast extract (BCYE; Oxoid Ltd., Basingstoke, United Kingdom) agar plates.

From nasopharyngeal samples, viral antigens for respiratory viruses (influenza A and B viruses, RSV, PIV types 1-3, and adenovirus) were examined by time-resolved fluoroimmunometry with monoclonal antibodies (Arstila 1988). Unconcentrated urine samples were tested with the immunochromatographic assay Binax NOW *S. pneumoniae* antigen (Binax, Portland, Maine, USA), and when considered clinically indicated, Binax NOW *L. pneumophila* antigen (Binax, Portland, Maine, USA) was used. Previously described methods were used for the *M. pneumoniae*, *C. pneumoniae*, and *Legionella* spp. PCR tests, as well as for the influenza A and B viral PCR tests (Cheng et al. 2004, Rantakokko-Jalava and Jalava 2001, Tondella et al. 2002, Waris et al. 1998). Throat swab specimens were examined for the presence of rhinoviruses and enteroviruses using previously described reverse-transcriptase (RT)-PCR assays (Vuorinen et al. 2003). Antigen detection and PCR tests were defined as rapid methods.

M. pneumoniae and *C. pneumoniae* IgG and IgM antibodies were measured with commercial enzyme immunoassay kits (IgG-EIA, and IgM-EIA, AniLabsystems, Helsinki, Finland). Legionella IgG and IgM antibodies were determined by enzyme immunoassay with *L. pneumophila* 1-4 and *L. micdadei* as antigens and virus-specific IgG antibody titers by enzyme immunoassay with influenza A and B viruses, PIV types 1-3, adenovirus, and RSV as antigens.

4.4. Bronchoscopy and BAL (V)

Bronchoscopy and BAL were done in local anesthesia with a solution of 4% lidocain using a fiberoptic flexible videobronchoscope. BAL was taken from the lobe in which the chest radiograph showed infiltration. The BAL samples were taken by introducing the tip of the scope into the respective lobe and by irrigating the lobe with 0.9% sterile NaCl using an amount of 20 ml and then suctioning with low suction power.

Aliquots of lavage fluid were submitted for cytological and microbiological examinations. The samples were examined microscopically after staining with the gram stain, acid-fast stain and the calcofluor white stain. Standard methods were used for the culturing of aerobic and anaerobic bacteria, fungi, mycobacteria and viruses. For the detection of *Legionella* spp., specimens were cultured as described above. The detection of *Legionella* spp. and *M. pneumoniae* by PCR was performed as described (Rantakokko-Jalava and Jalava 2001, Waris et al. 1998). Methods to identify *Pneumocystis jirovecii* included microscopical examination of the lavage fluid after staining with a silver stain or with immunofluorescence or both and, in addition, detection of the organism by a PCR method

as described (Wakefield et al. 1990). Methods to identify cytomegalovirus included cytopathological evaluation, viral culture or PCR (Demmler et al. 1988, Hukkanen et al. 2000). Detection and identification of mycobacteria were based on described methods (Rantakokko-Jalava et al. 2001, Soini et al. 1992).

4.5. Definitions for etiological diagnosis (I-V)

The etiology of the CAP was classified as definitive if one of the following criteria were met: 1. identification of an etiological agent from blood or pleural fluid, 2. detection of *M. pneumoniae* or *C. pneumoniae* by PCR from a throat swab sample, 3. demonstration of *Legionella* spp. by culture or PCR from a sputum sample, 4. detection of a respiratory virus from a nasopharyngeal sample by antigen detection or viral isolation or detection of influenza A or B virus by PCR from a throat swab sample, 5. a 1.5-fold increase (according to manufacturer's interpretation) of enzyme immunounits (EIU) of *M. pneumoniae* IgG or *C. pneumoniae* IgG, or a 4–fold titer increase in antibody level of *Legionella* spp., or a 4–fold titer increase of IgG antibody levels of respiratory viruses.

The etiology of pneumonia was defined as probable when the identification of the etiological agent was based on 1. detection of *S. pneumoniae* antigen or *L. pneumophila* antigen in urine, 2. sputum culture, or 3. detection of IgM antibodies for *M. pneumoniae* or for *C. pneumoniae*. Patients in whom more than one etiological agent was detected were classified as having mixed infections.

In BAL samples bacterial and fungal culture was considered positive when the organism was isolated in numbers greater than 10^4 colony-forming unit/ml. Demonstration of *Legionella* spp., *M. pneumoniae* and mycobacteria by culture or PCR were considered positive findings. Detection of a respiratory virus from the BAL fluid by culture was classified as clinically significant.

4.6. Determination of CRP (III, IV)

CRP levels were determined by an immunoturbidometric method (Tina-quant®) on Hitachi 917 automated biochemistry analyzer (Roche Diagnostics GmbH, Mannheim, Germany). In **Study III**, the CRP levels were examined on admission (CRP1), on a daily basis until the rising tendency of CRP turned into a declining tendency and later as clinically indicated 2-5 times a week during the hospitalization. In addition, CRP was examined at the time of clinical stability (CRP2). In **Study IV**, CRP was examined on the same day as the leukocyte receptor expression.

4.7. Measurement of leukocyte receptor expression (IV)

For the measurement of leukocyte receptor expression, 10 ml of heparin anticoagulated blood was collected from the patients within two days of hospital admission. The procedure was performed as described using fluorescence-labelled receptor-specific monoclonal antibodies (Grönlund et al. 1999). FITC-conjugated anti-FcγRI (CD64; mouse IgG1 isotype, clone 22), anti-FcγRIII (CD16; mouse IgG1 isotype, clone 3G8), anti-CR1 (CD35; mouse

IgG1 isotype, clone J3D3) and mouse IgG1 isotype control (clone 679.1Mc7) as well as PE-conjugated anti-FcγRII (CD32; mouse IgG2a isotype, clone 2E1), anti-CR3 (CD11b; mouse IgG1 isotype, clone Bear1), mouse IgG1 isotype control (clone 679.1Mc7) and mouse IgG2a isotype control (clone U7.27) were purchased from Immunotech (Marseille, France). A relative measure of receptor expression was obtained by determining the mean fluorescence intensity (MFI) of 5000 leukocytes by flow cytometer.

4.8. Statistical analyses (I, III, IV)

Study I. The two-sample t-test was used for the comparison of the mean ages of the patient groups. The differences in category variables between patient groups were compared with Pearson's χ^2 test with exact *P*-values. Values of $P<0.05$ were considered significant.

Study III. The associations of CRP values with baseline characteristics other than age were statistically tested using the two sample t-test. Associations between age and CRP values were studied using Pearson's correlation coefficient. The two sample t-test was applied also with other two group comparisons of the CRP mean values. One way analysis of variance (ANOVA) with Tukey's adjustment for post hoc comparisons was used when comparing more than two groups of CRP mean values. In multivariate analysis, when covariate adjusted group comparisons were done, the analysis was carried out using linear model techniques. Predictive associations of CRP values and dichotomic clinical classifications were analyzed using logistic regression models and ROC-analysis. The predictive associations of CRP with the time of stabilization were examined using Cox's proportional hazard's regression analysis. The quantifications of the analyses were done by giving the mean values or differences in the mean values in the case of t-test and ANOVA or by giving the odds ratios (OR), specificity, sensitivity and area under ROC-curve in the case of logistic analyses or by giving hazard ratios (HR). Statistical confidence was reported by giving the 95% confidence intervals (95% CI) for the estimates. Values of $P<0.05$ were considered significant. Statistical computing was performed with SAS System for Windows, Release 9.1.3/2004.

Study IV. The group differences were tested using ANOVA. Pairwise, group comparisons after ANOVA were carried out using Tukey's multiple comparison technique. Values of $P<0.05$ were considered significant.

4.9. Ethics

Patients gave written informed consent and the study was approved by the Ethics Committee of the Turku University Hospital.

5. RESULTS

5.1. Etiological diagnosis of CAP (I, II, V)

5.1.1. Rapid diagnostic methods for etiological diagnosis of CAP (I)

Among the 384 patients included between 1999 and 2004, a microbial etiology of CAP was established in 209 (54.4%) (**Table 11**). A total of 230 etiological agents were identified consisting of 15 various agents. In 21 episodes more than one etiological agent was identified. *S. pneumoniae* was the most common pathogen being detected in 107 (27.9%) patients followed by *M. pneumoniae* in 41 (10.7%) patients and *C. pneumoniae* in 28 (7.3%) patients. A respiratory virus was detected as the only etiological agent in 27 patients (7.0%) and in combination with *S. pneumoniae* or atypical pathogens in 15 patients (3.9%).

Of the 230 etiological agents, 135 (58.7%) were identified by antigen detection or PCR (**Table 11**). Of these 135 microbial agents, 95 (70.4%) were identified only by these rapid methods. The identification of the etiological agent led to a change of therapy for 26 (12.4%) of the 209 patients.

Table 11. Etiological agents in 384 patients with community-acquired pneumonia according to definitions and identification in the acute or convalescent phase (Study I)

Micro-organism	Definitive[a] Number	Probable[a] Number	Total[a] Number (%)	Identified by a rapid method[a] Number (%)
Bacterial				
S. pneumoniae	52	55	107 (27.9)	81 (75.7)
S. aureus	2	3	5 (1.3)	0
H. influenzae	0	2	2 (0.5)	0
S. pyogenes	1	0	1 (0.3)	0
Fusobacterium sp.	1	0	1 (0.3)	0
Atypical				
M. pneumoniae	38	3	41 (10.7)	13 (34.2)
C. pneumoniae	21	7	28 (7.3)	1 (4.8)
L. pneumophila	2	0	2 (0.5)	1 (50.0)
Viral				
Influenza A	23	0	23 (6.0)	19 (82.6)
Influenza B	7	0	7 (1.8)	7 (100)
Adenovirus	3	0	3 (0.8)	3 (100)
RSV	3	0	3 (0.8)	3 (100)
PIV type 1	3	0	3 (0.8)	3 (100)
PIV type 3	3	0	3 (0.8)	3 (100)
PIV type 2	1	0	1 (0.3)	1 (100)
Total	160	70	230 (54.4)	135 (58.7)
Unknown			175 (45.6)	

[a]Numbers include 21 episodes with dual organisms (*S. pneumoniae* with a respiratory virus, 11 cases; *S. pneumoniae* with *C. pneumoniae*, 1 case; *M. pneumoniae* with influenza A, 3 cases; *M. pneumoniae* with *H. influenzae*, 2 cases; *C. pneumoniae* with *S. aureus*, 2 cases; *C. pneumoniae* with adenovirus, 1 case; and influenza B virus with parainfluenza 3 virus, 1 case).

5.1.2. Rhinoviruses and enteroviruses as causative agents of CAP (II)

In the subset of 231 patients, who had their throat swab specimens examined also for the presence of rhinoviruses and enteroviruses, the total proportion of viruses as causative agents of CAP was 20%. Of the 46 patients with viral infection, 19 (41%) were positive for respiratory picornaviruses by RT-PCR. Among the 12 patients with enteroviruses, additional etiological agents were detected in 7 (58%), including 3 (25%) cases of *S. pneumoniae*. Among the 7 patients with rhinoviruses, a concomitant *S. pneumoniae* infection was detected in 4 (57%) (**Table 12**).

Table 12. Clinical characteristics and microbiological findings of 231 patients with community-acquired pneumonia who had a throat swab taken for viral analysis (Study II)

	Enterovirus (n=12)[a]	Rhinovirus (n=7)	Influenza A (n=17)[a]	Other respiratory viruses (n=12)	Other or undetermined etiology (n=184)
Age (years) (mean ± SD)	45.9 ± 18.5	44.6 ± 19.9	64.2±16.7	50.3±23.4	48.8±17.7
Male	6 (50)	6 (86)	5 (29)	7 (58)	103 (56)
Underlying disease	4 (33)	4 (57)	9 (53)	3 (25)	73 (40)
COPD or asthma	2 (17)	3 (43)	4 (24)	1 (8)	23 (13)
Cardiovascular disease	2 (17)	1 (14)	3 (18)	2 (17)	29 (16)
Smoker	4 (33)	4 (57)	3 (18)	1 (8)	59 (32)
PSI class IV-V	2 (17)	2 (29)	8 (47)	2 (17)	35 (19)
Died	0	1 (14)	3 (18)	0	1 (5)
S. pneumoniae	3 (25)	4 (57)	6 (35)	2 (17)	50 (27)

Data are shown as number (%) unless otherwise indicated.
COPD, chronic obstructive pulmonary disease; PSI, Pneumonia Severity Index
[a]2 patients with enterovirus and influenza A.

5.1.3. Diagnostic yield of various microbiological methods (I)

5.1.3.1. Antigen detection

The *S. pneumoniae* urinary antigen detection test was positive in 24.3% (81/333) of the patients. Among the 52 patients with bacteremic pneumococcal pneumonia, the antigen test was negative in 9 (17.3%) cases. In 51 (47.7%) of the 107 patients with pneumococcal pneumonia *S. pneumoniae* was identified only by the urinary antigen detection test.The results of the antigen tests with respect to the results of blood cultures are presented in **Table 13**. All of the 15 *L. pneumophila* antigen detection tests performed from urine samples were negative. Respiratory viral antigen detection from a nasopharyngeal sample was positive in 11.1% (35/314) of the patients.

Table 13. Comparison between the results of *Streptococcus pneumoniae* urinary antigen detection and blood culture (Study I)

Blood culture	*Streptococcus pneumoniae* urinary antigen detection		
	positive (n = 81)	negative (n = 252)	ND (n = 51)
Streptococcus pneumoniae (n = 52)	27	9	16
Negative for *Streptococcus pneumoniae* (n = 327)	54	239	34
ND (n = 5)	0	4	1

ND, test was not done.

5.1.3.2. PCR

A total of 619 PCR tests from 291 patients were performed including 553 PCR tests from throat swab specimens and 66 PCR tests from sputum specimens. The 553 PCR tests from throat swab specimens consisted of 174 tests for *M. pneumoniae*, 195 tests for *C. pneumoniae* and 184 tests for influenza A and B viruses. *M. pneumoniae* PCR was positive in 7.5% (13/174) and influenza A viral PCR was positive in 1.6% (3/184) of the patients. *C. pneumoniae* PCR was positive in one patient (0.5%). PCR test identified 13 (34.2%) of the 41 cases of *M. pneumoniae* and 1 (4.8%) of the 21 cases of *C. pneumoniae*. There was no positive finding for influenza B by PCR. The *M. pneumoniae* PCR findings with respect to the serological findings are presented in **Table 14**. Of the 66 *Legionella* spp. PCR tests from sputum samples, one positive result was obtained.

Table 14. Comparisons between the results of *Mycoplasma pneumoniae* polymerase chain reaction (PCR) and antibody tests (Study I)

	Acute phase serology of *Mycoplasma pneumoniae*			Convalescent phase serology of *Mycoplasma pneumoniae*		
Mycoplasma pneumoniae PCR	positive	negative	ND	positive	negative	ND
positive (n = 13)	2	10	1	8[a]	0	5
negative (n = 161)	9	139	13	19[b]	90	52
ND (n = 210)	4	156	50	5[c]	66	139

PCR, polymerase chain reaction; ND, test was not done.
[a]In 1 case antibody test was positive also in acute phase.
[b]In 7 cases antibody test was positive also in acute phase.
[c]In 2 cases antibody test was positive also in acute phase.

5.1.3.3. Other methods

Blood cultures were positive in 14.5% (55/379) of the patients yielding 52 cases of *S. pneumoniae*, 2 cases of *S. aureus*, and 1 case of *S. pyogenes*. Of the 119 sputum samples obtained between 1999 and 2002 for culture, the quality of the sputum was adequate in 68 (57.1%) patients. A microorganism classified as significant was isolated in 11 (9.2%) of these patients including 6 cases of *S. pneumoniae*, 3 cases of *S. aureus*, and 2 cases of *H. influenzae*. Of the 23 virus isolations performed on nasopharyngeal samples, 3 (13.0%) were positive for influenza A. In 1 patient, this was the only method to diagnose influenza A infection.

5.1.4. Diagnostic yield according to severity of pneumonia and preceding antimicrobial therapy (I)

The total diagnostic yield was significantly higher in PSI risk classes III-V than in risk classes I-II (61.9% vs. 49.8%; *P*=0.021). Among the diagnostic methods, the yields of blood culture, urinary pneumococcal antigen test and viral antigen detection were significantly higher in PSI risk classes III-V than in risk classes I-II (**Table 15**). In 14 (77.8%) of the 18 patients with a positive respiratory viral antigen detection in PSI risk classes III-V, the virus was the only etiological agent identified. In 8 (47.0%) of the 17 patients with positive respiratory viral antigen detection in PSI classes I-II, the virus was the only etiological agent identified. With one exception, all patients with a positive *M. pneumoniae* PCR test or positive serology for *M. pneumoniae* were stratified in PSI risk groups I and II (**Table 15)**. There were no significant differences between the PSI groups in the diagnostic yield of sputum culture or *C. pneumoniae* serogy (**Table 15)**. There were no significant differences in the total diagnostic yields between the patients with or without prior antimicrobial therapy (51.8% vs. 55.5%; *P*=0.571). Yet, significant differences were observed between these 2 patient groups in the diagnostic yields of the various methods (**Table 16**).

Table 15. Diagnostic yield of microbiological examinations by PSI risk classes I-V (Study I)

Microbiological examination	Number of positive/total tests (%) per PSI risk class				*P* value	
	I (*n* = 124)	II (*n* = 113)	III(*n* = 59)	IV-V (*n* = 88)	group	I-II vs. III-V
S. pneumoniae antigen detection	12/112 (10.7)	25/106 (23.6)	20/53 (37.7)	24/62 (38.7)	<0.001	<0.001
Virus antigen detection	8/111 (7.2)	9/97 (9.3)	6/46 (13.0)	12/59 (20.3)	0.064	0.017
M. pneumoniae PCR test	7/64 (10.9)	6/50 (12.0)	0/22 (0)	0/38 (0)	0.048	0.007
Blood culture	6/122 (4.9)	12/112 (10.7)	11/59 (18.6)	26/86 (30.2)	<0.001	<0.001
Sputum culture	2/35 (5.7)	4/38 (10.5)	2/16 (12.5)	3/30 (10.0)	0.802	0.627
M. pneumoniae serology	28/114 (24.6)	8/100 (8.0)	0/47 (0)	1/59 (1.7)	<0.001	<0.001
C. pneumoniae serology	8/114 (7.0)	9/100 (9.0)	6/47 (12.8)	5/59 (8.5)	0.708	0.468

PSI, pneumonia severity index; PCR, polymerase chain reaction.

Table 16. Diagnostic yield of microbiological methods in patients with or without prior antibiotic treatment (Study I)

	With prior antimicrobial treatment	Without prior antimicrobial treatment	*P* value
Microbiological investigation	No positive/tested (%)	No positive/tested (%)	
S. pneumoniae antigen detection in urine sample	14/103 (13.6)	67/230 (29.1)	0.002
Virus antigen detection in nasopharyngeal sample	8/91 (8.8)	27/223 (12.1)	0.437
M. pneumoniae PCR test	9/58 (15.5)	4/116 (3.4)	0.011
Blood culture	3/108 (2.8)	52/271 (19.2)	<0.001
Sputum culture	1/34 (2.9)	10/85 (11.8)	0.175
Serology[a]	32/101 (31.7)	32/219(14.6)	<0.001

[a]Including serology for *M. pneumoniae* and *C. pneumoniae*.

Table 17. Comparison of the mean values of C-reactive protein on admission (CRP1) with respect to the etiology of community-acquired pneumonia in 384 patients. Below diagonal, 95% confidence limits for differences between means; above diagonal, p-values. Overall ANOVA p-value <0.001 in CRP1 (Study III)

	CRP 1 (SD) (range) mg/l	*Streptococcus pneumoniae* *n* = 95	*Chlamydophila pneumoniae* *n* = 24	*Mycoplasma pneumoniae* *n* = 36	Respiratory virus *n* = 27	Other agent[a] *n* = 7	Dual infection[b] *n* = 20	Unknown *n* = 175
Streptococcus pneumoniae	291 (142) (4-650)		0.003	<0.001	<0.001	0.452	0.009	<0.001
Chlamydophila pneumoniae	200 (110) (25-531)	20 to 162		0.200	0.055	1.000	1.000	0.981
Mycoplasma pneumoniae	133 (53) (28-225)	97 to 219	-16 to 149		0.990	0.562	0.259	0.175
Respiratory virus	113 (71) (12-248)	109 to 245	-1 to 174	-60 to 99		0.310	0.081	0.034
Other agent	210 (84) (70-350)	-42 to 202	-145 to 123	-206 to 51	-230 to 35		1.000	0.990
Dual infection	200 (109) (27-445)	14 to 168	-95 to 94	-154 to 20	-178 to 6	-126 to 148		0.987
Unknown	181 (92) (1-560)	70 to 150	-49 to 87	-105 to 10	-132 to -3	-90 to 150	-54 to 93	

[a]*Staphylococcus aureus,* 3 cases; *Legionella pneumophila*, 2 cases; *Streptococcus pyogenes,* 1 case; *Fusobacterium* sp, 1 case.
[b]*S. pneumoniae* with a respiratory virus, 11 cases; *S. pneumoniae* with *C. pneumoniae*, 1 case; *M. pneumoniae* with influenza A, 3 cases; *M. pneumoniae* with *H. influenzae*, 2 cases; *C. pneumoniae* with *S. aureus*, 2 cases; *C. pneumoniae* with adenovirus, 1 case

5.2. Usefulness of CRP in assessing CAP severity, complications and etiology (III)

5.2.1. CRP on admission

CRP on admission (CRP1) was significantly higher in patients with alcoholism or smoking, and lower in patients with preceding antibiotic treatment compared to those without these baseline characteristics.

The mean value of CRP1 was significantly higher among the patients who were transferred to the ICU than among those who were not (P<0.001). No significant difference was observed in the mean values of CRP1 between the patients who died and those who survived (P=0.998). Significant (P<0.001) differences in mean CRP1 were observed between the patients belonging to various etiological groups (**Table 17**).

By univariate analysis, significant (P<0.001) differences were observed in CRP1 between the patients belonging to different PSI classes (**Table 18**). The differences in CRP1 were also significant (P<0.001), when PSI groups I-II combined were compared to PSI groups III-V combined. By multivariate analysis, the differences between the PSI groups remained significant after adjusting for age, sex, cardiovascular disease, bacteremic pneumonia and etiological agents (P=0.004). An increment of 50 mg/l of the CRP1 value was associated with a 1.22-fold odds for a patient to be in PSI class III-V as compared to being in class I-II (OR 1.22, 95% CI 1.11 to 1.34; P<0.001).

Table 18. Mean (SD) values of C-reactive protein on admission (CRP1) according to Pneumonia Severity Index (PSI) classes (Study III)

	PSI I *n* = 124	PSI II *n* = 113	PSI III *n* = 59	PSI IV *n* = 73	PSI V *n* = 15	*P* value
CRP1 mg/l						
Mean (SD)	163 (88)	200 (104)	232 (139)	220 (140)	320 (126)	<0.001
Range	1-445	4-502	8-650	13-560	129-491	
		PSI I-II combined		PSI III-V combined		*P* value
CRP1 mg/l						
Mean (SD)		180 (97)		236 (139)		<0.001
Range		1-502		8-650		

The mean (SD) values of CRP1 were significantly higher in the patients with bacteremic pneumonia than in those with nonbacteremic pneumonia (P<0.001). The difference was significant between the patients with bacteremic and nonbacteremic pneumococcal pneumonia (P<0.001). An increment of 50 mg/l of the CRP1 value was associated with a 1.67-fold odds for a patient to be bacteremic (OR 1.67, 95% CI 1.46 to 1.92; P<0.001).

ROC analysis was used to establish a cut-off point for CRP1 that would predict bacteremia with a sensitivity of at least 75% combined with the best possible specificity. A concentration of 230 mg/L was identified as such a cut-off point. CRP1 was ≥230 mg/l for 43 of the 55 patients with bacteremic pneumonia (sensitivity 78%) and <230 mg/l for 243 of the 324 patients with nonbacteremic pneumonia (specificity 75%) (area under the ROC curve 0.812).

5.2.2. CRP and clinical stabilization

The mean (SD) duration between the admission and the day when the patient was stabilized was 4.6 (3.2) days (range 1-27). There were significant differences in the CRP values at the time of clinical stability (CRP2) between the different PSI classes (*P*=0.022) and the etiology of CAP (*P*=0.029). By pairwise comparisons of the etiological agents, the difference was significant only between the patients with pneumococcal pneumonia and those with mycoplasma pneumonia.

There was a trend for an association between the level of CRP1 and the time to reach clinical stability**.** In the Cox's regression analysis, it was found that for an increment of 50 mg/l of the CRP1 value, the risk for the patient to remain unstabilized increased by 6% (HR 1.06, 95% CI 1.02 to 1.11; *P*=0.005).

5.2.3. CRP during the follow-up

Figure 3 shows the CRP values during the first five days of hospitalization with respect to PSI classes (A), etiology of CAP (B), bacteremic and nonbacteremic disease (C) and the development of complications (D).

The risk to be admitted to an ICU or the need to change the initial antimicrobial treatment was significantly higher in the patients with CRP values >100 mg/l on day 4 after admission and a significantly smaller number of these patients had been stabilized at that time point than of those whose CRP values were ≤100 mg/l (**Table 19**). The CRP value was >100 mg/L in all 4 patients who developed empyema.

Table 19. Comparison between patients with community-acquired pneumonia who had C-reactive protein (CRP) values ≤ 100 mg/l and > 100 mg/l on day 4 after admission (Study III)

	CRP ≤ 100 mg/l *n* = 172	CRP >100 mg/l *n* = 100	*P* value	CRP missing *n* = 82
Mean (SD) duration of hospitalization (range)	7.2 (4.0) (4-36)	13.1 (7.8) (6-42)	<0.001	8.2 (4.4) (4-29)
Patients clinically stabilized	130 (75.6)	19 (19.0)	<0.001	56 (68.3)
Patients admitted to the ICU	12 (7.0)[a]	21 (21.0)[b]	<0.001	2 (2.4)
Change of antimicrobial treatment	41 (23.8)	63 (63.0)	<0.001	13 (15.8)
Death	4 (2.3)[c]	4 (4.0)[d]	0.425	2 (2.4)[d]
Empyema	0	4 (4.0)	0.008	0

Data are number (%) of patients, unless otherwise indicated.
[a]None of the patients were admitted to the ICU later than day 4
[b]Two patients were admitted to the ICU later than day 4.
[c]Two patients died on day 4 and two patients later.
[d]All died later than day 4.
ICU, intensive care unit.

5.3. Usefulness of complement receptor 1, complement receptor 3, and FcγR1 on neutrophils in differentiating between bacterial and viral pneumonia (IV)

The mean expression of complement receptor 1 (CR1) on neutrophils was significantly higher in the patients with pneumococcal pneumonia than in those with influenza A

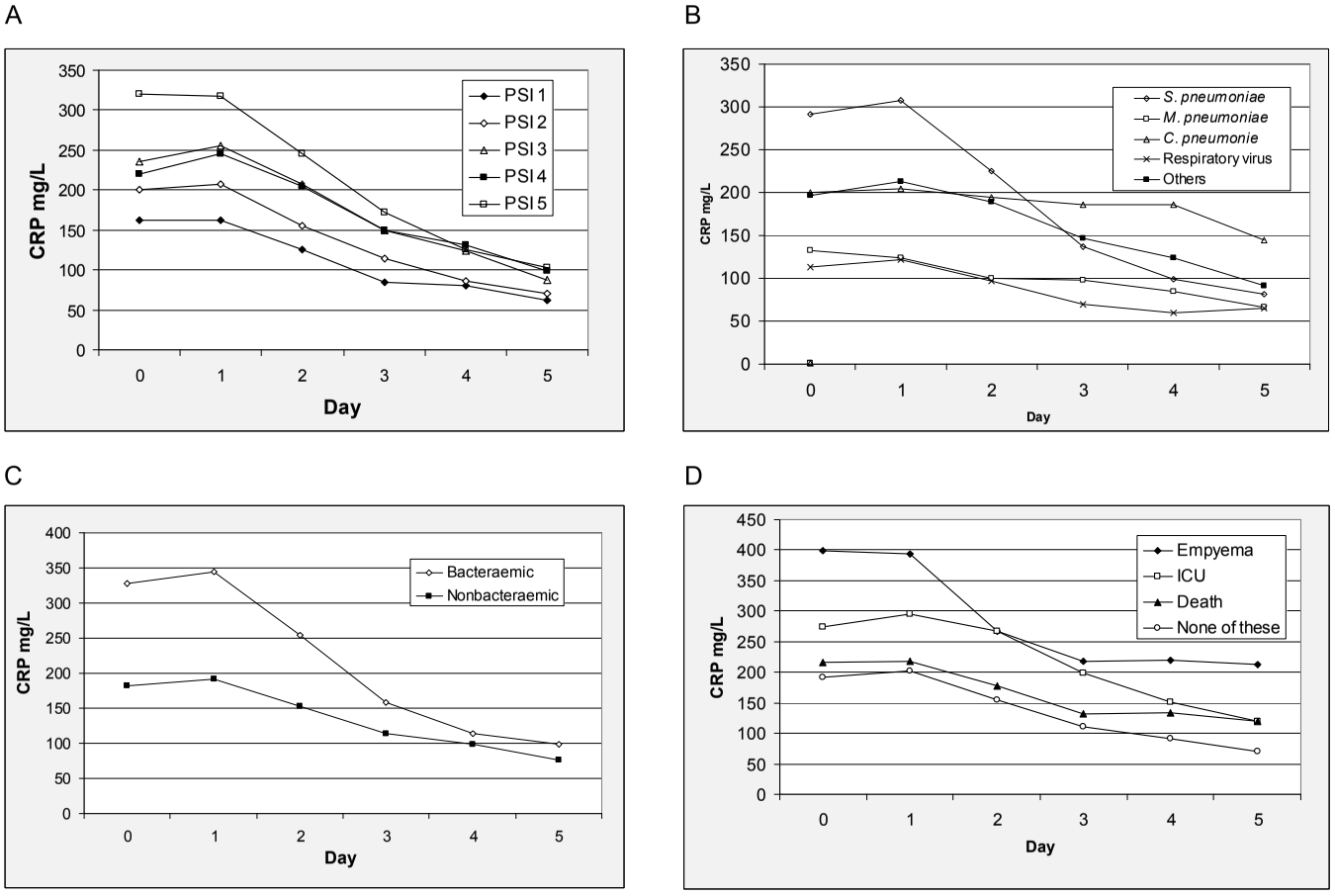


Figure 3. Mean values of C-reactive protein (CRP) in patients with community-acquired pneumonia on admission and during first five days of hospitalization with respect to Pneumonia Severity Index (PSI) classes (A), etiology (B), bacteremic and nonbacteremic disease (C) and the development of complications (D). Number of observations: 384 on day 0 (admission), 371 on day 1, 343 on day 2, 323 on day 3, 272 on day 4 and 233 on day 5. (Study III)

pneumonia. The mean expression of CR1 was also significantly higher in etiologically undefined pneumonia than in influenza A pneumonia, but there was no difference between pneumococcal and undefined pneumonia. Patients with influenza A could be divided in 2 subgroups by the expression of CR1 on neutrophils: 8 patients with CR1 ranging from 2.09 to 5.32, and 5 patients with CR1 ranging from 11.1 to 15.5. The CRP values ranged from 44 to 178 mg/l and from 120 to 300 mg/l in these subgroups, respectively. The expression of neutrophil CR1 in the patients and controls is presented in **Figure 4**.

The expression of CR1 ranged from 3.3 to 8.6 in the 3 patients with *M. pneumoniae* infection, was 6.8 in the patient with *C. pneumoniae* infection, and 33.6 in the patient with *L. pneumophila* infection.

There was no difference in the expression of CR3 on neutrophils in patients with pneumococcal pneumonia compared to patients with influenza A infection (63.1 vs. 80.7, *P*= 0.619). Instead there was a significant difference in expression between patients with pneumococcal pneumonia and patients with etiologically undefined pneumonia (63.1 vs. 107.2 *P*= 0.002).

The expression of receptors for the Fc portion of IgG, designated FcγR1, was significantly higher in all etiological groups of CAP compared to control group (*P* values <0.001-0.005) while no significant difference was detected between the three etiological groups.

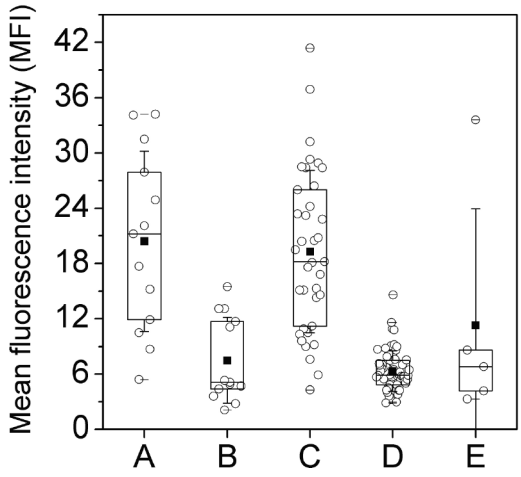


Figure 4. Expression of complement reseptor 1 on neutrophils in patients with pneumonia and controls. A: *Streptococcus pneumoniae* pneumonia; B: influenza A pneumonia; C: etiologically undefined pneumonia; D: controls; E: atypical pneumonia. (Study IV)

5.4. Value of BAL for etiological diagnosis of CAP in a routine setting (V)

An indication for the performance of BAL was severe CAP on admission or rapid deterioration in condition in 36 episodes, and slowly responding pneumonia in 35 episodes. The mean (SD) duration of the hospitalization before BAL was 9.9 (8.5) days (range 1 to 41 days). All BALs were performed during antimicrobial treatment of the patient.

5.4.1. BAL cultures

A total of 71 conventional bacterial cultures, 71 mycobacterial cultures, 69 *Legionella* spp. cultures, 66 fungal cultures, 61 viral cultures and 40 *Nocardia* spp. cultures were performed on the 71 BAL samples. The findings designated as significant for CAP are presented in **Table 20**.

Eight (11.2%) BALs produced $>10^4$ cfu/ml in terms of quantitative bacterial cultures. Only one (1.4%) of them was classified as clinically significant and yielded *P. aeruginosa;* it was obtained 2 days after the patient had been admitted. One mycobacterial culture yielded *Mycobacterium avium* which was considered significant. One *Legionella* spp. culture from a previously healthy patient grew *L. pneumophila*.

In 2 (3.0%) fungal cultures, *Candida* spp. grew in significant amounts. These specimens were obtained 3 and 4 days after admission and were considered to represent either colonization or contamination from the upper airways.

Seven (11.5%) BAL fluid samples referred for viral culture were positive. In 3 cases of positive respiratory virus isolations, the patient had been admitted 2 to 4 days previously (one case each of RSV, influenza A and adenovirus), and the virus was classified as the etiological agent of CAP. All of these patients had respiratory failure requiring treatment in an ICU and 2 of them needed mechanical ventilation. Two patients had COPD, and one had no underlying disease. One of the patients with COPD and adenoviral infection died after 7 days of hospitalization.

5.4.2. BAL PCR assays

In 68 (95.7%) of the 71 BAL samples studied, one or several specific PCR tests were performed. The results were positive for 4 (5.9%) samples. PCR for *M. pneumoniae* was positive in one patient and for *Legionella* spp. in one patient; both had severe pneumonia requiring treatment in an ICU. *L. pneumophila* was concurrently isolated from *Legionella* spp. culture. The PCR samples positive for *P. jirovecii* (1) and cytomegalovirus (1) were classified as clinically not significant.

5.4.3. Total diagnostic yield and impact of BAL on therapy

BAL was diagnostic in 7 (9.8%) of the 71 patients with CAP. In the 36 patients with severe CAP, who had BAL performed within 7 days after the admission, 6 (16.7%) findings were considered diagnostic for CAP. In the 35 patients with slowly resolving pneumonia, who had BAL performed later than 7 days after the admission, 1 finding was considered diagnostic for CAP (2.8%). In this patient group, bronchoscopy and BAL revealed also 3 cases of hospital-acquired respiratory virus infections, 2 cases of malignancy and 1 case of secondary, ventilatory-associated pneumonia. Thus, the total diagnostic yield in slowly resolving pneumonia was 20.0% (7/35).

The result of BAL indicated a change of the antimicrobial treatment of CAP in 1 of the 7 patients with a diagnostic BAL (**Table 20**).

Table 20. Etiology of community-acquired pneumonia, diagnostic methods and impact of bronchoalveolar lavage (BAL) on therapy (Study V)

			Diagnostic method		Chest radiograph findings		Change of Rx[b]	Mortality
Etiological agent	Cases	BAL	Noninvasive[a]	Both	Local	Diffuse		
S. pneumoniae	10	0	10		3	7		2
M. pneumoniae	1	1	0		0	1	-[c]	0
L. pneumophila	2	1	2[d]	1	0	2	-[c]	1
P. aeruginosa	2	1	1		1	1	1	2
C. pneumoniae	1		1[e]		1	0		0
S. aureus	1	0	1		0	1		0
Bacteroides fragilis	1	0	1		0	1		1
Mycobacterium avium	1	1			0	1	-[f]	1
Respiratory virus	3	3			0	3	-[c]	1

[a] Blood culture or serology
[b] Result of BAL indicated a change of antimicrobial treatment.
[c] Result of BAL did not indicate a change of treatment.
[d] High titers of IgM and IgG antibodies in one patient, and high titers of IgM antibodies in the other.
[e] A significant increase in IgG titers in paired samples.
[f] The patient died before the result of the mycobacterial culture was available.

6. DISCUSSION

6.1. Etiological diagnosis of CAP (I, II, V)

6.1.1. Rapid diagnostic methods for etiological diagnosis of CAP (I, II)

During the last decade, new microbiological techniques have provided an opportunity to rapidly determine the etiology of CAP (Cheng et al. 2004, Rantakokko-Jalava and Jalava 2001, Roson et al. 2004[b], Strålin et al. 2006, Tondella et al. 2002, Waris et al. 1998). One of the main purposes of this study was to evaluate the usefulness of these rapid methods to provide an early etiological diagnosis. Although clinically this is the most essential objective of the etiological diagnostics, previous studies have seldom focused on this question.

In this study, a wide range of rapid microbiological methods, including pneumococcal urinary antigen detection, antigen detection of respiratory viruses and PCR tests for *M. pneumoniae*, *C. pneumoniae* and influenza A and B viruses, were applied. The total diagnostic yield was 54.4%, which is comparable to previous reports (Bohte et al. 1995, Luna et al. 2000, Roson et al. 2001, Socan et al. 1999). Higher diagnostic yields (60.0-80.6%) have been obtained in studies which have put more emphasis on the serological diagnosis of etiological agents including conventional bacterial pathogens (Jokinen et al. 2001, Lieberman et al. 1996, Lim et al. 2001, Örtqvist et al. 1990) and in studies with more extensive use of invasive methods, e.g., BAL or transthoracic needle aspiration (Ishida et al. 1998, van der Eerden et al. 2005). In one of these studies, the diagnostic yield was no less than 76%; here, a wide range of PCR methods was used, which increased the diagnostic yield of viral agents, in particular (Templeton et al. 2005). In the clinical setting of the Department of Infectious Diseases of Turku University Hospital, more than half (58.7%) of the confirmed etiological agents were identified in the acute phase by antigen detection or gene amplification.

6.1.1.1. CAP due to bacterial pathogens (I)

In the patients of Study I, *S. pneumoniae* was the most common etiological agent of CAP and accounted for 28% of all cases. Thus, the most valuable rapid diagnostic method was the *S. pneumoniae* urinary antigen test which doubled the cases that were identified as pneumococcal pneumonia based on blood and sputum cultures. Unlike in children, the specificity of this test in adults is high – 92-100% (Roson et al. 2004[b], Strålin et al. 2004) – and a positive test can be considered diagnostic. The sensitivity of the *S. pneumoniae* urinary antigen test is between 54% and 94%, higher in patients with bacteremic pneumococcal pneumonia – 75% in this material - and in patients with severe disease (Roson et al. 2004[b], Strålin et al. 2004).

The combined proportion of *M. pneumoniae* and *C. pneumoniae* as the etiological agents of CAP was 18% of all 384 cases. Both *M. pneumoniae* and *C. pneumoniae* have traditionally been diagnosed by serology, but recently PCR tests have been introduced. Although the sensitivity of *M. pneumoniae* PCR in patients with CAP has generally been low (Räty et al. 2005, Strålin et al. 2006, Waris et al. 1998), in Study I the results of PCR

and acute phase serology complemented each other in the detection of *M. pneumoniae* in an early phase of CAP. Thirteen cases were diagnosed with *M. pneumoniae* by PCR from throat swab specimens, while only 2 of these patients had IgM antibodies in their acute phase serum samples. The use of sputum rather than nasopharyngeal specimens might increase the sensitivity of PCR (Räty et al. 2005, Strålin et al. 2006), but not many patients are able to produce an adequate sputum specimen at examination. Only one case of *C. pneumoniae* in our patients was diagnosed by PCR. The low yield of the *C. pneumoniae* PCR test is in agreement with earlier reports (Strålin et al. 2006, Templeton et al. 2005). Combined with previous findings, our results indicate that more reliable rapid tests for the detection of both *M. pneumoniae* and *C. pneumoniae* are needed (Räty et al. 2005, Strålin et al. 2006, Templeton et al. 2005, Waris et al. 1998).

Consistent with some earlier studies, there was no difference in the total diagnostic yield between the patients with and without antimicrobial treatment before admission (Luna et al. 2000, van der Eerden et al. 2005). On the other hand, the diagnostic yield of blood culture and pneumococcal antigen detection was lower among those who had received antibiotics beforehand. A similar observation has been made by van der Eerden *et al*. (van der Eerden et al. 2005). However, in the group of patients with prior antibiotics there were significantly more patients from PSI groups I-II, more patients with mycoplasma pneumonia and fewer patients with pneumococcal pneumonia. This variation in the patient characteristics may partly explain the differences in the diagnostic yield of various methods between these two groups.

6.1.1.2. Viral agents associated with CAP (I, II)

In the whole population of 384 patients (Study I), respiratory viruses accounted for 10.9% of the etiology of CAP. This finding is comparable to previous reports showing frequencies of 4% to 16% of respiratory viruses (Bohte et al. 1995, Ishida et al. 1998, Jokinen et al. 2001, Lieberman et al. 1996, Luna et al. 2000, van der Eerden et al. 2005, Örtqvist et al. 1990). Here, the recognition of respiratory viruses was based mainly on antigen detection from nasopharyngeal samples. The method is rapid and provides a diagnosis in a few hours. The viral antigen tests can be recommended for the etiological diagnostics of CAP also based on the relatively high diagnostic yield obtained by these methods in the present study. Yet, apart from detection of influenza virus, a positive antigen test seldom has an impact on treatment.

In most previous studies, the diagnosis of respiratory viruses has been based on serological tests (Bohte et al. 1995, Ishida et al. 1998, Jokinen et al. 2001, Lieberman et al. 1996, Lim et al. 2001, Roson et al. 2001, van der Eerden et al. 2005), and viral antigen detection has only been used in few studies (Luna et al. 2000, Socan et al. 1999). The incidence of positive antigen detection was somewhat higher in our study (11.1%) than in the previous studies (4.3-5.0%) which was probably due to the high sensitivity of the TR-FIA method used here (Waris et al. 1988). In addition, the significantly shorter 12 month study period (compared to our 5 years) and variable epidemiological situations in the earlier series might be partly responsible for the different findings.

In Study I, only the influenza A and influenza B PCR tests were targeted on viruses. Both tests were performed on samples from 184 patients. The diagnostic yield of these PCR

methods was very low, with only 3 samples (1.6%) positive for influenza A and none positive for influenza B. Due to the relatively small percentage of the patients in whom these tests were performed, no definite conclusions can be drawn on their usefulness based on the results of the present study. It is plausible that a more extensive use of PCR might allow the recognition of a wider range of respiratory viruses and increase the yield in viral diagnostics (Templeton et al. 2005). Even so, antigen detection may still be more often available in clinical practice and more economical than PCR.

In Study II, the role of respiratory picornaviruses as causative agents of CAP in adults and their contribution to disease severity was examined in a subset of 231 patients who had their throat swab specimens examined also for the presence of rhinoviruses and enteroviruses. Previously, Jennings *et al*. have reported on the common nature of mixed viral/bacterial etiology in patients with CAP. They found an association between mixed rhinovirus/pneumococcal infection and severe disease (Jennings et al. 2008). Experimental studies have shown that the adherence of *S. pneumoniae* to human tracheal epithelial cells is increased in the presence of rhinovirus (Ishizuka et al. 2003). The results of Jennings *et al*. prove this association in vivo by showing that 39% of their patients with rhinovirus identified from a nasopharyngeal sample had concurrent *S. pneumoniae* infection (Jennings et al. 2008).

In this subset of 231 patients, 19 were positive for respiratory picornaviruses, including 12 patients with enteroviruses and 7 patients with rhinoviruses. Additional etiological agents were identified in more than half of these cases. In agreement with the results of Jennings *et al*., as many as 57% of our patients with rhinovirus also had *S. pneumoniae* infection. Rhinovirus was associated with severe disease (PSI IV-V) in 29% of the cases, and one of the patients with mixed rhinovirus/pneumococcal infection died. The percentage of severe disease in our patients was somewhat lower than the 39% of severe rhinovirus-associated infections reported by Jennings *et al*. (Jennings et al. 2008).

Only one previous study has included the enterovirus PCR test in the diagnostic array of CAP in non-immunocompromised adult patients (Angeles Marcos et al. 2006). Moreover, only one (0.5%) of the 198 patients in that study had enterovirus infection. In the present study, on the other hand, enterovirus was the second most common viral agent after influenza A and was detected in 5% of our patients. This percentage is similar to that observed in association with lower respiratory tract infection in children (Jennings et al. 2004), in whom enteroviruses are among the most important viruses causing this disease. Collectively, our findings corroborate those of Jennings *et al*. and support their conclusion that the importance of both viral pneumonia and mixed viral/bacterial pneumonia may be greater than previously assumed.

6.1.1.3. Limitations of epidemiological information (I)

The present study has limitations as regards the epidemiology of CAP. First, the number of patients with CAP included during the study period is rather small considering that our university hospital serves as a primary-care facility for infectious diseases in a catchment area of about 200,000 inhabitants. The modest number of patients is due to the fact that in our area, patients with CAP are treated in 2 additional hospitals. Those with asthma or COPD as an underlying disease are often referred to the Respiratory Diseases Unit of

the Turku University Hospital. In addition, elderly patients with CAP may be admitted to the Turku City Hospital. This may have caused some bias, e.g., as regards the relative proportions of various causative agents of CAP. The main focus of this study was not, however, epidemiological.

Also, it is of note that not all of the diagnostic tests were carried out on every patient included in the study. At least to some extent, this impedes the conclusions made on the performance of different diagnostic methods. It is plausible that a higher total diagnostic yield may have been achieved had the diagnostic tests been performed uniformly in all patients. The diagnostic yield might have increased also by the application of a larger array of PCR tests especially for viral pathogens, as illustrated by the results of Study II. In that study, the proportion of viruses as etiological agents was 20% in the subset of 231 patients who were examined by PCR also for the presence of rhinoviruses and enteroviruses, while the proportion of viruses in Study I was only 10.9%.

6.1.1.4. Usefulness of rapid microbiological methods with respect to disease severity (I)

Only a few previous studies have concurrently analyzed the diagnostic yield of multiple rapid microbiological methods in different PSI groups, and the differences detected have been minor (Roson et al. 2001, van der Eerden et al. 2005). In the present study, the yield of the urinary pneumococcal antigen test increased significantly between PSI groups I and IV-V (from 11% to 37%). A somewhat unexpected finding was that the diagnostic yield of viral antigen detection from the nasopharyngeal sample was highest in patients in PSI groups IV-V. On the other hand, the PCR tests positive for mycoplasma were concentrated in the PSI groups I-II.

The yield of *S. pneumoniae* from blood cultures increased with PSI grade from 4.9% in PSI group I to 30.2% in PSI groups IV-V. Similar results have been previously reported (Waterer and Wunderink 2001). On the other hand, there are reports in which no or only insignificant differences were found (Roson et al. 2001, van der Eerden et al. 2005). At least two factors can contribute to the association between the PSI grade and the yield of blood culture. First, in pneumococcal and other bacterial pneumonias, bacteremia may be more common in severe than in mild cases. Second, compared to milder cases, a larger proportion of severe pneumonias may be caused by microbes detectable in blood cultures. Evidently, both of these factors were pertinent in our patients since 68.0% of all pneumococcal pneumonias in PSI classes IV-V were blood culture positive, while *M. pneumoniae* dominated as the etiological agent in PSI class I.

6.2. Usefulness of CRP to assess disease severity, complications and etiology (III)

The usefulness of the CRP values in the evaluation of patients with CAP was analyzed with special reference to disease severity. In this respect, the positive correlation between high CRP values and severe disease was one of the main findings. This was manifested, e.g., by the significantly higher CRP1 values in the patients who were transferred to an ICU than in those who were not, as well as by the significant association between the CRP1 levels and PSI class. Moreover, the CRP1 levels in PSI classes I-II differed

significantly from those in PSI classes III-V. These are clinically the 2 most important groups to be differentiated, since the risk of mortality and ICU admission rates are highest in the high PSI classes (Fine et al. 1997[a], Garau et al. 2008, Man et al. 2007). In a clinical setting, assessment of the PSI provides valuable help for the attending physician who must decide on whether a patient can be treated as an outpatient or whether he should be admitted to hospital. The PSI is not routinely determined in Finland, while CRP is. Based on the results of Study III, the higher the CRP1 value, the greater were the odds for a patient to belong to PSI class III-V as compared to class I-II. The significant correlation between PSI and CRP1 supports the concept that very early during patient evaluation, both of these parameters can be used towards the same end. However, the high range of the CRP values in different PSI classes makes the interpretation of CRP in respect to the disease severity even more complex. Consequently, it is evident that determination of the CRP on admission does not replace the assessment of the PSI, but that these 2 parameters are useful when used to complement each other.

Several studies have shown that mortality and admission rates to an ICU are higher among patients with bacteremic pneumonia than nonbacteremic pneumonia (Fine et al. 1996, Garau et al. 2008, Musher et al. 2000). Consequently, it is important that patients with bacteremia are rapidly recognized, since they should be admitted to hospital. In the present study, bacteremic patients had significantly higher CRP1 levels compared to nonbacteremic patients: an increment of 50 mg/l on admission was associated with a 1.67-fold odds for bacteremia. Furthermore, a cut-off point of 230 mg/l of CRP1 predicted bacteremia with a sensitivity of 78% and specificity of 75%. The use of this cut-off point may be especially useful in patients of PSI class I-II, for whom outpatient treatment is usually considered appropriate (Fine et al. 1997[a], Mandell et al. 2007). Still, high CRP values on admission should raise a suspicion of bacteremia even in this group of patients. In the present work, CRP1 was >230 mg/l in 13 (72%) of the 18 bacteremic patients in PSI class I-II. The result suggests that the CRP value may provide additional information on the likelihood of bacteremia. Also in this respect the CRP value is complementary to PSI classification. Recently Falguera *et al.* have developed a simple score based entirely on epidemiological and clinical variables to stratify patients with CAP into groups with a low or high risk of developing bacteremia (Falguera et al. 2009).

CRP proved valuable as a follow-up test: the CRP levels fell rapidly in accordance with the clinical recovery of the patient. This is in line with the few previous reports that have studied the usefulness of CRP as a follow-up test (Bruns et al. 2008, Chalmers et al. 2008[a], Menendez et al. 2008, Smith et al. 1995[b]). An important finding in our patients was that CRP levels of >100 mg/l on day 4 after admission suggest treatment failure or complications. It is of note that only 19% of the patients with CRP levels >100 mg/l on day 4 were in a stable condition, and 21% of them needed treatment in an ICU and 63% a change of antimicrobial treatment. The CRP value was >100 mg/l in all 4 patients who developed empyema.

As far as we know, the present study is the first to evaluate the level of CRP at the time of clinical stabilization, with the finding of a significant association between CRP2 and PSI classes. The significant association observed between CRP1 and the duration of time to reach clinical stability further corroborates the connection between high CRP values and severe disease.

CRP1 values were associated with alcohol abuse and smoking but not with any of the underlying diseases studied. The only two earlier studies on CAP focusing on the association between CRP and underlying diseases have yielded discordant results (Almirall et al. 2004, Bruns et al. 2008). On the other hand, the finding of a significantly lower level of CRP1 in the patients with preceding antibiotic treatment compared to those without antimicrobial treatment is consistent with some other studies (Smith et al. 1995[a], Örtqvist et al. 1995). This may be due to the antimicrobial treatment as such or, alternatively, to the patient population. As shown in Study I, significantly more patients in the antimicrobial treatment group were of PSI class I-II or had mycoplasma pneumonia, while significantly fewer had pneumococcal pneumonia.

Previous studies evaluating the changes in the concentrations of CRP in the serum with respect to etiology have yielded somewhat inconsistent results. A few studies have found no difference in the CRP levels between different etiologies, while others have found significantly higher CRP levels only in bacteremic pneumococcal pneumonia (Hedlund and Hansson 2000, Kosmas et al. 1997, Kragsbjerg et al. 1995, Örtqvist et al. 1995). In one study, the CRP values were significantly higher in *L. pneumophila* pneumonia than in any other group (García-Vázquez et al. 2003). In another study, significantly higher CRP levels were reported in pneumococcal or *L. pneumophila* pneumonia compared to pneumonia of other etiologies (Almirall et al. 2004). Here, CRP1 values in pneumococcal pneumonia differed significantly from all other etiologies but in a small group of patients with a pyogenic bacterial pathogen no difference was observed. The CRP1 values were lowest in viral and mycoplasma pneumonia but the differences between these and other groups were not throughout significant. Although there were significant differences in the CRP1 values between the different causative agents, the range within each group was wide with the lowest values less than 80 mg/l and highest values above 200 mg/l. The high variation and large overlap in the values of the patients with different microbial pathogens indicate that the CRP concentration is not a reliable guide as to decisions regarding the etiology of CAP in an individual patient.

6.3. Usefulness of CR1, CR3, and FcγR1 on neutrophils for differentiation between bacterial and viral pneumonia (IV)

Phagocytosis is an important part of the cellular defence system. The first step in phagocytosis is adherence of a particle onto a phagocyte membrane via complement receptors or Fc-receptors or both. In this study, we examined if the expression of complement receptors (CR1 and CR3) and Fcγ-receptors (FcγRI, FcγRII, and FcγRIII) on neutrophils or monocytes are of value in differentiating between bacterial and viral pneumonia in CAP patients.

The expression of CR1 on neutrophils was significantly higher in patients with pneumococcal pneumonia than in those with influenza A pneumonia. This suggests, on a more general level, that the expression of neutrophil CR1 is higher in classical bacterial pneumonia than in viral pneumonia. The high level of CR1 in etiologically undefined pneumonia is consistent with this finding, since one can speculate on an epidemiological basis (Almirall et al. 2000, de Roux et al. 2004, Jokinen et al. 2001) that most of these

patients probably did have bacterial pneumonia. In the patients with *M. pneumoniae* or *C. pneumoniae* infection, the expression of CR1 was low, although their number was too small to allow any distinct conclusions to be drawn from this finding. It is of note that there was a subgroup of patients with influenza A pneumonia who had CR1 levels ≥ 11.1. One explanation for this finding could be concomitant bacterial pneumonia.

Unlike the expression of CR1, the expression of CR3 and FcγR1 on neutrophils could not differentiate patients with bacterial and viral pneumonia. The finding of a significantly higher expression of FcγR1 on neutrophils in patients with bacterial or viral infection compared to healthy controls was later shown in another study of 89 patients with bacterial infection and 46 patients with viral infection (Nuutila et al. 2007). However, in clinical practice, the differentiation of the patients with fever caused by infection from the patients with fever caused by non-infective causes of inflammation is a major challence. The ability of the expression of any of these receptors or a combination of the results of the expression of these receptors to differentiate these patient groups is an objective for further research.

Although the high expression of neutrophil CR1 is suggestive of classical bacterial pneumonia, it is unlikely that any single parameter of inflammation alone can reliably differentiate between bacterial and viral pneumonia. Rather, it is possible that the diagnostic accuracy could be improved by a combination of the results of CRP, erythrocyte sedimentation rate and several cell receptors. Studies are presently underway to determine whether the diagnostic yield provided by the measured individual variables increase when they are combined (Nuutila et al. 2006, Nuutila and Lilius 2007).

6.4. Value of BAL in etiological diagnosis of CAP in a routine setting (V)

Although bronchoscopy and BAL have been widely used, e.g., in Finnish university hospitals as a diagnostic aid in patients with CAP when therapeutic difficulties have become manifest during empiric antimicrobial treatment, its value in such conditions has not been critically evaluated. In the present study, we retrospectively assessed the patients treated between 1996 and 2000 for CAP in the infectious diseases unit and medical ICU of a university hospital in Finland, and who had undergone BAL when it was considered clinically indicated. The purpose was to assess the value of BAL in CAP in routine clinical conditions. All patients had received antimicrobial therapy before performance of BAL

6.4.1. Quantitative bacterial culture of the BAL fluid

Quantitative bacterial culture, which has been the diagnostic cornerstone in most of the earlier studies on CAP, was performed on all BAL specimens also here. It is noteworthy that only 1 of the 71 (1.4%) quantitative bacterial cultures of the BAL fluid yielded a microbe that was considered diagnostic for the etiology of CAP. This finding clearly indicates that quantitative bacterial culture of the BAL fluid is of negligible usefulness, if taken during antibiotic treatment. In many of the former studies, the scientific goal has led to examination of the patients before commencement of antimicrobial treatment, in fact before any clinical need for performing bronchoscopy and BAL has become

manifest. In these studies the diagnostic yield of BAL has been up to 77-94% and the result of BAL indicated therapeutic consequences in up to 39% of the patients (Dalhoff et al. 1993, Jimenez et al. 1993, Rasmussen et al. 2001).

Consistent with our results, earlier studies have shown that the recovery of bacteria from the BAL fluid by culture declines after commencement of antimicrobial treatment. In one of these studies, 18 of the 36 (50%) untreated patients exhibited positive quantitative bacterial cultures, as compared to only 6 of the 31 (19%) patients with prior antimicrobial therapy with positive cultures (Rasmussen et al. 2001). In another study, BAL cultures revealed the etiological diagnosis in 4 of the 21 (19%) patients with severe CAP who had received antimicrobial therapy (Sörensen et al. 1989). In a retrospective analysis of 20 patients with severe CAP who had undergone BAL during antimicrobial therapy, Ewig *et al*. considered only 1 (5%) examination as having provided a definite etiological diagnosis. The findings of the present work support their conclusion that the diagnostic yield of routine microbiological investigation in pretreated patients is low (Ewig et al. 1996).

6.4.2. Viral isolations from BAL fluid

A respiratory viral infection was identified by virus isolation from the BAL fluid in 3 (6.0%) patients, in whom the respiratory virus was the only agent identified as being the causative agent of CAP. All of these patients with severe CAP were admitted to the ICU and 2 of them required respiratory support. This is consistent with the severe picture of viral CAP in the patients described in Study II. Consequently, also viral culture of the BAL fluid may sometimes provide valuable clinical data, although it is still possible that some of the patients who merely had a viral infection actually had a mixed infection with some unidentified bacterial pathogen.

In previous studies on BAL in CAP, viral culture has been performed only rarely, and the results have usually been negative (Feinsilver et al. 1990, Sörensen et al. 1989, Thorpe et al. 1987). This may be due to the nature of the patient populations studied, as one of those studies focused on acute bacterial pneumonia and another on nonresolving pneumonia (Feinsilver et al. 1990, Thorpe et al. 1987). However, since respiratory viruses have been detected in a considerable part of patients with CAP with other diagnostic methods (Ruiz et al. 1999[a], Socan et al. 1999), viral culture of BAL fluid seems to be recommendable, at least for selected patients. Identification of viruses may turn out to be even more important in the future, along with the advances in the viral chemotherapy.

6.4.3. Specific PCR assays on BAL fluid

The present study is one of the first to apply several specific PCR assays simultaneously on BAL fluid to identify the etiology of CAP. During the study period, BAL fluid from these immunocompetent patients was analyzed by the same diagnostic assays as BAL fluid from immunocompromised patients according to the routine protocol in the Turku University Hospital. This large array of assays was evidently not cost-effective in CAP. Of the total of 212 PCR tests performed on these BAL specimens, only 2 (1%) positive findings were designated as significant: 1 case of *Legionella* spp. and 1 case of *M. pneumoniae*. On the other hand, the 1 positive *P. jirovecii* PCR test and the 1

positive cytomegalovirus PCR test were designated as clinically not significant. This finding clearly shows that these 2 PCR tests have no value in the assessment of CAP in immunocompetent hosts and should, therefore, not be performed on their BAL fluids.

PCR tests were not applied to identify respiratory viruses on the BAL specimens. In recent studies, PCR tests have proved more sensitive than virus isolation to diagnose, e.g., rhinovirus infections (Vuorinen et al. 2003). Inclusion of a PCR test for rhinoviruses in the analysis of BAL fluids in CAP patients might be reasonable considering that recent literature has implicated rhinoviruses as important in respiratory infections of patients with COPD (Greenberg 2002).

6.4.4. Diagnostic yield of BAL and etiology of CAP

The detection of only 1 (1.4%) etiological diagnosis of CAP based on quantitative bacterial cultures of the BAL fluid clearly indicates the minimal value of this diagnostic method during antibiotic treatment. Other methods, i.e., special bacterial cultures, viral cultures and specific PCR assays, provided the etiological diagnosis only seldom, but increased the total diagnostic yield for CAP to 9.8%. The result of BAL indicated a change of antimicrobial treatment for only 1 patient. Thus, in the current clinical setting, the total diagnostic yield of BAL in CAP is very low and its therapeutic implications minimal. It is evident that in order to provide a better diagnostic yield, BAL should be made earlier in the clinical course. This approach may be justified if the patient has a very severe clinical presentation of disease or when unusual pathogens are suspected.

6.4.5. Slowly responding pneumonia and BAL

When the patients who responded slowly to antimicrobial treatment of CAP were analyzed separately, the total diagnostic yield was 20%. In addition to one finding that was considered diagnostic for CAP, also hospital-acquired pathogens and malignancies were identified in these patients. Thus, in this particular subgroup, bronchoscopy and BAL may have some usefulness even after antimicrobial treatment has been initiated. The value of fiberoptic bronchoscopy in nonresolving pneumonia has been previously demonstrated by Feinsilver *et al*. who showed in a series of 35 consecutive patients treated with antimicrobials for at least one week before the procedure that it was diagnostic in 86% (12/14) (Feinsilver et al. 1990). However, the number of patients in both of these studies is too small to make any firm conclusions on the usefulness of BAL in patients with nonresolving pneumonia.

7. SUMMARY AND CONCLUSIONS

CAP is a major cause of morbidity and mortality in adult patients. The etiological diagnosis of the disease is problematic, since the causative agents are seldom identified by noninvasive methods, e.g., blood cultures, sputum cultures and serology. During the last decade, new microbiological identification techniques such as antigen detection and gene amplification have provided an opportunity to rapidly diagnose the etiology of CAP. This study was made to evaluate to what extent it is possible to diagnose the etiological agent in the acute phase of CAP using these rapid, novel methods.

More than half (58.7%) of the etiological agents of CAP were identified in the acute phase by antigen detection or gene amplification. The most valuable of the rapid methods was the *S. pneumoniae* urinary antigen test: it doubled the cases that were identified as pneumococcal pneumonia based on blood and sputum cultures. The diagnostic yield of blood culture was significantly higher in PSI classes III-V compared to PSI classes I-II. On the other hand, though the diagnostic yield of *S. pneumoniae* urinary antigen test was higher in patients with severe pneumonia, it was considerably high also in patients with non-severe pneumonia (17%). Thus, it might be cost effective to use the pneumococcal urinary antigen detection as the primary test for pneumococcal pneumonia and take blood cultures only from patients with severe pneumonia. Moreover, the higher diagnostic yield of various microbiological tests in severe pneumonia should encourage clinicians in active searching of the etiological agents in these patients.

Positive *M. pneumoniae* PCR and serology were detected here almost exclusively in mild cases. Since the diagnostic yield of the PCR tests also was considerably low, its clinical usefulness remains questionable. A new rapid method, more useful than the present PCR test, would also be needed for the diagnostics of *C. pneumoniae* which may even cause severe pneumonia. For viral diagnosis, antigen detection offers a good, rapid alternative which could be adjusted by season and epidemiology. A more extensive use of PCR would make it possible to recognize a wider range of viral agents and to increase the yield in viral diagnostics. Still, diagnosing a viral agent seldom has an impact on antimicrobial treatment.

There is only limited data on the role of respiratory picornaviruses as causative agents of CAP in adult patients and on their contribution to disease severity. In this study, enterovirus was the second most common viral agent after influenza A virus in patients with CAP. Additional etiological agents were indentified in more than half of the cases with enteroviruses or rhinoviruses. Moreover, the rhinovirus was associated with severe disease in 29% of the patients. Corroborating some earlier findings of others, our results indicate that the importance of both viral pneumonia and mixed viral/bacterial pneumonia may be greater than previously realized. Identification of viruses may turn out to be even more important in the future, along with the advances in the viral chemotherapy.

Previous studies on the usefulness of CRP in patients with CAP have yielded somewhat inconsistent results. In this study, the usefulness of CRP in estimating the severity and complications as well as etiology of CAP was evaluated with comprehensive statistical analyses. The results of Study III show that the CRP test may be valuable as a tool to

identify emerging complications during treatment of patients with CAP. The positive correlation between high CRP values and serious illness suggests that CRP may also be useful as a method to assess disease severity. Towards this end, CRP complements the PSI. High CRP values were associated with a failure to reach clinical stability. The results presented here show that the CRP test is not reliable to guide decisions regarding the etiology of CAP in an individual patient.

On admission, it may be difficult to differentiate between bacterial and viral CAP. The aim of Study IV was to test whether the measurement of the expression of complement receptors or Fcγ receptors on neutrophils and monocytes is a useful preliminary test to differentiate between bacterial and viral pneumonia. The results of this study suggest that the expression of CR1 is higher in classical bacterial pneumonia than in viral pneumonia. Thus, determination of the expression of CR1 may be of value as an additional rapid tool for differentiating between bacterial and viral CAP. These results are preliminary and more research is needed to assess the ultimate usefulness of this novel method in the diagnostics of pneumonia. The diagnostic accuracy might be improved by a combination of several cell receptors with other markers of inflammation.

Only a few previous studies have focused on the use of BAL in patients with CAP. The aim of Study V was to evaluate the diagnostic value of BAL in CAP in a routine clinical setting. All BALs were requested by the attending physicians and performed during antimicrobial treatment of the patient. Only one (1.4%) quantitative bacterial culture was considered diagnostic for CAP. The diagnostic yield increased to 9.8%, when other methods were used. The result of BAL indicated a change of antimicrobial treatment in only one patient with CAP. In slowly responding pneumonia, on the other hand, hospital-acquired pathogens and malignancies were identified, and this resulted in a total diagnostic yield of 20.0%. Based on these results, it is meaningless to perform BAL for discovering the potential causative agents of CAP if the patient has already received antimicrobials. In a subgroup of slowly responding pneumonia, bronchoscopy and BAL may, however, have some usefulness even after antimicrobial therapy has been started, since the procedure may reveal reasons other than the initial causative pathogens for the delayed improvement of the disease. However, a reason for a treatment failure may also be found by easier, noninvasive methods.

8. ACKNOWLEDGEMENTS

This study was carried out at the Department of Medicine of the University of Turku during the years 1999-2009.

I express my deep gratitude to Professor Jorma Viikari, Head of the Department of Medicine, for making the completion of this study possible. I warmly thank him for his kind support to me whenever I needed it. I am privileged to work under his leadership.

I owe my sincere thanks to Professor Emerita Auli Toivanen, former Head of the Department of Medicine, for her strong support during the preparation of my thesis. I remember with warmth the time of my specialization in internal medicine under her kind and encouraging guidance.

I owe my appreciation to Professor Emeritus Paavo Toivanen, former Head of the Department of Medical Microbiology, for his positive attitude towards my research and for the challenging opportunity to work in his laboratory.

I express my warmest gratitude to my supervisor Professor Pirkko Kotilainen for her excellent guidance and patience during these years. Her vast knowledge of science and continuous encouragement have been invaluable to me and my work. I am deeply grateful to Pirkko for her suggestion to me to specialize in infectious diseases and for everything she has taught to me in this intriguing field of clinical medicine. Her logical way of thinking, and warm-hearted support have guided and helped me in numerous situations throughout the years.

I also express my warm gratitude to my supervisor Professor Emeritus Jukka Nikoskelainen for his enthusiastic support for this study. His optimistic attitude and contribution have been priceless for this study. I warmly thank him for his expert guidance and his encouraging support during my years of specialization in infectious diseases.

I thank the reviewers of this thesis, Docent Asko Järvinen and Docent Markku Koskela for their valuable and constructive comments on my work. I appreciate their thorough work reviewing this thesis during busy days of an ongoing pandemic – and during summer holidays.

I express my deepest thanks to Docent Raija Vainionpää. Her precious support and enormous knowledge of virological diagnostics were essential for the initiation and completion of this thesis.

I owe my special thanks to Docent Olli Meurman, Head of the Laboratory of Clinical Microbiology, for his invaluable contribution to my thesis. With kindness and patience he offered much of his time and placed his expertise in methodology at my disposal. I also thank Kaisu Rantakokko-Jalava, MD, PhD, from the Laboratory of Clinical Microbiology for offering her expertise in microbiological methods. It has been is a pleasure to collaborate with Olli and Kaisu not only with this thesis but also in clinical work.

I greatly appreciate Docent Esa-Matti Lilius and Docent Jari Nuutila from the Department of Biochemistry of the University of Turku, for fruitful co-operation. They have shown me the interesting and complex world of the immunological science.

I owe my gratitude to Hans Helenius, MSc and Saija Hurme, MSc for their invaluable help with statistics of this work.

I thank my co-authors Docent Jukka Sipilä, Maija Heiro, MD, PhD, Terhi Katiskalahti, MSc, Iina Laitinen, MSc, and Anna Vahtera, MD.

I thank Taina Löytölä, Sari Rautio, Pirkko Aarikka, and all the personnel of the Department of Infectious Diseases for their warm support and invaluable contribution for this study. It has been a privilege to work with you.

I thank Docent Robert Paul, for revising the language of this thesis.

I express my thanks to Docent Ilkka Kantola and Docent Rauli Leino, my present and former superiors, and to all senior and junior colleagues at the Department of Medicine. I owe my warm thanks to Taina Mertamo for her kind help with practical and computer problems. I express my special thanks to my nearest colleagues Docent Jarmo Oksi, Päivi Jalava-Karvinen, MD, PhD, and Taru Finnilä, MD, for the enjoyable partnership.

My sincere thanks are due to Docent Reijo Peltonen, Head of the Department of Infection Control and Hospital Hygiene for all he has taught me about infectious diseases and hospital hygiene. I also thank my colleague Harri Marttila, MD, PhD, Marianne Routamaa, MNSc, and all the personnel of the Department of Infection Control and Hospital Hygiene for pleasant collaboration.

I owe my deepest gratitude to my mother Marjatta Rauhala, to my sisters Kaisa and Inkeri and to my nephew Akseli. I thank my husband Risto for his support during these years. Finally, I express my deepest and warmest thanks to my dear daughter Ilona, the most valuable thing in my life.

This work has been financially supported by the Turku University Foundation, the Maud Kuistila Foundation, the Research Foundation of Orion Corporation and the Turku University Hospital Research Fund.

Turku, September 2009

Ulla Hohenthal

9. REFERENCES

Ajello GW, Bolan GA, Hayes PS, Lehmann D, Montgomery J, Feeley JC, Perlino CA, and Broome CV. Commercial latex agglutination tests for detection of Haemophilus influenzae type b and Streptococcus pneumoniae antigens in patients with bacteremic pneumonia. J Clin Microbiol 25:1388-91, 1987

Albaum MN, Hill LC, Murphy M, Li YH, Fuhrman CR, Britton CA, Kapoor WN, and Fine MJ. Interobserver reliability of the chest radiograph in community-acquired pneumonia. PORT Investigators. Chest 110:343-50, 1996

Almirall J, Bolibar I, Vidal J, Sauca G, Coll P, Niklasson B, Bartolome M, and Balanzo X. Epidemiology of community-acquired pneumonia in adults: a population-based study. Eur Respir J 15:757-63, 2000

Almirall J, Bolibar I, Toran P, Pera G, Boquet X, Balanzo X, and Sauca G. Contribution of C-reactive protein to the diagnosis and assessment of severity of community-acquired pneumonia. Chest 125:1335-42, 2004

Angeles Marcos M, Camps M, Pumarola T, Antonio Martinez J, Martinez E, Mensa J, Garcia E, Penarroja G, Dambrava P, Casas I, Jimenez de Anta MT, and Torres A. The role of viruses in the aetiology of community-acquired pneumonia in adults. Antivir Ther 11:351-9, 2006

Angus DC, Marrie TJ, Obrosky DS, Clermont G, Dremsizov TT, Coley C, Fine MJ, Singer DE, and Kapoor WN. Severe community-acquired pneumonia: use of intensive care services and evaluation of American and British Thoracic Society Diagnostic criteria. Am J Respir Crit Care Med 166:717-23, 2002

Andreo F, Prat C, Ruiz-Manzano J, Lores L, Blanco S, Cuesta MA, Gimenez M, and Dominguez J. Persistence of Streptococcus pneumoniae urinary antigen excretion after pneumococcal pneumonia. Eur J Clin Microbiol Infect Dis 2008

Arancibia F, Ewig S, Martinez JA, Ruiz M, Bauer T, Marcos MA, Mensa J, and Torres A. Antimicrobial treatment failures in patients with community-acquired pneumonia: causes and prognostic implications. Am J Respir Crit Care Med 162:154-60, 2000

Arancibia F, Bauer TT, Ewig S, Mensa J, Gonzalez J, Niederman MS, and Torres A. Community-acquired pneumonia due to gram-negative bacteria and pseudomonas aeruginosa: incidence, risk, and prognosis. Arch Intern Med 162:1849-58, 2002

Arstila PP, Halonen, PE. Direct antigen detection Laboratory Diagnosis of Infectious Diseases. Principle and Practice. New York Springer Verlag 60-75 1988

Balis E, Boufas A, Iliopoulos I, Legakis NJ, and Zerva L. Severe community-acquired pneumonia with acute hypoxemic respiratory failure due to primary infection with Chlamydia pneumoniae in a previously healthy adult. Clin Infect Dis 36:e155-7, 2003

Barlow G, Nathwani D, and Davey P. The CURB65 pneumonia severity score outperforms generic sepsis and early warning scores in predicting mortality in community-acquired pneumonia. Thorax 62:253-9, 2007

Bartlett JG, and Mundy LM. Community-acquired pneumonia. N Engl J Med 333:1618-24, 1995

Bartlett JG, Dowell SF, Mandell LA, File Jr TM, Musher DM, and Fine MJ. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. Clin Infect Dis 31:347-82, 2000

Bartlett JG. Is activity against "atypical" pathogens necessary in the treatment protocols for community-acquired pneumonia? Issues with combination therapy. Clin Infect Dis 47 Suppl 3:S232-6, 2008

Baughman RP, and Conrado CE. Diagnosis of lower respiratory tract infections: what we have and what would be nice. Chest 113:219S-223S, 1998

Baum SG. Adenovirus. In: Mandell GL, Douglas RG, Bennett JE, and Dolin R. Mandell, Douglas, and Bennett's principles and practice of infectious diseases 6th edn. Philadelphia: Churchill Livingstone; 2005; p. 1835-1841.

Beersma MF, Dirven K, van Dam AP, Templeton KE, Claas EC, and Goossens H. Evaluation of 12 commercial tests and the complement fixation test for Mycoplasma pneumoniae-specific immunoglobulin G (IgG) and IgM antibodies, with PCR used as the "gold standard". J Clin Microbiol 43:2277-85, 2005

Beigel JH. Influenza. Crit Care Med 36:2660-6, 2008

Benenson RS, Kepner AM, Pyle DN, 2nd, and Cavanaugh S. Selective use of blood cultures in emergency department pneumonia patients. J Emerg Med 33:1-8, 2007

Bengtsson J, Wahl M, and Larsson P. Assessment of the BacT/Alert blood culture system: rapid bacteremia diagnosis with loading throughout the 24 h. Clin Microbiol Infect 4:33-37, 1998

Benin AL, Benson RF, and Besser RE. Trends in legionnaires disease, 1980-1998: declining mortality and new patterns of diagnosis. Clin Infect Dis 35:1039-46, 2002

Beovic B, Bonac B, Kese D, Avsic-Zupanc T, Kreft S, Lesnicar G, Gorisek-Rebersek J, Rezar L, and Letonja S. Aetiology and clinical presentation of mild community-acquired bacterial pneumonia. Eur J Clin Microbiol Infect Dis 22:584-91, 2003

Bisharat N, Omari H, Lavi I, and Raz R. Risk of infection and death among post-splenectomy patients. J Infect 43:182-6, 2001

Blount RE, Jr., Morris JA, and Savage RE. Recovery of cytopathogenic agent from chimpanzees with coryza. Proc Soc Exp Biol Med 92:544-9, 1956

Bochud PY, Moser F, Erard P, Verdon F, Studer JP, Villard G, Cosendai A, Cotting M, Heim F, Tissot J, Strub Y, Pazeller M, Saghafi L, Wenger A, Germann D, Matter L, Bille J, Pfister L, and Francioli P. Community-acquired pneumonia. A prospective outpatient study. Medicine (Baltimore) 80:75-87, 2001

Boersma WG, Lowenberg A, Holloway Y, Kuttschrutter H, Snijder JA, and Koeter GH. Pneumococcal capsular antigen detection and pneumococcal serology in patients with community acquired pneumonia. Thorax 46:902-6, 1991

Boersma WG, Lowenberg A, Holloway Y, Kuttschrutter H, Snijder JA, and Koeter GH. Rapid detection of pneumococcal antigen in pleural fluid of patients with community acquired pneumonia. Thorax 48:160-2, 1993

Boersma WG, Daniels JM, Lowenberg A, Boeve WJ, and van de Jagt EJ. Reliability of radiographic findings and the relation to etiologic agents in community-acquired pneumonia. Respir Med 100:926-32, 2006

Bogaert D, De Groot R, and Hermans PW. Streptococcus pneumoniae colonisation: the key to pneumococcal disease. Lancet Infect Dis 4:144-54, 2004

Bohte R, van Furth R, and van den Broek PJ. Aetiology of community-acquired pneumonia: a prospective study among adults requiring admission to hospital. Thorax 50:543-7, 1995

Boivin G, De Serres G, Hamelin ME, Cote S, Argouin M, Tremblay G, Maranda-Aubut R, Sauvageau C, Ouakki M, Boulianne N, and Couture C. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility. Clin Infect Dis 44:1152-8, 2007

Boussekey N, Leroy O, Alfandari S, Devos P, Georges H, and Guery B. Procalcitonin kinetics in the prognosis of severe community-acquired pneumonia. Intensive Care Med 32:469-72, 2006

Bouza E, Pintado V, Rivera S, Blazquez R, Munoz P, Cercenado E, Loza E, Rodriguez-Creixems M, and Moreno S. Nosocomial bloodstream infections caused by Streptococcus pneumoniae. Clin Microbiol Infect 11:919-24, 2005

Brandenburg JA, Marrie TJ, Coley CM, Singer DE, Obrosky DS, Kapoor WN, and Fine MJ. Clinical presentation, processes and outcomes of care for patients with pneumococcal pneumonia. J Gen Intern Med 15:638-46, 2000

Bratzler DW, Ma A, and Nsa W. Initial antibiotic selection and patient outcomes: observations from the National Pneumonia Project. Clin Infect Dis 47 Suppl 3:S193-201, 2008

Breiman RF, Spika JS, Navarro VJ, Darden PM, and Darby CP. Pneumococcal bacteremia in Charleston County, South Carolina. A decade later. Arch Intern Med 150:1401-5, 1990

Brown PD, and Lerner SA. Community-acquired pneumonia. Lancet 352:1295-302, 1998

Brown RB, Iannini P, Gross P, and Kunkel M. Impact of initial antibiotic choice on clinical outcomes in community-acquired pneumonia: analysis of a hospital claims-made database. Chest 123:1503-11, 2003

Brunkhorst FM, Al-Nawas B, Krummenauer F, Forycki ZF, and Shah PM. Procalcitonin, C-reactive protein and APACHE II score for risk evaluation in patients with severe pneumonia. Clin Microbiol Infect 8:93-100, 2002

Bruns AH, Oosterheert JJ, Hak E, and Hoepelman AI. Usefulness of consecutive C-reactive protein measurements in follow-up of severe community-acquired pneumonia. Eur Respir J 32:726-32, 2008

BTS Guidelines for the Management of Community Acquired Pneumonia in Adults. Thorax 56 Suppl 4:IV1-64, 2001

Buchbinder S, Leitritz L, Trebesius K, Banas B, and Heesemann J. Mixed lung infection by Legionella pneumophila and Legionella gormanii detected by fluorescent in situ hybridization. Infection 32:242-5, 2004

Carpenter AB. Immunoassays for diagnosis of infectious diseases. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, and Pfaller MA. Manual of Clinical Microbiology 9th Edition 2007;p. 257-270

Castro-Guardiola A, Armengou-Arxe A, Viejo-Rodriguez A, Penarroja-Matutano G, and Garcia-Bragado F. Differential diagnosis between community-acquired pneumonia and non-pneumonia diseases of the chest in the emergency ward. Eur J Intern Med 11:334-339, 2000

Chalmers JD, Singanayagam A, and Hill AT. C-reactive protein is an independent predictor of severity in community-acquired pneumonia. Am J Med 121:219-25, 2008[a]

Chalmers JD, Singanayagam A, Murray MP, and Hill AT. Prior statin use is associated with improved outcomes in community-acquired pneumonia. Am J Med 121:1002-1007 e1, 2008[b]

Chanock RM. Mycoplasma pneumoniae: proposed nomenclature for atypical pneumonia organism (Eaton agent). Science 140:662, 1963

Charles PG, Whitby M, Fuller AJ, Stirling R, Wright AA, Korman TM, Holmes PW, Christiansen KJ, Waterer GW, Pierce RJ, Mayall BC, Armstrong JG, Catton MG, Nimmo GR, Johnson B, Hooy M, and Grayson ML. The etiology of community-acquired pneumonia in Australia: why penicillin plus doxycycline or a macrolide is the most appropriate therapy. Clin Infect Dis 46:1513-21, 2008[a]

Charles PG, Wolfe R, Whitby M, Fine MJ, Fuller AJ, Stirling R, Wright AA, Ramirez JA, Christiansen KJ, Waterer GW, Pierce RJ, Armstrong JG, Korman TM, Holmes P, Obrosky DS, Peyrani P, Johnson B, Hooy M, and Grayson ML. SMART-COP: a tool for predicting the need for intensive respiratory or vasopressor support in community-acquired pneumonia. Clin Infect Dis 47:375-84, 2008[b]

Cheng SM, Vainionpaa R, Zhao P, Li F, Hu A, Forrest B, and Rappaport R. Detection of influenza B in clinical specimens: comparison of high throughput RT-PCR and culture confirmation. Virus Res 103:85-90, 2004

Christ-Crain M, Stolz D, Bingisser R, Muller C, Miedinger D, Huber PR, Zimmerli W, Harbarth S, Tamm M, and Muller B. Procalcitonin guidance of antibiotic therapy in community-acquired pneumonia: a randomized trial. Am J Respir Crit Care Med 174:84-93, 2006

Christ-Crain M, and Muller B. Biomarkers in respiratory tract infections: diagnostic guides to antibiotic prescription, prognostic markers and mediators. Eur Respir J 30:556-73, 2007

Clyde WA, Jr. Clinical overview of typical Mycoplasma pneumoniae infections. Clin Infect Dis 17 Suppl 1:S32-6, 1993

Clyne B, and Olshaker JS. The C-reactive protein. J Emerg Med 17:1019-25, 1999

Cockerill FR, 3rd, Reed GS, Hughes JG, Torgerson CA, Vetter EA, Harmsen WS, Dale JC, Roberts GD, Ilstrup DM, and Henry NK. Clinical comparison of BACTEC

9240 plus aerobic/F resin bottles and the isolator aerobic culture system for detection of bloodstream infections. J Clin Microbiol 35:1469-72, 1997

Cockerill FR, 3rd, Wilson JW, Vetter EA, Goodman KM, Torgerson CA, Harmsen WS, Schleck CD, Ilstrup DM, Washington JA, 2nd, and Wilson WR. Optimal testing parameters for blood cultures. Clin Infect Dis 38:1724-30, 2004

Colice GL, Morley MA, Asche C, and Birnbaum HG. Treatment costs of community-acquired pneumonia in an employed population. Chest 125:2140-5, 2004

Cox NJ, and Subbarao K. Influenza. Lancet 354:1277-82, 1999

Dalhoff K, Braun J, Hollandt H, Lipp R, Wiessmann KJ, and Marre R. Diagnostic value of bronchoalveolar lavage in patients with opportunistic and nonopportunistic bacterial pneumonia. Infection 21:291-6, 1993

Dallo SF, and Baseman JB. Intracellular DNA replication and long-term survival of pathogenic mycoplasmas. Microb Pathog 29:301-9, 2000

Daneman N, McGeer A, Green K, and Low DE. Macrolide resistance in bacteremic pneumococcal disease: implications for patient management. Clin Infect Dis 43:432-8, 2006

Daxboeck F, Krause R, and Wenisch C. Laboratory diagnosis of Mycoplasma pneumoniae infection. Clin Microbiol Infect 9:263-73, 2003

de Roux A, Marcos MA, Garcia E, Mensa J, Ewig S, Lode H, and Torres A. Viral community-acquired pneumonia in nonimmunocompromised adults. Chest 125:1343-51, 2004

Declerck P, and Ollevier F. Detection of legionella in various sample types using whole-cell fluorescent in situ hybridization. Methods Mol Biol 345:175-83, 2006

Demmler GJ, Buffone GJ, Schimbor CM, and May RA. Detection of cytomegalovirus in urine from newborns by using polymerase chain reaction DNA amplification. J Infect Dis 158:1177-84, 1988

Den Boer JW, Nijhof J, and Friesema I. Risk factors for sporadic community-acquired Legionnaires' disease. A 3-year national case-control study. Public Health 120:566-71, 2006

Diaz A, Barria P, Niederman M, Restrepo MI, Dreyse J, Fuentes G, Couble B, and Saldias F. Etiology of community-acquired pneumonia in hospitalized patients in chile: the increasing prevalence of respiratory viruses among classic pathogens. Chest 131:779-87, 2007

Diederen BM. Legionella spp. and Legionnaires' disease. J Infect 56:1-12, 2008

Diederen BM, Kluytmans JA, Vandenbroucke-Grauls CM, and Peeters MF. Utility of real-time PCR for diagnosis of Legionnaires' disease in routine clinical practice. J Clin Microbiol 46:671-7, 2008

Dominguez J, Gali N, Matas L, Pedroso P, Blanco S, Gimenez M, Prat C, Sopena N, Sabria M, and Ausina V. PCR detection of Streptococcus pneumoniae DNA in serum samples for pneumococcal pneumonia diagnosis. Clin Microbiol Infect 7:164-6, 2001

Dorigo-Zetsma JW, Verkooyen RP, van Helden HP, van der Nat H, and van den Bosch JM. Molecular detection of Mycoplasma pneumoniae in adults with community-acquired pneumonia requiring hospitalization. J Clin Microbiol 39:1184-6, 2001[a]

Dorigo-Zetsma JW, Wilbrink B, van der Nat H, Bartelds AI, Heijnen ML, and Dankert J. Results of molecular detection of Mycoplasma pneumoniae among patients with acute respiratory infection and in their household contacts reveals children as human reservoirs. J Infect Dis 183:675-8, 2001[b]

Dowell SF, Anderson LJ, Gary HE, Jr., Erdman DD, Plouffe JF, File TM, Jr., Marston BJ, and Breiman RF. Respiratory syncytial virus is an important cause of community-acquired lower respiratory infection among hospitalized adults. J Infect Dis 174:456-62, 1996

Dowell SF, Peeling RW, Boman J, Carlone GM, Fields BS, Guarner J, Hammerschlag MR, Jackson LA, Kuo CC, Maass M, Messmer TO, Talkington DF, Tondella ML, and Zaki SR. Standardizing Chlamydia pneumoniae assays: recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). Clin Infect Dis 33:492-503, 2001

Dremsizov T, Clermont G, Kellum JA, Kalassian KG, Fine MJ, and Angus DC. Severe sepsis in community-acquired pneumonia: when does it happen, and do systemic inflammatory response syndrome criteria help predict course? Chest 129:968-78, 2006

Eaton MD, Meikejohn G, and Van Herick W. Studies on the etiology of primary atypical pneumonia: a filterable agent transmissible to cotton rats, hamsters, and chick embryos. J Exp Med 79:649-667, 1944

Einarsdottir HM, Erlendsdottir H, Kristinsson KG, and Gottfredsson M. Nationwide study of recurrent invasive pneumococcal infections in a population with a low prevalence of human immunodeficiency virus infection. Clin Microbiol Infect 11:744-9, 2005

Ekdahl K, Ahlinder I, Hansson HB, Melander E, Molstad S, Söderström M, and Persson K. Duration of nasopharyngeal carriage of penicillin-resistant Streptococcus pneumoniae: experiences from the South Swedish Pneumococcal Intervention Project. Clin Infect Dis 25:1113-7, 1997

Ekman MR, Leinonen M, Syrjälä H, Linnanmäki E, Kujala P, and Saikku P. Evaluation of serological methods in the diagnosis of Chlamydia pneumoniae pneumonia during an epidemic in Finland. Eur J Clin Microbiol Infect Dis 12:756-60, 1993

El Solh AA, Aquilina AT, Gunen H, and Ramadan F. Radiographic resolution of community-acquired bacterial pneumonia in the elderly. J Am Geriatr Soc 52:224-9, 2004

Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, Yao JD, Wengenack NL, Rosenblatt JE, Cockerill FR, 3rd, and Smith TF. Real-time PCR in clinical microbiology: applications for routine laboratory testing. Clin Microbiol Rev 19:165-256, 2006

Ewig S, Bauer T, Hasper E, Marklein G, Kubini R, and Luderitz B. Value of routine microbial investigation in community-acquired pneumonia treated in a tertiary care center. Respiration 63:164-9, 1996

Ewig S, Ruiz M, Mensa J, Marcos MA, Martinez JA, Arancibia F, Niederman MS, and Torres A. Severe community-acquired pneumonia. Assessment of severity criteria. Am J Respir Crit Care Med 158:1102-8, 1998

Ewig S, Schlochtermeier M, Goke N, and Niederman MS. Applying sputum as a diagnostic tool in pneumonia: limited yield, minimal impact on treatment decisions. Chest 121:1486-92, 2002

Ewig S, de Roux A, Bauer T, Garcia E, Mensa J, Niederman M, and Torres A. Validation of predictive rules and indices of severity for community acquired pneumonia. Thorax 59:421-7, 2004

Ewig S, Birkner N, Strauss R, Schaefer E, Pauletzki J, Bischoff H, Schraeder P, Welte T, and Hoeffken G. New perspectives on community-acquired pneumonia in 388,406 patients. Thorax 2009

Falguera M, Sacristan O, Nogues A, Ruiz-Gonzalez A, Garcia M, Manonelles A, and Rubio-Caballero M. Nonsevere community-acquired pneumonia: correlation between cause and severity or comorbidity. Arch Intern Med 161:1866-72, 2001

Falguera M, Lopez A, Nogues A, Porcel JM, and Rubio-Caballero M. Evaluation of the polymerase chain reaction method for detection of Streptococcus pneumoniae DNA in pleural fluid samples. Chest 122:2212-6, 2002

Falguera M, Trujillano J, Caro S, Menendez R, Carratala J, Ruiz-Gonzalez A, Vila M, Garcia M, Porcel JM, and Torres A. A prediction rule for estimating the risk of bacteremia in patients with community-acquired pneumonia. Clin Infect Dis 49:409-16, 2009

Falsey AR, and Walsh EE. Respiratory syncytial virus infection in adults. Clin Microbiol Rev 13:371-84, 2000

Farr BM, Kaiser DL, Harrison BD, and Connolly CK. Prediction of microbial aetiology at admission to hospital for pneumonia from the presenting clinical features. British Thoracic Society Pneumonia Research Subcommittee. Thorax 44:1031-5, 1989

Feikin DR, and Klugman KP. Historical changes in pneumococcal serogroup distribution: implications for the era of pneumococcal conjugate vaccines. Clin Infect Dis 35:547-55, 2002

Feinsilver SH, Fein AM, Niederman MS, Schultz DE, and Faegenburg DH. Utility of fiberoptic bronchoscopy in nonresolving pneumonia. Chest 98:1322-6, 1990

Fine MJ, Orloff JJ, Rihs JD, Vickers RM, Kominos S, Kapoor WN, Arena VC, and Yu VL. Evaluation of housestaff physicians' preparation and interpretation of sputum Gram stains for community-acquired pneumonia. J Gen Intern Med 6:189-98, 1991

Fine MJ, Smith MA, Carson CA, Mutha SS, Sankey SS, Weissfeld LA, and Kapoor WN. Prognosis and outcomes of patients with community-acquired pneumonia. A meta-analysis. JAMA 275:134-41, 1996

Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, Singer DE, Coley CM, Marrie TJ, and Kapoor WN. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med 336:243-50, 1997[a]

Fine MJ, Hough LJ, Medsger AR, Li YH, Ricci EM, Singer DE, Marrie TJ, Coley CM, Walsh MB, Karpf M, Lahive KC, and Kapoor WN. The hospital admission decision for patients with community-acquired pneumonia. Results from the pneumonia Patient Outcomes Research Team cohort study. Arch Intern Med 157:36-44, 1997[b]

Fine MJ, Stone RA, Singer DE, Coley CM, Marrie TJ, Lave JR, Hough LJ, Obrosky DS, Schulz R, Ricci EM, Rogers JC, and Kapoor WN. Processes and outcomes of care for patients with community-acquired pneumonia: results from the Pneumonia Patient Outcomes Research Team (PORT) cohort study. Arch Intern Med 159:970-80, 1999

Flanders SA, Stein J, Shochat G, Sellers K, Holland M, Maselli J, Drew WL, Reingold AL, and Gonzales R. Performance of a bedside C-reactive protein test in the diagnosis of community-acquired pneumonia in adults with acute cough. Am J Med 116:529-35, 2004

Foy HM, Grayston JT, Kenny GE, Alexander ER, and McMahan R. Epidemiology of Mycoplasma pneumoniae infection in families. Jama 197:859-66, 1966

Foy HM, Kenny GE, Cooney MK, and Allan ID. Long-term epidemiology of infections with Mycoplasma pneumoniae. J Infect Dis 139:681-7, 1979

Foy HM. Infections caused by Mycoplasma pneumoniae and possible carrier state in different populations of patients. Clin Infect Dis 17 Suppl 1:S37-46, 1993

Franquet T. Imaging of pneumonia: trends and algorithms. Eur Respir J 18:196-208, 2001

Gambotto A, Barratt-Boyes SM, de Jong MD, Neumann G, and Kawaoka Y. Human infection with highly pathogenic H5N1 influenza virus. Lancet 371:1464-75, 2008

Garau J, Baquero F, Perez-Trallero E, Perez JL, Martin-Sanchez AM, Garcia-Rey C, Martin-Herrero JE, and Dal-Re R. Factors impacting on length of stay and mortality of community-acquired pneumonia. Clin Microbiol Infect 14:322-9, 2008

García-Vázquez E, Martínez JA, Mensa J, Sánchez F, Marcos MA, de Roux A, and Torres A. C-reactive protein levels in community-acquired pneumonia. Eur Respir J 21:702-5, 2003

Garcia-Vazquez E, Marcos MA, Mensa J, de Roux A, Puig J, Font C, Francisco G, and Torres A. Assessment of the usefulness of sputum culture for diagnosis of community-acquired pneumonia using the PORT predictive scoring system. Arch Intern Med 164:1807-11, 2004

Garcia-Vidal C, Fernandez-Sabe N, Carratala J, Diaz V, Verdaguer R, Dorca J, Manresa F, and Gudiol F. Early mortality in patients with community-acquired pneumonia: causes and risk factors. Eur Respir J 32:733-9, 2008

Garnett P, Brogan O, Lafong C, and Fox C. Comparison of throat swabs with sputum specimens for the detection of Chlamydia pneumoniae antigen by direct immunofluorescence. J Clin Pathol 51:309-11, 1998

Genne D, Kaiser L, Kinge TN, and Lew D. Community-acquired pneumonia: causes of treatment failure in patients enrolled in clinical trials. Clin Microbiol Infect 9:949-54, 2003

Genne D, Siegrist HH, and Lienhard R. Enhancing the etiologic diagnosis of community-acquired pneumonia in adults using the urinary antigen assay (Binax NOW). Int J Infect Dis 10:124-8, 2006[a]

Genne D, Sommer R, Kaiser L, Saaidia A, Pasche A, Unger PF, and Lew D. Analysis of factors that contribute to treatment failure in patients with community-acquired pneumonia. Eur J Clin Microbiol Infect Dis 25:159-66, 2006[b]

Gennis P, Gallagher J, Falvo C, Baker S, and Than W. Clinical criteria for the detection of pneumonia in adults:

guidelines for ordering chest roentgenograms in the emergency department. J Emerg Med 7:263-8, 1989

Ginocchio CC. Detection of respiratory viruses using non-molecular based methods. J Clin Virol 40 Suppl 1:S11-4, 2007

Gleason PP, Meehan TP, Fine JM, Galusha DH, and Fine MJ. Associations between initial antimicrobial therapy and medical outcomes for hospitalized elderly patients with pneumonia. Arch Intern Med 159:2562-72, 1999

Glerant JC, Hellmuth D, Schmit JL, Ducroix JP, and Jounieaux V. Utility of blood cultures in community-acquired pneumonia requiring hospitalization: influence of antibiotic treatment before admission. Respir Med 93:208-12, 1999

Gnarpe H, Gnarpe J, and Lundback A. Evidence of 2 waves of Chlamydia pneumoniae infection in Gavle, Sweden, 1990-96. Scand J Infect Dis 31:83-6, 1999

Grandien M. Viral diagnosis by antigen detection techniques. Clin Diagn Virol 5:81-90, 1996

Grau I, Pallares R, Tubau F, Schulze MH, Llopis F, Podzamczer D, Linares J, and Gudiol F. Epidemiologic changes in bacteremic pneumococcal disease in patients with human immunodeficiency virus in the era of highly active antiretroviral therapy. Arch Intern Med 165:1533-40, 2005

Grayston JT, Alexander ER, Kenny GE, Clarke ER, Fremont JC, and Maccoll WA. Mycoplasma Pneumoniae Infections. Clinical and Epidemiologic Studies. Jama 191:369-74, 1965

Greenberg D, Givon-Lavi N, Broides A, Blancovich I, Peled N, and Dagan R. The contribution of smoking and exposure to tobacco smoke to Streptococcus pneumoniae and Haemophilus influenzae carriage in children and their mothers. Clin Infect Dis 42:897-903, 2006

Greenberg SB. Viral respiratory infections in elderly patients and patients with chronic obstructive pulmonary disease. Am J Med 112 Suppl 6A:28S-32S, 2002

Grönlund MM, Nuutila J, Pelto L, Lilius EM, Isolauri E, Salminen S, Kero P, and Lehtonen OP. Mode of delivery directs the phagocyte functions of infants for the first 6 months of life. Clin Exp Immunol 116:521-6, 1999

Guest JF, and Morris A. Community-acquired pneumonia: the annual cost to the National Health Service in the UK. Eur Respir J 10:1530-4, 1997

Gutierrez F, Masia M, Rodriguez JC, Ayelo A, Soldan B, Cebrian L, Mirete C, Royo G, and Hidalgo AM. Evaluation of the immunochromatographic Binax NOW assay for detection of Streptococcus pneumoniae urinary antigen in a prospective study of community-acquired pneumonia in Spain. Clin Infect Dis 36:286-92, 2003

Gutierrez F, Masia M, Rodriguez JC, Mirete C, Soldan B, Padilla S, Hernandez I, De Ory F, Royo G, and Hidalgo AM. Epidemiology of community-acquired pneumonia in adult patients at the dawn of the 21st century: a prospective study on the Mediterranean coast of Spain. Clin Microbiol Infect 11:788-800, 2005

Gutierrez F, Masia M, Mirete C, Soldan B, Rodriguez JC, Padilla S, Hernandez I, Royo G, and Martin-Hidalgo A. The influence of age and gender on the population-based incidence of community-acquired pneumonia caused by different microbial pathogens. J Infect 53:166-74, 2006

Hakim FA, and Tleyjeh IM. Severe adenovirus pneumonia in immunocompetent adults: a case report and review of the literature. Eur J Clin Microbiol Infect Dis 27:153-8, 2008

Hall CB. Respiratory syncytial virus and parainfluenza virus. N Engl J Med 344:1917-28

Hall CB, Long CE, and Schnabel KC. Respiratory syncytial virus infections in previously healthy working adults. Clin Infect Dis 33:792-6, 2001

Halm EA, Fine MJ, Marrie TJ, Coley CM, Kapoor WN, Obrosky DS, and Singer DE. Time to clinical stability in patients hospitalized with community-acquired pneumonia: implications for practice guidelines. JAMA 279:1452-7, 1998

Halm EA, Fine MJ, Kapoor WN, Singer DE, Marrie TJ, and Siu AL. Instability on hospital discharge and the risk of adverse outcomes in patients with pneumonia. Arch Intern Med 162:1278-84, 2002

Hansson LO, Axelsson G, Linne T, Aurelius E, and Lindquist L. Serum C-reactive protein in the differential diagnosis of acute meningitis. Scand J Infect Dis 25:625-30, 1993

Hauksdottir GS, Love A, Sigurdardottir V, and Jonsson T. Outbreaks of Mycoplasma pneumoniae infections in Iceland 1987 to 1997 - a ten and a half years review. Eur J Epidemiol 15:95-6, 1999

Hausdorff WP, Bryant J, Paradiso PR, and Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. Clin Infect Dis 30:100-21, 2000

Hayden RT, Uhl JR, Qian X, Hopkins MK, Aubry MC, Limper AH, Lloyd RV, and Cockerill FR. Direct detection of Legionella species from bronchoalveolar lavage and open lung biopsy specimens: comparison of LightCycler PCR, in situ hybridization, direct fluorescence antigen detection, and culture. J Clin Microbiol 39:2618-26, 2001

Hedlund J, and Hansson LO. Procalcitonin and C-reactive protein levels in community-acquired pneumonia: correlation with etiology and prognosis. Infection 28:68-73, 2000

Hedlund J, Strålin K, Örtqvist A, and Holmberg H. Swedish guidelines for the management of community-acquired pneumonia in immunocompetent adults. Scand J Infect Dis 37:791-805, 2005

Heiro M, Helenius H, Sundell J, Koskinen P, Engblom E, Nikoskelainen J, and Kotilainen P. Utility of serum C-reactive protein in assessing the outcome of infective endocarditis. Eur Heart J 26:1873-81, 2005

Henrichsen J. Six newly recognized types of Streptococcus pneumoniae. J Clin Microbiol 33:2759-62, 1995

Henrickson KJ. Parainfluenza viruses. Clin Microbiol Rev 16:242-64, 2003

Herrmann B, Larsson C, and Zweygberg BW. Simultaneous detection and typing of influenza viruses A and B by a nested reverse transcription-PCR: comparison to virus isolation and antigen detection by immunofluorescence and optical immunoassay (FLU OIA). J Clin Microbiol 39:134-8, 2001

Hicks LA, Shepard CW, Britz PH, Erdman DD, Fischer M, Flannery BL, Peck AJ, Lu X, Thacker WL, Benson RF, Tondella ML, Moll ME, Whitney CG, Anderson

LJ, and Feikin DR. Two outbreaks of severe respiratory disease in nursing homes associated with rhinovirus. J Am Geriatr Soc 54:284-9, 2006

Hohenadel IA, Kiworr M, Genitsariotis R, Zeidler D, and Lorenz J. Role of bronchoalveolar lavage in immunocompromised patients with pneumonia treated with a broad spectrum antibiotic and antifungal regimen. Thorax 56:115-20, 2001

Holm A, Pedersen SS, Nexoe J, Obel N, Nielsen LP, Koldkjaer O, and Pedersen C. Procalcitonin versus C-reactive protein for predicting pneumonia in adults with lower respiratory tract infection in primary care. Br J Gen Pract 57:555-60, 2007

Honkanen P, Jartti A, Järvinen A, Korppi M, Patja K, Puolijoki H, Ruuskanen O, Syrjälä H, Timonen O, and Vaara M. Keuhkokuumeen hoito. Duodecim 124:2030-2039, 2008

Hoymans VY, Bosmans JM, Van Renterghem L, Mak R, Ursi D, Wuyts F, Vrints CJ, and Ieven M. Importance of methodology in determination of Chlamydia pneumoniae seropositivity in healthy subjects and in patients with coronary atherosclerosis. J Clin Microbiol 41:4049-53, 2003

Huang AH, Yan JJ, and Wu JJ. Comparison of five days versus seven days of incubation for detection of positive blood cultures by the Bactec 9240 system. Eur J Clin Microbiol Infect Dis 17:637-41, 1998

Hukkanen V, Rehn T, Kajander R, Sjöroos M, and Waris M. Time-resolved fluorometry PCR assay for rapid detection of herpes simplex virus in cerebrospinal fluid. J Clin Microbiol 38:3214-8, 2000

Hulkko T, Lyytikäinen O, Kuusi M, Iivonen J, and Ruutu P. Infectious Diseases in Finland 2007. 9, 2008

Ieven M. Currently used nucleic acid amplification tests for the detection of viruses and atypicals in acute respiratory infections. J Clin Virol 40:259-76, 2007

Ingram JG, and Plouffe JF. Danger of sputum purulence screens in culture of Legionella species. J Clin Microbiol 32:209-10, 1994

Ishida T, Hashimoto T, Arita M, Ito I, and Osawa M. Etiology of community-acquired pneumonia in hospitalized patients: a 3-year prospective study in Japan. Chest 114:1588-93, 1998

Ishida T, Hashimoto T, Arita M, Tojo Y, Tachibana H, and Jinnai M. A 3-year prospective study of a urinary antigen-detection test for Streptococcus pneumoniae in community-acquired pneumonia: utility and clinical impact on the reported etiology. J Infect Chemother 10:359-63, 2004

Ishizuka S, Yamaya M, Suzuki T, Takahashi H, Ida S, Sasaki T, Inoue D, Sekizawa K, Nishimura H, and Sasaki H. Effects of rhinovirus infection on the adherence of Streptococcus pneumoniae to cultured human airway epithelial cells. J Infect Dis 188:1928-39, 2003

Ison MG, Hayden FG, Kaiser L, Corey L, and Boeckh M. Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia. Clin Infect Dis 36:1139-43, 2003

Jansson E, von Essen R, and Tuuri S. Mycoplasma pneumoniae pneumonia in Helsinki 1962-1970. Epidemic pattern and autoimmune manifestations. Scand J Infect Dis 3:51-4, 1971

Jartti T, Lehtinen P, Vuorinen T, Österback R, van den Hoogen B, Osterhaus AD, and Ruuskanen O. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. Emerg Infect Dis 10:1095-101, 2004

Jasti H, Mortensen EM, Obrosky DS, Kapoor WN, and Fine MJ. Causes and risk factors for rehospitalization of patients hospitalized with community-acquired pneumonia. Clin Infect Dis 46:550-6, 2008

Jennings LC, Anderson TP, Werno AM, Beynon KA, and Murdoch DR. Viral etiology of acute respiratory tract infections in children presenting to hospital: role of polymerase chain reaction and demonstration of multiple infections. Pediatr Infect Dis J 23:1003-7, 2004

Jennings LC, Anderson TP, Beynon KA, Chua A, Laing RT, Werno AM, Young SA, Chambers ST, and Murdoch DR. Incidence and characteristics of viral community-acquired pneumonia in adults. Thorax 63:42-8, 2008

Jimenez P, Saldias F, Meneses M, Silva ME, Wilson MG, and Otth L. Diagnostic fiberoptic bronchoscopy in patients with community-acquired pneumonia. Comparison between bronchoalveolar lavage and telescoping plugged catheter cultures. Chest 103:1023-7, 1993

Johansson N, Kalin M, Giske CG, and Hedlund J. Quantitative detection of Streptococcus pneumoniae from sputum samples with real-time quantitative polymerase chain reaction for etiologic diagnosis of community-acquired pneumonia. Diagn Microbiol Infect Dis 60:255-61, 2008

Johnstone J, Eurich DT, Majumdar SR, Jin Y, and Marrie TJ. Long-term morbidity and mortality after hospitalization with community-acquired pneumonia: a population-based cohort study. Medicine (Baltimore) 87:329-34, 2008[a]

Johnstone J, Majumdar SR, Fox JD, and Marrie TJ. Viral infection in adults hospitalized with community-acquired pneumonia: prevalence, pathogens, and presentation. Chest 134:1141-8, 2008[b]

Jokinen C, Heiskanen L, Juvonen H, Kallinen S, Karkola K, Korppi M, Kurki S, Rönnberg PR, Seppä A, Soimakallio S, and et al. Incidence of community-acquired pneumonia in the population of four municipalities in eastern Finland. Am J Epidemiol 137:977-88, 1993

Jokinen C, Heiskanen L, Juvonen H, Kallinen S, Kleemola M, Koskela M, Leinonen M, Rönnberg PR, Saikku P, Sten M, Tarkiainen A, Tukiainen H, Pyörälä K, and Mäkelä PH. Microbial etiology of community-acquired pneumonia in the adult population of 4 municipalities in eastern Finland. Clin Infect Dis 32:1141-54, 2001

Jordano Q, Falco V, Almirante B, Planes AM, del Valle O, Ribera E, Len O, Pigrau C, and Pahissa A. Invasive pneumococcal disease in patients infected with HIV: still a threat in the era of highly active antiretroviral therapy. Clin Infect Dis 38:1623-8, 2004

Jover F, Cuadrado JM, Andreu L, Martinez S, Canizares R, de la Tabla VO, Martin C, Roig P, and Merino J. A comparative study of bacteremic and non-bacteremic pneumococcal pneumonia. Eur J Intern Med 19:15-21, 2008

Juven T, Mertsola J, Waris M, Leinonen M, Meurman O, Roivainen M, Eskola J, Saikku P, and Ruuskanen O. Etiology of community-acquired pneumonia in 254

hospitalized children. Pediatr Infect Dis J 19:293-8, 2000

Kahn JS. Epidemiology of human metapneumovirus. Clin Microbiol Rev 19:546-57, 2006

Kaiser L, Keene ON, Hammond JM, Elliott M, and Hayden FG. Impact of zanamivir on antibiotic use for respiratory events following acute influenza in adolescents and adults. Arch Intern Med 160:3234-40, 2000

Kallen AJ, Brunkard J, Moore Z, Budge P, Arnold KE, Fosheim G, Finelli L, Beekmann SE, Polgreen PM, Gorwitz R, and Hageman J. Staphylococcus aureus community-acquired pneumonia during the 2006 to 2007 influenza season. Ann Emerg Med 53:358-65, 2009

Kang CI, Song JH, Oh WS, Ko KS, Chung DR, and Peck KR. Clinical outcomes and risk factors of community-acquired pneumonia caused by gram-negative bacilli. Eur J Clin Microbiol Infect Dis 27:657-61, 2008

Karvonen M, Tuomilehto J, Pitkaniemi J, and Saikku P. The epidemic cycle of Chlamydia pneumoniae infection in eastern Finland, 1972-1987. Epidemiol Infect 110:349-60, 1993

Kauppinen M, and Saikku P. Pneumonia due to Chlamydia pneumoniae: prevalence, clinical features, diagnosis, and treatment. Clin Infect Dis 21 Suppl 3:S244-52, 1995

Kauppinen MT, Saikku P, Kujala P, Herva E, and Syrjälä H. Clinical picture of community-acquired Chlamydia pneumoniae pneumonia requiring hospital treatment: a comparison between chlamydial and pneumococcal pneumonia. Thorax 51:185-9, 1996

Kim PE, Musher DM, Glezen WP, Rodriguez-Barradas MC, Nahm WK, and Wright CE. Association of invasive pneumococcal disease with season, atmospheric conditions, air pollution, and the isolation of respiratory viruses. Clin Infect Dis 22:100-6, 1996

King MD, Whitney CG, Parekh F, and Farley MM. Recurrent invasive pneumococcal disease: a population-based assessment. Clin Infect Dis 37:1029-36, 2003

Kirchberger S, Majdic O, and Stockl J. Modulation of the immune system by human rhinoviruses. Int Arch Allergy Immunol 142:1-10, 2007

Kleemola M, Saikku P, Visakorpi R, Wang SP, and Grayston JT. Epidemics of pneumonia caused by TWAR, a new Chlamydia organism, in military trainees in Finland. J Infect Dis 157:230-6, 1988

Klement E, Talkington DF, Wasserzug O, Kayouf R, Davidovitch N, Dumke R, Bar-Zeev Y, Ron M, Boxman J, Lanier Thacker W, Wolf D, Lazarovich T, Shemer-Avni Y, Glikman D, Jacobs E, Grotto I, Block C, and Nir-Paz R. Identification of risk factors for infection in an outbreak of Mycoplasma pneumoniae respiratory tract disease. Clin Infect Dis 43:1239-45, 2006

Klemets P, Lyytikäinen O, Ruutu P, Kaijalainen T, Leinonen M, Ollgren J, and Pekka Nuorti J. Trends and geographical variation in invasive pneumococcal infections in Finland. Scand J Infect Dis 40:621-8, 2008[a]

Klemets P, Lyytikäinen O, Ruutu P, Ollgren J, and Pekka Nuorti J. Invasive pneumococcal infections among persons with and without underlying medical conditions: implications for prevention strategies. BMC Infect Dis 8:96, 2008[b]

Koivula I, Sten M, and Mäkelä PH. Prognosis after community-acquired pneumonia in the elderly: a population-based 12-year follow-up study. Arch Intern Med 159:1550-5, 1999

Korppi M, Leinonen M, and Ruuskanen O. Pneumococcal serology in children's respiratory infections. Eur J Clin Microbiol Infect Dis 27:167-75, 2008

Korsgaard J, Rasmussen TR, Sommer T, Moller JK, Jensen JS, and Kilian M. Intensified microbiological investigations in adult patients admitted to hospital with lower respiratory tract infections. Respir Med 96:344-51, 2002

Kosmas EN, Baxevanis CN, Papamichail M, and Kordossis T. Daily variation in circulating cytokines and acute-phase proteins correlates with clinical and laboratory indices in community-acquired pneumonia. Eur J Clin Invest 27:308-15, 1997

Kragsbjerg P, Jones I, Vikerfors T, and Holmberg H. Diagnostic value of blood cytokine concentrations in acute pneumonia. Thorax 50:1253-7, 1995

Krüger S, Ewig S, Marre R, Papassotiriou J, Richter K, von Baum H, Suttorp N, and Welte T. Procalcitonin predicts patients at low risk of death from community-acquired pneumonia across all CRB-65 classes. Eur Respir J 31:349-55, 2008

Kumar S, and Hammerschlag MR. Acute respiratory infection due to Chlamydia pneumoniae: current status of diagnostic methods. Clin Infect Dis 44:568-76, 2007

Kuo CC, Jackson LA, Campbell LA, and Grayston JT. Chlamydia pneumoniae (TWAR). Clin Microbiol Rev 8:451-61, 1995

Kutlin A, Roblin PM, Kumar S, Kohlhoff S, Bodetti T, Timms P, and Hammerschlag MR. Molecular characterization of Chlamydophila pneumoniae isolates from Western barred bandicoots. J Med Microbiol 56:407-17, 2007

Lagerström F, Bader M, Foldevi M, Fredlund H, Nordin-Olsson I, and Holmberg H. Microbiological etiology in clinically diagnosed community-acquired pneumonia in primary care in Orebro, Sweden. Clin Microbiol Infect 9:645-52, 2003

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

Larinkari U, and Valtonen VV. Comparison of anti-alpha-haemolysin and teichoic acid antibody tests in patients with Staphylococcus aureus endocarditis or bacteraemia. J Infect 8:221-6, 1984

Laterre PF. Monotherapy or combination therapy for hospitalized patients with community-acquired pneumonia: not yet the end of the story? Clin Infect Dis 46:1510-2, 2008

Lauderdale TL, Chang FY, Ben RJ, Yin HC, Ni YH, Tsai JW, Cheng SH, Wang JT, Liu YC, Cheng YW, Chen ST, Fung CP, Chuang YC, Cheng HP, Lu DC, Liu CJ, Huang IW, Hung CL, Hsiao CF, and Ho M. Etiology of community acquired pneumonia among adult patients requiring hospitalization in Taiwan. Respir Med 99:1079-86, 2005

Le Monnier A, Carbonnelle E, Zahar JR, Le Bourgeois M, Abachin E, Quesne G, Varon E, Descamps P, De Blic J,

Scheinmann P, Berche P, and Ferroni A. Microbiological diagnosis of empyema in children: comparative evaluations by culture, polymerase chain reaction, and pneumococcal antigen detection in pleural fluids. Clin Infect Dis 42:1135-40, 2006

Lee A, Mirrett S, Reller LB, and Weinstein MP. Detection of bloodstream infections in adults: how many blood cultures are needed? J Clin Microbiol 45:3546-8, 2007

Leinonen M, and Mäkelä PH. Diagnosis of pneumococcal pneumonia. Clin Infect Dis 33:1440-1, 2001

Leland DS, and Ginocchio CC. Role of cell culture for virus detection in the age of technology. Clin Microbiol Rev 20:49-78, 2007

Leroy O, Santre C, Beuscart C, Georges H, Guery B, Jacquier JM, and Beaucaire G. A five-year study of severe community-acquired pneumonia with emphasis on prognosis in patients admitted to an intensive care unit. Intensive Care Med 21:24-31, 1995

Li J, Plorde JJ, and Carlson LG. Effects of volume and periodicity on blood cultures. J Clin Microbiol 32:2829-31, 1994

Liapikou A, Ferrer M, Polverino E, Balasso V, Esperatti M, Piner R, Mensa J, Luque N, Ewig S, Menendez R, Niederman MS, and Torres A. Severe Community-Acquired Pneumonia: Validation of the Infectious Diseases Society of America/American Thoracic Society Guidelines to Predict an Intensive Care Unit Admission. Clin Infect Dis 2009

Lidman C, Burman LG, Lagergren A, and Ortqvist A. Limited value of routine microbiological diagnostics in patients hospitalized for community-acquired pneumonia. Scand J Infect Dis 34:873-9, 2002

Lieberman D, Schlaeffer F, Boldur I, Horowitz S, Friedman MG, Leiononen M, Horovitz O, Manor E, and Porath A. Multiple pathogens in adult patients admitted with community-acquired pneumonia: a one year prospective study of 346 consecutive patients. Thorax 51:179-84, 1996

Lim WS, Macfarlane JT, Boswell TC, Harrison TG, Rose D, Leinonen M, and Saikku P. Study of community acquired pneumonia aetiology (SCAPA) in adults admitted to hospital: implications for management guidelines. Thorax 56:296-301, 2001

Lim WS, van der Eerden MM, Laing R, Boersma WG, Karalus N, Town GI, Lewis SA, and Macfarlane JT. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. Thorax 58:377-82, 2003

Loeb M. Pneumonia in older persons. Clin Infect Dis 37:1335-9, 2003

Loens K, Ursi D, Goossens H, and Ieven M. Molecular diagnosis of Mycoplasma pneumoniae respiratory tract infections. J Clin Microbiol 41:4915-23, 2003

Loens K, Ursi D, Goossens H, and Ieven M. Molecular diagnosis of Mycoplasma pneumoniae respiratory tract infections. J Clin Microbiol 41:4915-23, 2003

Lopez AD, Mathers CD, Ezzati M, Jamison DT, and Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet 367:1747-57, 2006

Luna CM, Famiglietti A, Absi R, Videla AJ, Nogueira FJ, Fuenzalida AD, and Gene RJ. Community-acquired pneumonia: etiology, epidemiology, and outcome at a teaching hospital in Argentina. Chest 118:1344-54, 2000

Macfarlane JT, Miller AC, Roderick Smith WH, Morris AH, and Rose DH. Comparative radiographic features of community acquired Legionnaires' disease, pneumococcal pneumonia, mycoplasma pneumonia, and psittacosis. Thorax 39:28-33, 1984

Macfarlane J, Holmes W, Gard P, Macfarlane R, Rose D, Weston V, Leinonen M, Saikku P, and Myint S. Prospective study of the incidence, aetiology and outcome of adult lower respiratory tract illness in the community. Thorax 56:109-14, 2001

Mahony JB. Detection of respiratory viruses by molecular methods. Clin Microbiol Rev 21:716-47, 2008

Maimon N, Nopmaneejumruslers C, and Marras TK. Antibacterial class is not obviously important in outpatient pneumonia: a meta-analysis. Eur Respir J 31:1068-76, 2008

Man SY, Lee N, Ip M, Antonio GE, Chau SS, Mak P, Graham CA, Zhang M, Lui G, Chan PK, Ahuja AT, Hui DS, Sung JJ, and Rainer TH. Prospective comparison of three predictive rules for assessing severity of community-acquired pneumonia in Hong Kong. Thorax 62:348-53, 2007

Mandell LA, Marrie TJ, Grossman RF, Chow AW, and Hyland RH. Canadian guidelines for the initial management of community-acquired pneumonia: an evidence-based update by the Canadian Infectious Diseases Society and the Canadian Thoracic Society. The Canadian Community-Acquired Pneumonia Working Group. Clin Infect Dis 31:383-421, 2000

Mandell LA. Epidemiology and etiology of community-acquired pneumonia. Infect Dis Clin North Am 18:761-76, vii, 2004

Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM, Jr., Musher DM, Niederman MS, Torres A, and Whitney CG. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 44 Suppl 2:S27-72, 2007

Mansel JK, Rosenow EC, 3rd, Smith TF, and Martin JW, Jr. Mycoplasma pneumoniae pneumonia. Chest 95:639-46, 1989

Marrie TJ. Community-acquired pneumonia. Clin Infect Dis 18:501-13; quiz 514-5, 1994

Marrie TJ, Peeling RW, Fine MJ, Singer DE, Coley CM, and Kapoor WN. Ambulatory patients with community-acquired pneumonia: the frequency of atypical agents and clinical course. Am J Med 101:508-15, 1996

Marrie TJ. Pneumococcal pneumonia: epidemiology and clinical features. Semin Respir Infect 14:227-36, 1999

Marrie TJ. Community-acquired pneumonia in the elderly. Clin Infect Dis 31:1066-78, 2000

Marrie TJ, Lau CY, Wheeler SL, Wong CJ, and Feagan BG. Predictors of symptom resolution in patients with community-acquired pneumonia. Clin Infect Dis 31:1362-7, 2000[a]

Marrie TJ, Lau CY, Wheeler SL, Wong CJ, Vandervoort MK, and Feagan BG. A controlled trial of a critical pathway for treatment of community-acquired pneumonia.

CAPITAL Study Investigators. Community-Acquired Pneumonia Intervention Trial Assessing Levofloxacin. JAMA 283:749-55, 2000[b]

Marston BJ, Lipman HB, and Breiman RF. Surveillance for Legionnaires' disease. Risk factors for morbidity and mortality. Arch Intern Med 154:2417-22, 1994

Marston BJ, Plouffe JF, File TM, Jr., Hackman BA, Salstrom SJ, Lipman HB, Kolczak MS, and Breiman RF. Incidence of community-acquired pneumonia requiring hospitalization. Results of a population-based active surveillance Study in Ohio. The Community-Based Pneumonia Incidence Study Group. Arch Intern Med 157:1709-18, 1997

Martinez JA, Pozo L, Almela M, Marco F, Soriano A, Lopez F, Balasso V, Aguilar J, and Mensa J. Microbial and clinical determinants of time-to-positivity in patients with bacteraemia. Clin Microbiol Infect 13:709-16, 2007

Marx A, Gary HE, Jr., Marston BJ, Erdman DD, Breiman RF, Torok TJ, Plouffe JF, File TM, Jr., and Anderson LJ. Parainfluenza virus infection among adults hospitalized for lower respiratory tract infection. Clin Infect Dis 29:134-40, 1999

Masiá M, Gutiérrez F, Shum C, Padilla S, Navarro JC, Flores E, and Hernández I. Usefulness of procalcitonin levels in community-acquired pneumonia according to the patients outcome research team pneumonia severity index. Chest 128:2223-9, 2005

McDade JE, Shepard CC, Fraser DW, Tsai TR, Redus MA, and Dowdle WR. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. N Engl J Med 297:1197-203, 1977

Menendez R, Torres A, Rodriguez de Castro F, Zalacain R, Aspa J, Martin Villasclaras JJ, Borderias L, Benitez Moya JM, Ruiz-Manzano J, Blanquer J, Perez D, Puzo C, Sanchez-Gascon F, Gallardo J, Alvarez CJ, and Molinos L. Reaching stability in community-acquired pneumonia: the effects of the severity of disease, treatment, and the characteristics of patients. Clin Infect Dis 39:1783-90, 2004[a]

Menendez R, Torres A, Zalacain R, Aspa J, Martin Villasclaras JJ, Borderias L, Benitez Moya JM, Ruiz-Manzano J, Rodriguez de Castro F, Blanquer J, Perez D, Puzo C, Sanchez Gascon F, Gallardo J, Alvarez C, and Molinos L. Risk factors of treatment failure in community acquired pneumonia: implications for disease outcome. Thorax 59:960-5, 2004[b]

Menendez R, and Torres A. Treatment failure in community-acquired pneumonia. Chest 132:1348-55, 2007

Menendez R, Cavalcanti M, Reyes S, Mensa J, Martinez R, Marcos MA, Filella X, Niederman M, and Torres A. Markers of treatment failure in hospitalised community acquired pneumonia. Thorax 63:447-52, 2008

Metersky ML, Ma A, Bratzler DW, and Houck PM. Predicting bacteremia in patients with community-acquired pneumonia. Am J Respir Crit Care Med 169:342-7, 2004

Metlay JP, Kapoor WN, and Fine MJ. Does this patient have community-acquired pneumonia? Diagnosing pneumonia by history and physical examination. JAMA 278:1440-5, 1997[a]

Metlay JP, Schulz R, Li YH, Singer DE, Marrie TJ, Coley CM, Hough LJ, Obrosky DS, Kapoor WN, and Fine MJ. Influence of age on symptoms at presentation in patients with community-acquired pneumonia. Arch Intern Med 157:1453-9, 1997[b]

Metlay JP, and Fine MJ. Testing strategies in the initial management of patients with community-acquired pneumonia. Ann Intern Med 138:109-18, 2003

Mills GD, Oehley MR, and Arrol B. Effectiveness of beta lactam antibiotics compared with antibiotics active against atypical pathogens in non-severe community acquired pneumonia: meta-analysis. BMJ 330:456, 2005

Minogue MF, Coley CM, Fine MJ, Marrie TJ, Kapoor WN, and Singer DE. Patients hospitalized after initial outpatient treatment for community-acquired pneumonia. Ann Emerg Med 31:376-80, 1998

Mittl RL, Jr., Schwab RJ, Duchin JS, Goin JE, Albeida SM, and Miller WT. Radiographic resolution of community-acquired pneumonia. Am J Respir Crit Care Med 149:630-5, 1994

Miyashita N, Niki Y, Nakajima M, Fukano H, and Matsushima T. Prevalence of asymptomatic infection with Chlamydia pneumoniae in subjectively healthy adults. Chest 119:1416-9, 2001

Miyashita N, Fukano H, Okimoto N, Hara H, Yoshida K, Niki Y, and Matsushima T. Clinical presentation of community-acquired Chlamydia pneumoniae pneumonia in adults. Chest 121:1776-81, 2002

Miyashita N, Fukano H, Mouri K, Fukuda M, Yoshida K, Kobashi Y, Niki Y, and Oka M. Community-acquired pneumonia in Japan: a prospective ambulatory and hospitalized patient study. J Med Microbiol 54:395-400, 2005

Miyashita N, Shimizu H, Ouchi K, Kawasaki K, Kawai Y, Obase Y, Kobashi Y, and Oka M. Assessment of the usefulness of sputum Gram stain and culture for diagnosis of community-acquired pneumonia requiring hospitalization. Med Sci Monit 14:CR171-6, 2008

Modlin JF Introduction to the enteroviruses. In: Mandell GL, Douglas RG, Bennett JE, and Dolin R. Mandell, Douglas, and Bennett's principles and practice of infectious diseases 6th edn. Philadelphia: Churchill Livingstone; 2005; p. 2133-2140

Mokdad AH, Marks JS, Stroup DF, and Gerberding JL. Actual causes of death in the United States, 2000. JAMA 291:1238-45, 2004

Mordhorst CH, Wang SP, and Grayston JT. Outbreak of Chlamydia pneumoniae infection in four farm families. Eur J Clin Microbiol Infect Dis 11:617-20, 1992

Morens DM, Taubenberger JK, and Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. J Infect Dis 198:962-70, 2008

Morozumi M, Hasegawa K, Chiba N, Iwata S, Kawamura N, Kuroki H, Tajima T, and Ubukata K. Application of PCR for Mycoplasma pneumoniae detection in children with community-acquired pneumonia. J Infect Chemother 10:274-9, 2004

Morozumi M, Nakayama E, Iwata S, Aoki Y, Hasegawa K, Kobayashi R, Chiba N, Tajima T, and Ubukata K. Simultaneous detection of pathogens in clinical samples from patients with community-acquired pneumonia by real-time PCR with pathogen-specific molecular beacon probes. J Clin Microbiol 44:1440-6, 2006

Mortensen EM, Kapoor WN, Chang CC, and Fine MJ. Assessment of mortality after long-term follow-up of patients with community-acquired pneumonia. Clin Infect Dis 37:1617-24, 2003

Muder RR, and Yu VL. Infection due to Legionella species other than L. pneumophila. Clin Infect Dis 35:990-8, 2002

Murdoch DR, Laing RT, Mills GD, Karalus NC, Town GI, Mirrett S, and Reller LB. Evaluation of a rapid immunochromatographic test for detection of Streptococcus pneumoniae antigen in urine samples from adults with community-acquired pneumonia. J Clin Microbiol 39:3495-8, 2001

Murdoch DR. Diagnosis of Legionella infection. Clin Infect Dis 36:64-9, 2003[a]

Murdoch DR. Nucleic acid amplification tests for the diagnosis of pneumonia. Clin Infect Dis 36:1162-70, 2003[b]

Murdoch DR, Anderson TP, Beynon KA, Chua A, Fleming AM, Laing RT, Town GI, Mills GD, Chambers ST, and Jennings LC. Evaluation of a PCR assay for detection of Streptococcus pneumoniae in respiratory and nonrespiratory samples from adults with community-acquired pneumonia. J Clin Microbiol 41:63-6, 2003

Murphy TF. Lung infections. 2. Branhamella catarrhalis: epidemiological and clinical aspects of a human respiratory tract pathogen. Thorax 53:124-8, 1998

Murphy TF, Sethi S, Klingman KL, Brueggemann AB, and Doern GV. Simultaneous respiratory tract colonization by multiple strains of nontypeable haemophilus influenzae in chronic obstructive pulmonary disease: implications for antibiotic therapy. J Infect Dis 180:404-9, 1999

Musher DM, Groover JE, Reichler MR, Riedo FX, Schwartz B, Watson DA, Baughn RE, and Breiman RF. Emergence of antibody to capsular polysaccharides of Streptococcus pneumoniae during outbreaks of pneumonia: association with nasopharyngeal colonization. Clin Infect Dis 24:441-6, 1997

Musher DM, Alexandraki I, Graviss EA, Yanbeiy N, Eid A, Inderias LA, Phan HM, and Solomon E. Bacteremic and nonbacteremic pneumococcal pneumonia. A prospective study. Medicine (Baltimore) 79:210-21, 2000

Musher DM, Mediwala R, Phan HM, Chen G, and Baughn RE. Nonspecificity of assaying for IgG antibody to pneumolysin in circulating immune complexes as a means to diagnose pneumococcal pneumonia. Clin Infect Dis 32:534-8, 2001

Musher DM. How contagious are common respiratory tract infections? N Engl J Med 348:1256-66, 2003

Musher DM, Montoya R, and Wanahita A. Diagnostic value of microscopic examination of Gram-stained sputum and sputum cultures in patients with bacteremic pneumococcal pneumonia. Clin Infect Dis 39:165-9, 2004

Musher DM, Rueda AM, Kaka AS, and Mapara SM. The association between pneumococcal pneumonia and acute cardiac events. Clin Infect Dis 45:158-65, 2007

Müller B, Harbarth S, Stolz D, Bingisser R, Mueller C, Leuppi J, Nusbaumer C, Tamm M, and Christ-Crain M. Diagnostic and prognostic accuracy of clinical and laboratory parameters in community-acquired pneumonia. BMC Infect Dis 7:10, 2007

Mäkelä MJ, Puhakka T, Ruuskanen O, Leinonen M, Saikku P, Kimpimäki M, Blomqvist S, Hyypiä T, and Arstila P. Viruses and bacteria in the etiology of the common cold. J Clin Microbiol 36:539-42, 1998

Nakashima K, Tanaka T, Kramer MH, Takahashi H, Ohyama T, Kishimoto T, Toshima H, Miwa S, Nomura A, Tsumura N, Ouchi K, and Okabe N. Outbreak of Chlamydia pneumoniae infection in a Japanese nursing home, 1999-2000. Infect Control Hosp Epidemiol 27:1171-7, 2006

Nato F, Contini C, Zamora-Zavala C, Viscardi P, Delia S, Mojon M, and Mazie JC. Production and characterization of monoclonal antibodies to human Pneumocystis carinii for the diagnosis of P. carinii pneumonia. Eur J Med 1:132-8, 1992

Niederman MS, Bass JB, Jr., Campbell GD, Fein AM, Grossman RF, Mandell LA, Marrie TJ, Sarosi GA, Torres A, and Yu VL. Guidelines for the initial management of adults with community-acquired pneumonia: diagnosis, assessment of severity, and initial antimicrobial therapy. American Thoracic Society. Medical Section of the American Lung Association. Am Rev Respir Dis 148:1418-26, 1993

Niederman MS. Community-acquired pneumonia: a North American perspective. Chest 113:179S-182S, 1998

Niederman MS, Mandell LA, Anzueto A, Bass JB, Broughton WA, Campbell GD, Dean N, File T, Fine MJ, Gross PA, Martinez F, Marrie TJ, Plouffe JF, Ramirez J, Sarosi GA, Torres A, Wilson R, and Yu VL. Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. Am J Respir Crit Care Med 163:1730-54, 2001

Niederman MS. Recent advances in community-acquired pneumonia: inpatient and outpatient. Chest 131:1205-15, 2007

Nilsson AC, Bjorkman P, and Persson K. Polymerase chain reaction is superior to serology for the diagnosis of acute Mycoplasma pneumoniae infection and reveals a high rate of persistent infection. BMC Microbiol 8:93, 2008

Nuorti JP, Butler JC, Crutcher JM, Guevara R, Welch D, Holder P, and Elliott JA. An outbreak of multidrug-resistant pneumococcal pneumonia and bacteremia among unvaccinated nursing home residents. N Engl J Med 338:1861-8, 1998

Nuorti JP, Butler JC, Farley MM, Harrison LH, McGeer A, Kolczak MS, and Breiman RF. Cigarette smoking and invasive pneumococcal disease. Active Bacterial Core Surveillance Team. N Engl J Med 342:681-9, 2000

Nuovo GJ. The utility of in situ--based methodologies including in situ polymerase chain reaction for the diagnosis and study of viral infections. Hum Pathol 38:1123-36, 2007

Nuutila J, Hohenthal U, Laitinen I, Kotilainen P, Rajamäki A, Nikoskelainen J, and Lilius EM. Quantitative analysis of complement receptors, CR1 (CD35) and CR3 (CD11b), on neutrophils improves distinction between bacterial and viral infections in febrile patients: comparison with standard clinical laboratory data. J Immunol Methods 315:191-201, 2006

Nuutila J, Hohenthal U, Laitinen I, Kotilainen P, Rajamäki A, Nikoskelainen J, and Lilius EM. Simultaneous quantitative analysis of FcgammaRI (CD64) expression on neutrophils and monocytes: a new, improved way

to detect infections. J Immunol Methods 328:189-200, 2007

Nuutila J, and Lilius EM. Distinction between bacterial and viral infections. Curr Opin Infect Dis 20:304-10, 2007

Oliveira EC, Marik PE, and Colice G. Influenza pneumonia: a descriptive study. Chest 119:1717-23, 2001

Oliver BG, Lim S, Wark P, Laza-Stanca V, King N, Black JL, Burgess JK, Roth M, and Johnston SL. Rhinovirus exposure impairs immune responses to bacterial products in human alveolar macrophages. Thorax 63:519-25, 2008

Ortega L, Sierra M, Dominguez J, Martinez J, Matas L, Bastart F, Gali N, and Ausina V. Utility of a pneumonia severity index in the optimization of the diagnostic and therapeutic effort for community-acquired pneumonia. Scand J Infect Dis 37:657-63, 2005

Paganin F, Lilienthal F, Bourdin A, Lugagne N, Tixier F, Genin R, and Yvin JL. Severe community-acquired pneumonia: assessment of microbial aetiology as mortality factor. Eur Respir J 24:779-85, 2004

Parody R, Rabella N, Martino R, Otegui M, del Cuerpo M, Coll P, and Sierra J. Upper and lower respiratory tract infections by human enterovirus and rhinovirus in adult patients with hematological malignancies. Am J Hematol 82:807-11, 2007

Pastor P, Medley F, and Murphy TV. Invasive pneumococcal disease in Dallas County, Texas: results from population-based surveillance in 1995. Clin Infect Dis 26:590-5, 1998

Peiris JS, Yuen KY, Osterhaus AD, and Stohr K. The severe acute respiratory syndrome. N Engl J Med 349:2431-41, 2003

Peltola V, Waris M, Österback R, Susi P, Hyypiä T, and Ruuskanen O. Clinical effects of rhinovirus infections. J Clin Virol 43:411-4, 2008

Peltola VT, and McCullers JA. Respiratory viruses predisposing to bacterial infections: role of neuraminidase. Pediatr Infect Dis J 23:S87-97, 2004

Peralta G, Rodriguez-Lera MJ, Garrido JC, Ansorena L, and Roiz MP. Time to positivity in blood cultures of adults with Streptococcus pneumoniae bacteremia. BMC Infect Dis 6:79, 2006

Pether JV, Wang SP, and Grayston JT. Chlamydia pneumoniae, strain TWAR, as the cause of an outbreak in a boys' school previously called psittacosis. Epidemiol Infect 103:395-400, 1989

Petitjean J, Vabret A, Gouarin S, and Freymuth F. Evaluation of four commercial immunoglobulin G (IgG)- and IgM-specific enzyme immunoassays for diagnosis of Mycoplasma pneumoniae infections. J Clin Microbiol 40:165-71, 2002

Plouffe JF, Breiman RF, and Facklam RR. Bacteremia with Streptococcus pneumoniae. Implications for therapy and prevention. Franklin County Pneumonia Study Group. Jama 275:194-8, 1996

Porcel JM, Ruiz-Gonzalez A, Falguera M, Nogues A, Galindo C, Carratala J, and Esquerda A. Contribution of a pleural antigen assay (Binax NOW) to the diagnosis of pneumococcal pneumonia. Chest 131:1442-7, 2007

Povoa P. C-reactive protein: a valuable marker of sepsis. Intensive Care Med 28:235-43, 2002

Priner M, Cornillon C, Forestier D, Valero S, and Paccalin M. Might Streptococcus pneumoniae urinary antigen test be positive because of pneumococcal vaccine? J Am Geriatr Soc 56:170-1, 2008

Pönkä A. Clinical and laboratory manifestations in patients with serological evidence of Mycoplasma pneumoniae infection. Scand J Infect Dis 10:271-5, 1978

Pönkä A. Occurrence of serologically verified Mycoplasma pneumoniae infections in Finland and in Scandinavia in 1970--1977. Scand J Infect Dis 12:27-31, 1980

Ramirez J, Aliberti S, Mirsaeidi M, Peyrani P, Filardo G, Amir A, Moffett B, Gordon J, Blasi F, and Bordon J. Acute myocardial infarction in hospitalized patients with community-acquired pneumonia. Clin Infect Dis 47:182-7, 2008

Rantakokko-Jalava K, and Jalava J. Development of conventional and real-time PCR assays for detection of Legionella DNA in respiratory specimens. J Clin Microbiol 39:2904-10, 2001

Rantakokko-Jalava K, Marjamäki M, Marttila H, Mäkelä L, Valtonen V, and Viljanen MK. LCx Mycobacterium tuberculosis assay is valuable with respiratory specimens, but provides little help in the diagnosis of extrapulmonary tuberculosis. Ann Med 33:55-62, 2001

Rasmussen TR, Korsgaard J, Moller JK, Sommer T, and Kilian M. Quantitative culture of bronchoalveolar lavage fluid in community-acquired lower respiratory tract infections. Respir Med 95:885-90, 2001

Regev-Yochay G, Raz M, Dagan R, Porat N, Shainberg B, Pinco E, Keller N, and Rubinstein E. Nasopharyngeal carriage of Streptococcus pneumoniae by adults and children in community and family settings. Clin Infect Dis 38:632-9, 2004

Rello J, Bodi M, Mariscal D, Navarro M, Diaz E, Gallego M, and Valles J. Microbiological testing and outcome of patients with severe community-acquired pneumonia. Chest 123:174-80, 2003

Renaud B, Coma E, Hayon J, Gurgui M, Longo C, Blancher M, Jouannic I, Betoulle S, Roupie E, and Fine MJ. Investigation of the ability of the Pneumonia Severity Index to accurately predict clinically relevant outcomes: a European study. Clin Microbiol Infect 13:923-31, 2007[a]

Renaud B, Coma E, Labarere J, Hayon J, Roy PM, Boureaux H, Moritz F, Cibien JF, Guerin T, Carre E, Lafontaine A, Bertrand MP, Santin A, Brun-Buisson C, Fine MJ, and Roupie E. Routine use of the Pneumonia Severity Index for guiding the site-of-treatment decision of patients with pneumonia in the emergency department: a multicenter, prospective, observational, controlled cohort study. Clin Infect Dis 44:41-9, 2007[b]

Retalis P, Strange C, and Harley R. The spectrum of adult adenovirus pneumonia. Chest 109:1656-7, 1996

Richter SS, Beekmann SE, Croco JL, Diekema DJ, Koontz FP, Pfaller MA, and Doern GV. Minimizing the workup of blood culture contaminants: implementation and evaluation of a laboratory-based algorithm. J Clin Microbiol 40:2437-44, 2002

Ricketts KD, and Joseph CA. Legionnaires disease in Europe: 2005-2006. Euro Surveill 12:E7-8, 2007

Riedel S, Bourbeau P, Swartz B, Brecher S, Carroll KC, Stamper PD, Dunne WM, McCardle T, Walk N, Fiebelkorn K, Sewell D, Richter SS, Beekmann S, and

Doern GV. Timing of specimen collection for blood cultures from febrile patients with bacteremia. J Clin Microbiol 46:1381-5, 2008

Roson B, Carratala J, Verdaguer R, Dorca J, Manresa F, and Gudiol F. Prospective study of the usefulness of sputum Gram stain in the initial approach to community-acquired pneumonia requiring hospitalization. Clin Infect Dis 31:869-74, 2000

Roson B, Carratala J, Dorca J, Casanova A, Manresa F, and Gudiol F. Etiology, reasons for hospitalization, risk classes, and outcomes of community-acquired pneumonia in patients hospitalized on the basis of conventional admission criteria. Clin Infect Dis 33:158-65, 2001

Roson B, Carratala J, Fernandez-Sabe N, Tubau F, Manresa F, and Gudiol F. Causes and factors associated with early failure in hospitalized patients with community-acquired pneumonia. Arch Intern Med 164:502-8, 2004[a]

Roson B, Fernandez-Sabe N, Carratala J, Verdaguer R, Dorca J, Manresa F, and Gudiol F. Contribution of a urinary antigen assay (Binax NOW) to the early diagnosis of pneumococcal pneumonia. Clin Infect Dis 38:222-6, 2004[b]

Ruiz-Gonzalez A, Falguera M, Nogues A, and Rubio-Caballero M. Is Streptococcus pneumoniae the leading cause of pneumonia of unknown etiology? A microbiologic study of lung aspirates in consecutive patients with community-acquired pneumonia. Am J Med 106:385-90, 1999

Ruiz M, Ewig S, Marcos MA, Martinez JA, Arancibia F, Mensa J, and Torres A. Etiology of community-acquired pneumonia: impact of age, comorbidity, and severity. Am J Respir Crit Care Med 160:397-405, 1999[a]

Ruiz M, Ewig S, Torres A, Arancibia F, Marco F, Mensa J, Sanchez M, and Martinez JA. Severe community-acquired pneumonia. Risk factors and follow-up epidemiology. Am J Respir Crit Care Med 160:923-9, 1999[b]

Räty R, Rönkkö E, and Kleemola M. Sample type is crucial to the diagnosis of Mycoplasma pneumoniae pneumonia by PCR. J Med Microbiol 54:287-91, 2005

Sahn SA. Diagnosis and management of parapneumonic effusions and empyema. Clin Infect Dis 45:1480-6, 2007

Saikku P, Wang SP, Kleemola M, Brander E, Rusanen E, and Grayston JT. An epidemic of mild pneumonia due to an unusual strain of Chlamydia psittaci. J Infect Dis 151:832-9, 1985

Sanchez-Vargas FM, and Gomez-Duarte OG. Mycoplasma pneumoniae-an emerging extra-pulmonary pathogen. Clin Microbiol Infect 14:105-17, 2008

Saubolle MA, and McKellar PP. Laboratory diagnosis of community-acquired lower respiratory tract infection. Infect Dis Clin North Am 15:1025-45, 2001

Schildgen O, Muller A, Allander T, Mackay IM, Volz S, Kupfer B, and Simon A. Human bocavirus: passenger or pathogen in acute respiratory tract infections? Clin Microbiol Rev 21:291-304, table of contents, 2008

Schmitz H, Wigand R, and Heinrich W. Worldwide epidemiology of human adenovirus infections. Am J Epidemiol 117:455-66, 1983

Scott JA, Hall AJ, and Leinonen M. Validation of immune-complex enzyme immunoassays for diagnosis of pneumococcal pneumonia among adults in Kenya. Clin Diagn Lab Immunol 7:64-7, 2000[a]

Scott JA, Hall AJ, Muyodi C, Lowe B, Ross M, Chohan B, Mandaliya K, Getambu E, Gleeson F, Drobniewski F, and Marsh K. Aetiology, outcome, and risk factors for mortality among adults with acute pneumonia in Kenya. Lancet 355:1225-30, 2000[b]

Seppä Y, Bloigu A, Honkanen PO, Miettinen L, and Syrjälä H. Severity assessment of lower respiratory tract infection in elderly patients in primary care. Arch Intern Med 161:2709-13, 2001

Shariatzadeh MR, Huang JQ, Tyrrell GJ, Johnson MM, and Marrie TJ. Bacteremic pneumococcal pneumonia: a prospective study in Edmonton and neighboring municipalities. Medicine (Baltimore) 84:147-61, 2005

Shefet D, Robenshtok E, Paul M, and Leibovici L. Empirical atypical coverage for inpatients with community-acquired pneumonia: systematic review of randomized controlled trials. Arch Intern Med 165:1992-2000, 2005

Sjögren AM, Holmberg H, and Krook A. Etiologic diagnosis of pneumonia by antigen detection: crossreactions between pneumococcal C-polysaccharide and oral microorganisms. Diagn Microbiol Infect Dis 6:239-48, 1987

Smith MD, Derrington P, Evans R, Creek M, Morris R, Dance DA, and Cartwright K. Rapid diagnosis of bacteremic pneumococcal infections in adults by using the Binax NOW Streptococcus pneumoniae urinary antigen test: a prospective, controlled clinical evaluation. J Clin Microbiol 41:2810-3, 2003

Smith RP, and Lipworth BJ. C-reactive protein in simple community-acquired pneumonia. Chest 107:1028-31, 1995[a]

Smith RP, Lipworth BJ, Cree IA, Spiers EM, and Winter JH. C-reactive protein. A clinical marker in community-acquired pneumonia. Chest 108:1288-91, 1995[b]

Socan M, Marinic-Fiser N, Kraigher A, Kotnik A, and Logar M. Microbial aetiology of community-acquired pneumonia in hospitalised patients. Eur J Clin Microbiol Infect Dis 18:777-82, 1999

Soini H, Skurnik M, Liippo K, Tala E, and Viljanen MK. Detection and identification of mycobacteria by amplification of a segment of the gene coding for the 32-kilodalton protein. J Clin Microbiol 30:2025-8, 1992

Sopena N, Sabria-Leal M, Pedro-Botet ML, Padilla E, Dominguez J, Morera J, and Tudela P. Comparative study of the clinical presentation of Legionella pneumonia and other community-acquired pneumonias. Chest 113:1195-200, 1998

Sopena N, Sabria M, Pedro-Botet ML, Manterola JM, Matas L, Dominguez J, Modol JM, Tudela P, Ausina V, and Foz M. Prospective study of community-acquired pneumonia of bacterial etiology in adults. Eur J Clin Microbiol Infect Dis 18:852-8, 1999

Sopena N, Pedro-Botet ML, Sabria M, Garcia-Pares D, Reynaga E, and Garcia-Nunez M. Comparative study of community-acquired pneumonia caused by Streptococcus pneumoniae, Legionella pneumophila or Chlamydia pneumoniae. Scand J Infect Dis 36:330-4, 2004

Sopena N, and Sabria M. Multicenter study of hospital-acquired pneumonia in non-ICU patients. Chest 127:213-9, 2005

Strålin K, Kaltoft MS, Konradsen HB, Olcen P, and Holmberg H. Comparison of two urinary antigen tests for establishment of pneumococcal etiology of adult community-acquired pneumonia. J Clin Microbiol 42:3620-5, 2004

Strålin K, Törnqvist E, Kaltoft MS, Olcen P, and Holmberg H. Etiologic diagnosis of adult bacterial pneumonia by culture and PCR applied to respiratory tract samples. J Clin Microbiol 44:643-5, 2006

Syrjälä H, Kujala P, Myllylä V, and Salminen A. Airborne transmission of tularemia in farmers. Scand J Infect Dis 17:371-5, 1985

Syrjälä H, Broas M, Suramo I, Ojala A, and Lähde S. High-resolution computed tomography for the diagnosis of community-acquired pneumonia. Clin Infect Dis 27:358-63, 1998

Säynäjäkangas P, Keistinen T, Honkanen PO, and Kivelä SL. Hospital discharges for pneumonia in Finland between 1972 and 1993 in the population aged 65 years or over. Age Ageing 26:269-73, 1997[a]

Säynäjäkangas P, Keistinen T, Honkanen PO, and Kivelä SL. Hospitalization for pneumonia in the Finnish working-age population. Cent Eur J Public Health 5:27-9, 1997[b]

Sörensen J, Forsberg P, Håkanson E, Maller R, Sederholm C, Sören L, and Carlsson C. A new diagnostic approach to the patient with severe pneumonia. Scand J Infect Dis 21:33-41, 1989

Talbot TR, Poehling KA, Hartert TV, Arbogast PG, Halasa NB, Edwards KM, Schaffner W, Craig AS, and Griffin MR. Seasonality of invasive pneumococcal disease: temporal relation to documented influenza and respiratory syncytial viral circulation. Am J Med 118:285-91, 2005

Talkington DF, Shott S, Fallon MT, Schwartz SB, and Thacker WL. Analysis of eight commercial enzyme immunoassay tests for detection of antibodies to Mycoplasma pneumoniae in human serum. Clin Diagn Lab Immunol 11:862-7, 2004

Tan YK, Khoo KL, Chin SP, and Ong YY. Aetiology and outcome of severe community-acquired pneumonia in Singapore. Eur Respir J 12:113-5, 1998

Tanaka H, Koba H, Honma S, Sugaya F, and Abe S. Relationships between radiological pattern and cell-mediated immune response in Mycoplasma pneumoniae pneumonia. Eur Respir J 9:669-72, 1996

Tanaka H, Narita M, Teramoto S, Saikai T, Oashi K, Igarashi T, and Abe S. Role of interleukin-18 and T-helper type 1 cytokines in the development of Mycoplasma pneumoniae pneumonia in adults. Chest 121:1493-7, 2002

Tarver RD, Teague SD, Heitkamp DE, and Conces DJ, Jr. Radiology of community-acquired pneumonia. Radiol Clin North Am 43:497-512, viii, 2005

Templeton KE, Scheltinga SA, Graffelman AW, Van Schie JM, Crielaard JW, Sillekens P, Van Den Broek PJ, Goossens H, Beersma MF, and Claas EC. Comparison and evaluation of real-time PCR, real-time nucleic acid sequence-based amplification, conventional PCR, and serology for diagnosis of Mycoplasma pneumoniae. J Clin Microbiol 41:4366-71, 2003

Templeton KE, Scheltinga SA, van den Eeden WC, Graffelman AW, van den Broek PJ, and Claas EC. Improved diagnosis of the etiology of community-acquired pneumonia with real-time polymerase chain reaction. Clin Infect Dis 41:345-51, 2005

Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, and Fukuda K. Mortality associated with influenza and respiratory syncytial virus in the United States. Jama 289:179-86, 2003

Thompson WW, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox NJ, and Fukuda K. Influenza-associated hospitalizations in the United States. Jama 292:1333-40, 2004

Thomsen RW, Riis A, Norgaard M, Jacobsen J, Christensen S, McDonald CJ, and Sorensen HT. Rising incidence and persistently high mortality of hospitalized pneumonia: a 10-year population-based study in Denmark. J Intern Med 259:410-7, 2006

Thorpe JE, Baughman RP, Frame PT, Wesseler TA, and Staneck JL. Bronchoalveolar lavage for diagnosing acute bacterial pneumonia. J Infect Dis 155:855-61, 1987

Tondella ML, Talkington DF, Holloway BP, Dowell SF, Cowley K, Soriano-Gabarro M, Elkind MS, and Fields BS. Development and evaluation of real-time PCR-based fluorescence assays for detection of Chlamydia pneumoniae. J Clin Microbiol 40:575-83, 2002

Torres A, and el-Ebiary M. Invasive diagnostic techniques for pneumonia: protected specimen brush, bronchoalveolar lavage, and lung biopsy methods. Infect Dis Clin North Am 12:701-22, 1998

Torres A, and El-Ebiary M. Bronchoscopic BAL in the diagnosis of ventilator-associated pneumonia. Chest 117:198S-202S, 2000

Torres JM, Cardenas O, Vasquez A, and Schlossberg D. Streptococcus pneumoniae bacteremia in a community hospital. Chest 113:387-90, 1998

Treanor JJ, Hayden FG, Vrooman PS, Barbarash R, Bettis R, Riff D, Singh S, Kinnersley N, Ward P, and Mills RG. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. US Oral Neuraminidase Study Group. JAMA 283:1016-24, 2000

Trifonov V, Khiabanian H, and Rabadan R. Geographic Dependence, Surveillance, and Origins of the 2009 Influenza A (H1N1) Virus. N Engl J Med 2009

Trotter CL, Stuart JM, George R, and Miller E. Increasing hospital admissions for pneumonia, England. Emerg Infect Dis 14:727-33, 2008

Tuomanen EI, Austrian R, and Masure HR. Pathogenesis of pneumococcal infection. N Engl J Med 332:1280-4, 1995

Turett GS, Blum S, and Telzak EE. Recurrent pneumococcal bacteremia: risk factors and outcomes. Arch Intern Med 161:2141-4, 2001

Tuuminen T, Varjo S, Ingman H, Weber T, Oksi J, and Viljanen M. Prevalence of Chlamydia pneumoniae and Mycoplasma pneumoniae immunoglobulin G and A antibodies in a healthy Finnish population as analyzed by quantitative enzyme immunoassays. Clin Diagn Lab Immunol 7:734-8, 2000

Vainio A, Lyytikäinen O, Sihvonen R, Kaijalainen T, Teirilä L, Rantala M, Lehtinen P, Ruuska P, and Virolainen A. An outbreak of pneumonia associated with S. pneumoniae at a military training facility in Finland in 2006. APMIS 117:488-91, 2009

Waites KB, and Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. Clin Microbiol Rev 17:697-728, table of contents, 2004

Wakefield AE, Pixley FJ, Banerji S, Sinclair K, Miller RF, Moxon ER, and Hopkin JM. Detection of Pneumocystis carinii with DNA amplification. Lancet 336:451-3, 1990

Wald TG, Shult P, Krause P, Miller BA, Drinka P, and Gravenstein S. A rhinovirus outbreak among residents of a long-term care facility. Ann Intern Med 123:588-93, 1995

Valencia M, Badia JR, Cavalcanti M, Ferrer M, Agusti C, Angrill J, Garcia E, Mensa J, Niederman MS, and Torres A. Pneumonia severity index class v patients with community-acquired pneumonia: characteristics, outcomes, and value of severity scores. Chest 132:515-22, 2007

Valtonen VV. Role of infections in atherosclerosis. Am Heart J 138:S431-3, 1999

van der Eerden MM, Vlaspolder F, de Graaff CS, Groot T, Jansen HM, and Boersma WG. Value of intensive diagnostic microbiological investigation in low- and high-risk patients with community-acquired pneumonia. Eur J Clin Microbiol Infect Dis 2005

van der Meer V, Neven AK, van den Broek PJ, and Assendelft WJ. Diagnostic value of C reactive protein in infections of the lower respiratory tract: systematic review. BMJ 331:26, 2005

Waring AL, Halse TA, Csiza CK, Carlyn CJ, Arruda Musser K, and Limberger RJ. Development of a genomics-based PCR assay for detection of Mycoplasma pneumoniae in a large outbreak in New York State. J Clin Microbiol 39:1385-90, 2001

Waris M, Halonen P, Ziegler T, Nikkari S, and Obert G. Time-resolved fluoroimmunoassay compared with virus isolation for rapid detection of respiratory syncytial virus in nasopharyngeal aspirates. J Clin Microbiol 26:2581-5, 1988

Waris M. Pattern of respiratory syncytial virus epidemics in Finland: two-year cycles with alternating prevalence of groups A and B. J Infect Dis 163:464-9, 1991

Waris ME, Toikka P, Saarinen T, Nikkari S, Meurman O, Vainionpää R, Mertsola J, and Ruuskanen O. Diagnosis of Mycoplasma pneumoniae pneumonia in children. J Clin Microbiol 36:3155-9, 1998

Waterer GW, Baselski VS, and Wunderink RG. Legionella and community-acquired pneumonia: a review of current diagnostic tests from a clinician's viewpoint. Am J Med 110:41-8, 2001

Waterer GW, and Wunderink RG. The influence of the severity of community-acquired pneumonia on the usefulness of blood cultures. Respir Med 95:78-82, 2001

Watson DA, Musher DM, Jacobson JW, and Verhoef J. A brief history of the pneumococcus in biomedical research: a panoply of scientific discovery. Clin Infect Dis 17:913-24, 1993

Watt JP, O'Brien KL, Katz S, Bronsdon MA, Elliott J, Dallas J, Perilla MJ, Reid R, Murrow L, Facklam R, Santosham M, and Whitney CG. Nasopharyngeal versus oropharyngeal sampling for detection of pneumococcal carriage in adults. J Clin Microbiol 42:4974-6, 2004

Wattanathum A, Chaoprasong C, Nunthapisud P, Chantaratchada S, Limpairojn N, Jatakanon A, and Chanthadisai N. Community-acquired pneumonia in southeast Asia: the microbial differences between ambulatory and hospitalized patients. Chest 123:1512-9, 2003

Weber TP, and Stilianakis NI. Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. J Infect 57:361-73, 2008

Weinstein MP, Klugman KP, and Jones RN. Rationale for revised penicillin susceptibility breakpoints versus Streptococcus pneumoniae: coping with antimicrobial susceptibility in an era of resistance. Clin Infect Dis 48:1596-600, 2009

Venkatesan P, and Macfarlane JT. Role of pneumococcal antigen in the diagnosis of pneumococcal pneumonia. Thorax 47:329-31, 1992

Werno AM, and Murdoch DR. Medical microbiology: laboratory diagnosis of invasive pneumococcal disease. Clin Infect Dis 46:926-32, 2008

Vikerfors T, Brodin G, Grandien M, Hirschberg L, Krook A, and Pettersson CA. Detection of specific IgM antibodies for the diagnosis of Mycoplasma pneumoniae infections: a clinical evaluation. Scand J Infect Dis 20:601-10, 1988

Vila-Corcoles A, Ochoa-Gondar O, Rodriguez-Blanco T, Raga-Luria X, and Gomez-Bertomeu F. Epidemiology of community-acquired pneumonia in older adults: a population-based study. Respir Med 103:309-16, 2009

Wilkinson M, and Woodhead MA. Guidelines for community-acquired pneumonia in the ICU. Curr Opin Crit Care 10:59-64, 2004

Wimberley NW, Bass JB, Jr., Boyd BW, Kirkpatrick MB, Serio RA, and Pollock HM. Use of a bronchoscopic protected catheter brush for the diagnosis of pulmonary infections. Chest 81:556-62, 1982

Wipf JE, Lipsky BA, Hirschmann JV, Boyko EJ, Takasugi J, Peugeot RL, and Davis CL. Diagnosing pneumonia by physical examination: relevant or relic? Arch Intern Med 159:1082-7, 1999

von Baum H, Ewig S, Marre R, Suttorp N, Gonschior S, Welte T, and Luck C. Community-acquired Legionella pneumonia: new insights from the German competence network for community acquired pneumonia. Clin Infect Dis 46:1356-64, 2008

von Baum H, Welte T, Marre R, Suttorp N, Luck C, and Ewig S. Mycoplasma pneumoniae pneumonia revisited within the German Competence Network for Community-acquired pneumonia (CAPNETZ). BMC Infect Dis 9:62, 2009

Woodhead MA, Macfarlane JT, McCracken JS, Rose DH, and Finch RG. Prospective study of the aetiology and outcome of pneumonia in the community. Lancet 1:671-4, 1987

Woodhead M. Community-acquired pneumonia in Europe: causative pathogens and resistance patterns. Eur Respir J Suppl 36:20s-27s, 2002

Woodhead M. Community-acquired pneumonia: severity of illness evaluation. Infect Dis Clin North Am 18:791-807; viii, 2004

Woodhead M, Blasi F, Ewig S, Huchon G, Ieven M, Örtqvist A, Schaberg T, Torres A, van der Heijden G, and Verheij TJ. Guidelines for the management of adult lower respiratory tract infections. Eur Respir J 26:1138-80, 2005

Woodhead M, Welch CA, Harrison DA, Bellingan G, and Ayres JG. Community-acquired pneumonia on the intensive care unit: secondary analysis of 17,869 cases in the ICNARC Case Mix Programme Database. Crit Care 10 Suppl 2:S1, 2006

Vuorinen T, Vainionpää R, and Hyypiä T. Five years' experience of reverse-transcriptase polymerase chain reaction in daily diagnosis of enterovirus and rhinovirus infections. Clin Infect Dis 37:452-5, 2003

Yang S, and Rothman RE. PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. Lancet Infect Dis 4:337-48, 2004

Yavlovich A, Tarshis M, and Rottem S. Internalization and intracellular survival of Mycoplasma pneumoniae by non-phagocytic cells. FEMS Microbiol Lett 233:241-6, 2004

Yende S, D'Angelo G, Kellum JA, Weissfeld L, Fine J, Welch RD, Kong L, Carter M, and Angus DC. Inflammatory markers at hospital discharge predict subsequent mortality after pneumonia and sepsis. Am J Respir Crit Care Med 177:1242-7, 2008

Young M, and Marrie TJ. Interobserver variability in the interpretation of chest roentgenograms of patients with possible pneumonia. Arch Intern Med 154:2729-32, 1994

Zalacain R, Torres A, Celis R, Blanquer J, Aspa J, Esteban L, Menendez R, Blanquer R, and Borderias L. Community-acquired pneumonia in the elderly: Spanish multicentre study. Eur Respir J 21:294-302, 2003

Örtqvist Å, Grepe A, Julander I, and Kalin M. Bacteremic pneumococcal pneumonia in Sweden: clinical course and outcome and comparison with non-bacteremic pneumococcal and mycoplasmal pneumonias. Scand J Infect Dis 20:163-71, 1988

Örtqvist Å, Hedlund J, Grillner L, Jalonen E, Kallings I, Leinonen M, and Kalin M. Aetiology, outcome and prognostic factors in community-acquired pneumonia requiring hospitalization. Eur Respir J 3:1105-13, 1990

Örtqvist Å, Hedlund J, Wretlind B, Carlström A, and Kalin M. Diagnostic and prognostic value of interleukin-6 and C-reactive protein in community-acquired pneumonia. Scand J Infect Dis 27:457-62, 1995

Örtqvist Å, Hedlund J, and Kalin M. Streptococcus pneumoniae: epidemiology, risk factors, and clinical features. Semin Respir Crit Care Med 26:563-74, 2005