Community acquired infections in adult and elderly airways; an evaluation of diagnostic harvesting techniques

Cand.med Susanne M Sørensen Hernes

Department of Geriatrics and Internal Medicine

Sorlandet Hospital Arendal

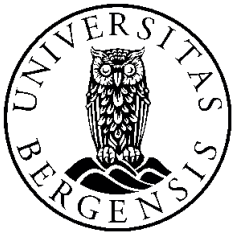
NORWAY

and

Department of Clinical Science

University of Bergen

Norway


UNIVERSITAS
BERGENSIS


SØRLANDET SYKEHUS

Table of Contents

1. Acknowledgements

The present work was carried out at the Department of Medicine, Sorlandet Hospital Arendal during the years of 2005, 2006-2008, 2010-2012. It was funded by the South-Eastern Norway Regional Health Authority, by the Norwegian Heart and Lung Patient Organization through the Norwegian Extra-Foundation for Health and Rehabilitation through the EXTRA funds, and by Sorlandet Hospital HF.

This thesis would never have been initiated nor completed without my supervisors in Arendal and Bergen, Professor Per Bakke, Professor Bjarne Bjorvatn and dr.med Egil Hagen. With your expertise in pulmonary medicine, infectious diseases, microbiology and internal medicine, you have provided invaluable knowledge, assistance and inspiration throughout this work and I have been honored to work with you.

The Medical Department at Sorlandet Hospital, with Head Physicians Geir Rørbakken and Ole Høie has given me excellent working facilities and invaluable support. A special thanks to Geir Rørbakken for letting me postpone my specialization into geriatric medicine, thus increasing his personal workload for several years, and for all the valuable knowledge he has passed my way over these years. A warm thanks to Professor Svein Gunnar Gundersen, Sissel Ledang and Sandrine Schuhler Slotten for their continuous facilitation, help and support.

This thesis would not have been completed without the assistance and encouragements from my colleagues at the Medical Department of Sorlandet Hospital Arendal. A special thanks to Endre Røynstrand for his encouragements, assistance with study planning as well as study execution, and to Terje Torp for giving me time off to finalize this thesis.

I would like to thank the Department of Microbiology at SSK, the Department of Laboratory Sciences at SSA and the Department of Radiology for skilled help and a generous attitude, even in periods of high-activity. To Ståle and Hanne, without you this thesis would never have happened. May we get many more research ideas in the

future! Thanks to Thomas, Nazeer, Eirin and Sunniva for their patience and help with the TFNA procedure in patients with CAP. You took on an extra workload on my behalf, I owe you greatly. Thanks to the skilful help from Andreas Christensen at the Department of Microbiology at St.Olavs Hospital. Furthermore, thanks to Truls Leegard and his team at the Microbiology Department at Oslo University Hospital (formerly Rikshospitalet University Hospital) for skilful help during initial PCR try-outs in paper I. Sadly, the sentence displaying our gratitude in the acknowledgement section of paper I got lost in the revision, but here it is ☺!

The extended multidisciplinary research group in Arendal; thanks for lunches, ideas, help and lots of fun. A special thanks to the researchers at the Medical Department, Benthe, Daniela, Bjørnar, Petter, Sigve, Thomas and Runar. To my team at the Memory Clinic, Anne Liv and Bengt Ove, geriatric research at SSA would not be the same without you.

Thanks to my parents for their care and love, and for letting me ask the question "why" a million times during my childhood, followed by conducting experiments to help me answer those questions. Thanks to my brother Kaare, who never misses the opportunity to put more laughter into my life. Thanks to my mother- and father-in-law for encouragement and a tremendous amount of baby-sitting hours so I could finish my research. Immensely grateful!

Finally, to my wonderful family, Knut, Sarah and David: Every day, you show me the importance of living my life outside the office, I am so happy to spend my days with you!

Arendal, June 22. 2013

Susanne M Sørensen Hernes

2. List of papers

Paper I:
Hernes SS, Hagen E, Tofteland S, Finsen NT, Christensen A, Giske CG, Bakke PS, Bjorvatn B. (2010). Transthoracic fine-needle aspiration in the aetiological diagnosis of community-acquired pneumonia. Clinical Microbiology and Infection, 16(7), 909-911.

Paper II:
Hernes SS, Quarsten H, Hagen E, Lyngroth AL, Pripp AH, Bjorvatn B, Bakke PS (2011) Swabbing for respiratory viral infections in older patients: a comparison of rayon and nylon flocked swabs. Eur J Clin Microbiol Infect Dis 30 (2):159-165

Paper III:
Hernes SS, Quarsten H, Hamre R, Hagen E, Bjorvatn B, Bakke PS (2013) A comparison of nasopharyngeal and oropharyngeal swabbing for the detection of influenza virus by real-time PCR. Eur J Clin Microbiol Infect Dis 32 (3):381-385

Paper IV
Hernes SS, Hagen E, Quarsten H, Bjorvatn B, Bakke PS
No impact of early real time PCR screening for respiratory viruses on length of stay and use of antibiotics in elderly patients hospitalized with symptoms of a respiratory tract infection. Submitted Eur J Clin Microbiol Infect Dis May 2013.

3. Abbreviations

ARIs	Acute respiratory infections
BAL	Bronchoalveolar lavage
CAP	Community acquired pneumonia
HMPV	Human Metapneumovirus
InfA	Influenza A virus
InfB	Influenza B virus
LRTI(s)	Lower respiratory tract infections
MCat	Moraxella catharralis
NP	Nasopharynx
OP	Oropharynx
PBS	Protected brush specimen (bronchoscopic sampling)
PCR	Polymerase chain reaction
PCV7	7-valent pneumococcal conjugate vaccine
PCV13	13-valent pneumococcal conjugate vaccine
PIV 1-4	Parainfluensavirus 1-4
PSSV23	23-valent pneumococcal capsular polysaccharide vaccine
RSV	Respiratory Syncytial Virus
RVI	Respiratory viral infection
TFNA	Transthoracic fine needle aspiration
TTA	Trans-tracheal aspiration

4. Background

Pneumonia and other lower respiratory tract infections (LRTI) are major public health problems[1]. World-wide, LRTIs are the third leading cause of death and the mortality rates have remained unchanged for decades[1]. Furthermore, community acquired pneumonia (CAP) is a major cause of hospital admission, especially in the elderly[2]. At the current level of methodological development, the frequent lack of exact etiological information to guide a rational choice of treatment is a major concern, especially when considering the high mortality rates associated with CAP[3, 4]. Another consequence of insufficient etiological diagnosis in LRTIs is that the true efficacy of vaccines against respiratory tract infections is impossible to assess directly [5, 6].

Norway is one of the few countries in the world where microbial resistance to major antibiotic groups such as penicillins, cephalosporins and macrolides is rare. For example, in 2010, 3% of *Streptococcus pneumoniae* isolates from Norwegian blood or spinal fluid culture were resistant to penicillin G, and concomitant resistance to cephalosporines was even more unusual (3/730 isolates) [7]. In most European countries, resistance to antibiotics is common among respiratory pathogens and therefore, Norwegian guidelines for antibiotic treatment in respiratory infections differ from most of those provided in the rest of Europe [8, 9].

Elderly patients are at increased risk for acquiring a respiratory infection. For patients above 65 years of age, the incidence of pneumonia is four times higher than in the population at large [10]. With a rapidly growing geriatric population, the number of patients suffering from pneumonia is estimated to double during the next thirty years [11]. This will have a strong impact on the Norwegian health care system, including far-reaching logistical and economic consequences.

4.1. Bacterial infections

Severe pneumonia most commonly affects the oldest, the youngest and the immune-suppressed individuals in our community. The mortality in pneumonia is high, from 2006 to 2010 almost 9000 individuals died from LRTI in Norway[12]. In 2008, approximately 200 000 died from LRTI in Europe[13], whereas worldwide, respiratory tract infections are responsible for an estimated 3.6 million deaths each year [14].

In order to ensure specific and timely antimicrobial therapy, etiological diagnosis is essential for a successful outcome in pneumonia [15]. However, the anatomically shielded location of the lungs prevents direct access to the infected area using conventional tools for microbial sampling. Thus, the pathogen remains unknown in 80% of hospitalized patients and 90 % of outpatients [16]. Insufficient knowledge of the pneumonia etiology frequently results in inappropriate treatment, which is a problem not only for the individual patient, but ultimately also for the society as a whole. In fact, the major driving force for the evolution of antimicrobial resistance is the indiscriminate use of broad spectrum antibiotics[17]. For example, multi-resistant strains of *S.pneumoniae* are an increasing concern globally [18].

In CAP the efficacy of conventional methods rarely exceeds 50 % [1, 19].
This etiological problem related to CAP has clinical implications as the value of treatment recommendations depends on type of prevailing pathogens and their resistance to antimicrobials. Furthermore, the current limitations in our diagnostic arsenal results in inadequate knowledge on the extent and importance of mixed viral and bacterial infections and on the role of viruses alone in the etiology of CAP.

Lung infections caused by anaerobes are now believed to be the result of aspiration, or bacterial colonization while on respirator or occasionally the cause of pulmonary abscesses [20, 21]. However, data obtained in the late 80's using trans-tracheal aspiration (TTA) revealed that anaerobes were responsible for at least 30 % of CAPs[22]. Some authors believe anaerobic lung infections often are misdiagnosed as "atypical bacterial infections" such as *Mycoplasma pneumoniae*, *Chlamydophila penumoniae* or Legionella-species[22, 23].

In Norway, only a few studies on respiratory pathogens have been performed (Table I) in adults and elderly individuals. The majority of these studies have been based on agent identification through culture or anti-body analysis. All but two of the studies were performed more than 30 years ago. To our knowledge, no such etiological studies have been conducted using real-time PCR-methods.

Table I. Norwegian epidemiological studies in adults on lower respiratory tract infections with pathogen identification

Author	Year[a]	Population	Age and Sex	Methods	Microbiological assays	Etiological diagnosis in percentage of the patients	Most common pathogens isolated
Schreiner [24]	1972	87 patients with pneumonia or bronchopneumonia [b]	Mean age not given 67% males	TTA Sputum	Culture	35 % (36/103)	TTA: 16 % *S.pneumoniae*, 8 % Enterobacteriaceae 6 % *H.influenzae*
Schreiner [25]	1973	341 patients with pneumonia, asthma, chronic bronchitis or emphysema, pulmonary embolism, non-pulmonary disease [b]	Mean age not given. 55% males	TTA Sputum	Culture	34% (117/341)	TTA: 18 % *S.pneumoniae* 8 % *H.Influenzae* 6% Enterobacteriaceae
Bjerkestrand [26]	1975	34 hospitalized patients with bronchiectasis and chronic bronchitis	Mean age not given Sex not given	TTA	Culture	85% (29/34)	23 % Enterobacteriaceae 17% *H.influenzae* 15% *S.pneumoniae*

Table I continued

Author	Year[a]	Population	Age and Sex	Methods	Microbiological assays	Etiological diagnosis in percentage of the patients	Most common pathogens isolated
Schreiner [27]	1978	87 hospitalized patients with acute exacerbation of chronic bronchitis	Mean 61 years 71% males	TTA	Culture	60% (62/103)	26 % *H.influenzae* 22% *S.pneumoniae* 8% *Neisseria meningitidis*
Schreiner [28]	1979	569 patients with acute pneumonia, chronic bronchitis, non-pulmonary disease or pulmonary embolism (552 hospitalized) [c]	Mean age not given Sex not given	TTA Sputum	Culture	Pneumonia: 46% Chronic bronchitis: 72% (63/87)	Pneumonia: 19 % *S.pneumoniae* 10 % *H.Influenzae* 4 % *Neisseria meningitides* Chronic bronchitis: 25% *Haemophilus species* 15% *S.pneumoniae* 7% *Neisseria meningitides*
Schreiner [29]	1979	15 hospitalized patients with empyema and 11hospitalized patients with lung abscess	Age and sex not given	Aspiration from pleural space TTA	Anaerobic culture	40 % (6/15) of empyema patients 64 % (7/11) of lung abscess patients.	Empyema: 20 % anaerobic Gram-positive cocci Lung abscess 36 % *Fusobacterium nucleatum*

Table I continued

Author	Year[a]	Population	Age and Sex	Methods	Microbiological assays	Etiological diagnosis in percentage of the patients	Most common pathogens isolated
Melby [30]	1992	153 non-hospitalized patients with lower respiratory tract infections	Median age 35 years 46 % males	Serology Spirometry Clinical laboratory tests	Serology for *S.pneumoniae*, Chlamydia species, *M. pneumonia,* Legionella species, influenzavirus A/B, parainfluenzavirus l and 3, adenovirus, Epstein Barr virus and RSV	37 % (44/153)	11% Influenza A and B 8 % RSV 6% *S.pneumoniae*
Norrby [31]	1997	411 patients with fever, x-ray verified pulmonary infiltrate and non-productive cough from Sweden, Norway, Denmark and Finland	Median age 33 years 69 % males	NP swab Serum, Sputum, Culture of blood	Bacterial culture Serum analysis for *M.pneumoniae, C.pneumoniae* and *L.pneumophila*	66% (270/411)	16% *M.pneumoniae*, 13% *C.pneumoniae*. 6% adenovirus

[a] Year of study publication, [b] No information of hospital admittance, [c] Data from [24],[25],[27] are by author incorporated into this study.

Overview of Norwegian epidemiological studies on etiological diagnosis of lower respiratory tract infections in adults. Percentages are calculated based on included patients in the study, not by retrospective analyses of infected patients.

4.2. Viral infections.

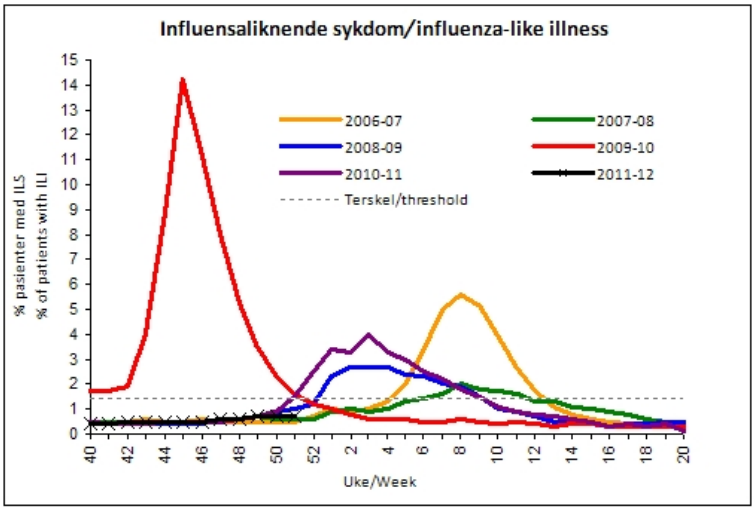
More than twenty viral species are currently known to cause acute respiratory tract infections in humans. The incidence rates of these viruses vary by season and may assume a biennial fashion [32]. Respiratory viral infections (RVIs) are potentially serious illnesses with mortality rates up to 55% in the elderly, particularly in patients with underlying heart or lung disease [33]. In the older age groups, influenza virus, Respiratory syncytial virus (RSV) and Human metapneumovirus (HMPV) are responsible for the most serious infections [34].

However, - the clinical characteristics of RVIs in the elderly may not be representative of typical disease manifestations in younger age groups. It is conceivable that a silent epidemic could remain unrecognized in long term care facilities, as symptomatic patients are rarely examined for respiratory viruses. American and Canadian researchers have performed a few studies showing that 36-41% of the residents in long term care facilities undergo at least one RVI each year [34, 35]. Several papers describe limited outbreaks with mortality rates of 0-50% [34, 36-39]. In Norway, no epidemiological, clinical or virological studies have been conducted to investigate the impact of respiratory virus infections in long time care facilities.

Of the RVIs, influenza virus is most extensively studied and influenza A and B viruses (InfA and InfB) are among the few respiratory viruses for which specific treatment is available. The incidence of influenza-like illness varies from season to season; as much as 40 % of the Norwegian population might have been infected with InfA and InfB in years with high influenza activity (Figure 1) [40]. Mortality rates (Figure 2) depends on the virulence of the seasonal influenza subtype, as well as on the level of specific immunity in the population. Disregarding the H1N1 pandemic where mainly individuals under 60 years of age were infected and on rare occasions died, the majority of influenza-related deaths are found in the older age groups [41]. For other types of respiratory viruses, less is known about incidence and mortality in different age groups, in particular among elderly individuals.

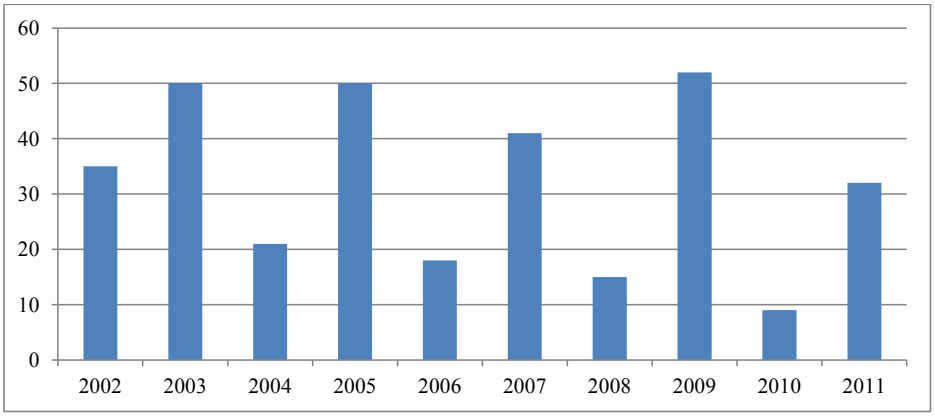
Vaccines are available for influenza virus, but the number of elderly patients achieving vaccine induced protection could be as low as 30 % [42] or even virtually absent [43]. This is mainly due to altered t-cell function (see section 4.4.). Vaccines for RSV have been in development since the 1960's, but several trials have stranded either because of safety concerns or due to low vaccine efficacy[44]. A vaccine against respiratory infections caused by adenovirus was recently introduced, primarily for use among US military recruits[45]. For other viruses, no vaccines appear to be in advanced stages of development[46].

Figure 1. Yearly incidence of influenza-like illnesses in Norway 2006-2012, as reported by the Norwegian Institute of Public Health.



[47]

Figure 2. Number of registered deaths from influenza infections in Norway 2002-2011, as reported by Statistics Norway.



[48]

4.3. Sample harvesting

Essential for the etiological diagnosis of an ARI is recovery of representative test samples without contamination. Several possible sampling methods are available (Table II).

In most cases of LRTIs, specimen recovered through the mouth or nose substitute for specimens collected directly from the affected area. Unfortunately, the diagnostic value of such samples is dubious, as in the nose and mouth contamination by potential irrelevant pathogens such as *Haemophilus influenzae, Moraxella catharralis, Streptococcus pneumoniae* and anaerobes is practically unavoidable [49-51].

Although in CAP, a positive blood culture is the most important etiological proof, blood cultures are positive only in approximately 30 % of the cases [52]. Paired serum antibody tests are sometimes helpful, but usually results are available only after a couple of weeks, which is too late for guiding the initial choice of antibiotic treatment. Urinary antigen tests are available for *S. pneumoniae* and *Legionella pneumophila* serotype 1. Although the sensitivity of such tests is high [53, 54], interpretation of positive results may be difficult as the actual urinary antigens may be detectable for up to a year after the initial infection [55, 56].

Conventional diagnostic measures including culture of blood and sputum, urinary antigen tests, upper respiratory swabs and serology are all indirect approaches to the etiological diagnosis of ARIs and both positive and negative results obtained with these methods require skilled interpretation[4].

Transthoracic fine needle aspiration (TFNA), first published in 1883, is an old method for diagnosis of pulmonary infections [57]. The technique was in common use in the pre-antibiotic era when treatment of pulmonary infections was mainly by pneumococcal antiserum. During the 1980-90s, the introduction of polymerase chain reaction (PCR) methods stimulated new interest in TFNA (Table III). Having localized the pneumonic infiltrate radiologically, TFNA is performed with a syringe and an ultrathin needle that is inserted directly into the infectious area in the lung, thus bypassing potential contamination from the oropharynx. Most TFNAs have been done

bedside, without radiological guidance [58]. The main adverse events of TFNA are pneumothorax and hemoptysis. Two large reviews of TFNA, one including 3560 children and the other over 3000 adults, reported incidences of pneumothorax of 3.2% and 3.3% respectively[52, 58]. Chest drainage was needed in 0.5% of the cases [52, 58]. Minor hemoptysis was by some authors reported as common[52], but was not mentioned in other studies. There was no report of empyema in any of these studies.

Trans-tracheal aspiration (TTA) as a method of specimen collection in lower respiratory airways was first published by Pecora in 1959[59]. The method was later modified by Kalinske[60] and was mainly use between 1960-1980 for research purposes. With the patient in a supine position, local anesthetics was applied to the cricothyroid membrane and the aspiration carried out using a needle guided catheter[61]. In Norway, this method was used by Schreiner et al. in the 1970s and 1980s to investigate the etiologies of CAP, anaerobic respiratory infections, chronic bronchitis and bronchiectasies, respectively [24-27]. The major side effects of this procedure are coughing, subcutaneous emphysema and hemoptysis [22, 62]. In one study of 25 patients with CAP, microbiological specimens were sampled with both TTA and TFNA[63]. In 20/25 of these patients, an etiological diagnosis was established by TFNA, whereas TTA and TFNA revealed the same pathogens in only 9/22 (41%) of the patients. Contaminations by oral flora was suspected in 11/22(50%) of the TTA samples [63]. This finding is in contrast to other reports claiming tracheal sterility when not suffering from a respiratory infection [62, 64, 65]. The patients who underwent both TFNA and TTA, preferred TFNA due to its lower incidence of pain and side-effects[63].

Table II. Assessment of diagnostic samples for respiratory infections in adults

Sample material	Analysis	Advantages	Disadvantages
Nasal • Midturbinate swab • wash	Culture, PCR, Rapid antigen test	Easily accessible with a minimum of training	Not sufficiently validated against upper respiratory swabs.
Nasopharyngeal • swab • wash • aspiration	Culture, PCR, Rapid antigen test	Efficient, easy acquisition PCR is efficient	Requires instruction to perform appropriately. NP washes are difficult to obtain in frail elderly[32]. Rapid antigen tests have low sensibility in the elderly in influenza/RS [66-68].
Oropharyngeal • swab	Culture, PCR, Rapid antigen test	Easily accessible with a minimum of training. Preferred site for diagnosis of *M. pneumoniae*.	Rapid antigen tests have low sensitivity in the elderly [66-68].
Transtracheal • aspiration	Culture, PCR, Rapid antigen test	Sample free from oral contamination.	Representability of the specimen Painful [63]? Risk of subcutaneous emphysema.
Bronchial • lavage • brush	Culture, PCR Rapid antigen tests	In hospitals usually easy to obtain	Contamination of the sample from the oropharynx. Requires equipment and trained personnel
Transthoracic • aspiration	Culture, PCR Rapid antigen tests	Samples directly from the infectious area.	Not possible to perform on patients using anticoagulants or with serious COPD. Risk of pneumothorax.
Sputum	Culture, PCR	Easily available in most patients, with proper assistance from physical therapists Excellent for legionella species (PCR)	Difficulties obtaining a sample of good quality[69] Possible contamination by irrelevant colonization in the oro/nasopharynx of HI, SP or MC [4]
Blood	Blood culture	Efficient and easily available	Only 30 % of bacterial pneumonias produce a positive blood culture[52]. Only available for bacteria capable of causing bacteremia.
Serum • Venous sample	Antibodies Pneumolysin	No discomfort to patient	Four weeks delay for obtaining paired tests Pneumolysin sensitivity still not assessed.
Urine • Urinary antigen	L.pneumophila serotype 1 S. pneumoniae	High sensitivity (>87%) [54] Sensitivity 65.9-82 % [53, 70, 71]	Does not reliably detect other serotypes. Remains positive for up to 362 days after illness[55]. Test might be negative the first days after subsidence[72]. Remains positive for up to a year later in concentrated urine[56]

HI=Hemophilus influenzae, SP=Streptococcus pneumoniae, MC=Moraxella catharralis

Assessment of diagnostic samples for pathogen identification in adult respiratory infections

Table III. Overview of studies from 1975-2005 using TFNA for etiological diagnosis of pneumonia

Author	Country	Year	Population	Age and Sex	Methods	Microbiological assays	Efficacy of diagnostic measure	Adverse effects
Davidson [73]	USA	1976	25 adults with CAP	Range 20-57 Sex not given	TFNA TTA Sputum Blood	Culture	All measures combined Not given. TFNA sensitivity 80 % (22/25)	Pneumothorax in 20% (5/25), no drainage needed.
Castellino [74]	USA	1979	82 immuno-compromised patients (108 TFNAs)	Median 47 years Sex not given	TFNA TTA Broncho-scopy Thoracotomy Autopsy	Culture	All measures combined 87% (94/108) TFNA sensitivity 73 % (79/108)	Hemoptysis in 3% (3/108). Pneumothorax in 26% (28/108), drainage needed in 14 patients
Zavala [75]	Spain	1981	25 lung infections 25 malignant lesions	Mean 55 years Male 74%	TFNA Sputum Blood	Gram, Giemsa, Grocott-Gomori, Auramine-Rhodamine stains. Immunoflourescence against *L.pneumophila.* Culture	All measures combined 84% (21/25) TFNA sensitivity 60% (15/25)	Hemoptysis in 4% (2/50) Pneumothorax in 8% (4/50), drainage needed in 2 patients
Wallace [76]	USA	1985	14 patients, 16 aspirations	Age not given Sex not given	TFNA Sputum	Culture Gram, GMS, Ziehl-Neelsen, PAS stains.	All measures combined 94% (15/16) TFNA sensitivity 88% (14/16)	Hemoptysis in 13% (2/16) Pneumothorax in 44% (7/16), drainage needed in 3 patients

Table III continued

Author	Country	Year	Population	Age and Sex	Methods	Microbiological assays	Efficacy of diagnostic measure	Adverse effects
Barnes [77]	Australia	1988	144 patients with pneumonia	Age not given Sex not given	TFNA Blood Sputum	Culture Gram stain	All measures combined 78% (112/144) TFNA sensitivity 63% (90/144)	Hemoptysis in 4% (6/144) Chest- wall hematoma in 0.4% (1/144) Pneumothorax not given.
Conces [78]	USA	1989	76 patients with suspected pulmonary infection. (80 TFNAs)	Mean 49 years Male 67%	TFNA Blood	Culture Cytology Gram, Giemsa, Methenamine-silver, acid-fast, Auramine-rhodamine stain. Legionella direct fluorescent stain.	All measures combined 56% (45/80) TFNA sensitivity 52% (35/67)	Pneumothorax in 18% (14/80) , 4 patients needed drainage
Torres [79]	Spain	1990	41 children and adult patients: 23 CAP 18 nosocomial pneumonia, 8 of these mechanical ventilated	Mean 51 years Male 83%	TFNA Blood Pleural fluid Bronchio-alveolar lavage	Gram, Ziehl-Neelsen, Methenamine-silver staining Culture: Aerobe, anaerobe, fungal. Immunofluorecence against *L.pneumophila.* Serology: *L.pneumophila, M.pneumoniae, C. psittachi, C.burnettii*	All measures combined 73% (30/41) TFNA sensitivity 41% (18/41)	Pneumothorax in 7% (3/41), 2 patients needed drainage

Table III continued

Author	Country	Year	Population	Age and Sex	Methods	Microbiological assays	Efficacy of diagnostic measure	Adverse effects
Bella [80]	Spain	1993	18 adults with severe community acquired pneumonia.	Mean 59 years Male 78%	TFNA Blood Urine	Culture, Gram stain. Serology: *L.pneumophila, M.pneumoniae, C.burnettii* Pneumococcal capsular antigen test (urine) Pneumococcal and *H.influenzae* antisera (lung aspirate)	All measures combined 89 % (16/18) TFNA sensitivity 78 % (14/18)	Pneumothorax in 6% (1/18), no drainage needed
Chen [81]	Taiwan	1993	18 adults with pneumonia, 16 adults with lung abscesses	Mean 62 years Male 94%	TFNA	Culture Papanicolau, Giemsa, acid fast and Gram stain	All measures combined not given TFNA sensitivity 76% (26/34)	Pneumothorax in 3% (1/34), 1 patient needed drainage
Falguera [82]	Spain	1994	45 adult HIV patients with CAP (47 TFNA)	Mean 32 years Male 78%	TFNA Blood	Gram and Ziehl-Neelsen stain Culture: Bacterial, fungal, *M.pneumoniae* and viral culture. PCR: *P. Carinii*	All measures combined. 87% (41/47) TFNA sensitivity 62% (29/47)	Pneumothorax in17% (8/47) , 1 patient needed drainage.
Dorca [83]	Spain	1995	97 adult patients with nosocomial pneumonia	Mean 54 years Male 64%	TFNA Sputum Blood Pleural fluid Protected specimen brush	Culture Gram stain Immunofluorescence for Legionella sp. Serology*: L.pneumophila, M.pneumoniae*, *C. psitacci, C. burnetii.*	All measures combined 71% (69/97) TFNA sensitivity 43% (42/97)	Hemoptysis in 5% (5/97) Pneumothorax in 3% (3/97), no drainage needed

Table III continued

Author	Country	Year	Population	Age and Sex	Methods	Microbiological assays	Efficacy of diagnostic measure	Adverse effects
Zalacain [84]	Spain	1995	91 adult patients with pneumonia	Mean 61 years Male 82%	TFNA Sputum Blood BAL	Culture Gram stain, Ziehl-Neelsen Immunofluroescence for Legionella sp. Serology*: L.pneumophila, M.pneumoniae, C. burnetti.*	All measures combined not given TFNA sensitivity 34% (31/91)	Hemoptysis in 1/91 (1%) Pneumothorax in 4% (4/91), no drainage needed
Falguera [85]	Spain	1996	101 adults with CAP	Mean 41 years Sex not given	TFNA Blood Sputum Pleural fluid	*M.pneumoniae* serology and PCR. Culture	All measures combined 83% (77/93) TFNA sensitivity not given.	Hemoptysis in 8% (8/101) Pneumothorax in 2% (2/101), no drainage needed.
Torres [86]	Spain	1996	123 COPD with CAP	Mean 67 years Male 93%	TFNA (41patients) Sputum (97) Blood (123) Pleural fluid (14) Protected specimen brush (17)	Culture Gram stain Serology: Influenza A/B, parainfluenza, RSV, adeno-virus, *M.pneumoniae, C.psitacci, C.burnetti.* Indirect fluorescence *C.pneumoniae.* ELISA for *L.pneumophila*.	All measures combined. 64% (80/123) TFNA sensitivity not given.	Hemoptysis in 41% (5/123) Pneumothorax in 7% (3/41), no drainage needed
Ruiz-Gonzalez [87]	Spain	1997	110 patients with CAP	Age not given Sex not given	TFNA Blood	Culture Serology: *M.pneumoniae, C.pneumoniae, C.psitacci, L.pneumophila, C.burnettii,* Influenza A. Antisera for *S.pneumoniae* PCR for *S.pneumoniae*	All measures combined 85% (94/110) TNFA sensitivity 77% (85/110)	Hemoptysis in 4% (5/110) Pneumothorax in 3% (3/110), no drainage needed.

Table III continued

Author	Country	Year	Population	Age and Sex	Methods	Microbiological assays	Efficacy of diagnostic measure	Adverse effects
Garcia [88]	Spain	1999	95 adult patients hospitalized with CAP	Mean 65 years Males 73%	TFNA Sputum Blood Serum	Bacterial and fungal culture , PCR and latex agglutination for *S.pneumoniae*	All measures combined 60% (57/95) patients TFNA sensitivity 34% (32/95)	Not given
Ruiz-Gonzalez [89]	Spain	1999	109 adults with CAP	Mean 51 years Males 62%	TFNA Sputum Blood	Culture. Antigen test PCR *S.pneumoniae, M.pneumoniae*, *C.Pneumoniae, L.pneumophila.* Serology: *M.pneumoniae, C.pneumoniae,* Influensa A, RSV, adenovirus, *C.burnettii, C.psitacci* Gram, Ziehl-Neelsen stain	All measures combined 83 % (90 /109) of the patients TFNA sensitivity 51% (56/109)	Hemoptysis in 6% (7/109) Pneumothorax in 3% (3/109), no drainage needed
Ishida [90]	Japan	2001	60 adults with CAP	Mean 64 years Male 80%	TFNA Blood Sputum Pleural fluid Broncho-alveolar lavage Protected specimen brush	Culture (incl anaerobic, mycobacterium and legionella) Serology: *L.pneumophila, M.pneumoniae, C.pneumoniae, C.psittachi*, virus	All measures combined 80% (48/60) TFNA sensitivity 50% (30/60)	Pneumothorax in 5% (3/60), 1 patient needed drainage
Clark [91]	USA	2002	45 patients with infection or malignancy	Age not given Sex not given	TFNA Blood Broncho-alveolar lavage	Cytology Pap, silver and acid-fast stains	All measures combined 42 % (19/45) TFNA sensitivity 40% (18/45)	Not given

Table III continued

Author	Country	Year	Population	Age and Sex	Methods	Microbiological assays	Efficacy of diagnostic measure	Adverse effects
Vuori - Holopainen [92]	Finland	2002	34 children with CAP	Mean 5 years Male 59%	TFNA Blood NP swab Blood	Aerobic and viral culture. Gram staining PCR for *S.pneumoniae, C.pneumoniae, H.influenzae*, enterovirus, rhinovirus. Immunofluroescence Nasopharyngeal and blood culture	All measures combined 62% (21/34) TFNA sensitivity 59% (20/34)	Pneumothorax in 18% (6/34), no drainage needed Pneumo-mediastinum in 1 patient
Kumar [93]	India	2004	27 children with pneumonia while treated with chemo-therapy (30 TFNA)	Mean 5 years	TFNA Blood Naso-paryngeal swab Oro-pharyngeal swab	Bacterial and fungal culture Gram, Giemsa, Ziehl-Neelson, Toulidine blue stains	All measures combined not given TFNA sensitivity 53% (16/30)	Pneumothorax in 3% (1/30), no drainage needed
Thanos [94]	Greece	2004	48 patients	Mean 50 years Male 60%	TFNA Automated core needle biopsy	Culture Cytology Histology Stains	All measures combined 88 % (42/48) TFNA sensitivity 21% (10/48)	Hemoptysis in 2 patients Pneumothorax in 8 % (4/48), no drainage needed

Overview of published studies from 1975-2005 using TFNA in etiological diagnosis of pneumonia. Studies with less than three participants are excluded.

4.4. Infection and immunity in the elderly

Elderly individuals have a higher susceptibility to, and are at increased risk of dying from infections such as pneumonia, urinary tract infections and skin infections[95]. An atypical clinical presentation, such as lack of fever is a common feature of infections in the elderly [96], and the causative pathogens are more diverse in older age groups than in younger individuals. This emphasizes the importance of arriving at an etiological diagnosis and to initiate proper and timely treatment in patients aged > 65 years.

Aging of the immune system, also known as immunosenescence, is characterized by unfavorable alterations in numbers, types, receptor density and cytokine production of the immune cells [97-99]. However, biological age does not seem to be the only indication of an immunosenescent state. Individual genetic factors, frailty, persistent cytomegalovirus infection and comorbidities may all influence the risk of reduced immune function [99, 100]. In practical terms, immunosenescence is reflected by the fact that in at least 50-70% of the influenza vaccinated elderly above 75 years of age protection is not achieved against the actual influenza strains [101] and that the widely used 23-valent pneumococcal capsular polysaccharide vaccines is hardly of any use in this age group [102]. On the other hand, approximately 30% of the elderly display T-cell populations (CD8+CD45RO+CD25+) where the T-cell functions are at the same level as in younger adults, including retained ability to diversify and mount a sufficient immune response when infected[99].

The concept of inflamm-aging was proposed in 2000 by Franceschi et al[103], recognizing that factors other than age may have significant influence on the immune system. Inflamm-aging is a condition whereby chronic activation of the innate immune system leads to several specific changes such as increased levels of MK cells and interleukins. These alterations are beyond and above the effects of immunosenescence. Inflamm-aging is claimed to have a genetic and an environmental component, rendering a potential for improvement by environmental interventions [103].

The ability to mount a sufficient antibody response and sustain durable antibody levels above a certain threshold after vaccination is crucial for vaccine efficacy. Following vaccination, the early protective efficacy is primarily conferred by the induction of antigen-specific antibodies. Antibody avidity is another important factor of efficacy and long term protection requires the generation of immune memory cells capable of rapid and effective reactivation upon subsequent microbial exposure. The predominant role of B cells in the efficacy of current vaccines should not overshadow the importance of T cells responses: T cells are essential to the induction of high-affinity antibodies and immune memory effectors [104].

The outcome of booster vaccination is in part dependent on the level of pre-booster antibodies [101] [105]. Ideally, in order to obtain long term protection in the elderly, vaccination against novel pathogens should be performed in the pre-immunosenescent state, when an adequate antibody-response can be mounted[101]. However, such preemptive priming of the immune system would be particularly problematic against agents such as InfA virus, where frequent antigenic changes could render previously obtained protection useless. Novel approaches such as vaccines based on protein antigens shared by all InfA strains, increased dose of vaccine antigens, intradermal vaccinations and optimal adjuvants might improve protective immunity in the elderly[106-110]. In addition, alternative vaccination schedules, primarily an increased number of injections for the elderly, are being tested [111]. Today, apart from high age and documented immunodeficiency, there are no markers able to identify likely non-responders to immunization.

5. Status of knowledge before study initiation

Since the late 1990'ies, real time PCR has been frequently used for identification of respiratory pathogens. More recently 16S rDNA/rRNA PCR methods were introduced for the identification of unknown pathogens. Compared with traditional methods such as bacterial and viral cultures, these PCR techniques allow rapid and in some cases more sensitive pathogen identification.

At the time of study initiation, samples for investigating lower respiratory tract infections were harvested mainly by the use of bronchoscopic techniques, swabs or by the induced sputum method. Using these methods, there is a high risk of sample contamination by irrelevant oropharyngeal flora and therefore interpretation of the results could be difficult. During the 1990's several studies were published on the use of TFNA in combination with real time PCR to increase the diagnostic gain in cases of CAP [82, 87, 88, 92, 112, 113]. However, no studies had used 16S rDNA/rRNA PCR techniques together with TFNA.

For decades, nasopharyngeal and oropharyngeal swabbings to recover the infectious agents combined with paired blood samples for antibody analysis have been the conventional tools to obtain an etiological diagnosis in upper respiratory tract infections. For RVIs, the WHO guidelines recommended a combination of nasopharyngeal(NP) and oropharyngeal (OP) sampling [114]. Alternative sample harvesting procedures included nasal washes and aspiration, but these methods have been difficult to implement in frail elderly [32].

At the time of study initiation no age-specific harvesting recommendations for respiratory viruses were available. In addition, the number of publications on viral infections in elderly subjects was scarce, and in most cases studies were performed by the use of immune-fluorescence techniques, rapid serological tests or serum antibody measurements. Little was known on the incidence and importance of respiratory viral infections in older age groups and in particular, no published studies had compared the viral load in the OP and NP of elderly individuals using swabbed mucosal specimens.

At the time of study initiation, two structurally different swab types were available for viral and bacterial sampling; flocked swabs and rayon swabs. Theoretically, the type of swab could influence both the ability to retain pathogens at the actual anatomical site, and subsequently to release the pathogens from the swab for further analysis in the laboratory. No comparative studies on viral yield had previously been performed between these swabs.

Early diagnosis of influenza based on rapid laboratory tests had been shown to shorten the length of stay among hospitalized children and middle aged adults [115] [116]. Additionally, in children early diagnosis of influenza was found to decrease the use of antibiotics [115]. No such studies had been performed in elderly age groups.

6. Objectives

The main objectives of this thesis were to assess whether:

1. investigational trans-thoracic fine needle aspiration for the etiological diagnosis of pneumonia is feasible and safe in adults with confirmed CAP.
2. there is a difference in respiratory viral load between mucosal specimens collected from the nasopharynx and the oropharynx.
3. the two swab types differs in viral retaining capacity when investigating respiratory viral infections in the elderly
4. routine real time PCR for respiratory viruses influences length of stay and antibiotic treatment in hospitalized elderly.

7. Methods

7.1. Population

Aust-Agder County, located in southern Norway, has approximately 111 000 inhabitants. Approximately 67 % of the population is living in densely populated areas and 21 % are 60 years or older [117]. In Aust-Agder, the mean individual income after taxes was NOK 283 600 in 2001 [117].

Vest-Agder County has a population of 175 000 inhabitants. Approximately 81% of the inhabitants are living in densely populated areas, 20% are 60 years or older. In Vest-Agder, the mean individual income after taxes was NOK 281 300 in 2001 [117].

Paper I: The 20 study subjects were recruited from patients hospitalized with CAP at the Medical Department, Sorlandet Hospital Arendal HF. An average of 212 patients were admitted to the Medical Department each year in the period 2008-2013 with lower respiratory tract infections[118].

Paper II and IV: The 223 study subjects were recruited from patients aged above 60 years and admitted to the Medical Department of Sorlandet Hospital Arendal. Approximately 5714 patients are admitted each year to the Medical Department[118].

Paper III: The 32 study subjects were recruited from the population of patients testing positive for Influenza A or B at Sorlandet Hospital Arendal HF and Sorlandet Hospital Kristiansand HF during the study period.

7.2. Obtaining brush specimens for viral diagnosis

The following procedures were used when swabbing for respiratory viruses.

Oropharyngeal swab samples were collected from the tonsils or posterior OP area by applying medium pressure on the swab. A tongue depressor was used during the procedure to avoid touching the tongue with the swabs.

Nasopharyngeal swab samples were collected by carefully inserting the swab as far as possible into one nostril, rotate 360 degrees and then withdraw.

The patients were informed in advance that local irritation from the swab could result in sneezing or tear-formation. In patients with international normalized ratio (INR) above 3, NP samples were not harvested due to increased risk of nasal bleeding.

Table IV. Types of swabs used for viral specimen harvesting

	Oropharynx	**Nasopharynx**
Paper I	MW950[1,a]	None
Paper II and Paper IV	MW950[1,a] 502CS01 regular[2,b]	MW975 [1,a] 503CS01 nasopharyngeal[2,b]
Paper III	502CS01 regular[2,b]	503CS01 nasopharyngeal[2,b]

[1]Virocult, Medical Wire & Equipment, England,
[2]Copan Italia, Brescia, Italy
[a] Rayon swab
[b] Flocked swab

Immediately after the procedure, the rayon swabs were inserted into the Virocult medium and the tip of the tube squeezed as instructed by the manufacturer. The nylon flocked swabs were inserted into Universal Transport Medium (UTM) tubes (1.5 ml medium, no beads, Copan Italia) and the tubes were shaken for 10 seconds. Only one swab was used per transport tube, regardless of type of swab or location swabbed. The

tubes were stored at room temperature and transported to the microbiological laboratory within 48 hours.

The study subjects in paper I underwent mucosal swabbing in the oropharynx at the time of inclusion into the study (within 36 hours after hospitalization). A total of two OP swabs were used, one on each tonsillary area.

The study population of paper II and IV underwent mucosal swabbing the day after hospitalization. Four weeks later, all patients testing positive for a respiratory virus were retested using the same procedure. A total of four swabs were used per patient. The swabbing was performed in the following order: Right oropharyngeal area, left oropharyngeal area, left NP and right NP. The order of application of the two types of swabs used was decided by block randomization.

The patients included in paper III were swabbed on average (SD) 1.5 (±0.8) days after hospital admission. In this study, only patients testing positive for influenza virus at the time of hospital admission were eligible for inclusion. For each patient, one flocked swab was used in the NP and one flocked swab in the OP.

7.3. Viral load

Isolation of nucleic acids and the subsequent real time PCR examinations are described in details in paper I-III.

Viral load is the estimated viral concentration in a sample. The viral load is dynamic and varies throughout the course of the infection. Studies on influenza reveals variable viral load- and shedding patterns depending on the patients' age, comorbidities and vaccination status, as well as on influenza subtype. The viral concentration in a sample may be measured directly by the use of flow-cytometric methods, enzyme-linked immunosorbent assays (ELISA) or by the use of fluorescent assays or culture [119]. Another possibility is an indirect estimate by calculating the difference between two or more samples using cycle threshold (CT) values. During PCR amplification the amount of DNA is doubled in each cycle until the components in the reaction reaches a critical level. There is an exponential relationship between the initial amount of template DNA and the CT values. A high CT value represents a low microbial load in the specimen and vice versa. The differences in CT-values obtained from different samples included in the same experimental run demonstrate a relative difference in concentration between samples. Samples that differ by a factor of two in the original DNA concentration would be expected to be one cycle apart in the run, whereas samples that differ by a factor of ten would be approximately 3.3 cycles apart.

Viral load calculations by cycle threshold comparisons was our method of choice as it is precise, cost effective and can be performed locally.

7.4. Transthoracic fine needle aspiration

Transthoracic fine needle aspiration was used in paper I as a method for harvesting microbiological samples from the infectious area of the lung.

Routine chest x-ray upon admission and subsequent fluoroscopy guided the choice of a suitable site for the needle puncture. The puncture site was anaesthetised for one hour using a lidocain 25mg/prilocain 25 mg patch (Emla, AstraZeneca AB Södertälje, Sweden). The area was then washed aseptically with iodide solution and a sterile dressing was applied. A 22 gauge needle (Spinocan-R, B Braun Melsungen AB, Melsungen, Germany), stylet removed, was attached to a 10 ml syringe containing 5 ml isotonic saline.

Under fluoroscopic guidance the needle was introduced into the pulmonary infiltrate (common location 3-6 cm from lung surface) while the patient was breathing shallowly or briefly suspending breathing. Four ml of isotonic saline (sterile water was used in the first 6 cases) were injected into the area of pulmonary inflammation, leaving 1 ml as carrier fluid in the syringe to safeguard diagnostic minimum procedures even with sparse material. Negative pressure was applied while the needle was slowly retracted. Chest x-rays in both inspiration and expiration were taken 1-2 hours after the TFNA-procedure to detect possible pneumothorax or intrapulmonary bleeding. The procedure was performed within 36 hours of hospital admission, ongoing or previous antibiotic treatment were not a reason for exclusion.

7.5. Assessment of comorbidity

Multimorbidity is common in the geriatric population. To establish a robust measure of the study participants coexisting illnesses, two comorbidity scales were used in paper IV.

Charlsons comorbidity index (CCI), originally developed in 1984 as "taxonomy for comorbid conditions that might alter short term mortality" [120]. CCI is the single most used comorbidity scale in the world; however generalization based on CCI may be difficult due to the composition of the initial study population and the elapsed time since its scale development [121].

Cumulative illness rating scale (CIRS-G) was developed in the 1960's[122], and subsequently modified several times for geriatric use (CIRS-G) [121, 123, 124]. Initially CIRS were designed to assess physical impairment and as a tool for medical prognosis [122].

Whereas the CIRS-G is based on the rater's judgment of severity based on instructions from the accompanying manual, the CCI uses explicit criteria for rating. The definition of comorbid illnesses differs between the scales, resulting in different ratings for the same patients.

8. Statistics

In this thesis, SPSS 16.0 or higher were used for statistical analysis.

Throughout the papers, Students t-test was used when comparing continuous data, Chi-square when comparing categorical data.

In paper II, linear mixed model analysis was used when comparing calculated viral load in sampling sites and different swab types as the variables were not independent of each other.

In paper IV, Cox regression was used for length of stay analysis. CIRS-G domain scores were analyzed as categorical variables, whereas total CIRS-G and CCI scores were analyzed as continuous variables [121].

9. Synopsis of the papers

Paper I: Transthoracic fine-needle aspiration in the aetiological diagnosis of community-acquired pneumonia

Objectives:	To assess safety and feasibility of TFNA in CAP
Population:	20 patients with CAP admitted to Sorlandet Hospital Arendal HF; Norway.
Inclusion criteria:	1. Chest x-ray upon admission showed infiltrate suggestive of pneumonia and 2. One or more of the following findings: cough, fever > 38°C, productive sputum, dyspnoea or CRP > 50 g/L.
Exclusion criteria:	One or more of the following: Age <18 years, pregnancy, INR > 1.8 or platelets < 50 G/L, chronic lung disease with FEV1 < 50% of predicted value, large emphysematous bullae, pO2 on arterial blood gas below 8 kPa, inability to give informed consent or cooperate, incontrollable cough, patients in need of respiratory assistance. Patient unwilling to participate.

We conducted TFNA under fluoroscopic guidance on 20 CAP patients within 36 hours of hospitalization. The procedure was easy to perform and the discomfort to the patients was minimal. There were no serious adverse events. Two patients developed minor, self-limiting pneumothorax and seven patients reported pin-point sized haemoptysis.

The CAP-aetiology was considered definite if based on 1) a positive culture from lung aspirate or blood and/or 2) a positive result of PCR on lung-aspirate and/or 3) a significant serological rise in the IgM/IgG levels, according to the respective manufacturers' definition of a positive test. The aetiology was considered presumptive when 1) an established lung pathogen was found by sputum culture, and/or b) an established lung pathogen was detected by PCR on oropharyngeal swabs, and/or a positive antigen test for *L. pneumophila* or *S. pneumoniae* were obtained in the urine.

A definite aetiology for CAP was established in 12/20 (60%) of the patients; in 8/20 (40%) by TFNA alone. When including presumptive aetiologies we established an aetiological diagnosis in 14/20 (70 %) of the patients.

The main conclusion from this study is that TFNA is a safe sampling procedure. Combined with modern microbiological methods TFNA may provide important additional information on the aetiology of CAP.

Paper II: Swabbing for respiratory viral infections in older patients: a comparison of rayon and nylon flocked swabs

Objectives:	To compare the sampling efficacy of rayon swabs and nylon flocked swabs, and of oropharyngeal and nasopharyngeal specimens for the detection of respiratory viruses in elderly patients.
Population:	223 patients hospitalized at Sorlandet Hospital Arendal HF, Norway
Inclusion criteria:	1. Born 1948 or earlier (60 years or older). 2. At least one of the following symptoms with onset less than three weeks ago: Nasal congestion or runny nose, throat pain, fever, malaise, muscle pain, self-diagnosis of "the common cold", diarrhea or eye-infection combined with laboratory values supporting an infection.
Exclusion criteria:	INR 3.0 or more Patient (or next of kin on behalf of the patient) unwilling to participate.

In recent years, respiratory viruses have been established as significant causes of mortality and morbidity in the older population [36, 39, 125-127]. However, as different respiratory pathogens may cause similar clinical pictures etiological diagnosis depends on laboratory confirmation [128].

As demonstrated with RSV, viral detection is more demanding in the elderly than in young individuals, as older people tend to shed less virus and have shorter viral-shedding periods [129]. Hence, rapid viral antigen tests that are useful in children may fail in the elderly[66]. Due to its high sensitivity and specificity, real-time PCR on

respiratory viral samples represents an important diagnostic opportunity also in the older age groups.

Two structurally different swabs are available for microbial sampling in the upper airways: rayon swabs and nylon flocked swabs. A few studies comparing the respective efficacies of these two swabs in providing viral diagnostic specimens have been performed in children, but to our knowledge, no such studies have been conducted in patients 60 years or older. in the elderly.

Samples were obtained from patients 60 years of age or above, who were admitted within the last 24 hours to Sorlandet Hospital Arendal, Norway. The patients were interviewed for current symptoms of a respiratory tract infection after previously established criteria. Using rayon swabs and nylon flocked swabs comparable sets of mucosal samples were harvested from the nasopharynx and the oropharynx. The samples were analysed using real-time PCR methods for RSV, HMPV, InfA, InfB, Adenovirus and Parainfluenza virus 1-4. To achieve a more complete diagnostic coverage of respiratory pathogens, PCR assays were added for the detection of *M.pneumoniae*, *C.pneumoniae*, and *B.pertussis*.

223 patients (mean age 74,9 years, SD 9.0 years) were swabbed and a virus was recovered from 11% of the symptomatic patients. Regardless of sampling site, a calculated 4.8 times higher viral load (95% CI 1.3-17, p=0.017) was obtained using the nylon flocked swabs, as compared to the rayon swabs. Also, regardless of type of swab, a calculated 19 times higher viral load was found in the samples from the nasopharynx, as compared to the oropharynx (95 % CI 5.4-67.4, $p<0.001$).

We conclude that when swabbing for respiratory viruses in elderly patients nasopharyngeal rather than oropharyngeal samples should be obtained. Nylon flocked swabs appears to be more efficient than rayon swabs.

Paper III: A comparison of nasopharyngeal and oropharyngeal swabbing for the detection of influenza virus by real-time PCR

Objectives:	To assess the diagnostic yield of real time PCR for influenza virus infection following oropharyngeal as compared with nasopharyngeal swabbing.
Population:	32 patients admitted to Sorlandet Hospital Arendal HF and Sorlandet Hospital Kristiansand HF, Norway
Inclusion criteria:	Positive real-time PCR for influenza at the time of hospitalization. Patients above 18 years old.
Exclusion criteria:	INR 3.0 or more Patient or (next of kin on behalf of the patient) unwilling to participate.

During the local influenza season of 2009-2010 hospitalized patients who upon admission had tested positive for presence of influenza virus by real time PCR methods were prospectively asked to participate in this study. Using flocked swabs, oropharyngeal and nasopharyngeal samples were harvested from all patients as soon as possible and no later than 3 days after the initial detection of influenza virus. Thirty-two patients were included in the study, mean age 50.9 (± 22.8) years, median duration of symptoms prior to hospitalization 3.3 (±2.7) days. The patients were swabbed on average 1.5 (±0.8) days after hospital admission. Seventeen patients were diagnosed with Influenza B, fourteen patients with Influenza A (H1N1) and one patient with Influenza A(H3N2).

The influenza seasons 2009-2011 were dominated by influenza B and influenza A(H1N1)pdm2009. In general, nasopharyngeal samples were positive at a lower CT value than oropharyngeal samples (mean difference CT 5.75 (95% CI 3.8-7.7) $p<0.01$) rendering a calculated 54 times higher (95% CI 13.7- 210.8) viral concentration in the nasopharyngeal samples. Calculations based exclusively on influenza A H1N1 strains suggests a 23 (95% CI 3.8-136.2, $p<0.01$) times higher mean viral concentration in the nasopharynx than in the oropharynx. For influenza B the calculated difference in mean viral concentration was 80 (95% CI 9.3 -694.6, $p<0.01$) times higher.

We conclude that in patients with acute influenza nasopharyngeal swabbing is superior to oropharyngeal swabbing in terms of diagnostic yield as assessed by real time PCR.

Paper IV: No impact of early real time PCR screening for respiratory viruses on length of stay and use of antibiotics in elderly patients hospitalized with symptoms of a respiratory tract infection

Objectives:	To examine whether a positive real-time polymerase chain reaction test for respiratory viruses influenced factors such as length of hospitalization and antibiotic prescription in patients above 60 years of age.
Population:	223 patients hospitalized at Sorlandet Hospital Arendal HF, Norway. (Same as paper II)
Inclusion criteria:	1. Born 1948 or earlier (60 years or older) 2. At least one of the following symptoms with onset less than three weeks ago: Nasal congestion or runny nose, throat pain, fever, malaise, muscle pain, self-diagnosis of "the common cold", diarrhea or eye-infection combined with laboratory values supporting an infection.
Exclusion criteria:	INR 3.0 or more Patient (or next of kin on behalf of the patient) unwilling to participate.

The burden of diseases caused by RVIs among elderly both in the community and in hospitals is difficult to establish as symptoms often are nonspecific[130]. The high diagnostic sensitivity of real time PCR may be of special value in the elderly, who seem to be characterized by low viral shedding, at least in the case of RS-virus[129, 130]. In children, rapid tests for respiratory viruses have reduced the duration of

hospitalization, antibiotic prescription, and the cost of hospitalization [115, 131]. Similar findings have been observed in young and middle aged adults [116]. Limited data is available on the effect of improved microbiological diagnostics in the elderly population [132].

A total of 922 patients aged ≥ 60 years were interviewed for symptoms of an ongoing respiratory tract infection and 147 patients and the 56 controls were allocated into 1) symptomatic and PCR positive (S/PCR+); 2) symptomatic and PCR negative(S/PCR-); or 3) no symptoms and PCR negative (Control) dependent on symptoms and microbiological findings.

The frequency of antibiotic treatment during hospitalization did not differ between the S/PCR+ and S/PCR- symptomatic cohorts (X^2=0.49). In the S/PCR+ group sixteen out of nineteen patients received antibiotics during hospitalization whereas in the S/PCR- cohort 99 out of 128 received antibiotics. When the PCR results became available, only two patients in the S/PCR+ cohort had their antimicrobial treatment discontinued. Four additional patients had CRP values below 100 mg/L and negative chest x-rays, and hence, the likelihood of an ongoing bacterial infection was low. In the remaining ten patients, four had CRP values above 100 mg/L, two were diagnosed with chest x-ray findings consistent with pneumonia, and four patients presented with both findings. In those patients, the possibility of an ongoing bacterial infection was relatively high.

In the length-of-stay analysis, a significant difference was found between the two symptomatic cohorts and the controls (p<0.001), but no difference were observed between the S/PCR- and S/PCR+ cohorts (p=0.39). When comparing total CIRS-G scores and CCI scores, no difference between the three cohorts were found (p=0.29 and p=0.15 respectively). A significantly higher proportion of category four CIRS-G domain scores were found in the S/PCR- cohort (p=0.032) compared to the S/PCR+ cohort. When comparing the self-reported symptoms between the two symptomatic cohorts, a significant difference in self-reported symptoms between the two symptomatic cohort were found only for coughing (p=0.003) which was reported more often in the S/PCR+ than in the S/PCR- cohort. No significant difference between the

cohorts was found when comparing the number of days in intensive care and deaths during the next 12 months.

We conclude that in our general hospital setting, access to an early viral diagnosis had little impact on the antimicrobial treatment or length of hospitalization of elderly patients.

10. Discussion

This section will first discuss methodological aspects of performing the study and then our main findings. Finally, some ethical aspects of studying illness in acute ill elderly persons will be discussed.

10.1. Methodological aspects

10.1.1 Subjects included in the study The involved patients in this thesis were mainly recruited from hospitalized individuals at Sorlandet Hospital Arendal HF, although in paper III, sixteen patients from Sorlandet Hospital Kristiansand HF were included as well. Sorlandet Hospital Arendal HF is the only general hospital in Aust-Agder County, Sorlandet Hospital Kristiansand HF is one of two general hospitals in Vest-Agder County. In 2008, the number of hospitalization days per 1000 inhabitants was 175.9 in Aust-Agder, and 168.1 in Vest-Agder. For Norway as a whole, the corresponding figure was 171.8[133].

In paper I, approximately 13 percent of the then hospitalized patients with radiographically confirmed CAP during the study period were included in the investigation. The majority of the non-eligible patients suffered from cognitive impairment, used anticoagulants or was diagnosed with COPD. Patients with cognitive impairment and/or using anticoagulants might be more susceptible for CAP but the general belief is that the pathogens are similar to those found in this study. COPD patients treated with steroids or who frequently receive antibiotics may have increase susceptibility for uncommon CAP-pathogens. Throughout this study, inability to give informed consent was a reason for exclusion. As elderly patients more often suffer from delirium both from the infection and from hospitalization itself, the proportion of elderly patients included in this study differs from the general population of hospital admitted elderly patients with CAP[134]. This methodological challenge is difficult to approach as long as TFNA is viewed as an experimental diagnostic measure. Studies

have revealed that dementia increases the risk of acute organ dysfunction and sepsis [135], which might explain why patients in advanced stages of dementia frequently dies from pneumonia[136]. Our results are thus representative only to the group of cognitively preserved elderly.

In paper II and IV the study population is likely to be representative for the hospital population as a whole as the patients included were hospitalized for a variety of reasons. Paper III might represent a subgroup of the general population, as the included patients were already hospitalized for influenza infections.

We conclude that the subjects included in the current studies are representative of those eligible. As Norway is relatively uniform in terms of ethnicity and socioeconomic conditions, and the two involved hospitals are the only major hospitals in their respective counties, we expect the observations made in the present studies to be quite representative of the country at large.

10.1.2 Sampling

Viral sampling: The sampling procedures were performed by local technicians, after proper training. Care was taken to minimize the number of personnel included in sampling, in order to possibly reduce inadequate sampling techniques.

TFNA: This procedure was performed by one doctor only to avoid inter-personnel variation.

10.1.3 Analysis: The biochemical analyses were performed by the Laboratory Department at Sorlandet Hospital Arendal and the Department of Microbiology Sorlandet Hospital Kristiansand, both departments accredited by the International Organization for Standardization. The amplification efficiency of the PCR assays performed at St.Olavs Hospital was estimated to be above 95 %. The amplification efficiency of the PCR assays used in paper II was estimated to be above 94 % and in paper III to be above 98%. Hence, it may be concluded that the quality of the specimen sampling and their subsequent analysis were adequate.

10.2. Safety and feasibility of TFNA

Our knowledge of the microbiological flora in normal lungs and in lungs with ARIs is limited. The previous belief of a sterile lung below carina is now refuted and an increased focus has been put on the local microbial flora (microbiota) of the lower respiratory tract, both as immunomodulators and as pathogens [137-141].

Better sample harvesting techniques in combination with multiplex PCR or 16 S rDNA/rRNA improves the possibility of detecting microorganisms. Harvesting techniques, such as bronchoscopic procedures, increases the risk of irrelevant colonization from the oro/nasopharynx being transported to the lung parenchyma, thus leading to difficulties in distinguishing contaminants from pathogens[50]. This is especially important in CAP etiology research, where possibly irrelevant upper airway colonization especially by *H.influenza, M.catharrhalis* and *S.pneumoniae* could lead to an erroneous etiological conclusion [49, 51]. The type of colonization might be dependent on age, comorbidities and immune status; elderly patients have surprisingly low carriage rates of pneumococcus [51], but no thorough consensus has been reached on actual figures.

No studies have been performed comparing the microbiological yield between samples obtained by bronchoscopic procedures and lung aspirates in combination with novel PCR methods. TFNA and TTA are the only available sampling methods bypassing the oro/nasopharynx. With increasing microbial resistance to antibiotics, better diagnostic methods for CAP are in high demand. As shown in paper I, TFNA is a feasible and efficient method to obtain material for the etiological diagnosis of radiographically confirmed CAP. By combining conventional analyses of blood, urine and sputum with TFNA, the ability to etiologically diagnose a pulmonary infection increases from 50 to 80% [89]. In addition, mixed pulmonary infections including those involving anaerobes are more likely to be identified with this method. Although novel PCR methods such as 16 S rRNA/rDNA in theory increase diagnostic yield, in our experience the method is still not sufficiently sensitive at least as applied to lung aspirates. The amount of aspirate is small, and due to the intra-syringeal dilution of the

sample by the 1 ml sodium chloride carrier, the analysis is performed near the PCR's lower detection limit.

Our study has shown that TFNA is both safe and applicable also in our hospital setting, and should be considered in clinically difficult cases including treatment failure. The currently low level of antibiotic resistance in pathogens in Norway may imply that in our country, TFNA will be used mainly for research purposes. We suggest that new methods for the etiological diagnosis of CAP should be validated against clinical material recovered by TFNA.

10.3. Superiority of nasopharyngeal swabs

At the time of our viral studies, a combination of intra-nasal and throat swabs were the internationally recommended sampling methods[142]. In 2012, the WHO guidelines were altered to recognize the higher influenza viral load in nasopharyngeal samples by recommending the NP as the primary sampling site[143]. No recommendations are available with regard to swab type.

We found significant differences between sampling sites as well as between types of swabs, favoring nasopharyngeal swabbings and flocked swabs [144]. However, the optimal sampling site, swab type and specimen material seem to vary with age. In children, the sensitivity of nasopharyngeal aspirates and mid-turbinate intranasal swabs are comparable for identification of common respiratory viruses, RSV excluded [145, 146]. In patients aged 18-69, no difference between intranasal flocked swabs and nasopharyngeal swabs were found, making self-sampling a possibility in this age group [147]. To our knowledge, no studies in the elderly comparing mid-turbinate intranasal swabs and nasopharyngeal swabs have yet been published.

Our finding that in elderly patients, the viral concentration in samples harvested with flocked swabs was 4.8 times higher than in samples harvested with rayon swabs is of considerable interest. The fact that in the elderly, the viral load is lower than in younger individuals [148] may possibly enhance the difference between these swab types. This hypothesis is supported by a study in children with influenza A and B, where rayon swabs and flocked swabs used in the nasopharynx performed equally well[149]. By recommending flocked swabs only, one ensures the best possible sampling method for the whole population

The difference between the NP and OP in terms of concentrations of influenza virus is intriguing. Influenza-hemagglutinin binds with a sialic acid (SA) galactose (GAL) linkage; influenza A seem to prefer SA-alpha2,6-Gal linkage[150]. These receptors are mainly found on ciliated goblet cells distributed from the nasal cavity to the bronchi [151]. No studies have compared the receptor density between the OP and the NP where theoretically a higher density might be found. Age differences have been found

with regard to expression of different receptor types in the airways, but no such studies have been performed solely on the elderly[150]. Transfer serum IgG antibodies across mucous surfaces could possibly reduce the viral concentration, especially in the oropharynx [104]. In addition, other antiviral properties in saliva such as salivary agglutinin (gp340, DMBT1), lactoferrins, lysozymes and mucins could be partly responsible for the lower viral concentration in the oropharyngeal cavity [152]. Dilution and mechanical cleansing through food and drink might also contribute to the lower viral load of the OP as compared with the NP.

In paper II and IV the number of patients interviewed was appropriate, but unfortunately the number of patients with a positive real time PCR for the included viruses was relatively low. However, in spite of the low numbers, we found a significant difference in viral load both between the swab types and between the anatomical sites. In paper III, the patients were reswabbed a mean (SD) 1.5 ± 0.8 days after the initial swabbing. As expected, due to increasing viral clearance with time, some of the previous influenza virus positive patients tested negative at the time of the reswabbing.

Our results have implications for clinical practice. First, when harvesting samples for respiratory viral diagnostics, NP samples are superior. The difference in viral load between the sites is so large that throat swabs should be recommended only in extraordinary cases such as in patients with increased risk of bleeding or with anatomical malformations. Swab type has an impact on viral load, and flocked swabs retain more virus than rayon swabs and should be preferred over rayon swabs.

Secondly, routine testing for respiratory viruses are not recommended in elderly patients, as apparently, there is no impact on duration of stay or antibiotic use. Routine testing, during the influenza season is one exception, as available antiviral therapy is recommended in influenza patients with serious illness, regardless of age.

10.4. Ethical consideration

The studies in this thesis were approved by the Regional Committee for Medical and Health Research Ethics (REK SØRØST).

Elderly individuals are in general positive to study inclusion [153], but are systematically underrepresented in clinical trials [154-156]. Non-inclusion of elderly study-participants is problematic, as age-associated physiological and biological alterations make extrapolation of scientific results between age groups difficult [157]. Comorbidities are a major reason for exclusion from clinical trials [158]; most patients above 70 years of age suffer from at least two comorbid conditions [159]. In order to accommodate for the heterogeneity in terms of general health, comorbidities and the possibility of death from non-study related causes in studies with mainly elderly participants, elderly study populations should in general be larger than corresponding populations of younger participants[160].

Variable ability to provide informed consent is a challenge when conducting research in the elderly [161]. Acute confusional states are common in hospitalized elderly populations [162] and these states are in some cases difficult to diagnose [163]. Non-acknowledged acute confusion or cognitive impairment could result in inaccurate self-reported patient data and ethically invalid informed consents.
In accordance with the Norwegian Law of Medical Research, research involving vulnerable patients groups should only be performed where the risk of the procedure for the patients is minimal and the person does not object to it[164]. Furthermore, acquisition of the desired scientific data should be impossible through studies of less vulnerable patient groups[164]. In this thesis, ethical considerations were important both in study planning and execution. Immunosenescence renders the elderly particularly vulnerable to infections, and hence, scientific data acquired from studies in younger individuals may be less relevant for senior citizens.

In this thesis, the level of cognitive impairment acceptable for inclusion varies with the different studies. TFNA is an invasive procedure, where the risk of pneumothorax is small, but still present. Thus, only patients able to give valid informed consent were included in the study. To ensure the validity of the consent, the patients were approached twice by a physician experienced in diagnosis of cognitive disorders. In addition, the attending nurses were interviewed and charts were reviewed for signs of confusion or cognitive impairment in the patient during hospitalization. For the studies involving NP and OP swabbing, the procedure itself is not harmful. The patients were approached by study technicians experienced in diagnosing cognitive impairment, and in cases where doubts of the patients' cognitive status, next of kin were asked to provide informed consent on behalf of the patient before sample harvesting. If the patients orally or by body language signaled unwillingness to participate, sample harvesting were not performed.

11. Perspectives

There is worldwide concern over increasing bacterial antibiotic resistance resulting mainly from unnecessary use of broad-spectrum antimicrobial drugs. Antibiotic treatment guided by exact etiological information would significantly reduce use of inappropriate antibiotics. Mixed infections are probably more common than realized, and improved diagnostics could reduce this probably underestimated cause of treatment failures. Respiratory viral infections are frequent, and in the elderly viral infections might be overlooked due to atypical presentations. Some of these, notably influenza, can now be successfully treated by specifically designed drugs.

Lately, the scientific community has shown increasing interest in the microbiome of the human body, including the lower airways[165]. Sampling of these microorganisms in the lungs by bronchoschopic methods is hampered by the risk of microbial contamination from the upper airways. To our knowledge no study has compared the feasibility of TNFA to that of bronchoalveolar lavage or protected sterile brush for mapping of the pulmonary microbiome.

Open lung biopsy is the gold standard for etiological diagnosis of CAP, but ethical as well as medical considerations make this approach impossible in daily practice. Bridging the gap between the gold standard and the diagnostics methods in ordinary use, TFNA might have an important place in validation of new diagnostic tools. This is especially important when introducing new genetic assays, such as PCR targeting spn9802 or lytA in CAP [166, 167]. The dynamic nature of the microbiological flora and of drug resistance necessitates improved diagnostic tools for the etiology of CAP.

For respiratory viral infections, little is known about the incidence in the oldest patients, mostly because adequate diagnostic measures are rarely applied. Thus, increased epidemiological research is important, especially in care facilities. Within the next few years, a RS virus and probably also an adenovirus vaccine may become widely available. The prevalence and impact of RS and adenovirus infections in elderly patients should be assessed prior to the introduction of these vaccines in Norway.

12. Conclusions

1. TFNA was found to be feasible and safe in a Norwegian clinical research setting.

2. Nasopharyngeal specimens contained a higher viral load than specimens obtained from the oropharynx for the respiratory viruses in question.

3. Flocked swabs retained more virus than fiber swabs when harvesting samples for respiratory virus detection in the elderly.

4. Routine viral real time PCR in hospital admitted patients does not necessarily alter length of stay or antibiotic treatment in hospitalized patients.

13. Erratum

Paper II: The data for the study population are erroneous described in paper II. A total of 147 out of 173 symptomatic patients and 56 non-symptomatic patients (controls) agreed to participate in the study. This had no impact on the results of the study.

14. References

[1] Polverino E, Torres Marti A (2011) Community-acquired pneumonia. Minerva Anestesiol 77 (2):196-211

[2] Marrie TJ (2000) Community-acquired pneumonia in the elderly. Clin InfectDis 31 (4):1066-1078

[3] File TM, Jr. (2004) Streptococcus pneumoniae and community-acquired pneumonia: a cause for concern. Am J Med 117 Suppl 3A:39S-50S

[4] Bartlett JG (2011) Diagnostic tests for agents of community-acquired pneumonia. Clin Infect Dis 52 Suppl 4:S296-304

[5] Moberley S, Holden J, Tatham DP, Andrews RM (2013) Vaccines for preventing pneumococcal infection in adults. Cochrane database of systematic reviews 1:CD000422

[6] Jefferson T, Di Pietrantonj C, Al-Ansary LA, Ferroni E, Thorning S, Thomas RE (2010) Vaccines for preventing influenza in the elderly. Cochrane database of systematic reviews (2):CD004876

[7] 2010 NNV (2011) Usage of Antimicrobial Agents and Occurrence of AntimicrobialResistance in Norway., Tromsø/Oslo

[8] Woodhead M, Blasi F, Ewig S, Garau J, Huchon G, Ieven M, Ortqvist A, Schaberg T, Torres A, van der Heijden G, Read R, Verheij TJ, Joint Taskforce of the European Respiratory S, European Society for Clinical M, Infectious D (2011) Guidelines for the management of adult lower respiratory tract infections--full version. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 17 Suppl 6:E1-59

[9] Haug JBea (2013) Nasjonal faglig retningslinje for bruk av antibiotika i sykehus, https://sites.google.com/site/abretlininnspill/home/detaljert-innholdsoversikt/administrative-og-metodekapitler/a02-redaksjon-og-arbeidsgrupper2013

[10] Klugman KP, Low DE, Metlay J, Pechere JC, Weiss K (2004) Community-acquired pneumonia: new management strategies for evolving pathogens and antimicrobial susceptibilities. IntJAntimicrobAgents 24 (5):411-422

[11] Wroe PC, Finkelstein JA, Ray GT, Linder JA, Johnson KM, Rifas-Shiman S, Moore MR, Huang SS (2012) Aging population and future burden of pneumococcal pneumonia in the United States. J Infect Dis 205 (10):1589-1592

[12] Health NIoP (2012) Norwegian Cause of Death statistics 2006-2010, www.norgeshelsa.no2012

[13] Geneve W (2011) CAUSES OF DEATH 2008 SUMMARY TABLES, http://apps.who.int/ghodata/?vid=10012

[14] WHO (2011) The top ten causes of death, http://www.who.int/mediacentre/factsheets/fs310/en/index.html. Cited 23.08.12 2012

[15] Stralin K (2008) Usefulness of aetiological tests for guiding antibiotic therapy in community-acquired pneumonia. Int J Antimicrob Agents 31 (1):3-11

[16] File TM, Jr. (2011) New diagnostic tests for pneumonia: what is their role in clinical practice? Clin Chest Med 32 (3):417-430

[17] Cornick JE, Bentley SD (2012) Streptococcus pneumoniae: the evolution of antimicrobial resistance to beta-lactams, fluoroquinolones and macrolides. Microbes Infect

[18] Linares J, Ardanuy C, Pallares R, Fenoll A (2010) Changes in antimicrobial resistance, serotypes and genotypes in Streptococcus pneumoniae over a 30-year period. Clinical

microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 16 (5):402-410
[19] Cilloniz C, Ewig S, Polverino E, Marcos MA, Esquinas C, Gabarrus A, Mensa J, Torres A (2011) Microbial aetiology of community-acquired pneumonia and its relation to severity. Thorax 66 (4):340-346
[20] Robert R, Grollier G, Frat JP, Godet C, Adoun M, Fauchere JL, Dore P (2003) Colonization of lower respiratory tract with anaerobic bacteria in mechanically ventilated patients. Intensive Care Med 29 (7):1062-1068
[21] Agvald-Ohman C, Wernerman J, Nord CE, Edlund C (2003) Anaerobic bacteria commonly colonize the lower airways of intubated ICU patients. ClinMicrobiolInfect 9 (5):397-405
[22] Ries K, Levison ME, Kaye D (1974) Transtracheal aspiration in pulmonary infection. ArchInternMed 133 (3):453-458
[23] Pollock HM, Hawkins EL, Bonner JR, Sparkman T, Bass JB, Jr. (1983) Diagnosis of bacterial pulmonary infections with quantitative protected catheter cultures obtained during bronchoscopy. JClinMicrobiol 17 (2):255-259
[24] Schreiner A, Digranes A, Myking O (1972) Transtracheal aspiration in the diagnosis of lower respiratory tract infections. Scandinavian journal of infectious diseases 4 (1):49-52
[25] Schreiner A, Digranes A, Myking O, Solberg CO (1973) [Value of transtracheal aspiration in bronchopulmonary infections in adults]. Infection 1 (3):137-143
[26] Bjerkestrand G, Digranes A, Schreiner A (1975) Bacteriological findings in transtracheal aspirates from patients with chronic bronchitis and bronchiectasis: a preliminary report. Scand J Respir Dis 56 (4):201-207
[27] Schreiner A, Bjerkestrand G, Digranes A, Halvorsen FJ, Kommedal TM (1978) Bacteriological findings in the transtracheal aspirate from patients with acute exacerbation of chronic bronchitis. Infection 6 (2):54-56
[28] Schreiner A, Diganes, A. (1979) Bacteriology of Acute Lower Respiratory Tract Infections. J Infect 1 (Suppl 2)
[29] Schreiner A (1979) Anaerobic pulmonary infections. Scand J Infect Dis Suppl (19):77-79
[30] Melbye H, Berdal BP, Straume B, Russell H, Vorland L, Thacker WL (1992) Pneumonia--a clinical or radiographic diagnosis? Etiology and clinical features of lower respiratory tract infection in adults in general practice. Scandinavian journal of infectious diseases 24 (5):647-655
[31] Ragnar Norrby S (1997) Atypical pneumonia in the Nordic countries: aetiology and clinical results of a trial comparing fleroxacin and doxycycline. Nordic Atypical Pneumonia Study Group. J Antimicrob Chemother 39 (4):499-508
[32] Falsey AR, Walsh EE (2006) Viral pneumonia in older adults. Clin InfectDis 42 (4):518-524
[33] Ellis SE, Coffey CS, Mitchel EF, Jr., Dittus RS, Griffin MR (2003) Influenza- and respiratory syncytial virus-associated morbidity and mortality in the nursing home population. J AmGeriatrSoc 51 (6):761-767
[34] Falsey AR, Dallal GE, Formica MA, Andolina GG, Hamer DH, Leka LL, Meydani SN (2008) Long-term care facilities: a cornucopia of viral pathogens. J AmGeriatrSoc 56 (7):1281-1285
[35] Loeb M, McGeer A, McArthur M, Peeling RW, Petric M, Simor AE (2000) Surveillance for outbreaks of respiratory tract infections in nursing homes. CMAJ 162 (8):1133-1137
[36] Boivin G, de SG, Hamelin ME, Cote S, Argouin M, Tremblay G, Maranda-Aubut R, Sauvageau C, Ouakki M, Boulianne N, Couture C (2007) An outbreak of severe respiratory

tract infection due to human metapneumovirus in a long-term care facility. Clin InfectDis 44 (9):1152-1158
[37] Hui DS, Woo J, Hui E, Foo A, Ip M, To KW, Cheuk ES, Lam WY, Sham A, Chan PK (2008) Influenza-like illness in residential care homes: a study of the incidence, aetiological agents, natural history and health resource utilisation. Thorax 63 (8):690-697
[38] Louie JK, Yagi S, Nelson FA, Kiang D, Glaser CA, Rosenberg J, Cahill CK, Schnurr DP (2005) Rhinovirus outbreak in a long term care facility for elderly persons associated with unusually high mortality. Clin InfectDis 41 (2):262-265
[39] Louie JK, Schnurr DP, Pan CY, Kiang D, Carter C, Tougaw S, Ventura J, Norman A, Belmusto V, Rosenberg J, Trochet G (2007) A summer outbreak of human metapneumovirus infection in a long-term-care facility. J InfectDis 196 (5):705-708
[40] folkehelseinstitutt N (2010) Influensa-faktaark. Elektronisk publikasjon, http://www.fhi.no/artikler/?id=51012. Cited 03.05. 2012
[41] Lemaitre M, Carrat F (2010) Comparative age distribution of influenza morbidity and mortality during seasonal influenza epidemics and the 2009 H1N1 pandemic. BMCInfectDis 10:162
[42] Weinberger B, Herndler-Brandstetter D, Schwanninger A, Weiskopf D, Grubeck-Loebenstein B (2008) Biology of immune responses to vaccines in elderly persons. Clin Infect Dis 46 (7):1078-1084
[43] Osterholm MT, Kelley NS, Sommer A, Belongia EA (2012) Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. The Lancet infectious diseases 12 (1):36-44
[44] research WIfv (2009) Acute respiratory infections, http://www.who.int/vaccine_research/diseases/ari/en/index2.html
[45] Armed Forces Health Surveillance C (2013) Incidence of acute respiratory illnesses among enlisted service members during their first year of military service: Did the 2011 resumption of adenovirus vaccination of basic trainees have an effect? Msmr 20 (5):14-18
[46] Diseases NFfI (2013) New and Emerging Vaccines for Respiratory Infections, https://custom.cvent.com/02EBD9C538FD49B7B1B863AD5F02EB85/files/b6092a8c88df4406a8fa2e22f95b39df.pdf2013
[47] Health NIoP (2012) Weekly influenza surveillance reports, www.fhi.no2012
[48] Norway S (2013) Influenza deaths in Norway, www.ssb.no/statistikkbanken
[49] Stralin K, Korsgaard J, Olcen P (2006) Evaluation of a multiplex PCR for bacterial pathogens applied to bronchoalveolar lavage. EurRespirJ 28 (3):568-575
[50] Kirkpatrick MB, Bass JB, Jr. (1989) Quantitative bacterial cultures of bronchoalveolar lavage fluids and protected brush catheter specimens from normal subjects. Am Rev Respir Dis 139 (2):546-548
[51] Garcia-Rodriguez JA, Fresnadillo Martinez MJ (2002) Dynamics of nasopharyngeal colonization by potential respiratory pathogens. J AntimicrobChemother 50 Suppl S2:59-73
[52] Scott JA, Hall AJ (1999) The value and complications of percutaneous transthoracic lung aspiration for the etiologic diagnosis of community-acquired pneumonia. Chest 116 (6):1716-1732
[53] Gutierrez F, Masia M, Rodriguez JC, Ayelo A, Soldan B, Cebrian L, Mirete C, Royo G, Hidalgo AM (2003) 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 InfectDis 36 (3):286-292
[54] Waterer GW, Baselski VS, Wunderink RG (2001) Legionella and community-acquired pneumonia: a review of current diagnostic tests from a clinician's viewpoint. Am J Med 110 (1):41-48

[55] Kohler RB, Winn WC, Jr., Wheat LJ (1984) Onset and duration of urinary antigen excretion in Legionnaires disease. J Clin Microbiol 20 (4):605-607
[56] Andreo F, Prat C, Ruiz-Manzano J, Lores L, Blanco S, Cuesta MA, Gimenez M, Dominguez J (2009) Persistence of Streptococcus pneumoniae urinary antigen excretion after pneumococcal pneumonia. Eur J Clin Microbiol Infect Dis 28 (2):197-201
[57] Leyden I (1883) Verhandlungen des Vereins for innere Medicin: Ueber infectiose penumonie. Deutsc Med Wschr 9:52-54
[58] Vuori-Holopainen E, Peltola H (2001) Reappraisal of lung tap: review of an old method for better etiologic diagnosis of childhood pneumonia. ClinInfectDis 32 (5):715-726
[59] Pecora DV (1959) A method of securing uncontaminated tracheal secretions for bacterial examination. The Journal of thoracic surgery 37 (5):653-654
[60] Kalinske RW, Parker RH, Brandt D, Hoeprich PD (1967) Diagnostic usefulness and safety of transtracheal aspiration. N Engl J Med 276 (11):604-608
[61] Manresa F, Dorca J (1991) Needle aspiration techniques in the diagnosis of pneumonia. Thorax 46 (8):601-603
[62] Bartlett JG, Rosenblatt JE, Finegold SM (1973) Percutaneous transtracheal aspiration in the diagnosis of anaerobic pulmonary infection. Ann Intern Med 79 (4):535-540
[63] Davidson M, Tempest B, Palmer DL (1976) Bacteriologic diagnosis of acute pneumonia. Comparison of sputum, transtracheal aspirates, and lung aspirates. JAMA 235 (2):158-163
[64] Hahn HH, Beaty HN (1970) Transtracheal aspiration in the evaluation of patients with pneumonia. Ann Intern Med 72 (2):183-187
[65] Pecora DV, Yegian D (1958) Bacteriology of the lower respiratory tract in health and chronic diseases. N Engl J Med 258 (2):71-74
[66] Casiano-Colon AE, Hulbert BB, Mayer TK, Walsh EE, Falsey AR (2003) Lack of sensitivity of rapid antigen tests for the diagnosis of respiratory syncytial virus infection in adults. J Clin Virol 28 (2):169-174
[67] Talbot HK, Williams JV, Zhu Y, Poehling KA, Griffin MR, Edwards KM (2010) Failure of routine diagnostic methods to detect influenza in hospitalized older adults. Infect Control Hosp Epidemiol 31 (7):683-688
[68] Monto AS, Rotthoff J, Teich E, Herlocher ML, Truscon R, Yen HL, Elias S, Ohmit SE (2004) Detection and control of influenza outbreaks in well-vaccinated nursing home populations. Clin Infect Dis 39 (4):459-464
[69] Roson B, Carratala J, Verdaguer R, Dorca J, Manresa F, Gudiol F (2000) Prospective study of the usefulness of sputum Gram stain in the initial approach to community-acquired pneumonia requiring hospitalization. Clin Infect Dis 31 (4):869-874
[70] Roson B, Fernandez-Sabe N, Carratala J, Verdaguer R, Dorca J, Manresa F, Gudiol F (2004) Contribution of a urinary antigen assay (Binax NOW) to the early diagnosis of pneumococcal pneumonia. Clin Infect Dis 38 (2):222-226
[71] Smith MD, Derrington P, Evans R, Creek M, Morris R, Dance DA, Cartwright K (2003) Rapid diagnosis of bacteremic pneumococcal infections in adults by using the Binax NOW Streptococcus pneumoniae urinary antigen test: a prospective, controlled clinical evaluation. JClinMicrobiol 41 (7):2810-2813
[72] Bernander S, Gastrin B, Lofgren S, Olinder-Nielsen AM (1994) Legionella urinary antigen in early disease. Scandinavian journal of infectious diseases 26 (6):777-778
[73] Winther FO, Horthe K, Lystad A, Vellar OD (1974) Pathogenic bacterial flora in the upper respiratory tract of healthy students. Prevalence and relationship to nasopharyngeal inflammatory symptoms. J Laryngol Otol 88 (5):407-412

[74] Castellino RA, Blank N (1979) Etiologic diagnosis of focal pulmonary infection in immunocompromised patients by fluoroscopically guided percutaneous needle aspiration. Radiology 132 (3):563-567
[75] Zavala DC, Schoell JE (1981) Ultrathin needle aspiration of the lung in infectious and malignant disease. AmRevRespirDis 123 (1):125-131
[76] Wallace JM, Batra P, Gong H, Jr., Ovenfors CO (1985) Percutaneous needle lung aspiration for diagnosing pneumonitis in the patient with acquired immunodeficiency syndrome (AIDS). Am Rev Respir Dis 131 (3):389-392
[77] Barnes DJ, Naraqi S, Igo JD (1988) The role of percutaneous lung aspiration in the bacteriological diagnosis of pneumonia in adults. Aust N Z J Med 18 (6):754-757
[78] Conces DJ, Jr., Clark SA, Tarver RD, Schwenk GR (1989) Transthoracic aspiration needle biopsy: value in the diagnosis of pulmonary infections. AJR Am J Roentgenol 152 (1):31-34
[79] Torres A, Jimenez P, Puig de la BJ, Celis R, Gonzalez J, Gea J (1990) Diagnostic value of nonfluoroscopic percutaneous lung needle aspiration in patients with pneumonia. Chest 98 (4):840-844
[80] Bella F, Tort J, Morera MA, Espaulella J, Armengol J (1993) Value of bacterial antigen detection in the diagnostic yield of transthoracic needle aspiration in severe community acquired pneumonia. Thorax 48 (12):1227-1229
[81] Chen CH, Kuo ML, Shih JF, Chang TP, Perng RP (1993) Etiologic diagnosis of pulmonary infection by ultrasonically guided percutaneous lung aspiration. Zhonghua yi xue za zhi = Chinese medical journal; Free China ed 51 (5):333-339
[82] Falguera M, Nogues A, Ruiz-Gonzalez A, Garcia M, Puig T, Rubio-Caballero M (1994) Transthoracic needle aspiration in the study of pulmonary infections in patients with HIV. Chest 106 (3):697-702
[83] Dorca J, Manresa F, Esteban L, Barreiro B, Prats E, Ariza J, Verdaguer R, Gudiol F (1995) Efficacy, safety, and therapeutic relevance of transthoracic aspiration with ultrathin needle in nonventilated nosocomial pneumonia. AmJRespirCrit Care Med 151 (5):1491-1496
[84] Zalacain R, Llorente JL, Gaztelurrutia L, Pijoan JI, Sobradillo V (1995) Influence of three factors on the diagnostic effectiveness of transthoracic needle aspiration in pneumonia. Chest 107 (1):96-100
[85] Falguera M, Nogues A, Ruiz-Gonzalez A, Garcia M, Puig T (1996) Detection of Mycoplasma pneumoniae by polymerase chain reaction in lung aspirates from patients with community-acquired pneumonia. Chest 110 (4):972-976
[86] Torres A, Dorca J, Zalacain R, Bello S, El-Ebiary M, Molinos L, Arevalo M, Blanquer J, Celis R, Iriberri M, Prats E, Fernandez R, Irigaray R, Serra J (1996) Community-acquired pneumonia in chronic obstructive pulmonary disease: a Spanish multicenter study. AmJRespirCrit Care Med 154 (5):1456-1461
[87] Ruiz-Gonzalez A, Nogues A, Falguera M, Porcel JM, Huelin E, Rubio-Caballero M (1997) Rapid detection of pneumococcal antigen in lung aspirates: comparison with culture and PCR technique. RespirMed 91 (4):201-206
[88] Garcia A, Roson B, Perez JL, Verdaguer R, Dorca J, Carratala J, Casanova A, Manresa F, Gudiol F (1999) Usefulness of PCR and antigen latex agglutination test with samples obtained by transthoracic needle aspiration for diagnosis of pneumococcal pneumonia. JClinMicrobiol 37 (3):709-714
[89] Ruiz-Gonzalez A, Falguera M, Nogues A, Rubio-Caballero M (1999) Is Streptococcus pneumoniae the leading cause of pneumonia of unknown etiology? A microbiologic study of lung aspirates in consecutive patients with community-acquired pneumonia. AmJMed 106 (4):385-390

[90] Ishida T, Hashimoto T, Arita M, Osawa M, Tachibana H, Nishioka M, Ito I (2001) Efficacy of transthoracic needle aspiration in community-acquired pneumonia. InternMed 40 (9):873-877
[91] Clark BD, Vezza PR, Copeland C, Wilder AM, Abati A (2002) Diagnostic sensitivity of bronchoalveolar lavage versus lung fine needle aspirate. Mod Pathol 15 (12):1259-1265
[92] Vuori-Holopainen E, Salo E, Saxen H, Hedman K, Hyypia T, Lahdenpera R, Leinonen M, Tarkka E, Vaara M, Peltola H (2002) Etiological diagnosis of childhood pneumonia by use of transthoracic needle aspiration and modern microbiological methods. ClinInfectDis 34 (5):583-590
[93] Kumar KG, Bakhshi S, Samantaray JC, Banerjee U, Arya LS (2004) Transthoracic lung aspiration in etiology of pneumonia. Indian JPediatr 71 (2):129-132
[94] Thanos L, Galani P, Mylona S, Pomoni M, Mpatakis N (2004) Percutaneous CT-guided core needle biopsy versus fine needle aspiration in diagnosing pneumonia and mimics of pneumonia. Cardiovasc Intervent Radiol 27 (4):329-334
[95] Yoshikawa TT (1981) Important infections in elderly persons. West J Med 135 (6):441-445
[96] Norman DC (2000) Fever in the elderly. Clin Infect Dis 31 (1):148-151
[97] Gomez CR, Nomellini V, Faunce DE, Kovacs EJ (2008) Innate immunity and aging. Exp Gerontol 43 (8):718-728
[98] Shaw AC, Joshi S, Greenwood H, Panda A, Lord JM (2010) Aging of the innate immune system. CurrOpinImmunol 22 (4):507-513
[99] Weiskopf D, Weinberger B, Grubeck-Loebenstein B (2009) The aging of the immune system. TransplInt 22 (11):1041-1050
[100] Yao X, Li H, Leng SX (2011) Inflammation and immune system alterations in frailty. Clin Geriatr Med 27 (1):79-87
[101] Weinberger B, Herndler-Brandstetter D, Schwanninger A, Weiskopf D, Grubeck-Loebenstein B (2008) Biology of immune responses to vaccines in elderly persons. ClinInfectDis 46 (7):1078-1084
[102] World Health O (2012) Pneumococcal vaccines, WHO position paper–2012. Wkly Epidemiol Rec 87:129-144
[103] Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908:244-254
[104] C-A. S (2008) Vaccine Immunology.In: Plotkin SA, Orenstein WA, Offit P, eds. Vaccines, 5th ed. Philadelphia, PA. WB Saunders Company, 2008: 258-290.
. In: Plotkin SA OW, Offit P, eds. (ed) Vaccines, 5th ed. WB Saunders Company, 2008: 258-290, Philadelphia, PA..
[105] Kaml M, Weiskirchner I, Keller M, Luft T, Hoster E, Hasford J, Young L, Bartlett B, Neuner C, Fischer KH, Neuman B, Wurzner R, Grubeck-Loebenstein B (2006) Booster vaccination in the elderly: their success depends on the vaccine type applied earlier in life as well as on pre-vaccination antibody titers. Vaccine 24 (47-48):6808-6811
[106] Keitel WA, Atmar RL, Cate TR, Petersen NJ, Greenberg SB, Ruben F, Couch RB (2006) Safety of high doses of influenza vaccine and effect on antibody responses in elderly persons. Arch Intern Med 166 (10):1121-1127
[107] Holland D, Booy R, De Looze F, Eizenberg P, McDonald J, Karrasch J, McKeirnan M, Salem H, Mills G, Reid J, Weber F, Saville M (2008) Intradermal influenza vaccine administered using a new microinjection system produces superior immunogenicity in elderly adults: a randomized controlled trial. J Infect Dis 198 (5):650-658

[108] Mannino S, Villa M, Apolone G, Weiss NS, Groth N, Aquino I, Boldori L, Caramaschi F, Gattinoni A, Malchiodi G, Rothman KJ (2012) Effectiveness of adjuvanted influenza vaccination in elderly subjects in northern Italy. Am J Epidemiol 176 (6):527-533
[109] Gilbert SC (2012) Advances in the development of universal influenza vaccines. Influenza and other respiratory viruses
[110] McElhaney JE, Zhou X, Talbot HK, Soethout E, Bleackley RC, Granville DJ, Pawelec G (2012) The unmet need in the elderly: how immunosenescence, CMV infection, co-morbidities and frailty are a challenge for the development of more effective influenza vaccines. Vaccine 30 (12):2060-2067
[111] Weinberger B, Grubeck-Loebenstein B (2012) Vaccines for the elderly. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 18 Suppl 5:100-108
[112] Falguera M, Lopez A, Nogues A, Porcel JM, Rubio-Caballero M (2002) Evaluation of the polymerase chain reaction method for detection of Streptococcus pneumoniae DNA in pleural fluid samples. Chest 122 (6):2212-2216
[113] Vuori-Holopainen E, Salo E, Saxen H, Vaara M, Tarkka E, Peltola H (2001) Clinical "pneumococcal pneumonia" due to Moraxella osloensis: case report and a review. ScandJInfectDis 33 (8):625-627
[114] WHO (2011) WHO information for molecular diagnosisn in humans update, http://www.who.int/influenza/resources/documents/molecular_diagnosis_influenza_virus_humans_update_201108.pdf. Cited 23.08 2012
[115] Bonner AB, Monroe KW, Talley LI, Klasner AE, Kimberlin DW (2003) Impact of the rapid diagnosis of influenza on physician decision-making and patient management in the pediatric emergency department: results of a randomized, prospective, controlled trial. Pediatrics 112 (2):363-367
[116] Barenfanger J, Drake C, Leon N, Mueller T, Troutt T (2000) Clinical and financial benefits of rapid detection of respiratory viruses: an outcomes study. JClinMicrobiol 38 (8):2824-2828
[117] Statistics Norway www.ssb.no/befolkning, www.ssb.no/befolkning
[118] Marianne Ekre MD, SSA (2013)
[119] Barnett JM, Cadman A, Gor D, Dempsey M, Walters M, Candlin A, Tisdale M, Morley PJ, Owens IJ, Fenton RJ, Lewis AP, Claas EC, Rimmelzwaan GF, De Groot R, Osterhaus AD (2000) Zanamivir susceptibility monitoring and characterization of influenza virus clinical isolates obtained during phase II clinical efficacy studies. Antimicrob Agents Chemother 44 (1):78-87
[120] Charlson ME, Pompei P, Ales KL, MacKenzie CR (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 40 (5):373-383
[121] Hall SF (2006) A user's guide to selecting a comorbidity index for clinical research. JClinEpidemiol 59 (8):849-855
[122] Linn BS, Linn MW, Gurel L (1968) Cumulative illness rating scale. J Am Geriatr Soc 16 (5):622-626
[123] Salvi F, Miller MD, Grilli A, Giorgi R, Towers AL, Morichi V, Spazzafumo L, Mancinelli L, Espinosa E, Rappelli A, Dessi-Fulgheri P (2008) A manual of guidelines to score the modified cumulative illness rating scale and its validation in acute hospitalized elderly patients. JAmGeriatrSoc 56 (10):1926-1931
[124] Rognstad GP, R. (2009) The modified cumulative illness rating scale, Norwegian version.
[125] Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE (2005) Respiratory syncytial virus infection in elderly and high-risk adults. NEnglJ Med 352 (17):1749-1759

[126] Han LL, Alexander JP, Anderson LJ (1999) Respiratory syncytial virus pneumonia among the elderly: an assessment of disease burden. J InfectDis 179 (1):25-30
[127] Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, Fukuda K (2003) Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 289 (2):179-186
[128] Walsh EE, Peterson DR, Falsey AR (2007) Is clinical recognition of respiratory syncytial virus infection in hospitalized elderly and high-risk adults possible? J InfectDis 195 (7):1046-1051
[129] Falsey AR, McCann RM, Hall WJ, Criddle MM (1996) Evaluation of four methods for the diagnosis of respiratory syncytial virus infection in older adults. J AmGeriatrSoc 44 (1):71-73
[130] Talbot HK, Falsey AR (2010) The diagnosis of viral respiratory disease in older adults. ClinInfectDis 50 (5):747-751
[131] Mahony JB, Blackhouse G, Babwah J, Smieja M, Buracond S, Chong S, Ciccotelli W, O'Shea T, Alnakhli D, Griffiths-Turner M, Goeree R (2009) Cost analysis of multiplex PCR testing for diagnosing respiratory virus infections. JClinMicrobiol 47 (9):2812-2817
[132] Falsey AR, Murata Y, Walsh EE (2007) Impact of rapid diagnosis on management of adults hospitalized with influenza. ArchInternMed 167 (4):354-360
[133] SSB.no (2008)
Døgnopphold, dagbehandlinger og liggedager ved somatiske sykehus, etter region, diagnose, kjønn, alder, tid og statistikkvariabel
[134] Riquelme R, Torres A, el-Ebiary M, Mensa J, Estruch R, Ruiz M, Angrill J, Soler N (1997) Community-acquired pneumonia in the elderly. Clinical and nutritional aspects. Am J Respir Crit Care Med 156 (6):1908-1914
[135] Shen HN, Lu CL, Li CY (2012) Dementia increases the risks of acute organ dysfunction, severe sepsis and mortality in hospitalized older patients: a national population-based study. PloS one 7 (8):e42751
[136] van der Steen JT, Ooms ME, van der WG, Ribbe MW (2002) Pneumonia: the demented patient's best friend? Discomfort after starting or withholding antibiotic treatment. JAmGeriatrSoc 50 (10):1681-1688
[137] Charlson ES, Bittinger K, Haas AR, Fitzgerald AS, Frank I, Yadav A, Bushman FD, Collman RG (2011) Topographical continuity of bacterial populations in the healthy human respiratory tract. Am J Respir Crit Care Med 184 (8):957-963
[138] D'Journo XB, Bittar F, Trousse D, Gaillat F, Doddoli C, Dutau H, Papazian L, Raoult D, Rolain JM, Thomas PA (2012) Molecular detection of microorganisms in distal airways of patients undergoing lung cancer surgery. Ann Thorac Surg 93 (2):413-422
[139] Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, Iwasaki A (2011) Microbiota regulates immune defense against respiratory tract influenza A virus infection. Proc Natl Acad Sci U S A 108 (13):5354-5359
[140] Reynolds HY (2011) Bronchoalveolar lavage and other methods to define the human respiratory tract milieu in health and disease. Lung 189 (2):87-99
[141] Zemanick ET, Wagner BD, Sagel SD, Stevens MJ, Accurso FJ, Harris JK (2010) Reliability of quantitative real-time PCR for bacterial detection in cystic fibrosis airway specimens. PloS one 5 (11):e15101
[142] (2011) WHO Regional Office for Europe guidance for sentinel influenza surveillance in humans.
[143] WHO (2012) WHO interim global epidemiological surveillance standards for influenza (July 2012). WHO,
http://www.who.int/influenza/resources/documents/INFSURVMANUAL.pdf. Cited 23.08 2012

[144] Hernes SS, Quarsten H, Hagen E, Lyngroth AL, Pripp AH, Bjorvatn B, Bakke PS (2011) Swabbing for respiratory viral infections in older patients: a comparison of rayon and nylon flocked swabs. EurJClinMicrobiolInfectDis 30 (2):159-165
[145] bu-Diab A, Azzeh M, Ghneim R, Zoughbi M, Turkuman S, Rishmawi N, Issa AE, Siriani I, Dauodi R, Kattan R, Hindiyeh MY (2008) Comparison between pernasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. J Clin Microbiol 46 (7):2414-2417
[146] Heikkinen T, Marttila J, Salmi AA, Ruuskanen O (2002) Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. J Clin Microbiol 40 (11):4337-4339
[147] Larios OE, Coleman BL, Drews SJ, Mazzulli T, Borgundvaag B, Green K, Group ST-FS, McGeer AJ (2011) Self-collected mid-turbinate swabs for the detection of respiratory viruses in adults with acute respiratory illnesses. PloS one 6 (6):e21335
[148] She RC, Polage CR, Caram LB, Taggart EW, Hymas WC, Woods CW, Schmader K, Petti CA (2010) Performance of diagnostic tests to detect respiratory viruses in older adults. Diagn Microbiol Infect Dis 67 (3):246-250
[149] Esposito S, Molteni CG, Daleno C, Valzano A, Cesati L, Gualtieri L, Tagliabue C, Bosis S, Principi N (2010) Comparison of nasopharyngeal nylon flocked swabs with universal transport medium and rayon-bud swabs with a sponge reservoir of viral transport medium in the diagnosis of paediatric influenza. J Med Microbiol 59 (Pt 1):96-99
[150] Nicholls JM, Bourne AJ, Chen H, Guan Y, Peiris JS (2007) Sialic acid receptor detection in the human respiratory tract: evidence for widespread distribution of potential binding sites for human and avian influenza viruses. Respiratory research 8:73
[151] Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y (2006) Avian flu: influenza virus receptors in the human airway. Nature 440 (7083):435-436
[152] Malamud D, Abrams WR, Barber CA, Weissman D, Rehtanz M, Golub E (2011) Antiviral activities in human saliva. Adv Dent Res 23 (1):34-37
[153] Peterson ED, Lytle BL, Biswas MS, Coombs L (2004) Willingness to participate in cardiac trials. The American journal of geriatric cardiology 13 (1):11-15
[154] McMurdo ME, Witham MD, Gillespie ND (2005) Including older people in clinical research. BMJ 331 (7524):1036-1037
[155] Townsley CA, Chan KK, Pond GR, Marquez C, Siu LL, Straus SE (2006) Understanding the attitudes of the elderly towards enrolment into cancer clinical trials. BMC cancer 6:34
[156] Witham MD, McMurdo ME (2007) How to get older people included in clinical studies. Drugs Aging 24 (3):187-196
[157] Bell JA, May FE, Stewart RB (1987) Clinical research in the elderly: ethical and methodological considerations. Drug Intell Clin Pharm 21 (12):1002-1007
[158] Van Spall HG, Toren A, Kiss A, Fowler RA (2007) Eligibility criteria of randomized controlled trials published in high-impact general medical journals: a systematic sampling review. JAMA 297 (11):1233-1240
[159] Marengoni A, Winblad B, Karp A, Fratiglioni L (2008) Prevalence of chronic diseases and multimorbidity among the elderly population in Sweden. Am J Public Health 98 (7):1198-1200
[160] Habicht DW, Witham MD, McMurdo ME (2008) The under-representation of older people in clinical trials: barriers and potential solutions. J Nutr Health Aging 12 (3):194-196
[161] Kim SY, Karlawish JH, Caine ED (2002) Current state of research on decision-making competence of cognitively impaired elderly persons. Am J Geriatr Psychiatry 10 (2):151-165
[162] Siddiqi N, House AO, Holmes JD (2006) Occurrence and outcome of delirium in medical in-patients: a systematic literature review. Age Ageing 35 (4):350-364

[163] Johnson J (1999) Identifying and recognizing delirium. Dement Geriatr Cogn Disord 10 (5):353-358
[164] ACT 2008-06-20 no. 44: Act on medical and health research (the Health Research Act).
[165] Beck JM, Young VB, Huffnagle GB (2012) The microbiome of the lung. Translational research : the journal of laboratory and clinical medicine 160 (4):258-266
[166] Abdeldaim G, Herrmann B, Molling P, Holmberg H, Blomberg J, Olcen P, Stralin K (2010) Usefulness of real-time PCR for lytA, ply, and Spn9802 on plasma samples for the diagnosis of pneumococcal pneumonia. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 16 (8):1135-1141
[167] Klugman KP, Madhi SA, Albrich WC (2008) Novel approaches to the identification of Streptococcus pneumoniae as the cause of community-acquired pneumonia. Clin Infect Dis 47 Suppl 3:S202-206

15. Appendix

Paper I	Letter of consent
	Blood sample reminder (letter)
	Patient letter regarding shipment of biological materials outside EU.
Paper II/IV	Letter of consent.
	List of variables
Paper III	Letter of consent
	List of variables
	Blood sample reminder (letter)

Prosjektleder Lege Susanne S. Hernes
Medisinsk Avdeling
Sørlandet Sykehus Arendal
Tlf 37014200

Sørlandet sykehus HF

Forespørsel om deltagelse i forskningsprosjekt.

Vi ønsker herved å spørre deg om du vil delta i forskningsprosjektet **"Diagnostisk finnålsaspirasjon ved pneumoni oppstått utenfor sykehus".**

Lungebetennelse er en alminnelig sykdom som rammer de aller fleste i løpet av livet. I Norge behandles pasienter med denne sykdommen med antibiotika, og de fleste kommer seg fint ved denne behandlingen.
Imidlertid er hvilken bakterie eller virus som gir hver enkelt lungebetennelse mindre kjent, og vi finner ut dette hos mindre enn 50 % av pasientene som legges inn på sykehus.

I prosjektet "**Diagnostisk finnålsaspirasjon ved pneumoni oppstått utenfor sykehus**" ønsker vi å kartlegge hvilken bakterie eller virus som har gitt infeksjonen hos hver enkelt pasient. Funn av bakterier eller virus vil gi oss mulighet til å gi en mer målrettet og dermed mer effektiv behandling.
For å kunne delta i studien må man ha en lungebetennelse som er synlig på røntgenbildet, som ikke har oppstått på sykehus, og hvor man i tillegg har funn i blodprøver som støtter at dette er en lungebetennelse. Man kan ikke være med i studien dersom man har forlenget blødningstid (pga medisiner eller sykdom), eller hvis man har ekstremt dårlig lungefunksjon i utgangspunktet.

I tillegg til blodprøver, urinundersøkelser, halsprøver og slimprøver som gjøres av alle personer med lungebetennelse ønsker vi å utføre en lungeaspirasjon og en ekstra blodprøve 6 uker etter at du har vært innlagt ved sykehuset.

Lungeaspirasjon (finnålsaspirasjon) foregår ved at man under røntgengjennomlysning stikker en ultratynn nål inn i området hvor lungebetennelsen sitter, og suger ut litt vevsvæske. Selve innstikket varer i ca 20 sekunder, og er like ubehagelig som en blodprøve. Vevsvæsken blir sendt til undersøkelser ved mikrobiologisk avdeling for at man skal kunne bestemme hvilken bakterie eller virus som er i væsken. Når svaret foreligger vil man sammenholde dette med den behandlingen du allerede har fått, og så endre behandlingen hvis det viser seg at en annen behandling er mere hensiktsmessig.
Vi trenger også å ta en ekstra blodprøve ca 6 uker etter starten på din lungebetennelse for å se etter om du har fått antistoffer mot enkelte virus (som kan ha forårsaket lungebetennelsen).

Lungeaspirasjon er en metode som er benyttet i flere europeiske land de siste 20 år, og har en lav bivirkningsprosent. Pneumothorax (luft i lungesekken som kan gjøre at deler av lungen trykkes noe sammen) oppstår hos ca 5 % av pasientene. Denne går som oftest over av seg selv. 1 av 200 pasienter kan komme til å trenge behandling for dette (i form av et tynt rør som suger luften ut).

Prosjektleder Lege Susanne S. Hernes
Medisinsk Avdeling
Sørlandet Sykehus Arendal
Tlf 37014200

Sørlandet sykehus HF

Deltagelse i prosjektet er frivillig, og selv om man takker ja kan man på et hvilket som helst tidspunkt trekke seg uten å oppgi grunn til dette. Dette vil ikke ha konsekvenser for ditt forhold til Sørlandet Sykehus HF.

Dersom du ikke ønsker å delta vil du få vår standard behandling ved lungebetennelser. Vi kan da ikke skreddersy behandlingen din ytterligere etter funn ved undersøkelsen.

Det vil bli opprettet en forskningsbiobank hvor man avidentifisert oppbevarer 2 blodprøver og noe av vevsvæsken fra lungebetennelsen i ti år. Prøvene i forskningsbiobanken vil bli undersøkt på et senere tidspunkt. Ut i fra Biobankloven har du mulighet til å kalle tilbake ditt samtykke til bruk av disse prøvene når du måtte ønske dette. Beskjed om dette gis til prosjektleder (se telefonnummer på toppen av arket) som også er ansvarshavende for forskningsbiobanken. Etter ti år vil prøvene bli destruert.

Det vil bli opprettet et helseregister hvor prøveresultatene vil bli oppbevart og hvor hver deltager vil bli identifisert med et nummer, og intet navn. Koden til dette registeret er det bare prosjektleder, Susanne S. Hernes som har tilgang til. Resultatene av prøvene dine vil også bli oppbevart i din pasientjournal, slik at du selv har innsyn i disse. Vi vil fra din pasientjournal kun innhente opplysninger om hvor lenge du var innlagt på sykehuset under denne lungebetennelsen, samt resultater av de rutinemessige infeksjonsblodprøvene dine. Opplysningene vil bli behandlet konfidensielt, og de involverte parter har taushetsplikt. Det vil ikke være mulig å identifisere deltagere i prosjektet når forskningsresultatet publiseres.

Prosjektet har blitt tilrådd av Regional Etisk Komite og er meldt til Personvern-ombudet for forskning, Norsk samfunnsvitenskapelig datatjeneste AS. Det er søkt om, og fått tillatelse til oppretting av en forskningsbiobank fra Sosial- og Helsedepartementet.

Med vennlig hilsen

Sørlandet Sykehus Arendal, februar 2005.

Susanne S. Hernes
Medisinsk avdeling
Sørlandet Sykehus Arendal
Sykehusvn 1
4838 ARENDAL
tlf 37014200

Prosjektleder Lege Susanne S. Hernes
Medisinsk Avdeling
Sørlandet Sykehus Arendal
Tlf 37014200

Sørlandet sykehus HF

Samtykkeerklæring:

Jeg har mottatt skriftlig og muntlig informasjon om forskningsprosjektet **"Diagnostisk finnålsaspirasjon ved pneumoni oppstått utenfor sykehuset"** og har sagt meg villig til å delta i dette.

_____ _____

Dato/Sted Navn

Prosjektleder Lege Susanne S. Hernes
Medisinsk Avdeling
Sørlandet Sykehus Arendal
Tlf 37014200

Sørlandet sykehus HF

”Diagnostisk finnålsaspirasjon ved pneumoni oppstått utefor sykehus”.

Hei !!

Håper du har kommet deg fint etter lungebetennelsen som du var innlagt på Sykehuset i Arendal med nylig.

Det er nå tid for en blodprøve i forbindelse med studien *”Diagnostisk finnålsaspirasjon ved pneumoni oppstått utenfor sykehus”*. Blodprøven må tas en gang i tidsperioden uke _____ til _____

Denne blodprøven kan du enten ta ved sykehusets laboratorium (i vanlig arbeidstid mandag til fredag klokken 0800-1500) eller hos din fastlege. Dersom du ønsker å ta blodprøven ved sykehuset i Arendal trenger du ikke å bestille time til dette. Ønsker du å ta den hos din fastlege bør du ta telefonisk kontakt med legekontoret for å høre om du trenger time til dette.

Blodprøven skal være gratis. Dersom du blir bedt om å betale for denne ber du legekontoret sende regningen direkte til meg (adressen står i vedlagte brev som er stilet til laboratoriet).

Jeg har fått resultatet på de prøvene som vi analyserer i forbindelse med innleggelsen din. Disse gir ikke noe sikkert svar på hvilken bakterie eller virus som ligger bak lungebetennelsen din. De resterende prøvene vil bli analysert i løpet av 2007.

Vedlagt ligger to ark som du skal levere på laboratoriet/hos fastlegen når du skal ta blodprøven.

Tusen takk for deltagelse i prosjektet!

Prosjektleder Lege Susanne S. Hernes
Medisinsk Avdeling
Sørlandet Sykehus Arendal
Tlf 37014200

Sørlandet sykehus HF

”Diagnostisk finnålsaspirasjon ved pneumoni oppstått utefor sykehus”.

Til laboratoriet:

Denne pasienten er med i forskningsprosjektet ” Diagnostisk finnålsaspirasjon ved pneumoni oppstått utenfor sykehus”.
I forbindelse med prosjektet skal det tas en prøve med tanke på atypiske lungebetennelsesbakterier 4-6 uker etter sykehusinnleggelsen.

Vedlagt ligger ferdig utfylt rekvisisjon til prøveanalysering ved Mikrobiologisk laboratorium i Kristiansand. Det er særs viktig at prøvene sendes dit, og at man benytter vedlagte rekvisisjonsskjema.

Dersom ditt laboratorium tar egenandel for å ta denne blodprøven skal ikke pasienten selv betale dette. I stedet ber jeg om at man sender en giro til:

Susanne Hernes
Medisinsk Avdeling
Sørlandet Sykehus Arendal
Serviceboks 605
4809 ARENDAL

Dersom det oppstår andre spørsmål rundt dette kan jeg kontaktes på telefon 37014200.

Med vennlig hilsen

Susanne M. Sørensen Hernes

Sørlandet sykehus HF

”Diagnostisk finnålsaspirasjon ved pneumoni oppstått utenfor sykehus”

Sørlandet Sykehus Arendal, 30 november 2007

Hei

Tusen takk for at du i 2007 deltok i forskningsprosjektet ”Diagnostisk finnålsaspirasjon ved pneumoni oppstått utenfor sykehus” i forbindelse med at du hadde en lungebetennelse og var innlagt ved medisinsk avdeling, Sørlandet Sykehus Arendal. Prosjektet er nå avsluttet, og vi holder på å analysere de siste prøvene.
Foreløpig har vi funnet ansvarlig bakterie eller virus hos 70 prosent av lungebetennelse, et resultat som vi er god fornøyde med.

Det gjenstår imidlertid enda noen analyser som vi vil gjøre på vevsvæsken fra lungen og blodprøvene. Det er dessverre ikke mulig å få utført disse analysene i Norge, og av den grunn må vi da sende prøvene ut av landet.

Biobanklovens § 10 gjør det klart at dersom man vil sende prøver fra et forskningsprosjekt til utlandet, må hver enkelt deltager i prosjektet bli forespurt om tillatelse til dette.
Jeg vil med dette spørre deg om din tillatelse til at jeg kan sende prøvene dine til utlandet for analyse. Prøvene er avidentifisert, slik at det bare er jeg som kjenner til dine personlige data.

En videre analyse i utlandet vil kunne gi oss verdifull informasjon om flere bakterier, som igjen vil gi oss uvurderlig informasjon.

Vedlagt ligger et samtykkeskjema for forsendelse av prøvene ut av landet. Dersom du gir din tillatelse er jeg takknemlig for at du returnerer dette skjemaet så raskt du kan. Samtykket må være skriftlig, men du står fritt til å bruke vedlagte frankerte svarkonvolutt, faks eller skanne det inn som et mailvedlegg.

Dersom du har spørsmål kan jeg nås på telefon 37 01 42 00.

De aller beste ønsker om en riktig god jul!

Med vennlig hilsen

Susanne Sørensen Hernes
Prosjektleder

Prosjektleder Lege Susanne S. Hernes
Medisinsk Avdeling
Sørlandet Sykehus Arendal
Sykehusveien 1
4809 Arendal

Tlf 37014200

Sørlandet sykehus HF

”Diagnostisk finnålsaspirasjon ved pneumoni oppstått utenfor sykehus”

Samtykkeerklæring:

Jeg er deltager i forskningsprosjektet ”Diagnostisk finnålsaspirasjon ved pneumoni oppstått utenfor sykehus” og gir herved min tillatelse til at blodprøver og vevsvæske tatt i forbindelse med dette forskningsprosjektet kan sendes ut av Norge for analyse.

Prøvene sendes avidentifisert, slik at ingen andre enn prosjektleder har tilgang til mine personlige data.

_____ _____

Dato/Sted Navn

Dette skjemaet kan returneres på følgende måter:

i vedlagte frankerte svarkonvolutt.
Per faks til Susanne Hernes, Medisinsk ekspedisjon faksnr:
Som vedlegg til e-post til: Susanne.Sorensen.Hernes@sshf.no

Prosjektleder Lege Susanne S. Hernes
Medisinsk Avdeling
Sørlandet Sykehus Arendal
Sykehusveien 1
4809 Arendal

Tlf 37014200

Prosjektleder Lege Susanne S. Hernes
Medisinsk Avdeling
Sørlandet Sykehus Arendal
Tlf 37014200

Sørlandet sykehus HF

Forespørsel om deltagelse i forskningsprosjekt.

Vi ønsker herved å spørre deg om du vil delta i forskningsprosjektet **"Lungebetennelse, veien videre" – virologi og "atypiske agens".**
De fleste av oss blir syke av et luftveisvirus i løpet av livet. Vi vet at personer over 60 år er spesielt utsatt. Imidlertid er det vanskelig å med sikkerhet diagnostisere denne virusinfeksjonen. Vi vet ikke om man bør ta prøver fra hals eller nese, og heller ikke hvilken prøvepinne som bør benyttes. I denne studien ønsker vi å se om vi kan klare å finne svar på dette.

Alle pasienter født i 1948 eller før som blir innlagt ved medisinsk avdeling SSA en bestemt dag i uken vil bli forespurt om å bli med i studien, uavhengig av om man har tegn på en luftveisinfeksjon eller ikke.

Vi ønsker å ta en dyp neseprøve av deg og en halsprøve utført med to forskjellige prøvepinner. Denne undersøkelsen er ikke farlig, og gjøres rutinemessig ved de fleste sykehus i landet. I tillegg ønsker vi å ta en blodprøve for å undersøke om du har tegn på infeksjon med luftveisvirus. I tillegg vil 30 pasienter hvor vi finner virus, og 30 pasienter uten virus bli forespurt om frivillig videre oppfølgning etter 4 uker og 4 måneder. Prøvene vil kunne bli analysert i utlandet (innenfor EU) .

Deltagelse i prosjektet er frivillig, og selv om man takker ja kan man på et hvilket som helst tidspunkt trekke seg uten å oppgi grunn til dette. Dette vil ikke ha konsekvenser for ditt forhold til Sørlandet Sykehus HF.
Dersom du ikke ønsker å delta vil du få vår standard behandling under oppholdet.

Det vil bli opprettet et helseregister hvor prøveresultatene vil bli oppbevart og hvor hver deltager vil bli identifisert med et nummer, og intet navn. Koden til dette registeret er det bare prosjektleder, Susanne S. Hernes som har tilgang til. Resultatene av prøvene dine vil bli tilført din pasientjournal, slik at du selv har innsyn i disse. Vi vil fra din pasientjournal kun innhente opplysninger vedrørende denne sykehusinnleggelsen. Opplysningene vil bli behandlet konfidensielt, og de involverte parter har taushetsplikt. Det vil ikke være mulig å identifisere deltagere i prosjektet når forskningsresultatet publiseres.

Prosjektet avsluttes senest ved utgangen av 2009.
Prosjektet har blitt tilrådd av Regional Etisk Komite og er meldt til Personvernombudet for forskning, Norsk samfunnsvitenskapelig datatjeneste AS.

Med vennlig hilsen

Lege Susanne S. Hernes
Medisinsk avdeling
Sørlandet Sykehus Arendal
tlf 37014200

Sørlandet Sykehus Arendal, januar 2008.

Prosjektleder Lege Susanne S. Hernes
Medisinsk Avdeling
Sørlandet Sykehus Arendal
Tlf 37014200

Sørlandet sykehus HF

Samtykkeerklæring:

Jeg har mottatt skriftlig og muntlig informasjon om forskningsprosjektet **"Lungebetennelse, veien videre- virologi og "atypiske agens" "** og har sagt meg villig til å delta i dette.

_____ _____

Dato/Sted Navn

”Lungebetennelse veien videre”- Virologiske og ”atypiske” agens

1. Pasientnr_____

Sett merkelapp her

2. Inklusjonsdato _____

3. Alder _____

4.Kjønn □M □ K

5.Kommer fra: □ Eget hjem □ Omsorgsbolig □ Sykehjem □ Annet_____
□ Eget hjem/omsorgsbolig uten hjelp
□ Eget hjem/omsorgsbolig med hjelp mindre enn 1 time per uke
□ Eget hjem/omsorgsbolig med kun hjelp til utdeling av medisiner
□ Eget hjem/omsorgsbolig med to tilsyn eller mindre per dag.
□ Eget hjem/omsorgsbolig med 2-4 tilsyn per dag.
□ Eget hjem/omsorgsbolig med over 4 tilsyn per dag.
□ Har dagsenter ___ dager per uke.

6. Innlagt av: □ Egen lege □ Annen lege ved samme legekontor □ Tilsynslege □ Legevakt
□ AMK

7. Legeskriv ved innleggelsen: □ JA □NEI

8. Innleggelsesdiagnose: _____

9. Tidspunkt for innleggelse på sykehus: kl (0-24) ___:___

10. Innlagt på □ OBS-posten □ UC □1C □1CC □2C □ 4EI

11 . Rtg thorax v innkomst:
Pneumoni □ H. overlapp □V.overlapp □ Stuvning □ Emfysem □Cancer
□ H.midtlapp □ Cancermistanke
□ H.underlapp □ V.underlapp

12. Sykdomsforløp: Antall dager syk _____

13. Dobbelt innsykning □ J □ N
Hvis J: lengde på siste sykdomsperiode_____

14. Symptomer: □Hals □ Nese □ Bihuler □Hoste □Feber □Ekspektorat
□Pleurittsmerter □Hemoptyse
Annet _____

15.Tidligere sykdommer:
□ Hjerte □Lunge □Diabetes □Cancer □Autoimmun □ Infeksjonssykdommer
Spesifiser:_____

”Lungebetennelse veien videre”- Virologiske og ”atypiske” agens

16.Medikamenter:

17. Røyk: □J □N. 18. Filter □J □N 19.Antall per uke_____

20. Alkohol. □J □N 21. Mengde per uke_____

Vaksinasjonsstatus:

22. Influensa sist _____ 23. Pneumokokkvaksine_____

24).CRP_____ 25). Høyeste under oppholdet_____

26).Kreatinin_____ 27). Hvite _____ 28). Urea_____

29. VEKT _____ 30.Høyde _____

31.Vektendring siste 6 mnd _____

32. EPIKRISEDIAGNOSE

Diagnoser ved utreise: _____

_____

33 Antall dager innlagt: _____
34 Antall dager på intensivavdeling (4ei eller 1cc) _____

35. Utskrevet til: □ Eget hjem □ Omsorgsbolig □ Korttidsplass □ Sykehjem

Fiberswab	**Nese**	
	Hals	
Flocked swab	**Nese**	
	Hals	

”Lungebetennelse veien videre”- Virologiske og ”atypiske” agens

36. Sannsynlig infeksjon: □ JA □ NEI

37. Bærertilstand: □ JA □ NEI

VIDERE OPPFØLGNING □ JA □ NEI

Sørlandet sykehus HF

Forespørsel om deltakelse i forskningsprosjektet

"Prøvetagning ved Influensa A infeksjon"

Bakgrunn og hensikt
Dette er et spørsmål til deg om å delta i en forskningsstudie for å undersøke om det er best å ta prøver fra nese eller hals når man har influensa. Dette er et viktig spørsmål for at vi skal kunne tilby pasienter med mistenkt influensasykdom best mulig helsehjelp.

Hva innebærer studien?
Det vil bli tatt en penselprøve fra hals og en fra nesen i dag. I tillegg ønsker vi å undersøke blodprøven som ble tatt av deg ved innkomst til sykehuset, samt spørre deg om du kan ta en ny blodprøve etter fire uker (hos din egen lege). Kun personer som allerede har fått påvist en influensainfeksjon kan være med i studien.

Studien vil ikke påvirke den behandlingen du allerede får ved sykehuset. Dersom du velger å ikke være med vil dette ikke påvirke ditt forhold til sykehuset.

Mulige fordeler og ulemper

Penselprøver fra hals og nese er begge rutineundersøkelser ved norske sykehus i dag. Studien vil ikke medføre noen ulemper for deltagerne.

Hva skjer med prøvene og informasjonen om deg
Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste
Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Prøvene og informasjonen om deg vil bli slettet senest i 2025.

Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Frivillig deltakelse
Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for din videre behandling. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte prosjektleder Susanne Hernes (37014200)

Ytterligere informasjon om studien finnes i kapittel *A* – *utdypende forklaring av hva studien innebærer.*
Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel B – *Personvern, biobank, økonomi og forsikring.*

Samtykkeerklæring følger etter kapittel B.

Kapittel A- utdypende forklaring av hva studien innebærer

Personer som er innlagt med Influensa ved Sørlandet Sykehus HF vil bli forespurt om å delta i studien. Man kan ikke delta dersom man er under 18 år, eller dersom man har forlenget blødningstid (INR>3)

Ved prøvetagning for influensa er det anbefalt enten penselprøve fra hals eller nese. Vi vet ikke med sikkerhet om disse to prøvene er like gode, og det er det vi ønsker å undersøke i denne studien.

Deltagelse i studien medfører kun at det tas en penselprøve fra hals og en fra nese som en engangsforeteelse under sykehusinnleggelsen. Penselprøver fra hals og nese er rutineundersøkelser på norske sykehus og medfører ingen bivirkninger.

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern
Opplysninger som registreres om deg er opplysninger om den aktuelle sykehistorien din (dvs hvor lenge du har vært syk, hvilke symptomer du har hatt, resultater av blodprøver, hvor lenge du var innlagt på sykehus og hvordan det har gått med deg etter at du har blitt skrevet ut). Navnelisten vil bli koblet mot Dødsårsaksregisteret.

Formålet er å kontrollere at studieopplysningene stemmer overens med tilsvarende opplysninger i din journal. Alle som får innsyn har taushetsplikt.

Sørlandet Sykehus HF ved administrerende direktør er databehandlingsansvarlig.

Biobank

Pensel-prøvene og blodprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en forskningsbiobank ved Sørlandet Sykehus HF. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Susanne Hernes er ansvarshavende for forskningsbiobanken. Biobanken planlegges å vare til 2025. Etter dette vil materialet og opplysninger bli destruert og slettet etter interne retningslinjer.

Utlevering av materiale og opplysninger til andre
Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver og avidentifiserte opplysninger utleveres til andre forskere innen for EU hvor formålet er influensaforskning.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver
Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi

Studien og biobanken er finansiert gjennom forskningsmidler fra Sørlandet Sykehus HF.

Informasjon om utfallet av studien
Du har rett til å få informasjon om utfallet i studien. Dette kan du få ved å kontakte prosjektleder.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Stedfortredende samtykke når berettiget, enten i tillegg til personen selv eller istedenfor

---

(Signert av nærstående, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

04.11.2009

PASIENTARK

PRØVETAGNING VED INFLUENSA A INFEKSJON

(fylles ut av den som tar prøven)

Pasientnr

Pasientmerkelapp

Prøven tatt: _____(dato) Tatt av HQ/ RH

Prøve ved innkomst tatt fra (ring rundt) Hals Nese Husker ikke

H1N1 vaksine tatt: J/N Dato dose 1_____ Dato dose 2 _____

Sesonginfluensavaksine tatt J/N Dato/årstall _____

Pneumokokkvaksine tatt J/N Dato/årstall_____

SENDES SUSANNE I INTERNPOSTEN (lukket konvolutt)

Susanne Hernes, Medisinsk avdeling SSA

Sørlandet sykehus HF

"Prøvetagning ved Influensa infeksjon"

Kjære deltager!

Jeg håper du har kommet deg etter sykehusinnleggelsen og har gjenopptatt normal aktivitet.

I forbindelse med deltagelsen din i forskningsprosjektet er det nå tid for å ta en siste blodprøve.
Vi ønsker å se på dannelsen av antistoffer mot influensa, noe vi gjør når vi sammenligner blodprøven ved sykehusinnleggelsen med denne siste blodprøven. Dette vil gi oss verdifull informasjon om beskyttelse mot influensainfeksjoner, og også effekt av eventuell vaksine. Kunnskap om beskyttende antistoffer vil komme alle influensapasienter til god.

Jeg vil av den grunn be deg om å ta en ny blodprøve i løpet av en uke. Blodprøven kan tas hos din fastlege eller ved sykehusets laboratorieavdeling (åpningstid 0800-1500 alle hverdager). Prøven er gratis, og skal ikke koste deg noe.

Ta med dette arket (med klistremerker) til laboratoriet, slik at de som tar prøven får den informasjonen de trenger.

Jeg vil med dette takke deg for deltagelse i studien.
Dersom du har noen spørsmål kan jeg nås på telefon 37 01 42 00.

Med vennlig hilsen

Susanne S Hernes, prosjektleder.
Medisinsk avdeling
Sørlandet Sykehus Arendal.

Sørlandet sykehus HF

Til laboratoriet

Pasienten er deltager i et forskningsprosjekt vedrørende prøvetagning ved Influensa infeksjon.
I den forbindelse skal det tas blodprøve ca fire uker etter sykehusinnleggelsen.

1.Ta to gelglass og sentrifuger disse innen to timer. Merk glassene med pasientens navn, fødselsnummer og dato for prøvetagning. I tillegg merkes glassene med vedlagte klistrelapper (sett de på tvers).

2. Send prøvene til :

Gunn Berge/ Kirsti Holden
Klinisk kjemisk avdeling
Sørlandet Sykehus Arendal
Serviceboks 605
4809 Arendal

Eller via hentetjenesten dersom dere er tilknyttet denne (som går til SSA)

3. Legg ved dette skjemaet.

På forhånd takk for hjelpen.

Dersom det påløper noen kostnader for pasienten i forbindelse med prøvetagningen ber jeg om at regning sendes til meg.

Med vennlig hilsen

Susanne Hernes
Medisinsk avdeling
Sørlandet Sykehus Arendal

Til Laboratorieavdelingen SSA

Sett prøvene kaldt.
Fryses av Gunn/Kirsti.