On community acquired infections requiring intensive care

Magnus Brink

Department of Infectious Diseases
Institute of Biomedicine
Sahlgrenska Academy at University of Gothenburg

UNIVERSITY OF GOTHENBURG

Gothenburg 2014

Cover illustration: Magnus Brink

On community acquired infections requiring intensive care

magnus.brink@gu.se

ISBN 978-91-628-9188-6 (printed)
ISBN 978-91-628-9189-3 (pdf) http://hdl.handle.net/2077/37107

Printed in Gothenburg, Sweden 2014
Ineko AB

"Ingenting är så svårt som att inte bedra sig."
Ludwig Wittgenstein

ABSTRACT

Acute bacterial meningitis (ABM), influenza, and necrotizing soft-tissue infections (NSTIs) are diseases that in a short period of time can progress to become life threatening. Individuals with severe forms of these infections must be treated in an intensive care unit were monitoring and support of failing organs improve the chances of survival. The overall aims of this thesis were to elucidate some aspects of the clinical presentation, diagnosis and intensive care treatment of ABM, severe influenza, and NSTIs.

In **paper I**, we investigated the outcome of 79 episodes of adult ABM. All patients were given β-lactam antibiotics according to the Swedish tradition with 8-hour intervals between the doses. This is less frequent compared with recommendations in most international guidelines. We found a high survival rate (94%), which suggests that other factors than antibiotic dosing intervals are more important. *Streptococcus pneumoniae* was the most common pathogen (48%).

In **paper II**, we explored the over-time performance for ABM diagnosis with broad-range polymerase chain reaction and immunochromatographic test. Both tests were highly sensitive for detection of bacteria in cerebrospinal fluid sampled up to one week into antibiotic therapy.

In **paper III**, we investigated the clinical characteristics and outcomes among the 126 Swedish cases of pandemic influenza A (H1N1) that required intensive care treatment. Risk factors were obesity, chronic pulmonary disease, and diabetes. The mortality was similar to what has been reported from other comparable countries. The use of non-invasive ventilation was not associated with improved outcomes compared with immediate invasive ventilation.

In **paper IV**, we studied patients with NSTIs treated at Sahlgrenska University Hospital/East during the period 2008–2011. The 30-day mortality was 14% and the incidence of amputation 24%. Group A streptococcus was the most common pathogen followed by Enterobacteriacae and colonic anaerobe bacteria. Inter-hospital transfer was not associated with a delay in key interventions and could not be identified as a risk factor for adverse outcome.

Keywords: intensive care, acute bacterial meningitis, β-lactam antibiotics, cerebrospinal fluid, polymerase chain reaction, immunochromatographic test, influenza A H1N1, pandemic, non-invasive ventilation, necrotizing soft-tissue infection, inter-hospital transfer

ISBN: 978-91-628-9188-6 (printed)

SAMMANFATTNING PÅ SVENSKA

Akut bakteriell meningit, influensa och vävnadsdestruerande mjukdelsinfektioner är exempel på sjukdomar som snabbt kan bli livshotande. Personer som drabbas av allvarliga former av dessa infektioner behöver som regel vårdas på en intensivvårdsavdelning där övervakning och behandling av sviktande organfunktioner är avgörande för chanserna till överlevnad. Det övergripande syftet med denna avhandling är att belysa olika aspekter på sjukdomsbild, diagnostik och behandlingar vid bakteriell meningit, svår influensa och vävnadsdestruerande mjukdelsinfektioner.

I Sverige ges ofta intravenösa doser av β-laktamantibiotika med 8-timmars intervall vid behandling av akut bakteriell meningit. Detta skiljer sig från vad som är praxis i de flesta andra länder där man ger antibiotika var 4:e eller var 6:e timma. I **delarbete I** undersökte vi behandlingseffekten av antibiotikabehandling med 8-timmars doseringsintervall. Vi fann att den svenska traditionen med 8-timmars doseringsintervall ger behandlingsresultat som är likvärdiga med vad som observerats med tätare dosering. I **delarbete II** studerade vi diagnostik av akut bakteriell meningit med nyare, icke odlingsberoende metoder: PCR och immunkromatografisk bakteriedetektion. Den förra metoden kan identifiera flera bakteriearter medan den senare enbart kan diagnostisera pneumokocker som dock är den vanligaste bakterien vid bakteriell meningit. Vi har nu visat att båda metoderna fungerar väl för diagnostik av bakteriell meningit på prov från ryggvätska som tagits upp till en vecka efter insatt antibiotikabehandling. Detta är viktigt eftersom de för många patienter finns faktorer som gör att provtagning inte kan genomföras tidigt i sjukdomsförloppet. **Delarbete III** omfattade 126 av de 136 patienter med influensa A (H1N1) som vårdades på svenska intensivvårdsavdelningar under pandemin 2009–2010. Merparten av dessa patienter var mycket svårt sjuka och krävde omfattande och långvariga intensivvårdsinsatser. Vi undersökte särskilt effekter av en relativt ny teknik av respiratorbehandling, så kallad non-invasiv ventilation (NIV). Vi kunde inte se några fördelar i form av högre överlevnad eller förkortad intensivvård för patienter som behandlades med NIV jämfört med dem som behandlats med konventionell invasiv ventilation. I **delarbete IV** studerade vi patienter med svåra vävnadsdestruerande mjukdelsinfektioner. *Streptococcus pyogenes* var den vanligaste bakterien och orsakade 41 % av fallen. Vi jämförde behandlingsresultaten mellan patienter som primärt vårdats på Sahlgrenska Universitetssjukhuset/Östra och de patienter som överfördes från mindre sjukhus i regionen. Förflyttning innebar inte någon försening i handläggningen och ökade inte risken för död eller amputation.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals (I–IV).

I. Brink M, Hagberg L. Outcome of 8-hour dosing intervals with beta-lactam antibiotics in adult bacterial meningitis. Scand J Infect Dis. 2006; 38(9): 772-7.

II. Brink M, Welinder-Olsson C, Hagberg L. Time window for positive cerebrospinal fluid broad-range bacterial PCR and streptococcus pneumoniae immunochromatographic test in acute bacterial meningitis.
Submitted.

III. Brink M, Hagberg L, Larsson A, Gedeborg R. Respiratory support during the influenza A (H1N1) pandemic flu in Sweden.
Acta Anaesthesiol Scand. 2012 Sep; 56(8): 976-86.

IV. Brink M, Arnell P, Lycke H, Rosemar A, Hagberg L. A series of severe necrotising soft-tissue infections in a regional centre in Sweden.
Acta Anaesthesiol Scand. 2014 Aug; 58(7): 882-90.

CONTENT

ABBREVIATIONS

ABM	Acute bacterial meningitis
AIDS	Acquired immune deficiency syndrome
APACHE 2	Acute physiology and chronic health evaluation 2
BBB	Blood brain barrier
CNS	Central nervous system
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
ECMO	Extra corporeal membrane oxygenation
ED	Emergency department
FiO_2	Fraction of oxygen in inspired gas mixture
HA	Hemagglutinin
HBO	Hyperbaric oxygen
HIV	Human immunodeficiency virus
ICP	Intracranial pressure
ICT	Immunochromatographic test
ICU	Intensive care unit
IQR	Interquartile range
IVIG	Intravenous immunoglobulin
LP	Lumbar puncture

LRINEC	Laboratory risk indicator for necrotising fasciitis
MIC	Minimum inhibitory concentration
NA	Neuraminidase
NIV	Non-invasive ventilation
NSTI	Necrotizing soft-tissue infection
PaO_2	Partial pressure of oxygen in arterial blood
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SAPS 3	Simplified acute physiology score 3
VAP	Ventilator associated pneumonia

1 INTRODUCTION

History of man – history of infections

Microorganisms such as bacteria, fungi, protozoa, and viruses have been our companions since the dawn of mankind. Our long-lasting and close relationship with microbes is a most complicated and multifaceted story. The symbiosis with the bacterial flora in our gut is one example of the positive sides of our encounter; we provide bacteria with accommodation and regular feeding and in exchange they help us digest the food and make various nutrients available for us to absorb. In fact, microorganisms help us with our meals even before consumption. Staple food and delights such as bread, yoghurt, beer, and fermented herring would not exist without the aid from domesticated bacteria and fungi. On the other hand, if we do not consume our food in due time it will be reclaimed by the microorganisms by rancidity, souring, or putrefaction. This could have been the end of a rather pleasant story if it was not for the capacity of many microorganisms to aggressively invade our bodies and cause diseases. Documents from our past tell us about devastating epidemics with cholera, plague, and smallpox.

Figure 1. Left: Plague doctor, Etching by Paulus Furst of Nuremberg, Germany, 1656. Right: A Mesoamerican infected with smallpox, From the Florentine Codex, by Bernardino de Sahagún, a 16th-century Spanish Franciscan missionary. (Both pictures are reproduced in accordance with "Public domain" legislation.)

The Spanish flu that coincided with the end of the First World War killed many more people than the war itself. Old acquaintances such as malaria, tuberculosis, hepatitis B, and leishmania are still killing hundreds of thousands of people every year. We have recently seen new, previously unknown infections, which in a short space of time have spread and caused major impacts in our societies. The human immunodeficiency virus (HIV)

has since its discovery in the mid 1980s spread to all continents and killed more than 35 million individuals. HIV is at present the leading cause of death among young adults in many sub-Saharan African countries. The on-going Ebola epidemic in West Africa gives us a striking example of the devastating consequences of a microorganism that combines high contagiousness with high lethality. In addition to all these fearsome microbes with strong epidemic potential there are several other organisms that almost never cause epidemic spread but substantially contribute to the total burden of infections by their vast number of sporadic cases. Examples of this are bacterial diseases such as pneumonia, urinary tact infections, and skin and soft tissue infections.

Our efforts to avoid or cure infections were for a long period of time ineffective and more or less futile. The growth of scientific knowledge and systematic medical science from the 1800 century and onwards paved the way for the breakthroughs in diagnostics, prevention, and treatment of infectious diseases to come during the following centuries. Edward Jenner's finding that inoculation with cowpox was protective against smallpox was a significant milestone to be followed by many essential discoveries. The identification of bacteria as the cause of many diseases by Robert Koch, the understanding of the importance of antiseptics by Ignaz Semelweis, and the invention of pasteurisation of milk by Louis Pasteur are some other prominent examples of scientific successes in understanding and battling diseases caused by microorganisms.

Alexander Fleming's discovery of penicillin in 1928 is probably the number one pioneering achievement in our struggle against infections. The following decades, a vast number of antibiotics with different antibacterial spectra were discovered. This rapid development continued until the 1980s and equipped us with an armoury where we had antibiotics with different mechanisms of action against next to all bacteria known to cause disease in humans. In parallel, there were discoveries of compounds against fungal infections and protozoa diseases such as malaria. The development of efficient drugs against viral infections started with Gertrud Ellison's synthesis of the first anti-herpes drug, acyclovir, in the late 1970s. The effective combination treatment of HIV that has been developed since the late 1990s represents another major leap forward in antiviral treatment. Modern combination antiretroviral therapy has changed the prospects of HIV-infected people from a previously inevitable death in AIDS to a chronic disease with a life span comparable to uninfected people. At present we see very promising results with new efficient drugs against hepatitis C virus.

In spite of all these achievements, infections are still among the leading causes of disease and death all around the world. This is, to a large extent, a consequence of global inequality with the majority of the worlds population still living under inadequate sanitary conditions and without access to efficient health care. The on-going epidemics of deadly diarrhoea in children, endemic malaria, and the continued spread of HIV are examples of health problems in resource poor settings that mainly have socioeconomic causes and thus cannot be efficiently met without substantial political and economical development. In resource rich countries the recent advances in medicine such as organ transplantation, potent anti-inflammatory medication, cancer treatment, and intensive care treatment have created opportunities for new types of infections, so called opportunistic infections. Otherwise harmless microbes find previously inaccessible ecological niches in immune-compromised hosts and give rise to dangerous and difficult to manage infections. An ageing population is another factor that increase the prevalence and severity of infections.

The biggest threat and the greatest challenge in today's medicine is the rapid emergence of bacteria resistant to antibiotic therapy [1]. Antibiotics, that until recently were the leading "game changers" in medicine, are now at risk of loosing their effect which would bring us back to the situation of the pre-antibiotic era. Common infections, such as pneumonias, urinary tract infections, and wound infections could once again become problematic to treat. Furthermore, the most advanced achievements in modern medicine such as major surgery, organ transplantation, cancer chemotherapy, and intensive care would probably not be possible without effective antibacterial treatments.

1.1 Intensive care

Intensive care can be defined as the use of medical technologies and medications to support or even to temporarily replace failing organs vital for immediate survival. The most central supportive strategies are mechanical ventilation in patients with respiratory failure and intravenous infusions of fluids and vasoactive drugs in patients with failing circulation. Continuous hemodialysis is another common intervention in patients with acute renal dysfunction. Safe care of a critically ill patient is dependent on close attention by highly qualified personnel as well as continuous monitoring of heart function, blood pressure, blood oxygenation, ventilation, and respiratory gas exchange. In addition, there is need for several other interventions such as enteral or parenteral feeding, pain management and sedation, thrombosis prophylaxis, corrections of disturbances in salt-balance and blood glucose,

and in most cases surgical and/or medical treatment for the condition that precipitated the need for intensive care.

History of intensive care

With just 60 years from its inception, intensive care is one of the youngest disciplines in clinical medicine. It all started with the Scandinavian polio epidemic in 1952–1953 [2]. During the first four weeks of the epidemic there had already been 27 deaths among young patients with bulbo-spinal paralysis at the Department of Communicable Diseases, the Blegdam Hospital in Copenhagen. Progression to death could not be avoided by the use of negative pressure respirators (tank or cuirass). The prevailing concept at that time was that fatal outcome was the result of massive cerebral infection, polio-encephalitis, and thus unavoidable in severe cases. A senior registrar, Mogens Bjørnebo, challenged this paradigm.

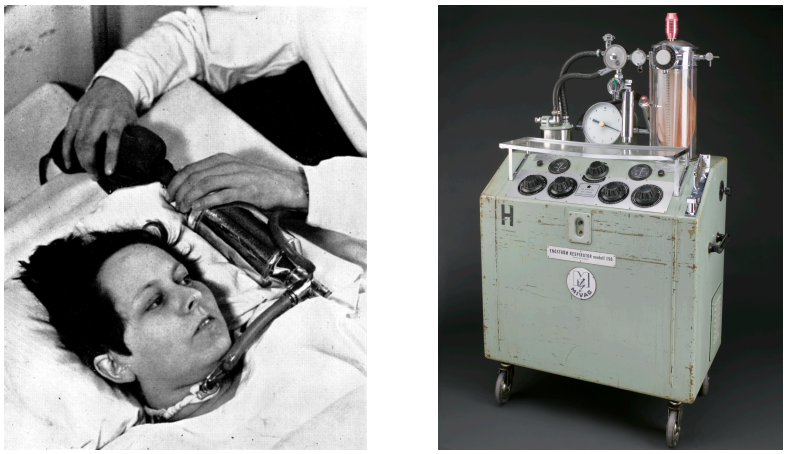

Figure 2. Left: A young woman during the 1952 Copenhagen polio epidemic getting respiratory support by hand-ventilation through a tracheostomy cannula. Right: The Engstrom model 150 ventilator introduced 1950. (Both pictures are reproduced in accordance with "Public domain" legislation.)

He hypothesized that the cyanosis, somnolence, and hypertension observed in severely ill patients could be caused by the respiratory failure in itself, leading to hypoxia and accumulation of carbon dioxide in the blood. He convinced his superior, professor H.C.A. Lassen, to engage a young anesthesiologist, Bjørn Ibsen, who successfully tracheotomied a young girl who then could be provided intermittent positive pressure hand-ventilation until her paralysis finally vanished. This successful outcome was followed by

a massive effort where 1500 volunteers, mostly medical students, were recruited to hand-ventilate all in all 300 polio victims.

In December 1953 Bjørn Ibsen founded the first multidisciplinary intensive care unit (ICU) in the world at the Kommune Hospital in Copenhagen. The concept was soon widely adopted with ICUs established in hospitals all over the world. Another coinciding breakthrough, essential for the development of intensive care, was the invention of the first mechanical ventilator in 1950 by the Swedish physician Carl Gunnar Engström [3].

Intensive care today

During the six decades since the dawn of intensive care in Copenhagen there has been an enormous development of the knowledge base and technologies used in intensive care [4]. Bedside monitors provide continuous information about oxygenation, arterial blood pressure, body temperature, exhaled carbon dioxide concentration and the electrical activity of the heart. Modern ventilators are highly sophisticated electronic machines with very little resemblance to the bulky and crude giants from the past. We have learnt to adopt lung protective ventilation with small tidal volumes and the use of alveolar recruitment maneuvers together with a graded use of positive end expiratory pressure [5]. The development of non-invasive mechanical ventilation (NIV) has decreased mortality and days spent in the ICU for patients with hypercapnic respiratory failure [6]. NIV has also been used in hypoxic respiratory failure although its feasibility is not yet fully established in this type of patients [7]. Machines for continuous veno-venous dialysis has it made possible to efficiently replace failing kidney function even in patients with unstable hemodynamics [8]. We have learnt to avoid excessive sedation [9] and are more restrictive with blood transfusions [10]. Large efforts have been directed towards improving survival in septic shock [11]. Attempts with different immunnomodulatory therapies in septic chock have this far been without success but new therapeutic approaches are under investigation [12]. Despite the absence of a “magic bullet” therapy against the excessive inflammation in sepsis, the mortality in severe sepsis/septic chock has decreased substantially during the last decade, in some studies down to 18% from 35% [13].

In Sweden, there are at present 84 ICUs with approximately 250 000 admissions per year. The size of the ICUs varies widely, from four up to 20 beds. All hospitals with emergency departments have general ICUs and third level teaching hospitals often have specialized ICUs, such as neuro-ICU, cardiothoracic-ICU, and paediatric ICU. Some centres also have a separate burns-ICU. In Sweden there are two ICUs incorporated in the departments of

infectious diseases. One is located in Gothenburg and the other one in Malmö. Both units have historical roots in the polio-epidemic of the 1950s and have gradually evolved according to the evolution of infectious diseases and intensive care.

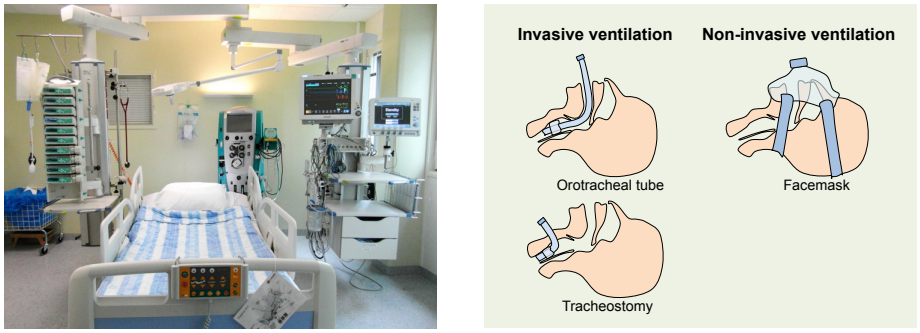

Figure 3. Left: Bed with equipment in a modern intensive care unit. Right: Schematic illustration of invasive ventilation (orotracheal tube, tracheostomy) and non-invasive ventilation (facemask).

Infections in the intensive care unit

The majority of patients in an ICU are treated with at least one and often several different antibiotics [14]. The high proportion of serious infections among ICU admissions can largely explain the high antibiotic utilization in ICUs. Data from the Swedish Intensive Care Registry show that (post-operative ICU-care excluded) the three most prevalent infectious conditions; severe sepsis, septic chock, and bacterial pneumonia, account for 20% of all ICU admissions and 30% of total days spent in Swedish ICUs [15]. Several less common infections such as endocarditis, necrotizing soft tissue infections, bacterial meningitis, and malaria can be severe and render the patients in need of intensive care.

Not only do patients get admitted to an ICU because of a critical infection, but they also have a high risk of acquiring an infection during intensive care (21%) with an estimated attributable mortality of 14% [16]. Many interventions lead to brakes of normal skin and mucosal barriers with an increased risk for infections caused by the patient's own bacterial flora or from microorganisms in the hospital. Ventilator associated pneumonia (VAP) is the most common nosocomial infection with an incidence ranging from two to 16 episodes per 1000 ventilator-days [17]. The pathogenesis of VAP is complex with the presence of an endotracheal tub as the major risk factor, but depressed cough reflexes and decreased muco-cilliary clearance probably

also play a role. Early VAP, with onset within 48 hours after intubation is often caused by the same bacteria as in community-acquired pneumonia while later onset VAP tends to be caused by gram-negative bacteria with an increased risk of multidrug resistance [18].

Other frequently occurring nosocomial infections in the ICU are urinary tract infections related to urinary catheters and blood stream infections related to central venous lines used for drug infusions, nutrition, and blood sampling [19]. The increasing prevalence of infections caused by multiresistant bacteria is problematic and has forced the ICU community to re-evaluate routines for antibiotic usage and stimulated research about optimal antibiotic dosing regimens. Regular, often daily, rounds by a specialist in infectious diseases is praxis in most Swedish ICUs and increasingly important given the rapid increase of antibiotic-resistant bacteria [20, 21].

After this broad, but short, overview of intensive care in the past and in the present, I will now discuss the three severe infections in focus of my research project. In the subsequent sections of the thesis, I will go into some details of acute bacterial meningitis, influenza A, and necrotizing soft-tissue infections.

1.2 Acute bacterial meningitis

Epidemiology of ABM

The incidence of ABM varies greatly by geographical regions and has undergone significant changed over time. In USA the annual number of ABM cases per 100 000 population in all age groups dropped from 2.0 in 1998–1999 to 1.38 in 2006–2007 [22]. Introduction of the 7-valent conjugate vaccine against *S. pneumoniae* is probably the main explanation for this reduction. There is no official statistics on the incidence of ABM in Sweden, but based on the cases of ABM in Gothenburg between 1999 and 2004, the annual incidence of community-acquired ABM in adults has been estimated to be 2.6 per 100 000 population. The incidence of ABM is much higher in most middle- and low-income countries and is especially high in sub-Saharan African countries with a mean annual incidence of 101 cases per 100 000 population in the worst affected areas [23].

The causing bacteria of ABM vary with the patients' age and the geographical region. In new-borns there is a predominance of Group B streptococci, gram-negative rods, and *Listeria monocytogenes (L. monocytogenes)* [24]. For older children *Haemophilus influenzae (H. influenzae)* type B has historically been the predominant agent but today it has virtually disappeared in countries were conjugate Hib-vaccine has been

included in general childhood vaccination programs [25]. Nowadays, *Streptococcus pneumoniae (S. pneumoniae)* and *Neisseria meningitidis (N. meningitidis)* are the dominating bacteria for children after the neonatal period and for adults up to 50 years of age [26, 27].

Elderly people and individuals with impaired immunity are at greater risk for ABM compared with the general population. In these patients *L. monocytogenes* replaces *N. meningitidis* as the second most predominant bacteria while *S. pneumoniae* remains the most common agent. The recent inclusion of a conjugate vaccine against *S. pneumoniae* in the Swedish childhood vaccination program will probably reduce the incidence of pneumococcal meningitis in children as well as in adults. This trend has already been observed in USA, where children have been vaccinated against pneumococci since 1998. The all-age incidence of pneumococcal meningitis in USA has been reduced by 26% [22]. This so-called "herd-immunity" effect with a reduced incidence of an infection in unvaccinated groups (older individuals) is possible because vaccination of children effectively eradicates asymptomatic carriage of *S. pneumoniae* and thus eliminates the major reservoir for further spread to others.

Figure 4. Left: the meningococcal belt. Right: Poster for meningococcal vaccine in Burkina Faso. (Photographs downloaded from open domain.)

The major burden of ABM is carried by low- and middle-income countries where meningitis is one of the leading causes of death and permanent disability among children. In addition to a high incidence of ABM caused by *S. pneumoniae* and *H. influenza* there are also recurrent epidemics with *N. meningitidis* in the "meningitis belt" in sub-Saharan, West-, Central-, and East Africa. Routine vaccine programs against *H. influenzae*, and in some countries also against *S. pneumoniae,* have recently been launched in most low- and middle-income countries but surveillance data of long-term effects are this far scarce [25]. Since 2010, a conjugate vaccine against *N.*

meningitidis group A (MenAfriVac®) is available and presently distributed in most countries within the African meningitis belt [28, 29].

Pathophysiology of ABM

The central nervous system (CNS) is comparatively well protected against invasion of microorganisms. This is mainly because of the special properties of the capillaries in the CNS. The endothelial cells in the CNS capillaries are, in contrast to elsewhere in the body, firmly fused by tight junctions. These junctions together with a thick basal membrane and an almost complete coverage of the outside of the capillaries with astrocyte foot processes, make a strong barrier that prevents large molecules and microorganisms from entering the brain and cerebrospinal fluid (CSF). CNS capillaries also have fewer intracellular transport vesicles.

CSF is formed as an ultra-filtrate of blood plasma by the choroid plexus in the side ventricles of the brain. It slowly circulates through the ventricular system and along the outer surfaces of the spinal cord and the cerebral cortex, and is finally resorbed by arachnoid granulations protruding from meningeal veins. The total volume of CSF is approximately 125 ml in adults and the CSF turnover rate is ~ 0.5 ml/minute. This means that there is a complete exchange of CSF four to five times a day. The CSF acts as a cushion protecting the brain from mechanical trauma and it has important, but still poorly understood, regulatory functions for the chemical environment in the CNS [30]. The filter function of the choroid plexus makes it a weak point in the barrier between blood and CSF and the plexus has been hypothesised as a possible route for bacterial invasion from the blood stream [31]. There are also other potential routes of bacterial entry to the CNS. Animal studies have demonstrated the possibility of direct bacterial spread to CSF from the mucosal membranes in the upper airways [32].

Once bacteria have entered the CSF, they meet an almost ideal environment with plenty of substrate and a weak immunologic response because of the absence of complement and opsonising antibodies [33]. The bacteria can thus thrive and replicate at almost the same speed as in the laboratory [34]. Subsequently, the delayed immune response becomes activated, and the degranulation of activated neutrophils will cause a massive local inflammation and cerebral oedema with increased intracranial pressure (ICP) [35]. High ICP reduces the cerebral blood perfusion, ultimately resulting in destruction of nerve cells, and in the worst-case scenario cerebral herniation and death [36].

Clinical presentation and diagnosis of ABM

The clinical debut of meningitis is sudden with typically less than 24 hours of

symptoms at arrival to hospital. Longer duration of symptoms should lead to consideration of other diagnoses but could also indicate a concomitant focus of infection. Media otitis, sinusitis, or pneumonia is present in up to 40% of adults with ABM [26].

The classic symptoms with fever, headache, and neck stiffness is absent in more than half of patients but 95% have at least two out of the symptoms fever, headache, neck stiffness, and altered mental status [26].

There is however an overlap in the clinical picture of ABM with viral meningitis and several other conditions why a lumbar puncture (LP) is essential to definitively establish or reject the diagnosis [37]. The general rule is that LP should be performed promptly in cases of suspected ABM. One important exception is patients with focal neurological signs since this could indicate the presence of a space-occupying lesion such as a cerebral abscess or an intracranial haematoma. In these cases sampling of CSF by LP could cause a pressure gradient with the potential of increasing the existing brain shift caused by the space-occupying lesion [38]. Other contraindications for LP are signs of impeding cerebral herniation, on-going epileptic seizures, severe coagulopathy, and infection at the site of the LP [39].

Typical CSF findings in patients with ABM include an elevated opening pressure and visible CSF turbidity. If the latter one is present, it provides immediate bedside confirmation of the diagnosis of ABM. Cloudy CSF is highly specific for ABM but clear CSF does not rule out meningitis. Biochemical analyses of CSF can be determined within one hour and the characteristic findings in ABM are pleocytosis with neutrophil dominance, low glucose level, raised albumin, and elevated lactate [40]. Lactate is the biomarker with the highest sensitivity and it has the diagnostic advantage, in contrast to glucose, not to cross the BBB [41]. A high CSF lactate should therefore not be suspected to represent spill over from the blood in cases of concomitant sepsis. Normal or modestly elevated neutrophil count occur in 5–10% of patients and is associated with worse prognosis [26]. Administration of antibiotics before LP influences the CSF analyses with higher glucose levels, lower lactate, and lower protein levels but does cause any immediate changes in the CSF cell count [42, 43].

A rapid, cheap, and well-established method to identify bacteria in CSF is by microscopy after gram-staining. This has, in general, a high sensitivity for detection of bacteria in untreated cases of ABM although the diagnostic yield varies with different microorganisms. For example, the reported sensitivity for CSF gram-staining varies from 25%–65% for *H. influenzae*, 60–93% for *S. pneumoniae*, and 30–89% for *N. meningitidis* [44]. The sensitivity for

detection of *L. monocytogenes* is lower, ranging from 10 to 35% [44]. A Danish study on ABM of different etiological agents reported a slight decrease in diagnostic yield from 56% in untreated to 52% in patients who had been treated with antibiotics a short time before the LP [45].

CSF culture is the golden standard for the diagnosis of ABM. Cultures also provide important information about antibiotic susceptibility. If LP is performed before initiation of antibiotics, CSF cultures are positive in up to 66% of patients with ABM [45]. The diagnostic sensitivity is lower for patients who have already received antibiotic treatment. One study of meningococcal meningitis in children found no bacterial growth if LP was performed more than five hours after starting parenteral antibiotics [46]. Another retrospective study in children showed no bacterial growth later than four hours after antibiotic commencement except for cases of pneumococci with reduced antibiotic susceptibility [47].

Latex agglutination tests utilize antisera directed against capsular polysaccharides of meningeal pathogens. They are, however, of limited additional value in the diagnosis of ABM because the diagnostic yield of the tests for common pathogens is not higher than for CSF cultures, the sensitivity is dramatically reduced by antibiotic pre-treatment, and is very low in culture negative cases [48-50].

Blood cultures are a valuable contribution in the diagnostic arsenal of ABM and are especially important in cases where LPs are postponed until after antibiotic initiation. The sensitivity of blood cultures varies for each causative organism; 50–90% for *H. influnezae* meningitis [51, 52], 75% for pneumococcal meningitis [53-55], and 40–60% for meningococcal meningitis [56, 57]. As for CSF cultures, the diagnostic yield of blood cultures is also reduced with antibiotic pre-treatment. One study reported a decline from 66% in untreated patients to 48% in patients who had been given antibiotics [42].

The limited sensitivity of CSF cultures once antibiotics have been given leaves a certain proportion of patients without etiological diagnosis. There is therefore a need for supplementary diagnostic methods that are rapid, sensitive, accurate, and not affected by antibiotic therapy.

Polymerase chain reaction (PCR) techniques are now available for the detection of bacterial deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). There are currently two different approaches in use for microbiological diagnosis. The broad-range bacterial detection strategy uses PCR that targets gene sequences encoding the 16S region of bacterial RNA,

while multiplex PCR consists of multiple primer sets directed towards predefined pathogens. The broad-range strategy can further be combined with sequencing that allows identification of the bacterial species. A recent meta-analysis based on 14 studies on broad-range PCR reported a pooled sensitivity of 92% and a specificity of 94% for culture proven ABM [58]. Multiplex PCR studies have demonstrated sensitivity between 88% and 100%, and a specificity of 100% [59, 60]. Up until now, there has been a lack of studies investigating the influence of antibiotic pre-treatment on the sensitivity of PCR diagnosis of ABM. In a prospective study from the Czech Republic including 28 patients with meningococcal meningitis, PCR was positive in 81% of pre-treated patients, compared with 100% in patients without pre-treatment [61]. Major disadvantages of PCR assays are the complexity of the equipment and high costs that hamper the availability in resource poor settings.

An immunochromatographic test (ICT) for the identification of pneumococcal cell wall antigen (BinaxNOW®, Portland, ME, USA) is available for the detection of *S. pneumoniae* in CSF. The test is simple, quick, and cheap, which makes it suitable as a diagnostic tool also in resource limited settings. There are favourable results from studies in Africa and Asia showing sensitivity up to 95%–99% and specificity of 100% for children with culture-positive pneumococcal meningitis [62, 63]. A rapid diagnostic test based on immunochromatography for detection of *N. meningitidis* has recently been developed. It is highly sensitive and specific for the identification of serogroup A, C, Y, and Z but it cannot detect group B and X [64].

Treatment of ABM

ABM is a medical emergency that requires rapid administration of antibiotics. A delay in antibiotic administration of longer than three hours in patients with pneumococcal meningitis has been associated with increased mortality [65]. There are few randomized controlled trials of the efficacy of different antibiotic regimens in adult ABM. Antibiotic recommendations for treatment of ABM are therefore mostly based on general knowledge of pharmacological properties of the different antibiotics, animal experiments, case series, and on clinical experience of established treatment regimens. Regional differences in the incidence of different pathogens in ABM as well as the prevalence of bacterial strains with reduced antibiotic susceptibility are important factors in the choice of empirical treatment. Most recommendations and guidelines include a third generation cephalosporin (e.g. cefotaxime or ceftriaxone) as empirical therapy [66-68]. The cephalosporines lack activity against *L. monocytogenes*, which is why

ampicillin should be added when this agent can be suspected. A less established but theoretically good alternative is mono-therapy with meropenem [69-72]. Inclusion of vancomycin in the empirical treatment for ABM is recommended in geographical regions where the prevalence of cephalosporin resistant *S. pneumoniae* is above 1% [68]. Once the bacterial species has been identified and the pattern of susceptibility established, antibiotic therapy can be further modified, as suggested below.

S. pneumoniae. Benzylpenicillin is the standard therapy for meningitis with fully susceptible strains of pneumococci. Cefotaxime is effective in cases with intermediate penicillin susceptibility (MIC > 0.06–1.0 mg/L) and vancomycin can be added in cases of high-grade penicillin resistance (MIC > 2 mg/L) [67, 68].

N. meningitidis. As for pneumococci benzylpenicillin is well established as first hand therapy but strains with reduced penicillin susceptibility should be treated with cefotaxime or ceftriaxone [67, 68].

H. influenzae. Penicillin susceptible strains can be treated with ampicillin, whereas third-generation cephalosporines are recommended for β-lactamase producing strains [67, 68].

L. monocytogenes. Since Listeria species are intrinsically resistant against cephalosporines the recommended first line treatment is ampicillin or according to some guidelines benzylpenicillin [67, 68]. There have been conflicting results regarding the effects of adding aminoglycosides to ampicillin in the treatment of Listeria meningitis. One study demonstrated better survival when ampicillin was combined with gentamycin [73], whereas another report found no reduction of mortality by adding an aminoglycoside but found increased rates of kidney injuries instead [74].

Staphylococcus aureus. For methicillin sensitive strains some guidelines recommend high doses with isoxazolyl-penicillin. The passage of isoxazolyl-penicillins across the BBB is, however, poorer compared with other β-lactam antibiotics, and it is therefore theoretically better to give a cephalosporin. Good treatment results have been documented with the use of cefuroxime for nosocomial *S. aureus* meningitis [75]. For methicillin resistant strains, linezolid or vancomycin in combination with rifampicin, are the most commonly recommended therapies [67, 68].

Aerobic gram-negative bacteria are mainly found in cases of health-care associated meningitis. The emergence of multi-drug resistance in this group

of bacteria is worrying. Meningitis caused by *Acinetobacter baumanii* can normally be treated with meropenem. For patients with carbapenem resistant acinetobacter meningitis, the best-documented treatment is colistin. Colistin is normally administered intravenously, but can also be administrated intraventricularly [76]. Meningitis caused by *Psedomonas aerguninosa* can in most instances be treated with ceftazidime or meropenem [77].

The antibacterial activity of ß-lactam antibiotics is dependent on the time their concentration exceeds the minimum inhibitory concentration (MIC) for the infecting organism, so called time-dependent killing [78]. They should therefore be administered in a way as to optimize the time with concentrations above MIC at the site of infection. As mentioned earlier, the CNS has several unique features to be kept in mind when deciding antibiotic doses and dosing intervals for the treatment of ABM. The ß-lactam antibiotics are highly hydrophilic agents that diffuse poorly through the BBB, but their CNS penetration is significantly increased in the presence of inflammation [79]. Still, ß-lactam antibiotics must be administered in higher than normal doses in order to reach bactericidal concentrations in CSF. Luckily, most ß-lactam antibiotics are relatively non-toxic, why high systemic doses are generally well tolerated.

The concentration-time curves in CSF lag behind those in serum, even with the high doses used in the treatment of ABM, and the peak CSF concentration with a single dose of antibiotics will be reached first after approximately three hours [80]. This phenomenon makes it difficult to estimate antibiotic penetration into CSF by measuring CSF concentrations at single time points [81].

The mechanisms for elimination of ß-lactam antibiotics from CSF differ between antibiotics. The main route of elimination for benzylpenicillin is by active transport across the choroid plexus [82]. This mechanism accounts for approximately two-thirds of the elimination from CSF. Diffusion across ependymal surfaces into brain parenchyma and CSF turnover account for the remaining elimination [82]. The active removal of benzylpenicillin is inhibited by meningeal inflammation but returns to normal within days of antibiotic therapy [83]. The elimination of other ß-lactam antibiotics (e.g. cephalosporines, carbapenems, and amoxicillin) is mainly by passive diffusion. As a consequence, these agents reach efficient CSF concentrations for a longer time than benzylpenicillin [84, 85].

Animal experiments have demonstrated a detrimental effect of steroid therapy on antibiotic penetration into CSF [86, 87]. Two small clinical

studies in infants and children who received high doses of cefotaxime for the treatment of ABM, found similar median CSF concentrations of cefotaxime regardless of whether dexamethasone was used [88] or not [89]. So, the effect of steroids on the CSF permeability for antibiotics in humans is still unclear. Since ß-lactam antibiotics have significantly prolonged half-lives in CSF compared with serum, it has been proposed that dosing intervals longer than those traditionally used could be effective in the treatment of ABM [90]. An alternative way of administering antibiotics is by continuous infusion. This has been studied in 723 African children with ABM, where participants were randomised to either conventional intermittent administration or continuous infusion with cefotaxime. There were no differences in outcome between the two treatment arms, but a predefined subgroup analysis showed that children with pneumococcal meningitis treated with continuous cefotaxime where less likely to die than those given intermittent treatment [91]. This difference in survival could, speculatively, be an effect of longer time over MIC and more efficient bacterial killing with infusion compared to intermittent antibiotic treatment.

Dosing recommendations for ß-lactam antibiotics vary between different countries. In Sweden, the ß-lactam antibiotics benzylpenicillin, ampicillin, cefuroxime, and cefotaxime have traditionally been administered at longer intervals than in most other countries. The Swedish dosing of meropenem, 2 grams 8-hourly, does not differ from international praxis. Table 1 shows the antibiotic recommendations for ABM as presented in some recent reviews and guidelines, together with the Swedish dosing regimens that were used in paper I.

Table 1. Doses and dosing intervals for β-lactam antibiotics in adult bacterial meningitis.

	Benzylpenicillin			Ampicillin			Cefotaxime		
	dose (g)	interval (h)	dose/ day (g)	dose (g)	interval (h)	dose/ day (g)	dose (g)	interval (h)	dose/ day (g)
EFNS[1]-guideline Chandhuri 2008 [68]	2.4	4	14.4	2	4	12	2	6-8	6-8
van den Beek 2013 [66]	4	4	24	2	4	12	2-3	4-6	8-12
IDSA[2]-guidelines Tunkel 2004 [67]	4	4	24	2	4	12	2-3	4-6	8-12
Brink 2006 [92]	**4**	**8**	**12**	**4**	**8**	**12**	**3**	**8**	**9**

[1] EFSN, European federation of neurological societies. [2] IDSA, infectious diseases society of America

Animal studies have demonstrated that the outcome of ABM can be improved by modulating the meningeal inflammation with corticosteroid treatment [93]. A large European randomised controlled study showed that adjunctive therapy with dexamethasone was associated with reduction in mortality from 15% to 7% among adults with ABM [94]. The beneficial effect was most pronounced for patients with pneumococcal meningitis where the mortality decreased from 34% to 14% with steroid treatment. Subsequent clinical trials in Africa and Asia, however, could not demonstrate any benefit from steroid treatment in adult ABM [95-97]. A meta-analysis on individual patient data from all the above-mentioned clinical trials on adjuvant steroid treatment could not identify any beneficial effect from dexamethasone treatment on survival, neurological sequels, or severe hearing loss [98]. Based on this, current treatment guidelines recommend adjunctive steroid treatment for cases of ABM in high-income countries only [66].

In the early phase of treatment of ABM, admission to an ICU is recommended. ICU monitoring is essential for the detection of changes in the patient's mental status, development of seizures, for treatment of agitation, and initiation of mechanical ventilation in patients with hypoventilation or compromised airways [99]. For severe cases, with impending or manifest brain herniation, neurosurgical intervention with placement of intra-ventricular drain followed by continuous monitoring and lowering of the ICP can be considered [36].

1.3 Influenza

The influenza virus

There are three distinctive genera of influenza viruses; influenza A, B, and C. Influenza A virus is the most important human pathogen as it can cause severe disease and rapid worldwide spread: an influenza pandemic. It is also the most common type in seasonal influenza. Wild aquatic birds are the natural host for a wide spectrum of influenza A viruses but the virus can cause disease in several other animal species such as swine, horses, ferrets, and seals [100, 101]. Influenza B is an almost exclusively human pathogen. It usually causes mild disease and lacks pandemic potential. Influenza C is the genus with the least significance as a human pathogen. It can infect humans, dogs, and pigs. Human disease caused by influenza C is most often mild but severe disease can occur and it can infrequently cause local epidemics.

All influenza viruses have a similar overall structure [102]. The virus particle consists of a spherical envelope enclosing a nucleus of RNA and proteins.

The host-cell derived lipid membrane is studded with surface glycoproteins, the hemagglutinin (HA) and the neuraminidase (NA). HA mediates viral binding to the host cell and entry of viral RNA into the cytoplasm, while NA is involved in the release of virus from infected cells. HA and NA are antigens to which antibodies can be raised and are also targets for antiviral drugs. Influenza A viruses are divided into subtypes according to the antigenic characteristics of HA and NA. The standard nomenclature for influenza A viruses includes virus type, species from which is was isolated, isolate number, isolate year, and for influenza A, HA and NA subtyps [103]. The influenza A and B viral genome each consist of eight separated viral RNA segments, whereas the genome of influenza C is divided into seven segments. Segmentation of the genome makes the basis for *antigenic shift*, in which a whole RNA segment can be passed over from one influenza A strain to another. This antigenic shift can result in a virus with radically new pathogenic and antigenic properties that could give rise to an influenza pandemic. *Antigenic drift* involves the accumulation of mutations within genes that code for the antibody binding sites in HA resulting in minor and gradual changes of antigenic characteristics.

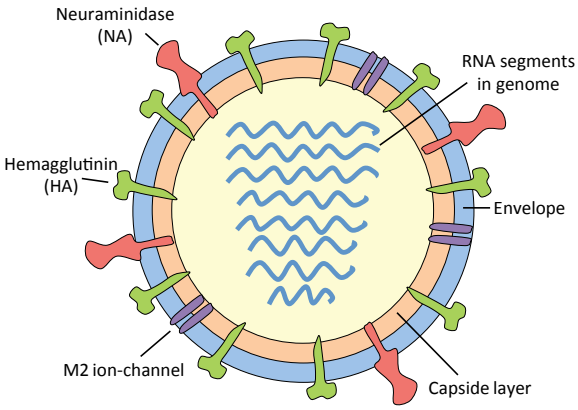

Figure 5. Influenza A virus

Human influenza infection

Shedding of influenza virus begins one day before onset of symptoms and last for five to seven days. People with the flu are generally most infective on the second and third day after onset of symptoms [104]. Virus can spread by direct person-to-person transmission, by the air-born route (inhalation of

droplets generated by sneezing people), and indirectly, by contact with contaminated surfaces [105].

The classic clinical picture of influenza is a sudden onset of fever, headache, cough, sore throat, nasal congestion, myalgia, weakness, and loss of appetite [106]. Seasonal influenza mostly gives rise to mild or moderately severe disease. Severe influenza is characterized by rapid destruction of the respiratory epithelium and profound lung pathology with development of respiratory failure. In addition, there is often an extensive systemic inflammatory response with the development of severe sepsis and multi-organ failure [107]. Secondary bacterial infections are common [108, 109]. The pathogenicity of influenza viruses is a complex phenomenon determined by the interplay of several genes involved in the virus interaction with the host [110]. Changes of the affinity for HA to the human respiratory epithelium, adjustments of virus polymerase activity, and mutations in the viral protein PB1-F2 promoting secondary bacterial infections, are examples of factors that may affect the pathogenicity of an influenza A strain [111]. The most important host factor is the degree of pre-existing immunity. Low immunity is associated with worse prognosis and furthermore, if a characteristic of the general population, a critical factor for the emergence of pandemic spread. Individual host factors associated with adverse outcome are: high age, chronic circulatory and respiratory diseases, diabetes mellitus, and conditions with impaired immunity. In fact, more than 90% of seasonal influenza-associated deaths occur in people of 65 years of age or older [112, 113].

There are at present two main groups of drugs for treatment of influenza. The adamantanes, amantadine and rimantadine, act as blockers of the influenza A M2 envelope protein. Because of previously extensive use of adamantanes, in humans as well as in poultry farming, there is now widespread resistance towards these drugs in most influenza strains [114]. The currently most used influenza treatment regimens, oseltamivir and zanamivir, are blockers of NA. Oseltamivir is available as a capsule for oral treatment while zanamivir must be delivered by inhalation. The oral route of administration has favoured the use of oseltamivir even if adverse drug effects, including nausea, diarrhoea, and neuropsychiatric events, are more common compared with zanamivir. A recent meta-analysis investigated the effects of NA-inhibitors in influenza A. It included all, published and unpublished, randomised placebo-controlled clinical trials. The final analysis comprised 42 studies with more than 25 000 patients. Confirmed cases of influenza that received treatment had an approximately half a day reduction of the time to first alleviation of

symptoms. Treatment had no effect on hospitalizations and there was no reduction in complications classified as serious [115].

Every year, the seasonal influenza causes a high number of hospital admissions. Respiratory failure with the need for mechanical ventilation is the predominant factor precipitating ICU admission. Respiratory support can be provided either by NIV or by invasive ventilation requiring intubation or tracheostomy (Figure 3). In case of critical hypoxic failure in mechanically ventilated patients there are several adjunctive therapeutic strategies to improve oxygenation. Treatment with neuro-muscular blockers can reduce the overall oxygen consumption and decrease the resistance in the thoracic wall and the diaphragm, thereby improving oxygenation and reducing the risk for alveolar baro-trauma. Inhalational therapy with locally acting vasodilators, such as nitric oxide and prostacyclins, can adjust impaired pulmonary auto-regulation, resulting in better perfusion in aerated alveoli and thereby an overall more efficient gas-exchange [116, 117]. Prone positioning can also improve the matching of pulmonary circulation and perfusion [118]. All these rescue strategies can be off limited effectiveness and for really severe cases, extra corporeal membrane oxygenation (ECMO) might be the only way to achieve sufficient oxygenation [119].

Influenza pandemics

The biology and ecology of influenza A virus, especially its ability to exchange whole gene segments between different strains following mixed infections and the ability of the virus to adapt to several avian and mammalian species, forms the basis for the capacity of the virus to cause devastating pandemics. For at least five centuries, there have been recurrent epidemics and pandemics of human influenza [120]. The 1918–1919 influenza A (H1N1) pandemic is estimated to have caused at least 50 millions of deaths worldwide, mostly among young and previously healthy individuals [121]. The later pandemics; the 1957–1958 ‘Asian’ (H2N2) influenza and the 1968–1969 ‘Hong Kong’ (H3N2) influenza were not close to being as severe as the Spanish flu, but they are calculated to have caused more than one million deaths each [122]. It has been estimated that if a strain with virulence similar to the 1918 influenza emerged today, the global death toll could be between 50 and 80 million people [123].

Figure 6. Soldiers from Fort Riley, Kansas, ill with Spanish influenza at a hospital ward at Camp Funston. (Photograph downloaded from open domain)

The ever-present threat of a new severe influenza A pandemic motivates global influenza surveillance programs and costly international and national plans for healthcare, vaccine production, and stockpiling of antivirals and antibiotics.

In the beginning of 2009, countries in the southern hemisphere observed a high and rapidly rising incidence of severe influenza [124]. A previously unseen strain of influenza A (H1N1) was isolated. Within a few months it had spread to all continents with cases reported from more than 70 countries and an early report from Mexico indicated a high rate of severe illness among previously healthy young adults [125]. On June 11, 2009, the Director-General of the World Healthcare Organisation declared the novel influenza A (H1N1) virus a pandemic [126].

This was the starting signal for the Swedish Pandemrix® vaccination program and for extensive planning and preparation of activities involving the entire Swedish health care sector. The Swedish College for Anaesthesia and Intensive Care appointed an expert group with the mandate to formulate guidelines for treatment of severe influenza in ICUs. This group also initiated internet-based collection of data on patients with influenza A (H1N1) treated in ICUs. The register was funded by The Swedish Association of Local Authorities and Regions (SKL) and the National Board of Health and Welfare. All Swedish ICUs were approached and recruited on a voluntary basis. The register was used for continuous reports to the funding authorities. In addition, it formed the basis for a scientific report (paper III) that is part of this thesis.

1.4 Necrotizing soft tissue infections

Definition and pathophysiology of NSTIs

Infections of the skin are among the most frequent conditions to be treated by general practitioners. Some infections, like superficial furuncles and cutaneous abscesses can often be cured with simple surgical drainage while others, such as erysipelas and infected animal bites often need antibiotics. More widespread cutaneous infections as well as post-operative wound infections are generally accompanied with fever and malaise, and often require hospital care with intravenous antibiotic treatment. Underlying conditions like diabetes mellitus, peripheral vascular occlusive diseases, and the use of intravenous drugs are risk factors for skin and soft tissue infections. Rarely, an infection of the soft-tissues can take a dramatic course with rapid local tissue destruction accompanied by a severe systemic inflammatory response. Hippocrates wrote the first medical text on severe skin infections: *"...would quickly spread widely in all directions. Flesh, sinews, and bones fell away in large quantities... There were many deaths. The course of the disease was the same to whatever part of the body it spread."* [127]. This is still an adequate description of the clinical picture for this type of infections. Since Hippocrates time, there have been many reports and publications on fulminant soft-tissue infections, and based on the causing organism, affected body parts, and other characteristics a wide number of definitions and classifications have been used [128, 129]. Many diagnostic terms have been suggested including: flesh-eating bacteria syndrome, suppurative fasciitis, streptococcal gangrene, necrotizing erysipelas, and necrotizing fasciitis. It has recently been suggested that the name necrotizing soft tissue infections (NSTIs) is the most comprehensive and clinically most useful term since diagnostic procedures and treatments are similar for all infections included in this entity. In a recent review NSTI was defined as: "… infections of any of the layers within the soft tissue compartment (dermis, subcutaneous tissue, superficial fascia, deep fascia, or muscle) that are associated with necrotizing changes" [130]. The predominant causing agent in NSTIs is *Streptococcus pyogenes (S. pyogenes)*. It is remarkable that this bacterium, typically causing superficial skin infections of limited severity, in rare cases can give rise to such a rapid and life-threatening disease progression. Since the mid 1980s there has been a worldwide increase in severe manifestations of *S. pyogenes* infections. The factors underlying this surge remain unknown [131].

The Lancefield classification divides *S. pyogenes* into different subclasses on the basis of serologic differences in the M protein. Some M-types are known to cause severe infections and the serotypes M1 and M3 are particularly associated with severe disease (NSTI and streptococcal toxic shock syndrome) [132]. The bacterium expresses a variety of different virulence factors contributing to the severe tissue destruction that is characteristic of NSTIs. Virulence mechanisms include host cell adhesion, immune evasion, and tissue destruction by bacterial enzymes [133]. Production of proteins acting as super-antigens with the capacity to non-specifically activate a large T-cell population precipitating the *"Streptococcal toxic shock syndrome"* is another potent virulence factor [134]. Host derived molecules can also be commandeered by bacteria to further contribute to tissue damage [133]. Less is known about specific virulence characteristics for colonic bacteria that comprise the second most prevalent bacterial agent in NSTIs. Host factors likely play an important role because many of the affected patients have diabetes mellitus and/or local compromising factors such as abdominal surgery, trauma, perianal abscess, or abdominal malignancy [135].

Clinical presentation and diagnosis of NSTIs

The early clinical picture of NSTIs includes local swelling, erythema and tenderness, and is often indistinguishable from a benign skin infection. Once the infection progresses, the signs and symptoms get more typical with development of tense oedema, pain disproportionate to appearance, skin discoloration, necrosis, and sometimes crepitus. These symptoms are strong warning signals that motivate urgent action. The patients often also have signs of a systemic inflammatory response including high fever, tachycardia, hypotension, and shock. Importantly, although the mentioned local findings are typical and quite specific for NSTI, their sensitivity is low, and they are initially present in less than half of patients with NSTI [136, 137].

Routine inflammatory markers, such as C-reactive protein (CRP) and white blood cell count are typically elevated. Based on the values of six common laboratory parameters: CRP, white blood cell count, haemoglobin, sodium, creatinin, and glucose, Wong and colleagues have constructed a simple scoring system to help discriminate between superficial and deep necrotizing soft-tissue infections [138]. In the original study this "Laboratory risk indicator for necrotising fasciitis" (LRINEC) a score ≥ 6 had a positive predictive value of 92% and a negative predictive value of 96% for NSTI.
The key diagnostic element once NSTI has been suspected is surgical exploration for inspection of underlying tissues and to secure samples for microbiological analyses [130]. If the initial suspicion of NSTI is low or moderate it is recommended to perform a bedside surgical incision in local anaesthesia. Such incision allows for a "finger test" whereby necrotic tissues

can be identified by an abnormally loose consistence and the possibility to dissect fascia planes with the finger [139]. In addition to cultures from the affected site and blood it is advisable to perform a bedside streptococcal test, using the standard rapid streptococcal diagnostic kit on extracted fluid [140].

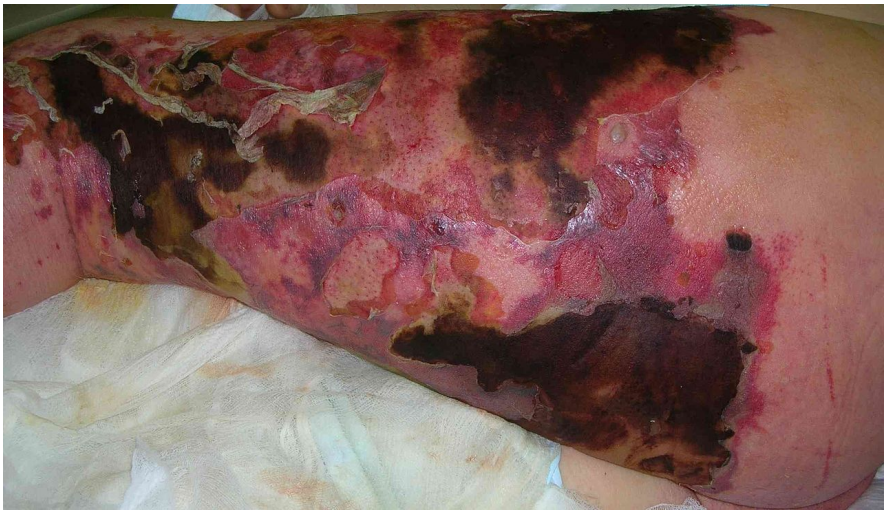

Figure 7. Person with necrotizing soft-tissue infection. The left leg shows extensive redness and necrosis. (Photography downloaded from open domain)

Treatment of NSTIs

The treatment of NSTI is based on three essential components: rapid antibiotic treatment, early surgical debridement, and monitoring and care in an ICU. Empiric antimicrobial therapy must cover a wide range of bacteria and a broad-spectrum β-lactam antibiotic, such as piperazillin/tazobactam or meropenem, is the first hand alternative [141]. For streptococcal infections, and in all cases before the aetiology is established, additional antimicrobial treatment with clindamycin is recommended [130, 141]. This is motivated by suboptimal activity of β-lactam antibiotics in very dense populations of bacteria. In this situation bacteria tend to divide at a slower rate and are as a consequence less accessible to cell wall antibiotics such as β-lactams [142, 143]. Clindamycin is an inhibitor of bacterial protein synthesis and its effect is therefore not dependent on bacterial division and cell wall synthesis. Furthermore, clindamycin may help by inhibiting bacterial toxin production, which can be crucial for controlling tissue destruction and the systemic inflammatory response [143]. There are however no human trials confirming an additional effect of clindamycin.

Early and aggressive surgical debridement of all devitalized tissue is a fundamental part of the therapy. The time from hospital admission to surgery is the strongest determinant for outcome. In an analysis of 65 NSTI patients, the average time to surgery was 90 hours in non-survivors compared with 25 hours in survivors [136]. A more recent study identified surgery within 24 hours after hospital admission as the only predictor of survival in a multivariate analyse [137]. Surgery has to be repeated daily until no devitalized tissue can be found [141]. After the acute phase of illness, most patients will need reconstructive surgery including skin grafting.

The third decisive component in the care of NSTI is treatment in an ICU where adequate monitoring, treatment of septic shock, and support of failing organs are essential for patients chances of survival.

A less established adjunctive therapy is the administration of high dose intravenous immunoglobulin (IVIG). There is experimental evidence for IVIGs capacity to neutralize streptococcal super-antigens, thereby protecting against the development of streptococcal toxic shock syndrome [144]. There is, however, limited support for IVIG in clinical trials; one case series has shown better survival with IVIG compared with untreated historical controls [145], while the only randomized controlled trial this far showed a tendency towards reduced mortality with IVIG, but the study was under-powered and did not allow for any general conclusions [146].

Another adjunctive therapy is hyperbaric oxygen (HBO) therapy in which the patient is enclosed in a chamber and breathes 100% oxygen at pressure > 1 atmosphere absolute. HBO increases the oxygen partial pressure in blood and tissues. Experimentally HBO has been associated with positive effects in infected tissues including: enhanced neutrophil bacterial killing, reduction of tissue oedema, increased local antibiotic activity, and improved survival of ischemic tissue [147]. Clinical data for a role of HBO as part of NSTI treatment are of very poor quality and based merely on uncontrolled, observational case series [148].

Mortality among patients with NSTI is primarily a consequence of early therapy-resistant septic shock while later deaths are caused by multi-organ failure or complications such as secondary hospital-acquired infections [136]. There is a large variation in the NSTI mortality rate, ranging from 8% to 30% [136, 149-153]. Caution is warranted when comparing treatment results from different studies because of large inter-study variations of important variables such as demographic characteristics, causative agents, and initial severity of illness. There is also a disparity in how mortality is registered in different

studies, some use in-hospital mortality and some use time limited (28 days, 30 days, 90 days) mortality.

As for many other rare and complex conditions, it is common to centralize the care of patients with severe NSTI to large tertiary hospitals, often with HBO facilities. Sahlgrenska University Hospital/East serves as a regional centre for the treatment of patients with NSTI in the Western part of Sweden. Treatment with HBO is available around the clock and transport of patients from other hospitals in the region is facilitated by an ambulance helicopter service. All patients with NSTIs treated at this unit are included in a quality register. There are many potential benefits of centralizing care for patients with NSTIs, but this concept has also been questioned. One aspect of centralized care is that patients with a severe infection, in this case NSTI, have to be transferred from one hospital to another, and moving a critically ill patient is not entirely without risk. This early inter-hospital transfer has been identified as a risk factor for increased mortality in patients with NSTI in two studies from the USA [154, 155].

2 AIM

The overall aim of this thesis was to elucidate some defined aspects of the diagnosis and treatment of three very severe infections requiring intensive care, infections that we see on a regular basis in our ICU at the Department of Infectious Diseases.

The specific aims were:

- To investigate the outcome of acute bacterial meningitis in adult patients treated with β-lactam antibiotics in 8-hour dosing intervals.

- To define the time windows for positive CSF PCR and CSF ICT in community-acquired acute bacterial meningitis.

- To determine the characteristics of patients with Influenza A (H1N1) during the 2009–2010 pandemic treated in Swedish ICUs, and particularly, to describe the clinical course and outcome depending on respiratory support strategy.

- To analyse patient characteristics, inter-hospital transfer, treatment, and outcome of patients with necrotizing soft tissue infections at Sahlgrenska University Hospital/East from 2008 to 2011.

3 PATIENTS AND METHODS

3.1 Study participants

Paper I

All adult patients (≥16 years of age) with community-acquired ABM treated at Sahlgrenska University Hospital, Gothenburg, Sweden during a 6-year period (1999–2004) were included. For inclusion, the clinical diagnosis had to be supported by CSF neutrophilic pleocytosis, and/or a positive culture from CSF or blood. Patients also had to be treated with β-lactam antibiotics. Patients with infections related to ventricular shunts or other CNS indwelling devices were excluded. We also omitted patients having primarily epidural or intra-cerebral infections with meningeal involvement and patients with an ABM caused by *Mycoplasma pneumoniae*. Patient characteristics and data on treatment and outcome of therapy were retrospectively extracted from medical charts. We identified possible participants by searching the hospital database of medical diagnosis according to International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10). The final analysis included 77 patients with total 79 episodes of ABM.

Paper II

This prospective study recruited patients 16 years of age or older, with a medical history and clinical signs compatible with community-acquired ABM. Participants had to have a bacterium identified by CSF culture, CSF PCR, or CSF ICT, or a positive blood culture in combination with CSF pleocytosis defined as a leukocyte count of more than 100 x 10^6/L. Patients with indwelling CNS devices with community-acquired ABM were accepted for the study, but patients with post-operative infections following neurosurgery were excluded. We also excluded patients with epidural or intra-cerebral infections and secondary meningeal involvement.

In total, CSF was collected from 25 subjects with ABM admitted to the Department of Infectious Diseases, Sahlgrenska University Hospital, Gothenburg, Sweden, between January 2007 and April 2014. Informed consent was obtained from all study participants or, for unconscious subjects, from a relative.

Paper III

This study comprised 126 out of the 136 patients with laboratory-confirmed novel influenza A (H1N1), who were treated in Swedish ICUs during the period August 2008 to March 2009. Ninety-five of the patients were included via the national registry of patients with the pandemic flu. Another 31 patients were identified and included by information provided from the Swedish Institute for Communicable Disease Control. For the latter cases data collection was based on copies of medical charts. Analyses of ventilatory support strategies were only conducted on adult patients (≥16 years of age).

Paper IV

All individuals with NSTIs admitted to the ICU at Sahlgrenska University Hospital/East during the period 1 January 2008 to 31 December 2011 were included in the study. Study inclusion was based on the surgeon's description of necrosis engaging the dermis, subcutaneous tissue, fascia, or muscle during primary surgical revision. Study criteria where met by 29 patients. Patient data was extracted from the local quality register of patients with NSTIs at the Sahlgrenska University Hospital/East. For transferred patients, data concerning the care in other hospitals was extracted from copies of medical charts.

3.2 Methods

Paper I

Level of consciousness was assessed using the Reaction Level Scale. Outcomes were graded according to the Glasgow Outcome Scale. Bacterial cultures of CSF and blood were performed according to hospital routines. Resistance testing was carried out with a standardized disc diffusion method. Mortality was defined as in-hospital mortality.

Paper II

In addition to the first, clinically justified LP, participants underwent one or two more LPs during the first 10 days after initiation of antibiotic treatment. CSF was analysed for concentrations of polymorphonuclear and mononuclear leukocytes, glucose, albumin, and lactate. Microbiological analyses performed on CSF samples were cultures, broad-spectrum PCR, and ICT. Blood cultures were drawn on the day of admission only.

Bacterial cultures of CSF and blood were performed according to hospital routines. Resistance testing was carried out with a standardized disc diffusion method.

For broad-range PCR the CSF was centrifuged, after which DNA was extracted. Forward and reverse primers were used to amplify a segment of the 16s rRNA gene. A positive finding was followed by genome sequencing. The bacterial species was determined by comparing the sequencing products with sequences in a database. The method is described in detail in paper II.

The CSF *S. pneumoniae* ICT (BinaxNow®) was performed according to instructions by the manufacturer.

Paper III

NIV was defined as continuous positive airway pressure with or without pressure support ventilation delivered through a facial mask or another external connection device. Participants were classified into three groups: (a) supplementary oxygen only, (b) NIV only or followed by invasive ventilation, or (c) invasive ventilation without preceding NIV. Patients who were treated with NIV for less than one hour prior to intubation were classified as treated with invasive ventilation without preceding NIV (group c). The primary outcomes were mortality at 28 and 90 days following hospital admission. We also determined time with invasive ventilation, length of ICU stay, length of hospital stay, and complications such as radiographically diagnosed pulmonary barotrauma, central venous catheter-related blood stream infections, and VAP. Severity of illness was estimated in adults (>16 years) using Simplified Acute Physiology Score 3 (SAPS 3).

Paper IV

The main outcomes were mortality at 30 and 90 days following hospital admission. We also used a composite outcome, 'adverse outcome', which was defined as the 30-day mortality or amputation of an extremity. Other outcomes were time on mechanical ventilation, length of ICU stay, and length of hospital stay. General severity of illness was estimated using the Acute Physiology and Chronic Health Evaluation 2 (APACHE 2). The score was calculated for the first 24 hours after admission to the ICU at Sahlgrenska University Hospital/East. The LRINEC score was calculated for all patients.

3.3 Statistics

Descriptive analyses included frequency analysis (percentage) for categorical variables and medians [with interquartile range (IQR)] or means [with standard deviation (SD)] for continuous variables. In paper II comparison of CSF levels of cells, glucose, albumin, and lactate at different time periods was performed with the Kruskal-Wallis one-way analysis of variance. In paper III, relative risk of death, with the 95% confidence interval (CI), was calculated for patients treated with NIV compared with immediately intubated patients. Kaplan-Meier curves were used for time to event analyses. In paper IV comparison between two independent groups was performed with Fisher´s exact test for categorical variables and with the Mann-Whitney U-test for continuous variables.

3.4 Ethics

Ethical approvals were obtained from the Regional Ethical Review board at the University of Gothenburg: Dnr 074-06 (paper II), Dnr 557-10 (paper III), Dnr 866-12 (paper IV). Written informed consent was obtained from patients participating in paper II.

4 RESULTS AND DISCUSSIONS

4.1 Paper I

Results

During the six-year study period, a total of 80 adult cases of community-acquired ABM were identified. Out of these, 79 were mainly treated with a β-lactam antibiotic, dosed 8-hourly or less frequently, and therefore included in the final analysis. Median age of the patients was 49.5 years (range 16–93) and 50% were female. Compromising conditions (e.g. malignancy, autoimmune disorders, immunosuppression, diabetes mellitus, alcoholism, or historical neuro-trauma) were present in 35%.

S. pneumoniae was the most prevalent pathogen, identified in 47.5%, followed by *N. meningitidis* (12.5%) and *L. monocytogenes* (5.0%) (Table 2).

Table 2. Aetiology and outcome of acute bacterial meningitis.

Bacteria	No. of episodes (%)	No. of deaths	No. of sequels
S. pneumoniae	37 (47.5)	2	11
N. meningitidis	10 (12.5)	1	
L. monocytogenes	4 (5.0)		1
S. aureus	3 (3.8)	1	
K. pneumoiae	3 (3.8)		2
H. Influenzae	3 (3.8)		
E. coli	2 (2.5)		
S. pyogenes	1 (1.2)		1
Coag. neg. staph.	1 (1.2)		
Unknown	15 (18.8)	1	4
Total	**79**	**5 (6.3%)**	**19 (24.1%)**

The most frequently used empirical antibiotics were: cefotaxime + ampicillin (35%), ampicillin (22%), and cefotaxime (13%). Adjunctive therapy with high dose corticosteroids was given in 59% of the patients. Benzylpenicillin was the most frequently used antibiotic after culture results were obtained and it was overall the most used antibiotic (Table 3). Seventy-two patients (91%) were treated in an ICU and one patient was supplied with a device for intracranial pressure monitoring. In hospital mortality was 6.3% and 24.1% of the patients developed neurological sequels (Table 2).

Table 3. Usage of β-lactam antibiotics.

Antibiotic	No. of treatment courses	Median dose and range (g)	Dose interval (h)	Median days and range
Benzylpenicillin	43	4 (3[a]–4)	8	8 (1–15)
Ampicillin	54	4 (2[a]–4)	8 (8–12[b])	2 (1–18)
Cefotaxime	56	3 (2[a]–3)	8 (8–24[b])	4 (1–14)
Cefuroxime	13	1.5[a] (0.75[a]–3)	8	2 (1–10)
Ceftazidime	2	0.5[a] and 2	12	2 and 5
Meropenem	14	2 (0.5[a]–2)	8 (8–24[b])	3 (1–13)

[a] Lower than recommended doses given as follows: in 10 episodes before correct diagnosis established, in 3 episodes because of renal failure, in 1 episode for subsequent nosocomial infection, and in 2 episodes for unknown reasons. [b] Dose intervals longer than 8 h used at some phase of the treatment in 3 episodes of meningitis with acute renal failure, and in 1 episode for unknown reasons.

Discussion

The mortality was low (6.3%) among our patients with community-acquired ABM. At the time this study was performed most comparable publications reported mortalities ranging from 20% to 30% and there were only two studies with similar low mortalities, 11% and 8.7%, respectively [94, 156]. Mortality in later studies from high-income countries varies between 9.9% and 23% [157-163]. The Swedish national quality register for adult ABM reported an in hospital mortality of 6.1% for the 132 patients registered 2013 [164].

The incidence of neurological sequels among patients in our study is rather high (24%) compared to the studies mentioned above (8–24%). One possible explanation for this discrepancy could theoretically be that an increased number of survivors of such a severe infection in the CNS could lead to a higher incidence of neurological deficits. It is also probable that the inter-study variation could, at least partly, be due to methodological differences in parameters such as follow up times and diagnostic criteria for sequels.

Based on published data, it is not possible to determine the reasons for the low mortality among our patients. One of the most important risk factors for death in ABM is the level of consciousness at the time of hospital admission [165]. The proportion of patients with decreased level of consciousness was in the same range among our patients as in studies with higher mortality [92]. One difficulty is that we cannot compare the proportions of patients with *deep* coma (RLS $\geqq$ 4), since this information is not stated in most studies. In our study, 28% of the patients had RLS 4 or more on arrival to the hospital.

We can therefore not exclude that this important risk factor associated with a severe outcome has varied between studies. There may also be important differences in the overall care of patients between different sites. The incidence of patients monitored in an ICU is often not reported, but the proportion of ICU-admissions (91%) among our patients is probably unusually high and could be a factor that favours survival.

The crucial question is if our results allow for general statements regarding the optimal dosing of β-lactam antibiotics in patients with ABM. The pharmacodynamics and pharmacokinetics of antibiotics in bacterial CNS infections are as described in the introduction, highly complicated, incompletely understood, and to a large extent not confirmed in humans. We know that the BBB constitutes an obstacle for the diffusion of β-lactam antibiotics into CSF, but we also know that the elimination of β-lactams is slower from CSF than from most other body compartments. Meningeal inflammation causes an increase of BBB permeability with increased influx of β-lactam antibiotics but this increased permeability is possibly attenuated by steroid treatment and the permeability gradually normalizes during the course of antibiotic treatment. The net effect of all these parameters on the total CSF exposure over-time of β-lactams is not known. Additionally, immunity inside the CNS is in many ways less effective compared with other compartments but if, and to what existent, this has implications on required "time over MIC" for optimal antimicrobial effect is unclear.

Our results cannot prove that a regimen with 8-hourly dosing of β-lactam antibiotics is equally effective as the more widely adopted regimens with 6 or 4-hourly dosing. In the light of our exceptionally good treatment results it can nevertheless be stated that the potential difference in clinical effect between 8-hour dosing and more frequent dosing regimens could at the most be of minor clinical significance. Rapid diagnosis, prompt antibiotic treatment, and adequate monitoring in an ICU, remain the central elements in the treatment of patients with bacterial meningitis.

4.2 Paper II

Results

This study was based on 70 prospectively collected CSF samples from 25 individuals with community-acquired ABM. All patients were treated with high-dose β-lactam antibiotics and all but one patient received adjunctive therapy with corticosteroids. LP was performed prior to the first dose of

antibiotics in 40% of the patients and blood cultures were drawn before initiation of antibiotics in all but three participants.

Table 4. Results of PCR analysis for all patients during the study period.

Patient code	Bacteria	CSF PCR day 0	CSF PCR days 1-3	CSF PCR days 4-6	CSF PCR days 7-10
07-4	*S. pneumoniae*	+	+	+	
09-3	*S. pneumoniae*	+	+	+	
11-1	*S. pneumoniae*	+	+		
11-6	*S. pneumoniae*	+	+	+	
12-11	*S. pneumoniae*	+	+	+	
12-15	*S. pneumoniae*	+	+	+	
13-1	*S. pneumoniae*	+		+	+
13-3	*S. pneumoniae*	+	+		+
13-6	*S. pneumoniae*		+	+	–
13-8	*S. pneumoniae*	+	+	–	
13-11	*S. pneumoniae*		+	+	
14-2	*S. pneumoniae*	+		+	–
08-5	*N. meningitidis*	+	+		
11-2	*N. meningitidis*	+	+	+	
12-1	*N. meningitidis*	+	–	–	
08-2	*N. meningitidis*		+	+	
10-6	*H. influenzae*	+	+	–	
11-5	*H. influenzae*	+	–		
13-4	*S. pyogenes*			+	–
14-4	*S. pyogenes*	+		+	
08-7	*L. monocytogenes*	+	+	–	
07-1	Strep. group C	+	+	+	
12-12	*P. multocida*	+		+	
12-10	*E. faecalis*	+		–	–
07-5	Gemella specie	+	+	–	
		Day 0	Days 1-3	Days 4-6	Days 7-10
	Positive/total	21/21	17/19	15/21	2/6
	Sensitivity	100 %	89 %	71 %	33 %

Grey areas indicate that the analysis has not been performed. CSF, cerebrospinal fluid; PCR, polymerase chain reaction; ICT, immunochromatographic test

We identified nine different bacterial species. The most frequently identified bacteria were *S. pneumoniae* (48%), *N. meningitidis* (16%), *H. influenza* (8%), and *S. pyogenes* (8%). There were no contradictory findings between the different diagnostic methods. Of all samples the infecting bacteria could

be identified by CSF culture in 44%, by blood culture in 58%, and by CSF PCR in 100% of the patients. The ICT was positive in 92% of the cases with pneumococcal meningitis.

There were no positive CSF cultures in samples from day 1 or later. The diagnostic sensitivity of CSF PCR was 89% on days 1–3, 71% on days 4–6, and 33% on days 7–10 (Table 4). In patients with pneumococcal meningitis, the ICT was positive in 88% on days 1–3, 90% on days 4–6, and 75% on days 7–10. The PCR performance over time in identifying *S. pneumoniae* was 100% on days 1–3, 90% on days 4–6, and 50% on days 7–10.

All CSF chemical analyses changed significantly from highly pathological levels towards normal during the study period (Figure 8).

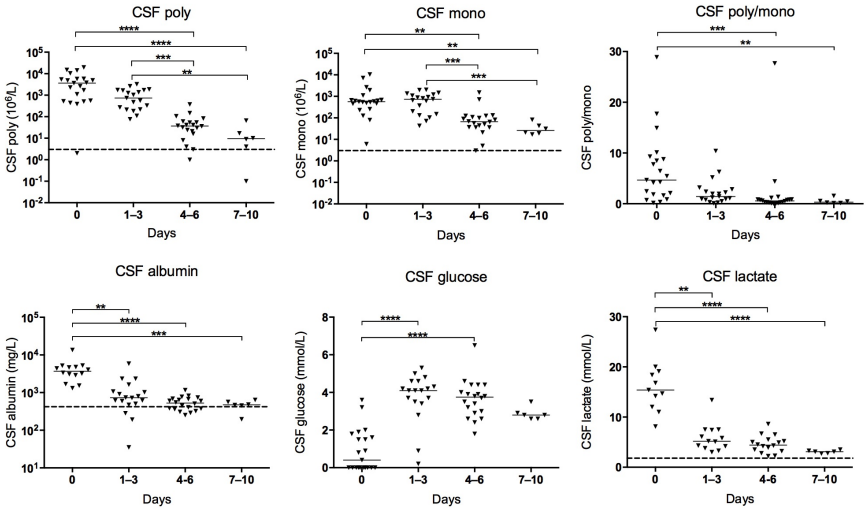

Figure 8. CSF levels of biochemical markers in different time periods during the treatment of bacterial meningitis.

Discussion

Our study shows that the diagnostic sensitivity of broad-range PCR is high for CSF samples taken during the first week of treatment. This is in agreement with a smaller previous study of 11 cases of pneumococcal meningitis in infants where PCR and ICT remained positive for up to 20 days after the initiation of antibiotics [63].

Culture negative ABM is common among adults and is most often the result of antibiotic administration before LP. PCR is much less affected by antibiotic pre-treatment and our study provides evidence to perform a diagnostic LP for PCR up to one week after initiation of therapy in ABM without positive cultures. This long time window for PCR is of particular clinical relevance in patients where early LP is contraindicated because of septic shock, signs of impeding cerebral herniation, severe coagulopathy, or infection at the site of the LP. For these patients it will often take several days before it is safe to perform a LP.

There is one published study comparing the over-time performance of CSF cultures with broad-range PCR for patients with shunt-associated bacterial meningitis [166]. CSF cultures from patients with shunt-associated meningitis were to a large extent culture positive several days after the start of antibiotic treatment. Broad-range PCR increased the probability in finding an aetiological agent for these patients at every time-point, but there was no difference in the incidence of positive findings over time when comparing cultures with PCR. The divergence of results for neurosurgical patients compared with our patients with community-acquired ABM is very interesting and we recognise two plausible explanations. Firstly, in the neurosurgical patients, the predominant bacteria were opportunistic agents, such as coagulase-negative staphylococci and gram-negative enteric bacteria. Some of these bacteria are less susceptible to antibiotics compared with the bacterial species commonly found in community-acquired ABM (i.e. *S. pneumoniae, N. meningitides, and H. influenzae*), which could result in slower bacterial killing and extended culture positivity. Secondly, the intra-ventricular shunts provide surfaces for bacterial biofilm formation and may thereby contribute to prolonged bacterial survival among neurosurgical patients.

The major drawback of PCR is that it does not readily provide information about antibiotic susceptibility for the identified bacteria. This problem is particularly important in meningitis caused by *S. pneumoniae* in parts of the world where the prevalence of penicillin-resistant strains is high. Although there are methods for detecting the most common resistance genes in *S. pneumoniae* there is always the possibility that new mutations will appear. This is why cultures with susceptibility testing provide the only absolutely safe information on patterns of resistance. The identification of the bacterial species is, however, in most cases enough for safe narrowing of the antibiotic therapy and for guiding of the length of treatment.

The sensitivity and time window for positivity of the ICT for *S. pneumoniae* was similar to the broad-range PCR. Remarkably though, there was one case of pneumococcal meningitis, confirmed by both CSF culture and PCR that was negative in ICT. This finding of positive PCR but negative ICT persisted in all samples taken at three different time points during the course of treatment. This could suggest a pneumococcal strain with a variety of the polysaccharide c antigen not detected by the ICT. We have not been able to find any observations of this kind in the literature. We have this *S. pneumoniae* strain stored in the laboratory and plan to investigate it further.

With the obvious limitation of only detecting *S. pneumoniae*, ICT has several advantages. It is inexpensive and does not require advanced laboratory techniques, which makes it suitable for use in low-income countries with limited health-care resources. It has been tested in some African and Asian countries and has been shown to have additional diagnostic value even in environments where CSF cultures are available [167]. Our study demonstrates persistent sensitivity for the ICT during the first week of antibiotic treatment, which justifies a late LP with ICT analysis in ABM of unknown aetiology.

Because of the excellent performance of broad-range PCR there is however little if any additional diagnostic value of ICT, why the use of ICT only can be recommended in settings where PCR not is available.

It is important to be cautious when deciding how to use new diagnostic tools and to consider to what extent they could and should change the total diagnostic process. Potentially, there is a risk that the high probability for a positive PCR result even with antibiotic pre-treatment could influence the diagnostic routine towards postponing LP. Since LP and CSF analysis is the only way to definitively establish the ABM diagnosis it is probable that such a tendency would lead to an increase in both under- and over-diagnosis of ABM. The old rule to always, unless clearly contraindicated, perform an immediate LP in cases of suspected ABM is still valid.

Repeated CSF samples also gave us the opportunity to analyse the over time changes of chemical routine analyses (cell count, glucose, albumin, and lactate) performed for the diagnosis of CNS infections. Normalisation of these parameters is rapid and for most patients the typical bacterial pattern has disappeared within the first days of antibiotic treatment, which explains why a delayed LP could easily be misinterpreted. The observation that, after a few days of antibiotic treatment, the mononuclear leukocytes typically outnumber the polymorphonuclear leukocytes is especially important, as this

could be mistaken for a sign of a viral CNS infection. The over time change towards mononuclear dominance has previously been described in children [168], but to our knowledge, not previously in adult ABM.

4.3 Paper III

Results

During the period August 2009 to February 2010, 136 cases of laboratory verified influenza A (H1N1) were admitted to Swedish ICUs. A population based surveillance reported that around 500 000 individuals fell ill with influenza during the pandemic in Sweden [169]. The incidence of intensive care can then be determined to 27 per 100 000 infected individuals. We could retrieve detailed enough data for 126 out of the 136 individuals admitted to ICUs, and these were included in the study. The median age was 44 years (IQR 28–56) and 66% were male. The age distribution was: < 16 years 13%, 16–65 years 80%, and > 65 years 7%. Major co-morbidities were present in 41%. Chronic pulmonary disease (26%) and diabetes mellitus (16%) were the two most prevalent co-morbidities. Obesity (BMI > 30 kg/m^2) was observed in 39% of adult patients. This is twice as high as the prevalence in the general adult Swedish population. The median time from onset of influenza symptoms to hospital admission was six days (IQR 3–7) and the median time from arrival in the emergency department to ICU admission was 10 hours (IQR 1–28). The majority of patients (56%) had visited a hospital or a primary health-care facility because of their influenza symptoms before hospital admission. Of these, 54% had been prescribed antibiotics, while only three individuals (5%) had been prescribed oseltamivir.

The treatments provided were: mechanical ventilation (NIV and/or invasive ventilation) in 85%, renal replacement therapy in 21%, and circulatory support with vasopressors or inotropes in 56%. The median time on mechanical ventilation was 13 days (IQR 6–24) for the 76 patients (63%) requiring invasive ventilation. Respiratory rescue therapies (muscle relaxants, nitric oxide inhalation, prostacyclin inhalation, prone positioning, and ECMO) were used in 29%. Among these, 16/126 (13%) were treated with ECMO.

Corticosteroids were given to 52% of the patients. There was no difference in the 90-day mortality between patients that had been treated with (11%) and without (10%) corticosteroids. Antibiotics were given to 98% of the patients and 91% were given oseltamivir. Four of these patients were also given inhalation therapy with zanamivir.

Fourteen patients (11%) died within 28 days and 21 patients (17%) died within 90 days after ICU admission. Non-survivors where generally older, had a higher prevalence of co-morbidities, and had signs of more severe respiratory disease on ICU admission compared with survivors. The median BMI was higher for survivors than for non-survivors (28 vs. 26 kg/m^2). Median time from ICU admission to death was 19 days (IQR 6–37). The overall median length of stay in ICU for all patients was 9 days (IQR 3–21), and the median length of stay in hospital was 19 days (IQR 9–36).

Detailed information about respiratory support was available for 109 of the 110 adult patients. Sixteen of these (15%) were treated with supplementary oxygen via a facial mask or nasal catheter only, while 67 (61%) were treated with NIV. Forty-five (67%) patients receiving NIV were later intubated for invasive ventilation. Twenty-six patients (24%) were intubated and started on invasive ventilation without any preceding NIV. Median time from arrival in the ICU to intubation was 12 h (IQR 5–23) for patients treated with NIV before intubation and 2.5 h (IQR 1–10) for those started on invasive ventilation without preceding NIV.

Patients treated with supplementary oxygen only were younger, had fewer co-morbidities, and less severe acute illness in terms of SAPS 3 compared with the other two groups. Patients directly started on invasive ventilation were older (median age 52 versus 49 years), a higher prevalence for major co-morbidities (50% versus 44%), and lower PaO_2/FiO_2 ratios at ICU admission (80 versus 86 mmHg) compared with patients started on NIV. The 28-day mortality did not differ for patients started on NIV compared with patients who were intubated directly, but the 90-day mortality was 25% and 12% (relative risk 2.2, 95% CI 0.7–6.9), respectively (Figure 9 and Table 5).

Discussion

The incidence of ICU admissions in Sweden during the pandemic phase of Influenza A (H1N1) – 1.5/100 000 inhabitants – was less than half compared with incidences reported from Australia and New Zealand but in the same range as reported from Denmark [170, 171]. One explanation for this discrepancy could be how the countries approached the controversial issue regarding vaccination during the pandemic. Vaccination could have a major impact on the need for ICU treatment during influenza pandemics. In Sweden, vaccination of the general population was initiated in mid-October 2009. According to official statistics from the Swedish Institute for Communicable Disease Control, around 60% of the population was vaccinated with at least one dose of Pandemrix® vaccine. Pandemrix® has

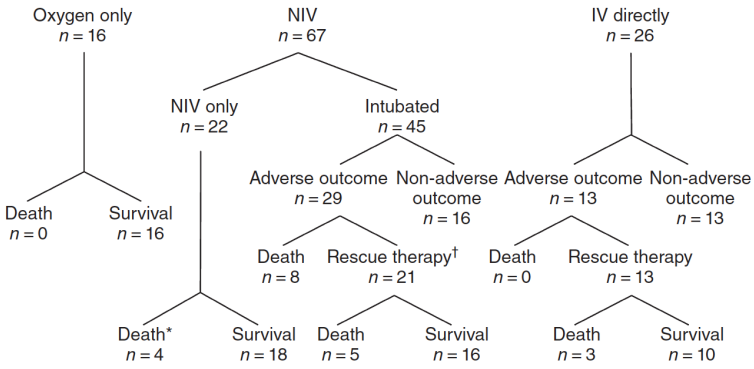

*Figure 9. Outcome for adult patients grouped after level of respiratory support. *Death defined as 90-day mortality. Rescue therapy includes nitric oxide inhalation, treatment with muscle relaxants, and extracorporeal membrane oxygenation. NIV, non-invasive ventilation, IV, invasive ventilation.*

Table 5. Treatment and outcome for adult patients grouped according to level of respiratory support.

	All adult patients (n=110)	**Oxygen only** (n=16)	**NIV**[1] (n=67)	**IV**[2] **directly** (n=26)
Treatments % (no./total no.)				
Oseltamivir	92% (99/107)	81% (13/16)	94% (61/65)	96% (24/25)
Antibiotics	98% (105/107)	93% (13/14)	98% (65/66)	100% (26/26)
Vasopressors or inotropes	60% (65/108)	0% (0/16)	63% (41/67)	88% (23/26)
Corticosteroids	52% (55/105)	31% (5/16)	56% (35/62)	54% (14/26)
Dialysis	24% (26/106)	7% (1/15)	20% (13/64)	46% 12/26
Respiratory rescue therapy				
Any rescue therapy	32% (35/107)	n.a.	31% (21/67)	54% (13/24)
Nitric oxide	4% (4/102)	n.a.	3% (2/63)	9% (2/22)
Prostacycline	5% (5/102)	n.a.	6% (4/62)	4% (1/23)
Prone positioning	9% (9/104)	n.a.	8% (5/63)	17% (4/24)
Muscle relaxation	15% (15/103)	n.a.	16% (10/63)	17% (4/23)
ECMO[3]	15% (16/110)	n.a.	15% (10/67)	23% (6/26)
Outcomes %, (no./total no.)				
28-days mortality	12% (13/110)	0% (0/16)	15% (10/67)	12% (3/26)
90-days mortality	18% (20/110)	0% (0/16)	25% (17/67)	12% (3/26)
Days with IV, median (IQR[5])	15 (7-24)	n.a.	14 (8-24)	16 6-33
Days in ICU, median (IQR)	11 (4-25)	1 (1-3)	12 (5-26)	17 8-32
Days in hospital, median (IQR)	23 (11-40)	6 (4-13)	24 (12-39)	24 16-57
Duration, ED to death[6] - days, median (IQR)	20 (6-38)	No deaths	28 (12-39)	7 n.a.

[1] NIV, non-invasive ventilation; [2] IV, invasive ventilation; [3] ECMO, extra corporeal membrane oxygenation; [4] adverse outcome defined as dead within 90-days or usage of any rescue therapy; [5] IQR, inter-quartile range; [6] based on 90-day mortality. n.a.: not applicable

since then been shown to increase the risk for developing narcolepsy [172, 173]. A recent epidemiological survey estimated the Swedish Pandemrix® vaccination campaign to have caused 84 extra cases of narcolepsy among individuals younger than 20 years of age [174]. The tragedy of the vaccine-caused narcolepsy cases combined with the fact that the pandemic turned out to be only of moderate severity has led to questioning whether it was right or not to mass vaccinate. On the other hand, vaccination might have reduced the number of individuals with severe influenza in need of ICU treatment. Speculatively, it is possible that without the vaccination programme, Sweden would have reached the same incidence of ICU admissions as the un-vaccinated populations in Australia and New Zealand. In that scenario, Swedish ICUs would have faced a doubling of severe influenza cases and presumably another 20 influenza deaths.

Almost all patients in our study where treated with oseltamivir. It is largely unknown if oseltamivir is beneficial in cases of influenza with manifest respiratory failure, but some reports indicate a reduced risk for ICU admission with early oseltamivir treatment in hospitalized patients [175, 176].

Since the lung injury in influenza to a large extent is a result of excess local inflammation there is theoretically a potential for protective effects from immunomodulatory therapy with corticosteroids, but there is no clinical evidence that corticosteroids are of benefit in severe influenza. On the contrary, the use of steroids has been associated with worse outcomes in patients with influenza [177, 178]. We could not confirm these findings, but our results do not indicate any obvious benefit of corticosteroids either.

The optimal ventilatory strategies in cases of hypoxic respiratory failure are not fully known. The use of NIV may avoid intubation but could also delay the initiation of invasive ventilation, thereby making the patient more vulnerable to complications and adverse outcomes. Among mechanically ventilated patients with pandemic influenza A H1N1, NIV was used in 19–41% of the patients [125, 179, 180]. In our study, 61% of the ICU patients received respiratory support with NIV. For approximately one third of the patients this was a sufficient support strategy, but the rest had to be converted to invasive ventilation. The mortality among patients treated with NIV tended to be higher than for those started on invasive ventilation directly. Similar results have been demonstrated by other studies regarding the possible adverse effects associated with delayed initiation of invasive ventilation [181, 182]. A recently published prospective multicentre study from Italy evaluated the use of NIV in 98 ICU patients with influenza A H1N1 during the year following the pandemic [183]. They found that NIV was effective in

preventing invasive ventilation in 48% and they also saw a reduced number of secondary infections. There are, however, important differences between the Italian patients and the patients in our study. Firstly, in the Italian study only patients with less sever respiratory impairment were selected for initial NIV. Secondly, monitoring during NIV was protocolized with prompt, early intubation in patients that did not respond with respiratory improvement. The Swedish participants treated with NIV, had very severe respiratory impairment and for patients failing NIV, time to intubation was noticeably long (median 22 hours, IQR 8–44).
The overall conclusion is that if NIV is to be used in patients with hypoxic influenza it should be applied early and in those with a disease of little or moderat severity only. The treatment must be monitored carefully with a readiness to intubate if there is no improvement during the first few hours with NIV. With these limitations NIV could probably be a safe strategy for a selected minority of influenza patients but it does not seem to have the potential for being used as a large-scale ventilatory strategy that could be relocated to wards outside ICUs.

4.4 Paper IV

Results

During the four-year study period from January 2008 to December 2011, 29 patients with NSTIs were included. More than half of the patients, 59% (17/29) were referred from other hospitals. Patient characteristics are shown in table 6. NSTI was correctly diagnosed in only 36% of the patients during their stay in the emergency department (ED). The most prevalent bacterium was *S. pyogenes*, diagnosed in 41% while aerobic or anaerobic enteric bacteria were fond in 34% of the patients. *S. pyogenes* was more common among transferred patients (53%) compared with directly admitted patients (25%). Treatments, timing of treatments, and outcome are shown in Table 7. All patients were treated with antibiotics and surgical debridement. Among the transferred patients 88% were surgically debrided before transportation. HBO was used in 86% and IVIG was given to 52% of the patients. For the 12 patients with confirmed *S. pyogenes* infection, IVIG was given to 83%. There was a high usage of advanced intensive care therapies: mechanical ventilation in 83%, vasopressors and inotropes in 90%, and renal replacement therapy in 24% of the patients. The median times from arriving in the ED to key interventions were five hours for first dose of antibiotics, 12 hours to ICU admission, and 16 hours for primary surgery. The overall 30-day mortality was 14% and amputations were performed in 24% of the patients.

Table 6. Baseline characteristics of all patients and by admission source.

	All patients (n=29)		Emergency dep. SUH/E (n=12)		Inter-hospital transfer (n=17)	
Age – years, median (IQR)	54	(40-64)	58	(49-69)	52	(36-62)
Male, – % (no/total no.)	69%	(20/29)	75%	(9/12)	65%	(11/17)
Number of chronic morbidity – % (no/total no.)						
Cardiovascular disease excl. hypertension	14%	(4/29)	25%	(3/12)	6%	(1/17)
Diabetes mellitus	14%	(4/29)	8%	(1/12)	18%	(3/17)
Malignancy	10%	(3/29)	8%	(1/12)	12%	(2/17)
Immunosuppression	14%	(4/29)	17%	(2/12)	6%	(1/17)
Intravenous drug abuse	10%	(3/29)	25%	(3/12)	0	
Alcohol abuse	7%	(2/29)	17%	(2/12)	0	
Time from onset of sympt. to hospital, – days, median (IQR)	3	(1-3)	3	(2-4)	2	(0-3)
SBP in ED – mmHg, median (IQR)	115	(94-134)	115	(91-138)	115	(98-134)
SBP in ED $\leq$ 90 mmHg, % (no./total no.)	23%	(6/26)	25%	(3/12)	18%	(3/14)
LRINEC score in ED, median (IQR)	8	(6-9)	8,5	(6,5-10)	7	(6-9)
LRINEC score in ED $\geq$6 – % (no./total no.)	92%	(22/24)	100%	(12/12)	86%	(12/14)
APACHE 2 at ICU SUH/E, median (IQR)	17	(11-23)	17,5	(12-28)	16,5	9-23)

SUH/E Sahlgrenska University Hospital/East; IQR, inter-quartile range; ED, emergency department; LRINEC score, the laboratory risk indicator for necrotising fasciitis score; APACHE 2, acute physiology and chronic health evaluation 2; ICU, intensive care unit; SBP, systolic blood pressure

The 30-day mortality was 25% for directly admitted patients and 6% for patients transferred from other hospitals. The difference was not statistically significant. Patients who died, were older, had a higher prevalence of co-morbidities and a significantly higher APACHE-2 score, as compared to survivors. We did not find any difference in 30-day mortality or amputation rate related to appropriateness of diagnoses in the ED despite that patients with early NSTI-diagnosis were treated more promptly, with shorter times to antibiotics and primary surgery.

Discussion

The 14% mortality and 24% amputation rate in this study compares well to other published case series. NSTIs are uncommon infections with complex treatment requirements and it is therefore common to centralize the care of these patients to large centres with sufficient experience and skills. The drawbacks of transfers are the risks inherent in the transport of critically ill

Table 7. Treatment provided, timing of key interventions and outcome for all study patients and by admission source.

	All patients (n=29)		Emergency department, SUH/E (n=12)		Inter-hospital transfer (n=17)	
Treatment – % (no./total no.)						
Surgery	100%	(29/29)	100%	(12/12)	100%	(17/17)
Vasopressors or inotropes	90%	(26/29)	91%	(11/12)	88%	(15/17)
Mechanical ventilation	83%	(24/29)	83%	(10/12)	82%	(14/17)
Dialyss	24%	(7/29)	17%	(2/12)	29%	(5/17)
Intravenous immunoglobulin	52%	(15/29)	33%	(4/12)	65%	(11/17)
Hyperbaric oxygen	86%	(25/29)	92%	(11/12)	82%	(14/17)
Number of operations, median (IQR)	5	(4-10)	4	(3-9)	7	(4-11)
Number of HBO sessions, median (IQR)	5	(4-8)	4	(3-5)	5	(4-8)
Time[#] from arrival at ED to key interventions – hours, median (IQR)						
First dose of antibiotics	5	(2-10)	7	(5-11)	5	(2-12)
Intensive care unit admission	12	(6-35)	12	(8-39)	7	(6-35)
First surgery	16	(6-31)	14	(6-32)	16	(8-30)
First hyperbaric oxygen session	38	(22-60)	32	(11-51)	46	(25-64)
Outcome						
Days on mech. ventilation, median (IQR)	10	(4-14)	10	(4-14)	10	(4-28)
Days in ICU, median (IQR)	12	(4-20)	13	(4-21)	12	(4-25)
Days in hospital, median (IQR)	33	(16-62)	26	(15-62)	36	(22-85)
30-day mortality –% (no./total no.)	14%	(4/29)	25%	(3/12)	6%	(1/17)
90-day mortality – % (no./total no.)	21%	(6/29)	33%	(4/12)	12%	(2/17)
Amputation – % (no./total no.)	24%	(7/29)	33%	(4/12)	18%	(3/17)
Adverse outcome*– % (no./total no.)	34%	(10/29)	42%	(5/12)	24%	(4/17)

*Adverse outcome defined as mortality within 30 days or amputation

[#] Relevant time reports could be retrieved for 13 of the 17 patients transferred from other hospitals

SUH/E, Sahlgrenska University Hospital/East; IQR, inter-quartile range; HBO, hyperbaric oxygen; ED, emergency department; ICU, intensive care unit; n.a. not applicable

patients and the potential for delay in primary surgery. Two recently published studies identify the inter-hospital transfer of patients with NSTIs as an independent risk factor for mortality [154, 155]. Both these studies are, however, based on administrative databases only and do not contain any information concerning baseline patient characteristics or details in the provided therapy. It is therefore not possible to evaluate whether the observed differences in relation to transfer status can be attributed to transfer as such or to uncontrolled co-variation in baseline characteristics and/or severity of disease. There might also have been a selection of more severe and complicated cases among the transfers. Another important factor to notice is that in one of these studies all the transferred patients had their first surgical debridement after transportation [155]. The study does not provide data concerning the timing of surgery but most likely there was a significant delay of primary surgery in the transferred group. In our cohort, 88% of transferred

patients had their first operation before transportation and the median transport time was less than one hour. Even if our material with just 29 individuals is to small to allow for a definitive judgement, and taking into account the possibility for a selection bias favouring survival for patients transferred from other hospitals, we still think that the excellent results for transferred patients is to the advantage of our regional model with centralized care of patients with severe NSTIs.

The majority of our cases (68%) were not identified as NSTIs during their stay at the ED. Incorrect diagnosis in the ED was a strong determinant when it came to delays in initiation of antibiotic treatment and primary surgery. The difficulties associated with diagnosing NSTIs are well known, as the physical findings can be diffuse and non-specific at an early stage of the disease. In our study, the retrospectively calculated LRINEC score was on or above the diagnostic cut-off for NSTIs ($\geq$ 6) in 92% (22/24) of the patients. The LRINEC score could be calculated for six of the eight cases that were mistaken for superficial skin infections in the ED and they all had a score of $\geq$ 6. This suggests that use of the LRINEC-score potentially could have speeded up diagnosis and start of therapy in those cases. However, the overall rarity of NSTIs makes the feasibility of LRINEC-scoring as a diagnostic aid in the ED questionable. From a broader perspective, it might therefore be of greater value if all severely ill patients with suspected infections, upon arrival at the ED, were systematically assessed for physical signs and laboratory markers indicating severe infection and consequently given prompt antibiotic treatment, immediate evaluation of the need for surgical source control and appropriate levels of monitoring and support for failing organs.

5 CONCLUSIONS

- Treatment with β-lactam antibiotics in 8-hour dosing intervals in acute bacterial meningitis resulted in high survival.

- Broad range PCR used in CSF is highly sensitive for the detection of bacteria in acute bacterial meningitis in samples taken up to one week into antibiotic therapy. ICT is also a highly sensitive diagnostic method for the detection of *S. pneumoniae* in CSF samples taken during the first week of treatment.

- During the 2009–2010 influenza A (H1N1) pandemic, 136 patients were admitted to Swedish ICUs because of severe respiratory symptoms. Our study of 126 of these patients identified obesity, chronic pulmonary disease, and diabetes as risk factors for intensive care. The use of NIV was not associated with improved outcomes compared with immediate invasive ventilation.

- 29 patients with NSTIs were treated at Sahlgrenska University Hospital/East during the period 2008 to 2011. *S. pyogenes* was the predominant bacterium, detected in 41%. The 30-day mortality was 14% and the incidence of amputation 24%. Inter-hospital transfer was not associated with a delay in key interventions and could not be identified as a risk factor for adverse outcome.

FUTURE PERSPECTIVES

Regarding ABM, there is a need for more research focusing on methods to control inflammation and thereby counteract oedema formation, elevated ICP, and cell death. β-lactam antibiotics kill bacteria by rupturing their cell walls, resulting in an abrupt release of sub capsular bacterial components that cause a peek in the release of inflammatory mediators. Animal experiments have shown that ABM treatment with non-bacteriolytic antibiotics such as rifampicin, daptomycin, and moxifloxacin cause less inflammation and, in some studies, higher survival compared to standard treatment with β-lactam antibiotics [31]. There is a potential role for directly acting immuno-modulatory drugs other than corticosteroids. Granulocytes release many of the mediators responsible for the local inflammation. Roscovitine is a substance that can induce apoptosis of granulocytes. In a mouse model of pneumococcal meningitis, adjuvant treatment with roscovitine was associated with reduced mortality and reduced neuronal damage in brain histopathology [33].

The currently most used influenza drugs, zanamivir and oseltamivir, have a limited protective effect and virus strains resistant to these drugs occur sporadically. There is therefore a need for new and more potent treatment options. One possibility is to combine drugs with different mechanisms of action. The combination of amantadine, oseltamivir, and ribavirin has shown synergistic effects in vitro and trends towards better outcome in severe cases of pandemic influenza A (H1N1) [184] and is currently being tested in a randomized controlled trial (NCT01227967).

Recent research has characterized several monoclonal antibodies that efficiently neutralize influenza viruses. Some of these antibodies are directed towards the highly conserved stem region of hemagglutinin that is shared among several different subtypes of influenza A viruses. There are furthermore defined antibodies with universal activity towards all influenza A subtypes as well as toward the two varieties of influenza B. These discoveries can hopefully give rise to monoclonal antibody preparations for therapeutic use and moreover new possibilities for future construction of universally active influenza vaccines [185]. As for ABM, another option for treatment of severe influenza pneumonitis would theoretically be to modulate the host inflammatory response. Our understanding of the heterogeneity and dynamics of immune responses in serious human influenza disease is still

very limited. Further investigation in these complicated mechanisms is therefore needed.

A large proportion of the costs and general resources currently used in the treatment of NSTIs are of uncertain value. We still do not know if HBO improves patient survival and we also do not know if IVIG improves outcome in severe infections with *S. pyogenes*. Both of these treatments need to be evaluated in sufficiently powered randomized clinical trials.

ACKNOWLEDGEMENT

I would like to thank:

Lars Hagberg, my supervisor, for continuous support, bright ideas, and never failing enthusiasm.

Aylin Yilmaz, for helping me to think scientifically and to dress these thoughts in intelligible English.

Rolf Gedeborg, whose dual expertise in critical care and epidemiology was invaluable for the influenza study.

All my other co-authors: Christina Welinder-Olsson at the Department of Clinical Microbiology for knowing everything worth knowing about bacterial PCR. Anders Larsson at the Department of Anaesthesiology and Intensive Care at Uppsala University Hospital for providing specialist knowledge in mechanical ventilation. Per Arnell and Hans Lycke at the Department of Anaesthesia and Intensive Care, together with Anders Rosemar at the Department of Surgery, whose collective expertise was essential for our study on patients with severe soft-tissue infections.

Those patients with bacterial meningitis who volunteered for extra lumbar punctures.

The staff at the Department of Clinical Microbiology, for help with microbiological analyses.

Eva Karlsson at our research laboratory, for keeping the CSF samples in perfectly deep-frozen order.

My boss, Lars-Magnus Andersson and all other colleges at the Department of Infectious Diseases for being such good and competent colleges.

Laila and all staff in ward 302, a highly professional team with whom I have the privilege to cooperate in the caring for patients with severe infections.

My colleges in the working groups for *Sepsis* and *Bacterial CNS infections*, for all inspiration and knowledge that I get in the process of writing national guidelines.

My parents, Jörgen and Anita, who told me that school is important.

My brother Gunnar and my sister Josefin, who I hope to see more often.

My friends inside and outside the hospital walls. No one mentioned, no one forgotten. I will be back.

My dear Aylin for love, support and joy.

Hugo and Nora, for welcoming me as half time bonus parent.

My daughters, Ella and Tove. I love you.

REFERENCES

1. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, Vlieghe E, Hara GL, Gould IM, Goossens H *et al*: Antibiotic resistance-the need for global solutions. *The Lancet Infectious diseases* 2013, 13(12):1057-1098.
2. Berthelsen PG: Manual positive pressure ventilation and the Copenhagen poliomyelitis epidemic 1952: An attempt at setting the record straight. *Acta anaesthesiologica Scandinavica* 2014, 58(5):503-507.
3. Engstrom CG: Treatment of severe cases of respiratory paralysis by the Engstrom universal respirator. *British medical journal* 1954, 2(4889):666-669.
4. Vincent JL, Fink MP, Marini JJ, Pinsky MR, Sibbald WJ, Singer M, Suter PM, Cook D, Pepe PE, Evans T: Intensive care and emergency medicine: progress over the past 25 years. *Chest* 2006, 129(4):1061-1067.
5. Biehl M, Kashiouris MG, Gajic O: Ventilator-induced lung injury: minimizing its impact in patients with or at risk for ARDS. *Respiratory care* 2013, 58(6):927-937.
6. Ward NS, Dushay KM: Clinical concise review: Mechanical ventilation of patients with chronic obstructive pulmonary disease. *Critical care medicine* 2008, 36(5):1614-1619.
7. Nava S, Schreiber A, Domenighetti G: Noninvasive ventilation for patients with acute lung injury or acute respiratory distress syndrome. *Respiratory care* 2011, 56(10):1583-1588.
8. Galvagno SM, Jr., Hong CM, Lissauer ME, Baker AK, Murthi SB, Herr DL, Stein DM: Practical considerations for the dosing and adjustment of continuous renal replacement therapy in the intensive care unit. *Journal of critical care* 2013, 28(6):1019-1026.
9. Reade MC, Finfer S: Sedation and delirium in the intensive care unit. *The New England journal of medicine* 2014, 370(5):444-454.
10. Athar MK, Puri N, Gerber DR: Anemia and blood transfusions in critically ill patients. *Journal of blood transfusion* 2012, 2012:629204.
11. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R *et al*: Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive care medicine* 2013, 39(2):165-228.
12. Shukla P, Rao GM, Pandey G, Sharma S, Mittapelly N, Shegokar R, Mishra PR: Therapeutic interventions in sepsis: current and

anticipated pharmacological agents. *British journal of pharmacology* 2014.
13. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R: Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. *JAMA : the journal of the American Medical Association* 2014, 311(13):1308-1316.
14. Erlandsson M, Burman LG, Cars O, Gill H, Nilsson LE, Walther SM, Hanberger H: Prescription of antibiotic agents in Swedish intensive care units is empiric and precise. *Scandinavian journal of infectious diseases* 2007, 39(1):63-69.
15. Svenska intensivvårdsregistret. Available from: http://www.icuregswe.org/sv/Utdata
16. Januel JM, Harbarth S, Allard R, Voirin N, Lepape A, Allaouchiche B, Guerin C, Lehot JJ, Robert MO, Fournier G *et al*: Estimating attributable mortality due to nosocomial infections acquired in intensive care units. *Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America* 2010, 31(4):388-394.
17. Barbier F, Andremont A, Wolff M, Bouadma L: Hospital-acquired pneumonia and ventilator-associated pneumonia: recent advances in epidemiology and management. *Current opinion in pulmonary medicine* 2013, 19(3):216-228.
18. Kalanuria AA, Zai W, Mirski M: Ventilator-associated pneumonia in the ICU. *Critical care (London, England)* 2014, 18(2):208.
19. Timsit JF: Diagnosis and prevention of catheter-related infections. *Current opinion in critical care* 2007, 13(5):563-571.
20. Arnold HM, Micek ST, Skrupky LP, Kollef MH: Antibiotic stewardship in the intensive care unit. *Seminars in respiratory and critical care medicine* 2011, 32(2):215-227.
21. Udy AA, Roberts JA, Lipman J: Clinical implications of antibiotic pharmacokinetic principles in the critically ill. *Intensive care medicine* 2013, 39(12):2070-2082.
22. Thigpen MC, Whitney CG, Messonnier NE, Zell ER, Lynfield R, Hadler JL, Harrison LH, Farley MM, Reingold A, Bennett NM *et al*: Bacterial meningitis in the United States, 1998-2007. *The New England journal of medicine* 2011, 364(21):2016-2025.
23. Campagne G, Schuchat A, Djibo S, Ousseini A, Cisse L, Chippaux JP: Epidemiology of bacterial meningitis in Niamey, Niger, 1981-96. *Bulletin of the World Health Organization* 1999, 77(6):499-508.
24. Berardi A, Lugli L, Rossi C, China MC, Vellani G, Contiero R, Calanca F, Camerlo F, Casula F, Di Carlo C *et al*: Neonatal bacterial meningitis. *Minerva pediatrica* 2010, 62(3 Suppl 1):51-54.
25. McIntyre PB, O'Brien KL, Greenwood B, van de Beek D: Effect of vaccines on bacterial meningitis worldwide. *Lancet* 2012, 380(9854):1703-1711.

26. van de Beek D, de Gans J, Spanjaard L, Weisfelt M, Reitsma JB, Vermeulen M: Clinical features and prognostic factors in adults with bacterial meningitis. *The New England journal of medicine* 2004, 351(18):1849-1859.
27. Kim KS: Acute bacterial meningitis in infants and children. *The Lancet Infectious diseases* 2010, 10(1):32-42.
28. Bishai DM, Champion C, Steele ME, Thompson L: Product development partnerships hit their stride: lessons from developing a meningitis vaccine for Africa. *Health affairs (Project Hope)* 2011, 30(6):1058-1064.
29. Djingarey MH, Barry R, Bonkoungou M, Tiendrebeogo S, Sebgo R, Kandolo D, Lingani C, Preziosi MP, Zuber PL, Perea W *et al*: Effectively introducing a new meningococcal A conjugate vaccine in Africa: the Burkina Faso experience. *Vaccine* 2012, 30 Suppl 2:B40-45.
30. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA *et al*: A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. *Science translational medicine* 2012, 4(147):147ra111.
31. Mook-Kanamori BB, Geldhoff M, van der Poll T, van de Beek D: Pathogenesis and pathophysiology of pneumococcal meningitis. *Clinical microbiology reviews* 2011, 24(3):557-591.
32. Zwijnenburg PJ, van der Poll T, Florquin S, van Deventer SJ, Roord JJ, van Furth AM: Experimental pneumococcal meningitis in mice: a model of intranasal infection. *The Journal of infectious diseases* 2001, 183(7):1143-1146.
33. Koedel U, Klein M, Pfister HW: New understandings on the pathophysiology of bacterial meningitis. *Current opinion in infectious diseases* 2010, 23(3):217-223.
34. Small PM, Tauber MG, Hackbarth CJ, Sande MA: Influence of body temperature on bacterial growth rates in experimental pneumococcal meningitis in rabbits. *Infection and immunity* 1986, 52(2):484-487.
35. Tauber MG, Sande MA: Pathogenesis of bacterial meningitis: contributions by experimental models in rabbits. *Infection* 1984, 12 Suppl 1:S3-10.
36. Lindvall P, Ahlm C, Ericsson M, Gothefors L, Naredi S, Koskinen LO: Reducing intracranial pressure may increase survival among patients with bacterial meningitis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2004, 38(3):384-390.
37. Brouwer MC, Thwaites GE, Tunkel AR, van de Beek D: Dilemmas in the diagnosis of acute community-acquired bacterial meningitis. *Lancet* 2012, 380(9854):1684-1692.

38. Jim KK, Brouwer MC, van der Ende A, van de Beek D: Cerebral abscesses in patients with bacterial meningitis. *The Journal of infection* 2012, 64(2):236-238.
39. Glimaker M, Johansson B, Bell M, Ericsson M, Blackberg J, Brink M, Lindquist L, Sjolin J: Early lumbar puncture in adult bacterial meningitis--rationale for revised guidelines. *Scandinavian journal of infectious diseases* 2013, 45(9):657-663.
40. Lindquist L, Linne T, Hansson LO, Kalin M, Axelsson G: Value of cerebrospinal fluid analysis in the differential diagnosis of meningitis: a study in 710 patients with suspected central nervous system infection. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 1988, 7(3):374-380.
41. Huy NT, Thao NT, Diep DT, Kikuchi M, Zamora J, Hirayama K: Cerebrospinal fluid lactate concentration to distinguish bacterial from aseptic meningitis: a systemic review and meta-analysis. *Critical care (London, England)* 2010, 14(6):R240.
42. Nigrovic LE, Malley R, Macias CG, Kanegaye JT, Moro-Sutherland DM, Schremmer RD, Schwab SH, Agrawal D, Mansour KM, Bennett JE *et al*: Effect of antibiotic pretreatment on cerebrospinal fluid profiles of children with bacterial meningitis. *Pediatrics* 2008, 122(4):726-730.
43. Sakushima K, Hayashino Y, Kawaguchi T, Jackson JL, Fukuhara S: Diagnostic accuracy of cerebrospinal fluid lactate for differentiating bacterial meningitis from aseptic meningitis: a meta-analysis. *The Journal of infection* 2011, 62(4):255-262.
44. Brouwer MC, Tunkel AR, van de Beek D: Epidemiology, diagnosis, and antimicrobial treatment of acute bacterial meningitis. *Clinical microbiology reviews* 2010, 23(3):467-492.
45. Bohr V, Rasmussen N, Hansen B, Kjersem H, Jessen O, Johnsen N, Kristensen HS: 875 cases of bacterial meningitis: diagnostic procedures and the impact of preadmission antibiotic therapy. Part III of a three-part series. *The Journal of infection* 1983, 7(3):193-202.
46. Crosswell JM, Nicholson WR, Lennon DR: Rapid sterilisation of cerebrospinal fluid in meningococcal meningitis: Implications for treatment duration. *J Paediatr Child Health* 2006, 42(4):170-173.
47. Kanegaye JT, Soliemanzadeh P, Bradley JS: Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics* 2001, 108(5):1169-1174.
48. Nigrovic LE, Kuppermann N, McAdam AJ, Malley R: Cerebrospinal latex agglutination fails to contribute to the microbiologic diagnosis of pretreated children with meningitis. *The Pediatric infectious disease journal* 2004, 23(8):786-788.

49. Tarafdar K, Rao S, Recco RA, Zaman MM: Lack of sensitivity of the latex agglutination test to detect bacterial antigen in the cerebrospinal fluid of patients with culture-negative meningitis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2001, 33(3):406-408.
50. Kennedy WA, Chang SJ, Purdy K, Le T, Kilgore PE, Kim JS, Anh DD, Huong PL, Dong BQ, Tan DM *et al*: Incidence of bacterial meningitis in Asia using enhanced CSF testing: polymerase chain reaction, latex agglutination and culture. *Epidemiology and infection* 2007, 135(7):1217-1226.
51. Nigrovic LE, Kuppermann N, Malley R: Children with bacterial meningitis presenting to the emergency department during the pneumococcal conjugate vaccine era. *Academic emergency medicine : official journal of the Society for Academic Emergency Medicine* 2008, 15(6):522-528.
52. Pedersen TI, Howitz M, Ostergaard C: Clinical characteristics of Haemophilus influenzae meningitis in Denmark in the post-vaccination era. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2010, 16(5):439-446.
53. Bruyn GA, Kremer HP, de Marie S, Padberg GW, Hermans J, van Furth R: Clinical evaluation of pneumococcal meningitis in adults over a twelve-year period. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 1989, 8(8):695-700.
54. Durand ML, Calderwood SB, Weber DJ, Miller SI, Southwick FS, Caviness VS, Jr., Swartz MN: Acute bacterial meningitis in adults. A review of 493 episodes. *The New England journal of medicine* 1993, 328(1):21-28.
55. Sigurdardottir B, Bjornsson OM, Jonsdottir KE, Erlendsdottir H, Gudmundsson S: Acute bacterial meningitis in adults. A 20-year overview. *Archives of internal medicine* 1997, 157(4):425-430.
56. Heckenberg SG, de Gans J, Brouwer MC, Weisfelt M, Piet JR, Spanjaard L, van der Ende A, van de Beek D: Clinical features, outcome, and meningococcal genotype in 258 adults with meningococcal meningitis: a prospective cohort study. *Medicine* 2008, 87(4):185-192.
57. Stephens DS, Greenwood B, Brandtzaeg P: Epidemic meningitis, meningococcaemia, and Neisseria meningitidis. *Lancet* 2007, 369(9580):2196-2210.
58. Srinivasan L, Pisapia JM, Shah SS, Halpern CH, Harris MC: Can broad-range 16S ribosomal ribonucleic acid gene polymerase chain reactions improve the diagnosis of bacterial meningitis? A systematic review and meta-analysis. *Annals of emergency medicine* 2012, 60(5):609-620 e602.

59. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB: Simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and Streptococcus pneumoniae in suspected cases of meningitis and septicemia using real-time PCR. *Journal of clinical microbiology* 2001, 39(4):1553-1558.
60. Drakopoulou Z, Kesanopoulos K, Sioumala M, Tambaki A, Kremastinou J, Tzanakaki G: Simultaneous single-tube PCR-based assay for the direct identification of the five most common meningococcal serogroups from clinical samples. *FEMS immunology and medical microbiology* 2008, 53(2):178-182.
61. Bronska E, Kalmusova J, Dzupova O, Maresova V, Kriz P, Benes J: Dynamics of PCR-based diagnosis in patients with invasive meningococcal disease. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2006, 12(2):137-141.
62. Moisi JC, Saha SK, Falade AG, Njanpop-Lafourcade BM, Oundo J, Zaidi AK, Afroj S, Bakare RA, Buss JK, Lasi R *et al*: Enhanced diagnosis of pneumococcal meningitis with use of the Binax NOW immunochromatographic test of Streptococcus pneumoniae antigen: a multisite study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2009, 48 Suppl 2:S49-56.
63. Saha SK, Darmstadt GL, Yamanaka N, Billal DS, Nasreen T, Islam M, Hamer DH: Rapid diagnosis of pneumococcal meningitis: implications for treatment and measuring disease burden. *The Pediatric infectious disease journal* 2005, 24(12):1093-1098.
64. Collard JM, Wang X, Mahamane AE, Idi I, Issaka B, Ousseni M, Mayer LW, Nato F, Moulia-Pelat JP: A five-year field assessment of rapid diagnostic tests for meningococcal meningitis in Niger by using the combination of conventional and real-time PCR assays as a gold standard. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2014, 108(1):6-12.
65. Auburtin M, Wolff M, Charpentier J, Varon E, Le Tulzo Y, Girault C, Mohammedi I, Renard B, Mourvillier B, Bruneel F *et al*: Detrimental role of delayed antibiotic administration and penicillin-nonsusceptible strains in adult intensive care unit patients with pneumococcal meningitis: the PNEUMOREA prospective multicenter study. *Critical care medicine* 2006, 34(11):2758-2765.
66. van de Beek D, Brouwer MC, Thwaites GE, Tunkel AR: Advances in treatment of bacterial meningitis. *Lancet* 2012, 380(9854):1693-1702.
67. Tunkel AR, Hartman BJ, Kaplan SL, Kaufman BA, Roos KL, Scheld WM, Whitley RJ: Practice guidelines for the management of bacterial meningitis. *Clinical infectious diseases : an official*

publication of the Infectious Diseases Society of America 2004, 39(9):1267-1284.

68. Chaudhuri A, Martinez-Martin P, Kennedy PG, Andrew Seaton R, Portegies P, Bojar M, Steiner I: EFNS guideline on the management of community-acquired bacterial meningitis: report of an EFNS Task Force on acute bacterial meningitis in older children and adults. *European journal of neurology : the official journal of the European Federation of Neurological Societies* 2008, 15(7):649-659.
69. Capitano B, Nicolau DP, Potoski BA, Byers KE, Horowitz M, Venkataramanan R, Paterson DL: Meropenem administered as a prolonged infusion to treat serious gram-negative central nervous system infections. *Pharmacotherapy* 2004, 24(6):803-807.
70. Gerber CM, Cottagnoud M, Neftel KA, Tauber MG, Cottagnoud P: Meropenem alone and in combination with vancomycin in experimental meningitis caused by a penicillin-resistant pneumococcal strain. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 1999, 18(12):866-870.
71. Odio CM, Puig JR, Feris JM, Khan WN, Rodriguez WJ, McCracken GH, Jr., Bradley JS: Prospective, randomized, investigator-blinded study of the efficacy and safety of meropenem vs. cefotaxime therapy in bacterial meningitis in children. Meropenem Meningitis Study Group. *The Pediatric infectious disease journal* 1999, 18(7):581-590.
72. van de Beek D, Hensen EF, Spanjaard L, de Gans J, Enting RH, Dankert J: Meropenem susceptibility of Neisseria meningitidis and Streptococcus pneumoniae from meningitis patients in The Netherlands. *The Journal of antimicrobial chemotherapy* 1997, 40(6):895-897.
73. Mylonakis E, Hohmann EL, Calderwood SB: Central nervous system infection with Listeria monocytogenes. 33 years' experience at a general hospital and review of 776 episodes from the literature. *Medicine* 1998, 77(5):313-336.
74. Mitja O, Pigrau C, Ruiz I, Vidal X, Almirante B, Planes AM, Molina I, Rodriguez D, Pahissa A: Predictors of mortality and impact of aminoglycosides on outcome in listeriosis in a retrospective cohort study. *The Journal of antimicrobial chemotherapy* 2009, 64(2):416-423.
75. Norgaard M, Gudmundsdottir G, Larsen CS, Schonheyder HC: Staphylococcus aureus meningitis: experience with cefuroxime treatment during a 16 year period in a Danish region. *Scandinavian journal of infectious diseases* 2003, 35(5):311-314.
76. Moon C, Kwak YG, Kim BN, Kim ES, Lee CS: Implications of postneurosurgical meningitis caused by carbapenem-resistant Acinetobacter baumannii. *Journal of infection and chemotherapy :*

official journal of the Japan Society of Chemotherapy 2013, 19(5):916-919.
77. Alestig K, Olaison L, Rylander M: Ceftazidime for Pseudomonas meningitis. *Lancet* 1985, 1(8421):161-162.
78. Craig WA: Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 1998, 26(1):1-10; quiz 11-12.
79. Scheld WM: Drug delivery to the central nervous system: general principles and relevance to therapy for infections of the central nervous system. *Reviews of infectious diseases* 1989, 11 Suppl 7:S1669-1690.
80. Nau R, Prange HW, Muth P, Mahr G, Menck S, Kolenda H, Sorgel F: Passage of cefotaxime and ceftriaxone into cerebrospinal fluid of patients with uninflamed meninges. *Antimicrobial agents and chemotherapy* 1993, 37(7):1518-1524.
81. Dagan R, Velghe L, Rodda JL, Klugman KP: Penetration of meropenem into the cerebrospinal fluid of patients with inflamed meninges. *The Journal of antimicrobial chemotherapy* 1994, 34(1):175-179.
82. Ogawa M, Suzuki H, Sawada Y, Hanano M, Sugiyama Y: Kinetics of active efflux via choroid plexus of beta-lactam antibiotics from the CSF into the circulation. *The American journal of physiology* 1994, 266(2 Pt 2):R392-399.
83. Spector R, Lorenzo AV: Inhibition of penicillin transport from the cerebrospinal fluid after intracisternal inoculation of bacteria. *The Journal of clinical investigation* 1974, 54(2):316-325.
84. Suzuki H, Sawada Y, Sugiyama Y, Iga T, Hanano M, Spector R: Transport of imipenem, a novel carbapenem antibiotic, in the rat central nervous system. *The Journal of pharmacology and experimental therapeutics* 1989, 250(3):979-984.
85. Spector R: Advances in understanding the pharmacology of agents used to treat bacterial meningitis. *Pharmacology* 1990, 41(3):113-118.
86. Scheld WM, Brodeur JP: Effect of methylprednisolone on entry of ampicillin and gentamicin into cerebrospinal fluid in experimental pneumococcal and Escherichia coli meningitis. *Antimicrobial agents and chemotherapy* 1983, 23(1):108-112.
87. Cabellos C, Martinez-Lacasa J, Martos A, Tubau F, Fernandez A, Viladrich PF, Gudiol F: Influence of dexamethasone on efficacy of ceftriaxone and vancomycin therapy in experimental pneumococcal meningitis. *Antimicrobial agents and chemotherapy* 1995, 39(9):2158-2160.
88. Friedland IR, Klugman KP: Cerebrospinal fluid bactericidal activity against cephalosporin-resistant Streptococcus pneumoniae in children

with meningitis treated with high-dosage cefotaxime. *Antimicrobial agents and chemotherapy* 1997, 41(9):1888-1891.
89. Doit C, Barre J, Cohen R, Bonacorsi S, Bourrillon A, Bingen EH: Bactericidal activity against intermediately cephalosporin-resistant Streptococcus pneumoniae in cerebrospinal fluid of children with bacterial meningitis treated with high doses of cefotaxime and vancomycin. *Antimicrobial agents and chemotherapy* 1997, 41(9):2050-2052.
90. Lutsar I, McCracken GH, Jr., Friedland IR: Antibiotic pharmacodynamics in cerebrospinal fluid. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 1998, 27(5):1117-1127, quiz 1128-1119.
91. Pelkonen T, Roine I, Cruzeiro ML, Pitkaranta A, Kataja M, Peltola H: Slow initial beta-lactam infusion and oral paracetamol to treat childhood bacterial meningitis: a randomised, controlled trial. *The Lancet Infectious diseases* 2011, 11(8):613-621.
92. Brink M, Hagberg L: Outcome of 8-hour dosing intervals with beta-lactam antibiotics in adult acute bacterial meningitis. *Scandinavian journal of infectious diseases* 2006, 38(9):772-777.
93. Tauber MG, Khayam-Bashi H, Sande MA: Effects of ampicillin and corticosteroids on brain water content, cerebrospinal fluid pressure, and cerebrospinal fluid lactate levels in experimental pneumococcal meningitis. *The Journal of infectious diseases* 1985, 151(3):528-534.
94. de Gans J, van de Beek D: Dexamethasone in adults with bacterial meningitis. *The New England journal of medicine* 2002, 347(20):1549-1556.
95. Molyneux EM, Walsh AL, Forsyth H, Tembo M, Mwenechanya J, Kayira K, Bwanaisa L, Njobvu A, Rogerson S, Malenga G: Dexamethasone treatment in childhood bacterial meningitis in Malawi: a randomised controlled trial. *Lancet* 2002, 360(9328):211-218.
96. Nguyen TH, Tran TH, Thwaites G, Ly VC, Dinh XS, Ho Dang TN, Dang QT, Nguyen DP, Nguyen HP, To SD *et al*: Dexamethasone in Vietnamese adolescents and adults with bacterial meningitis. *The New England journal of medicine* 2007, 357(24):2431-2440.
97. Scarborough M, Gordon SB, Whitty CJ, French N, Njalale Y, Chitani A, Peto TE, Lalloo DG, Zijlstra EE: Corticosteroids for bacterial meningitis in adults in sub-Saharan Africa. *The New England journal of medicine* 2007, 357(24):2441-2450.
98. van de Beek D, Farrar JJ, de Gans J, Mai NT, Molyneux EM, Peltola H, Peto TE, Roine I, Scarborough M, Schultsz C *et al*: Adjunctive dexamethasone in bacterial meningitis: a meta-analysis of individual patient data. *The Lancet Neurology* 2010, 9(3):254-263.

99. van de Beek D, de Gans J, Tunkel AR, Wijdicks EF: Community-acquired bacterial meningitis in adults. *The New England journal of medicine* 2006, 354(1):44-53.
100. Olsen B, Munster VJ, Wallensten A, Waldenstrom J, Osterhaus AD, Fouchier RA: Global patterns of influenza a virus in wild birds. *Science* 2006, 312(5772):384-388.
101. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y: Evolution and ecology of influenza A viruses. *Microbiological reviews* 1992, 56(1):152-179.
102. Bouvier NM, Palese P: The biology of influenza viruses. *Vaccine* 2008, 26 Suppl 4:D49-53.
103. A revision of the system of nomenclature for influenza viruses: a WHO memorandum. *Bulletin of the World Health Organization* 1980, 58(4):585-591.
104. Carrat F, Luong J, Lao H, Salle AV, Lajaunie C, Wackernagel H: A 'small-world-like' model for comparing interventions aimed at preventing and controlling influenza pandemics. *BMC medicine* 2006, 4:26.
105. Hall CB: The spread of influenza and other respiratory viruses: complexities and conjectures. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007, 45(3):353-359.
106. Cate TR: Clinical manifestations and consequences of influenza. *The American journal of medicine* 1987, 82(6A):15-19.
107. Florescu DF, Kalil AC: The complex link between influenza and severe sepsis. *Virulence* 2014, 5(1):137-142.
108. Metzger DW, Sun K: Immune dysfunction and bacterial coinfections following influenza. *Journal of immunology* 2013, 191(5):2047-2052.
109. van der Sluijs KF, van der Poll T, Lutter R, Juffermans NP, Schultz MJ: Bench-to-bedside review: bacterial pneumonia with influenza - pathogenesis and clinical implications. *Critical care (London, England)* 2010, 14(2):219.
110. Taubenberger JK, Kash JC: Insights on influenza pathogenesis from the grave. *Virus research* 2011, 162(1-2):2-7.
111. Tscherne DM, Garcia-Sastre A: Virulence determinants of pandemic influenza viruses. *The Journal of clinical investigation* 2011, 121(1):6-13.
112. Sprenger MJ, Mulder PG, Beyer WE, Van Strik R, Masurel N: Impact of influenza on mortality in relation to age and underlying disease, 1967-1989. *International journal of epidemiology* 1993, 22(2):334-340.
113. Centers for Disease C, Prevention: Estimates of deaths associated with seasonal influenza --- United States, 1976-2007. *MMWR Morbidity and mortality weekly report* 2010, 59(33):1057-1062.

114. Astrahan P, Arkin IT: Resistance characteristics of influenza to amino-adamantyls. *Biochimica et biophysica acta* 2011, 1808(2):547-553.
115. Jefferson T, Jones MA, Doshi P, Del Mar CB, Hama R, Thompson MJ, Spencer EA, Onakpoya I, Mahtani KR, Nunan D *et al*: Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children. *The Cochrane database of systematic reviews* 2014, 4:CD008965.
116. Adhikari NK, Burns KE, Friedrich JO, Granton JT, Cook DJ, Meade MO: Effect of nitric oxide on oxygenation and mortality in acute lung injury: systematic review and meta-analysis. *Bmj* 2007, 334(7597):779.
117. Walmrath D, Schneider T, Pilch J, Grimminger F, Seeger W: Aerosolised prostacyclin in adult respiratory distress syndrome. *Lancet* 1993, 342(8877):961-962.
118. Gattinoni L, Tognoni G, Pesenti A, Taccone P, Mascheroni D, Labarta V, Malacrida R, Di Giulio P, Fumagalli R, Pelosi P *et al*: Effect of prone positioning on the survival of patients with acute respiratory failure. *The New England journal of medicine* 2001, 345(8):568-573.
119. Peek GJ, Mugford M, Tiruvoipati R, Wilson A, Allen E, Thalanany MM, Hibbert CL, Truesdale A, Clemens F, Cooper N *et al*: Efficacy and economic assessment of conventional ventilatory support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR): a multicentre randomised controlled trial. *Lancet* 2009, 374(9698):1351-1363.
120. Morens DM, Taubenberger JK: Pandemic influenza: certain uncertainties. *Reviews in medical virology* 2011.
121. Johnson NP, Mueller J: Updating the accounts: global mortality of the 1918-1920 "Spanish" influenza pandemic. *Bulletin of the history of medicine* 2002, 76(1):105-115.
122. Potter CW: A history of influenza. *Journal of applied microbiology* 2001, 91(4):572-579.
123. Murray CJ, Lopez AD, Chin B, Feehan D, Hill KH: Estimation of potential global pandemic influenza mortality on the basis of vital registry data from the 1918-20 pandemic: a quantitative analysis. *Lancet* 2006, 368(9554):2211-2218.
124. Lopez-Cervantes M, Venado A, Moreno A, Pacheco-Dominguez RL, Ortega-Pierres G: On the spread of the novel influenza A (H1N1) virus in Mexico. *Journal of infection in developing countries* 2009, 3(5):327-330.
125. Dominguez-Cherit G, Lapinsky SE, Macias AE, Pinto R, Espinosa-Perez L, de la Torre A, Poblano-Morales M, Baltazar-Torres JA, Bautista E, Martinez A *et al*: Critically Ill patients with 2009

influenza A(H1N1) in Mexico. *JAMA : the journal of the American Medical Association* 2009, 302(17):1880-1887.
126. World now at the start of 2009 influenza pandemic. Statement to the press by WHO Director-General Dr Margaret Chan, 11 June 2009. http://www.who.int/mediacentre/news/statements/2009/h1n1_pandemic_phase6_20090611/en/
127. Descamps V, Aitken J, Lee MG: Hippocrates on necrotising fasciitis. *Lancet* 1994, 344(8921):556.
128. Dellinger EP: Severe necrotizing soft-tissue infections. Multiple disease entities requiring a common approach. *JAMA : the journal of the American Medical Association* 1981, 246(15):1717-1721.
129. Patino JF, Castro D: Necrotizing lesions of soft tissues: a review. *World journal of surgery* 1991, 15(2):235-239.
130. Anaya DA, Dellinger EP: Necrotizing soft-tissue infection: diagnosis and management. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2007, 44(5):705-710.
131. Efstratiou A: Group A streptococci in the 1990s. *The Journal of antimicrobial chemotherapy* 2000, 45 Suppl:3-12.
132. Luca-Harari B, Darenberg J, Neal S, Siljander T, Strakova L, Tanna A, Creti R, Ekelund K, Koliou M, Tassios PT *et al*: Clinical and microbiological characteristics of severe Streptococcus pyogenes disease in Europe. *Journal of clinical microbiology* 2009, 47(4):1155-1165.
133. Olsen RJ, Musser JM: Molecular pathogenesis of necrotizing fasciitis. *Annual review of pathology* 2010, 5:1-31.
134. McCormick JK, Yarwood JM, Schlievert PM: Toxic shock syndrome and bacterial superantigens: an update. *Annual review of microbiology* 2001, 55:77-104.
135. Eke N: Fournier's gangrene: a review of 1726 cases. *The British journal of surgery* 2000, 87(6):718-728.
136. McHenry CR, Piotrowski JJ, Petrinic D, Malangoni MA: Determinants of mortality for necrotizing soft-tissue infections. *Ann Surg* 1995, 221(5):558-563; discussion 563-555.
137. Wong CH, Chang HC, Pasupathy S, Khin LW, Tan JL, Low CO: Necrotizing fasciitis: clinical presentation, microbiology, and determinants of mortality. *J Bone Joint Surg Am* 2003, 85-A(8):1454-1460.
138. Wong CH, Khin LW, Heng KS, Tan KC, Low CO: The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: a tool for distinguishing necrotizing fasciitis from other soft tissue infections. *Critical care medicine* 2004, 32(7):1535-1541.
139. Childers BJ, Potyondy LD, Nachreiner R, Rogers FR, Childers ER, Oberg KC, Hendricks DL, Hardesty RA: Necrotizing fasciitis: a

fourteen-year retrospective study of 163 consecutive patients. *The American surgeon* 2002, 68(2):109-116.
140. Ault MJ, Geiderman J, Sokolov R: Rapid identification of group A streptococcus as the cause of necrotizing fasciitis. *Annals of emergency medicine* 1996, 28(2):227-230.
141. Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, Hirschmann JV, Kaplan SL, Montoya JG, Wade JC: Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014, 59(2):e10-52.
142. Eagle H: Experimental approach to the problem of treatment failure with penicillin. I. Group A streptococcal infection in mice. *The American journal of medicine* 1952, 13(4):389-399.
143. Stevens DL, Gibbons AE, Bergstrom R, Winn V: The Eagle effect revisited: efficacy of clindamycin, erythromycin, and penicillin in the treatment of streptococcal myositis. *The Journal of infectious diseases* 1988, 158(1):23-28.
144. Norrby-Teglund A, Kaul R, Low DE, McGeer A, Newton DW, Andersson J, Andersson U, Kotb M: Plasma from patients with severe invasive group A streptococcal infections treated with normal polyspecific IgG inhibits streptococcal superantigen-induced T cell proliferation and cytokine production. *Journal of immunology* 1996, 156(8):3057-3064.
145. Kaul R, McGeer A, Norrby-Teglund A, Kotb M, Schwartz B, O'Rourke K, Talbot J, Low DE: Intravenous immunoglobulin therapy for streptococcal toxic shock syndrome--a comparative observational study. The Canadian Streptococcal Study Group. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 1999, 28(4):800-807.
146. Darenberg J, Ihendyane N, Sjolin J, Aufwerber E, Haidl S, Follin P, Andersson J, Norrby-Teglund A: Intravenous immunoglobulin G therapy in streptococcal toxic shock syndrome: a European randomized, double-blind, placebo-controlled trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2003, 37(3):333-340.
147. Jallali N, Withey S, Butler PE: Hyperbaric oxygen as adjuvant therapy in the management of necrotizing fasciitis. *American journal of surgery* 2005, 189(4):462-466.
148. Hassan Z, Mullins RF, Friedman BC, Shaver JR, Brandigi C, Alam B, Mian MA: Treating necrotizing fasciitis with or without hyperbaric oxygen therapy. *Undersea Hyperb Med* 2010, 37(2):115-123.
149. Dworkin MS, Westercamp MD, Park L, McIntyre A: The epidemiology of necrotizing fasciitis including factors associated

with death and amputation. *Epidemiology and infection* 2009, 137(11):1609-1614.
150. Frazee BW, Fee C, Lynn J, Wang R, Bostrom A, Hargis C, Moore P: Community-acquired necrotizing soft tissue infections: a review of 122 cases presenting to a single emergency department over 12 years. *The Journal of emergency medicine* 2008, 34(2):139-146.
151. Khanna AK, Tiwary SK, Kumar P, Khanna R, Khanna A: A case series describing 118 patients with lower limb necrotizing fasciitis. *The international journal of lower extremity wounds* 2009, 8(2):112-116.
152. Massey PR, Sakran JV, Mills AM, Sarani B, Aufhauser DD, Jr., Sims CA, Pascual JL, Kelz RR, Holena DN: Hyperbaric oxygen therapy in necrotizing soft tissue infections. *The Journal of surgical research* 2012, 177(1):146-151.
153. Nisbet M, Ansell G, Lang S, Taylor S, Dzendrowskyj P, Holland D: Necrotizing fasciitis: review of 82 cases in South Auckland. *Internal medicine journal* 2011, 41(7):543-548.
154. Endorf FW, Klein MB, Mack CD, Jurkovich GJ, Rivara FP: Necrotizing soft-tissue infections: differences in patients treated at burn centers and non-burn centers. *Journal of burn care & research : official publication of the American Burn Association* 2008, 29(6):933-938.
155. Holena DN, Mills AM, Carr BG, Wirtalla C, Sarani B, Kim PK, Braslow BM, Kelz RR: Transfer status: a risk factor for mortality in patients with necrotizing fasciitis. *Surgery* 2011, 150(3):363-370.
156. Flores-Cordero JM, Amaya-Villar R, Rincon-Ferrari MD, Leal-Noval SR, Garnacho-Montero J, Llanos-Rodriguez AC, Murillo-Cabezas F: Acute community-acquired bacterial meningitis in adults admitted to the intensive care unit: clinical manifestations, management and prognostic factors. *Intensive care medicine* 2003, 29(11):1967-1973.
157. Dauchy FA, Gruson D, Chene G, Viot J, Bebear C, Maugein J, Bezian MC, Dutronc H, Dupon M: Prognostic factors in adult community-acquired bacterial meningitis: a 4-year retrospective study. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2007, 26(10):743-746.
158. Domingo P, Pomar V, Benito N, Coll P: The changing pattern of bacterial meningitis in adult patients at a large tertiary university hospital in Barcelona, Spain (1982-2010). *The Journal of infection* 2013, 66(2):147-154.
159. Dzupova O, Rozsypal H, Prochazka B, Benes J: Acute bacterial meningitis in adults: predictors of outcome. *Scandinavian journal of infectious diseases* 2009, 41(5):348-354.

160. Ishihara M, Kamei S, Taira N, Morita A, Miki K, Naganuma T, Minami M, Shiota H, Hara M, Mizutani T: Hospital-based study of the prognostic factors in adult patients with acute community-acquired bacterial meningitis in Tokyo, Japan. *Internal medicine (Tokyo, Japan)* 2009, 48(5):295-300.
161. Moon SY, Chung DR, Kim SW, Chang HH, Lee H, Jung DS, Kim YS, Jung SI, Ryu SY, Heo ST *et al*: Changing etiology of community-acquired bacterial meningitis in adults: a nationwide multicenter study in Korea. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2010, 29(7):793-800.
162. Sakata H, Sato Y, Nonoyama M, Haruta T, Ouchi K, Yamaguchi S, Sunakawa K: Results of a multicenter survey of diagnosis and treatment for bacterial meningitis in Japan. *Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy* 2010, 16(6):396-406.
163. Stockdale AJ, Weekes MP, Aliyu SH: An audit of acute bacterial meningitis in a large teaching hospital 2005-10. *QJM : monthly journal of the Association of Physicians* 2011, 104(12):1055-1063.
164. 2013 annual report on adult acute bacterial meningitis in Sweden. Available from: http://www.infektion.net/sites/default/files/bakteriell_meningit_2013.pdf
165. Aronin SI, Peduzzi P, Quagliarello VJ: Community-acquired bacterial meningitis: risk stratification for adverse clinical outcome and effect of antibiotic timing. *Annals of internal medicine* 1998, 129(11):862-869.
166. Deutch S, Dahlberg D, Hedegaard J, Schmidt MB, Moller JK, Ostergaard L: Diagnosis of ventricular drainage-related bacterial meningitis by broad-range real-time polymerase chain reaction. *Neurosurgery* 2007, 61(2):306-311; discussion 311-302.
167. Samra Z, Shmuely H, Nahum E, Paghis D, Ben-Ari J: Use of the NOW Streptococcus pneumoniae urinary antigen test in cerebrospinal fluid for rapid diagnosis of pneumococcal meningitis. *Diagnostic microbiology and infectious disease* 2003, 45(4):237-240.
168. Bonadio WA, Smith D: Cerebrospinal fluid changes after 48 hours of effective therapy for Hemophilus influenzae type B meningitis. *American journal of clinical pathology* 1990, 94(4):426-428.
169. Report by the Public Health Agency of Sweden, The Influenza A (H1N1) 2009 Pandemic in Sweden, 2009-2010. Available from: http://www.folkhalsomyndigheten.se/publicerat-material/publikationer/The-Influenza-AH1N12009-Pandemic-in-Sweden-2009-2010/

170. Investigators AI, Webb SA, Pettila V, Seppelt I, Bellomo R, Bailey M, Cooper DJ, Cretikos M, Davies AR, Finfer S *et al*: Critical care services and 2009 H1N1 influenza in Australia and New Zealand. *The New England journal of medicine* 2009, 361(20):1925-1934.
171. Gubbels S, Perner A, Valentiner-Branth P, Molbak K: National surveillance of pandemic influenza A(H1N1) infection-related admissions to intensive care units during the 2009-10 winter peak in Denmark: two complementary approaches. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2010, 15(49).
172. Miller E, Andrews N, Stellitano L, Stowe J, Winstone AM, Shneerson J, Verity C: Risk of narcolepsy in children and young people receiving AS03 adjuvanted pandemic A/H1N1 2009 influenza vaccine: retrospective analysis. *Bmj* 2013, 346:f794.
173. Partinen M, Saarenpaa-Heikkila O, Ilveskoski I, Hublin C, Linna M, Olsen P, Nokelainen P, Alen R, Wallden T, Espo M *et al*: Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. *PloS one* 2012, 7(3):e33723.
174. Persson I, Granath F, Askling J, Ludvigsson JF, Olsson T, Feltelius N: Risks of neurological and immune-related diseases, including narcolepsy, after vaccination with Pandemrix: a population- and registry-based cohort study with over 2 years of follow-up. *Journal of internal medicine* 2014, 275(2):172-190.
175. Hiba V, Chowers M, Levi-Vinograd I, Rubinovitch B, Leibovici L, Paul M: Benefit of early treatment with oseltamivir in hospitalized patients with documented 2009 influenza A (H1N1): retrospective cohort study. *The Journal of antimicrobial chemotherapy* 2011, 66(5):1150-1155.
176. Launes C, Garcia-Garcia JJ, Jordan I, Martinez-Planas A, Selva L, Munoz-Almagro C: 2009 Influenza A H1N1 infections: delays in starting treatment with oseltamivir were associated with a more severe disease. *The Pediatric infectious disease journal* 2011, 30(7):622-625.
177. Brun-Buisson C, Richard JC, Mercat A, Thiebaut AC, Brochard L: Early corticosteroids in severe influenza A/H1N1 pneumonia and acute respiratory distress syndrome. *American journal of respiratory and critical care medicine* 2011, 183(9):1200-1206.
178. Kim SH, Hong SB, Yun SC, Choi WI, Ahn JJ, Lee YJ, Lee HB, Lim CM, Koh Y, Korean Society of Critical Care Medicine HNC: Corticosteroid treatment in critically ill patients with pandemic influenza A/H1N1 2009 infection: analytic strategy using propensity scores. *American journal of respiratory and critical care medicine* 2011, 183(9):1207-1214.

179. Estenssoro E, Rios FG, Apezteguia C, Reina R, Neira J, Ceraso DH, Orlandi C, Valentini R, Tiribelli N, Brizuela M *et al*: Pandemic 2009 influenza A in Argentina: a study of 337 patients on mechanical ventilation. *American journal of respiratory and critical care medicine* 2010, 182(1):41-48.
180. Kumar A, Zarychanski R, Pinto R, Cook DJ, Marshall J, Lacroix J, Stelfox T, Bagshaw S, Choong K, Lamontagne F *et al*: Critically ill patients with 2009 influenza A(H1N1) infection in Canada. *JAMA : the journal of the American Medical Association* 2009, 302(17):1872-1879.
181. Demoule A, Girou E, Richard JC, Taille S, Brochard L: Benefits and risks of success or failure of noninvasive ventilation. *Intensive care medicine* 2006, 32(11):1756-1765.
182. Rana S, Jenad H, Gay PC, Buck CF, Hubmayr RD, Gajic O: Failure of non-invasive ventilation in patients with acute lung injury: observational cohort study. *Critical care (London, England)* 2006, 10(3):R79.
183. Nicolini A, Tonveronachi E, Navalesi P, Antonelli M, Valentini I, Melotti RM, Pigna A, Carrassi A, Righini P, Ferrari Bravo M *et al*: Effectiveness and predictors of success of noninvasive ventilation during H1N1 pandemics: a multicenter study. *Minerva anestesiologica* 2012, 78(12):1333-1340.
184. Dunning J, Baillie JK, Cao B, Hayden FG: Antiviral combinations for severe influenza. *The Lancet Infectious diseases* 2014.
185. Laursen NS, Wilson IA: Broadly neutralizing antibodies against influenza viruses. *Antiviral research* 2013, 98(3):476-483.