Aspects of neonatal septicaemia – prevention and complications
To all children born early into this world.
Louise Björkman Hjalmarsson

Aspects of neonatal septicaemia – prevention and complications
ABSTRACT


Study I was part of the Extremely Preterm Infants in Sweden Study (EXPRESS), a prospective national study including all infants born <27 weeks in Sweden in 2004-2007 that survived their first year of life (n=497). Neonatal sepsis was evaluated as a risk factor for neonatal morbidities. Definite sepsis was associated with severe bronchopulmonary dysplasia and prolonged hospital stay, but not with a higher risk of retinopathy of prematurity or intraventricular haemorrhage.

Study II was a non-randomized single-centre intervention study evaluating possible preventive effects on coagulase-negative staphylococci (CoNS) sepsis when the scrub the hub method was used. During the intervention period, the incidence of CoNS sepsis decreased from 1.5% to 0% (CI: 0.53-2.58%, p=0.06).

Study III was an in-vitro study evaluating leakage of isopropanol (IPA) and ethanol when alcohol caps and scrub the hub were used to disinfect hubs. Alcohol leakage was measured using gas chromatography. IPA was detected in all samples from cap circuits, and mean leakage increased over time. Ethanol levels were low, and scrub the hub therefore seems safe to use.

Study IV was a survey study evaluating reported hygiene routines from Swedish neonatal intensive care units (NICUs) included in the EXPRESS study. Routines were compared between the EXPRESS period (2004-2007) and 2013. Improvements were seen regarding basic hygiene routines, routines for work clothing, and follow-up of compliance. Antibiotic prophylaxis decreased while fungal prophylaxis increased, but the empiric treatment of suspected late-onset sepsis (LOS) showed heterogeneity.

Study V investigated the association between incidence in LOS in the EXPRESS cohort and the hygiene routines previously evaluated in Study IV. Strict catheter routines, blood culture routines, and non-use of antibiotic prophylaxis were associated with decreased sepsis risk.

Keywords: neonatal sepsis, prevention, coagulase-negative staphylococci, nosocomial, hygiene, neonatal morbidity, alcohol toxicity.

Louise Björkman Hjalmarsson, Institution of Medical Sciences
Örebro University, SE-701 82 Örebro, Sweden,
louise.bjorkman-hjalmarsson@regionorebrolan.se
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### Abbreviations

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<td>CLD</td>
<td>Chronic lung disease</td>
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<td>CONS</td>
<td>Coagulase-negative staphylococci</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>CVC</td>
<td>Central venous catheter</td>
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<td>EOS</td>
<td>Early-onset sepsis</td>
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<td>EXPRESS</td>
<td>Extremely Preterm Infants in Sweden Study</td>
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<td>GBS</td>
<td>Group B streptococci</td>
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<td>IL-6</td>
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<td>IL-8</td>
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<td>IPA</td>
<td>Isopropanol</td>
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<td>IVH</td>
<td>Intraventricular haemorrhage</td>
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<td>LOS</td>
<td>Late-onset sepsis</td>
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<td>NEC</td>
<td>Necrotizing enterocolitis</td>
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<td>NICU</td>
<td>Neonatal intensive care unit</td>
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<td>NS</td>
<td>Neonatal sepsis</td>
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<td>OR</td>
<td>Odds ratio</td>
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<td>PCT</td>
<td>Procalcitonin</td>
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<td>PICC</td>
<td>Peripherally inserted central catheter</td>
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<td>PVL</td>
<td>Periventricular leukomalacia</td>
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<td>RDS</td>
<td>Respiratory distress syndrome</td>
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<td>ROP</td>
<td>Retinopathy of prematurity</td>
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<td>RR</td>
<td>Relative risk</td>
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<td>SIRS</td>
<td>Systemic inflammatory response syndrome</td>
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<td>TLR</td>
<td>Toll-like receptor</td>
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<td>TNF-α</td>
<td>Tumour necrosis factor α</td>
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<td>UAC</td>
<td>Umbilical arterial catheter</td>
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<td>UVC</td>
<td>Umbilical venous catheter</td>
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<td>VLBW</td>
<td>Very low birth weight</td>
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<td>NS</td>
<td>Neonatal sepsis</td>
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Original articles

This thesis is based on the following articles, which are referred to in the text by their respective Roman numerals (I-V):


Introduction

Epidemiology of neonatal sepsis

Neonatal sepsis (NS), defined as a systemic infection that occurs within the first 28 days of life,\(^1\) is the cause of approximately 1 million deaths per year worldwide.\(^2, 3\) It is one of the most common complications affecting infants in the neonatal period, and although mortality is decreasing it remains a major concern. The incidence of NS remains high, especially among preterm infants, and it is also associated with a high risk of other morbidities.\(^4-8\)

NS is normally classified according to the time of onset. Early-onset sepsis (EOS) occurs before 48-72 hours of age and is considered a vertical infection, while late-onset sepsis (LOS) occurs after that time.\(^9,10\) It is not only the time when the infection occurs that separates EOS from LOS, but also the causative pathogens, which have not been consistent over the last century.\(^11-15\)

Regarding EOS, Group B streptococci (GBS) were once the predominant causative pathogen, but prophylactic strategies such as the use of intrapartum antibiotics have led to decreased incidence both in EOS caused by GBS and in total EOS.\(^11,16,17\) Today, Escherichia coli is the most common pathogen in EOS.\(^3,18\)

LOS, which is considered a nosocomial infection, is the most prevalent form of neonatal sepsis. The most common causative agents in the developed world today are the coagulase-negative staphylococci (CoNS).\(^4,19,20\) Other important pathogens in this group are Staphylococcus aureus, Klebsiella pneumonia, Enterococcus spp., Pseudomonas aeruginosa, Candida albicans, and E. coli.\(^16\) These pathogens differ in virulence, with Pseudomonas\(^4,21\) and Klebsiella causing high mortality rates.\(^21\) CoNS are not considered as virulent as other pathogens in terms of toxin production, but can still cause long-term sequelae and mortality.\(^5,9,22,23\)

There are several reasons why nosocomial infections are especially common in very preterm neonates. One is these neonates’ need for intensive care, which entails mechanical ventilation, intravascular catheters with long dwell times, parenteral nutrition, delayed enteral feeding and colonization with nosocomial bacteria.\(^4,11\) They also have lower immune system function than infants born at term.\(^16,24\) Since LOS is mainly nosocomial, it is considered preventable, and so much time and effort have been put into
different preventive strategies.\textsuperscript{25-30} Several research groups have shown a decrease in LOS over the past decade,\textsuperscript{5, 31-35} and the observed decrease has been associated with successful preventive strategies and interventions.

It is not only bacteria that cause invasive infections in newborns. In the preterm group especially, invasive candida infections are a cause of both morbidity and mortality.\textsuperscript{4} The symptoms of invasive candida infections are diffuse, and late diagnosis is common.\textsuperscript{34}

**The neonatal immune system**

The skin and mucous membrane of the body constitute the first line of defence. The stratum corneum, which is the outmost layer of the skin, is not fully mature in preterm infants.\textsuperscript{1, 35} Another defence mechanism in the skin is the waxy coat called vernix caseosa, which is formed mainly during the last trimester.\textsuperscript{36}

The human immune system can be divided into two connected systems: the innate and the adaptive system. The innate system protects the body against invading pathogens, but is not antigen-specific and does not memorize the pathogens it has encountered. It consists of soluble proteins, peptides, and phagocytes. The phagocytes communicate with the adaptive immune system by presenting antigens, and constitute a link between the two systems.\textsuperscript{37}

The function of the innate immune system of the newborn differs from that of an adult. This is especially true for the immune system of the very preterm infant.\textsuperscript{38} It has been shown that preterm infants have a gestational age dependant deficiency in complement factors, antimicrobial proteins and peptides, and mannose-binding lectin, which affects opsonisation, phagocytosis, and the first line of defence in mucous and skin.\textsuperscript{24} The proper activation of pattern recognition receptors is also a crucial part of the surveillance properties of the innate immune system. One example of a pattern recognition receptor is the Toll-like receptor (TLR). It has been shown that the preterm immune system’s TLR cytokine responses are weaker than those in term infants and adults,\textsuperscript{39} and TLR2, which is especially important in the defence against CoNS, develops late in gestation.\textsuperscript{40}

Neutropenia is common in the preterm, and both the ability of neutrophils to transport themselves to the infection site and their phagocytic ability are insufficient,\textsuperscript{5, 24, 38, 41} probably as a result of low complement and immunoglobulin levels.\textsuperscript{52} Preterm infants also have a less efficient neutrophil oxidative burst, which affects neutrophil bacterial killing.\textsuperscript{43} Moreover,
there seems to be a deficiency in the ability of macrophages and monocytes to interact with the adaptive immune system.\textsuperscript{24}

The adaptive immune system of the preterm infant has several weaknesses and differences compared with a more mature immune system,\textsuperscript{40} and is still not fully developed and adapted to the early extra-uterine life. The infant therefore relies on its innate immune system in combination with antibodies from its mother. Antibodies are mainly transferred across the placenta during the third trimester, meaning that the preterm neonate is born with low levels.\textsuperscript{2, 37} The B-cell receptor signalling is less functional. The T-cells that are responsible for tolerance and anti-inflammation are higher in numbers than the T-cells responsible for defence, which means that the immune system of a newborn infant is directed against tolerance, rather than responding to a foreign threat. This tolerance is crucial for foetal life, but seemingly less so for extra uterine life.\textsuperscript{41}

**Coagulase-negative staphylococci**

Staphylococci are facultative, anaerobic, gram-positive cocci. They can be divided into two groups on the basis of their ability or inability to produce coagulase: coagulase-negative and coagulase-positive staphylococci.

*Staphylococcus epidermidis*, which is the most commonly isolated bacteria from the human skin, is coagulase-negative. *S. epidermidis* and *S. haemolyticus* are the most important CoNS species in neonatal infections.\textsuperscript{44, 45}

Neonates become colonized with microorganisms from their surroundings within the first week of life.\textsuperscript{44, 46} Infants admitted to a neonatal intensive care unit are at risk of becoming colonized with bacteria dwelling in the hospital environment instead of their parents’ normal flora, which results in carriership and potential infections caused by bacteria with multiresistance\textsuperscript{47} and the ability to form biofilm.\textsuperscript{44, 48-50}

Biofilm formation is one of *S. epidermidis*’ most important virulence factors. This ability makes it an important pathogen in catheter-related infections, since it can adhere to indwelling materials.\textsuperscript{51} In addition to enabling CoNS to dwell on surfaces, biofilm also acts as a barrier against antibiotics, antimicrobial therapy, and the immune system.\textsuperscript{52, 53} *S. epidermidis* uses toxins as virulence factors, with one of the most important being phenol-soluble modulins. These contribute to biofilm production, but also have the ability to lyse granulocytes.\textsuperscript{44} Further, *S. epidermidis* easily develops antimicrobial resistance through its staphylococcal cassette
chromosome (mec). This genetic flexibility further helps it to survive in the hospital environment.\textsuperscript{44}

The cytokine response in neonates is different from that in adults.\textsuperscript{38} When neonatal blood was compared with adult blood, a lower complement activation could be seen in response to \textit{S. epidermidis}. The neonatal blood also had lower levels of antibodies directed against both the bacteria itself and its biofilm.\textsuperscript{52} As mentioned previously, the production of proinflammatory cytokines is correlated to gestational age, and so extremely preterm infants are especially vulnerable.\textsuperscript{53}

Taking all these virulence factors into consideration, one of the strongest abilities of \textit{S. epidermidis} is to evade the human immune system. This is partly achieved by the lysis of neutrophils, but also by resistance to phagocytosis,\textsuperscript{44} making these pathogens challenging for the preterm immune system.

**Definition of neonatal sepsis**

In adult patients, sepsis is defined as “a life-threatening organ dysfunction caused by a dysregulated host response to infection”.\textsuperscript{54} However, neonatal sepsis does not have a widely accepted definition.\textsuperscript{55, 56} This creates problems both in the clinical setting and in research.

Systemic inflammatory response syndrome (SIRS) is a non-specific inflammatory process that can occur as a result of an infection. In 2005, a conference was held with the purpose of modifying the adult SIRS criteria for the paediatric patient, including term newborns (≥37 weeks), but premature infants were not included in this consensus. The paediatric consensus defined sepsis as SIRS in the presence of proven or suspected sepsis. A diagnosis of SIRS requires an abnormal white blood count, >10\% immature neutrophils, or an abnormal core temperature (>38.5 or <36°C).\textsuperscript{57} However, Hofer et al. examined almost 500 cases of neonatal sepsis retrospectively, and found that the definition applied to only 53\% of the term neonates with verified EOS.\textsuperscript{58}

There is also a special syndrome when it comes to neonatal infections, called “clinical sepsis”. It is unclear what the syndrome stands for, but it has often been described as involving symptoms associated with neonatal sepsis, but with negative blood cultures.\textsuperscript{56, 59}
Diagnosis of neonatal sepsis

Neonatal sepsis lacks specific symptoms, but can be suspected when a neonate exhibits a number of rather unspecific symptoms and clinical signs, such as apnoea, low blood pressure, lethargy, and bradycardia. Preterm infants may respond to infection with hypothermia instead of fever, due to lack of temperature control, especially during the first days of life. The symptoms can be especially discreet when it comes to sepsis caused by low-virulence species such as CoNS.

Blood culture

The gold standard for the diagnosis of NS is positive blood cultures, but there are several problems with this diagnostic tool. The first problem occurs in the clinical setting, since the results of blood cultures take time and so cannot be of any help initially to the clinician when the patient is acutely ill. This means that empiric antibiotic treatment must be initiated on suspicion, before the blood culture results are at hand.

Secondly, the sensitivity of blood cultures is unclear, and a false-negative rate of 20% has been suggested. Moreover, a majority of neonates treated for suspected sepsis have negative blood cultures. The lack of sensitivity can partly be explained by the difficulty of obtaining sufficient amounts of blood to reach above the lower detection level, which causes a high proportion of false negative results. It can also be a result of inappropriately obtained blood cultures, for example after antibiotic therapy has been initiated.

Thirdly, there is a problem with the specificity of blood cultures. It is difficult to draw conclusions from one positive blood culture, especially those with growth of commensal species such as CoNS. There are different methods to obtain blood samples for blood cultures. Arterial or venous punctures or samples from a newly placed catheter are often used. Sometimes a specimen is obtained from a catheter that is already in place, which might reflect colonization of the catheter rather than bacteraemia. It is common for extremely preterm infants to have at least one CoNS positive blood culture during their hospital stay, and a number of these are probably explained by contamination or colonization and not by bacteraemia. One could therefore suspect that a significant proportion of infants receiving antibiotics due to a positive blood culture during their stay in the NICU are given an unnecessary treatment. Struthers et al. showed that empirical treatment for suspected LOS could be decreased by taking two blood cultures instead of only one when sepsis was suspected.
If only one of the two cultures showed growth of CoNS, the result was considered as contamination and antibiotic treatment was ended after 48 hours of treatment.\textsuperscript{70}

**Haematological markers**

Several different haematological markers have been evaluated as a test for neonatal sepsis, such as white blood cell count, neutrophil count, and immature to total neutrophil ratio.\textsuperscript{16, 55, 71-75} These markers lack specificity, especially when analysed at only one time-point. Immature to total neutrophil ratio might be useful when measured repeatedly, primarily to indicate when sepsis is unlikely.\textsuperscript{71} Neutrophil to lymphocyte ratio and mean platelet volume have shown promising results in recently published studies.\textsuperscript{76-78}

**Acute phase proteins and cytokines**

A number of cytokines and acute phase proteins are used in the diagnosis of NS. C-reactive protein (CRP) and procalcitonin (PCT) are two of the most commonly used markers in the clinical setting.\textsuperscript{16, 79}

CRP is an acute phase reactant synthesized by hepatocytes in the liver. It is well studied, has a high positive predictive value, and is regarded as a specific marker for sepsis, but it has a low sensitivity in the early phases of infection.\textsuperscript{16, 65, 79, 80} especially in preterm infants.\textsuperscript{81} The level of specificity is affected by the fact that several non-infectious conditions can result in an increase,\textsuperscript{16} and levels are often elevated as a result of a normal birth.\textsuperscript{82, 83} CRP can be useful if it is measured repeatedly as an aid to rule out infection and possibly shorten unnecessary antibiotic treatment.\textsuperscript{9, 79, 84, 85} If CRP levels continue to be elevated during the course of antibiotic treatment, this can indicate that the treatment is not adequate or that complications such as abscess formation have occurred.\textsuperscript{86}

PCT is another acute phase reactant. It is produced by macrophages and hepatocytes, and responds to bacterial toxin with a few hours.\textsuperscript{85} PCT might be used as an early marker for sepsis.\textsuperscript{16} However, there are problems with both the sensitivity and specificity, since PCT is physiologically elevated during the first days of life\textsuperscript{82, 85} and the threshold value for confirmation of sepsis is unknown.\textsuperscript{65} Several perinatal factors also affect PCT levels.\textsuperscript{16} Physiologically elevated levels are not as high as the elevated levels caused by a bacterial infection, but false positive cases have been reported. Respiratory distress syndrome, for example, can cause significantly elevated levels of PCT.\textsuperscript{85}
Cytokines are elevated in response to infection, and the elevation occurs before the acute phase reactants respond. Several cytokines, for example interleukin-6 (IL-6), interleukin-8 (IL-8), and tumour necrosis factor α (TNF-α), have been shown to respond to neonatal sepsis. IL-6 responds very early to infection, and its sensitivity is high, but its half-life is short; it can therefore be used as an early marker of infection.

IL-8 also responds quickly to infection and has a high sensitivity and specificity, but normalizes quickly in serum and is therefore mainly used in combination with, for example, CRP. These cytokines are not considered ideal markers, and they are not as commonly used in the clinical setting as CRP or PCT.

An ideal marker for neonatal sepsis would be specific for neonatal sepsis and possible to use for early detection. It would require low blood volumes and be possible to use for monitoring progress of treatment.

In summary, it is not easy to exactly define which newborn has a “true” sepsis. Many different clinical and laboratory parameters are used, and most of them detect inflammation rather than infection. The experienced clinician needs to make a judgement based on both clinical and laboratory parameters for each case.

**Cell surface markers**

The most investigated cell surface antigens in neonatal sepsis are CD64 and CD11b. CD64 is expressed mainly by monocytes and macrophages, and also by neutrophils but to a smaller extent. Neutrophil expression of CD64 is elevated during bacterial infection in neonates, and this increased expression of CD64 can enable early differentiation between infected and non-infected neonates. Both sensitivity and specificity are highest 24 h after symptom onset.

CD11b is expressed on the surface of neutrophils, in lower concentrations when the neutrophil is non-activated and to a larger extent when activated. Its sensitivity has been found to be high in EOS, but less so in LOS. The clinical use of these analyses has mainly been limited by high prices and the requirement for advanced technology and analysts around the clock.
Neonatal sepsis as a risk factor for morbidity

Mortality from neonatal sepsis is decreasing, but morbidity is still a major concern. Several scientific reports describe bacterial infection as an important contributing factor in the development of acute and long-term complications in the neonatal period, but the mechanism behind this association is not entirely understood. The development of bronchopulmonary dysplasia (BPD), intraventricular haemorrhage (IVH), and ROP is thought to be aggravated by bacterial infections causing systemic inflammation. A recent meta-analysis concluded that infants who had suffered from neonatal sepsis had a higher risk of developing BPD, respiratory distress syndrome (RDS), IVH, and periventricular leukomalacia (PVL).

Even though CoNS is regarded as a pathogen with relatively low virulence, studies have shown that sepsis caused by CoNS can be associated with sequelae such as BPD and impaired neurodevelopmental outcome. When sepsis caused by CoNS was compared to sepsis caused by other pathogens, CoNS sepsis was associated with lower risks for complications and death. However, contradictory results have been found in studies of complications following sepsis caused by CoNS, showing similar risks for complications or death.

Bronchopulmonary dysplasia

BPD was first described as a result of pulmonary healing after premature birth, RDS, and its treatments. Since treatment of RDS has improved using treatments such as modern mechanical ventilation with less barotrauma, antenatal glucocorticoids and surfactant, the features of BPD have also changed to the extent that the syndrome is often referred to today as the “new BPD”. The new BPD is characterized by a delayed and impaired postnatal development of the lung, primarily affecting the most immature infants, and with a multifactorial aetiology.

BPD was first defined as the requirement for treatment with >21% oxygen for at least 28 days but in order to accommodate preterm infants, this was altered to oxygen use at 36 weeks postmenstrual age. Severe BPD is defined as treatment with 30% oxygen at 36 weeks postmenstrual age. A recent evaluation of this definition concluded that oxygen and/or respiratory support at 40 weeks postmenstrual age was the best predictor of respiratory morbidity after the neonatal period.

There is a widespread theory that inflammation is central to the development of BPD, although the pathogenesis is not fully understood. Before the surfactant era, chorioamnionitis was regarded a risk factor for devel-
development of BPD, but today it is instead associated with a decreased risk of acquiring BPD. The theory behind this decreased risk is that prenatal inflammation might mature the lung and therefore decrease the risk of BPD.

Several studies have reported neonatal sepsis to be a risk factor for developing BPD, and in some studies CoNS sepsis has been shown to be as strong a risk factor for BPD as other more virulent bacteria, or even stronger.

### Retinopathy of prematurity
Retinopathy of prematurity (ROP) is a vasoproliferative disorder affecting the normal development of the retina with excessive vascularisation, and is mainly seen in infants born preterm. Initially it was regarded as a syndrome caused by high oxygen exposure, but even though successful efforts have been made to reduce oxygen exposure, ROP is still common in extremely preterm infants, probably as a consequence of the higher survival rate in this group. Exposure to infection or inflammation during the perinatal period can be a risk factor for ROP, possibly due to disturbance of retinal angiogenesis.

There is a risk of visual disability and blindness following ROP.

### Intraventricular haemorrhage
IVH is the most common abnormal finding on ultrasound examinations among preterm infants. The severity of the bleeding can be graded into four stages according to Papile. IVH is a risk factor for neurodevelopmental impairment. Preterm infants who had neonatal sepsis or necrotizing enterocolitis (NEC) had a greater risk of impaired motor development at 2 years of age; this association is believed to be caused by white matter abnormality.

### Necrotizing enterocolitis
NEC lacks a clear definition, and covers several different diseases. It presents as an acute inflammatory necrosis of the bowel, is graded according to Bell, and is a common complication among preterm infants. The syndrome entails high risks of mortality or subsequent morbidity such as short bowel syndrome. In its severe form, it can be clearly associated with other neonatal conditions such as severe BPD, severe ROP, and cerebral white matter damage.
The microbiota appears to play an important role in the pathogenesis of NEC, in that differences in the faecal microbiota have been seen between preterm infants with NEC and infants without NEC. NEC has also been associated with antibiotic treatment; the theory behind this association is that antibiotics disturb the intestinal microbiota. Probiotics have been found to be protective against NEC. There also seems to be an association between infections and NEC, since several studies have shown concurrent infections or infections following the onset of NEC.

**Prevention of neonatal sepsis and prophylaxis**

Quality improvement projects worldwide have contributed to a decreased incidence of neonatal sepsis. Basic hygiene routines such as hand washing and hand disinfection play important roles in these projects. A review from 2017, based on three sets of evidence-based guidelines, found that all three sets of guidelines recommended health care workers to “decontaminate immediately after direct patient care [and] when hands are visibly soiled”, and to use “an alcohol-based rub for the decontamination of hands before and after patient contact and clinical care”.

Even though knowledge about the importance of hand hygiene is widespread and the method is simple, compliance remains a problem. Sometimes a lack of compliance may be due to health care workers lacking awareness of the routines. A systematic review investigating strategies used to improve hand hygiene compliance found that both individual intervention strategies and combinations of strategies resulted in increased compliance, but it was not clear what specific strategy or combination of strategies was the most effective. Performance feedback and education seemed to improve compliance, but the level of evidence was low. Other efforts have been made to identify successful hand hygiene educational interventions, without clear results. In summary, it seems that multiple interventions are more successful than single interventions in creating a long-term difference in compliance.

A recent study investigating the use of plastic gloves found that care with non-sterile gloves after hand hygiene was superior to hand hygiene alone in decreasing the incidence of LOS in extremely preterm infants, but the finding did not reach statistical significance. Similar results have been shown previously. Combining evidence-based strategies into bundles and using checklists have been shown to be efficient in decreasing nosocomial infections. Other factors crucial in prevention of neonatal sepsis are early enteral
feeding, use of breast milk, minimal invasiveness during intensive care, and correct use of antibiotics.\textsuperscript{1, 3, 30, 35, 135, 136}

**Prophylaxis**

Antibiotic prophylaxis is still used in some NICUs today. The use of vancomycin as a prophylactic treatment has been shown to decrease the incidence of total neonatal sepsis and sepsis caused by CoNS. However, the morbidity and mortality of the studied infants were not affected, and so this treatment is not recommended because its benefit does not exceed its harm.\textsuperscript{137} Several reviews and meta-analyses have been performed on antibiotic prophylaxis, antifungal prophylaxis, and immunoglobulin treatment during the last 10 years; as shown by the summary presented in Table 1, only antifungal prophylaxis has been recommended.\textsuperscript{138}

Since studies have shown an increased risk of sepsis in very low birth weight infants after the removal of a central catheter,\textsuperscript{139, 140} prophylactic antibiotic treatment before the removal of the catheter has been discussed. However, a systematic review reported that there were not enough studies to make a well-founded conclusion on the matter.\textsuperscript{141}

Prophylactic fluconazole to prevent invasive candida infection has been recommended in preterm infants since it has been found to decrease the incidence of invasive fungal infections.\textsuperscript{34, 138, 142} However, no significant difference in mortality has been shown.\textsuperscript{138} The development of resistance has been discussed, but not reported since the introduction of the treatment.\textsuperscript{138}
**Table 1. Summary of Cochrane reviews on antibiotic prophylaxis, antifungal prophylaxis, and immunoglobulin treatment.**

<table>
<thead>
<tr>
<th>Cochrane reviews</th>
<th>Year</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylactic antibiotics - UAC* 143</td>
<td>2007</td>
<td>No</td>
</tr>
<tr>
<td>Prophylactic antibiotics - UVC* 144</td>
<td>2005</td>
<td>No</td>
</tr>
<tr>
<td>Prophylactic antibiotics - CVC* 145</td>
<td>2008</td>
<td>No</td>
</tr>
<tr>
<td>Prophylactic antibiotics for ventilated newborns 146</td>
<td>2007</td>
<td>No</td>
</tr>
<tr>
<td>Prophylactic vancomycin for preterms 137</td>
<td>2000</td>
<td>No</td>
</tr>
<tr>
<td>Prophylactic antibiotics - intercostal catheters 147</td>
<td>2012</td>
<td>No</td>
</tr>
<tr>
<td>IVIG to prevent infections in preterms 148</td>
<td>2013</td>
<td>No</td>
</tr>
<tr>
<td>Antistaph. Ig to prevent staphylococcal infections 149</td>
<td>2009</td>
<td>No</td>
</tr>
<tr>
<td>Antimicrobial-impregnated catheters 150</td>
<td>2015</td>
<td>No</td>
</tr>
<tr>
<td>Prophylactic antifungals in VLBW* 138</td>
<td>2015</td>
<td>Yes</td>
</tr>
<tr>
<td>Antibiotics at the time of removal of CVC* 141</td>
<td>2018</td>
<td>No</td>
</tr>
</tbody>
</table>

NOTE: UAC, umbilical arterial catheter; UVC, umbilical venous catheter; CVC, central venous catheter; VLBW, very low birth weight

**Treatment of neonatal sepsis**

Neonatal sepsis is a serious complication in the neonatal period. Empiric treatment is used to treat the patient with a suspected infection as soon as the suspicion arises, and the treatment should be directed against the most probable and most virulent pathogens.\(^{15}\) It is common for preterm infants to receive antibiotic treatment as early as their first days of life, even though the incidence of EOS is low in this group.\(^{98, 151}\) This means that the overall antibiotic use in NICUs is high.\(^{152}\)

A 2004 Cochrane review of the empiric treatment of suspected EOS concluded that there was not enough evidence to support any particular antibiotic treatment.\(^{153}\) However, two commonly used combinations are benzylpenicillin and aminoglycoside,\(^{154}\) and ampicillin and aminoglycoside.\(^{155}\) These combinations cover both Gram-positive and Gram-negative organisms, and are thus well suited for the bacterial spectrum that causes EOS.\(^{156}\)

LOS is the most common type of neonatal sepsis, and CoNS is the major pathogen in the developed world today.\(^{6, 19, 20}\) There is no complete consensus regarding the empiric treatment of suspected LOS\(^{157}\), but the combination of an isoxazolyl-penicillin and aminoglycoside is recommended in Swedish guidelines.\(^{154, 158}\) Since CoNS is the most common
pathogen in LOS, and the strains that cause LOS are often multiresistant, vancomycin has become a more frequent choice, often in combination with another antibiotic.\textsuperscript{154, 159} Unfortunately, vancomycin-resistant CoNS are emerging, and therefore more focus has been directed toward decreasing the unnecessary use of vancomycin.\textsuperscript{96} CoNS sepsis is normally not considered a fulminant infection or associated with high morbidity,\textsuperscript{15, 96} and vancomycin has not been recommended as an empiric treatment for suspected LOS, but should instead be saved for treatment of multiresistant strains.\textsuperscript{15, 154, 160, 161} Empiric treatment with cephalosporins should be minimized as well, since it has been associated with development of multiresistance.\textsuperscript{162, 163} Cephalosporins have also been associated with an increased risk of mortality.\textsuperscript{164}

It is desirable to shorten antibiotic treatments as much as possible, and so it is important to cease antibiotic treatment when the infant is no longer suspected to have an infection. A recent meta-analysis showed an association between antibiotic treatment of uninfected infants and NEC and death.\textsuperscript{165} Other studies have shown associations between antibiotic prophylaxis and NEC\textsuperscript{166} and between prolonged antibiotic exposure and NEC\textsuperscript{167}, retinopathy of prematurity (ROP), and chronic lung disease.\textsuperscript{33}

There are also long-term risks of antibiotic exposure early in life, since disrupted microbiota can lead to increased risk for the development of asthma, infections, diabetes, and obesity as well as problems with absorption of nutrients.\textsuperscript{168, 169}

**Central catheters and infection risk**

The most common invasive procedure in neonatal intensive care is the use of central venous catheters (CVCs)\textsuperscript{170}, and the association between LOS and central catheters has been shown repeatedly.\textsuperscript{5, 22, 171-175} Pathogens reach the patient’s blood circulation via either an extraluminal or an intraluminal route.\textsuperscript{176} It is believed that an intraluminal route of infection is associated with longer dwell times, whereas the extraluminal route is more common in catheters with shorter dwell times.\textsuperscript{177}

To decrease the risk of catheter-related infections, the dwell time should be as short as possible.\textsuperscript{20, 136, 170, 178} In neonatal intensive care, two types of central catheters are commonly used; peripherally inserted central catheters (PICC) and umbilical catheters. The recommended dwell times of these catheters differ and no current consensus exists.\textsuperscript{179} The United States Centers for Disease Control and Prevention recommend limiting umbilical catheter dwell time to maximum 14 days.\textsuperscript{180}

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Recently Sanderson et al. recommended that umbilical catheters should be removed, and if necessary replaced with a PICC, within 3 days. There was however no clear cut-off for when PICCs should be removed with regard to risk of infection, and an evaluation of early planned removal of PICCs found no evidence for this strategy. Milstone et al. showed an increased risk of catheter-associated infections during the two weeks after PICC line insertion, and this risk remained increased during the time the catheters were in place; the authors therefore recommended daily reviews of the necessity of the catheter. Early planned removal versus a more expectant approach has also been reviewed, but not recommended since early planned removal did not show a decreased incidence of catheter-related infections.

The right technique and level of sterility when inserting and handling central catheters are both important, and the combination of these routines in a so-called bundle has also proved effective.

The removal of CVCs has been suspected to be a potential risk factor for sepsis. Antibiotic prophylaxis before the removal was recently investigated in a Cochrane meta-analysis, with the conclusion that there is no evidence for this recommendation today.

**Scrub the hub**

To minimize health risks for healthcare workers (e.g. risks associated with sharp injuries and transmission of blood-borne pathogens), needleless connectors/hubs have been introduced and recommended. Catheter hubs have been associated with an increased risk of infection due to contamination of the hub membrane, but when they are properly disinfected, the risk of infection is decreased. Several preventive methods have therefore been developed.

One method that can be used to disinfect a connector is called “scrub the hub”. The method entails scrubbing the connector membrane of the central line with a swab soaked in alcohol. The scrub time necessary to properly disinfect the hub membrane has been evaluated in several in vitro studies, with recommendations varying from 5 to 30 seconds. The United States Centers for Disease Control and Prevention recommend the use of scrub the hub, but do not specify the duration of the scrubbing. When investigated in a clinical setting, 15 seconds scrub time was found effective, and the same scrub time has been recommended in both the Epic3 study and a Swedish review.

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A recent evaluation of the ability of health care workers to perform the scrub method for the necessary amount of time found that aid with assessing time is important when using methods in which time is crucial. Munoz-Price et al. specified the scrub time used in their study at 15 seconds, but when they evaluated the actual scrub time used in the study, the median time was 9 seconds, implying compliance issues. Problems with compliance have also been observed in other studies.

**Alcohol caps/port protectors**

As an alternative to decrease contamination of the catheter hub, alcohol impregnated caps or port protectors have been developed. They are designed to be attached to the hub when the hub is not in use. The cap covers the membrane mechanically, and since the cap contains a sponge soaked in isopropanol or isopropyl alcohol (IPA) the membrane is continuously disinfected. Several studies have shown that alcohol caps are efficient in preventing hub contamination and sepsis. Most of these studies compared the disinfection capacity of the alcohol caps to a swipe with an alcohol-soaked swab, but one recent study comparing a specific type of cap with the scrub method using a scrub time of 15 seconds found that the cap was superior in terms of decontamination of the hub. However, there have been reports of the risk of alcohol leakage and thus the risk of alcohol toxicity.

**Isopropanol**

IPA is an alcohol commonly used as a disinfectant both in industry and in health care, typically in a concentration of 70% IPA in water. When used in concentrations between 60-80%, IPA is an efficient disinfectant and may have better bactericidal activity than ethanol.

In the human body, IPA is metabolised mainly into acetone and is eliminated through the renal system. The half-life in adults varies between 2.5 and 8 hours, with excretion mainly through the renal route. The exact mechanism of toxicity is not known, but its main metabolite, acetone, has several adverse effects on the human body such as metabolic acidosis. IPA itself causes an osmol gap and ketonaemia without metabolic acidosis, and this finding together with increased acetone levels in blood is typical of IPA intoxication. Suppression of the central nervous system is regarded as the most common finding in IPA intoxication, and previously this was regarded as an effect of its metabolite acetone. However, it...
seems as though isopropanol itself also has a suppressive effect on the central nervous system.\textsuperscript{209, 216} The effects of IPA on newborn infants and infants born preterm remain unclear.

Intoxication is uncommon, although serious intoxications have been reported.\textsuperscript{217, 218} IPA can be toxic through ingestion, inhalation, and dermal or rectal exposure. If high doses of IPA are ingested or injected, there is a risk of severe morbidity or death, but the lethal and toxic dose remains unclear and the effect of small repetitive doses is unknown.\textsuperscript{209} The toxic dose for paediatric and neonatal patients is not known, but based on known intoxications blood concentrations of 25mg/dL have been proposed as a toxic threshold.\textsuperscript{208} Levels of isopropanol blood concentration in previous intoxications have been in the range of 25–520 mg/dL.\textsuperscript{216-219}

**Metabolic acidosis**

Metabolic acidosis has several adverse effects on the human body; for example, it causes depressive effects on the cardiovascular system such as increased risk of arrhythmias and hypotension. It also affects the immune system negatively and increases inflammation.\textsuperscript{220, 221} Acute forms of metabolic acidosis often arise from the overproduction of organic acids such as lactic acids or ketoacids.\textsuperscript{220}

**Ethanol**

Ethanol is used as a disinfectant in concentrations of 60-85\%, and appears to be more efficient than IPA when used against viruses.\textsuperscript{210} Ethanol intoxications in infants are uncommon, but there are some known cases where accidental, iatrogenic, or deliberate (child abuse) intoxications have occurred, causing symptoms such as hypotension, depressed consciousness, tachycardia, tachypnoea\textsuperscript{222, 223} and even death.\textsuperscript{224} The blood alcohol levels in reported cases range between 43mg/dL and 440 mg/dL. The most common cause is accidental oral ingestion, but absorption through skin or mucous membranes has also occurred.\textsuperscript{222-224}
Aims of the thesis

I. To evaluate sepsis as a risk factor for neonatal morbidities, and to investigate the association between specific pathogens and neonatal morbidities in a national material covering all extremely pre-term infants.

II. To investigate if scrubbing the hub of intravenous catheters with an alcohol wipe for 15 seconds could reduce the incidence of neonatal CoNS sepsis.

III. To evaluate potential leakage from IPA caps and to test the scrub the hub method from this safety standpoint.

IV. To investigate which hygiene routines were used in Swedish neonatal units during 2004-2007 compared with 2013.

V. To investigate the association between self-reported hygiene procedures in Swedish NICUs and incidence of LOS.
Material and methods

Design
Study I was part of the EXPRESS study, a nationwide Swedish prospective cohort study of extremely preterm infants (born before 27 weeks of gestation) conducted between 2004 and 2007. All septic episodes as well as neonatal morbidities were prospectively recorded in the EXPRESS database. Multiple logistic regression was used to investigate associations between neonatal sepsis and neonatal morbidities.

Study II was a single-centre non-randomized intervention study performed at the NICU at Örebro University Hospital, Örebro, Sweden in 2011-2013, including all infants enrolled at the neonatal ward over a period of 25 months. The incidence of CoNS sepsis was compared before and after the scrub the hub method was introduced.

Study III was an in vitro study testing two caps together with two hubs to evaluate alcohol leakage. The scrub the hub method was also tested. Circuits consisting of a hub, an IPA cap, a catheter tube, and a needle in a glass vial were constructed and flushed with sodium chloride (Figure 1). After the hub had been exposed to an IPA cap for 1 hour, 24 hours, and 7 days, respectively, the fluid was collected and the amounts of IPA in it were measured using gas chromatography. Ethanol from the scrub circuits was measured using the same method.

Fig 1. Schematic of IPA cap circuit system.
Study IV was a survey study. Questionnaires were sent to all Swedish NICUs in order to assess the strategies implemented for preventing nosocomial infections when caring for extremely preterm infants. The questionnaires included questions on both the current hygiene routines and, retrospectively, hygiene routines in 2004-2007 (i.e. the EXPRESS period).

Study V used both data from the EXPRESS study and survey data from the retrospective hygiene survey performed in Study IV to analyse the associations between the hygiene routines the infants had been exposed to and their risk of acquiring LOS during their first month in the NICU.

Participants
The participants in Study I were drawn from the EXPRESS study. Between 1 April 2004 and 31 March 2007, perinatal data were collected on all 1001 infants born in Sweden before 27 completed gestational weeks. Study I included the 497 extremely preterm infants who were born alive and survived their first year of life.

Study II included all infants enrolled at the neonatal ward at Örebro University Hospital, Sweden, over a period of 25 months from 1 January 2011 to 31 January 2013. The control cohort consisted of 579 infants and the intervention cohort of 281.

Study III was an in vitro study, testing potential alcohol leakage. Six circuits of each cap combination were created for a total of 24 alcohol cap circuits (the Swan-lock hub with Swabcap, the Swan-lock hub with Curos, the Bionector hub with Swabcap and the Bionector hub with Curos). Six circuits of the scrub combinations were created (n=12)(Figure 2). No human participants were required.

![Fig 2. Circuit set-up for Study III: four circuits with IPA caps and two scrub circuits.](image)
Study IV was a survey study that invited all 36 paediatric units in Sweden, of which 27 (75%) responded. The participating units represented the Swedish NICUs that cared for 97.1% of the EXPRESS cohort.

The participants in Study V were drawn from the 707 live-born infants in the EXPRESS study. After the exclusion of 86 infants due to missing basic data, 626 infants were included in this study and constituted cohort 1 (Figure 3). In order to investigate the association between LOS incidence and hygiene routines, a subgroup analysis was conducted among the infants (n=244) alive at 28 days, without EOS, without transfers during the first 28 days, and without clinical sepsis. These infants, who comprised 102 with no sepsis and 142 with definite sepsis between day 3 and day 28, constituted cohort 2 (Figure 3). Demographic data on cohorts 1 and 2 are displayed in Table 2. The survey data from Study IV were used to stratify all units according to how strict their hygiene routines were during the EXPRESS study, and the association between the units’ hygiene domain grading and sepsis risk was investigated by matching survey data from the hospital units to sepsis incidence in cohort 2. For each sepsis diagnosis, the unit identification made it possible to infer what hygiene exposure the infants had.
<table>
<thead>
<tr>
<th>Demographics</th>
<th>Cohort 1, n=626</th>
<th>Cohort 2, n=244</th>
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</thead>
<tbody>
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<td></td>
<td>University unit, n=438</td>
<td>Non-university unit, n=188</td>
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<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>242 (55.3)</td>
<td>99 (52.7)</td>
</tr>
<tr>
<td>Female</td>
<td>196 (44.7)</td>
<td>89 (47.3)</td>
</tr>
<tr>
<td><strong>Gestational age</strong></td>
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<tr>
<td>21 weeks</td>
<td>1 (0.2)</td>
<td>0</td>
</tr>
<tr>
<td>22 weeks</td>
<td>13 (3.0)</td>
<td>7 (3.7)</td>
</tr>
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<td>23 weeks</td>
<td>54 (12.3)</td>
<td>27 (14.4)</td>
</tr>
<tr>
<td>24 weeks</td>
<td>107 (24.4)</td>
<td>28 (14.9)</td>
</tr>
<tr>
<td>25 weeks</td>
<td>124 (28.3)</td>
<td>63 (33.5)</td>
</tr>
<tr>
<td>26 weeks</td>
<td>139 (31.7)</td>
<td>63 (33.5)</td>
</tr>
<tr>
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<td></td>
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<td>112 (25.6)</td>
<td>53 (28.2)</td>
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<td>2005</td>
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<td>2006</td>
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<td>155 (83.3)</td>
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<tr>
<td>Twin</td>
<td>95 (21.9)</td>
<td>26 (14.0)</td>
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<td>Triplet</td>
<td>9 (2.1)</td>
<td>5 (2.7)</td>
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<tr>
<td><strong>Birth weight</strong></td>
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<td></td>
</tr>
<tr>
<td>Mean (g)</td>
<td>747.6</td>
<td>771.1</td>
</tr>
</tbody>
</table>
Definitions

In Study I, all septic episodes were stratified into two groups: definite sepsis and clinical sepsis.

Definite sepsis was defined as one episode with clinical symptoms consistent with sepsis judged by the neonatologist in charge and a positive blood culture.

Clinical sepsis was defined as one episode with clinical symptoms consistent with sepsis judged by the neonatologist in charge and antibiotic treatment for a minimum of five days, but a negative blood culture.

EOS was defined as sepsis <72 hours and LOS as sepsis >72 hours.

All infants were stratified into five different groups depending on what infections they contracted during their neonatal period: no sepsis, sepsis with CoNS without other bacteria, sepsis with CoNS with other bacteria, sepsis with other bacteria, and clinical sepsis. This stratification was performed to investigate any association between causative pathogen and morbidity.

The morbidities that were selected as outcomes were ROP, BPD, severe BPD, severe IVH, PVL, poor neonatal growth, and prolonged hospital stay.

ROP was defined according to the International Classification for Retinopathy of Prematurity.\textsuperscript{109}

BPD and severe BPD were defined according to the diagnostic criteria developed by the National Institutes of Health workshop.\textsuperscript{102} Severe BPD (30% oxygen at 36 weeks) was chosen over BPD (any level of oxygen for at least 28 days) as the main pulmonary outcome, since BPD can be diagnosed as early as 28 days and therefore might have occurred before the infant contracted neonatal sepsis.

IVH was graded from grade one to four in accordance with Papile et al.\textsuperscript{113}, and cystic PVL was defined in accordance with data from de Vries et al.\textsuperscript{225} In the analyses, severe IVH (grade three or more) was combined with cystic PVL into one outcome (IVH+PVL).

Growth failure was defined as body weight lower than two standard deviations below the mean at 36 weeks of postmenstrual age.\textsuperscript{226}

Prolonged hospital stay was defined as hospital stay that continued after 44 weeks of postmenstrual age.

In Study II, all blood cultures positive for CoNS were retrieved from the clinical microbiological laboratory. In order to separate true sepsis from positive cultures due to contamination, all blood cultures positive for
CoNS were assessed by an external senior neonatologist who was blinded to the period during which the infant acquired the suspected CoNS sepsis. The external neonatologist classified all episodes as contamination or sepsis on the basis of clinical signs, gestational age at birth, age at blood culture, CRP levels, blood culture results, antibiotic treatment, and diagnosis at discharge.

In Study III, the toxic level of IPA was calculated on the basis of previous studies of IPA intoxication, which have reported levels of IPA blood concentration ranged from 25mg/dL to 520 mg/dL. We chose the lowest blood concentration for our calculations, and performed the same calculation as Sauron et al. to estimate a critical amount for IPA if given to a preterm neonate weighing 500 grams; that is, 1130 μg per injection. The corresponding threshold for ethanol when calculated in a similar manner is 1943 μg per injection.

In Study IV, the NICUs were divided into two groups depending on their connection to a university: university units and non-university units.

Maximum barrier protection when inserting catheters was defined as the use of sterile drapes, surgical gown, surgical mask, and cap.

Regular controls were defined as regular controls regardless of frequency.

Staff assigned to work with hygiene was defined as there being any staff member given the specific task of working with hygiene-related matters, regardless of profession.

Working time was defined as any amount of time that was clearly assigned for any person to work with hygiene questions and routines.

Antibiotic prophylaxis was defined as the use of antibiotics based on risk factors such as level of prematurity or use of central catheters. Treatment of infants with clinical signs or symptoms of infection was not considered to be prophylactic. To prevent any bias, the units were asked to specify their antibiotic routines in free text.

Study V used the same definitions for hygiene routines as in Study V, and the same definitions for clinical and definite sepsis as in Study I.

EOS was defined as sepsis occurring before 48 hours, and LOS was defined as sepsis occurring after 48 hours.
Gas chromatography

Since its invention in 1941, gas chromatography has been developed into one of the most important techniques in analytical chemistry.\(^\text{227}\) The liquid sample that is to be analysed is injected through a septum into a heated port in which the sample evaporates. The vapour is then transported through a heated column by a carrier gas. The analytes separated from the original sample flow through a detector, and the results from the detector are displayed on a computer.\(^\text{228}\) The detector used in Study III was a flame ionization detector (GC-FID).

Statistics

In Study I, neonatal sepsis was evaluated as a risk factor for neonatal morbidity using multiple logistic linear regression analyses comparing risk for neonatal morbidities in infants with no sepsis and sepsis caused by different pathogens. All statistical analyses were performed using version 6.0 of the GAUSS software package. The results are presented as odds ratios (ORs).

Regression analysis is a statistical method used to analyse an association between an independent variable and a dependent variable. Multiple regression analysis makes it possible to adjust for factors that would affect the result; that is, confounding effects.\(^\text{229}\)

Odds ratio is a measure of association between an exposure and an outcome, and is used to compare the relative odds that a certain outcome will occur given the exposure to a variable.\(^\text{230}\)

Study II compared the incidence of CoNS sepsis between the control period and the intervention period. Relative frequencies of CoNS sepsis were calculated with 95% confidence intervals, using a normal approximation for difference of relative frequencies. A secondary analysis of sepsis incidence in all infants born preterm was conducted.

Version 11 of the STATA software package was used.

In Study III, mean and maximum leakage of alcohol per circuit was calculated in µg. For the cap circuits the amount of IPA leakage was measured and for the scrub circuits the amount of ethanol was measured.

Answers to the survey questions in Study IV were analysed both separately and by creating domains or groups of questions within the same area of
practices, for example catheter routines. The answers to the questions analysed by domain were recoded if necessary to values of 1 (favourable from a prevention standpoint) and 0 (less favourable). The sum of the answers within each domain was calculated and divided by the total number of questions answered within that domain, thus taking care of the necessary adjustments for the few missing answers (equivalent to a mean-value imputation technique). The average thus obtained was then converted to percentage points, where 100% indicated optimal hygiene practices within each domain, and the change over time was evaluated.

Study V used a Poisson regression model, which is suitable for outcomes that are counts of independent events. The number of sepsis episodes was the outcome, and the main determinants were different aspects of the hygiene routines of the treating unit. The outcome parameter of the Poisson regression was a relative risk (RR) reported with a 95% confidence interval (CI) and a p-value for the test of the hypothesis that RR=1.0; that is, no effect of hygiene on sepsis. Statistical significance was set at p<0.05.

The main explanatory factor was the hygiene factor, supplemented in the model by the demographic covariates sex, weeks of gestation, and year of birth. The hygiene factors were either chosen from the 26 individual questions in the survey study or, in the domain-oriented analysis, using the summarizing indices for each of the domains. The individual questions were treated as categorical variables, while the indices were treated in some models as continuous variables and in others as categorical variables with cut-off points chosen to roughly indicate the median.

Two statistical software packages were used: STATA (version 15.1) and SPSS (version 25).

**Ethical considerations**

The Central Ethical Review Board of Lund University (ETIKH4) gave ethical approval for Studies I and V as part of EXPRESS.

No ethical approval was sought for the remaining three studies. Study II was a report on a hygiene quality project. Study III only included laboratory material and no human or animal participants. Study IV was a survey study directed at heads of departments, examining routines used in NICUs, and did not involve human participants as defined in Swedish statute 2003:460.
Results

Neonatal sepsis as a risk factor for morbidity (Study I)

Of the 1011 infants born before 27 weeks of gestation during the EXPRESS study period, 707 were live-born. The 497 (70%) who survived until the age of one year formed the cohort of this study.

Among the study cohort, 326 (66%) had at least one sepsis episode; 92 had only clinical sepsis while 234 (47%) had at least one episode of definite sepsis. Of all newborns with septic episodes, 214 had one sepsis episode and 112 had multiple septic episodes. Hence a total of 324 definite sepsis episodes were recorded in 234 individuals; 212 of these episodes were caused by CoNS, making it the most common cause of neonatal septicaemia, followed by Candida and S. aureus. EOS (<72h) was seen in 20 infants.

Definite sepsis was associated with severe BPD (OR: 1.6, 95% CI: 1.0-2.7), but clinical sepsis was not (OR: 1.1, 95% CI: 0.6-2.0). There were no significant differences in severe BPD incidence between CoNS and the other causative pathogens.

No differences in the incidence of ROP were detected between CoNS and the other causative pathogens, or between clinical and definite sepsis. IVH+PVL was not significantly correlated to any sepsis. Growth failure was most common in the group with definite sepsis caused by CoNS and other bacteria (OR: 1.5, 95% CI: 1.2-2.9). Definite sepsis was associated with a prolonged hospital stay (OR: 1.6, 95% CI: 1.0-2.7).

Prevention of neonatal sepsis (Study II)

The neonatal ward admitted 579 newborn infants during the control period (1 January 2011 – 14 May 2012) and 281 during the intervention period (15 May 2012 – 31 January 2013).

Fourteen blood cultures were positive for CoNS during the control period. Nine of these were defined as sepsis and five as contamination, resulting in a CoNS sepsis incidence of 1.5%. Three blood cultures were positive for CoNS during the intervention period, but none of these was classified as true sepsis. Because no patient had CoNS sepsis during the intervention period, the risk reduction was 1.5% (95% CI: 0.53-2.48%, p=0.06). In the preterm infant population, the incidence of sepsis decreased from 3.6% to 0%. The risk difference was 3.6% (95% CI: 1.1-6.0%, p=0.11).
All nine sepsis patients had previously had umbilical catheters. Eight of the nine patients had a venous catheter at the time of infection.

**Alcohol toxicity (Study III)**

IPA was detected in all samples from circuits with alcohol caps, and ethanol was detected in all samples from circuits that had undergone the scrub the hub method. Mean and maximum leakage per circuit was calculated in µg. Mean leakage increased over time in the IPA circuits, but this trend was not observed in the scrub circuits.

On day 1, when the circuits had been exposed to alcohol caps for one hour, the IPA levels were below the critical amount for all circuits.

On day 2, when the hubs had been exposed to alcohol caps for 24 hours, higher amounts of IPA were detected. In the Swan-lock circuits, critical amounts (>1130 µg) were reached in the first injection, but the levels were lower for the following three injections and did not reach the critical cut-off value. The first injection in one of the Bionector circuits contained more IPA than the other identical circuits.

The same pattern, with the first injection from the Swan-lock circuits reaching critical amounts, could be observed on day 7, when the circuits had been exposed to an alcohol cap for another 6 days. This was not seen in the Bionector circuits (Figure 4).

The amounts of ethanol passing across the membrane in the scrub circuits were lower, and are presented in Figure 5 for days 1, 2, and 7, respectively. The maximum leakage was 85µg in the Swan-lock circuits and 73 µg in the Bionector circuits.
Fig 4. Amounts of isopropanol (IPA) measured from the cap circuits. The circuits with the same system are represented by the same colour and pattern, and each line illustrates four injections. The black dotted line represents the critical amount of 1130 μg.
Fig 5. Amounts of ethanol measured from the scrub circuits. The circuits with the same hub are represented by the same colour and pattern, and each line illustrates four injections.

Hygiene routines (Study IV)
A majority (75%) of the Swedish NICUs responded to the survey. The participating units represented the Swedish NICUs that cared for 97.1% of the participants in the EXPRESS study. Reported hygiene routines in 2004-2007 were evaluated and compared with reported hygiene routines in 2013.

Basic hygiene routines improved over time. The availability of hand sanitizers was high and stable over time, and the use of a disposable apron and gloves before opening an incubator became more common. In 2013,
no units allowed long-sleeved work clothing, wristwatches, rings, jewellery, and long hair worn down.

Regular controls of compliance with basic hygiene routines became a standard routine over time in all units, and the same tendency was seen regarding follow-up of compliance with work clothing routines.

University units improved their routines for inserting and maintaining central catheters, while the non-university units had already implemented strict routines in 2004-2007. The dwell times of umbilical catheters varied, with longer dwell times reported from the university units and a tendency towards shorter dwell times over time in the non-university units.

In 2004-2007, all university units and a majority (83.3%) of the non-university units took blood from a central catheter, but only at the time when the catheter was inserted. This routine was still common in 2013. Most of the units took only one blood culture when sepsis was suspected.

Antibiotic prophylaxis became less common, but was used by 25% of the university units and 31.6% of the non-university units in 2013. The use of fungal prophylaxis increased in the university units (50-85.7%) but remained uncommon (29.4%) in the non-university units.

Empiric treatment of suspected EOS was most commonly a combination of penicillin and an aminoglycoside in both periods and in all units. Empiric treatment of LOS showed more heterogeneity, with 10 different strategies reported in total. In 2004-2007, 50% of the university units used vancomycin together with another antibiotic to treat suspected LOS, and this choice was more common in 2013 (62.5%). The non-university units mostly used a penicillin/aminoglycoside combination, and this choice was again more common in 2013 (52.9%). The use of cephalosporins decreased somewhat over time in both groups.

Hygiene routines and sepsis risk (Study V)

Among the 626 infants constituting cohort 1, 776 sepsis episodes were registered; 658 (84.8%) of them occurred after 48 hours, making LOS the most common infection. A total of 224 infants had more than one sepsis episode, and up to five episodes were seen, although this was uncommon. Finally, 198 infants did not have sepsis.

Infants born in gestational weeks 25-26 were less likely to acquire sepsis than infants born in gestational weeks 22-24 (RR=0.71, 95% CI: 0.52-0.97, p=0.03). Infants born at the start of the EXPRESS study (2004) had the highest sepsis risk.
In order to study the impact of hygiene routines in detail, a subanalysis was performed on the 244 infants that saw no change in hygiene routines during their first 28 days (cohort 2). This analysis did not show an association with strict routines when all domains were studied, but when basic routines were excluded the association was strong (RR=1.74, 95% CI: 1.23-2.45, p=0.002).

There were higher risks for sepsis in units with less strict catheter routines (RR=1.70, 95% CI: 1.21-2.38, p=0.002) and units with low grading in blood culture routines (RR=2.02, 95% CI: 1.45-2.81, p=<0.001).

The basic hygiene domain, the compliance control domain, and the NICU design domain showed a lower association with sepsis in units with low scores in the domain (Table 3).

A further analysis was performed on the association between sepsis risk and specific questions included in the catheter and blood culture domain. The relative risk for sepsis was 1.88 (95% CI: 1.36-2.59, p=<0.001) when maximum barrier protection was not used in the insertion of CVCs/PICCs. There was also an increased risk when a single operator with maximum barrier protection but without assistants inserted umbilical catheters (RR=1.87, 95% CI: 1.08-3.25, p=0.03) and CVCs or PICCs (RR=2.05, 95% CI: 1.06-3.97, p=0.03).

Taking a blood sample from a central catheter for blood culture at the same time as inserting the catheter did not show a clear association with higher sepsis risk, but using the femoral vein for blood sampling did show an association (RR=1.71, 95% CI: 1.22-2.39, p=0.002). Sampling of 0.5–1ml showed a higher risk for sepsis than sampling of ≥1 ml.

The use of antibiotic prophylaxis was associated with an increased risk for sepsis (RR=1.68, 95% CI: 1.21-2.33, p=0.002).
Table 3. The associations between grading in each hygiene domain and sepsis risk for cohort 2 (n=244). Units stratified according to domain grading, high vs. low.

<table>
<thead>
<tr>
<th>Hygiene grading</th>
<th>0 sepsis, n</th>
<th>1 sepsis, n</th>
<th>2-3 sepsis, n</th>
<th>Relative Risk for sepsis (RR)</th>
<th>95% CI</th>
<th>p-value</th>
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<tr>
<td>All domains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>51</td>
<td>66</td>
<td>14</td>
<td>1.0 (reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>50</td>
<td>56</td>
<td>6</td>
<td>0.87</td>
<td>0.63 – 1.19</td>
<td>0.372</td>
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<tr>
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<td>58</td>
<td>40</td>
<td>3</td>
<td>1.0 (reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>43</td>
<td>82</td>
<td>17</td>
<td>1.74</td>
<td>1.23 – 2.45</td>
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<tr>
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<td>48</td>
<td>71</td>
<td>14</td>
<td>1.0 (reference)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>53</td>
<td>51</td>
<td>6</td>
<td>0.78</td>
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<td>Compliance routines</td>
<td>87</td>
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<tr>
<td>Low</td>
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<td>7</td>
<td>1</td>
<td>0.62</td>
<td>0.31 – 1.21</td>
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<td>42</td>
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<tr>
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<td>43</td>
<td>80</td>
<td>17</td>
<td>1.70</td>
<td>1.21 – 2.38</td>
<td>0.002</td>
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<tr>
<td>High</td>
<td>97</td>
<td>90</td>
<td>8</td>
<td>1.0 (reference)</td>
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<tr>
<td>Low</td>
<td>4</td>
<td>32</td>
<td>12</td>
<td>2.02</td>
<td>1.45 – 2.81</td>
<td>&lt;0.001</td>
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<td>NICU design</td>
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<tr>
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<td>35</td>
<td>56</td>
<td>13</td>
<td>1.0 (reference)</td>
<td></td>
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</tr>
<tr>
<td>Low</td>
<td>66</td>
<td>66</td>
<td>7</td>
<td>0.76</td>
<td>0.56 – 1.04</td>
<td>0.09</td>
</tr>
</tbody>
</table>


Discussion

A major problem concerning neonatal sepsis is the lack of a clear, useful, and widely accepted definition. This creates difficulties both in the clinical setting and in research.

Studies I and V used two separate definitions for sepsis: definite sepsis and clinical sepsis. These, or similar definitions are common in research on neonatal sepsis. Definite sepsis was a risk factor for increased morbidity in Study I, but clinical sepsis was not. This implies that the patients in the clinical sepsis group did not have the same disease as those in the definite sepsis group, but it remains unclear what clinical sepsis really is. Clinical sepsis could be a reflection of the poor sensitivity of blood cultures, but is more likely a clinical presentation of the patient’s immaturity. If we speculate that all negative blood cultures in patients with clinical sepsis in our study were falsely negative, then it would be reasonable for the infants with clinical sepsis to contract the same morbidities as the infants with definite sepsis. Since this was not the case, we suggest that a significant number of these infants had some other medical condition. The uncertainty of what clinical sepsis stands for was the reason for the exclusion of infants with clinical sepsis in Study V.

Study I showed weaker associations between neonatal sepsis and neonatal morbidities compared with previous studies.\(^4, 5, 90\) That CoNS\(^4\) and other pathogens provoke inflammation when causing sepsis is obvious, and the relationship between inflammation and different morbidities has been described in several other studies.\(^51, 93, 231\) The reason for the moderate associations shown in this study is not known, but one possible explanation might be that while the immature immune systems of premature infants fail to provide protection against infections, it will also be less able to harm the host, due to failure to launch a strong inflammatory response.\(^51\) Another possible explanation for the lower correlation between neonatal sepsis and morbidities found in Study I is that the plasticity of the affected organs may be sufficient to enable regeneration.

We only studied extremely preterm infants surviving to one year of age, as it is likely that the infants who did not survive had a higher rate of morbidity than the surviving infants.

It is obvious that there is an association between neonatal sepsis and neonatal morbidities, but it is more difficult to prove a causal relationship; that is, that neonatal sepsis in fact causes these morbidities. Several risk
factors for poor neonatal outcome are closely linked together, and the sickest infants often contract several complications during their neonatal period.232 These complications often require intensive and invasive treatments, and the treatments themselves are associated with potential side-effects. In Study I, the infants with sepsis also had a higher incidence of NEC, poor nutrition, and extensive ventilator treatment. These complications might be results of sepsis instead of risk factors for sepsis.

This study also showed that CoNS sepsis seemed to be associated with fewer morbidities than infections caused by other pathogens and similar results have been shown in recent studies.95, 96 However, contradictory results have been found in studies of complications following sepsis caused by CoNS, showing similar risks for complications or death92, 93, 98 which is interesting and important since sepsis caused by CoNS is very common and currently empiric treatment with vancomycin is not recommended.15, 154, 161

There is probably more than one explanation for the association between sepsis and neonatal morbidities, and in any case the importance of prevention remains.

We have shown that nosocomial sepsis is common among extremely pre-term infants in Sweden, and since nosocomial infections are preventable we also investigated hygiene routines used in Swedish NICUs during and after the EXPRESS period. In Study IV it was clear that the use of several simple and affordable procedures, such as basic hygiene routines, increased over time, and improvements in compliance control were also seen. Nevertheless, these reported improvements have not been reflected by a decreased incidence of LOS in Sweden.233, 234 Other studies have shown a decreasing incidence of LOS, and this has been explained by successful preventive interventions,8, 31, 33 but it is important to note that to our knowledge no national study has shown a decrease in LOS incidence. One possible explanation for why a similar trend has not been observed in Sweden might be poor compliance, which is a well-known problem regarding preventive hygiene strategies.123, 124

Reported hygiene procedures do not seem to fully reflect the procedures that are clinically used, and this study was not designed to evaluate compliance with hygiene routines. It is likely that Swedish NICUs use the procedures and routines that have been investigated and recommended, but the challenge ahead is to achieve a high level of compliance. It is possible that a nationwide initiative can make a difference in this respect.235 There
was however a tendency towards a decreased sepsis incidence during the EXPRESS study; one could speculate whether this positive change might have been associated with the EXPRESS study, which could have increased the units’ focus on prevention.

Antibiotics are commonly used in neonatal intensive care. The use of empiric treatment is crucial, since due to the vulnerability of these patients treatment must often be initiated as soon as sepsis is suspected. Both vancomycin and cephalosporins were reported as empiric treatments by the units in Study IV. Empiric treatment should be chosen to cover the most common and the most dangerous pathogens. Since not all pathogens are equally likely to cause complications and death, empiric treatment with vancomycin – a treatment directed against CoNS – is being questioned more and more strongly, since no decreased mortality has been observed with this strategy.\textsuperscript{35, 154, 156, 161} Cephalosporins are known to be a driving force behind the development of multiresistance, and so their use should be limited.\textsuperscript{156, 162} There is still no consensus on empiric treatment of suspected neonatal sepsis,\textsuperscript{153, 157} which might explain the heterogeneity of empiric antibiotic treatments that are used in neonatal care, which was shown in Study IV. Overall, there is clear room for improvement regarding the choices of empiric treatment for suspected sepsis.

The use of antibiotics has increased more for culture-negative sepsis than for culture-proven sepsis,\textsuperscript{236} threatening to aggravate the already problematic situation of multiresistance.\textsuperscript{47} It seems clinicians find it difficult to trust sterile cultures from an infant that appears unwell. There are few diagnostic markers to aid the clinician in determining the time at which sepsis should no longer be suspected, and so the clinician must rely on their own experience and intuition when treating sick patients. The experienced neonatologist is also aware of the low sensitivity of blood cultures, which is partly due to the challenge of sampling a sufficient blood volume; the sensitivity decreases by 10-40% if 0.5 ml is taken instead of 1 ml,\textsuperscript{237} and decreases even further if only one blood culture is collected.\textsuperscript{70} In Study IV, blood culture routines entailing the possibility to sample larger blood volumes (blood from CVC or femoral vein) were associated with increased sepsis risk, probably because of higher sensitivity of the blood cultures. However, this could not be shown for the separate analysis of volumes.
We found that the use of antibiotic prophylaxis was associated with higher sepsis risk. Previous evaluations of antibiotic prophylaxis given to infants with central catheters have concluded that there is insufficient evidence to recommend the practice.\textsuperscript{143-145}

A Swedish study recently showed an increased risk for sepsis in very low birth weight infants after the removal of central catheters. This was not seen in those infants receiving continuous antibiotic treatment after the catheter removal.\textsuperscript{140} However, when the question of antibiotic prophylaxis on removing central catheters was investigated in 2018, there were not enough studies to make a well-founded conclusion on the matter.\textsuperscript{141}

Study V investigated the association between reported hygiene routines during the EXPRESS study and sepsis risk, using the unique data from both EXPRESS and the retrospective hygiene survey. We were able to identify associations between sepsis risk and strict catheter insertion routines, blood culture routines, and antibiotic prophylaxis. In other words, the routines that Study IV revealed to have shown little improvement over time were the ones that had stronger correlations with sepsis risk.

The survey did not include questions on catheter maintenance routines such as the scrub the hub method, but only investigated insertion routines and line days allowed for umbilical catheters. However, there is reason to believe that not only catheter insertion routines, but also strict maintenance routines, are important in the prevention of LOS.\textsuperscript{134, 197, 238} Indeed, Miller et al. reported that daily maintenance routines were the key to reduction of catheter-related infections in paediatric intensive care units.\textsuperscript{239} This leaves room for improvement in Swedish NICUs.

As previously mentioned, the use of bundles and checklists has successfully decreased the incidence of neonatal sepsis.\textsuperscript{8, 31-33} Commonly, a bundle of strategies are implemented at the same time, meaning that it is not possible to gauge whether any components of the bundle are ineffective or even counter-effective. We therefore chose to study the effect of a single hygiene procedure implemented at a Swedish level-three NICU, namely scrub the hub. Our study showed a decreased sepsis incidence when the method was used, but statistical significance could not be achieved, probably because of lack of power. Despite the lack of statistical significance, we are convinced that reducing the incidence and risk of CoNS sepsis with a method as simple as scrub the hub is of great value in preventing nosocomial infection in the newborn infant.
It remains unknown which part of the scrub the hub routine is of greater importance: the use of alcohol solution or the scrubbing itself? As CoNS is known to form biofilm, it would seem logical that the mechanical scrubbing itself is important. Mechanical scrubbing alone has not yet been compared with mechanical scrubbing with an alcohol wipe, but since alcohol solution is an effective and cheap disinfectant it seems wise to use an alcohol-soaked wipe even though the mechanical scrubbing itself might be the most important part of the technique. The biofilm formation of bacteria has been found to increase after exposure to ethanol and isopropanol, which might strengthen the notion that the scrubbing part is crucial. The scrub time needed to achieve proper disinfection has been investigated and 15 seconds has recently been recommended in a Swedish review.

It is well known that compliance with routines in the health care setting can be low. A recent evaluation of compliance with scrub the hub showed that the method could benefit from aids to qualify the necessary scrub time.

Alcohol impregnated port protectors or caps were developed to decrease the risk of contamination of the hub as an alternative to other disinfection methods such as scrub the hub. They have shown very promising results regarding prevention of catheter-related infections. Since they were suspected of potentially resulting in alcohol leakage across the membrane we tested the alcohol-impregnated port protectors together with hubs commonly used in Swedish neonatal intensive care. Since our group had previously shown that scrub the hub seemed efficient as a preventive method, we believed it important to test it from the same safety standpoint as the port protectors. Alcohol leakage was apparent in all circuits that were tested, especially in the circuits containing Swan-lock hubs. As mentioned in the introduction, intoxication with isopropanol causes diffuse symptoms, and these symptoms might be particularly difficult to interpret in the extremely preterm population.

The reason why isopropanol was chosen over other alcohol solutions as the disinfectant in both alcohol port protectors is unknown to our research group. Isopropanol may be superior to ethanol in terms of bactericidal activity. The metabolites of ethanol are less toxic than those of isopropanol, and one could speculate if it might be possible to exchange the isopropanol for ethanol and still achieve proper disinfection of the hub membrane but with lower risks of toxicity. Regardless, we still consider...
the leakage of isopropanol as a problem, meaning that the tested port protectors are not safe to use on all hubs as described by the manufacturers. We therefore suggest that they should not be used in neonatal care until further safety precautions are undertaken and evaluated.

The alcohol leakage from the hubs disinfected with alcohol wipes for 15 seconds did not reach critical levels, and this method therefore seems safe to use.

Strengths and limitations

The results from Study I provide important information on the association between neonatal sepsis and morbidity. Due to the national nature of the study, the results could be extrapolated to other groups of premature infants treated under similar conditions, but should not be uncritically compared with other cohorts consisting of more mature infants.

Our study was a national study with a total coverage of all extremely preterm infants born in Sweden during three years, and might therefore be less prone to selection bias than studies performed at large referral centres.

In a large study with many participating centres, there is a risk that there are differences between units in the way study forms are filled out, among other potential differences. This could have affected the results in Studies I and V.

Multiple regression analysis was chosen as the statistical method in Study I, since this technique makes it possible to adjust for potential confounding factors such as gender and gestational age. It is for example well known that female infants are less vulnerable during their neonatal period. Low gestational age is also a risk factor for neonatal sepsis, and had we not adjusted for gestational age, neonatal sepsis could have become a confounding factor in this study, since less-mature infants are more likely to acquire both neonatal sepsis and other neonatal morbidities.

Only infants that survived their first year of life were included in the study. The reason for this choice was that the aim of the study was to investigate morbidity associated with neonatal sepsis, and not mortality. The original plan was to further study these surviving infants regarding their morbidities at follow-up at 2.5 years and 6 years respectively, but this was not possible because of problems with the acquisition of data from the EXPRESS database.

Study II was a small, single-centre, non-randomized study with historical controls, and originally a quality project at our NICU, which has obvious
limitations. Despite this, to our knowledge it was the first study to describe the clinical effect of a scrub the hub intervention in a neonatal intensive care setting. The results did not reach statistical significance, possibly due to lack of power. Another weakness was the failure to collect reliable data on line days; however, data we collected from the national quality register indicated similar rates of line days during the two periods in the study, and the clinical guidelines for central lines were unchanged during the study. In our opinion, the decrease in infection rate cannot be explained only by a potential decrease in the use of central lines. The incidence of CoNS sepsis in the control period was fairly high (1.5%), and some of the effect may be attributable to regression to mean. Nevertheless, this study showed a clinical effect for a method that had already been shown efficient in vitro.

Only sepsis caused by CoNS was studied. LOS is predominantly caused by CoNS, and since these infections are considered to be preventable with the right strategies, we decided it was most interesting to evaluate the possible prevention of this type of sepsis. It would also be logical to assume that the mechanical disinfection on which the scrub the hub method is based would be effective to prevent infection caused by a biofilm-forming pathogen such as CoNS. Yet another reason was that our NICU had a relatively high incidence of CoNS sepsis prior to the intervention (though, as mentioned above, it is important to remember that some of the effect seen in the study might be a result of regression to mean). Blood cultures contaminated with CoNS are common, and it was therefore interesting to evaluate if the intervention would affect both true sepsis and contamination of blood cultures.

One further limitation is the fact that we had only one neonatologist assessing the blood cultures positive for CoNS and deciding whether the infants had true sepsis or if the culture was most likely contaminated. This assessment can be partly subjective, and the use of several assessors would have limited this problem.

Study III was an in vitro study designed to investigate alcohol leakage. One strength of the study is that all circuits with the same cap and hub combination showed similar results, and so it is unlikely that there was a need to test more circuits of the same type. Moreover, our study showed similar results to the study by Sauron et al. However, there are more caps and hubs on the market than the four tested in our study. We can therefore not know if another cap and hub combination could cause a
leakage of alcohol or be free from leakage issues, which is a limitation of this study.

The true toxic level of isopropanol is not known. We chose the level in this study on the basis of previous studies and discussions with a toxicologist, but we cannot be sure that the amounts of IPA transferred over the membrane into what would have been the patient’s circulation would in fact harm the patient. Nevertheless, the difficulty of trusting the safety of these products remains.

Study IV had some weaknesses, one being the retrospective collection of data for the EXPRESS period (2004-2007). Retrospective studies always carry the risk of recall bias, which could be aggravated in this study since some of the responders could have joined the unit after 2007. We tried to adjust for this by encouraging the responders to both consult with other experienced staff members and go through old routine documents to make sure that their answers were as accurate as possible. Some questions had lower response rates, especially from the non-university units, which made it difficult to draw conclusions in some domains.

This study used self-reported responses from the neonatologist in charge, which is not a measure of true implementation or compliance. It is however a clear signal of what procedures had been set as standard at each unit.

The survey had a response rate of 75%, and the responding units had cared for 97.1% of the extremely preterm infants born during 2004-2007. We therefore believe that this study is representative of the Swedish NICUs caring for these children.

The strengths of Study V were the prospectively collected material on 626 extremely preterm infants from the national EXPRESS study, and the fact that the infants in cohort 2 had the same exposure time and type of neonatal care during their first 28 days.

A limitation was the self-reported retrospectively-collected exposure data on hygiene routines from the survey study. This meant that we could not be sure about each infant’s hygiene exposure, since the data could have been affected both by the retrospective collection of data and by compliance issues.
Conclusions

- Extremely preterm infants face a high risk of acquiring neonatal sepsis, with CoNS being the most common pathogen in this population. Definite sepsis seems to be a risk factor for severe BPD and prolonged hospital stay, but the associations in this national study were weaker than those reported by previous studies.

- Scrubbing the hub of intravenous catheters with an alcohol wipe seems to be an efficient method to prevent sepsis caused by CoNS in newborn infants.

- There is a risk of IPA leakage when IPA impregnated caps are used. This leakage increased the longer the port was exposed to an IPA cap, and a 30 second drying time was not sufficient to solve the problem. The different cap and hub combinations showed differences in alcohol leakage, and so there is a need to test all caps and hubs used in the clinical setting. We suggest that the use of IPA caps should be discontinued in neonatal and paediatric patients until further research is performed to further evaluate the safety of these products.

- Hygiene routines have improved in Swedish NICUs, but neonatology still faces a high incidence of neonatal sepsis. This means that there is more work to be done in order to prevent nosocomial infections. The empiric treatment of suspected LOS was not in line with international recommendations.

- Associations were seen between decreased sepsis risk and strict catheter routines, blood culture routines, and the non-use of antibiotic prophylaxis. Findings regarding basic hygiene, compliance control, and NICU design were contradictory.
SAMMANFATTNING

Bakgrund
Neonatal sepsis (NS) innebär risker för följdsjukdom och död hos nyfödda barn. Det är framförallt de barn som föds extremt tidigt, d.v.s. innan v 27, som riskerar att drabbas av NS.

NS definieras som early onset sepsis (EOS) om den inträffar inom de första 48-72 levnadstimmarna, och late onset sepsis (LOS) om den inträf-
far därefter. Bakterierna som orsakar EOS överförs vanligen från mor till barn innan eller i samband med förlossningen. LOS uppstår efter födseln, är i regel sjukhusförvärvad och är den vanligaste formen av NS. Över tid har LOS blivit den vanligaste formen av NS, troligen till följd av den ökade överlevnaden bland de för tidigt födda barnen med omoget immunförsvar och behov av långa vårdtider med invasiva åtgärder.

De bakterier som orsakar NS har ändrats över tid. Idag är den vanliga-
aste orsaken till LOS koagulasnegativa stafyloklocker (KNS).

Risken för extremt för tidigt födda barn att drabbas av sjukdom och/eller död har varit och är fortsatt hög. I takt med framsteg inom neo-
matologin har dödligheten sjunkit drastiskt men sjukligheten är fortfaran-

För att förebygga infektioner är väl fungerande basala hygienrutiner av hög vikt. Dessutom är god hygien vid anläggning och skötsel av centrala infarter viktig då användande av centrala infarter innebär risk för infektion. Behovet av kårinfarter är betydande och ofrånkomligt inom neonatal intensivvård och därför är förebyggande åtgärder för att minska risken för infektion mycket viktiga. Dessa förebyggande metoder får dock inte vara skadliga för patienten vilket kan vara en risk om metoderna som används inte är tillräckligt studerade.
Målsättning med studierna i avhandlingen

I. Att utvärdera sepsis som riskfaktor för neonatal sjukdom och undersöka sambandet mellan orsakande bakterie och neonatala sjukdomar.

II. Att undersöka om metoden ”scrub the hub” (mekanisk rengöring av infartskopplings membran) minskar risken att insjukna i neonatal sepsis orsakad av koagulasnegativa stafylokokker (KNS).

III. Att utvärdera potentiellt läckage av alkohol över membranet på intravenösa kopplingar vid användning av alkoholimpregnerade korkar samt att utvärdera metoden scrub the hub med avseende på risk för alkoholläckage.


V. Att undersöka om det fanns en koppling mellan de hygienrutiner som rapporterades från svenska neonatalenheter under EXPRESS-studien och risken att insjukna i LOS.

Studie I

Som en del av den nationella, prospektiva multicenterstudien EXPRESS som genomfördes i Sverige mellan åren 2004-2007 studerades alla extremt för tidigt födda barn som överlevde till 1 års ålder (n=497). Förekomsten av neonatal sepsis studerades hos dessa barn och genom multipel regressionsanalys undersöktes kopplingen mellan neonatal sepsis och förekomst av bestående sjukdomstillstånd såsom bronkopulmonell dysplasi (BPD), intraventrikulär blödning (IVH), påverkad tillväxt, förlängd sjukhusvistelse och retinopati (ROP) Sepsisepisoderna analyserades som definitiv sepsis och klinisk sepsis.

Resultat: 326 av 497 extremt för tidigt födda barn (66 %) hade minst en sepsisepisod under sin nyföddhetsperiod. KNS var den vanligaste orsaken till sepsis. Definitiv sepsis var associerad med svår BPD (OR 1.6) till skillnad från klinisk sepsis där inget samband kunde ses. Definitiv sepsis innebar en större risk för förlängd sjukhusvistelse (OR 1.6). Sepsis orsakad av KNS och annan bakterie var associerad med försämrad tillväxt (OR 1.5). Sepsis var inte associerat med en högre risk för ROP eller IVH.
**Studie II**

Scrubs the hub är en metod som innebär att man mekaniskt rengör membranet på infartskopplingen med en alkoholdränkt tuss i 15 sekunder. För att undersöka effektiviteten hos denna metod mätte vi incidens av KNS infektioner före och efter metoden infördes på vår neonatalenhet.

Resultat: 9 barn insjuknade i KNS sepsis under kontrollperioden (1 januari 2011-14 maj 2012) jämfört med 0 barn under interventionsperioden (15 maj 2012-31 januari 2013). Sepsisincidensen minskade från 1.5% till 0% vilket gav en riskreduktion på 1.5% (95% CI 0.53-2.58%) (p=0.06). Incidensen i den prematurfödda gruppen minskade från 3.6% till 0% vilket innebar en riskreduktion på 3.6% (p=0.11).

**Studie III**

Alkoholkorkar innehållande isopropanol (IPA) har utvecklats för att förhindra kontamination på intravenösa katetrens membran och deras effektivitet har visats i ett flertal studier. När alkoholkorkar införts på en neonatalenhet i Canada uppstod oro för alkoholläckage från korkarna vilket föranledde genomförandet av en in-vitrostudie som påvisade alkoholläckage.208 Vi genomförde en liknande studie med syfte att undersöka säkerheten hos alkoholkorkar och även scrub the hubs säkerhet gällande alkoholläckage. Vi utformade kretsar av en infartskoppling (hub) och en kateterslang och utifrån denna ”grundkrets” skapades fyra olika varianter för att testa två alkoholkorkar tillsammans med två olika infartskopplingar. Efter att varje krets exponerats för en alkoholkork i 1 h, 24 h och 7 dygn spolades kretsen med koaksalt och vätskan samlades upp och analyserades med gaskromatografi för att mäta eventuellt innehåll av IPA. Samma metod användes för kretsen som desiniferats med scrub the hub metoden, men då analyserades vätskan för etanolinnehåll. Medelläckage per krets beräknades i μg.

Resultat: Från all kretser som exponerats för spritkorkar återfanns IPA och etanol återfanns i alla prover från skrubbkretser. Medelläckage ökade över tid i IPA kretsdna men samma trend kunde inte ses i skrubbkretsarna. Mängden etanol var 10- till 100-falt lägre än mängden IPA.
Studie IV
Förebyggande av vårdrelaterade infektioner har varit i fokus under 2000-talet och många neonatalenheter har ändrat sina hygienrutiner utan att detta har utväckerats vetenskapligt. I denna studie kartlades de hygienrutiner som svenska neonatalenheter rapporterade som införda på sina enheter under EXPRESS perioden och jämförde med de rutiner som rapporterades som införda när enkäten skickades ut (2013).

Resultat: Positiva förändringar hade skett över tid avseende rapporterade hygienrutiner. Följande rutiner rapporterades från samtliga enheter 2013: engångsförkläde i plats och engångshandskar bars innan kuvösöpande, endast kortärmade arbetskläder var tillåtta, inga klockor eller ringar var tillåtna, regelbundna kontroller av efterföljande av hygienrutiner och klädrutiner genomfördes, full sterilitet användes vid insättning av centrala katetrar och alla enheter hade personal med ansvar för hygienfrågor, antibiotikaprofylax blev mindre vanligt och användande av svampprofylax hade ökat.

Universitetskliniker använde full sterilitet vid etablering av centrala infarter in mindre utsträckning än icke-universitetskliniker. Valet av empirisk antibiotikabehandling vid misstänkt LOS skiljde sig åt mellan enheterna och även från gällande rekommendationer. Det var vanligt att vancomycin användes som empirisk behandling vid LOS på universitetsklinikerna medan cefotaxim var vanligare på icke-universitetsklinikerna.

Studie V
Utifrån data från EXPRESS och hygienstudien (studie IV) genomfördes denna studie med målet att undersöka en eventuell koppling mellan rapporterade hygienrutiner och LOS-incidens.

En kohort 2 bildades genom att exkludera barn som avlidit inom 28 dagar, som transporterats inom 28 dagar, barn med EOS och barn med klinisk sepsis (n=244). Associationen mellan LOS incidens och hygienrutiner undersöcktes med Poisson regression och relativa risker för sepsis beräknades.

Resultat: LOS var den vanligaste infektionen (84.8% av all sepsis). När alla hygienrutiner studerades tillsammans fanns ingen tydlig koppling mellan hygienrutiner och sepsisrisk, men när basala hygienrutiner exkluderades sågs en stark association, RR=1.74, 95% CI (1.23-2.45), p=0.002.

Det fanns en stark koppling mellan barn som vårdats på enheter med låga betyg inom blododlingsdomänen och sepsisrisk (RR=2.02, 95% CI 1.45-2.81, p<0.001).
Risken för sepsis var förhöjd för de som vårdats på enheter där full sterilitet inte användes vid etablering av centrala katetrar. Det var också en ökad risk för sepsis när en ensam sterilklädd operator utan assistenter satte navelkatetrar och CVK/PICK.

Att använda femoralvenen för blodprovstaging för blododling visade en klar association till sepsisrisk och så även användande av antibiotikaprofylax.

**Slutsatser**

- Användande av metoden scrub the hub kan minska risken för KNS sepsis hos nyfödda barn.
- Det finns en risk för alkoholläckage när IPA impregnerade spritkorkar används. Läckaget ökade ju längre kopplingen var exponerad för en IPA kork och en 30 sekunders torktid var inte tillräcklig för att lösa problemet. Vi föreslår att spritkorkar inte används på barnpatienter till dess att säkerheten kan bevisas.
- Hygienrutinerna hade förbättrats över tid på svenska neonatalenheter mellan åren 2004 och 2013, men med fortsatt hög incidens av neonatala infektioner finns det mer att göra vad gäller preventivt arbete då de förbättrade rutinerna inte har gett ett tydligt avtryck i minskad infektionsincidens.
- Rutiner vid anläggande av centrala katetrar, blododlingsrutiner och användning av antibiotikaprofylax är kopplat till risk för insjuknande i LOS hos extremt för tidigt födda barn.
Acknowledgements

I wish to express my deepest gratitude to:

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My dear friend Ida Ellerström, and the fantastic women in Ladies Circle 33 Örebro; thank you for support, sisterhood, and good times.

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Region Örebro läns forskningskommitté
Örebro Universitet (ALF-medel).
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171. Salzman MB, Isenberg HD, Shapiro JF, Lipsitz PJ, Rubin LG. A prospective study of the catheter hub as the portal of entry for


80 Louise Björkman Hjalmarsson  *Aspects of neonatal sepsicaemia*


Appendix

Infection Express: a survey regarding hygiene routines

This survey is a part of the EXPRESS study, which includes all pregnancies that ended preterm in gestation weeks 22-26. Please answer the questions below regarding the hygiene routines used at your department during the period 1 April 2004 to 31 March 2007. If the hygiene routines changed during this time, please state which routines were used during the majority of the period. We are interested in studying how these routines have changed during the last ten years, and so we also ask you to state which hygiene routines you use today.

This survey only includes hygiene routines regarding infants born preterm in gestation weeks 22-26. If you have different routines for other patient groups, please do not include these in your answers to this survey.

Basic hygiene routines

1. Was there a gate with sink at the entrance of the NICU?

<table>
<thead>
<tr>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes, gate with sink</td>
<td></td>
</tr>
<tr>
<td>Yes, sink but no gate</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

2. How many bottles of hand sanitizers were there in each room?

<table>
<thead>
<tr>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 per room</td>
<td></td>
</tr>
<tr>
<td>1 per room</td>
<td></td>
</tr>
<tr>
<td>&gt;1 per room</td>
<td></td>
</tr>
</tbody>
</table>

3. What routines were used before health care staff opened the incubator and/or handled the patient (weighing, diaper change, etc.)?

<table>
<thead>
<tr>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand washing</td>
<td></td>
</tr>
<tr>
<td>Hand sanitizing</td>
<td></td>
</tr>
<tr>
<td>Apron</td>
<td></td>
</tr>
<tr>
<td>Plastic gloves</td>
<td></td>
</tr>
</tbody>
</table>
4. What routines were used regarding work clothing?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Only short-sleeved work clothing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long sleeved work clothing could be worn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watches could be worn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ring and jewellery could be worn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long hair could be worn down</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Compliance control**

5. How was compliance with hygiene routines controlled?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>No controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporadic controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular surveys</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. How was compliance with work clothing routines controlled?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>No controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporadic controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular surveys</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

7. Did your unit have staff assigned to work on hygiene matters?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. What possibility was staff given to work on hygiene matters?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>When time could be spared</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dedicated part time</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Catheter-routines

9. What level of barrier protection was used when inserting umbilical catheters?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile gloves, sterile drapes and sterile instruments</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Maximum barrier protection (sterile drapes, surgical gown, surgical mask and cap)</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

10. What level of barrier protection was used when inserting central catheters and peripherally inserted central catheters?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile gloves, sterile drapes and sterile instruments</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Maximum barrier protection (sterile drapes, surgical gown, surgical mask and cap)</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

11. How many people worked together when inserting umbilical catheters?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 person with maximum barrier protection and no assistants</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>1 person with maximum barrier protection and 1-2 assistants without maximum barrier protection</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>2 persons with maximum barrier protection</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
12. How many people worked together when inserting peripherally inserted central catheters?

<table>
<thead>
<tr>
<th>Description</th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 person with maximum barrier protection and no assistants</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>1 person with maximum barrier protection and 1-2 assistants without maximum barrier protection</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>2 persons with maximum barrier protection</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

**Blood culture routines**

13. How was blood for blood cultures taken?

<table>
<thead>
<tr>
<th>Description</th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>From central catheters, regardless of dwell time</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>From central catheters, only when inserted</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Peripheral vein punctures</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Puncture of a femoral vein</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Capillary blood sampling</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

14. How many blood cultures were taken when sepsis was suspected?

<table>
<thead>
<tr>
<th>Number of cultures</th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>2</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>&gt;2</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

15. What blood volume was taken for a blood culture?

2004-2007: _____________ml  
2013: _____________ml

16. Who took the blood cultures?

<table>
<thead>
<tr>
<th>Role</th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doctor</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Nurse</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
17. For how many days was an umbilical catheter allowed to be used?

<table>
<thead>
<tr>
<th>Duration</th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-10 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;10 days</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Antibiotics**

18. Was antibiotic prophylaxis used?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes, in all with central catheters</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Yes, in all born before this gestational week</td>
<td>_______v</td>
<td>_______v</td>
</tr>
<tr>
<td>Yes, with other indication (2004-2007)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, with other indication (2013)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

19. What antibiotic treatment was given when early onset sepsis (<72h) was suspected?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

20. What antibiotic treatment was given when late onset sepsis (>72h) was suspected?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-2007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

21. Was fungal prophylaxis used?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes, Fluconazol</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Yes, other</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>No</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
NICU design

22. How many patients were treated in each room when the patient needed intensive care?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

23. How many patients were treated in your unit?

<table>
<thead>
<tr>
<th></th>
<th>2004-2007</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>In total:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensive care:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-intensive care</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

24. Have you moved or rebuilt your NICU since 2004-2007?

Yes ☐ No ☐

If yes, please describe the differences in your new/rebuilt NICU:

________________________________________________________________
________________________________________________________________
________________________________________________________________


41. Gustafsson, Sanna Aila (2010). *The importance of being thin – Perceived expectations from self and others and the effect on self-evaluation in girls with disordered eating.*

42. Johansson, Bengt (2010). *Long-term outcome research on PDR brachytherapy with focus on breast, base of tongue and lip cancer.*

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