Neonatal Invasive Infections

Focused on Group B Streptococci

Margrét Johansson Guðjónsdóttir

Department of Pediatrics
Institute of Clinical Sciences
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

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Cover illustration: *Streptococcus agalactiae* by Alissa Eckert - Medical Illustrator.

A three-dimensional (3D), computer-generated image, of a group of Gram-positive, Streptococcus agalactiae (group B Streptococcus) bacteria. The artistic recreation was based upon scanning electron microscopic (SEM) imagery.

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Neonatal Invasive Infections – Focused on Group B Streptococci

margret.johansson.gudjonsdottir@vgregion.se

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"Happiness and bacteria have one thing in common;
they multiply by dividing!"
— **Rutvik Oza, mathematician**

Til fjölskyldunnar minnar
(To my family)

ABSTRACT

Invasive infections affect neonates with the risk of severe morbidity and death, and *Streptococcus agalactiae* (Group B streptococcus, GBS) remains one of the most common pathogens. The aim of this thesis was to assess infections among neonates and infants, focusing on GBS to better understand prevention and treatment. Clinical data and outcomes were collected from patients' medical records.

Paper I was a prospective cohort study of GBS isolates obtained from adults and children with an invasive GBS infection in the years between 2004 and 2009. The study showed that among infants, serotype III was the most prevalent (48%), but serotype V (39%) was most common among adults. Paper II and III were observational, retrospective studies on early-onset (EO) and late-onset (LO) invasive infections among infants living within Gothenburg or five surrounding municipalities, from whom a pathogenic organism was isolated from blood or cerebrospinal fluid during the years 1997–2017. The studies showed that EO infections decreased from 1.4 to 0.9 per 1000 live births from 1997–2007 to 2008–2017. During the same period, the incidence of LO infections increased from 2.0 to 3.1 per 1000 live births. The case fatality rate remained unchanged for both studies. Paper IV was a cohort study of GBS isolates obtained from pregnant or postpartum women and infants with an invasive GBS infection in Western Sweden during 1988-2001 and 2004-2009. The study showed that invasive isolates exhibited more pigmentation compared to commensal isolates.

Conclusions: These studies have shown that the incidence of EO infections has declined, but for LO infections, it has increased. The serotype distribution of invasive GBS strains has remained the same. The invasive strains exhibit more pigmentation, which provides the basis for additional studies to determine if routine laboratory testing can be safely used to identify the GBS strains that put the unborn child at risk.

Keywords: Neonatal sepsis, Group B streptococci, Virulence factors.
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SAMMANFATTNING PÅ SVENSKA

Mål: Att studera infektioner hos nyfödda med speciell fokus på grupp B streptokocker (GBS) för att få mer kunskap inför prevention och behandling.

Patienter och metoder: I studie I ingick patienter oavsett ålder från Västra Götaland och Halland där GBS odlats från normalt sterila kroppsvätskor. Bakteriestammarna samlades in under åren 2004-2009 och serotypades. Studie II och III var retrospektiva studier om tidiga (första levnadsveckan) respektive sena (från 3 till 120 dagars ålder) infektioner hos nyfödda som innefattade journalgenomgång av alla nyfödda vars mammor var bosatta i Göteborg, Mölndal, Härryda, Öckerö, Kungälv eller Partille under åren 1997-2017. Resultaten jämfördes med tidigare studier från samma befolkning från 1975. I studie IV ingick invasiva GBS stammar från nyfödda samt gravida eller nyförlösta kvinnor från 1988-2001 samt 2004-2009. Virulensfaktorer hos dessa GBS stammar jämfördes med GBS isolat från amerikanska gravida GBS bärare. Klinisk information samlades in från patientjournaler.

Resultat: Studierna har visat att incidensen av tidiga infektioner hade gått ner men incidensen av sena infektioner hade stigit och den största ökningen var inom gruppen som var födda extremt tidigt dvs. före 28 fulla graviditetsveckor. GBS orsakade 40% av tidiga infektioner. Fördelningen av polysackaridkapseln som utgör de olika serotyperna hos GBS bakterien hade inte ändrats jämfört med tidigare studier men de skiljde sig mellan nyfödda och vuxna. Detta är viktig information för utvecklingen av vaccin. Incidensen av tidiga GBS infektioner var högre i vårt material än i studier där man screenar för GBS bakterien hos kvinnor sent i graviditeten. Screening kan leda till ökad användning av profylaktisk antibiotika och därför vore det önskvärt att kunna identifiera vilka GBS stammar som orsakar sjukdom och vilka som inte gör det. I studie IV såg vi att isolat från svåra infektioner visade ökad pigmentering jämfört med isolat från gravida kvinnor i samhället. Detta är av intresse för fortsatta studier om vilka stammar som kan orsaka perinatal smitta till det ofödda barnet och på längre sikt identifiera vilka mödrar som har störst nytta av profylaktisk antibiotika under förlossningen.

LIST OF PAPERS

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

I. **Johansson Gudjonsdottir M**, Hentz E, Berg S, Backhaus E, Elfvin A, Kawash S, Trollfors B.

Serotypes of group B streptococci in western Sweden and comparison with serotypes in two previous studies starting from 1988. *BMC Infectious Diseases, 2015; 15(1), 507. DOI 10.1186/s12879-015-1266-4.*

II. **Johansson Gudjonsdottir M**, Elfvin A, Hentz E, Adlerberth I, Tessin I, Trollfors B.

Changes in incidence and etiology of early-onset neonatal infections 1997–2017 – a retrospective cohort study in western Sweden. *BMC Pediatrics, 2019; 19(1), 490. DOI: 10.1186/s12887-019-1866-z.*

III. **Johansson Gudjonsdottir M**, Elfvin A, Hentz E, Adlerberth I, Tessin I, Trollfors B.

Late-onset Neonatal Infections 1997 to 2017 Within a Cohort in Western Sweden—The Last 21 Years of a 43-Year Surveillance, *The Pediatric Infectious Disease Journal: April 2021 - Volume 40 - Issue 4 - p 359-364. DOI: 10.1097/INF.0000000000002987*

IV. Huebner E*, **Johansson Gudjonsdottir M***, Dacanay M*, Nguyen S, Brokaw A, Sharma K, Hentz E, Elfvin A, Rivera Y, Burd N, Coler B, Li M, Li A, Munson J, Orvis A, Coleman M, Jacobsson B*, Rajagopal L*, Adams Waldorf K*. **Equal contributions*

Virulence Factors of Invasive Group B Streptococcus Isolates Obtained from Swedish Pregnant Women and Neonates. *In manuscript.*

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TABLE OF CONTENTS

ABBREVIATIONS

GAS	Group A streptococci, *Streptococcus pyogenes*
GBS	Group B streptococci, *Streptococcus agalactiae*
NICU	Neonatal intensive care unit
CoNS	Coagulase-negative staphylococci
BBB	Blood-brain barrier
EO	Early-onset
LO	Late-onset
EOS	Early-onset sepsis
LOS	Late-onset sepsis
CSF	Cerebrospinal fluid
SIRS	Systemic inflammatory response syndrome
qSOFA	Quick Sequential Organ Failure Assessment
pSOFA	Pediatric Sequential Organ Failure Assessment
nSOFA	Neonatal Sequential Organ Failure Assessment
VLBW	Very low birth weight
WBC	White blood cells
CRP	C-reactive protein
PCT	Procalcitonin
IL	Interleukin
TNF	Tumor necrosis factor
PCR	Polymerase chain reaction

I:T	Immature to total neutrophil ratio
PAMP	Pathogen-associated molecular patterns
DAMP	Damage-associated molecular patterns
PRR	Pattern recognition receptors
IVH	Intraventricular hemorrhage
SAA	Serum amyloid A
LPB	Lipopolysaccharide binding proteins
LP	Lumbar puncture
IUFD	Intrauterine fetal demise
IAP	Intrapartum antibiotic prophylaxis
PROM	Premature rupture of membranes
PICC	Peripherally inserted central catheter
HvgA	Hypervirulent GBS adhesin
CPS	Capsular polysaccharide
CC	Clonal complex
CAMP	Christie Atkins Munch Peterson
CovR/S	Cov (control of virulence) R/CovS two-component system
HylB	GBS hyaluronidase enzyme
CI	Confidence interval
SD	Standard deviation
SE	Standard error

IQR	Interquartile range
RR	Relative risk
MRSA	Methicillin-resistant *S. aureus*
MSSA	Methicillin-sensitive *S. aureus*
NEC	Necrotizing enterocolitis
BPD	Bronchopulmonary dysplasia

THESIS AT A GLANCE

Paper	**I**	**II**	**III**	**IV**
Aims	To detect any changes in GBS serotype distribution	I - To assess the incidence, etiology, and case fatality rate of EO invasive infections II - To assess the timing of EO GBS infections	I - To assess the incidence, etiology, and case fatality rate of LO invasive infections II – To recalculate data on infections 3-27 days of age from 1975	To determine if high-risk GBS virulence factors correlate with an invasive GBS infection
Methods *Study design*	Prospective cohort surveillance study.	Retrospective observational epidemiological study.	Retrospective observational epidemiological study.	Case-control laboratory study based on a prospective cohort.
Population and inclusion criteria	All ages with the cultivation of GBS from an otherwise sterile site.	0-6 days of age with a pathogenic organism isolated from blood or CSF.	3-120 days of age with a pathogenic organism isolated from blood or CSF.	Infants and pregnant/ postpartum women with saved invasive GBS isolates.
Study period	2004-2009	1997-2017	1997-2017 (1975-2017)	1988-2001 & 2004-2009
Results	Serotype III was the most prevalent (48%) in neonates and Serotype V (39%) in adults	A total of 209 cases gave an incidence of 1.1/1000 live births. 15 (7%) died. GBS (40%) was the most common pathogen	A total of 473 cases gave an incidence of 2.6/1000 live births. 29 (6%) died. *S. aureus* (25%) and CoNS (17%) were the most common pathogens	The 233 invasive isolates had a mean±SE pigment score of 2.4±0.9 compared to 1.4±1.0 for the 51 commensal isolates
Conclusions	The serotype distribution remained unchanged, which is promising regarding GBS vaccination	The incidence has declined, but the case fatality rate remained unchanged. All EO GBS cases occurred within 72 hours of birth	The incidence increased in the last 10 years. The case fatality rate was lower compared to previous surveillance data (1975-1996)	The hemolytic potential differs and skews toward more significant hemolysis in the invasive isolates

INTRODUCTION

Infections at childbirth have been well known for centuries. Childbed fever with high maternal mortality was a dreaded complication of childbirth, causing almost one-third of every woman to die of puerperal sepsis in the early 19th century. Dr. Alexander Gordon, an obstetrician, working in Aberdeen, stated in the year 1795 that childbed fever was contagious and good hygiene was vital in its prevention (1). In 1847 the Hungarian obstetrician Ignaz Semmelweis recognized that medical students caused a spread of "decaying animal organic matter" to puerperal women by performing autopsies between deliveries while practicing in the Erste Wiener Gebärklinik in Vienna. Dr. Semmelwis received much criticism from his colleagues and supervisors after introducing antiseptic techniques with handwash and chlorine solution to prevent the spread. His contribution to science was not accepted until after his death in 1865 (2). "Decaying animal organic matter" was later diagnosed as *Streptococcus pyogenes*, or β-hemolytic streptococcus group A (GAS), which also causes scarlet fever, tonsillitis, impetigo, sepsis, and necrotizing fasciitis. Along with antiseptic techniques and potential lower virulence of the bacteria, cases of puerperal sepsis declined at the end of the 19th century (3). However, before the discovery of penicillin by Alexander Fleming in 1928, physicians were helpless against puerperal sepsis, and the mortality remained high.

Neonates are highly susceptible to infections due to an immature immune system. Along with changing etiology of puerperal sepsis, other bacterial pathogens, such as *Streptococcus agalactiae* (the Group B streptococcus (GBS)), *Listeria monocytogenes, Escherichia coli*, and other Gram-negatives emerged as pathogens.

This thesis includes studies covering neonatal infections from 1975 to 2017. The neonatal care and environment have changed tremendously during this long period. With improved care of pregnant women and proactive care involving resuscitation and neonatal intensive care

(NICU), fundamental improvements were made to increase preterm neonates' survival and survival at lower gestational ages (4).

The first study within this surveillance between 1975 and 1986, conducted by Tessin et al. (5), included neonates between days 0 and 27 (both days included). Possible commensal organisms like coagulase-negative staphylococci (CoNS) were not considered pathogens and, therefore, not included in the study. With improved survival, the care has evolved with extended hospital stays and more invasive procedures, exposing the neonates to medical interventions that put them at risk for infections with commensals organisms (6). That is why Persson et al., in the study - covering the years 1987 to 1996 - included CoNS and extended the surveillance to include neonatal infections up to 120 days of age (7).

This thesis covers the last 21 years of this 43-year long surveillance on neonatal infections, including 1997 to 2017. The studies include comparisons with results from the previous reviews as well as comparisons of the last ten years (2008-2017) with the 11 previous years (1997-2007).

This thesis's work describes the incidence of early- and late neonatal infections and various virulence factors in GBS, which has remained one of the leading pathogens for neonates.

BACKGROUND

NEONATAL INFECTIONS – AN OVERVIEW

Invasive infections remain one of the most detrimental risks for the neonate causing substantial morbidity and mortality. An invasive infection means that the infection, due to bacteria, virus, or fungi, has spread invasively to an otherwise sterile site like blood and can cause sepsis. In severe cases, the pathogen crosses the blood-brain barrier (BBB) and causes meningitis.

Neonatal infections are divided into groups based on the time of presentation. Early-onset (EO) neonatal infections are thought to be caused by vertical transmission of pathogens that may have contaminated the placenta, amniotic fluid, or the vagina and affect the neonate in the womb or during delivery (8, 9). The definition varies but refers to infections with symptoms within 72 hours of birth or during the first week of life (5, 7, 10-13). Late-onset (LO) neonatal infections are, on the other hand, thought to be caused by horizontal transmission from the environment or gut after birth. LO-infection has been variably defined as an infection that occurs after day 3 to 7 of life and before day 28, 90, and 120 (10, 14-19).

The main risk factors for infections are well studied in neonates. Maternal colonization with GBS is a significant risk factor for neonatal GBS infections of all gestational ages (20, 21). Preterm neonates are at higher risk for disease due to a less efficient immune system with decreased IgG antibodies, incompetent activation of the complement system, and opsonization of bacteria (22). The innate immune system is compromised, as well, with an immature epithelial barrier along with an increased need for invasive devices that may disrupt the mucosa (23). Neonatal sepsis (EOS = early-onset sepsis/LOS = late-onset sepsis) is often defined as a pathogen cultivated from blood and/or cerebrospinal fluid (CSF). Meningitis is therefore included in most reports on neonatal sepsis (24).

NEONATAL SEPSIS

Neonates have the highest incidence of sepsis within all age groups at 22 per 1000 live births, affecting around 3 million babies worldwide each year with a case fatality rate of 11-19%, according to a systematic review and meta-analysis covering the years 1979 to 2016 (25). The accuracy of these numbers is affected by the lack of standardization of diagnostic criteria. Even though consensus regarding pediatric sepsis was established over 15 years ago (26), no international consensus exists on the definition of neonatal sepsis.

The pediatric guidelines modified the systemic inflammatory response syndrome (SIRS)-criteria (26) based upon the previous consensus for adults (27). However, adults' criteria had an excessive focus on inflammation and the consensus changed in 2016 with the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) (28, 29). A linear relationship between the number of affected organs and sepsis-related mortality exists in adults (30). In the Sepsis-3 consensus, there is a clinical approach with less emphasis on traditional microbiological results. Sepsis is instead defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection (28). Organ dysfunction is rarely used as a diagnostic criterion for neonatal invasive infections, and the presentation of infection may differ for the term and preterm neonate. Organ dysfunction may be occult, and the neonates can deteriorate quickly from bacteremia to fulminant sepsis with septic shock.

There are screening tools for sepsis to enable the clinician to identify patients with suspected sepsis early. In adults, qSOFA (Quick Sequential Organ Failure Assessment) is used to identify organ dysfunction bedside taking into account increased respiratory rate, systolic blood pressure, and altered mentation (28). Pediatric SOFA (pSOFA) has adjusted physiological parameters to age-related cut-offs (31). Wynn et al. suggested a neonatal-specific SOFA – nSOFA, based on four different scorings (from 0 to 3 regarding the following six systems: 1. Respiratory-, 2. Cardiovascular-, 3. Renal- and 4. The central

nervous system, along with 5. Platelets- and 6. Absolute neutrophil counts (32). nSOFA might predict mortality in very low birth weight (VLBW) neonates with LO sepsis (33, 34).

The challenges for the assessments of these scores for neonates are multivariate. The baseline for neonates is often not known. Platelets, absolute neutrophil counts as well as the proportion of immature to total neutrophil ratio (I:T), have poor predictive value with low sensitivity and wide variations due to multiple clinical factors like gestational age, maternal hypertension, and induced labor affecting and limiting their validity (35-39).

The clinical diagnosis of neonatal sepsis is based on a combination of factors, including; clinical presentation of the neonate, perinatal history of risk factors, for example, maternal infection, and different biomarkers as white blood cells (WBC) count, C-reactive protein (CRP), procalcitonin (PCT) and cytokines, including interleukin (IL) – 6, IL-8 and tumor necrosis factor (TNF) – α, figure 1. Polymerase chain reaction (PCR) and cultures are used to identify a possible pathogen, but antibiotic treatment for culture-negative sepsis is frequent among neonates since up to 60% of blood cultures can be falsely negative (13, 40). No single biomarker fulfills the criteria of a perfect biomarker for neonatal invasive infection (41). Most biomarkers increase both due to a bacterial infection and other inflammations, figure 2 (42). That is why most biomarkers have a higher negative- than positive predictive value.

CRP and PCT are both acute-phase reactant proteins produced mainly by the liver when stimulated by cytokines. They correlate with the severity of the infection and can be used to monitor the response to antibiotics (43).

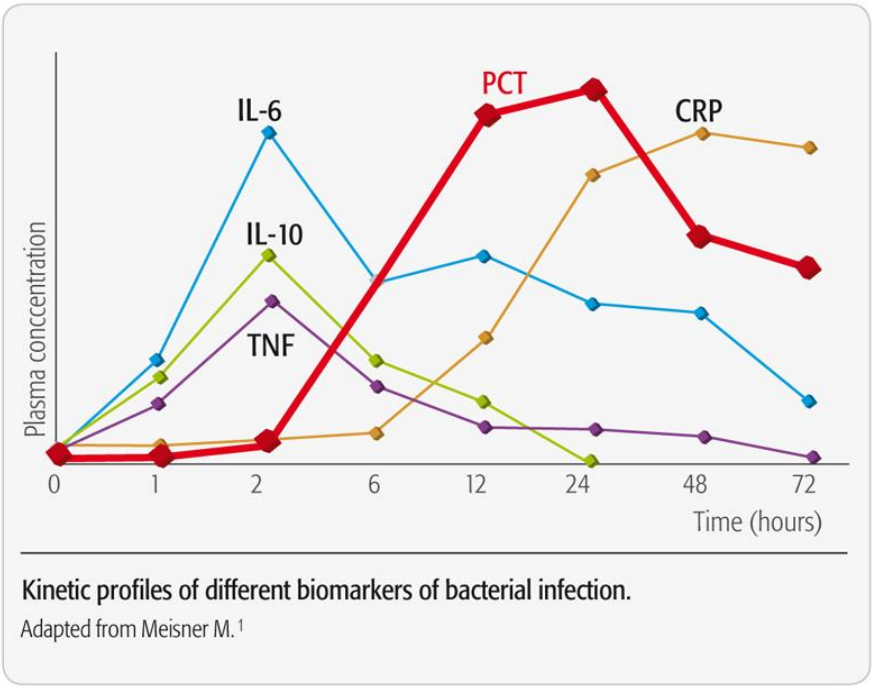

Figure 1. Kinetic profiles of biomarkers for bacterial infection. Downloaded and reproduced with courtesy of bioMérieux Sweden AB from http://www.biomerieux-diagnostics.com/vidas-brahms-pct, accessed on 13-Apr-2021. Adapted from Meisner M et al. Crit Care. 1999;3(1):45-50.

PCT is both detected, and returns earlier to normal as the infection resolves compared to CRP, figure 1. Meta-analysis have shown PCT to have higher sensitivity (79% vs. 69%) and specificity (84% vs. 77%) compared with CRP (44, 45). PCT is more specific in differentiating bacterial infection from other inflammations than CRP alone, and mode of delivery does not affect PCT in contrast to CRP (46, 47). However, CRP has better availability, and its predictive value increases when combined with a cytokine biomarker (41). Cytokines like IL-6, IL-8, and TNF-α are the precursors of acute-phase proteins and therefore have a quicker onset but also a shorter half-life. IL-6 has been shown to have a high sensitivity (89%) and negative predictive value (91%) in LO-sepsis among VLBW infants (48). However, they can be undetectable despite an ongoing infection if taken too late, making it essential to

combine cytokines with biomarkers with slower kinetics like CRP (49), figure 1.

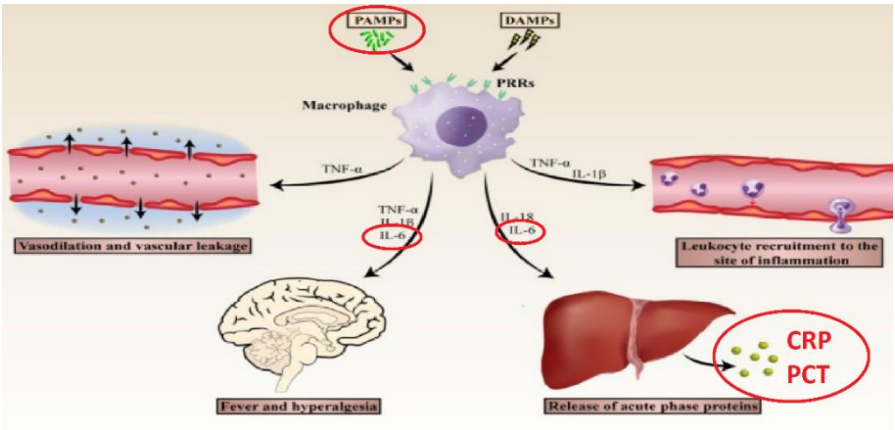

Figure 2. *The release of pro-inflammatory cytokines mediates the acute inflammatory response. PAMPs = pathogen-associated molecular patterns (for example bacteria). DAMPs = damage-associated molecular patterns (for example, injury). PRRs = pattern recognition receptors trigger pro-inflammatory and antimicrobial responses by inducing the release of a broad range of cytokines. Modified and reproduced from Slaats et al. 2016 (42) under the attribution 4.0 International of creative commons https://creativecommons.org/licenses/by/4.0/*

Most studies on biomarkers for infections are done by comparison of infection with otherwise healthy individuals. This scenario is not relevant for a NICU. The neonates may have other factors that cause an inflammatory response, like perinatal asphyxia, respiratory distress, intraventricular hemorrhage (IVH), pneumothorax, or tissue damages related to instrumental birth. A quick, affordable biomarker that would identify a bacterial infection in need of antibiotics utilizing small sample volumes in these settings would be of great value.

Serum amyloid A (SAA) and lipopolysaccharide binding proteins (LPB) are also biomarkers synthesized by the liver and have improved the diagnostics of neonatal sepsis in some studies (50-52). However, neither differentiate between infectious nor non-infectious systemic inflammatory response (41). Progress has been made in molecular diagnostics of infections with PCR technology. They are widely used in detecting pathogens in CSF but are now developed to detect pathogens

from whole blood (53). These non-culture-based methods on identifying a possible pathogen with antimicrobial resistance gene testing provide an opportunity to use antibiotics with a more narrow spectrum within hours. They have their limitations, but with further improvements and lower costs, molecular diagnostics are promising for future diagnostics and treatment for invasive infections.

Kaiser EO-sepsis calculator – http://newbornsepsiscalculator.org/ was designed to predict the risk of EO-sepsis based on maternal intrapartum risk factors and was later combined with clinical symptoms of the neonate (54-56). This calculator places the neonate into three risk-groups - 1) treat empirically, 2) observe and evaluate and 3) continued observation - and has been shown to reduce antibiotic use among neonates and especially among infants with low risk of EO-sepsis (57-59). However, this calculator has not been validated for infants born <34 weeks gestation and does not apply to LO-sepsis.

In papers II and III (on EO and LO neonatal invasive infections), the criteria for an invasive infection were the isolation of an infective organism from blood or CSF by culture with specific criteria for pathogens of unclear clinical relevance. This is one of the most common criteria used in diagnosing neonatal sepsis and meningitis (24). Australian and New Zealand Neonatal Network have added CSF PCR into their criteria (60). PCR techniques have a high sensitivity and specificity in diagnosing infection and are often relied upon in clinical settings (9).

Research on neonatal infections is even more challenging because the definition of time of onset varies between studies. Early-onset (EO) is defined variably as an infection within the first 48 hours of life (60-62), within the first 72 hours of life (10-12, 63)) or within the first week of life (day 0-6) (5, 7, 13). Some studies differ depending on if the neonates are born preterm/term or depending on the pathogen. Many studies, especially on GBS, report EO infection within the first week of life (9, 64-66).

These different definitions make the comparison of the outcomes from sepsis-related research more complicated than it has to be. The lack of standardization and international consensus makes it essential to follow the same population's incidence, etiology, and prognosis using the same criteria.

NEONATAL MENINGITIS

Meningitis is most common within the first month than at any other time of life (67). Reports often define neonatal sepsis as isolation of an infective organism from blood or CSF, including neonatal meningitis. Reports specified on meningitis have shown that the incidence is significantly higher among infants born preterm (2/1000 live births if born <32 weeks) compared to at term (0.3/1000 live births), and preterm infants are accounting for 30% of neonatal meningitis (68, 69).

Meningitis may occur in up to 15% of neonates with bacteremia. Among those with GBS, 5-10% of EO and about 25% of those with LO infections have meningitis (70-73). GBS and *E. coli* are the pathogens in 65-75% of EO meningitis cases, and *E. coli* is up to seven-fold more frequent in preterm than term infants (12, 69, 74-79).

Meningitis can occasionally occur with normal CSF parameters, especially if lumbar puncture (LP) is done early in the course. If an LP is repeated, there is always pleocytosis when the meninges are inflamed (80). Molecular methods are used increasingly to diagnose meningitis, and they have a high sensitivity as they are not affected by prior antibiotics (81, 82).

In this thesis, only infections confirmed by culture are included. CSF cultures are included in the calculations on neonatal sepsis incidence if not otherwise specified.

EARLY-ONSET NEONATAL INFECTIONS

Most cases of EO - infection occur among term infants, but the incidence and case fatality rate are higher among infants born preterm (12, 17, 83). As previously mentioned, the etiology can depend on the mothers' cultivation, as neonatal EO infections are often caused by the transmission of pathogens from the mothers' genitourinary tract. These pathogens can ascend the vagina and infect the amniotic fluid and cause a neonatal infection that sometimes leads to intrauterine fetal demise (IUFD). Additionally, the neonate can get exposed during delivery through the vaginal canal (figure 3). The most common pathogens are GBS and *E. coli* which have the highest case fatality rate (9). The incidence of EO neonatal infections due to GBS has declined by treating the mother with intrapartum antibiotics. However, GBS remains one of the most common pathogens (9). The increased use of intrapartum antibiotics may lead to increased cases of neonatal sepsis due to ampicillin-resistant *E. coli* (84). Some studies have reported a rise in infections caused by *E. coli* after the implementation of intrapartum antibiotic prophylaxis (IAP), especially among the neonates born extremely preterm. But the mortality has not increased despite that *E. coli* has higher case fatality rate compared to GBS (12, 17, 19, 66). Most EO cases are associated with preterm birth, premature rupture of membranes (PROM), and maternal chorioamnionitis (39, 56, 85).

The Gram-positive bacteria *Listeria monocytogenes*, responsible for listeriosis, and the parasitic protozoan eukaryote *Toxoplasma gondii*, causing toxoplasmosis, are food-borne, intracellular pathogens that are usually transmitted via transplacental hematogenous spread in utero (figure 3). The incidence of listeriosis and toxoplasmosis has decreased, possibly due to decreased consumption of high-risk foods by pregnant women due to better education (86-88).

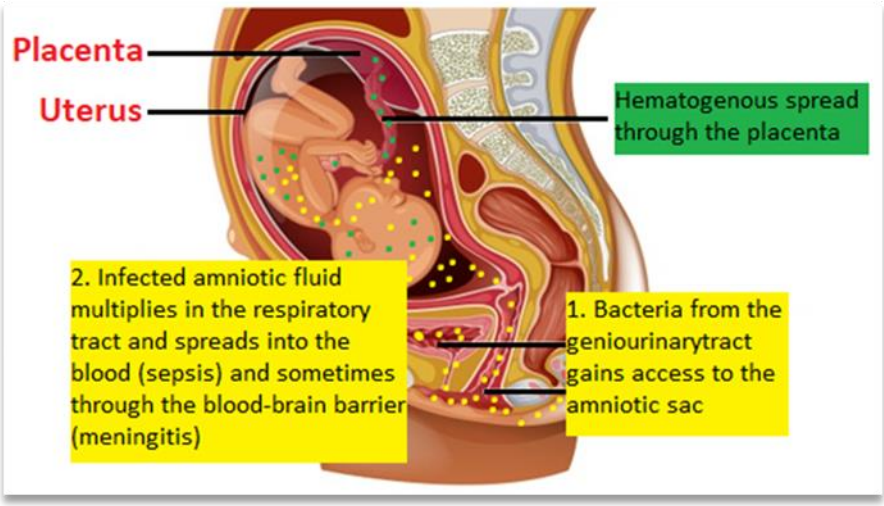

Figure 3. *Dissemination of infections during pregnancy can cause IUFD or early-onset invasive infection (ascending infection in yellow and hematogenous in green). Modified image reproduced with courtesy from www.freepik.com*

In pregnancy, untreated syphilis caused by Treponema pallidum leads to a fetal infection rate of almost 100%. The latent stage causes no symptoms for the mother but still can cause a transplacental transmission to the fetus at any time during gestation (89). Intrauterine herpes simplex virus (HSV) infection is usually transplacental but can be an ascending infection and infect the neonate vertically through exposure during labor (90).

Other viral infections during pregnancy can cause fetal demise or be transmitted to the neonate and cause an EO infection, such as hepatitis B and C, cytomegalovirus, human immunodeficiency virus, parvovirus B-19, and Zika virus (91). The studies in this thesis, however, only include bacterial and fungal pathogens.

LATE-ONSET NEONATAL INFECTIONS

Study III in this thesis includes infants with LO-infections from an out-of-hospital setting as well as VLBW infants within a NICU since it is population-based and includes all infants at the age of 3-120 days.

Infants with LO infections often have comorbid conditions, most common being respiratory, gastrointestinal, and cardiovascular (92). However, LO-sepsis occurs more frequently among preterm infants and especially among those born extremely preterm. Studies have shown that about 21-27% of surviving infants born earlier than 28 gestational weeks experience at least one episode of LO-sepsis before discharge from the NICU (14, 93).

Background causes of preterm birth may explain the increased risk of LO infections. Fetal growth restriction and/or maternal hypertensive disorder were strong risk factors for LO-sepsis irrespectively of the duration of central catheters, according to Letouzey et al. in the French EPIPAGE-2 cohort study (94). However, many studies have shown that central catheters constitute a significant risk factor for LO infections, especially since the most common pathogens are staphylococci (93, 95-99).

Implementing care bundles on caring for intravascular lines has had success in diminishing CoNS sepsis which further suggests that intravascular catheters are a major risk for LO infections (19, 100). If the mother has a hypertensive disorder and the fetus is growth-restricted, the choice of birth route is often cesarean. Olivier et al. found that the odds of CoNS sepsis were higher for neonates born before 32 weeks gestation if the birth route was cesarean vs. vaginal, possibly due to dysbiosis since the birth route influences the composition of neonatal gut microbiota (101, 102).

Intravascular catheters, especially central catheters like umbilical-, peripherally inserted central- (PICCs), and central venous catheters breach barriers and are associated with LO-infections (11, 14, 97). Both

S. aureus and CoNS produce a biofilm when in contact with a surface. These biofilms protect the bacteria against immune defenses and antibiotics and trigger dispersal mechanisms to other sites in the body where they can seed new biofilm production and thrive on another foreign device (103), figure 4. That is why foreign devices should promptly be removed when *S. aureus* is cultivated. Even though a central line can be salvaged in about half of CoNS cases, their removal is strongly recommended and essential if the bacteremia persists (104, 105).

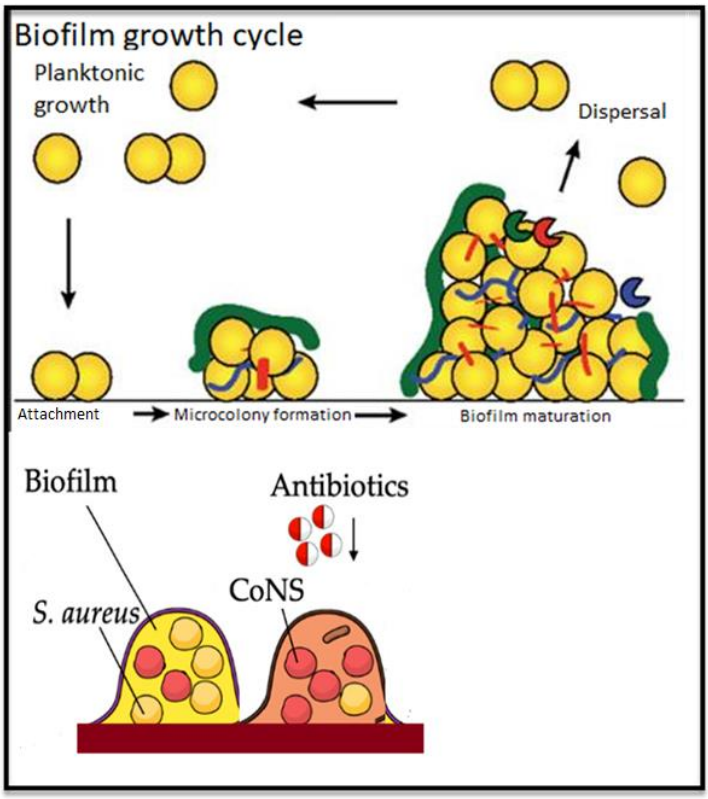

Figure 4. *Biofilm production in S. aureus and CoNS. Modified and reproduced from Lister et al. 2014 (103) and Di Domenico et al. 2019 (106) adapted from Mind the Graph (https://mindthegraph.com). Both under the attribution 4.0 International of creative commons https://creativecommons.org/licenses/by/4.0/.*

GROUP B STREPTOCOCCI

GBS is a β-hemolytic Gram-positive coccus first reported as a cause of cow mastitis in 1887 (107). It was differentiated from other streptococci by Rebecca Lancefield in the 1930s after isolation from the milk of affected cows - thereof its name; a-galactiae means no milk in Latin (108).

Human infections were first observed as sepsis, meningitis, and endocarditis and emerged as a leading cause of neonatal sepsis in the 1970s (109, 110).

INCIDENCE

GBS is a common asymptomatic colonizer of healthy adults' lower genitourinary- and gastrointestinal tracts (111-113). Among pregnant women, vaginal colonization rates of GBS vary from 11-35% worldwide (114). This asymptomatic harmless opportunistic organism colonizing otherwise healthy adults can subvert suboptimal host defenses to cause severe invasive disease and tissue damage (115-117).

ADULTS

Laboratory surveillance reports have shown a rise in invasive GBS infections among non-pregnant adults, with around 0.1 cases per 1000 persons and one-third of the patients needing intensive care (70, 118, 119). The incidence rises with age and is more than doubled in patients 65 years and older (70, 120, 121). Almost all adult cases have an underlying condition. The most common are obesity and diabetes, and their increase in prevalence might have contributed to the rise in invasive GBS infections (119, 122, 123). Obesity and diabetes, along with tobacco use, are also risk factors for the colonization of pregnant women (124, 125).

Skin and soft tissue infections, septic arthritis, septicemia without known focus, pneumonia, and endocarditis are the most common clinical presentations among non-pregnant adults (126-128). Pregnancy

is an immunosuppressed state, and GBS can cause endometritis, mastitis, and in rare cases, sepsis (129). The most significant risk with GBS vaginal colonization is the development of neonatal GBS disease and preterm birth (75, 117).

NEONATES AND INFANTS

Neonates are susceptible to GBS before, at, and after birth. If the mother is colonized with GBS, the bacteria can pass from the vagina into the amniotic fluid and cause IUFD and preterm birth (130, 131). A further risk for exposure happens during passage through the birth canal (132) and after birth via breast milk or the environment (133, 134).

The incidence of GBS infections among neonates and infants worldwide is about 0.5 per 1000 live births (135, 136). The incidence of EO infections has declined since studies from the 1980s showed that fewer neonates got EO GBS infections by administering antibiotics to the mothers at partus (132, 137, 138). In the United States, the incidence of EO infections declined from 1.8 per 1000 live births in the 1990s (139) to as low as 0.2 per 1000 live births in 2016 (72) due to tackling the most significant risk factor for neonates – the colonization of the mothers - by implementing IAP.

INTRAPARTUM ANTIBIOTIC PROPHYLAXIS – DIFFERENT STRATEGIES

The American College of Obstetricians and Gynecologists (ACOG) recommended IAP to prevent GBS disease in 1996 (21), and the American Academy of Pediatrics recommended it in 1997 (140). The Swedish National Board of Health and Welfare published guidelines on IAP more than ten years later in 2008 (141).

Universal screening for GBS with rectovaginal cultures late in pregnancy was recommended in the USA in 2002, after revising the guidelines (142). Screening is recommended at 36+0 to 37+6 weeks of pregnancy, except for women who have previously given birth to an infant with invasive GBS infection or have had GBS bacteriuria – they get IAP nevertheless, and screening is not needed (143, 144). The reason for a screening near term is that many women have transient or intermittent colonization, and GBS colonization status may not be correct if taken too early (111, 132, 145).

The Swedish recommendations have been unchanged since 2008 and still recommend a risk factor-based approach for identifying IAP candidates. The risk factor-based IAP is based on the presence of one or more following risk factors to identify parturient who should receive IAP during labor:

1. Intrapartum fever ≥38°C,
2. Preterm birth (<37 weeks),
3. Rupture of membranes ≥18 hours,
4. Previous delivery of an infant with invasive GBS infection or
5. GBS bacteriuria in the current pregnancy.

These risk factors are cumulative, with an increase in risk if more than one is present (54). The problem with the risk-based strategy is that some cases do not present with any risk factors or are not identified. The screening-based strategy seems to have a more significant impact than risk factor-based IAP on EO GBS infection. That is why many recommend IAP strategy based on screening (143, 146-153).

VIRULENCE OF GROUP B STREPTOCOCCI

GBS possesses several different virulence factors assisting the bacteria in adhesion, invasion of host epithelial cells and colonization, and immune evasions as well as adaptation to the host environment (154-156).

GBS have surface-associated proteins like adhesins, such as hyper virulent GBS adhesin (HvgA), fibrinogen-binding proteins, pili, plasminogen-binding proteins, and serine rich-repeat glycoproteins. These proteins make it possible for GBS to bind to and colonize epithelial cells and, in some cases, to cross the mucosal barrier of the host and become invasive pathogens (157).

GBS encodes many two-component signal transduction systems at cellular levels. The most studied is CovR/CovS (control of virulence) two-component system (CovR/S), where it serves as a regulatory system, either activating or repressing genes that may affect its virulence, such as the hemolysin operon *cyl* (157, 158). A transmembrane-bound protein known as Abx1 has been shown to form a signaling complex with the histidine kinase CovS in GBS (159).

SEROTYPES

GBS possesses two saccharides, the group B carbohydrate in the cellular wall and an extracellular capsular polysaccharide (CPS) which is rich in sialic acid and constitutes one of the main virulence factors of GBS. It helps GBS escape from the host defenses by interfering with the macrophages' phagocytic killing (160). The CPS's are classified into serotypes based upon structural differences. There are ten distinct CPS antigens described (Ia, Ib, II-IX) (161), and they are all antigenically distinct (162).

Serotype distribution differs between neonates and adults, with serotype III and Ia predominating among neonates and infants and serotype V being most common in infections among adults (112, 136, 163, 164).

A highly virulent clonal complex 17 (CC17) strains in serotype III, which possesses the adhesin HvgA has enhanced ability to penetrate the BBB and cause meningitis (165).

Studies have shown that serotype distribution differs between countries and changes over time (118, 119, 164, 166-169).

A successful GBS vaccine must be directed against the CPSs of the most common GBS serotypes. That is why it is vital to have ongoing surveillance and consider possible changes when GBS vaccines are formulated.

THE CAMP FACTOR

The Christie Atkins Munch Peterson (CAMP) factor is a protein exotoxin that forms oligomeric pores on susceptible cell membranes, promoting intracellular survival and systemic dissemination by entering the pathogen into host cells (170). It is not considered an essential virulence factor in humans (171). The CAMP factor has variable hemolytic activity and acts synergistically with sphingomyelinase, a protein secreted by *Staphylococcus aureus*. The CAMP reaction, shown as a triangular-hemolytic shape on blood agar when grown near the colonies of *S. aureus*, is used as a diagnostic tool for identifying GBS (172), figure 5.

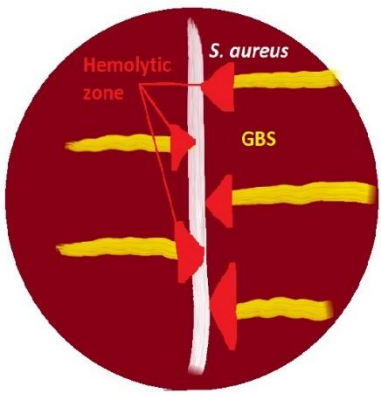

Figure 5. *A sketch of the CAMP reaction, showing an arrowhead where the zone of hemolysis occurs on blood agar when GBS is grown near S. aureus*

THE HEMOLYTIC PIGMENT

The β-hemolysis sometimes called complete hemolysis – compared to partial hemolysis with α-hemolysis – is one of the earliest identified virulence factors of GBS (173). It has been identified to be vital for the penetration of the BBB in GBS meningitis (174). Removal of the hemolytic pigment - which mediates the hemolysis - renders it avirulent and leads to inefficient vaginal colonization (175-178). The pigment is required for the bacteria to resist the host's immune response (177, 179). Low levels of pigment expression are favorable for vaginal colonization, and high levels are necessary to penetrate tissues (180, 181). Over-expression of the pigment induces an effective host immune response, diminishing colonization, which may be why hyper-pigmented GBS strains are seldom isolated from the vagina (180, 182, 183). A balance of the expression of hemolytic pigments is vital for the virulence of GBS (183). This hemolytic pigment can also be used to identify GBS as hemolytic GBS strains produce an orange-brick-red pigment (Granadaene) when cultivated on granada medium (184, 185).

CovR/S regulates the hemolytic pigment expression negatively by repressing the expression of the *cyl* operon, which is necessary for the biosynthesis of the hemolytic pigment (158, 186). Genetic ablation of *covR/S* makes GBS hyper-hemolytic and hyper-pigmented (175). Some hyperhemolytic clinical isolates have altered amino acid sequences in CovR/S, which might explain the phenotype of the hyperhemolytic GBS strains (187). A study on mice that were vaginally inoculated with non-hemolytic GBS had decreased bacterial dissemination and more minor fetal injury (179). GBS strains with increased hemolytic expression had accelerated GBS invasion of the amniotic cavity in a nonhuman primate model (178). CovR/S-deficient GBS strains are hyperhemolytic, and they have a better success of penetrating the BBB and have enhanced production of cytotoxins in the bloodstream. Still, most clinical GBS isolates have a functional CovR/S as the CovR/S seems to be critical for colonization and epithelial cell invasion, reflecting the importance of a balance of the expression of the hemolytic pigment (187).

Identifying the degree of hemolysis in GBS strains in human cases may lead to a better understanding of hemolysin's role in GBS infection in humans.

GBS HYALURONIDASE

Studies on mice have shown that cervical hyaluronan plays a role in epithelial barrier protection of the lower reproductive tract (188, 189). GBS hyaluronidase (HylB) is an enzyme that cleaves the extracellular matrix's hyaluronic acid into disaccharides (190). These disaccharides can then block the toll-like receptors critical for detecting bacteria and prevent the host immune system's response (191). GBS strains belonging to the same serotype exhibit varying hyaluronidase activity levels, and the activity is not correlated to specific capsular serotypes. Vornhagen et al. showed that hyaluronidase expression was higher in clinical GBS isolates than commensal isolates. GBS deficient of hyaluronidase had reduced ability to establish in utero infections due to increased immune recognition (192).

Defining mechanisms underlying different GBS virulence factors may lead to the development of strategies for identification and further intervention to battle GBS neonatal infections.

VACCINATION

Studies have shown that maternal antibodies against GBS CPS antigen as well as against some surface proteins may protect the neonate against perinatal infection (193-196). However, many GBS colonized women do not have sufficient antibody response at the time of delivery. Even though IAP may protect against neonatal disease, it is often missed (197, 198).

The group B carbohydrate non-CPS antigen common to all GBS strains is not a vaccine candidate as the antibodies induced are not protective (199).

Several different GBS antigens are included in potential vaccines (200). Vaccines based on serotypes (CPS) were the initial antigens included in developing a GBS vaccine and have come farthest in the development (200). Conjugation with protein antigens with tetanus toxoid or a genetically modified diphtheria toxin as the carrier protein was implemented to enable better immunogenic response and has been shown to prevent vaginal colonization in phase II trials (198, 201-203). This is based on the same principles as the conjugated vaccines against *Haemophilus influenzae* type b and pneumococcal vaccines (204). The development of a vaccine for elderly individuals is ongoing but with a focus on serotype V (205, 206).

Complete genome sequences for GBS allow vaccine development of a combination vaccine against different potential antigens, such as surface proteins combined with CPS (207-209). This offers advantages over vaccines containing irrelevant proteins as tetanus toxoid by combining antigens, not among the included serotypes, thereby providing a multivalent vaccine that protects against several serotypes.

AIMS

The studies incorporated in this thesis aimed to investigate neonatal invasive infections' epidemiology with a special focus on GBS to gain information for possible prevention and improvements on diagnostic sensitivity.

The specific aims of each study were:

I. To survey the serotype distribution of invasive GBS strains obtained prospectively between 2004 and 2009 and to compare it with previous studies (1998-2001) within the same area to detect any changes over time.

II. To assess the incidence, etiology, and any changes in the case fatality rate of neonatal invasive infections within the first week of life by comparing the last ten years (2008-2017) with 1997 – 2007 and two previous studies covering the period 1975 – 1996 (5, 7). A secondary aim was to evaluate if a change in EO neonatal infections' definition to <72 h after birth, instead of within the first week of life, would lead to missing cases of EO GBS infections.

III. To assess the incidence, etiology, and case fatality rate of LO neonatal invasive infections between 1997 and 2017, defining LO as infections occurring at 3-120 days of age. A secondary aim was to recalculate data on infections occurring between 3 and 27 days of age from 1975 and onwards to evaluate possible changes over a 43-year surveillance period.

IV. To determine if GBS isolates from pregnant or postpartum women and neonates with GBS invasive disease exhibit differences in virulence factor expression, strain genetics, and immune evasion compared to commensal isolates.

PATIENTS AND METHODS

PATIENTS AND METHODS

OVERVIEW OF THE METHODS

Paper	I	II	III	IV
Study design	Prospective cohort surveillance study	Retrospective observational epidemiological study	Retrospective observational epidemiological study.	Case-control laboratory study based on a prospective cohort
Population	Patients of all ages living within the counties Region Västra Götaland or Region Halland	Newborns at 0-6 days of age and their mothers living in Gothenburg or five surrounding municipalities at the time of birth	Infants 3-120 days of age and their mothers living in Gothenburg or five surrounding municipalities at the time of birth	Infants and pregnant/post-partum women living within the counties Region Västra Götaland or Region Halland and anonymous commensal isolates from the UW
Inclusion criteria	Invasive GBS infection in western Sweden	A pathogenic organism isolated from blood or CSF and mother living within the study area at the time of delivery	A pathogenic organism isolated from blood or CSF and mother living within the study area at the time of delivery	Saved invasive GBS isolates from neonates, pregnant/post-partum women, and commensal isolates from pregnant women.
Study period	2004-2009	1997-2017	1997-2017 (1975-2017)	1988-2001 & 2004-2009
Main research question	Serotype distribution of GBS	Incidence and etiology of early-onset neonatal infections	Incidence and etiology of late-onset neonatal infections	Is the expression of high-risk GBS virulence factors correlated with an invasive infection?

STUDY DESIGN

Paper I was a prospective cohort surveillance study of invasive GBS infection among all ages between January 1, 2004, and December 31, 2009, in two counties in western Sweden. Invasive infection was defined as isolation of the organism from blood, CSF, or synovial fluid. No GBS isolates came from the pleura, pericardium, peritoneum, or corpus vitreum.

Paper II and III were retrospective observational epidemiological studies on infants with a pathogenic organism isolated from blood or CSF during 1997 – 2017, both years included.

Their mothers had to be living in Gothenburg or five surrounding municipalities (Mölndal, Härryda, Partille, Öckerö or Kungälv) at time of birth. Flowcharts of included and excluded patients are shown in figures 6 and 7.

Paper IV was a case-control laboratory study based on a prospective cohort of invasive GBS strains collected from patients in western Sweden between 1988 – 2001 and 2004 – 2009. Strains from 214 infants and 19 pregnant or postpartum women were identified. Control group of 51 commensal GBS isolates were obtained from pregnant women through the University of Washington, Seattle, USA.

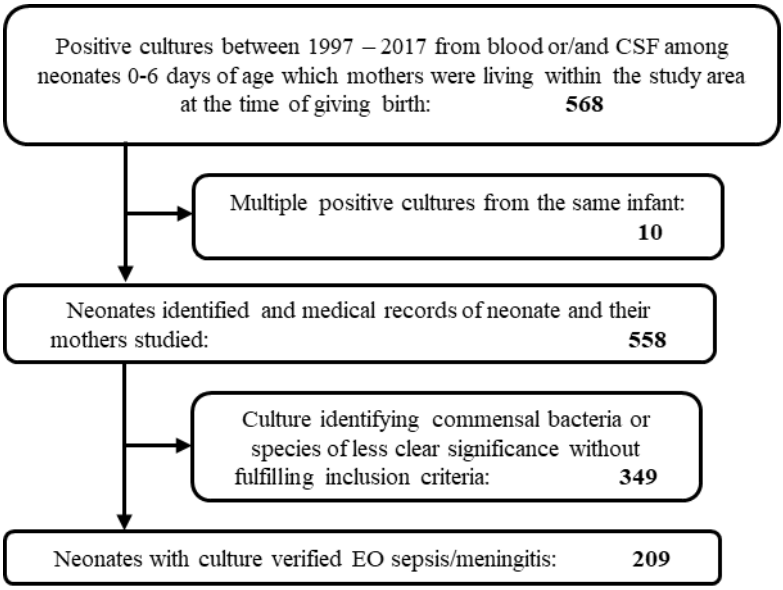

Figure 6. *Flowchart of included and excluded patients in Paper II. (Modified and reprinted under the terms of the Creative Common Attribution 4.0 International License http://creativecommons.org/licenses/by/4.0/: Gudjónsdóttir et al. BMC Pediatrics 2019, Vol. 19, Iss. 1.)*

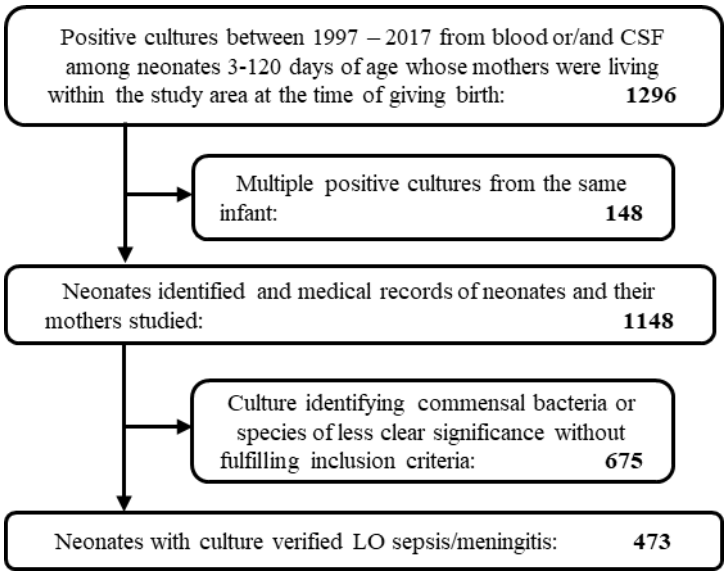

Figure 7. *Flowchart of included and excluded patients regarding the years 1997-2017 in Paper III*

DATA COLLECTION

In papers I and IV, invasive GBS isolates were collected prospectively from bacteriological laboratories that served the hospitals in Västra Götaland and Halland, two counties of western Sweden. Data on patient characteristics and outcomes were retrieved in retrospect from the medical records. In the paper IV control group, GBS clinical isolates were obtained from general screening. Rectovaginal swabs were obtained from women in their third trimester of pregnancy at the University of Washington Medical Center and Harborview Medical Center, without any identifiers or clinical information.

In papers II and III, data on positive cultures were obtained from the Clinical Microbiology laboratory at Sahlgrenska University Hospital, Gothenburg. We retrieved data on the mothers' ZIP code and possible risk factors from the electronic medical record Obstetrix (Cerner). Data on patient characteristics and outcomes we collected from the electronic medical record system Melior (Cerner).

DEFINITION OF A PATHOGENIC ORGANISM

GBS was the only organism included in **papers I and IV**. Invasive isolates were identified as GBS by colony morphology, microscopy of Gram-stained smears, and co-agglutination with group-specific reagents.

In **papers II and III,** organisms were defined either as a recognized pathogen or, if less clear clinical significance, as commensal species, table 1. The occurrence of recognized pathogens was, in all cases, regarded as the cause of infection. Species regarded as commensal were considered the cause of infection if both of the following criteria were fulfilled; otherwise, they were excluded from the studies:

1. At least one of the following signs and symptoms as a change from baseline: (1) Apnea, (2) Bradycardia, (3) Temperature <36.5° or >38°C.

2. Appropriate antibiotics were intended for >120 hours, and a central catheter in place within 72 (paper II) and 48 (paper III) hours before the culture was drawn.

Table 1. *Recognized pathogens and species of less clear clinical significance.*

1. Recognized pathogens:	
Staphylococcus aureus	*Escherichia coli,*
Group A,B and C streptococci,	*Klebsiella* spp.
Pneumococci	*Haemophilus influenzae,*
Enterococcus spp.	*Pseudomonas aeruginosa*
Actinomyces	*Enterobacter* spp
Listeria monocytogenes	*Proteus mirabilis*
Candida albicans	*Serratia marcescens*
2. Commensal species or species of less clear clinical significance:	
Coagulase-negative staphylococci (CoNS)	*Bacillus* spp
Viridans streptococci	*Bacteroides* spp*

*Was considered a recognized pathogen in the EO study (Paper II).

SEROTYPING OF GBS

The isolates were stored in a broth at -70°C until serotyping was performed. The latex agglutination test with type-specific antisera for serotypes Ia, Ib, II, III, IV, V, VI, VII, VIII, and IX was used for serotyping (Statens Serum Institut, Copenhagen, Denmark) as previously described (210).

CHARACTERISTICS TESTS OF GBS

The wild-type GBS strain COH1, a clinical isolate obtained from an infected newborn, is a capsular serotype III, hypervirulent ST-17 clone (211), and the hyper-pigmented GBS strain NCTC 10/84 (212) were used as controls in the characteristics tests of GBS as described below.

CAMP-FACTOR

First, *S. aureus* was streaked vertically down the agar plate. Then a single GBS colony was streaked horizontally near *S. aureus*. The plate was then incubated for 24 hours at 37°C, and the triangular hemolysis graded from 0 - ++++ and photographed for second grading.

Figure 8. *Control picture. On the left of the plates is GBS COH-1 replicated 3 times. This is equivalent to +++ for CAMP factor. On the right of the plate is NCTC 10/84. This is equivalent to 0 for CAMP factor.*

BETA-HEMOLYSIS

A single GBS colony was streaked in alternating directions on sheep blood agar and incubated for 24 hours at 37°C. Hemolysis was determined between 0 and +++ according to control, as is shown in figure 9.

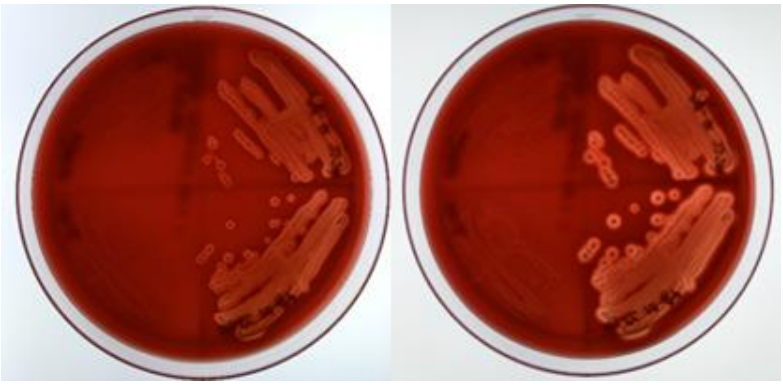

Figure 9. *On the right of the plate is NCTC 10/84. This is equivalent to +++ for hemolysis, and on the left of the plate is GBS COH-1 which is equivalent to ++ for hemolysis. The same plate but 24 hours later in the picture on the right.*

GRANADA

A single GBS colony was streaked on Granada media and incubated for 24 hours at 37°C. Pigment production was evaluated on a scale of 0 to ++++ in comparison with control, according to figure 10.

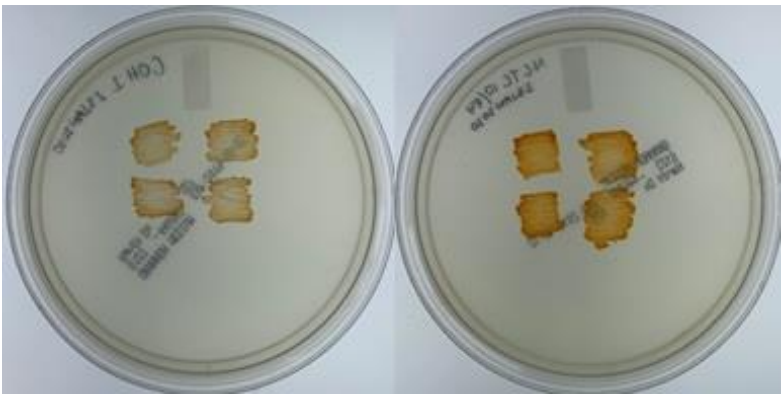

Figure 10. *On the left is GBS COH-1 WT on Granada, replicated four times. This is equivalent to +++ in terms of pigmentation and on the right is GBS NCTC 10/84 equivalent to ++++ in terms of pigmentation.*

COVR/COVS SEQUENCING

For each of the 284 GBS isolates, the entire *covR/S* region was amplified. Amplified samples were confirmed by gel electrophoresis and then column purified with a Genejet PCR purification kit (Thermofisher Scientific, Waltham, MA, USA) and prepared for Sanger Sequencing according to Genewiz recommendations (https://www.genewiz.com). Samples were diluted and mixed with seven different primers that anneal to multiple locations in c*ovR/S*. The sequences returned by Genewiz overlapped and encapsulated the entire *covR/S* region. Codon location and changes were recorded for non-synonymous mutations in *covR/S* for each isolate.
For further details, see the methods section in manuscript IV.

HYALURONIDASE EXPRESSION

GBS strains were centrifuged to cultured supernatants after growing them overnight in tryptic soy broth. Hyaluronic acid was added to each GBS culture. Hyaluronidase (Sigma-Aldrich, Saint Louis, USA, https://www.sigmaaldrich.com) was used to make standards at different concentrations. Following incubation, sodium tetraborate was added to the samples and hyaluronidase standard and 4-dimethylaminobenzaldehyde after further incubation. A color change to magenta was evaluated since that indicated hyaluronidase activity. Samples and standards were transferred to a 96-well plate to measure absorbance at 585 nm immediately following a color change. The GBS clinical wild-type strain GB37 (high activity) (213) and isogenic hyaluronidase deficient GB37Δ*hylB* (low activity) were used as positive and negative controls, respectively. For further details, see the methods section in manuscript IV.

STATISTICAL METHODS

In all papers, the characteristics of the data were explored. Distribution patterns were tested for normality with the Kolmogorov-Smirnov test. Demographic data were presented as mean with 95% confidence interval (CI), standard deviation (SD), or standard error (SE) when normally distributed and median with interquartile range (IQR) if otherwise.

In papers II and III, incidences were estimated as the number of infected infants overall or by subgroup, divided by the total number of live births within the population or the total number of live births reported for the same subgroup. Reports on the number of live births were retrieved from Statistics Sweden (www.scb.se/en), and the number of live births according to gestational age within the study population was retrieved from the Swedish Medical Birth Register/Medicinska födelseregistret (MFR) (www.socialstyrelsen.se) for study III. The data was retrieved according to the postal code of the maternal residence at the time of birth. Cases with missing or wrongly registered gestational age were included in calculations on overall incidence data but otherwise excluded.

Independent sample t-tests were used for normally distributed data. Non-normally distributed data were compared using Mann Whitney U Test. Correlations between continuous outcomes were analyzed using the Spearman test. Fisher's exact test (two-tailed) was used for comparisons of proportions (http://graphpad.com/quickcalcs/contingency1.cfm and https://www.socscistatistics.com/tests/fisher/default2. aspx).

For papers I, II, and III, statistical analyses were also performed using IBM SPSS Statistics version 25.0 and 26.0 (IBM corporation, Armonk, NY, USA). For paper IV, statistical analyses were performed by Dr. Jeff Munson by programming in R.

A p-value $<.05$ was considered significant in all papers.

ETHICAL CONSIDERATIONS

The concerned authorities approved all studies in this thesis.

Paper I was approved by the Ethics Committees of Gothenburg University (Registration nr: Ö 524-03) and Lund University (Registration nr: LU 855-03). The committees did not require individual consent because the clinical data were obtained in retrospect when many patients and/or relatives could not be found.

Paper II and III were retrospective studies spanning a significant time interval. Approval from each individual was not considered reasonable nor possible to obtain. It was approved by the Ethics Committees of Gothenburg University (Registration nr: Ö 020-03) and extended twice, once by the Ethics Committees of Gothenburg University (Registration nr: T760-16) and once by the Swedish Ethical Review Authority (Registration nr: EPM 2020-06340).

Paper IV included GBS strains collected in previous studies between 1988-2001 and strains collected for paper I with the approval from the Ethics Committees of the University of Gothenburg and Lund University. The committees did not require individual consent because the clinical data were all obtained in retrospect when many patients and/or relatives could not be found. The isolates were approved for shipping to the University of Washington and further study by The Swedish Ethical Review Authority on February 8, 2019, registration number EPM 2019-00549. Permit to Import Infectious Biological Agents was given from the Centers for Disease Control and Prevention (PHS Permit no. 20190529-3089A). GBS commensal isolates in the control group were collected without any identifiers or clinical information, and a waiver for written informed consent was obtained for testing anonymous samples.

No identifiable data can be linked to any study subject in any of the studies.

RESULTS

PAPER I

"Serotypes of group B streptococci in western Sweden and comparison with serotypes in two previous studies starting from 1988"

A total of 410 GBS strains were isolated from 398 patients. Ten patients had two infectious episodes, and one patient had three episodes with intervals between 10 and 25 months. All patients with recurrent infections were adults. A flowchart of the included isolates is shown in figure 11.

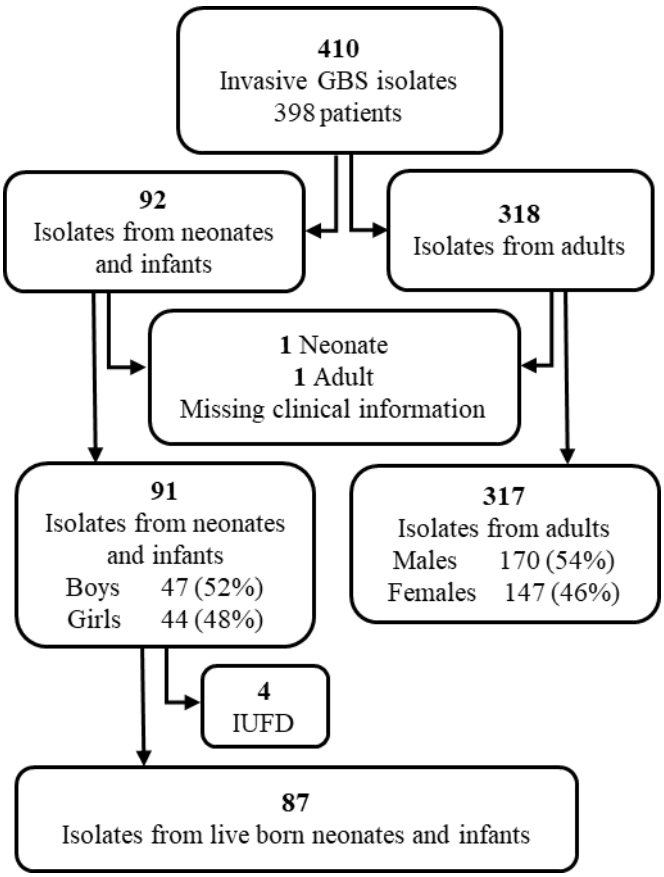

Figure 11. *Flowchart of 410 serotyped isolates*

NEONATES AND INFANTS

Among the 91 isolates from neonates or infants, the median age was one day (0 - 209 days). Unfortunately, we could not recover documents on clinical manifestation from one neonate with EO, serotype Ia. The clinical manifestation of the 87 live-born neonates and infants is shown in table 2.

Table 2. *Clinical manifestations and onset among 87 infants and neonates*

Clinical manifestation	Early-onset	Late-onset	No.	(%)
Sepsis of unknown focus	46	17	63	(72)
Meningitis	5	7	12	(14)
Pneumonia	6	2	8	(9)
Skin infection	0	2	2	(2)
Urosepsis	0	1	1	(1)
Septic arthritis	0	1	1	(1)
Total	57	30	87	(100)

Of the 87 live born infants were 33 (38 %) born preterm. Twenty of the infants born preterm had EO disease, and 13 had LO disease.

ADULTS

There were 318 isolates from adults, and the records of one adult patient could not be found. The median age of the remaining 317 adults was 73 years (23 – 103 years). Underlying medical conditions were documented in 259, with the most common being cardiovascular disease, 36% (114/317), diabetes, 25% (80/317), and malignant disease, 20% (62/317). Sepsis with unknown focus - with 29% (92/317) of the cases - was the most common clinical manifestations among the adults, followed by erysipelas 24% (75/317) and septic arthritis 16% (52/317).

SEROTYPE DISTRIBUTION

The results of the serotype distribution of invasive GBS infections showed that it had not changed compared to previous studies in the same region (164, 167). Serotype III continued to be the most common serotype in isolates from neonates and infants (48% (44/92)), followed by serotypes Ia (18% (17/92) and V (16% (15/92). In adults, serotype V was still the most prevalent (39% (124/318), followed by III (19% (62/318) and Ib (14% (45/318). Serotype distribution according to clinical manifestation among infants (upper panel) and adults (lower panel) is shown in figure 12.

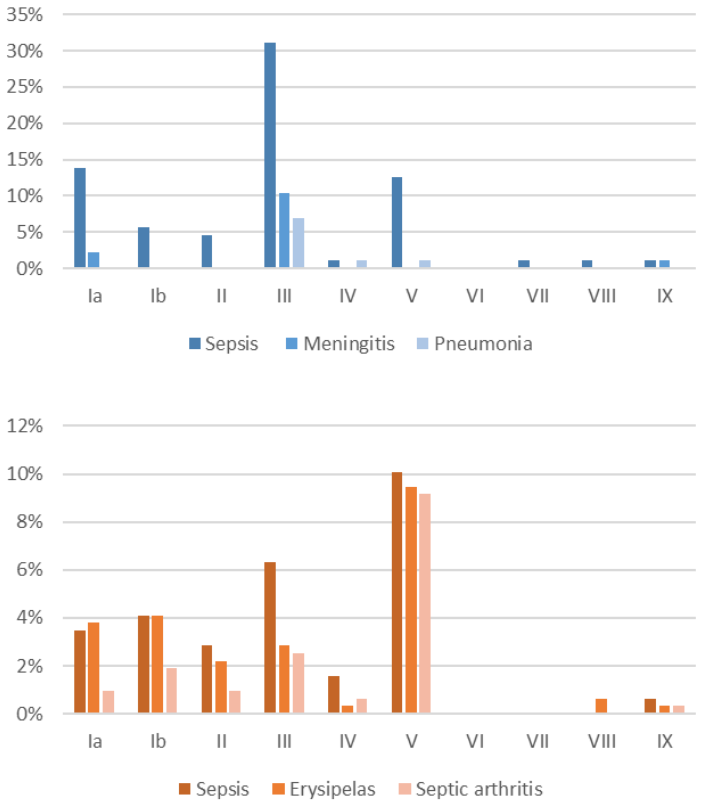

Figure 12. *Percentage of serotype distribution according to the three most common clinical manifestations among neonates and infants (upper figure) and adults (lower figure).*

PAPER II

"**Changes in incidence and etiology of early-onset neonatal infections 1997-2017 – a retrospective cohort study in western Sweden"**

INCIDENCE AND ETIOLOGY OF EO-INFECTIONS

The incidence of EO-invasive infections within the first week of life declined from 1.4 per 1000 live births in 1997 – 2007 to 0.9 per 1000 live births in 2008 – 2017 (p=.004), but the case fatality rate at 7% did not differ during the study period, table 3. There was no difference in patients characteristics during the study period except the mean birth weight was lower (2376g (CI 2095-2658) in period II compared to period I, (2874g (CI 2663-3085)) (p=.004), which was in agreement with a higher proportion of extremely preterm infants, born <28 weeks gestation, among the patients with EO infection (25/90 in 2008-2017 vs. 12/119 in 1997-2007, p=.002).

Table 3. *Incidence of EO invasive infections (per 1000 live births) and case fatality rate according to pathogen.*

Type of organism	Incidence / 1000 live births		*p-value*	Case fatality rate (%)
	Period 1 1997-2007	Period 2 2008-2017		
GBS	0.5	0.4	ns.	7/48 (8)
Staphyococcus aureus	0.3	0.1	.01	1/33 (3)
CoNS	0.08	0.1	ns.	0/18
Other Gram-positive bacteria*	0.2	0.08	.04	1/25 (4)
Gram-negative bacteria**	0.3	0.2	ns.	6/47 (13)
Candida albicans	0	0.02	ns.	0/2
Total	1.4	0.9	.004	15/209 (7)

*Enterococci, *Listeria monocytogenes*, pneumococci, beta-hemolytic streptococci group A and C, *Actinomyces* spp. ****E. coli, Klebsiella pneumoniae, K. oxytoca, Proteus mirabilis, Serratia Marcescens*, other *Enterobacter* spp. *Pseudomonas* spp., *Hemophilus influenzae, Burkholderia cepacia, Neisseria meningitides, Bacteroides* spp.

The most common organism was GBS (84/209), causing 40% of the cases with an incidence of 0.45 per 1000 live births. None of the cases between 72 hours and one week of life was caused by GBS.

FATAL CASES OF EO INFECTIONS

Among the 15 fatal cases, 13 were born before 37 weeks gestation, and 62% (8/13) of them were born extremely preterm (<28 weeks gestation), figure 13. The case fatality rate of 7% did not change between 1997-2007 and 2008-2017 (8/119 vs. 7/90)

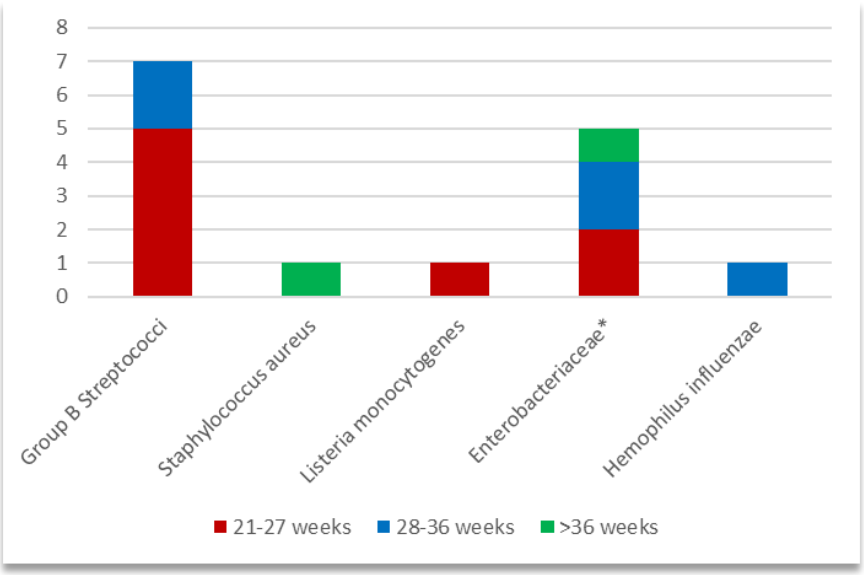

Figure 13. *Fatal EO-cases according to pathogen and gestational age group.* **E. coli* (n=2), *Klebsiella pneumoniae* (n=2), *Hemophilus influenzae* (n=1), *Proteus mirabilis* (n=1)

PAPER III

"Late-onset Neonatal Infections 1997 to 2017 within a cohort in western Sweden – the last 21 years of a 43-year surveillance"

INCIDENCE AND ETIOLOGY OF LO-INFECTIONS

The incidence of LO-infection between 3 and 120 days of age increased from 2.0 per 1000 live births in 1997–2007 to 3.1 per 1000 live births in 2008-2017 ($p<.001$). The timing of LO infections according to gestational age is shown in figure 14, and the distribution of pathogens is shown in figure 15.

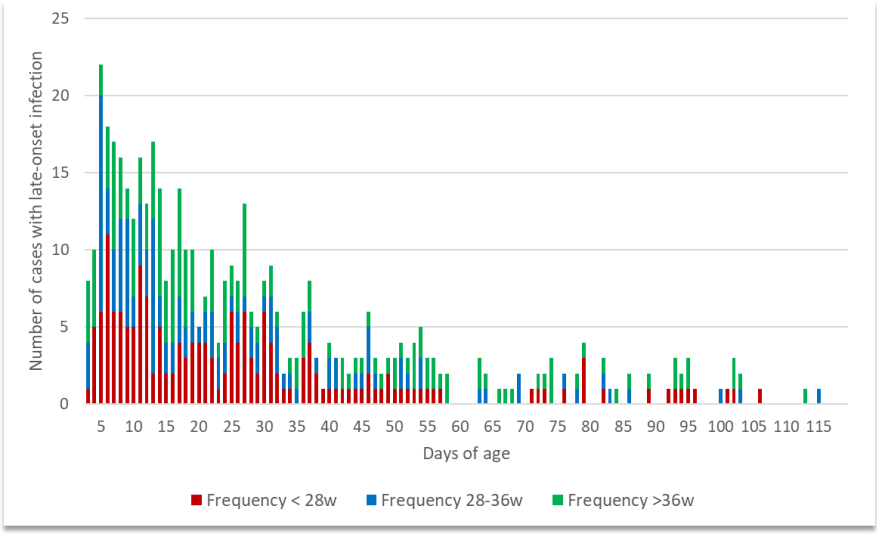

Figure 14. *The timing of late-onset infections according to gestational age among 469 infants with late-onset infection, 3-120 days of age during 1997-2017 (4 cases with unknown gestational age are excluded).*

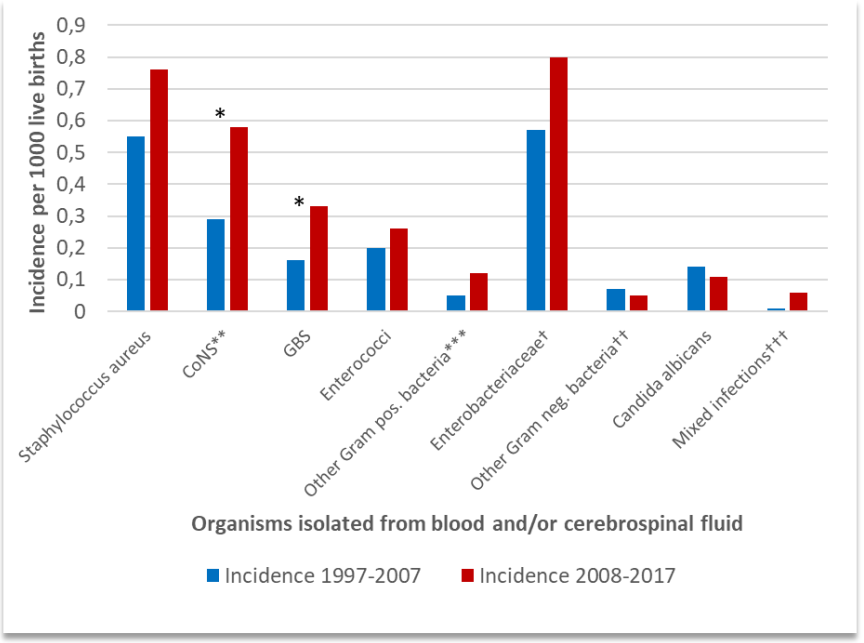

Figure 15. *The incidence of LO-infections per 1000 live births stratified by organisms isolated from blood and/or cerebrospinal fluid from 473 infants 3-120 days of age during 1997-2007 compared to 2008-2017.*
**p*-value < .05. ***Staphylococcus epidermidis, S. hominis, S. capitis.* ****S. parasanguinis, S. anginosus, S. pneumoniae, Bacillus cereus, Rothia mucilaginosa,* Beta-hemolytic streptococci group A. *†Escheria Coli, Klebsiella pneumonia, K. oxytoca, Serratia marcescens* other Enterobacter spp. *††Pseudomonas aeruginosa, Haemophilus influenzae* type b, *Acinetobacter* spp, *Moraxella* spp, *Stenotrophomonas maltophilia.* *†††S. aureus* and enterococci (n=3), *S. aureus* and *Acinetobacter* (n=1), *K. pneumoniae* and enterococci (n=1), *K. pneumoniae* and *Pseudomonas aeruginosa* (n=1), *K. pneumoniae*, and *E. coli* (n=1).

Term infants, among the infants with an infection after 28 days of age, were as many (66/176) as the infants born extremely preterm (66/176), figure 14, and the etiology differed between in- or out-of-hospital settings, table 4.

Table 4. *Cultivated pathogen in LO-infections, 3-120 days of age during 1997-2017, according to in-hospital settings and out-of-hospital settings.*

	Settings					
Pathogen	Neonatology	Cardiology	Surgery	Pediatrics	Out-of-hospital	Total
Staphylococcus aureus	89	2	7	1	21	120
CoNS*	59	4	17	1	0	81
GBS	19	0	1	0	26	46
Enterococcus	29	4	2	1	6	42
Other Gram pos bacteria**	3	1	0	0	12	16
Enterobacteriaceae***	57	6	12	2	50	127
Other Gram neg. bacteria†	5	2	0	0	4	11
Candida albicans	15	1	5	1	1	23
Mixed infections††	6	0	0	0	1	7
Total	282	20	44	6	121	473

**Staphylococcus epidermidis, S. hominis, S. capitis **S. parasanguinis, S. anginosus, S. pneumoniae, Bacillus cereus, Rothia mucilaginosa,* Beta-hemolytic streptococci group A. ****Escherichia coli, Klebsiella pneumonia, K. oxytoca, Serratia marcescens* other *Enterobacter* spp. *†Pseudomonas aeruginosa, Haemophilus influenzae* type B, *Acinetobacter* spp, *Moraxella* spp, *Stenotrophomonas maltophilia. ††S. aureus* and *Enterococcus* (n=3), *S. aureus* and *Acinetobacter* (n=1), *K. pneumoniae* and *Enterococcus* (n=1), *K. pneumoniae* and *Pseudomonas aeruginosa* (n=1), *K. pneumoniae, and E. coli* (n=1).

FATAL CASES OF LO INFECTIONS

The case fatality rate at 6% (29/473) did not differ during the study period, and the fatal cases according to pathogen and gestational age are shown in figure 16.

Enterobacteriaceae (13/29) were the most common pathogens cultivated from fatal LO infections. Five fatal cases had a positive culture with CoNS.

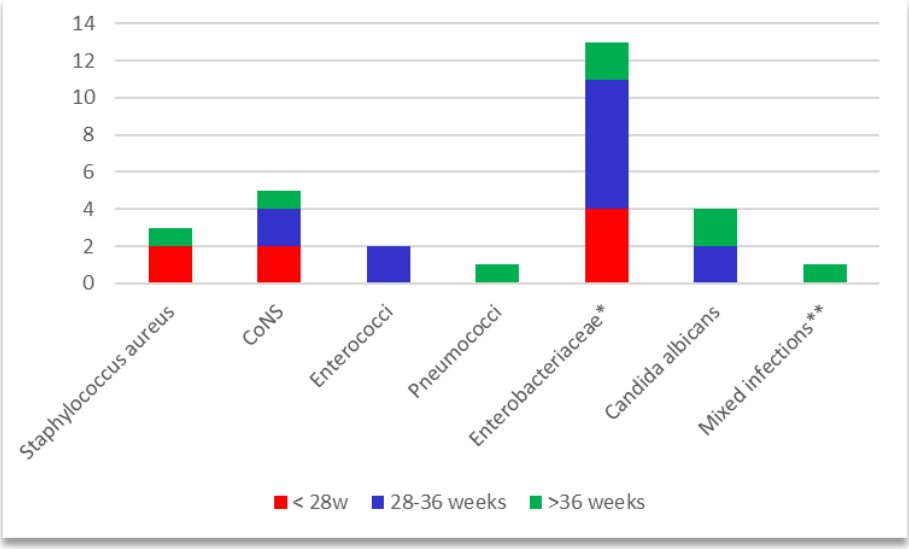

Figure 16. *Fatal LO – cases during the study period 1997-2017 according to pathogen and gestational age group.*
**Klebsiella pneumonia* (n=4), *Serratia marcescens* (n=3), *K. oxytoca* (n=2), *Escherichia coli* (n=1), other *Enterobacter* spp. (n=3). ***E. coli + K. pneumoniae* (n=1).

NEONATAL INFECTIONS <28 DAYS OF LIFE BETWEEN 1975 AND 2017

The incidence of neonatal sepsis <28 days of life (CoNS and viridans streptococci not included) decreased from 3.2 per 1000 live births during 1975-1996 to 2.1 per 1000 live births in 1997-2017 (p<.001), and the case fatality rate dimidiated from 12% (64/540) to 6% (23/380) during the same period, (p=.003). The incidence of invasive infections due to GBS, *S. aureus,* and *Enterobacteriaceae* <28 days of life between 1975 and 2017, both years included, is shown in figure 17.

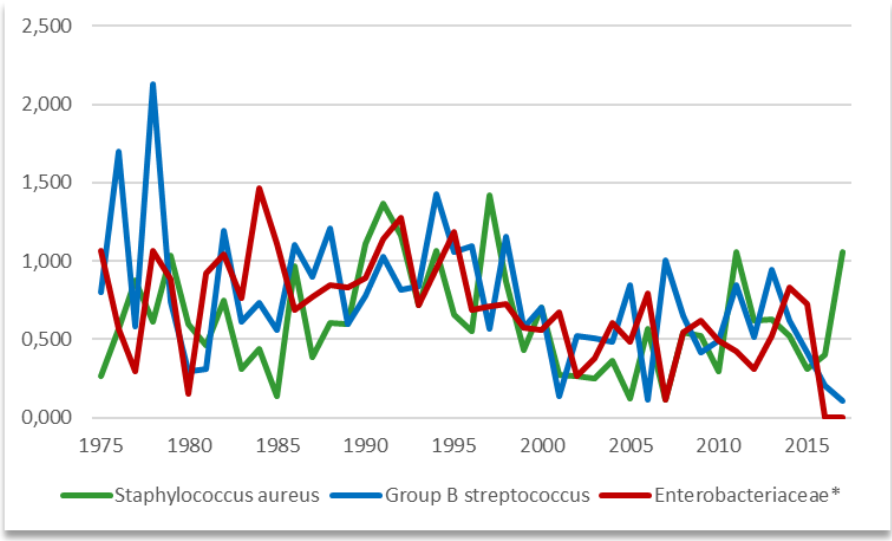

Figure 17. *Incidence per 1000 live births of GBS, S. aureus, and Enterobacteriaceae during the first 28 days of life between 1975 and 2017.*
**Escherichia coli, Klebsiella pneumoniae, K oxytoca,* other *Enterobacter* spp., *Salmonella, Serratia marcescens, Proteus mirabilis*

A total of 11% (97/920) had culture-confirmed meningitis among the invasive infections <28 days of life. The case fatality rate was 7% (7/97). The most common pathogens cultivated from CSF were GBS (34% (33/97)) and *Enterobacteriaceae* (36% (35/97)). The incidence of EO meningitis was lower (0.03 per 1000 live births) in 2008-2017 compared to 1975-1996 (0.16 per 1000 live births, $p<.001$) and LO meningitis (3-27 days of age) the incidence went from 0.3 to 0.1 per 1000 live births ($p<.001$).

PAPER IV

"Virulence Factors of Invasive Group B Streptococcus Isolates Obtained from Swedish Pregnant Women and Neonates"

A total of 233 invasive GBS isolates were identified and compared to 51 commensal isolates, figure 18.

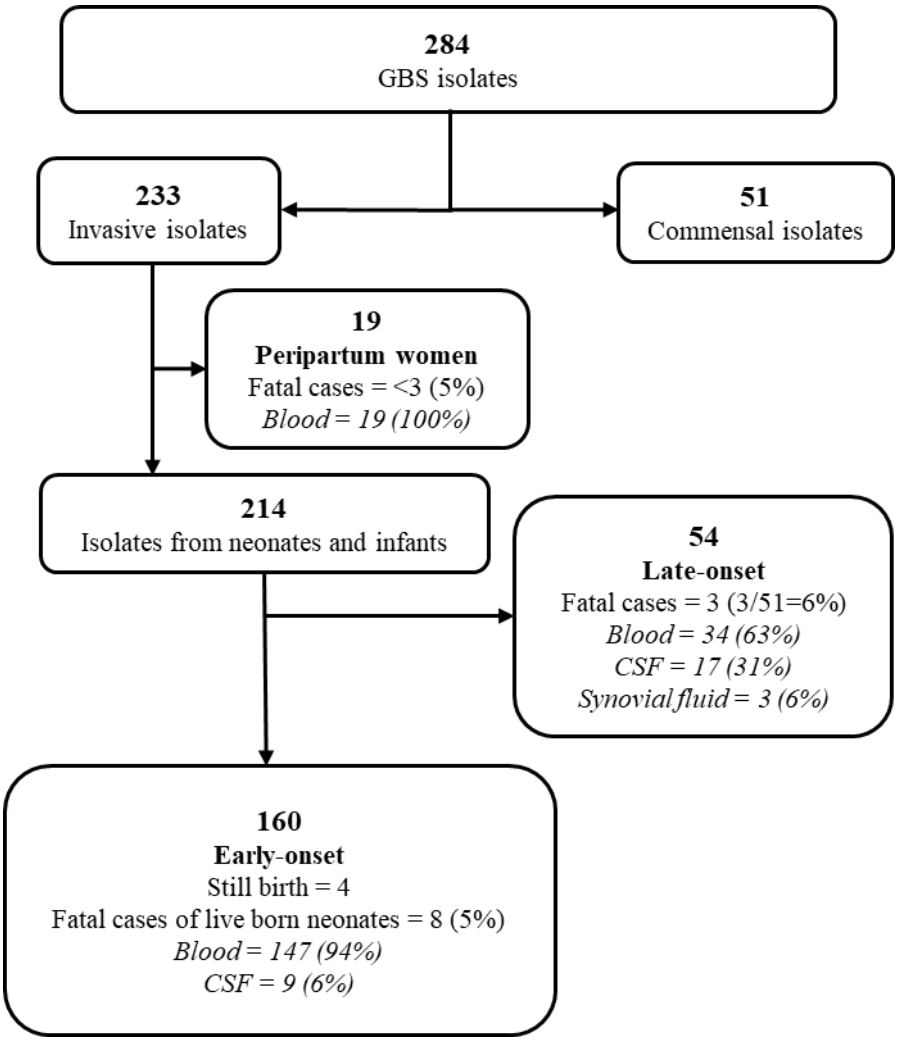

Figure 18. *GBS isolates included in the study and their site of origin.*

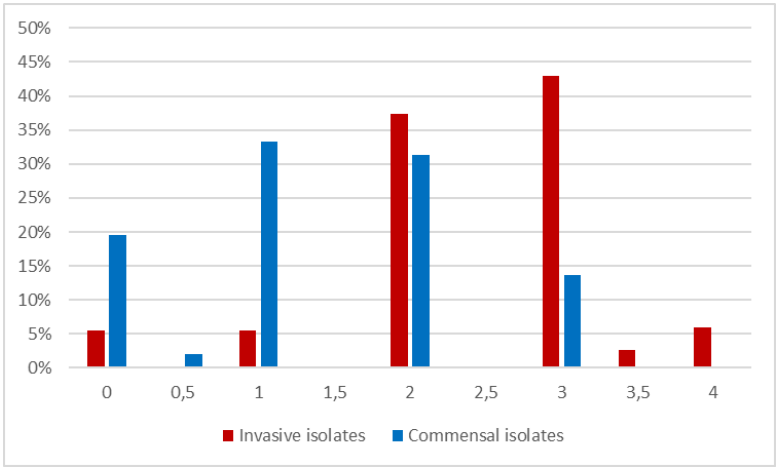

Figure 19. T*he percentage of scoring of hemolytic pigment expression between 0 and 4 on Granada media for the 233 invasive isolates (red) and the 51 commensal isolates (blue).*

The mean scoring of hemolytic pigment expression on Granada media was significantly higher for the invasive isolates (2.4 ± 0.1 SE) compared to commensal isolates (1.4 ± 0.1 SE), $p<.001$, figure 19. No difference was noted for the EO isolates compared to the LO isolates, figure 20.

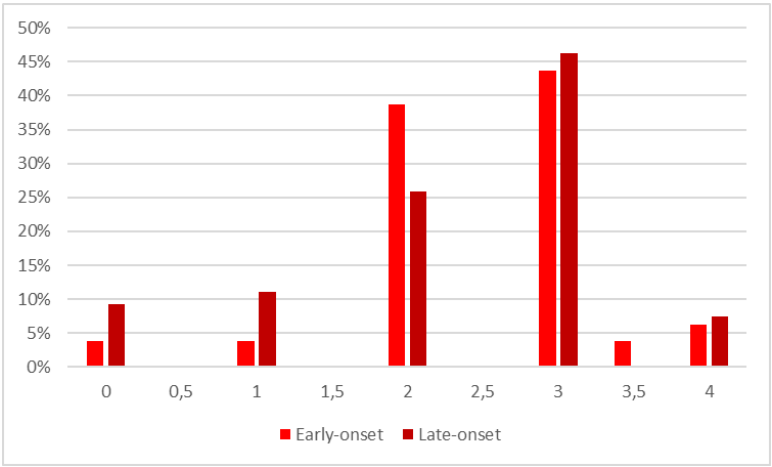

Figure 20. *The percentage of scoring of hemolytic pigment expression between 0 and 4 on Granada media for the 160 EO-invasive GBS isolates (4 cases of IUFD included) (lighter red) and the 54 LO-invasive GBS isolates (darker red).*

DISCUSSION

DISCUSSION

The studies presented in this thesis have advanced our knowledge of neonatal infections with a focus on GBS.

We found that there had not been any significant changes in the serotype distribution of invasive GBS infections compared to previous studies (164, 167). This is promising regarding a GBS vaccine since a successful CPS vaccine has to target the most common GBS serotypes.

We made observations on changes in incidence, etiology, and short-time prognosis over a long follow-up period of 43 years. We found that the incidence of EO invasive infections had decreased despite a proportional rise of extremely preterm infants. However, the incidence of LO invasive infections up to 120 days of age increased during the last ten years, especially among neonates born extremely preterm.

Infections after 72 hours are more likely acquired horizontally than vertically transmitted by exposure at birth, and that applies to GBS as well since all GBS infections <7 days in our study occurred within 72 hours.

We did not find that the incidence of EO GBS had decreased significantly after the implementation of IAP.

In studies on the virulence of GBS isolates, we found that the invasive isolates had more significant hemolytic potential compared to commensal isolates.

INCIDENCE OF INVASIVE NEONATAL INFECTIONS

During the study period 1997-2017, there were 181 928 live births within the study population, and 6% (10 221/181 928) were born preterm before gestational week 37. During the study period, infants born extremely preterm rose from 0.24% (208/85331) to 0.33% (317/96544). There was no change regarding infants born after 28 weeks gestation.

One of the limitations in papers II and III is the observational design and the inability to determine causality. We can only speculate on possible reasons for changes in incidence since the risk of bias is high (214). In addition, the study population was observed over 43 years, and therefore, many unknown factors may have contributed to possible differences.

Laboratory criteria were not included as criteria for neonatal sepsis in our studies. The treating physician has evaluated it as a true infection using the intention to treat with antibiotics >120 hours or five days as a criterion. Not surprisingly, after reviewing the cases, most of them had positive biomarkers like elevated CRP or IL-6, which are widely used as a screening tool for infection and symptoms in the clinical setting. Few or none of the cases would have been excluded if we also would have included laboratory criteria for a pathogenic infection depending on the biomarkers cut-off point.

The intention to treat for more than four days of antibiotics is a common criterion for the diagnosis of neonatal sepsis and, in some studies, regardless of the pathogen (24, 61-63). Thus, even though we only included this criterion regarding organisms that might otherwise be regarded as contaminants, it is unlikely that we have over-evaluated the incidence of recognized pathogens since these infections would seldom be treated for less than five days.

We did not exclude polymicrobial cultures in papers II and III. However, we evaluated every case, and cases fulfilling the inclusion criteria were presented as mixed-infections since more than one organism can cause clinical symptoms concurrently (24, 215). None of the mixed-infections included in our studies was caused by organisms that might otherwise be regarded as a contaminant.

Bacteroides spp. were considered a recognized pathogen in the EO study (paper II), in which there were only two cases. Both were cultivated within 24 hours of birth, and they had symptoms of an infection with elevated CRP. However, in paper III on LO infections, they were considered as possible commensal bacteria.

To keep consistency in the calculations on the incidence of neonatal infections <28 days of life from 1975 and onwards, CoNS and viridans streptococci were excluded in comparing the different study periods covering 1975-2017. Over the 43 years, there were 19 cases of EO meningitis (<72 hours), and 63% were due to GBS 12/19. The incidence among preterm infants was 0.2 per 1000 live births compared to 0.05 among the overall birth population. That the incidence of EO meningitis is higher among preterm infants agrees with other studies reporting 0.7 cases per 1000 live births at 22–28 weeks' gestation (216) and 0.02–0.04 cases per 1000 live births within the overall birth population (75, 83). Although infants with culture-confirmed meningitis often have blood cultures yielding the same organism, the concordance is not 100%, and CSF cell count parameters may not always identify meningitis (80).

PCR is more often relied upon in the clinical setting to rule out meningitis since an LP is often postponed until the patient has received antibiotics and is clinically stable enough for the procedure (39). Since our studies only included pathogens yielded by culture, the incidence of neonatal meningitis is most likely undervalued.

FATAL INFECTIONS

In our studies, the case fatality rate of neonatal sepsis was 7% (15/209) within the first week of life and 6% (29/473) for LO - infections after 72 hours of life. The overall case fatality rate for neonates with invasive infection within the first 120 days of life between 1997 and 2017 was 6.7% (42/626), and 32 of the 42 fatal cases (76%) were born preterm. This agrees with other studies reporting an overall case fatality rate of 5-10% (66, 83, 217).

Among extremely preterm infants, neonatal sepsis remains a major cause of death. Other studies have reported a 25-29% case fatality rate for preterm neonates with an EO infection and 18% with LO infections (14, 63, 218). Case fatality rate differs between studies, especially if the incidence of "low-mortality" pathogens like CoNS is high (219). Gram-negative sepsis is associated with a more fulminant course with septic shock that may result in death within 48 hours (63, 220-224). A recent Swedish study reported a 15% case fatality rate in EO Gram-negative infections, with the definition of EO being within 72 hours of life and more than doubled (34%) for LO Gram-negative infections (225). The case fatality rate in EO Gram-negative infections agrees with 13% (6/47) in our EO study, including infants within the first week of life. However, the case fatality rate among LO Gram-negative infections was much lower or 10% (14/138) (mixed infections not included). This discrepancy could be explained by our study being population-based, including all infants up to 120 days of age, whereas their study included only infants within a neonatal unit. A cohort that included neonates of all birth weights and gestational ages with LO sepsis had a frequency of fatal cases with *Pseudomonas* spp 56%, *E. coli* 19%, *Enterobacter* spp 14%, *Klebsiella* spp 13%, *S. aureus* 6%, and CoNS 1% (220). Our studies between 1997 and 2017 showed that 40% (17/42) of the fatal cases among neonates and infants before 120 days of age were due to Gram-negative infections.

Five of the 42 fatal cases among neonates and infants 0-120 days of age had a positive culture with CoNS. They were either born preterm

or/and had an underlying condition with more than one culture yielding CoNS and elevated biomarkers.

Neonatal invasive infection is a risk factor for long-term sequelae, especially for infants born extremely preterm, either by direct infection of the central nervous system (CNS) or indirectly due to inflammation, leading to cerebral palsy and vision impairment (226-228).

In our studies between 1997 and 2017, 62% (52/84) of EO GBS infections were born at term, and the case fatality rate was 8% (7/84) but all seven fatal cases were among preterm neonates. The case fatality rate among preterm infants was 22% (7/32), which agrees with other reports that the mortality is around 20-30 % in preterm infants and 1-3% in term infants (72, 229, 230). None of the 46 LO GBS infections was fatal, but half of the cases were infants born prematurely (22/44, two had missing data on gestational age). This agrees with other studies showing that preterm infants are more susceptible to LO GBS infection, and the rise in survival of preterm infants might explain the increase in incidence (72, 118, 163).

EARLY-ONSET INVASIVE INFECTIONS

Results from paper II showed that the incidence of EO infections significantly declined from 1.4 in 1997-2007 to 0.9 per 1000 live births in 2008-2017. Studies on EO sepsis (<72 hours) from the US have shown a similar incidence; A study from the San Francisco Bay area in California, representing ~ 5% of US live births between 2005 and 2014, reported an incidence of approximately 0.8 per 1000 live births (83). In a study from Stoll et al. covering the years 2015-2017 (12), the incidence was 1.08 per 1000 live births, and *E. coli* had succeeded GBS and was the most frequent pathogen in 37% of the cases. A report from the German Neonatal Network on VLBW infants *E. coli* was the most common pathogen in 35% of EO cases, and surprisingly CoNS was described as a pathogen in 24% of cases and GBS in 16% (231). Bizzarro et al. also reported *E. coli* as the most common cause of invasive EO-sepsis between 2004 and 2013 (19). A reason for *E. coli*

being more frequent than GBS might be that IAP has had more impact on reducing EO GBS infections among term neonates and *E. coli* infections are more common among preterm neonates (75, 83, 232). Other reports show the same as ours that GBS is still more common than *E. coli* (83, 233, 234). A Norwegian study found that *E. coli* was more common than GBS among neonates born extremely preterm, but for all neonates, GBS was the most common (235).

Our study on EO infections showed that most EO infections, including GBS, were within the first 48 h of life. No infection due to GBS within the first week of life occurred after 72 hours so we chose to have the LO cut-off point after 72 hours, even for GBS.

INTRAPARTUM ANTIBIOTIC PROPHYLAXIS

As previously mentioned, IAP based on screening seems to impact EO GBS infections more than IAP based on risk factors. The incidence of EO GBS at 0.45 per 1000 live births in our study was higher than in studies where screening has been implemented (12, 83).

Universal screening for GBS in pregnant women is performed in the United States, Canada, and many European countries like France, Italy, Germany, Spain, and Belgium (143, 236, 237). However, the United Kingdom, Ireland, the Netherlands, and all Nordic countries – except for Finland that screens for GBS with PCR at time of delivery (238) - still recommend risk-based IAP (153, 239-241).

Seedat et al. from the United Kingdom argued in a study published in 2019 that universal screening cannot be recommended as it may cause more harm than good (239). They pointed out that maternal culture does not accurately predict EO GBS in the neonate and that 99.8% of cases with positive screening would get unnecessary IAP. Excessive IAP may have adverse effects, like possible maternal anaphylaxis and an effect on the neonatal microbiota. The risk of maternal anaphylaxis and adverse effect of possible unnecessary IAP exists as well for risk-based strategy. Mothers may benefit from IAP as GBS colonized women who

receive IAP have a lower risk of chorioamnionitis and wound infection than non-colonized parturients (124).

A major argument for not starting screening is that more women would get unnecessary IAP. In Sweden, that would mean that 30% of pregnant women would receive IAP instead of 20% with one or more risk factors (112). However, since neonates born to non-colonized women with one risk factor seldom get EO GBS disease, the risk for unnecessary IAP is high (20). A retrospective study on introducing a <2 hours GBS PCR test on intrapartum mothers with risk factors and only offering IAP to the women with a positive test has shown to reduce the use of antibiotics by 40% without seeing an increase of EO GBS infections (242).

The quick real-time PCR assays for GBS are promising with high sensitivity and specificity (243). They provide information on GBS colonization status when needed (not several weeks prior) but are more expensive than IAP (244).

A systematic review and meta-analysis from 2020 by Hasperhoven et al. reported that IAP based on screening had a more significant impact on reducing EO GBS compared to risk-based strategy (relative risk (RR) 0.43 (95% CI 0.32-0.56)) (153). There was no significant reduction when comparing risk-based strategy vs. no strategy at all (RR 0.86 (95% CI 0.61-1.2)). No randomized controlled study was included in the review.

There was not a significant reduction after the implementation of risk-based IAP in our study. However, a significant bias in our and other observational studies is the timing of when IAP started. ACOG recommended IAP to prevent GBS disease in 1996 (21). Thus, there is a possibility that mothers with one or several risk factors received IAP from the attending physician before the publication of the Swedish guidelines in 2008 (141). In our unit, the Swedish guidelines were not formally implemented until 2011.

A randomized multi-center trial aiming to compare screening with the United Kingdom's current risk-based approach is ongoing. At least 320 000 women from 80 hospitals around the UK will be included in the GBS3 trial, https://www.gbs3trial.ac.uk/home.aspx (accessed 17th of May 2021). The trial will also compare screening with the quick real-time PCR assays for GBS. We wait with anticipation on the first results from this randomized trial which will hopefully provide additional guidelines in the debate whether universal screening for GBS saves more lives, and if so, what kind of screening would be most cost-effective.

IAP is missed in both strategies, and most of the neonates with EO GBS infection were born to GBS colonized mothers (21). The essential in this discussion regarding screening or risk-based strategy has to be that IAP should be given to the ones that benefit it the most.

LATE-ONSET INVASIVE INFECTIONS

The study in this thesis includes infants with LO-infections from an out-of-hospital setting as well as VLBW infants within a NICU since it is population-based and includes all infants at the age of 3-120 days.

The etiology for LO infections differs according to settings. In our study, Gram-negative bacteria were the most common pathogens cultivated from infants in an out-of-hospital setting. Within the neonatal department, the majority of infections were either due to *S. aureus* or CoNS.

The physical environment, health care workers, and parents may be reservoirs for *S. aureus* transmission within a NICU and after methicillin-resistant *S. aureus* (MRSA) emerged and spread across the globe in the 1990s, infection control policies like surveillance, isolation procedures, and decolonization strategies have been developed in an attempt to contain its spread (245). However, neonates are vulnerable due to the establishment of their microbiota, and routine chlorhexidine bathing is contraindicated due to the potential neurotoxicity from hexachlorophene (246). Studies have shown that transmission decreases by decolonizing parents and healthcare workers (247, 248). However, eradication seems challenging. Despite aggressive measures, transmission and subsequent infections still occur both for MRSA and methicillin-sensitive *S. aureus* (MSSA), and screening for both is becoming increasingly important within the NICU (245, 249).

Preterm neonates are at increased risk of LO infections because of multiple risk factors directly linked to each other. Due to maternal or fetal distress, the birth choice is often cesarean section, which affects the neonatal microbiota, leading to dysbiosis and a reservoir for LO sepsis with pathogens transmitting across the epithelial barrier (101, 250). Since the neonates have low birth weight and might even be growth restricted, they need parenteral nutrition through intravascular lines increasing the risk for infections due to staphylococci spp. Episodes with inflammation where sepsis cannot be ruled out lead to

antibiotic treatments affecting the establishment of the microbiota further. Not to mention the immature immune system of the preterm neonates to battle these risk factors.

Other studies agree with our finding that the incidence of LO infections has increased and a likely reason for this is that prematurity is a major risk factor for LO infection and the preterm neonates have increased survival rates.

We know that neonates and especially the ones born most preterm and VLBW infants with culture-proven invasive infection have an increased risk for various complications and diseases like retinopathy of prematurity, patent ductus arteriosus, necrotizing enterocolitis (NEC), IVH, bronchopulmonary dysplasia (BPD), and death (14, 17, 231, 251, 252). This is why caregivers often have a low threshold for treatment for possible infection in neonates and infants, leading to overtreatment with antibiotics and many cases of "culture-negative sepsis".

"CULTURE-NEGATIVE" INFECTIONS

Decreased incidence of EO GBS after IAP may be due to low likelihood of positive culture because of maternal antibiotics since most studies only include infections confirmed by culture. Seedat et al. speculate that this could explain the reduction in incidence between screening and risk-based IAP strategy without affecting the true incidence since neonatal sepsis mortality is no different (239). This speculation would mean that the incidence of culture-negative sepsis has increased after IAP implementation and that the incidence would be higher in the settings where screening is preferred over a risk-based strategy.

Studies have shown that empirical antibiotic treatment for very preterm infants is associated with increased rates of NEC, BPD, and death (216, 253-257). However, the sickest infants get the most antibiotics and have the highest risk for complications.

Even shorter periods (less than three days) with antibiotics affect the microbiota with decreased levels of intestinal bifidobacteria and this imbalance in gut microbiota, so-called dysbiosis, which may modulate vascular development and reduce immunity (258-260). Association has also been shown between antibiotics in infancy and health problems as asthma and obesity later in childhood (261, 262). Mode of delivery, type of feeding, and gestational age affect the microbiota as well (258).

The mothers of neonates with EO infection have a high rate of chorioamnionitis, but only a small portion of the neonates of mothers with chorioamnionitis develop an infection. A review estimated that around 450 neonates exposed to chorioamnionitis would have to be treated per case of confirmed EO-sepsis (85). Serial physical examinations within the first three days of life in term neonates with suspected EOS or exposed to maternal chorioamnionitis have been shown to reduce the burden of antibiotic exposure without delay of treatment or change in the outcome of infected neonates (263).

Inflammatory markers as CRP, PCT, and IL-6 increase in response to infection and other inflammatory stimuli as asphyxia, pneumothorax, and naturally after birth (47, 264). Invasive infection is unlikely if these values are consistently normal, but serial abnormal values should not be used to extend the antibiotic therapy in the absence of a positive culture (39). A German study from 1997 described that it was safe to stop antibiotics when CRP was <10 mg/L 24-48 hours after starting antibiotics for EO-infections within the first week of life (265). Infants with elevated CRP were called "probably infected," and antibiotic treatment continued until CRP normalized or completed at least five days of treatment. This study stated that this would shorten the days on antibiotics and became quite influential despite not reporting any clinical features. A randomized controlled intervention study performed in 18 hospitals in four countries reported that PCT-guided decision making was superior to standard care in reducing the antibiotic therapy in EO-sepsis <72 hours of age (266). In the study, discontinuation of antibiotic treatment was not recommended despite negative cultivation

if the following three factors existed; risk factors, clinical signs, and abnormal routine laboratory values, which meant at least seven days of antibiotics. The intervention was only in the group where infection was possible (2 of 3) or unlikely (<2) then antibiotic therapy could be stopped after at least 24 hours if two consecutive PCT values were within range (taken after 12 h, 24 h, 36 h and repeated every 24-48 h >72 h). Cessation of antibiotics for EOS guided by CRP and clinical symptoms has also been shown to reduce the duration of antibiotics (267). However, other studies have shown that it is safe to stop antibiotics after 48-72 hours if blood cultures are negative in a clinically well neonate without considering biomarkers (268, 269).

Three days of antibiotics – which is often practiced in the wait for a negative culture - might be enough in those cases when the bacterial load is so low that it is not captured in a blood culture. This can probably be shortened further to 36-48 hours of treatment if the neonate has improved since most cultures are positive within this time, thereby shortening the days with antibiotics further (270). Saturation screening has increased the detection of sepsis and could be of benefit if included in the Neonatal EO-Sepsis calculator along with other clinical presentation and possible risk factors (55, 271).

Culture-negative sepsis is impossible to compare between studies in lack of a proper definition of neonatal sepsis. The challenge with research on neonatal sepsis is multivariate; the gold standard of a positive culture does not mean that there is an infection, and the lack of a pathogen from a culture does not rule out an infection either.

The studies this thesis is based upon do not include suspected infections with negative cultures, but culture-negative sepsis is not an uncommon diagnosis in neonatal practice. Venipuncture of an infant can be demanding. You need to capture the pathogen in a small amount of blood, and the sensitivity of blood cultures is linked to the amount inoculated. Schelonka et al. found that as many as 60% would be falsely negative if only 0.5 ml would be sampled in low-colony-count sepsis, and obtaining 1-2 ml of blood for cultivation would improve the blood

culture yield in neonates (40). Another study by Kellogg et al. found that low-level bacteremia was common in neonates and infants up to 2 months of age and that up to 6 ml of blood (up to 4.5% of an infant's total blood volume!) was required for the detection of pathogens (272). However, quantitative blood culture studies have shown that neonates have high levels of bacteremia (273). The level of CoNS has shown correlations with whether it is a clinically significant infection or not as low-level bacteremia is often, but not always, clinically assessed as contaminants (99, 274-276). It is in the guidelines to achieve at least 1 ml of blood for culture at our neonatal unit, but we did not control or measure the amount of blood inoculated in our studies.

Viral infections and especially respiratory viruses were detected in 4% of suspected LO infections (277). In the same study, 11% of suspected LO-sepsis had blood culture-proven bacterial infection leaving 85% of suspected LO-sepsis at risk for evaluation as "culture-negative infection". The biggest problem with culture-negative sepsis is that studies have shown that more antibiotics are used (as much as 10-fold) when the culture is negative compared to sepsis verified by culture (13, 278). Culture-negative sepsis is most likely not sepsis. However, it might still be an infection, but without bacteremia such as pneumonia. Hence, it is essential to look for other signs of infection and evaluate the infant's clinical status before discontinuing the antibiotic treatment.

In our studies, we did not differ between the kind of central catheter in use at the time of infection. Studies have not found any significant differences in the incidence of nosocomial infections regarding the central catheter type. However, studies have shown that the longer they are in use, the increased risk of LO-sepsis (279-282).

Studies that have had success in decreasing the incidence of LO-infections have mostly been done by quality improvements with "bundles of care". Therefore, it is harder to pinpoint precisely what improvements make the difference (19, 283-286). Perhaps it is this kind of "bundles" that make the difference – "a chain is no stronger than its weakest link" (Thomas Reid 1786)…

GROUP B STREPTOCOCCI

The main aim of paper I was to survey the serotype distribution of invasive GBS infections and compare the results with previous studies to detect any changes over time. The first case of serotype V in our region appeared in 1993 in the study of Berg et al. and became the most common serotype among adults (42%, 47/111) in the study of Persson et al. Since then; there have not been any significant changes in the serotype distribution of invasive GBS infections within our study population. We serotyped fourteen isolates as serotype VII-IX in our study, and none was non-typeable. There were five non-typeable isolates in the previous studies, and none was serotyped above V (164, 167). Improved methods on serotyping with the GBS latex test published in 2003 (210) and the identification of serotype IX in 2007 (161) might explain the emergence of isolates VII-IX and that none of the isolates in our study was non-typeable.

In a study from the United States, serotypes Ib, II, and IV accounted for 75% of the increase in incidence between 2008 and 2016 (119). It has been speculated that these serotypes, along with an increase in the prevalence of underlying diseases as obesity and diabetes, might explain the increasing incidence of invasive GBS infections among non-pregnant adults (119, 122). We cannot speculate on changes in incidence since our study was not population-based. However, we did not observe a higher prevalence of these serotypes among adults with an invasive GBS infection than in previous studies (164, 167).

VIRULENCE OF GBS

One of the study's strengths in paper IV includes the repository of >200 GBS invasive isolates with corresponding clinical outcomes. The study provides the ability to test many virulence factors that would easily be replicable with standard laboratory techniques. We showed that the invasive isolates were more pigmented compared to the commensal isolates. We need to verify these results with quantitative hemolytic titer assays. Studies have demonstrated that the hemolytic pigment has a role in colonization, fetal damage (179, 181), and various clinical manifestations (176, 287) and that it is vital for the survival of GBS in the human host. This makes the pigment an exciting target for testing clinical relevance for IAP and a target for future vaccine development.

The CAMP factor is not considered an essential virulence factor in humans, and the in vivo significance of hemolysis only when red blood cells have been pre-sensitized to *staphylococcus* hemolysin is unknown (171). It is, however, thought to promote intracellular survival and systemic dissemination into host cells (170), and in our study, the mean CAMP activity was higher among the invasive isolates compared to the commensals

The majority or 62% (52/84) of EO GBS cases occurred within 24 hours, which agrees with other studies that most EO GBS cases occur in utero by ascending GBS (83, 115, 288). It is known that cervical viral infection (289) and lower cervical hyaluronic acid levels (188) predispose to ascending bacterial infection, and studies suggest that hyaluronidase plays a vital role in ascending GBS infection. Both by reducing anti-bacterial inflammation in uterine tissue as well as by dissemination and immune evasion (191, 192, 290). GBS with high hyaluronidase enzyme activity appears to be able to disseminate into deeper tissues such as fetal tissues that may result in IUFD or preterm birth (192).

In our study, the immune suppression achieved by increased hyaluronidase might promote virulence since known invasive strains

had higher levels of hyaluronidase enzyme activity than commensal strains. Further studies need to clarify its clinical relevance. If this would be the case, it might be of great benefit to evaluate HylB activity to identify the strains at high risk for invasive infection.

It is not only the infection itself that may be damaging. The host inflammatory response causes a big part of the CNS injury in cases of GBS meningitis. A study on mice showed that the pigment beta-hemolysin caused a part of the neuronal damage in GBS meningitis by neutrophil recruitment with chemokines and cerebral blood flow disturbances, and this pigment is believed to be crucial to GBS manifestation in the CNS (174).

Colonization with GBS can be transient (291), and its density can vary (292). Around 18-20% of pregnant women are transiently colonized with GBS with regional variations from 11% in Asia to 35% in African countries (112-114, 124). The variable colonization rates may be due to means of diagnosing GBS, cultivation vs. PCR-based methods but a systematic review and meta-analysis showed that the prevalence of colonized women and the serotype distribution vary, even after adjusting for laboratory methods (114). Previous GBS colonization, obesity, diabetes, and tobacco use are known risk factors for colonization (8, 116, 117). Studies have shown that relatively few clonal types cause a significant portion of invasive infection in humans (293, 294). Identification of these strains might lead to better prevention.

Since GBS colonization varies globally, relevant prevention efforts should be applied accordingly, as interventions need to have minimal detrimental effects on the microbiome and a low number needed to treat. The screening program likely misses a significant portion of colonization during pregnancy as ascending infection may occur at any time during pregnancy leading potentially to preterm birth or IUFD. IAP does not help in these situations nor with LO infections.

A GBS vaccine has been on the horizon for many years and has proven to be challenging. GBS vaccine has not had the shortcut as covid-19–

vaccines, and there is a reluctance to include pregnant women in vaccination research due to fear of large lawsuits in case of adverse events and since invasive GBS disease is that uncommon massive trials are needed to demonstrate efficacy (295). Anti-vax movements with a reluctance or refusal to be vaccinated are a growing problem worldwide. The World Health Organization (WHO) lists vaccine hesitancy as one of the top 10 risks to global public health (https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019, accessed 6th of April 2021). So challenges will exist even after a successful implementation of a vaccine.

LIMITATIONS

Some of the limitations of the studies have already been discussed to some extent in this thesis. One of the most important limitations is the observational design of the studies in papers II and III, making it impossible to conclude cause and effect. We can only speculate on possible causality regarding significant changes. In paper I, we compared the distribution of serotypes with previous studies, and we compared the last ten years with 11 years prior and previous studies in papers II and III. These historical controls lead to bias due to healthcare- and methodological improvements over time.

We did not define neonatal sepsis as occurring only within the first 28 days of life, as is the classical definition, which may affect the comparison with other studies. The reason for this is that we wanted to include the time of care that infants born extremely preterm have within a neonatal unit. For example, an infant born at 23 weeks of gestation is not ready for discharge four weeks later and may still need interventions at 27 weeks of gestational age, putting the infant at risk for infections. That is why we assess that later onset of infections should be added to the total burden of neonatal infections. However, since we did a population-based study including infants up to 120 days of age, we included healthy neonates with a community-acquired infection later on in life and infants with a nosocomial infection within a NICU. This heterogeneity limits the study's comparability in paper III with other studies that include only LO cases within a NICU.

Unfortunately, the commensal isolates used for control in paper IV were neither contemporary nor obtained from the same country as the invasive isolates. Since screening is not performed in Sweden, the controls were obtained from a universal screening of pregnant women in Washington State. The unequal sample size might have influenced the difference in the outcome of virulence factors, and some commensal strains were possibly invasive that could have led to an invasive infection. We had no information on the control group's

clinical outcome since the isolates were obtained anonymously, and if we had, the outcome would possibly be affected by IAP.

Finally, all studies in this thesis include invasive isolates collected in western Sweden, affecting the studies' generalizability.

SIGNIFICANCE AND CLINICAL RELEVANCE

This research project gives information on changes in incidence, etiology, risk factors, and short-time prognosis on invasive infections among neonates over a long follow-up period of 43 years. This is important for the initial treatment and prevention of neonatal infections (Paper II and III).

The best possible GBS vaccines must be directed against the polysaccharides of the most common GBS serotypes. Since changes occur over time, it is essential to have ongoing surveillance and consider possible changes when GBS vaccines are formulated (Paper I).

The collaboration with the research team at the University of Washington gives us the opportunity to compare the virulence factors of over 230 invasive GBS strains causing infection among neonates and postpartum women with commensal wild-type isolates. One of the study's strengths in paper IV includes the repository of >200 GBS invasive isolates with corresponding clinical outcomes. The study provides the ability to test virulence factors that would easily be replicable with standard laboratory techniques. We showed that the invasive isolates were more pigmented compared to the commensal isolates. Studies have demonstrated that the hemolytic pigment has a role in colonization, fetal damage (179, 181), and various clinical manifestations (176, 287) and that it is vital for the survival of GBS in the human host. This makes the pigment an exciting target for testing clinical relevance for IAP and a target for future vaccine development (296). Comparing the virulence factors between invasive and commensal isolates gives us information and a foundation on which

virulence factors to focus on in future research. Future research might elucidate GBS infection's pathogenesis and increase the diagnostic sensitivity to predict preterm delivery, neonatal GBS sepsis, intrauterine fetal death, and other pregnancy and child outcomes (Paper IV).

CONCLUSIONS

I. The serotype distribution remained unchanged between 2004 and 2009 compared to previous studies. This is promising regarding GBS vaccination.

II. The incidence of EO invasive infections had continued to decline the last ten years compared to 1997-2007, but the case fatality rate remained unchanged during 1997-2017. The case fatality rate was lower when compared to previous studies. There was no difference in EO GBS whether the definition of EO would be <3 days or <1 week of life since all EO GBS cases occurred within 72 hours.

III. The incidence of LO neonatal invasive infections increased in 2008-2017 compared to 1997-2007, especially among the extremely preterm neonates, but the case fatality rate remained unchanged. When comparing the whole 43-year period <28 days of life, the incidence and the case fatality rate had decreased.

IV. GBS isolates from pregnant or postpartum women and neonates with invasive GBS disease had significantly greater hemolytic potential and hyaluronidase activity than commensal isolates.

FUTURE PERSPECTIVES

Research is needed to identify neonates at risk for infection without overtreatment with antibiotics.

We need to abolish culture-negative sepsis. Use other microbiological identification measures like PCR to identify both bacterial and viral pathogens more quickly. If they are negative, the focus should be on non-infectious reasons for clinical symptoms and minimize the use of antibiotics as much as possible if not necessary.

There is a need for a validated consensus definition of neonatal sepsis to compare different studies and improve neonatal infection research.

Hopefully, we will get a GBS vaccine for pregnant women in the near future, but in the meantime, we should provide IAP to those that benefit from it the most. Better diagnostic tools to determine if a woman is colonized with a virulent GBS strain or not could help identify who should benefit from IAP. Thereby decreasing and pinpointing the number needed to treat to prevent neonatal disease.

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REFERENCES

REFERENCES

1. Gould IM. Alexander Gordon, puerperal sepsis, and modern theories of infection control--Semmelweis in perspective. Lancet Infect Dis. 2010;10(4):275-8.

2. Kadar N. Rediscovering Ignaz Philipp Semmelweis (1818-1865). Am J Obstet Gynecol. 2019;220(1):26-39.

3. Britannica, The Editors of Encyclopaedia. "Puerperal fever". Encyclopedia Britannica, 28 Nov. 2017. Available from: https://www.britannica.com/science/puerperal-fever. Accessed 19 April 2021.

4. Fellman V, Hellström-Westas L, Norman M, Westgren M, Källén K, Lagercrantz H, et al. One-year survival of extremely preterm infants after active perinatal care in Sweden. JAMA. 2009;301(21):2225-33.

5. Tessin I, Trollfors B, Thiringer K. Incidence and etiology of neonatal septicaemia and meningitis in western Sweden 1975-1986. Acta Paediatr Scand. 1990;79(11):1023.

6. Gladstone IM, Ehrenkranz RA, Edberg SC, Baltimore RS. A ten-year review of neonatal sepsis and comparison with the previous fifty-year experience. Pediatr Infect Dis J. 1990;9(11):819-25.

7. Persson E, Trollfors B, Brandberg LL, Tessin I. Septicaemia and meningitis in neonates and during early infancy in the Göteborg area of Sweden. Acta Paediatr Scand. 2002;91(10):1087.

8. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014;345(6198):760.

9. Simonsen KA, Anderson-Berry AL, Delair SF, Davies HD. Early-Onset Neonatal Sepsis. Clin Microbiol Rev. 2014;27(1):21.

10. Wynn JL, Hansen NI, Das A, Cotten CM, Goldberg RN, Sánchez PJ, et al. Early Sepsis Does Not Increase the Risk of Late Sepsis in Very Low Birth Weight Neonates. J Pediatr. 2013;162(5):942-8.

11. Bizzarro MJ, Sabo B, Noonan M, Bonfiglio M-P, Northrup V, Diefenbach K. A Quality Improvement Initiative to Reduce Central Line–Associated Bloodstream Infections in a Neonatal Intensive Care Unit. Infect Control Hosp Epidemiol. 2010;31(3):241-8.

12. Stoll BJ, Puopolo KM, Hansen NI, Sánchez PJ, Bell EF, Carlo WA, et al. Early-Onset Neonatal Sepsis 2015 to 2017, the Rise of Escherichia coli, and the Need for Novel Prevention Strategies. JAMA pediatr. 2020;174(7).

13. Fjalstad WJ, Stensvold JH, Bergseng SH, Simonsen EG, Salvesen EB, Rønnestad EA, et al. Early-onset Sepsis and Antibiotic Exposure in Term Infants: A Nationwide Population-based Study in Norway. Pediatr Infect Dis J. 2016;35(1):1-6.

14. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002;110(2):285.

15. Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. Lancet. 2017;390(10104):1770-80.

16. Schuchat A. Neonatal Group B Streptococcal Disease — Screening and Prevention. N Engl J Med. 2000;343(3):209-10.

17. Hornik CP, Fort P, Clark RH, Watt K, Benjamin DK, Smith PB, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early Hum Dev. 2012;88:S69-S74.

18. Bauserman MS, Laughon MM, Hornik CP, Smith PB, Benjamin Jr DK, Clark RH, et al. Group B Streptococcus and escherichia coli infections in the intensive care nursery in the era of intrapartum antibiotic prophylaxis. Pediatr Infect Dis J. 2013;32(3):208-12.

19. Bizzarro MJ, Shabanova V, Baltimore RS, Dembry L-M, Ehrenkranz RA, Gallagher PG. Neonatal Sepsis 2004-2013: The Rise and Fall of Coagulase-Negative Staphylococci. J Pediatr. 2015;166(5):1193-9.

20. Boyer KM, Gotoff SP. Strategies for chemoprophylaxis of GBS early-onset infections. Antibiot Chemother. 1985;35:267-80.

21. Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease-revised guidelines from CDC, 2010. MMWR Recomm Rep. 2010;59(RR-10):1-36.

22. Marodi L. Neonatal Innate Immunity to Infectious Agents. Infect Immun. 2006;74(4):1999.

23. Singh M AM, Gray CP. Neonatal Sepsis Treasure Island (FL): StatPearls Publishing; Updated 2020 Sep 4. Available from: https://www.ncbi.nlm.nih.gov/books/NBK531478/. Accessed 15 April 2021.

24. McGovern M, Giannoni E, Kuester H, Turner MA, van Den Hoogen A, Bliss JM, et al. Challenges in developing a consensus definition of neonatal sepsis. Pediatr Res. 2020;88(1):14.

25. Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N. The global burden of paediatric and neonatal sepsis: a systematic review. Lancet Respir Med. 2018;6(3):223-30.

26. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: Definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med. 2005;6(1):2-8.

27. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G; SCCM/ESICM/ACCP/ATS/SIS. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med. 2003 Apr;31(4):1250-6.

28. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-10.

29. Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):762-74.

30. Vincent LJ-L, Sakr MY, Sprung MC, Ranieri MV, Reinhart MK, Gerlach MH, et al. Sepsis in European intensive care units: Results of the SOAP study. Crit Care Med. 2006;34(2):344-53.

31. Matics TJ, Sanchez-Pinto LN. Adaptation and Validation of a Pediatric Sequential Organ Failure Assessment Score and Evaluation of the Sepsis-3 Definitions in Critically Ill Children. JAMA pediatr. 2017;171(10).

32. Wynn JL, Polin RA. Progress in the management of neonatal sepsis: The importance of a consensus definition. Pediatr Res. 2018;83(1):13-5.

33. Wynn J, Polin R. A neonatal sequential organ failure assessment score predicts mortality to late-onset sepsis in preterm very low birth weight infants. Pediatr Res. 2020;88(1):85-90.

34. James LW, Richard AP. Progress in the management of neonatal sepsis: the importance of a consensus definition. Pediatr Res. 2017;83(1-1).

35. Del Vecchio A. Evaluation and management of thrombocytopenic neonates in the intensive care unit. Early Hum Dev. 2014;90:S51-S5.

36. Hornik PC, Benjamin KD, Becker CK, Li HJ, Clark BR, Cohen-Wolkowiez BM, et al. Use of the Complete Blood Cell Count in Late-onset Neonatal Sepsis. Pediatr Infect Dis J. 2012;31(8):803-7.

37. Klingenberg C, Kornelisse RF, Buonocore G, Maier RF, Stocker M. Culture-negative early-onset neonatal sepsis - at the crossroad

between efficient sepsis care and antimicrobial stewardship. Front Pediatr. 2018;6.

38. Newman TB, Puopolo KM, Wi S, Draper D, Escobar GJ. Interpreting complete blood counts soon after birth in newborns at risk for sepsis. Pediatrics. 2010;126(5):903.

39. Puopolo KM, Benitz WE, Zaoutis TE. Management of Neonates Born at ≤34 6/7 Weeks' Gestation With Suspected or Proven Early-Onset Bacterial Sepsis. Pediatrics. 2018;142(6).

40. Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP. Volume of blood required to detect common neonatal pathogens. J Pediatr. 1996;129(2):275-8.

41. Sharma D, Farahbakhsh N, Shastri S, Sharma P. Biomarkers for diagnosis of neonatal sepsis: a literature review. J Matern Fetal Neonatal Med. 2018;31(12):1646-59.

42. Slaats J, Ten Oever J, van de Veerdonk FL, Netea MG. IL-1β/IL-6/CRP and IL-18/ferritin: Distinct Inflammatory Programs in Infections. PLoS Pathog. 2016;12(12).

43. Eschborn S, Weitkamp JH. Procalcitonin versus C-reactive protein: review of kinetics and performance for diagnosis of neonatal sepsis. J Perinatol. 2019;39(7):893-903.

44. Yu Z, Liu J, Sun Q, Qiu Y, Han S, Guo X. The accuracy of the procalcitonin test for the diagnosis of neonatal sepsis: a meta-analysis. Scand J Infect Dis. 2010;42(10):723-33.

45. Liu Y, Zhao L, Wu Z. Accuracy of C-Reactive Protein Test for Neonatal Septicemia: A Diagnostic Meta-Analysis. Med Sci Monit. 2019;25:4076-81.

46. Assumma M, Signore F, Pacifico L, Rossi N, Osborn JF, Chiesa C. Serum procalcitonin concentrations in term delivering mothers and their healthy offspring: a longitudinal study. Clin Chem. 2000;46(10):1583-7.

47. Chiesa C, Panero A, Rossi N, Stegagno M, De Giusti M, Osborn JF, et al. Reliability of Procalcitonin Concentrations for the Diagnosis of Sepsis in Critically Ill Neonates. Clin Infect Dis. 1998;26(3):664-72.

48. Ng PC, Cheng SH, Chui KM, Fok TF, Wong MY, Wong W, et al. Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecule, and C-reactive protein in preterm very low birthweight infants. Arch Dis Child Fetal Neonatal Ed. 1997;77(3).

49. Silveira RC, Procianoy RS. Evaluation of interleukin-6, tumour necrosis factor-alpha and interleukin-1beta for early diagnosis of neonatal sepsis. Acta Paediatr Scand.1999;88(6):647-50.

50. Arnon S, Litmanovitz I, Regev R, Bauer S, Lis M, Shainkin-Kestenbaum R, et al. Serum amyloid A protein is a useful inflammatory marker during late-onset sepsis in preterm infants. Biol Neonate. 2005;87(2):105-10.

51. Arnon S, Litmanovitz I, Regev RH, Bauer S, Shainkin-Kestenbaum R, Dolfin T. Serum amyloid A: an early and accurate marker of neonatal early-onset sepsis. J Perinatol. 2007;27(5):297-302.

52. Berner R, Fürll B, Stelter F, Dröse J, Müller HP, Schütt C. Elevated levels of lipopolysaccharide-binding protein and soluble CD14 in plasma in neonatal early-onset sepsis. Clin Diagn Lab Immunol. 2002;9(2):440-5.

53. Sinha M, Jupe J, Mack H, Coleman TP, Lawrence SM, Fraley SI. Emerging Technologies for Molecular Diagnosis of Sepsis. Clin Microbiol Rev. 2018;31(2).

54. Puopolo KM, Draper D, Wi S, Newman TB, Zupancic J, Lieberman E, et al. Estimating the Probability of Neonatal Early-Onset Infection on the Basis of Maternal Risk Factors. Pediatrics. 2011;128(5).

55. Escobar GJ, Puopolo KM, Wi S, Turk BJ, Kuzniewicz MW, Walsh EM, et al. Stratification of Risk of Early-Onset Sepsis in Newborns ≥34 Weeks' Gestation. Pediatrics. 2014;133(1):30-6.

56. Puopolo KM, Benitz WE, Zaoutis TE. Management of Neonates Born at ≥35 0/7 Weeks' Gestation With Suspected or Proven Early-Onset Bacterial Sepsis. Pediatrics. 2018;142(6).

57. Strunk T, Buchiboyina A, Sharp M, Nathan E, Doherty D, Patole S. Implementation of the Neonatal Sepsis Calculator in an Australian Tertiary Perinatal Centre. Neonatology. 2018;113(4):379-82.

58. Achten NB, Dorigo-Zetsma JW, Van Der Linden PD, Van Brakel M, Plötz FB. Sepsis calculator implementation reduces empiric antibiotics for suspected early-onset sepsis. Eur J Pediatr. 2018;177(5):741-6.

59. Achten NB, Klingenberg C, Benitz WE, Stocker M, Schlapbach LJ, Giannoni E, et al. Association of Use of the Neonatal Early-Onset Sepsis Calculator With Reduction in Antibiotic Therapy and Safety. JAMA pediatr. 2019;173(11):1032.

60. Chow S. S..W CP, Chambers G.M. and Lui K. Report of the Australian and New Zealand Neonatal Network 2018 Sydney: ANZNN; 2020. Available from: https://anznn.net/Portals/0/AnnualReports/Report%20of%20the%20Australian%20and%20New%20Zealand%20Neonatal%20Network%202018.pdf. Accessed 12 March 2021.

61. Vergnano S, Menson E, Kennea N, Embleton N, Russell AB, Watts T, et al. Neonatal infections in England: the NeonIN surveillance network. Arch Dis Child Fetal Neonatal Ed. 2011;96(1).

62. Oeser C, Vergnano S, Naidoo R, Anthony M, Chang J, Chow P, et al. Neonatal invasive fungal infection in England 2004–2010. Clin Microbiol Infect. 2014;20(9):936-41.

63. Stoll JB, Hansen IN, Higgins DR, Fanaroff AA, Duara AS, Goldberg AR, et al. Very Low Birth Weight Preterm Infants With Early Onset Neonatal Sepsis: The Predominance of Gram-Negative Infections Continues in the National Institute of Child Health and Human Development Neonatal Research Network, 2002–2003. Pediatr Infect Dis J. 2005;24(7):635-9.

64. Fluegge K, Siedler A, Heinrich B, Schulte-Moenting J, Moennig M-J, Bartels DB, et al. Incidence and clinical presentation of invasive neonatal group B streptococcal infections in Germany. Pediatrics. 2006;117(6).

65. Berardi A, Rossi C, Lugli L, Creti R, Bacchi Reggiani ML, Lanari M, et al. Group B streptococcus late-onset disease: 2003-2010. Pediatrics. 2013;131(2).

66. Weston JE, Pondo MT, Lewis IM, Martell-Cleary JP, Morin LC, Jewell JB, et al. The Burden of Invasive Early-onset Neonatal Sepsis in the United States, 2005–2008. Pediatr Infect Dis J. 2011;30(11):937-41.

67. Thigpen MC, Whitney CG, Messonnier NE, Zell ER, Lynfield R, Hadler JL, et al. Bacterial Meningitis in the United States, 1998–2007. N Engl J Med. 2011;364(21):2016-25.

68. Okike IO, Johnson AP, Henderson KL, Blackburn RM, Muller-Pebody B, Ladhani SN, et al. Incidence, Etiology, and Outcome of Bacterial Meningitis in Infants Aged <90 Days in the United Kingdom and Republic of Ireland: Prospective, Enhanced, National Population-Based Surveillance. Clin Infect Dis. 2014;59(10).

69. Romain A-S, Cohen R, Plainvert C, Joubrel C, Béchet S, Perret A, et al. Clinical and Laboratory Features of Group B Streptococcus Meningitis in Infants and Newborns: Study of 848 Cases in France, 2001–2014. Clin Infect Dis. 2018;66(6):857-64.

70. Phares CR, Lynfield R, Farley MM, Mohle-Boetani J, Harrison LH, Petit S, et al. Epidemiology of Invasive Group B Streptococcal Disease in the United States, 1999-2005. JAMA. 2008;299(17):2056-65.

71. Gaschignard J, Levy C, Romain O, Cohen R, Bingen E, Aujard Y, et al. Neonatal Bacterial Meningitis: 444 Cases in 7 Years. Pediatr Infect Dis J. 2011;30(3):212-7.

72. Nanduri S, Petit S, Smelser C, et al. Epidemiology of invasive early-onset and late-onset group b streptococcal disease in the united states, 2006 to 2015: Multistate laboratory and population-based surveillance. JAMA pediatr. 2019. Mar 1;173(3):224-233.

73. Jordan HT, Farley MM, Craig A, Mohle-Boetani J, Harrison LH, Petit S, et al. Revisiting the need for vaccine prevention of late-onset neonatal group B streptococcal disease: a multistate, population-based analysis. Pediatr Infect Dis J. 2008;27(12):1057-64.

74. Basmaci R, Bonacorsi S, Bidet P, Biran V, Aujard Y, Bingen E, et al. Escherichia ColiMeningitis Features in 325 Children From 2001 to 2013 in France. Clin Infect Dis. 2015;61(5):779-86.

75. Stoll BJ, Hansen NI, Sánchez PJ, Faix RG, Poindexter BB, Van Meurs KP, et al. Early onset neonatal sepsis: the burden of group B Streptococcal and E. coli disease continues. Pediatrics. 2011;127(5):817.

76. El-Naggar W, Afifi J, McMillan D, Toye J, Ting J, Yoon EW, et al. Epidemiology of Meningitis in Canadian Neonatal Intensive Care Units. Pediatr Infect Dis J. 2019;38(5):476-80.

77. Ouchenir L, Renaud C, Khan S, Bitnun A, Boisvert A-A, McDonald J, et al. The Epidemiology, Management, and Outcomes of Bacterial Meningitis in Infants. Pediatrics. 2017;140(1).

78. Leazer R, Erickson N, Paulson J, Zipkin R, Stemmle M, Schroeder AR, et al. Epidemiology of Cerebrospinal Fluid Cultures and Time to Detection in Term Infants. Pediatrics. 2017;139(5).

79. Martinez E, Mintegi S, Vilar B, Martinez MJ, Lopez A, Catediano E, et al. Prevalence and predictors of bacterial meningitis in young infants with fever without a source. Pediatr Infect Dis J. 2015;34(5):494-8.

80. Garges HP. Neonatal Meningitis: What Is the Correlation Among Cerebrospinal Fluid Cultures, Blood Cultures, and Cerebrospinal Fluid Parameters? Pediatrics. 2006;117(4):1094-100.

81. Graf EH, Farquharson MV, Cárdenas AM. Comparative evaluation of the FilmArray meningitis/encephalitis molecular panel in a pediatric population. Diagn Microbiol Infect Dis. 2017;87(1):92-4.

82. Arora HS, Asmar BI, Salimnia H, Agarwal P, Chawla S, Abdel-Haq N. Enhanced Identification of Group B Streptococcus and Escherichia Coli in Young Infants with Meningitis Using the Biofire Filmarray Meningitis/Encephalitis Panel. Pediatr Infect Dis J. 2017;36(7):685-7.

83. Schrag SJ, Farley MM, Petit S, Reingold A, Weston EJ, Pondo T, et al. Epidemiology of Invasive Early-Onset Neonatal Sepsis, 2005 to 2014. Pediatrics. 2016;138(6).

84. Bizzarro MJ, Dembry LM, Baltimore RS, Gallagher PG. Changing patterns in neonatal Escherichia coli sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. Pediatrics. 2008;121(4):689-96.

85. Wortham JM, Hansen NI, Schrag SJ, Hale E, Van Meurs K, Sánchez PJ, et al. Chorioamnionitis and Culture-Confirmed, Early-Onset Neonatal Infections. Pediatrics. 2016;137(1).

86. Jackson KA, Iwamoto M, Swerdlow D. Pregnancy-associated listeriosis. Epidemiol Infect. 2010;138(10):1503-9.

87. Lamont RF, Sobel J, Mazaki-Tovi S, Kusanovic JP, Vaisbuch E, Kim SK, et al. Listeriosis in human pregnancy: a systematic review. J Perinat Med. 2011;39(3):227-36.

88. Hampton MM. Congenital Toxoplasmosis: A Review. Neonatal netw. 2015;34(5):274-8.

89. Keuning MW, Kamp GA, Schonenberg-Meinema D, Dorigo-Zetsma JW, van Zuiden JM, Pajkrt D. Congenital syphilis, the great imitator-case report and review. Lancet Infect Dis. 2020;20(7).

90. Edwards MS, Popek EJ, Wise B, Hatzenbuehler L, Arunachalam AR, Hair AB. Ascending in utero herpes simplex virus infection in an initially healthy-appearing premature infant. Pediatr Dev Pathol. 2015;18(2):155-8.

91. Leeper C, Lutzkanin A. Infections During Pregnancy. Prim Care. 2018;45(3):567-86.

92. Weiss SL, Fitzgerald JC, Pappachan J, Wheeler D, Jaramillo-Bustamante JC, Salloo A, et al. Global epidemiology of pediatric severe sepsis: the sepsis prevalence, outcomes, and therapies study. Am J Respir Crit Care Med. 2015;191(10):1147-57.

93. Boghossian NS, Page GP, Bell EF, Stoll BJ, Murray JC, Cotten CM, et al. Late-Onset Sepsis in Very Low Birth Weight Infants from Singleton and Multiple-Gestation Births.(Report). J Pediatr. 2013;162(6).

94. Letouzey M, Foix-L'Hélias L, Torchin H, Mitha A, Morgan AS, Zeitlin J, et al. Cause of preterm birth and late-onset sepsis in very preterm infants: the EPIPAGE-2 cohort study. Pediatr Res. 2021.

95. Dong Y, Speer CP. Late-onset neonatal sepsis: recent developments. Arch Dis Child Fetal Neonatal Ed. 2015;100(3):F257.

96. Gowda H, Norton R, White A, Kandasamy Y. Late-onset Neonatal Sepsis—A 10-year Review From North Queensland, Australia. Pediatr Infect Dis J. 2017;36(9):883-8.

97. Ista E, van Der Hoven B, Kornelisse R, van Der Starre C, Vos M, Boersma E, et al. Effectiveness of insertion and maintenance bundles to prevent central-line-associated bloodstream infections in critically ill patients of all ages: a systematic review and meta-analysis. Lancet Infect Dis. 2016;16(6):724-34.

98. Hooven TA, Polin RA. Healthcare-associated infections in the hospitalized neonate: a review. Early Hum Dev. 2014;90.

99. Marchant EA, Boyce GK, Sadarangani M, Lavoie PM. Neonatal sepsis due to coagulase-negative staphylococci. Clin Dev Immunol. 2013;2013:586076.

100. Björkman L, Ohlin A. Scrubbing the hub of intravenous catheters with an alcohol wipe for 15 sec reduced neonatal sepsis. Acta Paediatr Scand. 2015;104(3):232-6.

101. Olivier F, Bertelle V, Shah PS, Drolet C, Piedboeuf B. Association between birth route and late-onset sepsis in very preterm neonates. J Perinatol. 2016;36(12):1083-7.

102. Shao Y, Forster SC, Tsaliki E, Vervier K, Strang A, Simpson N, et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. Nature. 2019;574(7776):117-21.

103. Lister JL, Horswill AR. Staphylococcus aureus biofilms: recent developments in biofilm dispersal. Front Cell Infect Microbiol. 2014;4:178.

104. Karlowicz MG, Furigay PJ, Croitoru DP, Buescher ES. Central venous catheter removal versus in situ treatment in neonates with coagulase-negative staphylococcal bacteremia. Pediatr Infect Dis J. 2002;21(1):22-7.

105. Benjamin Jr DK, Miller W, Garges H, Benjamin DK, McKinney Jr RE, Cotton M, et al. Bacteremia, Central Catheters, and Neonates: When to Pull the Line. Pediatrics. 2001;107(6):1272-6.

106. Di Domenico EG, Cavallo I, Capitanio B, Ascenzioni F, Pimpinelli F, Morrone A, et al. Staphylococcus aureus and the Cutaneous Microbiota Biofilms in the Pathogenesis of Atopic Dermatitis. Microorganisms. 2019;7(9).

107. Keefe GP. Streptococcus agalactiae mastitis: a review. Can Vet J. 1997;38(7):429-37.

108. Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. J Exp Med. 1933;57(4):571-95.

109. Franciosi RA, Knostman JD, Zimmerman RA. Group B streptococcal neonatal and infant infections. J Pediatr. 1973;82(4):707.

110. McCracken GH. Group B streptococci: The new challenge in neonatal infections. J Pediatr. 1973;82(4):703-6.

111. Regan AJ, Klebanoff AM, Nugent PR. The Epidemiology of Group B Streptococcal Colonization in Pregnancy. Obstet Gynecol. 1991;77(4):604-10.

112. Håkansson S, Axemo P, Bremme K, Bryngelsson AL, Wallin MC, Ekström CM, et al. Group B streptococcal carriage in Sweden: a national study on risk factors for mother and infant colonisation. Acta Obstet Gynecol Scand. 2008;87(1):50-8.

113. Kwatra G, Cunnington MC, Merrall E, Adrian PV, Ip M, Klugman KP, et al. Prevalence of maternal colonisation with group B streptococcus: a systematic review and meta-analysis. Lancet Infect Dis. 2016;16(9):1076-84.

114. Russell NJ, Seale AC, O'Driscoll M, O'Sullivan C, Bianchi-Jassir F, Gonzalez-Guarin J, et al. Maternal Colonization With Group B Streptococcus and Serotype Distribution Worldwide: Systematic Review and Meta-analyses. Clin Infect Dis. 2017;65(suppl_2).

115. Hillier SL, Krohn MA, Kiviat NB, Watts DH, Eschenbach DA. Microbiologic causes and neonatal outcomes associated with chorioamnion infection. Am J Obstet Gynecol. 1991;165(4 Pt 1):955-61.

116. Lawn JE, Gravett MG, Nunes TM, Rubens CE, Stanton C. Global report on preterm birth and stillbirth (1 of 7): definitions, description of the burden and opportunities to improve data. BMC Pregnancy Childbirth. 2010;10(Suppl 1).

117. Allen U, Nimrod C, Macdonald N, Toye B, Stephens D, Marchessault V. Relationship between antenatal group B streptococcal vaginal colonization and premature labour. Paediatr Child Health. 1999;4(7):465-9.

118. Alhhazmi A, Hurteau D, Tyrrell GJ. Epidemiology of Invasive Group B Streptococcal Disease in Alberta, Canada, from 2003 to 2013. J Clin Microbiol. 2016;54(7):1774.

119. Francois Watkins LK, McGee L, Schrag SJ, Beall B, Jain JH, Pondo T, et al. Epidemiology of Invasive Group B Streptococcal Infections Among Nonpregnant Adults in the United States, 2008-2016. JAMA Intern Med. 2019;179(4):479-88.

120. Skoff TH, Farley MM, Petit S, Craig AS, Schaffner W, Gershman K, et al. Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990-2007. Clin Infect Dis. 2009;49(1):85.

121. Dahl MS, Tessin I, Trollfors B. Invasive group B streptococcal infections in Sweden: incidence, predisposing factors and prognosis. Int J Infect Dis. 2003;7(2):113-9.

122. Pitts SI, Maruthur NM, Langley GE, Pondo T, Shutt KA, Hollick R, et al. Obesity, Diabetes, and the Risk of Invasive Group B Streptococcal Disease in Nonpregnant Adults in the United States. Open Forum Infect Dis. 2018;5(6).

123. Jump RLP, Wilson BM, Baechle D, Briggs JM, Banks RE, Song S, et al. Risk Factors and Mortality Rates Associated With Invasive Group B Streptococcus Infections Among Patients in the US Veterans Health Administration. JAMA Netw Open. 2019;2(12).

124. Edwards JM, Watson N, Focht C, Wynn C, Todd CA, Walter EB, et al. Group B Streptococcus (GBS) Colonization and Disease among Pregnant Women: A Historical Cohort Study. Infect Dis Obstet Gynecol. 2019;2019:5430493.

125. Venkatesh KK, Vladutiu CJ, Strauss RA, Thorp JM, Stringer JSA, Stamilio DM, et al. Association Between Maternal Obesity and Group B Streptococcus Colonization in a National U.S. Cohort. J Womens Health (Larchmt). 2020;29(12):1507-12.

126. Farley MM, Harvey C, Stull T, Smith JD, Schuchat A, Wenger JD, et al. A Population-Based Assessment of Invasive Disease Due to Group B Streptococcus in Nonpregnant Adults. N Engl J Med. 1993;328(25):1807-11.

127. High KP, Edwards MS, Baker CJ. Group B Streptococcal Infections in Elderly Adults. Clin Infect Dis. 2005;41(6):839-47.

128. Sendi P, Johansson L, Norrby-Teglund A. Invasive Group B Streptococcal Disease in Non-pregnant Adults. Infection. 2008;36(2):100-11.

129. Hall J, Adams NH, Bartlett L, Seale AC, Lamagni T, Bianchi-Jassir F, et al. Maternal Disease With Group B Streptococcus and Serotype Distribution Worldwide: Systematic Review and Meta-analyses. Clin Infect Dis. 2017;65(suppl2):S112-S24.

130. Seale AC, Blencowe H, Bianchi-Jassir F, Embleton N, Bassat Q, Ordi J, et al. Stillbirth With Group B Streptococcus Disease Worldwide: Systematic Review and Meta-analyses. Clin Infect Dis. 2017;65(suppl_2):S125-S32.

131. Bianchi-Jassir F, Seale AC, Kohli-Lynch M, Lawn JE, Baker CJ, Bartlett L, et al. Preterm Birth Associated With Group B Streptococcus Maternal Colonization Worldwide: Systematic Review and Meta-analyses. Clin Infect Dis. 2017;65(suppl_2):S133-S42.

132. Boyer KM, Gadzala CA, Kelly PD, Burd LI, Gotoff SP. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. II. Predictive value of prenatal cultures. J Infect Dis.1983;148(5):802.

133. Le Doare K, Kampmann B. Breast milk and Group B streptococcal infection: vector of transmission or vehicle for protection? Vaccine. 2014;32(26):3128-32.

134. Zimmermann P, Gwee A, Curtis N. The controversial role of breast milk in GBS late-onset disease. J Infect. 2017;74 Suppl 1:S34-s40.

135. Madrid L, Seale AC, Kohli-Lynch M, Edmond KM, Lawn JE, Heath PT, et al. Infant Group B Streptococcal Disease Incidence and Serotypes Worldwide: Systematic Review and Meta-analyses. Clin Infect Dis. 2017;65(suppl2):S160-S72.

136. Edmond KM, Kortsalioudaki C, Scott S, Schrag SJ, Zaidi AK, Cousens S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. Lancet. 2012;379(9815):547-56.

137. Boyer K, Gotoff S. Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. N Engl J Med. 1986 Jun 26;314(26):1665-9.

138. Easmon CSF, Anthony BF. Gastrointestinal Carriage of Group B Streptococci. J Infect Dis. 1983;148(2):361-2.

139. Zangwill KM, Schuchat A, Wenger JD. Group B streptococcal disease in the United States, 1990: report from a multistate active surveillance system. MMWR CDC Surveill Summ. 1992;41(6):25-32.

140. Revised Guidelines for Prevention of Early-onset Group B Streptococcal (GBS) Infection. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Fetus and Newborn. Pediatrics. 1997;99(3):489.

141. The Swedish National Board of Health and Welfare S. Prevention av tidiga infektioner med grupp B streptokocker (GBS) hos nyfödda [Prevention of early-onset GBS infection in newborns]. Stockholm: Socialstyrelsen; 2008 [In Swedish]. Available from: http://www.socialstyrelsen.se/Lists/Artikelkatalog/Attachments/8836/2008-130-7_20081307.pdf. Accessed 11 Jan 2021.

142. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recomm Rep. 2002;51(Rr-11):1-22.

143. Committee A. Prevention of Group B Streptococcal Early-Onset Disease in Newborns: ACOG Committee Opinion Summary, Number 782. Obstet Gynecol. 2019;134(1).

144. Horsley L. CDC updates guidelines for the prevention of perinatal GBS disease. Am Fam Physician. 2011;83(9):1106.

145. Virranniemi M, Raudaskoski T, Haapsamo M, Kauppila J, Renko M, Peltola J, et al. The effect of screening-to-labor interval on the sensitivity of late-pregnancy culture in the prediction of group B streptococcus

colonization at labor: A prospective multicenter cohort study. Acta Obstet Gynecol Scand. 2019;98(4):494-9.

146. Schrag SJ, Zell ER, Lynfield R, Roome A, Arnold KE, Craig AS, et al. A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. N Engl J Med. 2002;347(4):233.

147. Schrag SJ, Verani JR. Intrapartum antibiotic prophylaxis for the prevention of perinatal group B streptococcal disease: Experience in the United States and implications for a potential group B streptococcal vaccine. Vaccine. 2013;31:D20-D6.

148. Di Renzo GC, Melin P, Berardi A, Blennow M, Carbonell-Estrany X, Donzelli GP, et al. Intrapartum GBS screening and antibiotic prophylaxis: a European consensus conference. J Matern Fetal Neonatal Med. 2015, Vol28(7), p766-782. 2015;28(7):766-82.

149. Le Doare K, O'Driscoll M, Turner K, Seedat F, Russell NJ, Seale AC, et al. Intrapartum Antibiotic Chemoprophylaxis Policies for the Prevention of Group B Streptococcal Disease Worldwide: Systematic Review. Clin Infect Dis. 2017;65(suppl2):S143-S51.

150. Gopal Rao G, Townsend J, Stevenson D, Nartey G, Hiles S, Bassett P, et al. Early-onset group B (EOGBS) infection subsequent to cessation of screening-based intrapartum prophylaxis: findings of an observational study in West London, UK. BMJ open. 2017;7(11).

151. Gopal Rao G, Nartey G, McAree T, O'Reilly A, Hiles S, Lee T, et al. Outcome of a screening programme for the prevention of neonatal invasive early-onset group B Streptococcus infection in a UK maternity unit: an observational study. BMJ Open. 2017;7(4).

152. Khalil MR, Uldbjerg N, Thorsen PB, Møller JK. Risk-based approach versus culture-based screening for identification of group B streptococci among women in labor. Int J Gynaecol Obstet. 2019;144(2):187.

153. Hasperhoven GF, Al-Nasiry S, Bekker V, Villamor E, Kramer B. Universal screening versus risk-based protocols for antibiotic prophylaxis during childbirth to prevent early-onset Group B streptococcal disease: a systematic review and meta-analysis. BJOG. 2020 May;127(6):680-691.

154. Rajagopal L. Understanding the regulation of Group B Streptococcal virulence factors. Future Microbiol. 2009;4(2):201-21.

155. Burcham LR, Spencer BL, Keeler LR, Runft DL, Patras KA, Neely MN, et al. Determinants of Group B streptococcal virulence potential amongst vaginal clinical isolates from pregnant women. PLoS One. 2019;14(12).

156. Maisey HC, Doran KS, Nizet V. Recent advances in understanding the molecular basis of group B Streptococcus virulence. Expert Rev Mol Med. 2008;10:e27.

157. Armistead B, Oler E, Adams Waldorf K, Rajagopal L. The Double Life of Group B Streptococcus: Asymptomatic Colonizer and Potent Pathogen. J Mol Biol. 2019;431(16):2914-31.

158. Lamy MC, Zouine M, Fert J, Vergassola M, Couve E, Pellegrini E, et al. CovS/CovR of group B streptococcus: a two-component global regulatory system involved in virulence. Mol Microbiol. 2004;54(5):1250-68.

159. Firon A, Tazi A, Da Cunha V, Brinster S, Sauvage E, Dramsi S, et al. The Abi-domain protein Abx1 interacts with the CovS histidine kinase to control virulence gene expression in group B Streptococcus. PLoS Pathog. 2013;9(2).

160. Marques MB, Kasper DL, Pangburn MK, Wessels MR. Prevention of C3 deposition by capsular polysaccharide is a virulence mechanism of type III group B streptococci. Infect Immun. 1992;60(10):3986.

161. Slotved HC, Kong F, Lambertsen L, Sauer S, Gilbert GL. Serotype IX, a proposed new Streptococcus agalactiae serotype. J Clin Microbiol. 2007;45(9):2929-36.

162. Wessels MR, Paoletti LC, Rodewald AK, Michon F, Difabio J, Jennings HJ, et al. Stimulation of protective antibodies against type Ia and Ib group B streptococci by a type Ia polysaccharide-tetanus toxoid conjugate vaccine. Infect Immun. 1993;61(11):4760-6.

163. Lamagni TL, Keshishian C, Efstratiou A, Guy R, Henderson KL, Broughton K, et al. Emerging trends in the epidemiology of invasive group B streptococcal disease in England and Wales, 1991-2010. Clin Infect Dis. 2013;57(5):682.

164. Persson E, Berg S, Trollfors B, Larsson P, Ek E, Backhaus E, et al. Serotypes and clinical manifestations of invasive group B streptococcal infections in western Sweden 1998-2001. Clin Microbiol Infect. 2004;10(9):791-6.

165. Teatero S, Ramoutar E, McGeer A, Li A, Melano RG, Wasserscheid J, et al. Clonal Complex 17 Group B Streptococcus strains causing invasive disease in neonates and adults originate from the same genetic pool. Scientific Reports. 2016;6(1):20047.

166. Blumberg HM, Stephens DS, Modansky M, Erwin M, Elliot J, Facklam RR, et al. Invasive group B streptococcal disease: the emergence of serotype V. J Infect Dis. 1996;173(2):365.

167. Berg S, Trollfors B, Lagergård T, Zackrisson G, Claesson BA. Serotypes and clinical manifestations of group B streptococcal infections in western Sweden. Clin Microbiol Infect. 2000;6(1):9-13.

168. Ekelund K, Slotved H-C, Nielsen HU, Kaltoft MS, Konradsen HB. Emergence of Invasive Serotype VIII Group B Streptococcal Infections in Denmark. J Clin Microbiol. 2003;41(9):4442.

169. Zaleznik DF, Rench MA, Hillier S, Krohn MA, Platt R, Lee M-LT, et al. Invasive Disease Due to Group B Streptococcus in Pregnant Women and Neonates from Diverse Population Groups. Clin Infect Dis. 2000;30(2):276-81.

170. Lang S, Palmer M. Characterization of Streptococcus agalactiae CAMP factor as a pore-forming toxin. J Biol Chem. 2003;278(40):38167-73.

171. Hensler ME, Quach D, Hsieh CJ, Doran KS, Nizet V. CAMP factor is not essential for systemic virulence of Group B Streptococcus. Microb pathog. 2008;44(1):84-8.

172. Jin T, Brefo-Mensah E, Fan W, Zeng W, Li Y, Zhang Y, et al. Crystal structure of the Streptococcus agalactiae CAMP factor provides insights into its membrane-permeabilizing activity. J Biol Chem. 2018;293(30):11867-77.

173. Marchlewicz BA, Duncan JL. Properties of a hemolysin produced by group B streptococci. Infect Immun. 1980;30(3):805-13.

174. Doran KS, Liu GY, Nizet V. Group B streptococcal beta-hemolysin/cytolysin activates neutrophil signaling pathways in brain endothelium and contributes to development of meningitis. J Clin Invest. 2003;112(5):736-44.

175. Whidbey C, Harrell MI, Burnside K, Ngo L, Becraft AK, Iyer LM, et al. A hemolytic pigment of Group B Streptococcus allows bacterial penetration of human placenta. J Exp Med. 2013;210(6):1265.

176. Liu GY, Doran KS, Lawrence T, Turkson N, Puliti M, Tissi L, et al. Sword and shield: Linked group B streptococcal -hemolysin/cytolysin and carotenoid pigment function to subvert host phagocyte defense. Proc Natl Acad Sci U S A. 2004;101(40):14491-6.

177. Carey AJ, Tan CK, Mirza S, Irving-Rodgers H, Webb RI, Lam A, et al. Infection and Cellular Defense Dynamics in a Novel 17β-Estradiol Murine Model of Chronic Human Group B Streptococcus Genital Tract Colonization Reveal a Role for Hemolysin in Persistence and Neutrophil Accumulation. J Immunol. 2014;192(4):1718-31.

178. Boldenow E, Gendrin C, Ngo L, Bierle C, Vornhagen J, Coleman M, et al. Group B Streptococcus circumvents neutrophils and

neutrophil extracellular traps during amniotic cavity invasion and preterm labor. Sci immunol. 2016;1(4).

179. Randis TM, Gelber SE, Hooven TA, Abellar RG, Akabas LH, Lewis EL, et al. Group B Streptococcus β-hemolysin/cytolysin breaches maternal-fetal barriers to cause preterm birth and intrauterine fetal demise in vivo. J Infect Dis. 2014;210(2):265.

180. Gendrin C, Vornhagen J, Ngo L, Whidbey C, Boldenow E, Santana-Ufret V, et al. Mast cell degranulation by a hemolytic lipid toxin decreases GBS colonization and infection. Sci Adv. 2015;1(6).

181. Whidbey C, Vornhagen J, Gendrin C, Boldenow E, Samson JM, Doering K, et al. A streptococcal lipid toxin induces membrane permeabilization and pyroptosis leading to fetal injury. EMBO Mol Med. 2015;7(4):488-505.

182. Patras KA, Wang NY, Fletcher EM, Cavaco CK, Jimenez A, Garg M, et al. Group B S treptococcus CovR regulation modulates host immune signalling pathways to promote vaginal colonization. Cellular Microbiology. 2013;15(7):1154-67.

183. Vornhagen J, Adams Waldorf KM, Rajagopal L. Perinatal Group B Streptococcal Infections: Virulence Factors, Immunity, and Prevention Strategies. Trends Microbiol. 2017;25(11):919-31.

184. Rosa-Fraile M, Rodriguez-Granger J, Cueto-Lopez M, Sampedro A, Gaye EB, Haro JM, et al. Use of Granada medium to detect group B streptococcal colonization in pregnant women. J Clin Microbiol. 1999;37(8):2674-7.

185. Church DL, Baxter H, Lloyd T, Miller B, Elsayed S. Evaluation of StrepB carrot broth versus Lim broth for detection of group B Streptococcus colonization status of near-term pregnant women. J Clin Microbiol. 2008;46(8):2780-2.

186. Rajagopal L, Vo A, Silvestroni A, Rubens CE. Regulation of cytotoxin expression by converging eukaryotic-type and two-component signalling mechanisms in Streptococcus agalactiae. Mol Microbiol. 2006;62(4):941-57.

187. Zhu L, Beres SB, Yerramilli P, Pruitt L, Cantu CC, Olsen RJ, et al. Genetic Basis Underlying the Hyperhemolytic Phenotype of Streptococcus agalactiae Strain CNCTC10/84. J Bacteriol. 2020;202(23).

188. Akgul Y, Word RA, Ensign LM, Yamaguchi Y, Lydon J, Hanes J, et al. Hyaluronan in cervical epithelia protects against infection-mediated preterm birth. J Clin Invest. 2014;124(12):5481-9.

189. Mahendroo M. Cervical remodeling in term and preterm birth: insights from an animal model. Reproduction. 2012;143(4):429-38.

190. Baker JR, Pritchard DG. Action pattern and substrate specificity of the hyaluronan lyase from group B streptococci. Biochem J. 2000;348 Pt 2:465-71.

191. Kolar SL, Kyme P, Tseng CW, Soliman A, Kaplan A, Liang J, et al. Group B Streptococcus Evades Host Immunity by Degrading Hyaluronan. Cell Host Microbe. 2015;18(6):694-704.

192. Vornhagen J, Quach P, Boldenow E, Merillat S, Whidbey C, Ngo LY, et al. Bacterial Hyaluronidase Promotes Ascending GBS Infection and Preterm Birth. mBio. 2016;7(3).

193. Fabbrini M, Rigat F, Rinaudo CD, Passalaqua I, Khacheh S, Creti R, et al. The Protective Value of Maternal Group BStreptococcusAntibodies: Quantitative and Functional Analysis of Naturally Acquired Responses to Capsular Polysaccharides and Pilus Proteins in European Maternal Sera. Clin Infect Dis. 2016;63(6):746-53.

194. Dangor Z, Kwatra G, Izu A, Adrian P, Cutland CL, Velaphi S, et al. Correlates of protection of serotype-specific capsular antibody and invasive Group B Streptococcus disease in South African infants. Vaccine. 2015;33(48):6793-9.

195. Dangor Z, Kwatra G, Izu A, Adrian P, Cutland CL, Velaphi S, et al. Association between maternal Group B Streptococcus surface-protein antibody concentrations and invasive disease in their infants. Expert Rev Vaccines. 2015;14(12):1651-60.

196. Kwatra G, Adrian PV, Shiri T, Buchmann EJ, Cutland CL, Madhi SA. Natural acquired humoral immunity against serotype-specific group B Streptococcus rectovaginal colonization acquisition in pregnant women. Clin Microbiol Infect. 2015;21(6):568.

197. Vergnano S, Embleton N, Collinson A, Menson E, Russell AB, Heath P. Missed opportunities for preventing group B streptococcus infection. Arch Dis Child Fetal Neonatal Ed. 2010;95(1).

198. Heath PT. Status of vaccine research and development of vaccines for GBS. Vaccine. 2016;34(26):2876-9.

199. Marques MB, Kasper DL, Shroff A, Michon F, Jennings HJ, Wessels MR. Functional activity of antibodies to the group B polysaccharide of group B streptococci elicited by a polysaccharide-protein conjugate vaccine. Infect Immun. 1994;62(5):1593.

200. Atul Kumar J, Lawrence CP, Philippe G, Meenakshi D, Puja Kumari S, Guido G, et al. Group B Streptococcus: global incidence and vaccine development. Nat Rev Microbiol. 2006;4(12):932.

201. Kasper DL, Paoletti LC, Wessels MR, Guttormsen HK, Carey VJ, Jennings HJ, et al. Immune response to type III group B streptococcal polysaccharide-tetanus toxoid conjugate vaccine. Clin Infect Dis. 1996;98(10):2308.

202. Carol, Lawrence, Michael, Guttormsen HK, Marcia, Melissa, et al. Safety and Immunogenicity of Capsular Polysaccharide–Tetanus Toxoid Conjugate Vaccines for Group B Streptococcal Types Ia and Ib. J Infect Dis. 1999;179(1):142-50.

203. Hillier SL, Ferrieri P, Edwards MS, Ewell M, Ferris D, Fine P, et al. A Phase 2, Randomized, Control Trial of Group B Streptococcus (GBS) Type III Capsular Polysaccharide-tetanus Toxoid (GBS III-TT) Vaccine to Prevent Vaginal Colonization With GBS III. Clin Infect Dis. 2019;68(12):2079.

204. Knuf M, Kowalzik F, Kieninger D. Comparative effects of carrier proteins on vaccine-induced immune response. Vaccine. 2011;29(31):4881-90.

205. Debra, Marcia, Morven, Carol. Use of Type V Group B Streptococcal Conjugate Vaccine in Adults 65–85 Years Old. J Infect Dis. 2004;190(3):558-64.

206. Baker CJ, Rench MA, Paoletti LC, Edwards MS. Dose–response to type V group B streptococcal polysaccharide–tetanus toxoid conjugate vaccine in healthy adults. Vaccine. 2007;25(1):55-63.

207. Maione D. Identification of a Universal Group B Streptococcus Vaccine by Multiple Genome Screen. Science. 2005;309(5731):148-50.

208. Margarit I, Rinaudo CD, Galeotti CL, Maione D, Ghezzo C, Buttazzoni E, et al. Preventing Bacterial Infections with Pilus-Based Vaccines: the Group B Streptococcus Paradigm. J Infect Dis. 2009;199(1):108-15.

209. Madoff LC, Paoletti LC, Tai JY, Kasper DL. Maternal immunization of mice with group B streptococcal type III polysaccharide-beta C protein conjugate elicits protective antibody to multiple serotypes. J Clin Invest. 1994;94(1):286-92.

210. Slotved HC, Elliott J, Thompson T, Konradsen HB. Latex Assay for Serotyping of Group B Streptococcus Isolates. J Clin Microbiol. 2003;41(9):4445.

211. Martin TR, Rubens CE, Wilson CB. Lung antibacterial defense mechanisms in infant and adult rats: implications for the pathogenesis of group B streptococcal infections in the neonatal lung. J Infect Dis. 1988;157(1):91-100.

212. Wilkinson HW, Eagon RG. Type-Specific Antigens of Group B Type Ic Streptococci. Infection and Immunity. 1971;4(5):596-604.

213. Gendrin C, Vornhagen J, Armistead B, Singh P, Whidbey C, Merillat S, et al. A Nonhemolytic Group B Streptococcus Strain Exhibits Hypervirulence. J Infect Dis. 2018;217(6):983-7.

214. Grimes DA, Schulz KF. Bias and causal associations in observational research. Lancet. 2002;359(9302):248-52.

215. Tsai M-H, Chu S-M, Hsu J-F, Lien R, Huang H-R, Chiang M-C, et al. Polymicrobial Bloodstream Infection in Neonates: Microbiology, Clinical Characteristics, and Risk Factors.(Research Article). PLoS One. 2014;9(1).

216. Puopolo KM, Mukhopadhyay S, Hansen NI, Michael Cotton C, Stoll BJ, Sanchez PJ, et al. Identification of extremely premature infants at low risk for early-onset sepsis. Pediatrics. 2017;140(5).

217. Kalliola S, Vuopio-Varkila J, Takala AK, Eskola J. Neonatal group B streptococcal disease in Finland: a ten-year nationwide study. Pediatr Infect Dis J. 1999;18(9):806-10.

218. Bakhuizen SE, De Haan TR, Teune MJ, Van Wassenaer-Leemhuis AG, Van Der Heyden JL, Van Der Ham DP, et al. Meta-analysis shows that infants who have suffered neonatal sepsis face an increased risk of mortality and severe complications. Acta Paediatr Scand. 2014;103(12):1211-8.

219. Benjamin DK, Delong E, Cotten CM, Garges HP, Steinbach WJ, Clark RH. Mortality Following Blood Culture in Premature Infants: Increased with Gram-negative Bacteremia and Candidemia, but Not Gram-positive Bacteremia. J Perinatol. 2004;24(3):175-80.

220. Karlowicz MG, Buescher ES, Surka AE. Fulminant Late-Onset Sepsis in a Neonatal Intensive Care Unit, 1988-1997, and the Impact of Avoiding Empiric Vancomycin Therapy. Pediatrics 2000;106(6):1387-90.

221. Makhoul I, Smolkin T, Lusky A. Epidemiological, clinical, and microbiological characteristics of late-onset sepsis among very low birth weight infants in Israel: A national survey. Pediatrics. 2002;109(1):34-9.

222. Levit O, Bhandari V, Li FY, Shabanova V, Gallagher PG, Bizzarro MJ. Clinical and laboratory factors that predict death in very low birth weight infants presenting with late-onset sepsis. Pediatr Infect Dis J. 2014;33(2):143-6.

223. Gordon A, Isaacs D. Late onset neonatal Gram-negative bacillary infection in Australia and New Zealand: 1992-2002. Pediatr Infect Dis J. 2006;25(1):25-9.

224. Piening BC, Geffers C, Gastmeier P, Schwab F. Pathogen-specific mortality in very low birth weight infants with primary bloodstream infection. PLoS One. 2017;12(6).

225. Nordberg V, Iversen A, Tidell A, Ininbergs K, Giske CG, Navér L. A decade of neonatal sepsis caused by gram-negative bacilli—a retrospective matched cohort study. Eur J Clin Microbiol Infect Dis. 2021.

226. Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, et al. Neurodevelopmental and Growth Impairment Among Extremely Low-Birth-Weight Infants With Neonatal Infection. JAMA. 2004;292(19):2357-65.

227. van Vliet EO, de Kieviet JF, Oosterlaan J, van Elburg RM. Perinatal infections and neurodevelopmental outcome in very preterm and very low-birth-weight infants: a meta-analysis. JAMA pediatr. 2013;167(7):662-8.

228. Mitha A, Foix-L'Hélias L, Arnaud C, Marret S, Vieux R, Aujard Y, et al. Neonatal infection and 5-year neurodevelopmental outcome of very preterm infants. Pediatrics. 2013;132(2).

229. Yeo KT, Lahra M, Bajuk B, Hilder L, Abdel-Latif ME, Wright IM, et al. Long-term outcomes after group B streptococcus infection: a cohort study. Arch Dis Child. 2019;104(2):172.

230. Edwards MS, Gonik B. Preventing the broad spectrum of perinatal morbidity and mortality through group B streptococcal vaccination. Vaccine. 2012.

231. Köstlin-Gille N, Härtel C, Haug C, Göpel W, Zemlin M, Müller A, et al. Epidemiology of Early and Late Onset Neonatal Sepsis in Very Low Birthweight Infants: Data From the German Neonatal Network. Pediatr Infect Dis J. 2021;40(3):255-9.

232. Schrag SJ, Hadler JL, Arnold KE, Martell-Cleary P, Reingold A, Schuchat A. Risk factors for invasive, early-onset Escherichia coli infections in the era of widespread intrapartum antibiotic use. Pediatrics. 2006;118(2):570.

233. Bekker V, Bijlsma MW, Van de Beek D, Kuijpers TW, Van der Ende A. Incidence of invasive group B streptococcal disease and pathogen genotype distribution in newborn babies in the Netherlands over 25 years: a nationwide surveillance study. Lancet Infect Dis. 2014;14(11):1083.

234. Berardi A, Baroni L, Bacchi Reggiani ML, Ambretti S, Biasucci G, Bolognesi S, et al. The burden of early-onset sepsis in Emilia-Romagna (Italy): a 4-year, population-based study. J Matern Fetal Neonatal Med. 2016;29(19):3126-31.

235. Vatne A, Klingenberg C, Rettedal S, Øymar K. Early-Onset Sepsis in Neonates - A Population-Based Study in South-West Norway From 1996 to 2018. Front Pediatr. 2021;9:634798.

236. Rodriguez-Granger J, Alvargonzalez JC, Berardi A, Berner R, Kunze M, Hufnagel M, et al. Prevention of group B streptococcal neonatal disease revisited. The DEVANI European project. Eur J Clin Microbiol Infect Dis. 2012;31(9):2097-104.

237. Merviel PJGOF. Prévention anténatale du risque infectieux bactérien néonatal précoce. J Gynecol Obstet Biol Reprod (Paris). 2001;29:34045.

238. Finnish institute for health and welfare. Grupp B streptokock. Finnish institute for health and welfare; 2020. [Updated April 28, 2020. In Swedish]. Available from: https://thl.fi/fi/web/infektiotaudit-ja-rokotukset/taudit-jatorjunta/taudit-ja-taudinaiheuttajat-a-o/b-ryhman-streptokokki: Accessed 12 Feb 2021.

239. Seedat F, Geppert J, Stinton C, Patterson J, Freeman K, Johnson SA, et al. Universal antenatal screening for group B streptococcus may cause more harm than good. BMJ. 2019;364.

240. Brigsten AK. Gruppe B streptokokker hos gravide og fødende. Den norske legeforening; 2020. Available from: https://www.legeforeningen.no/foreningsledd/fagmed/norsk-gynekologisk-forening/veiledere/veileder-i-fodselshjelp/gruppe-b-streptokokker-hos-gravide-og-fodende/. Accessed 12 March 2021.

241. Óladóttir LG, Erlendsdóttir SH, Pálsson GG, Björnsdóttir GE, Kristinsson GK, Haraldsson GÁ. Increasing Incidence of Late-onset Neonatal Invasive Group B Streptococcal Infections in Iceland. Pediatr Infect Dis J. 2011;30(8):661-3.

242. Helmig RB, Gertsen JB. Intrapartum PCR-assay for detection of Group B Streptococci (GBS). Eur J Obstet Gynecol Reprod Biol X. 2019;4:100081.

243. Håkansson S, Källén K, Bullarbo M, Holmgren PÅ, Bremme K, Larsson A, Norman M, Norén H, Ortmark-Wrede C, Pettersson K, Saltvedt S, Sondell B, Tokarska M, von Vultee A, Jacobsson B. Real-time PCR-assay in the delivery suite for determination of group B streptococcal colonization in a setting with risk-based antibiotic prophylaxis. J Matern Fetal Neonatal Med. 2014 Mar;27(4):328-32.

244. Jacobsson Bo PK, Herbst Andreas, Håkansson Stellan, Ohlin Andreas, Lundberg Fredrik , Skiöld Beatrice. Replik till Claes Schalén och medförfattare: Viktigt att diskutera profylax mot neonatala GBS-infektioner. Lakartidningen. 2018;115:FDWX.

245. Carey AJ. War on Staphylococcus aureus. J Perinatol. 2014;34(11):803-4.

246. Bolon B, Butt MT, Garman RH, Dorman DC. Chapter 52 - Nervous System. In: Haschek WM, Rousseaux CG, Wallig MA, editors. Haschek and Rousseaux's Handbook of Toxicologic Pathology (Third Edition). Boston: Academic Press; 2013. p. 2005-93.

247. Milstone AM, Voskertchian A, Koontz DW, Khamash DF, Ross T, Aucott SW, et al. Effect of Treating Parents Colonized With Staphylococcus aureus on Transmission to Neonates in the Intensive Care Unit: A Randomized Clinical Trial. JAMA. 2020;323(4):319-28.

248. Popoola VO, Milstone AM. Decolonization to prevent Staphylococcus aureus transmission and infections in the neonatal intensive care unit. J Perinatol. 2014;34(11):805-10.

249. Popoola VO, Budd A, Wittig SM, Ross T, Aucott SW, Perl TM, et al. Methicillin-resistant Staphylococcus aureus transmission and infections in a neonatal intensive care unit despite active surveillance cultures and decolonization: challenges for infection prevention. Infect Control Hosp Epidemiol. 2014;35(4):412-8.

250. Shaw AG, Sim K, Randell P, Cox MJ, McClure ZE, Li MS, et al. Late-Onset Bloodstream Infection and Perturbed Maturation of the Gastrointestinal Microbiota in Premature Infants. PLoS One. 2015;10(7).

251. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Changes in Pathogens Causing Early-Onset Sepsis in Very-Low-Birth-Weight Infants. N Engl J Med. 2002;347(4):240-7.

252. Klinger G, Levy I, Sirota L, Boyko V, Lerner-Geva L, Reichman B. Outcome of Early-Onset Sepsis in a National Cohort of Very Low Birth Weight Infants. Pediatrics. 2010 Apr;125(4):e736-40.

253. Cotten CM, Taylor S, Stoll B, Goldberg RN, Hansen NI, Sánchez PJ, et al. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. Pediatrics. 2009;123(1):58.

254. Alexander VN, Northrup V, Bizzarro MJ. Antibiotic Exposure in the Newborn Intensive Care Unit and the Risk of Necrotizing Enterocolitis. J Pediatr. 2011;159(3):392-7.

255. Kuppala VS, Meinzen-Derr J, Morrow AL, Schibler KR. Prolonged Initial Empirical Antibiotic Treatment is Associated with Adverse Outcomes in Premature Infants. J Pediatr. 2011;159(5):720-5.

256. Ting JY, Synnes A, Roberts A, Deshpandey A, Dow K, Yoon EW, et al. Association Between Antibiotic Use and Neonatal Mortality and Morbidities in Very Low-Birth-Weight Infants Without Culture-Proven Sepsis or Necrotizing Enterocolitis. JAMA pediatrics. 2016;170(12):1181.

257. Greenwood C, Morrow AL, Lagomarcino AJ, Altaye M, Taft DH, Yu Z, et al. Early Empiric Antibiotic Use in Preterm Infants Is Associated with Lower Bacterial Diversity and Higher Relative Abundance of Enterobacter. J Pediatr. 2014 Jul;165(1):23-9.

258. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors Influencing the Composition of the Intestinal Microbiota in Early Infancy. Pediatrics. 2006 Aug;118(2):511-21.

259. Vangay P, Ward T, Jeffrey, Knights D. Antibiotics, Pediatric Dysbiosis, and Disease. Cell Host & Microbe. 2015;17(5):553-64.

260. Kinlay S, Michel T, Leopold JA. The Future of Vascular Biology and Medicine. Circulation. 2016;133(25):2603-9.

261. Ajslev TA, Andersen CS, Gamborg M, Sørensen TI, Jess T. Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. Int J Obes. 2011 Apr;35(4):522-9.

262. Risnes KR, Belanger K, Murk W, Bracken MB. Antibiotic exposure by 6 months and asthma and allergy at 6 years: Findings in a cohort of 1,401 US children. Am J Epidemiol. 2011 Feb 1;173(3):310-8.

263. Vatne A, Klingenberg C, Øymar K, Rønnestad AE, Manzoni P, Rettedal S. Reduced Antibiotic Exposure by Serial Physical Examinations in Term Neonates at Risk of Early-onset Sepsis. Pediatr Infect Dis J. 2020;39(5):438-43.

264. Benitz WE. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. Clin Perinatol. 2010;37(2):421-38.

265. Ehl S, Gering B, Bartmann P, Hogel J, Pohlandt F. C-Reactive Protein Is a Useful Marker for Guiding Duration of Antibiotic Therapy in Suspected Neonatal Bacterial Infection. Pediatrics. 1997;99(2):216-21.

266. Stocker M, Van Herk W, El Helou S, Dutta S, Fontana MS, Schuerman FABA, et al. Procalcitonin-guided decision making for duration of antibiotic therapy in neonates with suspected early-onset sepsis: a multicentre, randomised controlled trial (NeoPIns). Lancet. 2017;390(10097):871-81.

267. Gyllensvärd J, Ingemansson F, Hentz E, Studahl M, Elfvin A. C-reactive protein- and clinical symptoms-guided strategy in term neonates with early-onset sepsis reduced antibiotic use and hospital stay: a quality improvement initiative. BMC Pediatr. 2020;20(1).

268. Kaiser JR, Cassat JE, Lewno MJ. Should Antibiotics be Discontinued at 48 Hours for Negative Late-Onset Sepsis Evaluations in the Neonatal Intensive Care Unit? J Perinatol. 2002;22(6):445-7.

269. Saini SS, Dutta S, Ray P, Narang A. Short course versus 7-day course of intravenous antibiotics for probable neonatal septicemia: a pilot, open-label, randomized controlled trial. Indian Pediatr. 2011 Jan;48(1):19-24.

270. Cantey JB, Baird SD. Ending the Culture of Culture-Negative Sepsis in the Neonatal ICU. Pediatrics. 2017;140(4).

271. de-Wahl Granelli A, Wennergren M, Sandberg K, Mellander M, Bejlum C, Inganäs L, et al. Impact of pulse oximetry screening on the detection of duct dependent congenital heart disease: a Swedish prospective screening study in 39,821 newborns. BMJ. 2009;338:a3037.

272. Kellogg JA, Ferrentino FL, Goodstein MH, Liss J, Shapiro SL, Bankert DA. Frequency of low level bacteremia in infants from birth to two months of age. Pediatr Infect Dis J. 1997;16(4):381-5.

273. Buttery JP. Blood cultures in newborns and children: optimising an everyday test. Arch Dis Child Fetal Neonatal Ed.. 2002;87(1):25F-8.

274. St Geme JW, 3rd, Bell LM, Baumgart S, D'Angio CT, Harris MC. Distinguishing sepsis from blood culture contamination in young infants with blood cultures growing coagulase-negative staphylococci. Pediatrics. 1990;86(2):157-62.

275. Sabui T, Tudehope D, Tilse M. Clinical significance of quantitative blood cultures in newborn infants. J Paediatr Child Health. 1999;35(6):578-81.

276. Fortino Solorzano-Santos MG. A Blood Micro-Culture System for the Diagnosis of Bacteremia in Pediatric Patients. Scand J Infect Dis. 1998;30(5):481-3.

277. Kidszun A, Klein L, Winter J, Schmeh I, Gröndahl B, Gehring S, et al. Viral Infections in Neonates with Suspected Late-Onset Bacterial Sepsis—A Prospective Cohort Study. Am J Perinatol. 2017;34(01):01-7.

278. Cantey JB, Wozniak PS, Pruszynski JE, Sánchez PJ. Reducing unnecessary antibiotic use in the neonatal in Scand J Infect Dis tensive care unit (SCOUT): a prospective interrupted time-series study. Lancet Infect Dis. 2016;16(10):1178-84.

279. Shalabi M, Adel M, Yoon E, Aziz K, Lee S, Shah PS. Risk of Infection Using Peripherally Inserted Central and Umbilical Catheters in Preterm Neonates. Pediatrics. 2015;136(6):1073-9.

280. Konstantinidi A, Sokou R, Panagiotounakou P, Lampridou M, Parastatidou S, Tsantila K, et al. Umbilical Venous Catheters and Peripherally Inserted Central Catheters: Are They Equally Safe in VLBW Infants? A Non-Randomized Single Center Study. Medicina (Kaunas). 2019;55(8):442.

281. Dongara AR, Patel DV, Nimbalkar SM, Potana N, Nimbalkar AS. Umbilical Venous Catheter Versus Peripherally Inserted Central Catheter in Neonates: A Randomized Controlled Trial. J Trop Pediatr. 2017;63(5):374-9.

282. Butler-O'Hara M, Buzzard CJ, Reubens L, McDermott MP, Digrazio W, D'Angio CT. A randomized trial comparing long-term and short-term use of umbilical venous catheters in premature infants with birth weights of less than 1251 grams. Pediatrics. 2006;118(1).

283. Bowen JR, Callander I, Richards R, Lindrea KB. Decreasing infection in neonatal intensive care units through quality improvement. Arch Dis Child Fetal Neonatal Ed.. 2017;102(1)

284. Fisher D, Cochran KM, Provost LP, Patterson J, Bristol T, Metzguer K, et al. Reducing Central Line-Associated Bloodstream Infections in North Carolina NICUs. Pediatrics. 2013;132(6).

285. Kaufman DA, Blackman A, Conaway MR, Sinkin RA. Nonsterile Glove Use in Addition to Hand Hygiene to Prevent Late-Onset Infection in Preterm Infants. JAMA pediatr. 2014;168(10):909.

286. Polin RA, Denson S, Brady MT. Strategies for Prevention of Health Care–Associated Infections in the NICU. Pediatrics. 2012;129(4).

287. Lupo A, Ruppen C, Hemphill A, Spellerberg B, Sendi P. Phenotypic and molecular characterization of hyperpigmented group B Streptococci. Int J Med Microbiol. 2014;304(5-6):717-24.

288. Romero R, Gomez R, Chaiworapongsa T, Conoscenti G, Cheol Kim J, Mee Kim Y. The role of infection in preterm labour and delivery. Paediatr Perinat Epidemiol. 2001;15(s2):41-56.

289. Racicot K, Cardenas I, Wünsche V, Aldo P, Guller S, Means RE, et al. Viral Infection of the Pregnant Cervix Predisposes to Ascending Bacterial Infection. J Immunol. 2013;191(2):934-41.

290. Wang Z, Guo C, Xu Y, Liu G, Lu C, Liu Y. Two Novel Functions of Hyaluronidase from Streptococcus agalactiae Are Enhanced Intracellular Survival and Inhibition of Proinflammatory Cytokine Expression. Infect Immun. 2014;82(6):2615-25.

291. Anthony BF, Okada DM, Hobel CJ. Epidemiology of Group B Streptococcus: Longitudinal Observations during Pregnancy. J Infect Dis. 1978;137(5):524-30.

292. Hansen SM, Uldbjerg N, Kilian M, Sorensen UBS. Dynamics of Streptococcus agalactiae Colonization in Women during and after Pregnancy and in Their Infants. J Clin Microbiol. 2004;42(1):83-9.

293. Rolland K, Marois C, Siquier V, Cattier B, Quentin R. Genetic Features of Streptococcus agalactiae Strains Causing Severe Neonatal Infections, as Revealed by Pulsed-Field Gel Electrophoresis and hylB Gene Analysis. J Clin Microbiol. 1999;37(6):1892-8.

294. Musser JM, Mattingly SJ, Quentin R, Goudeau A, Selander RK. Identification of a high-virulence clone of type III Streptococcus agalactiae (group B Streptococcus) causing invasive neonatal disease. Proc Natl Acad Sci U S A. 1989 Jun;86(12):4731-5

295. Heath PT, Le Doare K, Khalil A. Inclusion of pregnant women in COVID-19 vaccine development. Lancet Infect Dis. 2020;20(9):1007-8.

296. Armistead B, Herrero-Foncubierta P, Coleman M, Quach P, Whidbey C, Justicia J, et al. Lipid analogs reveal features critical for hemolysis and diminish granadaene mediated Group B Streptococcus infection. Nat Commun. 2020;11(1).