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TUULI METSVAHT

Optimal antibacterial therapy of neonates at risk of early onset sepsis

TARTU UNIVERSITY
PRESS

Institute of Microbiology, University of Tartu, Estonia

Dissertation is accepted for the commencement of the degree of Doctor of Medical Sciences on March 17, 2009 by the Council of the Faculty of Medicine, University of Tartu, Tartu, Estonia

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Commencement: May 27, 2010

This research was supported by the European Regional Development Fund

Publication of this dissertation is granted by University of Tartu

ISSN 1024–395x
ISBN 978–9949–19–339–4 (trükis)
ISBN 978–9949–19–340–0 (PDF)

Tartu Ülikooli Kirjastus
www.tyk.ee
Tellimuse nr. 160

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2. T. Metsvaht, M.-L. Ilmoja, Ü. Parm, L. Maipuu, M. Merila, I. Lutsar. Comparison of ampicillin plus gentamicin vs. Penicillin plus gentamicin in empiric treatment of neonates at risk of early onset sepsis. Acta Paediatr 2010; 99: 665-672
3. T. Metsvaht, H. Pisarev, M.-L. Ilmoja, Ü. Parm, L. Maipuu, M. Merila, P. Müürsepp, I. Lutsar. Clinical parameters predicting failure of empirical antibacterial therapy in early onset neonatal sepsis, identified by classification and regression tree analysis. BMC Pediatr 2009; 9:72
4. T. Metsvaht, M.-L. Ilmoja, Ü. Parm, M. Merila, L. Maipuu, P. Müürsepp, K. Julge, E. Sepp, I. Lutsar. Comparison of ampicillin versus penicillin in the empiric therapy of extremely low birth weight neonates at risk of early onset sepsis. (submitted to J Perinatol)

Degree of the applicant's personal contribution to the publications: Tuuli Metsvaht participated in study design, data collection, analysis and interpretation of the pharmacokinetic and clinical efficacy study, the papers are based on. She wrote the first drafts of the manuscripts and was responsible for the responses throughout the review process.

ABBREVIATIONS

$A_e\tau$	the amount of unchanged drug excreted into urine (A_e) during the dosing interval (τ)
$AUC_{0–12}$	area under the concentration-time curve over the dosing interval of 0 to 12 h
BPD	bronchopulmonary dysplasia
BW	birth weight
CI	confidence interval
CL	clearance at steady state
CL_R	renal clearance
CL_{Cr}	creatinine clearance
CoNS	coagulase negative staphylococci
CRP	C-reactive protein
CSF	cerebrospinal fluid
ELBW	extremely low birth weight (birth weight below 1000g)
EOS	early onset neonatal sepsis
ESBL	extended spectrum β-lactamase
GA	gestational age
GBS	group B streptococci
GFR	glomerular filtration rate
IAP	intrapartum antibiotic prophylaxis
IL6	interleukin 6
IL8	interleukin 8
IQR	interquartile range
I/T ratio	immature to total neutrophil count ratio
IVH	intraventricular haemorrhage
LFT	liver function tests (aminotransferases)
LOS	late onset neonatal sepsis
MBC	minimal bactericidal concentration
MIC	minimal inhibitory concentration
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
NPV	negative predictive value
OR	odds ratio
PCT	procalcitonin
PD	pharmacodynamic
PDA	patent arterial duct
PK	pharmacokinetic
PK/PD	pharmacokinetic/ pharmacodynamic
PNA	postnatal age
PPV	positive predictive value
RDS	respiratory distress syndrome
ROP	retinopathy of prematurity
SIRS	systemic inflammatory response syndrome

$T_{1/2}$	half-life
TNFα	tumour necrosis factor α
TPN	total parenteral nutrition
V_{ss}	volume of distribution at steady state
VLBW	very low birth weight (below 1500 g)
WBC	white blood count

1. INTRODUCTION

More than one third of the estimated four million neonatal deaths around the world each year are caused by severe infections and a quarter – around one million deaths – are due to neonatal sepsis and/or pneumonia alone (WHO 2009). In developed countries, where perinatal mortality is around 4–5‰, infections are the third common cause of neonatal death (Schrag *et al.* 2006b). In the developing world the burden is even higher. Neonatal mortality ranges from 17‰ in Latin America and the Caribbean to 42‰ in Africa and infections are the commonest cause of neonatal death responsible for about one third of all cases (Vergnano *et al.* 2005). Furthermore, the data reported for Africa and Asia are likely to underestimate the true dimensions of the problem, as in many countries a vast majority of neonates never see a doctor, in others births are registered only after 7 days, excluding those who have succumbed by that time.

Although the outcome of neonatal sepsis has significantly improved over the last decades in terms of survival (Bizzarro *et al.* 2005), it still remains an important risk factor of neonatal death, impaired neurodevelopment (Stoll *et al.* 1996, Ng 2004, Stoll *et al.* 2004, Silveira *et al.* 2008, Bassler *et al.* 2009, Cohen-Wolkowiez *et al.* 2009) and chronic lung disease (Lahra *et al.* 2009). Preterm very low birth weight (VLBW; birth weight < 1500 g) neonates with early onset sepsis (EOS) have three times higher mortality, compared to those without (37% vs 13%) (McGuire *et al.* 2004, Fanaroff *et al.* 2007); up to one third of survivors of EOS due to *Escherichia coli* (Jones *et al.* 2004) and 73% of neonates with invasive *Candida* infection manifest neurodevelopmental impairment (Benjamin *et al.* 2006).

Neonates, especially preterm VLBW babies are unique in their developmental physiology and clinical pharmacology (Paap *et al.* 1990, Allegaert *et al.* 2008). Clinicians have only recently realised the shortcomings of extrapolating adult and paediatric pharmacology to neonates – grey baby syndrome associated with chloramphenicol (Mulhall *et al.* 1983) and gasping syndrome associated with benzyl alcohol preservative use in neonates (Brown *et al.* 1982, CDC 1982) are just a few examples. Although recognition of the need for pharmacokinetic and pharmacodynamic studies has stimulated further research in neonates (Paap *et al.* 1990), many fields including antibacterial therapy remain inadequately covered.

Although antibacterial therapy is one of the key issues in the treatment of neonatal sepsis, evidence based data in the field are scarce (Mtitimila *et al.* 2004). Most of the prospective studies comparing the clinical efficacy of different regimens date from times more than 20 years ago (Snelling *et al.* 1983, Miall-Allen *et al.* 1988, Wiese 1988, Hammerberg *et al.* 1989) and in some antibiotics not even used in present neonatal practice, are studied (Miall-Allen *et al.* 1988). Furthermore, this means that no studies in specific populations like extremely low birth weight (ELBW; birth weight < 1000 g) neonates, whose survival has significantly improved only over these last few decades, have ever been preformed. The potential side effects of early antibiotic treatment on gut

colonisation and subsequent disease development in short- and long-term are not well understood yet.

Early gut colonisation and sepsis in neonates has been one of the research directions in the Institute of Microbiology of the University of Tartu over the last decades (Mandar *et al.* 1996, Sepp *et al.* 1997a, Sepp *et al.* 1997b, Bjorksten *et al.* 2001, Mandar *et al.* 2001, Sepp *et al.* 2003). The thesis relies on and carries forward this expertise and experience.

2. REVIEW OF LITERATURE

2.1. Terminology and incidence of neonatal sepsis

Neonatal sepsis is defined as systemic inflammatory response syndrome (SIRS) occurring in the presence of or as a result of suspected or proven infection (Goldstein *et al.* 2005).

Most authors accept that the time of presentation is associated with the likely source of infection in neonatal sepsis (Klein *et al.* 1990, Schrag *et al.* 2006b). Still, as exceptions occur, the respective terms are based on the timing alone. EOS, i.e. disease manifesting within the first few days of life, more likely results from vertical transmission of bacteria from the mother during the intrapartum period, whereas late onset sepsis (LOS) originates from community or nosocomial source (Vergnano *et al.* 2005, Mishra *et al.* 2006, Schrag *et al.* 2006b). However, the time limit between the two entities is obscure, a range between 48 h to 7 days of age has been suggested, with no consensus achieved yet (Ronnestad *et al.* 2005, Vergnano *et al.* 2005, Schrag *et al.* 2006b). The most frequently applied age limit of 48–72 h between EOS and LOS (Stoll *et al.* 2002a, McGuire *et al.* 2004) likely rises from the understanding that 90–95% of neonates with EOS due to group B streptococci (GBS) present with clinical signs within 24 h of life, another 4% between 24 to 48 h and only about 1% after 48 h of age (Bromberger *et al.* 2000, Schuchat *et al.* 2000, Society 2007).

The reported incidence of neonatal sepsis (including both EOS and LOS) varies from 3 per 1000 live births in Northern Europe (Tessin *et al.* 1990) and 6–9 per 1000 live births in the United States and Australasia (Daley *et al.* 2004, Cohen-Wolkowiez *et al.* 2009) to 6.5–23 in Africa and 7.1–38 in Asia (Vergnano *et al.* 2005, Tiskumara *et al.* 2009).

No population-based statistics about the incidence of neonatal sepsis in Estonia is available.

2.2. Bacterial aetiology of neonatal sepsis

The bacterial aetiology of neonatal sepsis is related to the timing of disease onset and has changed significantly over the last century (Klein 1990, Bizzarro *et al.* 2005). In the early 1900s, group A streptococci and *S. aureus* were the leading causes of neonatal and maternal peripartum infections (Dunham 1933, Bizzarro *et al.* 2005, Schrag *et al.* 2006b) but *Bacillus coli* sepsis was also reported occasionally (Dunham 1933). However, because of the relative infrequency at which blood cultures were drawn from young infants at that time the true incidence of neonatal sepsis most probably remained unknown (Dunham 1933). From early 1940s the role of Gram-negative pathogens increased, with *E. coli* becoming the predominant cause of neonatal sepsis for more than three decades, till 1970s or even 1980s (Pryse-Davies *et al.* 1979, Baker *et al.* 1990, Bizzarro *et al.* 2005).

2.2.1. Early onset sepsis

From 1970s and early 1980s the incidence of GBS disease increased going up from 0.1 to 0.5 and further to 2.5 per 1000 live births by early 1990s (Sjoberg *et al.* 1990, Isaacs *et al.* 1999). Concomitant increase in the overall incidence of neonatal sepsis was seen (Freedman *et al.* 1981, Baker *et al.* 1990, Persson *et al.* 2002). Since 1980s GBS have outnumbered all other EOS causative pathogens in most industrial countries (Vesikari *et al.* 1985, Tessin *et al.* 1990, Isaacs *et al.* 1999, Persson *et al.* 2002, Dahl *et al.* 2003, Schrag *et al.* 2006b), being responsible for 47–55% of EOS cases. Other frequent isolates include *E. coli* (14–23%), *S. aureus (*7%), coagulase negative staphylococci (CoNS; 5%), *Haemophilus influenzae* (4.5–8%) and enterococci (4–5%) (Gladstone *et al.* 1990, Persson *et al.* 2002, Bizzarro *et al.* 2005, Cohen-Wolkowiez *et al.* 2009). Other Gram-negative rods are very rare in EOS and include *Pseudomonas aeruginosa*, *Acinetobacter spp.* and *Citrobacter freundii* (responsible for about 1% of cases, each) (Bizzarro *et al.* 2005). *Listeria monocytogenes* infection has become extremely unusual, counting also for less than 1% of EOS cases in most populations (Freedman *et al.* 1981, de Louvois *et al.* 1992, Bizzarro *et al.* 2005). Fungi, mostly *Candida species*, have been isolated in 1–2% of EOS cases, especially in ELBW infants (Gerberding *et al.* 1989, Bizzarro *et al.* 2005).

Most recent changes in the bacterial aetiology of EOS have been reinforced by health care interventions over the last two decades. Intrapartum antibiotic prophylaxis (IAP) with ampicillin or penicillin G for the prevention of GBS disease, was first suggested in early 1980s (Boyer *et al.* 1983a, Boyer *et al.* 1983b, Boyer *et al.* 1983c) and has been routinely used since 1990s (AAP 1997, Mtitimila *et al.* 2004). IAP has reduced the rate of EOS due to GBS by 50–80% (Stoll *et al.* 1996, Chen *et al.* 2001, Moore *et al.* 2003, Daley *et al.* 2004, Chen *et al.* 2005, Trijbels-Smeulders *et al.* 2007) and related mortality by up to 60% (Lukacs *et al.* 2004). Associated trends in the incidence of EOS caused by other microorganisms have varied between countries and study centres. In Australia decrease in EOS due to GBS has been accompanied by concomitant decrease in *E. coli* sepsis (Isaacs *et al.* 1999, Daley *et al.* 2004), while in US increasing incidence of Gram negative EOS in VLBW neonates has been reported (Hyde *et al.* 2002, Stoll *et al.* 2002a, Stoll *et al.* 2005, Bizzarro *et al.* 2008), although this trend has not been uniformly confirmed (Baltimore *et al.* 2001, Sutkin *et al.* 2005). While Gram-positive cocci still prevail among term and near-term infants (Cohen-Wolkowiez *et al.* 2009), the predominance of Gram-negative rods in the aetiology of EOS among VLBW neonates has been confirmed in recent studies from developed countries (Ronnestad *et al.* 2005, Stoll *et al.* 2005, Bizzarro *et al.* 2008, Klinger *et al.* 2009).

The most frequent isolates in EOS in developing countries are *S. aureus*, *E. coli*, *Klebsiella* and *Streptococcus pyogenes* (Al-Zwaini 2002, Waheed *et al.* 2003, Osrin *et al.* 2004, Vergnano *et al.* 2005, Ojukwu *et al.* 2006, Trotman *et al.* 2006, Litzow *et al.* 2009, Zaidi *et al.* 2009).

Data about the aetiology of EOS in Estonia are scarce. In a previous study by our group, including 23 cases of EOS (defined as sepsis diagnosed within the first four days of life) in 1994, CoNS were the predominant isolates, responsible for 12 cases (52%), followed by *S. aureus*, *E. coli*, *E. aerogenes* and *C. freudii* (two cases each) and *E. faecalis* and *P. mirabilis* (one case each) (Sepp *et al.* 1997b).

2.2.2. Late onset sepsis

The bacterial aetiology of LOS differs from that of EOS, with CoNS, being the most frequent isolate, accounting for 39–66% of cases (Stoll *et al.* 2002b, Bizzarro *et al.* 2005, Hira *et al.* 2007, Cohen-Wolkowiez *et al.* 2009). Gram-negative rods are responsible for about 26–36% of cases, with *K. pneumoniae* isolated in 10%, *E. coli* in 6–9%, *Serratia* spp and *Enterobacter cloacae* both in 3% of cases, respectively (Stoll *et al.* 2002b, Bizzarro *et al.* 2005, Larson *et al.* 2005). The incidence of Gram-negative infections seems to be increasing in many neonatal intensive care units (NICU) (Nambiar *et al.* 2002, Toltzis 2003, Bizzarro *et al.* 2008). *Candida* spp have been isolated in about 10% of LOS cases (Bizzarro *et al.* 2005), with about 7–10% of ELBW neonates developing invasive *Candida* infections (Saiman *et al.* 2001, Benjamin *et al.* 2006). Significant variation between centres with rates ranging from 2–20% has been described (Cotten *et al.* 2006).

In developing countries Gram-negative rods account for 52–62% of LOS cases, with *Klebsiella sp.* being the most frequently isolated microorganism (27–34%), followed by *E. coli* (10–17%), *Acinetobacter* (9%) and *Pseudomonas aeruginosa* (6%) (Kumhar *et al.* 2002, Waheed *et al.* 2003, Couto *et al.* 2007b, Litzow *et al.* 2009). Other Gram-negative isolates account for less than 2–8% of cases, each, and include *Alcaligenes faecalis*, *Proteus*, *Serratia* and *Enterobacter spp*. Gram-positive microorganisms account for around 20–37% of cases with CoNS isolated in 8–21%, followed by *S. aureus* (4–24%), *Enterococcus spp* (2–5%) and GBS (1%) (Kumhar *et al.* 2002, Couto *et al.* 2007b). High prevalence of fungal infections has been described, with *Candida spp.* isolated in 11–18% of cases (Kapoor *et al.* 2005).

In Estonia one study of LOS in 247 neonates treated in the Paediatric and Neonatal ICUs of Tallinn Children's Hospital and Tartu University Clinics from March to November, 2000 has identified CoNS as the most frequent cause, followed by *K. pneumoniae* (Sepp *et al.* 2003).

2.3. Antibacterial resistance of early onset sepsis causative pathogens

GBS have been found uniformly susceptible to penicillins with little change over the last 50 years, including almost two decades of extensive IAP (Jones *et*

al. 1957, Baker *et al.* 1976, Chen *et al.* 2005, Trijbels-Smeulders *et al.* 2006, Persson *et al.* 2008, Panda *et al.* 2009). However, the minimal inhibitory concentration (MIC) of penicillin G is 4- to 10-fold greater (range 0.01–0.4 mg/L) for GBS than that for group A streptococci (Eickhoff *et al.* 1964, Anthony *et al.* 1975, Baker *et al.* 1976). More recently single genetic lineages of penicillin insusceptible strains have independently emerged in Japan through accumulating mutations in their penicillin binding protein genes (Dahesh *et al.* 2008, Kimura *et al.* 2008, Nagano *et al.* 2008).

The inoculum size of GBS has been shown to significantly affect the in vitro susceptibility to penicillins. For example if the inoculum size is increased from 10^4 to 10^7 colony forming units (CFU) per ml, the minimal bactericidal concentration (MBC) of ampicillin is increased from 0.06 to 3.9 mg/L (Feldman 1976, Weeks *et al.* 1981). These findings, although not tested in vivo, may have clinical correlates, as neonates with GBS meningitis may have initial cerebrospinal fluid (CSF) bacterial concentrations of 10^7 to 10^8 CFU per ml (Feldman 1976, Fujita *et al.* 1977) and achievable levels of penicillin G or ampicillin in CSF are about 10 to 20 percent of serum levels (Hieber *et al.* 1977, Lutsar *et al.* 1998).

Increasing resistance of GBS isolates to antibiotics, occasionally used for IAP in penicillin allergic women, like macrolides (resistance reaching 2–25% and 32% for erythromycin and azithromycin, respectively), clindamycin (resistance 1–21%, increasing), tetracycline (resistance 23%) and doxycycline (resistance 68%, increasing) has been noted (Persson *et al.* 2008, Panda *et al.* 2009).

The antibiotic resistance of *E. coli* in the era of wide spread IAP is of even more concern, as both Gram-negative infections and higher resistance are associated with increased mortality rates in neonates (Joseph *et al.* 1998, Schuchat *et al.* 2000, Laugel *et al.* 2003, Mayor-Lynn *et al.* 2005, Schrag *et al.* 2006b, Sehgal *et al.* 2007, Bizzarro *et al.* 2008). Up to 85% of Gram-negative EOS isolates are resistant to ampicillin in USA (Stoll *et al.* 2005, Schrag *et al.* 2006b). Intrapartum ampicillin use has been suggested as a risk factor of resistant *E. coli* sepsis (Laugel *et al.* 2003), although an independent association has not been confirmed in a large study applying multivariate analysis (Stoll *et al.* 2002a, Schrag *et al.* 2006a).

Although ampicillin resistance is relatively common among Gram-negative EOS isolates, the prevalence of gentamicin resistance has remained relatively low in most centres (Jones *et al.* 2004). In a study of neonatal Gram-negative blood stream infections in two NICUs in USA 23% of *E. coli* strains isolated from neonates were resistant to gentamicin (Larson *et al.* 2005).

Extremely high antibiotic resistance rates of neonatal Gram-negative pathogens have been reported in the developing world (Waheed *et al.* 2003, Litzow *et al.* 2009). In a systematic review 72% of *E. coli* isolates were found resistant to ampicillin, 78% to cotrimoxazole and 19% to third generation cephalosporins (Thaver *et al.* 2009). Among *Klebsiella species* almost all isolates were resistant to ampicillin, 45% to cotrimoxazole and 66% to third

generation cephalosporins (Couto *et al.* 2007b, Thaver *et al.* 2009). Resistance to gentamicin was relatively low among *E. coli* (13%) but much higher among *Klebsiella species*.

In a recent study of nosocomial blood stream isolates from two referral centres and one central hospital in Estonia 45% of invasive *E. coli* strains were resistant to ampicillin, 26% to ampicillin-sulbactam, 3% to 2nd and 3rd generation cephalosporins and 4% to gentamicin (Mitt *et al.* 2009). The antibiotic resistance of *Klebsiella* spp. ranges from 93% for ampicillin; 31% and 16% for 2nd and 3rd generation cephalosporins to 10% and 2% for gentamicin and amikacin, respectively (Mitt *et al.* 2009). Among neonatal *K. pneumoniae* isolates in Estonia the resistance rate ranges from almost 98% for ampicillin to about 1% for carbapenems; about 50–60% are resistant to 3rd generation cephalosporins and 70% to gentamicin (Sepp *et al.* 2003).

Generally most CoNS strains are believed to be penicillin resistant. Early in vitro studies have found a variable degree of resistance (at MIC values of 12.5 mg/L or greater) to penicillin and ampicillin in staphylococci, with some methicillin resistant strains being more sensitive to penicillin and to certain semisynthetic penicillins than to methicillin (Zygmunt *et al.* 1968). More recent studies of staphylococci from neonatal bloodstream infections have shown penicillin resistance as high as 86–97%, with mecA gene found in up to 87% of CoNS strains (Ronnestad *et al.* 1999, Hira *et al.* 2007). In a large study, comparing isolates from clinically significant infections in 18 centres from five European countries 60% of CoNS strains were oxacillin resistant (Biedenbach *et al.* 2009). Aminoglycoside resistance, mediated by genes encoding aminoglycoside modifying enzymes, has been shown to vary from 1% to arbekacin to 35–66% to gentamicin in CoNS strains (Klingenberg *et al.* 2004, Biedenbach *et al.* 2009). Gentamicin resistance is accompanied by methicillin resistance, with rates of 4% vs 91% described in methicillin susceptible and –resistant CoNS strains, respectively (Klingenberg *et al.* 2004). Multiresistance has been found in 77% of CoNS isolates, being more common in *S. haemolyticus* compared to *S. epidermidis* (92% vs 67%, respectively) (Hira *et al.* 2007). In Estonia 83% of invasive CoNS strains were found to be resistant to oxacillin and 76% to gentamicin (Mitt *et al.* 2009). The prevalence of methicillin-resistant strains among neonatal invasive and non-invasive CoNS isolates is 88% with 81% being resistant to gentamicin (Sepp *et al.* 2003).

2.4. Diagnosis of neonatal sepsis

The ideal diagnosis of neonatal sepsis is to solve two key issues – fast and sensitive identification of infants in need of prompt antibiotic therapy and identification of the pathogen to ensure adequate treatment. However, neither of the two is well met based on the presently applied consensus criteria of neonatal sepsis.

Sepsis is generally defined as SIRS in the presence of or as a result of suspected or proven infection (Bone *et al.* 1992a, Bone *et al.* 1992b). An International Paediatric Sepsis Consensus Conference has delineated six paediatric age groups, with age specific vital signs and laboratory variable values of SIRS symptoms suggested (Goldstein *et al.* 2005). In newborns, aged 0–7 days, at least two of the following criteria, one of which has to be abnormal temperature or white blood count (WBC), are required for the diagnosis of SIRS: core temperature > 38.5^0C or < 36.0^0C; tachycardia >180 beats per minute or bradycardia <100 beats per minute; tachypnea >50 breaths per minute, WBC >34 x 10^9 per L and/or systolic blood pressure <65 mmHg (Goldstein *et al.* 2005).

The main problem lies in the non-specificity but also variable severity of initial clinical signs and symptoms (Dawodu *et al.* 1985, Klein *et al.* 1990, Gerdes 1991, Franz *et al.* 1999b, Mtitimila *et al.* 2004). In addition, clinical picture may vary significantly depending on the population studied (term vs preterm neonates), time of infection (early vs late onset), underlying bacterial aetiology (GBS vs CoNS vs Gram-negative or fungi) etc. Therefore, the presently applied strategy to reduce morbidity and mortality in newborns with EOS involves the combination of perinatal risk factors, clinical signs and haematological and biochemical markers for early identification of babies at risk.

2.4.1. Pre- and perinatal features

A variety of prenatal features like the presence of chorionamnionitis, premature rupture of membranes, intrapartum fever or preterm delivery are associated with increased risk of EOS in the neonate (Yancey *et al.* 1996, Schuchat *et al.* 2000, Klinger *et al.* 2009). Preterm delivery, intrapartum fever, or membrane rupture >/=18 hours are found in 49% of EOS due to GBS and 79% of other sepsis (Schuchat *et al.* 2000); 30–43% of neonates with EOS are born preterm (Waheed *et al.* 2003, Bizzarro *et al.* 2005).

In most cases of intrauterine infection pathogens arise from the ascending route, from maternal vaginal flora, causing chorionamnionitis. The release of inflammatory cytokines, prostaglandins, metalloproteinases in response to bacterial endo- and/or exotoxins may result in premature rupture of membranes and preterm delivery (Garland *et al.* 2002, Mishra *et al.* 2006). Intrapartum treatment of amniotic infection (Sperling *et al.* 1987) and premature rupture of membranes (Magwali *et al.* 1999, August Fuhr *et al.* 2006) has been shown to reduce the incidence of sepsis in neonates.

However, early diagnosis of intra-amniotic infection is problematic, because clinical signs and symptoms like premature rupture of membranes or preterm labour are non-specific and tend to be late manifestations (Gibbs 1990). Fulminant intra-amniotic infection without labour and premature rupture of membranes may be caused by *Listeria monocytogenes* (Halliday *et al.* 1979). Other more specific signs like foul-smelling amniotic fluid and uterine

tenderness, occur only in a minority of cases (Koh *et al.* 1979, Gibbs *et al.* 1980, Gibbs 1990); occurrence of maternal and/or foetal tachycardia has varied widely between series (Schiano *et al.* 1984, Gibbs 1990). Blood cultures are positive in only about 10% of mothers with intraamniotic infection (Gibbs 1990) and maternal C-reactive protein (CRP) measurement does not have a high sensitivity in predicting underlying asymptomatic intra-amniotic sepsis (Giles *et al.* 2005). Histological chorionamnionitis (Rao *et al.* 2001) and amniotic fluid tumour necrosis factor-α (Park *et al.* 2004) have been found more specific. However, for measurement of interleukins, polymerase chain reaction (PCR) tests, or microbial cultures of the amniotic fluid amniocentesis is required and the results, like for placental histology, are often delayed (Mishra *et al.* 2006).

Foetal distress and birth asphyxia with low Apgar scores may be the first signs of infection in a neonate, present in about 50% of cases (Waheed *et al.* 2003, Mayor-Lynn *et al.* 2005, Shah *et al.* 2006). Meconium stained amniotic fluid has been associated with adverse foetal outcome, including increased rate of EOS (Berkus *et al.* 1994, Rao *et al.* 2001, Shah *et al.* 2006). While amniotic fluid itself is a poor culture medium for bacteria (Bratlid *et al.* 1978), even small amounts of meconium in amniotic fluid can encourage bacterial growth especially for *E. coli* and *L. monocytogenes* (Rao *et al.* 2001).

2.4.2. Clinical signs and symptoms in the neonate

The first clinical symptoms of sepsis in a neonate may be minimal and similar to those observed in non-infectious processes (Klein *et al.* 1990, Ng 2004). As characterised in an early study by EC Dunham: "the first evidences of illness were manifold and as a rule suggested rather that the infant was acutely ill than that septicaemia might be the cause of illness" (Dunham 1933).

The most prominent signs of EOS are usually respiratory distress (present in 33%), lethargy (25–50%), irritability (16–32%), fever (51–75%) or hypothermia (15%), apnoea (10–22%), jaundice (28–35%), hepatomegaly (25–33%), vomiting (25–50%), abdominal distension (17%), diarrhoea (11–17%) and skin manifestations like petechiae, abscesses and sclerema (Dunham 1933, Nyhan *et al.* 1958, Klein *et al.* 1990). Skin symptoms, though rare nowadays, have been described in up to 77% of neonates with sepsis in early series when the diagnosis was probably made at a later stage (Dunham 1933). In a more recent series of 3303 infants, studied in developing countries, i.e. Ethiopia, Gambia, Papua New Guinea and the Philippines, Weber *et al.* identified a similar list of 14 signs or symptoms independently associated with severe disease, defined as sepsis, meningitis, hypoxemia or pneumonia: reduced feeding ability (prevalence 17%); no spontaneous movement (11%); body temperature >38°C (19%); drowsy/ unconscious (7%); history of feeding problem (16%); history of change of activity (21%); agitation (4%); digital capillary refill more than 2 sec (11%); lower chest wall in drawing (14%); respiratory rate > 60 per min (23%);

grunting (2%); cyanosis (4%); history of seizure (4%); bulging fontanel (2%) (Weber *et al.* 2003).

The wide list of signs and symptoms reflects well the diversity of the clinical picture of neonatal sepsis, as well as the fact that any individual sign by itself has only a limited value. The presence of any one of the signs, listed above, had a sensitivity of 87% and a specificity of 54% in predicting severe infection. Increasing the number of clinical signs required, caused major loss of sensitivity without significantly improving specificity in this analysis (Weber *et al.* 2003), pointing that application of strict rules for the diagnosis of neonatal sepsis in clinical practice carries high risk of missing several cases. For study purposes, requiring a more precise case definition, the presence of at least two clinical signs in combination with laboratory findings has been used (Auriti *et al.* 2005). The presence of three or more clinical signs has been found to have the best predictive accuracy for a positive blood culture (Modi *et al.* 2009).

Hemodynamic changes have been extensively studied as a possible early sign of sepsis in neonates. Pale or greyish skin colour, cyanosis or mottled skin and prolonged capillary refill have all been noted as signs of disturbed microcirculation. In a series of 17 newborns developing septic shock due to Gram-negative bacilli Töllner *et al.* has given the following description: "A skin color fading and changing from reddish-pink to yellow-green was the most early noticeable clinical symptom in all patients" (Tollner *et al.* 1976). Despite attempts of quantification of such hemodynamic response, no clinically applicable tools have been developed. Owing to a limited number of research studies in the very young, the hemodynamic response of premature infants and neonates is not well understood; the presenting hemodynamic abnormalities are more variable than in older children and adults (McKiernan *et al.* 2005). In comparison with adults, children more often present in a non-hyperdynamic circulatory state with decreased cardiac output and increased systemic vascular resistance; blood pressure has been found a poor indicator of systemic blood flow in neonates (Luce *et al.* 2007).

Over recent years a more mathematical approach to the analysis of hemodynamic indices based on computerised algorithms has emerged (Griffin *et al.* 2001, Lake *et al.* 2002, Griffin *et al.* 2003, Cao *et al.* 2004). Griffin *et al.* have analysed heart rate variability and asymmetry of frequency histograms of RR intervals and have shown significant difference between the values of skewness in infants subsequently developing sepsis or sepsis like illness already two to three days before sepsis (Griffin *et al.* 2001, Griffin *et al.* 2005). However such approach requires sophisticated analysis, not readily available in most NICUs.

2.4.3. Laboratory markers of inflammation

Lack of reliable clinical signs and symptoms has prompted a widespread search for additional laboratory markers for the diagnosis neonatal sepsis.

2.4.3.1. Haematological tests

Haematological tests alone or in various combinations, described in the early series already (Dunham 1933) have been the main adjunct diagnostic measure for decades. However, they have not proven sensitive enough as a single guide of treatment decisions in neonates at risk of sepsis. WBC, total and immature neutrophil count, immature to total neutrophil ratio (I/T ratio), immature to mature neutrophil ratio, morphological or degenerative changes in neutrophils (like vacuolization and toxic granulation) and platelet count have all been studied (Manroe *et al.* 1977, Manroe *et al.* 1979, Philip *et al.* 1980, Philip 1982, Engle *et al.* 1984, Gerdes *et al.* 1987, Rodwell *et al.* 1988). Systematic reviews of clinical studies (Gerdes 1991, Da Silva *et al.* 1995, Fowlie *et al.* 1998, Ng 2004) have underlined the higher sensitivity of neutrophil ratios, ranging from 58 to 90% with the cut-off point of 0.2 for I/T ratio; while WBC and neutrophil counts tend to have higher specificity – ranging from 81 to 98% for WBC of $< 5 \times 10^9$ /L or $> 20 \times 10^9$ /L and from 61 to 92% for neutropaenia $< 1{,}75 \times 10^9$ /L. However, the corresponding specificity for I/T ratio has varied between 31–95% and the corresponding sensitivity for WBC and neutrophil counts between 17–87% (Gerdes 1991, Da Silva *et al.* 1995). Several scoring systems including different WBC counts and indices have been developed to improve accuracy (Gerdes *et al.* 1987, Rodwell *et al.* 1988), but have not been widely adopted in clinical practice because of their unfavourable diagnostic values, complexity of the scoring method and high personnel training and labour requirements (Ng 2004). A wide inter-observer variability in the identification of immature or 'band' neutrophils has been demonstrated (Schelonka *et al.* 1995).

The individual finding of a clinical blood count with the highest positive predictive value (PPV) is total WBC count of less than 5.0×10^9/L; if present, there is a post-test probability of sepsis of approximately 10% to 20% (Fowlie *et al.* 1998). Such risk has been suggested to justify antibiotic treatment even in a well-appearing infant after a full diagnostic workup (Society 2007). However, only between 22% and 44% of infants with sepsis will have such a low total WBC count (Fowlie *et al.* 1998). A more recent study in 1665 healthy term infants at risk of sepsis (Ottolini *et al.* 2003) showed a PPV of 1.5% of an 'abnormal' clinical blood count, defined as WBC $\leq 5.0 \times 10^9$/L or $\geq 30 \times 10^9$/L or an absolute neutrophil count $< 1.5 \times 10^9$/L or an immature to mature neutrophil ratio > 0.2, in identifying the development of clinical sepsis. Of note, none of these infants developed a positive blood culture.

Platelet count $< 150 \times 10^{12}$ /L, often described as a late sign of sepsis (Ng 2004), has been found to have even lower diagnostic accuracy, with sensitivity ranging from 3 to 61% and specificity from 78 to 99% (Gerdes 1991, Fowlie *et al.* 1998). A wide variety of conditions, present at birth, including chronic intrauterine hypoxia, maternal preeclampsia and increased pulmonary platelet consumption in neonatal respiratory distress syndrome, may cause similar findings (Ng 2004).

Activation of the clotting and fibrinolytic systems has been described in both adults and neonates with severe infection (Ng 2004, Lauterbach *et al.* 2006). Circulating thrombin-antithrombin III complex, plasminogen activator inhibitor-1, plasminogen tissue activator, fibrinogen and D-dimer concentrations are significantly raised in infected compared to non-infected patients (Mautone *et al.* 1997, Ng 2004). However, preterm neonates with respiratory distress syndrome (RDS) may also develop deranged coagulation and fibrinolysis values.

2.4.3.2. Acute phase proteins: C-reactive protein, procalcitonin and others

Acute phase proteins are produced by the liver as part of an immediate inflammatory response to infection or tissue injury (Ng 2004). The most extensively used and studied is CRP, a protein with a half life of 19 hours, synthesized within six to eight hours of exposure to infection or tissue damage (Kushner *et al.* 1978). Although CRP has higher sensitivity and specificity than total neutrophil count and I/T ratio, the relatively slow response time allows only about 16–60% sensitivity at the time of initial sepsis evaluation (Mathers *et al.* 1987, Gerdes 1991, Fowlie *et al.* 1998, Ng 2004). Serial measurements at 24 and 48 hours after the onset of illness have been found to improve sensitivity to 82–92% (Mathers *et al.* 1987, Ng 2004), however, the specificity and PPV of CRP range from 41–100% and 13–100%, respectively (Gerdes 1991, Fowlie *et al.* 1998, Ng 2004). A variety of pre- and perinatal conditions, like premature rupture of membranes, maternal fever and chorionamnionitis, foetal distress, low 1st minute Apgar score, need for intubation in delivery room, gestational diabetes and maternal drug abuse (Ainbender *et al.* 1982, Forest *et al.* 1986, Schouten-Van Meeteren *et al.* 1992, Chiesa *et al.* 2003b), as well as postnatal problems, like meconium aspiration, tissue necrosis and surgery (Ng 2004), have been found to increase CRP at or immediately after birth. Life-threatening infections, like fungal meningitis, without elevated CRP levels, probably due to the localized chronic low grade nature of the infection, have been described (Ng 2004). In contrast, CRP has proven a useful guide for determining the response to antibacterial therapy and the duration of treatment (Sann *et al.* 1984, Gerdes 1991, Philip *et al.* 2000).

Another acute phase marker that has gained much attention more recently is procalcitonin (PCT). Although the exact site of PCT synthesis is not known, monocytes and hepatic cells are believed to be potential sources (Dandona *et al.* 1994). In sepsis PCT hyper secretion probably emanates from multiple tissues throughout the body (Becker *et al.* 2009). Serum PCT concentrations begin to rise four hours after exposure to bacterial endotoxin, peak at six to eight hours and remain raised for at least 24 hours (Ng 2004). The serum values correlate with disease severity; moreover, administration of PCT to septic animals has been found to increase mortality, suggesting its role in the pathogenesis of tissue damage (Becker *et al.* 2009). The prognostic value early in life, however,

is hampered by a mild physiological increase in serum PCT levels, from < 0.08 ng/ml at birth to 0.6 ng/ml at about 24 hours, most probably due to fast colonisation of the gastrointestinal tract and translocation of bacterial endotoxin through the bowel wall (Ng 2004). PCT diagnostic profile in neonatal sepsis has been claimed to be superior to that of other acute phase reactants, including CRP, with sensitivity and specificity ranging from 80–100% in term neonates (Chiesa *et al.* 1998, Ng 2004, Bender *et al.* 2008, Spada *et al.* 2009). However, although the mild physiological elevation can probably be differentiated from bacterial cause of PCT, specific cut-off values for the diagnosis of early-onset neonatal infection are required at each evaluation time point over the first 48 h of life (Chiesa *et al.* 2003b, Turner *et al.* 2006a). Studies in preterm neonates have yielded more conflicting results with suggested cut-off limits ranging from 0.5 to 2.3 ng/ml and the corresponding sensitivity and specificity varying from below 50 to 92% and 63 to 97%, respectively (Vazzalwar *et al.* 2005, Turner *et al.* 2006b, Spada *et al.* 2009). False negative cases have been reported (Lapillonne *et al.* 1998, Spada *et al.* 2009) and elevated serum levels have been detected in various perinatal conditions, like birth asphyxia, chorionamnionitis or preeclampsia (Assumma *et al.* 2000, Chiesa *et al.* 2003a, Chiesa *et al.* 2003b) and in patients with respiratory distress syndrome, hemodynamic failure and severe trauma without bacterial infection (Ng 2004). The added value of PCT when combined with other markers of sepsis like interleukin 6 (IL6) (Bender *et al.* 2008) or interleukin 10 and nCD64 has been suggested (Zeitoun *et al.*). PCT has proven useful in identifying neonates in whom antibiotic therapy can be limited to 72 h, i.e. those not developing EOS (Stocker *et al.* 2009).

Many other acute phase reactants, like α1 antitrypsin (Suri *et al.* 1991), fibronectin (Gerdes *et al.* 1983, Gerdes *et al.* 1987), haptoglobin (Salmi 1973, Speer *et al.* 1983), lactoferrin (Scott 1989, Thomas *et al.* 2002), neopterin (Jurges *et al.* 1996) and orosomucoid (Sann *et al.* 1984), have been evaluated in relation to neonatal sepsis. Although serum levels have been found to differ in neonates with and without infection, slow response time, poor response in specific infections (like orosomucoid in GBS infection) and inferior diagnostic accuracy in relation to already existing diagnostic tests have limited their clinical use (Gerdes 1991, Ng 2004).

2.4.3.3. Chemokines, cytokines, adhesion molecules and components of the immune pathway

In the mid and late 1990s a group of intercellular messengers has been extensively studied in an attempt to find earlier and more sensitive markers of infection, than the acute phase reactants, used so far. In neonatal sepsis most attention has focused on IL6, interleukin 8 (IL8) and TNFα.

Umbilical cord IL6 level has been identified as an excellent marker of early onset neonatal infection with a sensitivity of 87–100% and a negative predictive

value (NPV) of 93–100% in a number of studies (Smulian *et al.* 1997, Krueger *et al.* 2001, Hatzidaki *et al.* 2005). The diagnostic accuracy still probably depends on the sensitivity of the assay method (Ng 2004), as less promising results have been described in some earlier studies (Lehrnbecher *et al.* 1995, Lehrnbecher *et al.* 1996). Another problem is the very short half life of IL6, leading to undetectable levels in most infected patients within 24 hours (Buck *et al.* 1994). Correspondingly the sensitivity at 24 and 48 hours is reduced to 67% and 58%, respectively (Ng *et al.* 1997, Ng 2004). Concomitant measurement of CRP as a late but specific, and IL6 as an early and sensitive marker has yielded better performance than either marker alone (Buck *et al.* 1994). Kuster *et al.* have found elevated levels of circulating IL6 and IL1 receptor antagonist for up to 2 days before the clinical diagnosis of neonatal sepsis (Kuster *et al.* 1998).

The diagnostic and kinetic properties of IL8 are similar to those of IL6 (Ng 2004). The response pattern appears not to be affected by gestational age (GA), with elevated levels seen in both term and preterm neonates with infection. The reported sensitivity of 80–91% and specificity of 76–100% can be further improved by concomitant measurement of CRP or neutrophil cell surface marker CD11b (Franz *et al.* 1999a, Franz *et al.* 1999b, Nupponen *et al.* 2001). Application of IL8 in combination with CRP and/or WBC (leucopaenia) and elevated I/T ratio has been shown to reduce the number of term and near term neonates, considered to require antibiotic therapy, by about 14% without increasing the risk of missing any cases (Franz *et al.* 1999b; 2001). However, the present studies involve mostly stable infants; where there is time to wait for laboratory results.

The usefulness of TNFα as a diagnostic marker of neonatal sepsis has not been as good as that of IL6 or IL8 (Dollner *et al.* 2001, Santana *et al.* 2001, Ng 2004). Similarly elevated serum levels of other cytokines (interleukin1β, soluble interleukin 2 receptor, interferon γ) and adhesion molecules (ICAM-1, VCAM-1, E-selectin etc) and complement activation products (C3bBbP, sC5b-9, C3a-desArg) have been found during sepsis, but none of these markers has fulfilled the criteria to be considered suitable for clinical application in newborns (Ng 2004).

Granulocyte colony stimulating factor has been proposed as a reliable infection marker for early diagnosis of neonatal sepsis (Gessler *et al.* 1993, Kennon *et al.* 1996), however is not routinely available in most NICUs. The cut off value of 200 pg/ml has a sensitivity of 95% and a negative predictive value of 99% for predicting early neonatal bacterial and fungal infections (Kennon *et al.* 1996).

2.4.4. Identification of bacterial aetiology

Based on the definition of sepsis as SIRS in the presence of or as a result of suspected or proven infection, the identification of infection is equally important. Knowing the aetiology offers also the possibility of timely adequate

antibacterial therapy. The role of identifying the bacterial aetiology in the diagnosis of neonatal sepsis was recognised by EC Dunham as early as in 1933: "Many cases of septicaemia are overlooked or are diagnosed only when localised lesions appear unless blood cultures are made in all cases of obscure illness in the neonatal period." (Dunham 1933).

Superficial cultures like surface swabs (including ear, umbilical, axillar, nasal, nasopharyngeal and rectal swabs) and gastric aspirate cultures have been extensively studied in the identification of the bacterial aetiology of neonatal infections. The sensitivity has ranged from 48–88% for gastric aspirate cultures to 78–100 for ear swab cultures, with the corresponding specificities of 61–100% and 40–90%, respectively (Fowlie *et al.* 1998). Some studies have used the presence of polymorphonuclear leukocytes in smear light microscopy to improve diagnostic accuracy. For surface swab assessment, the EOS likelihood ratios range from 33.6 (2.1 to 519.8) for a positive gastric aspirate culture to 0.08 (0.006 to 1.12) for microscopy of ear swab material that does not show any neutrophils, reflecting only a limited value in the diagnosis of infection in neonates (Fowlie *et al.* 1998).

Deep site cultures, i.e. isolation of bacteria from a normally sterile body fluid has remained the gold standard of the diagnosis of neonatal sepsis (Ng 2004). However, up to 1/3 of positive blood cultures taken from term or near-term neonates yield non-typable Gram-positive cocci, likely reflecting contamination rather than infection (Cohen-Wolkowiez *et al.* 2009). On the other hand positive blood culture rates ranging from 8% to 73% have been reported in neonates with clinical signs suggestive of EOS (Nupponen *et al.* 2001, Buttery 2002, Chiesa *et al.* 2003b, Mishra *et al.* 2006). An additional drawback of culture based diagnosis is the 24–48 hour assay time (Mishra *et al.* 2006).

The reasons for negative blood cultures in neonates with EOS are multiple and include maternal antibiotic prophylaxis, small amounts of blood available for culturing and low counts of bacteria still leading to serious disease in relatively immunocompromised hosts like neonates. A recent prospective multicentre surveillance of 107,021 deliveries with IAP applied, found a prevalence of probable GBS sepsis exceeding that of proven (0.47 vs 0.39 per 1000 live births respectively) with no difference in the severity of the clinical picture (Carbonell-Estrany *et al.* 2008). Infants with probable GBS sepsis, i.e. with no positive deep site cultures, were significantly more likely to be born to mothers who had received either complete or partial course of IAP. Heimler has also found lower rate of positive blood cultures in infants born to mothers who have received IAP compared to those who have not (Heimler *et al.* 1995).

Strategies applied in adults to improve diagnostic accuracy of blood cultures, i.e. increasing the amount of blood cultured and taking multiple cultures from different sites simultaneously, are not readily applicable in neonates. The amount of blood available for culturing in a neonate is very limited – a volume of 4 ml for repeated culturing would mean about 7% blood loss in a neonate

with a birth weight (BW) of 600 g. Therefore blood culture volumes of 0.5 ml have been considered adequate in neonates with bacterial loads exceeding 10 CFU/ml (Brown *et al.* 1995, Connell *et al.* 2007) with still a significantly better yield achievable when volumes exceed 1 ml (Sarkar *et al.* 2006). In vitro blood culture volumes exceeding 0.5 ml have proven inadequate for sensitive and timely detection of bacteraemia with colony counts less than 4 CFU/ml (Schelonka *et al.* 1996), which is likely the case in many neonatal infections. In clinical studies about 60% of neonates and children with positive blood cultures have low level bacteraemia with pathogen counts less than 11 CFU/ml (Kellogg *et al.* 1997, Kellogg *et al.* 2000). A total blood culture volume of 2–6 ml is required to improve diagnostic accuracy in sepsis with low CFU counts (Schelonka *et al.* 1996, Kellogg *et al.* 2000). Simultaneous culturing from multiple sites does not ensure better yield of pathogens in initial evaluation of neonatal sepsis (Sarkar *et al.* 2006), although has been shown to improve sensitivity for follow-up of bacterial eradication during antibiotic therapy (Sarkar *et al.* 2007). Blood cultures taken before administration of antibiotics have a better yield rate, however neonates with high risk of quick deterioration tend to have less cultures taken prior to antibiotic administration (Connell *et al.* 2007).

The above discussed shortcoming of routine bacterial cultures have prompted a search for new **molecular techniques** to quickly point out infants with sepsis and identify the bacterial aetiology (Reier-Nilsen *et al.* 2009). For fast bacterial antigen detection target genes allowing reliable discrimination between bacterial species like 16S or 23S ribosomal RNA or the intervening spacer region have been used (Dark *et al.* 2009). Two technologies have been developed: (1) real-time PCR, in which amplified segments of DNA are being monitored quantitatively by fluorescent dyes or labelled hybridization probes and (2) DNA microarrays, in which labelled ribosomal RNA or genomic DNA is detected by hybridization with specific DNA probes spotted on a solid phase (Struelens 2009).

In adult studies the first commercially available test SeptiFast™ allowing detection of 25 bacterial and fungal species has shown a sensitivity of 60–95% and specificity of 74–83% in hemato-oncology, emergency and critical care settings. Although the concordance between multiplex PCR and blood cultures is moderate (Struelens 2009), in a recent multicentre study the clinical relevance of blood samples positive for bacterial PCR has been confirmed by correlation with disease severity (Bloos *et al.* 2009).

PCR studies in neonates have yielded the sensitivity, specificity, PPV, NPV and positive and negative likelihood ratios of 42–100%, 88–98%, 64–95%, 75–99%, 26.1, and 0.04, respectively (Jordan *et al.* 2006, Ohlin *et al.* 2008, Dutta *et al.* 2009, Reier-Nilsen *et al.* 2009) with the lower accuracy reported for plasma and higher for whole blood samples. Real-time PCR technique has been shown to allow fast discrimination between *S. aureus* and CoNS strains in blood cultures positive for clustered Gram-positive cocci on direct smear examination (Ruimy *et al.* 2008). However, the number of PCR negative/ culture positive cases has been a problem in a number of neonatal studies (Jordan *et al.* 2006,

Ohlin *et al.* 2008). Accuracy has been found to depend on material selection (plasma vs whole blood, heel prick vs sterile sample collection) and possibly bacterial load (Jordan *et al.* 2006). No study has demonstrated the clinical benefit of PCR guided antibiotic therapy so far (Struelens 2009).

Another approach includes identification of activation patterns of multiple biochemical markers for fast pathogen identification (Kingsmore *et al.* 2008), however none have come into clinical application yet.

As GBS has been the predominant pathogen in EOS, several specific tests for fast GBS antigen detection were developed in early 1990s. However, significant variation in the sensitivity (but not in the specificity) of the commercially available latex agglutination tests for identification of GBS in urine has been described (Ascher *et al.* 1991a, Greenberg *et al.* 1995); sensitivity and specificity as high as 88–90% and 70–98% has been reported in some studies (McIntosh *et al.* 1992, Williamson *et al.* 1995). Although negative test has proven useful in excluding GBS disease (NPV of 99%), the false positive rate has been found unacceptably high (30%) with a PPV of 12% (McIntosh *et al.* 1992, Williamson *et al.* 1995). Contamination of urine bag specimens with GBS from perineal and rectal colonization may produce positive test results without any sign of systemic infection (Sanchez *et al.* 1990), although this has not been confirmed by all studies (Harris *et al.* 1989). False positive urine tests have been observed in patients infected with other bacterial pathogens, for example *Proteus mirabilis* (Ingram *et al.* 1982). Maternal antibiotic treatment during labour has been suggested as an important cause of apparent false-positive results (Harris *et al.* 1989), however the final interpretation of positive results with a concomitant negative blood culture has remained controversial (Ascher *et al.* 1991b). Thus these tests have proven only limited value in clinical application and are not in routine use in most NICUs anymore.

2.5. Pharmacokinetics and safety of antibiotics in neonates

Neonatal drug dosing needs to be based on the physiological characteristics of the newborn and the pharmacokinetic (PK) parameters of the drug (Alcorn *et al.* 2003). Size-related changes can in part be modelled based on allometry and relate to the observation that metabolic rate relates to weight by a kg 0.75 trend (Allegaert *et al.* 2008). However, the PK of a drug in a neonate is not only an issue of size modelling.

2.5.1. General characteristics

Differences in body composition and ontogeny are most prominent in neonates (Allegaert *et al.* 2008). The body fat content is markedly lower and the body water content is markedly higher in neonates compared with adults and older

children (Alcorn *et al.* 2003, Allegaert *et al.* 2008). These findings have an impact on the Vd of both lipophilic and hydrophilic drugs (Paap *et al.* 1990). Altered protein binding due to different plasma protein profile (McNamara *et al.* 2002, Alcorn *et al.* 2003), competitive inhibition from endogenous compounds and differences in binding affinity also affect unbound concentrations and Vd (Paap *et al.* 1990). Until adult metabolic activity has been reached, hepatic isoenzyme-specific maturation and maturation of CL_R significantly contribute to differences in drug metabolism (de Wildt *et al.* 1999a; b, Alcorn *et al.* 2002a; b, Allegaert *et al.* 2008). Other covariables, like genetic polymorphisms, co-administration of drugs, first pass metabolism and disease characteristics further increase the interindividual variability in neonatal drug disposition (de Wildt *et al.* 1999a; b, Alcorn *et al.* 2002b; a, Allegaert *et al.* 2008). In addition several changes such as perfusion failure and capillary leakage, changes in hepatic metabolism and renal excretion, impairment of the gastrointestinal system and lung injury, all of which have influence on the pharmacokinetic/ pharmacodynamic (PK/ PD) characteristics of a drug, may occur in sepsis (Lutsar *et al.* 2010b).

2.5.2. Penicillins

A β-lactam antibiotic combination with an aminoglycoside has remained the cornerstone of empiric treatment in neonatal sepsis. Traditionally, the pharmacodynamics (PD) of β-lactams is determined by the fraction of time above MIC (f%T>MIC) with the therapeutic target of 50–100% suggested for immunocompromised hosts like neonates (de Hoog *et al.* 2005, Lutsar *et al.* 2010b). As penicillins are water soluble compounds mainly eliminated by the kidneys, significant differences from adult drug disposition can be expected in neonates due to higher body water content as well as lower glomerular filtration (GFR) and tubular secretion rate (Paap *et al.* 1990).

2.5.2.1. Penicillin G

The first data about the PK of penicillin G in neonates date from the late 1940s and early 1950s (Barnett *et al.* 1949, Huang *et al.* 1953). Linear PK has been described with an intramuscular dose of 22,000 IU/kg producing mean peak serum levels of 21.6 mg/L and 13.7 mg/L in term and preterm neonates, respectively (Huang *et al.* 1953), while administration of 50,000 IU/kg results in peak concentrations as high as 50–100 mg/L (Abramowicz *et al.* 1966). A more detailed study of penicillin G PK profile in neonates by McCracken *et al.* enlightened age-related differences and presented time-concentration curves (McCracken *et al.* 1973). In that study of 10–30 mg/kg per dose administered intramuscularly to 40 term and preterm neonates they showed that the $T_{1/2}$ of penicillin G in neonates exceeded that of adults and older children by more than

threefold with significant decrease from 3.2 h to 1.4 h over the first three weeks of life. The $T_{1/2}$ values of penicillin G were found to be independent of BW and dosage. The study included a few preterm neonates; however, no details about the GA of the study population were given. Considering the study time – early 1970s – it is unlikely that the population most distinct from the point of PK features, i.e.VLBW neonates, were included.

About 30% of the administered dose was excreted in urine within the following 8–12 h. In contrast to adults, in whom tubular secretion is the primary mechanism of penicillin G excretion into urine, with less than 10% excreted by GFR (Chambers 2005), in neonates penicillin G excretion in urine was correlated with creatinine clearance (CL_{Cr}), suggesting GFR as the predominant mechanism of penicillin G renal excretion (McCracken *et al.* 1973). The likely explanation lies in the relative immaturity of tubular function in neonates, improving significantly over the first weeks of life only.

Other elimination routes have not been studied in neonates, but adult studies have shown about 0.09–0.12% biliary recovery of parenterally administered dose (Acocella *et al.* 1968, Brogard *et al.* 1979) and in animal studies the penicillin G concentration in caecal fluid after i.v. administration remains below 0.6 mg/L (Horspool *et al.* 1995).

Penicillin G levels in foetal serum are 0.26–0.7 of the maternal levels (Wasz-Hockert *et al.* 1970, Charles 1977).

Penicillin G has a relatively favourable side effect profile with no serious adverse events described in neonatal studies (McCracken *et al.* 1973, Snelling *et al.* 1983, Haffejee 1984, Hall *et al.* 1988, Gokalp *et al.* 1990). Allergy occurs in 1–10% of the general population, but in the majority of cases is limited to skin reaction; only about 0.01% (15–40/100,000) of treated patients develop anaphylaxis (Kerr 1994, Karabus *et al.* 2009) with fatal outcome occurring in about 1.5–2/100,000 treated patients (Kerr 1994). Serious immediate reactions are mediated by IgE antibodies and can feature urticaria, angioedema, bronchospasm, laryngeal oedema, hypotension and cardiac arrhythmias (Karabus *et al.* 2009). True sensitivity reactions are rare in children and hardly ever occur in neonates (Le *et al.* 2006). The explanation lies probably in the immature immune system of the neonate and lack of prior sensitisation.

Another potentially serious adverse event is penicillin G seizure inducing activity, widely used in animal models of epilepsy (Fisher 1989). Human populations at risk of penicillin G induced seizures include those with impaired renal function, infants and the elderly, patients with meningitis or with a history of seizures (Barrons *et al.* 1992). At extremely high penicillin G serum concentrations (around 100 mg/L) seizures have been described in adults without prior risk factors (Raichle *et al.* 1971). Prolongation of bleeding time due to disturbance of platelet aggregation, increase of antithrombin III activity and inhibition of factor Xa activity have all been described after high dose penicillin G administration (40 million IU/day) in patients with normal GFR and after normal dose administration with pre-existing coagulation abnormalities, including uraemia (10 million IU/day) (Andrassy *et al.* 1976).

2.5.2.2. Ampicillin

Similar to that of penicillin G, ampicillin has linear PK with serum peak concentrations ranging from 16–60 mg/L after ampicillin doses of 5–25 mg/kg (Grossman *et al.* 1965, Axline *et al.* 1967, Boe *et al.* 1967) and doses of 50–100 mg/kg resulting in mean serum concentrations of 104 to 204 mg/L, respectively (Kaplan *et al.* 1974). Kaplan *et al.* found consistently higher peak serum concentrations in preterm neonates receiving large doses of ampicillin (50–100 mg/kg) compared to term infants and suggested a different V_d for ampicillin compared to penicillin G in premature infants (Kaplan *et al.* 1974). Later studies have shown that V_d alone cannot account for the peak concentrations reported. V_d is more closely correlated with postnatal age (PNA) than with GA; conversely, CL is significantly greater in full term than in preterm infants (Kaplan *et al.* 1974, Paap *et al.* 1990). Thus lower CL is the likely primary reason for high peak concentrations in premature neonates (Paap *et al.* 1990).

$T_{1/2}$ of ampicillin is inversely related to GA and PNA, being around 4 h in the first week of life in full-term neonates and decreasing to 1.6 h thereafter (Kaplan *et al.* 1974). Early studies in larger preterm neonates have yielded similar results with $T_{1/2}$ of ampicillin around 4–5.5 h during the first week of life, decreasing to 2.8 h in the second week and 1.7 h by 15–30 days of age (Axline *et al.* 1967, Paap *et al.* 1990, Pacifici *et al.* 2009). A more profound prolongation of steady state serum $T_{1/2}$ to around 9.5 h is seen in very preterm neonates with gestational age of 26–33 weeks (Dahl *et al.* 1986).

An average of 19–79% of the administered ampicillin dose is excreted in urine within 12 h in neonates, with significant correlation between the fraction of dose excreted and CL_{CR} (Kaplan *et al.* 1974). In animal and adult studies partial biliary excretion has been demonstrated. About 0.1–2.8% of the administered ampicillin dose is recovered in bile and the biliary concentrations (bile C_{max} 471 mg/L 0.5–1h after i.v. administration of 1g of ampicillin and 0.5g of sulbactam; C_{mean} 15.9 mg/L in gallbladder bile after the same dose given immediately prior to elective cholecystectomy) exceed significantly the MIC of most pathogens for several hours (Pinget *et al.* 1976, Morris *et al.* 1986).

Ampicillin concentration in CSF in 8 neonates with bacterial meningitis ranged from 1 to 28 mg/l (11–65% of the simultaneous serum levels) with highest individual values seen 3–7 h after administration (Kaplan *et al.* 1974). The mean peak concentrations of ampicillin in CSF at 2 and 6 h after administration were 13.6 and 15.2 mg/l, respectively.

Ampicillin crosses the placenta more readily than penicillin G with comparable concentrations seen in maternal and foetal sera within 60–90 minutes after administration (foetal to maternal serum concentration ratio 0.2–2.5) (Bray *et al.* 1966, Nau 1987).

Ampicillin, when parenterally administered, is a relatively safe drug. No serious adverse events described in neonatal studies (Marks *et al.* 1978, Hammerberg *et al.* 1989, Gokalp *et al.* 1990, Umana *et al.* 1990, de Louvois *et al.*

1992). Non-specific rashes, urticaria and mild eosinophilia have rarely been reported (McCracken *et al.* 1990) and enteral administration has been associated with diarrhoea and candidiasis (McCracken *et al.* 1990). A potential for central nervous system irritability or crystalliuria with extremely large doses has been suggested (Potter *et al.* 1971) but not confirmed in clinical studies (Kaplan *et al.* 1974, McCracken *et al.* 1990).

2.5.3. Gentamicin

Aminoglycoside PD is determined by the C_{max}>MIC (Lutsar *et al.* 2010b). In a recent PK/PD study in neonates, Sherwin *et al.* identified the C_{max}/MIC ratio of amikacin as the only independent predictor of treatment failure, with C_{max} /MIC ratio < 8 associated with increased relative risk of failure (Sherwin *et al.* 2009b).

Population PK studies have confirmed the role of current body weight and postconceptional age as explanatory factors for the variability in aminoglycoside PK in neonates (Sherwin *et al.* 2008, Begg *et al.* 2009). The use of GA and/or BW has been found most appropriate when characterising aminoglycoside PK during the first week of life (Arbeter *et al.* 1983, Koren *et al.* 1985, Nahata *et al.* 1986, Paap *et al.* 1990). Thereafter postconceptional age has been suggested as the best correlate with significant increase in aminoglycoside CL seen at 34 weeks (Kildoo *et al.* 1984, Miranda *et al.* 1985, Thomson *et al.* 1988, Paap *et al.* 1990).

Current body weight is the principal determinant of V_d (Sherwin *et al.* 2008, Begg *et al.* 2009). In general gentamicin V_d approximates the extracellular fluid volume in neonates (Paap *et al.* 1990). A decrease over the first 4 days of life has been described, reflecting probably the changes in body composition from the onset of diuresis and increased insensible water loss (Nakae *et al.* 1988). Higher V_d of gentamicin in septic neonates has been demonstrated (Lingvall *et al.* 2005, Sherwin *et al.* 2009a). Patent arterial duct has also been shown to increase Vd, probably due to increased extracellular fluid volume as a result of pulmonary shunting (Watterberg *et al.* 1987).

Gentamicin serum $T_{1/2}$ correlates inversely with CL_{CR}, GA, BW and PNA and is in term infants about 5 h during the first two days of life, decreasing to 3.4 h by the end of the first week of life and to approximately 3 h thereafter (Nelson *et al.* 1973, Pons *et al.* 1988, McCracken *et al.* 1990). As $T_{1/2}$ of gentamicin in VLBW neonates is approximately 11–13 h during the first week of life (Nelson *et al.* 1973, Kildoo *et al.* 1984, Landers *et al.* 1984, Nakae *et al.* 1988), decreasing to 10 h by the second to fourth week (Kildoo *et al.* 1984) and to 4.4 beyond one month of age (Kildoo *et al.* 1984), extended dosing interval of 18–24 h for VLBW neonates has been suggested in early studies already (Szefler *et al.* 1980, McCracken *et al.* 1990). Conditions associated with altered renal function, e.g. patent arterial duct or births asphyxia, have been associated with extended gentamicin $T_{1/2}$ (McCracken *et al.* 1990).

Today once daily dosing has become a generally accepted practice with safety and clinical efficacy at least equivalent to multiple daily dosing (Contopoulos-Ioannidis *et al.* 2004, Rao *et al.* 2006). More recently individualized dosing with therapeutic drug monitoring 24 to 48 (60) h after the first dose has been explored for improved therapeutic target attainment, especially in VLBW neonates (Sherwin *et al.* 2008, Begg *et al.* 2009, Sherwin *et al.* 2009b). Extending dosing interval to at least five half-lives (i.e. 60 h in neonates with current body weight < 1.5 kg, 48 h with 1.5–3.0 kg and 36 h with 3–5 kg) requires higher dose administration but results in a substantial improvement in achieving gentamicin target C_{max} (> 10 mg/L) and C_{min} (< 1 mg/L) values with no change in target AUC attainment (Stickland *et al.* 2001, Begg *et al.* 2009). The characteristics of aminoglycosides, making this approach particularly attractive include concentration-dependent bactericidal activity, postantibiotic effect and decreased risk of adaptive resistance (Contopoulos-Ioannidis *et al.* 2004, Begg *et al.* 2009). Cost-effectiveness analysis has pointed out reduced need for gentamicin concentration monitoring in short treatment courses (<72 h) and significant antibiotic associated hospital cost savings in neonates with GA >= 34 weeks (Thureen *et al.* 1999).

Aminoglycoside penetration into CSF has been variable and is generally considered inadequate to treat common pathogens causing meningitis (Tessin *et al.* 1989, Paap *et al.* 1990). CSF concentration of 1.6 mg/l has been observed in neonates after intravenous administration of 2.5 mg/kg per dose (McCracken *et al.* 1980). Ventricular and CSF concentrations of 10–130 and 8–86 mg/l, respectively, have been achieved 1–6 h after intraventricular administration of 2.5 mg of gentamicin, however at the cost of significant toxicity (McCracken *et al.* 1980). As aminoglycosides exhibit concentration-dependent bacterial killing, not surprisingly single daily dose treatment of experimental meningitis has been found as effective as divided dosing regimens (Lutsar *et al.* 1998).

Aminoglycoside therapy has been found to result in significant nephro- and ototoxicity in neonates (Adelman *et al.* 1987b; a), although once daily dosing regimen has been associated with diminished accumulation in renal tubules and the inner ear (Contopoulos-Ioannidis *et al.* 2004, Drusano *et al.* 2007). Given the wide interindividual variability, therapeutic drug monitoring is generally considered necessary in neonates.

In late pregnancy, gentamicin crosses the placenta rapidly but peak foetal levels may be low, especially if maternal levels are subtherapeutic (McCracken *et al.* 1990). Levels of aminoglycosides in amniotic fluid are usually below foetal serum levels and peak concentrations may be attained only after 2–6 hours (Bray *et al.* 1966, Weinstein *et al.* 1976).

2.5.4. Route of administration

Although parenteral antibiotic therapy is the standard of care for neonatal sepsis (Tessin *et al.* 1991, Yurdakok 1998, Mtitimila *et al.* 2004, WHO 2009), oral antibiotics have proven better than no antibiotic at all in the management of serious neonatal bacterial infections in developing country communities (Bang *et al.* 1999, Bang *et al.* 2005a, Bang *et al.* 2005b, Bhutta *et al.* 2009, Darmstadt *et al.* 2009). However, in a recent randomised controlled trial from Karachi, Pakistan, in neonates with suspected sepsis considered to require hospital management, mortality was significantly lower in those treated with parenteral procaine penicillin and gentamicin compared with those refusing hospital care and treated with oral cotrimoxazole and parenteral gentamicin (Zaidi *et al.* 2006, Darmstadt *et al.* 2009). These data strongly support the WHO recommendation about parenteral therapy being superior in neonates deemed sick enough to need in hospital treatment.

The wide variability and/or the incomplete data about bioavailability of enterally administered antibiotics has limited this route of administration to the no-seriously ill newborn only in most cases (Paap *et al.* 1990). Factors affecting the bioavailability of oral antibiotics in neonates include age dependant variation in the acidity of the stomach (Agunod *et al.* 1969, Euler *et al.* 1977), delayed gastric passage and intestinal transit time (Gupta *et al.* 1978, Berseth 1989), intestinal surface area, variations in bacterial flora (Alcorn *et al.* 2003), variable enzyme/transporter activity (Johnson *et al.* 2001, Alcorn *et al.* 2002a), food interference with absorption, gastrointestinal side effects and concentration of available suspensions (Besunder *et al.* 1988, Alcorn *et al.* 2003). The PK data of oral antibiotic administration in neonates are scarce. In a study by Gras–Le Guen *et al*. adequate serum concentrations of amoxicillin were achieved with oral doses of 200 and 300 mg/kg/day (in 4 divided doses) in 222 full-term neonates switched to oral therapy after 48 hours of parenteral amoxicillin (Gras-Le Guen *et al.* 2007). All neonates were treated for GBS disease and early switch to oral route was well tolerated and effective with favourable clinical outcome in all cases.

2.6. Antibacterial treatment of neonatal sepsis

The standard antimicrobial therapy of neonatal sepsis consists of a combination of two or more antibiotics administered parenterally for 10 to 14 days (or longer as in meningitis) (WHO 2003; 2009).

2.6.1. Terminology

Antibacterial therapy can be divided into (1) prophylactic, when antibiotic is administered to a group of clinically healthy subjects, either uniformly or based

on the presence of certain risk-factors; (2) pre-emptive, when the presence of a non-specific sign suggests high probability of infection; and (3) true treatment, when the diagnosis of infection has been established. When the bacterial aetiology is not known yet and the choice of drug(s) is based on the epidemiological data, antibiotic therapy is termed empiric. As it is difficult to draw a clear border between pre-emptive and true treatment of EOS in most studies, both will be handled together in this thesis.

2.6.2. Antibiotic prophylaxis in early onset sepsis

As delayed antibacterial treatment is associated with increased mortality (Lannering *et al.* 1983, Mtitimila *et al.* 2004) and clinical signs of EOS are non-specific and may be subtle initially, antibiotic prophylaxis of EOS has been extensively studied in neonates. Most studies have focused on the role of prophylaxis in the prevention of GBS disease. Before widespread application of maternal IAP two prophylactic approaches have been applied: (1) routine administration of intramuscular penicillin G immediately after birth to all neonates (Siegel *et al.* 1980, Patel *et al.* 1999) and (2) monitoring of neonates born to mothers with risk factors of infection and selective antibiotic administration only if superficial bacterial cultures (e.g. external auditory canal, gastric aspirate or foetal side of the placenta) reveal GBS colonisation or if infection is suspected (Gerard *et al.* 1979, Pyati *et al.* 1983).

Studies comparing universal administration of intramuscular penicillin G vs no treatment have proven the efficacy of treatment in terms of reduced colonisation as well as invasive infection rates. An early non-randomised study of a single dose of intramuscular penicillin G in 18,738 neonates showed significant reduction in the concordant colonisation rate of neonates (to 12.2% vs 50% in untreated infants; $p<0.001$) and a significant decrease in the incidence of disease caused by all penicillin susceptible organisms (to 0.64 vs 2.26 per 1000 live births in untreated infants; $p=0.005$) (Siegel *et al.* 1980). However, the disease caused by penicillin resistant pathogens was increased in the treatment group during the first period (year) of the study and therefore universal prophylaxis was not recommended. The efficacy of prophylaxis in the era of wide spread IAP has been confirmed by a later large study including 10,998 neonates assigned randomly in two to three months blocks to receive penicillin G prophylaxis vs no treatment. Significant reduction in clinical sepsis (1.7% vs 2.5%; $p<0.01$), GBS infection (0.4% vs 0.9%; $p<0.001$) and deaths from sepsis (0.1% vs 0.3%; $p<0.05$) was found in treated vs non-treated infants (Patel *et al.* 1999).

However, no advantage of universal prophylaxis over more expectant close monitoring has been proven. A randomised trial comparing early prophylactic penicillin G (100,000 IU/kg q12h for 3 days) vs close monitoring in 1187 preterm neonates with BW up to 2,000 g, found no difference in the incidence of GBS infection (RR 0.73; 95% CI 0.32–1.62) or GBS infection related or

overall mortality (RR 0.78; 95% CI 0.55–1.11) (Pyati *et al.* 1983). A small RCT comparing universal prophylaxis of infants born to GBS positive mothers (n=29) to expectant management based on superficial culture results (n=38), found both strategies to be equally effective with no cases of GBS infection or neonatal deaths in either group (Gerard *et al.* 1979).

Today universal antibiotic prophylaxis in neonates born to mothers with risk factors of infection has been abandoned, because clear advantage over more expectant management, exposing less neonates to antibacterial therapy has not been proven, widespread use of antibiotics carries the risk of increasing resistance and may cause diagnostic difficulties by increasing negative culture results (Ungerer *et al.* 2004, Woodgate *et al.* 2004). The risk of invasive early onset GBS disease even in a neonate of a GBS positive mother who has not received IAP is still very low, approximately 1% (Schrag *et al.* 2002).

In the present decision algorithms need for prophylactic antibiotic administration is limited to neonates born to GBS positive mothers without adequate IAP or to mothers who have received IAP for suspected chorionamnionitis (Schrag *et al.* 2002, Morantz 2003). Neonates, born near or at term from a pregnancy without any risk factors of infection and/or to GBS negative mothers or GBS positive mothers, who have received IAP with penicillin or ampicillin more than 4 h prior to delivery (termed adequate IAP), do not need early antibiotic prophylaxis (Boyer *et al.* 1983b, Boyer *et al.* 1983c, Boyer *et al.* 1986, AAP 1997, Schrag *et al.* 2002, Society 2007). However, IAP with drugs other than β-lactams, occasionally used for mothers at risk of anaphylaxis from penicillin, should be handled as incomplete (Society 2007).

2.6.3. Early empiric antibacterial therapy in early onset sepsis

Although IAP has dramatically reduced the frequency of EOS due to GBS, it has not affected the frequency of sepsis caused by other organisms (Baltimore *et al.* 2001, Hyde *et al.* 2002, Daley *et al.* 2004, Schrag *et al.* 2006b). Also, GBS disease is still possible, even if very rare, in infants born to mothers who have received adequate IAP (Pinto *et al.* 2003). Therefore, the focus has moved to early empiric antibiotic therapy of at risk neonates. All neonates developing any signs and symptoms suggestive of infection or born from pregnancies with risk factors of infection (maternal fever >= 38^{o}C, prolonged premature rupture of membranes for more than 18 h, preterm labour in <35 weeks of gestation) or with GBS colonisation of the genital tract with adequate IAP and developing at least one non-specific sign or symptom (including RDS), have antibiotic therapy initiated after full sepsis screen has been performed (Schuchat *et al.* 2000, Schrag *et al.* 2002).

The strategy of early empiric antibiotic therapy for suspected neonatal sepsis, adopted since 1970s has lead to dramatic improvement of survival rates of neonatal sepsis (Lannering *et al.* 1983, Stoll *et al.* 1998, Lukacs *et al.* 2004, Bizzarro *et al.* 2005) and overall infection related child mortality (Veldhoen *et*

al. 2009). However, with the present approach about 4% of live-born infants (Labenne *et al.* 2007), including about 60% of all preterm (Pacifici *et al.* 2009) and 94–100% of ELBW neonates receive antibiotic treatment within a few hours from birth (Cordero *et al.* 2003, Shani *et al.* 2008). In a large population-based study from Burgundy, France, including 25,480 births, 68% of treated infants never developed serious infections. The reported incidence of positive deep site cultures among antibiotic treated neonates ranges from 2–5% (Snelling *et al.* 1983, Miall-Allen *et al.* 1988, Stoll *et al.* 1996, Schrag *et al.* 2006b).

Only lately the disadvantages of this relatively non-selective approach like spread of antibacterial resistance, changes in host microbial ecology affecting bacterial aetiology of late onset infections but possibly also immune development and long-term morbidities like allergy, have become more recognised (Gordon *et al.* 2004, Furrie 2005, Labenne *et al.* 2007, Schelonka 2007, Vael *et al.* 2008).

2.6.3.1. Choice of drug

The widespread application of empiric antibiotic treatment of EOS in neonates prioritizes the safety and narrow spectrum of antibiotics used. Considering that 15–20% of neonates with EOS also have meningeal involvement (Ansong *et al.* 2009) and lumbar puncture may be deferred, the empiric regimen should ensure adequate CSF concentrations. Therefore a combination of a β-lactam and an aminoglycoside has remained the cornerstone of EOS antibiotic therapy in most NICUs with the initial choice of drugs based on the susceptibility of the predominant causative microorganisms (Society 2007). The scientific evidence of which treatment regimen should be preferred is poor, with only a few underpowered prospective trials comparing different a regimens in the treatment of neonatal sepsis.

Previous studies on antibacterial treatment of neonatal sepsis have been reviewed in a recent Cochrane meta- analysis (Mtitimila *et al.* 2004). The review of 15 randomised controlled studies identified only two studies that specifically compared antibiotic regimens for suspected EOS (Snelling *et al.* 1983, Miall-Allen *et al.* 1988), both performed more than 15 years ago, small in sample size and with antibiotics some of which are no longer in routine use (Miall-Allen *et al.* 1988, Mtitimila *et al.* 2004). Another meta-analysis focusing on LOS considered only one study to meet the predefined methodological criteria (Gordon *et al.* 2005).

We performed a Medline search of randomised clinical trials of antibiotic therapy in neonatal sepsis. Separate searches for "neonatal sepsis" and generic names of antibiotics were also reviewed to ensure better yield. In addition, all studies referred to were identified from references and published data accessed.

Altogether 18 **prospective comparative studies** were identified, 16 of them performed in neonates and two reporting inseparable outcomes for a paediatric

population, including 10% (Begue *et al.* 1997) and 43% of neonates (Haffejee 1984), respectively (Table 1). Major methodological differences between the studies were noted, making the results not applicable to the present population of EOS. Inclusion criteria varied from risk factors or suspicion of infection (Adelman *et al.* 1987b, Miall-Allen *et al.* 1988, de Louvois *et al.* 1992) to clinical (Fogel *et al.* 1983) and even culture proven sepsis (Bingen *et al.* 1987, Odio *et al.* 1987). One study, including only neonates, did not report on the procedure of treatment assignment (Gokalp *et al.* 1990). Two studies recruited neonates only up to 48 h (Snelling *et al.* 1983) and 7 days (Hammerberg *et al.* 1989), respectively, and another study reported separate outcomes for EOS (within 48 h) and LOS (Miall-Allen *et al.* 1988). All other studies did not outline separate outcomes for the two disease entities. However, as the etiologic structure of EOS and LOS differs significantly, aiming at a universally effective antibiotic regimen does not seem appropriate.

The three studies reporting outcomes for EOS up to 48h (Snelling *et al.* 1983, Miall-Allen *et al.* 1988) or 7 days of age (Hammerberg *et al.* 1989), compared ceftazidime, ticarcillin-clavulanic acid or piperacillin monotherapies to a combination of a penicillin (ampicillin, penicillin G or piperacillin) and aminoglycoside (gentamicin or amikacin). Cochrane meta-analysis defining EOS as sepsis occurring within the first 48 h of life and incorporating the two respective studies (Snelling *et al.* 1983, Miall-Allen *et al.* 1988) found no difference between monotherapy vs combination therapy in 28 day mortality (RR 0.75; 95% CI 0.19–2.9), treatment failure defined as change of antibiotic regimen (RR 1.25; 95% CI 0.19–8.39) or resistance of invasive strains to initial empiric regimen (no cases in both studies) (Mtitimila *et al.* 2004). The third study including neonates up to 7 days of age also found no difference between piperacillin vs ampicillin plus amikacin regimen in deaths from infection or side effect profile, although death within one week of antibiotic treatment tended to be more frequent in the combination therapy group (27/196 vs 17/200; p=0.11) (Hammerberg *et al.* 1989).

The remaining 15 studies compared a wide variety of combined regimens and monotherapies in a mixed population of neonates with suspected or clinical EOS and LOS (Table 1) and reported different outcomes: overall and infection related mortality, need for change of empiric regimen and antibacterial resistance, adverse effects like nephrotoxicity or incidence of systemic candidiasis, serum and CSF concentrations of antibiotics and minimal bactericidal time of serum. Although regimens containing third generation cephalosporins demonstrated superior clinical outcomes in terms of treatment success (Haffejee 1984, Odio *et al.* 1987, Gokalp *et al.* 1990, de Louvois *et al.* 1992) and survival (Haffejee 1984, Odio *et al.* 1987, Gokalp *et al.* 1990) compared to a penicillin and aminoglycoside regimen in four studies incorporating both EOS and LOS patients (total 1480 neonates), the only study comparing these two regimens in the treatment of EOS alone in 55 neonates (Snelling *et al.* 1983), reported no difference in mortality or treatment success. Two studies including both EOS and LOS patients (total 271 neonates) (Hall *et al.* 1988, Wiese 1988) also found

no difference in clinical outcomes between third generation cephalosporin containing regimen vs a penicillin and aminoglycoside combination. The different results from studies including LOS patients are attributable to differences in the rate and etiologic structure of culture proven infection. Studies having higher rates of proven infection (176/1316; 48/73; 9/72; 60/60 vs 5/222; not reported/49) with high proportion due to Gram-negative pathogens (46%; 29%; 56%; 72%), have reported superior clinical outcome for 3rd generation cephalosporins. One study of culture proven *E. coli* sepsis found no difference in clinical cure rate, but β-lactamase resistant penicillin (amoxicillin calvulanic acid) was used in the penicillin plus aminoglycoside arm.

However, the side effect profile does not favour the use 3rd generation cephalosporins. In one RCT 3rd generation cephalosporin use was associated with increase in resistance to cefotaxime over time (Hall *et al.* 1988) and another study found increased rate of invasive *Candida* infections in neonates receiving cephalosporin containing regimen (Odio *et al.* 1987). These findings have been confirmed by a large number of later studies (Leibovitz *et al.* 1992, Saiman *et al.* 2001, Benjamin *et al.* 2006, Cotten *et al.* 2006, Manzoni *et al.* 2006). Some data have also suggested increased resistance in association with the use of aminoglycosides in neonates (Toltzis 2003).

In conclusion, the insufficient statistical power as well as the wide methodological variation of the available RCTs does not allow drawing meaningful conclusions about the optimal treatment regimen of EOS for the presently treated population.

Table 1. Prospective randomized studies evaluating the efficacy of different antibiotic regimens in the treatment of neonatal sepsis.

Study Study design	Population N/ age/ inclusion criteria	Regimens (n)	proven sepsis N (%)	Bacterial aetiology	Clinical sepsis n (%)	Main results
Separable outcomes reported for EOS						
Snelling 1983 **open-label RCT**	**55** neonates/ **<48h**/ suspected infection	**CTZ** (24) vs **PEN +GEN** (31)	3 (5.5)	*S. aureus* 1; CONS 1; *P. maltophila* 1	11 (20)	No **deaths**; 11 full course treatment – all clinically improved, cultures negative; **resistance**: none to CTZ; all to PEN; 1 *Pseudomonas* to GEN;
Miall Allen 1988 **Open-label RCT**	**64** neonates/ **<48h**/ suspected & proven EOS	**TICCA** (32) vs **PIP +/-GEN** (32)	5 (7.8)	GBS 1; CONS 1; *S. faecalis* 1; *E. agglomerans* 1; *E. coli* 1	48 (75)	TICCA vs PIP (+GEN); n= **Deaths**: 3 vs 5; **treatment failures**: 2 vs 7; **resistance:** none to either regimen
Hammerberg 1989 **Blinded RCT excluded proven sepsis**	**396 premature** neonates/ **</= 7 days**/ risk factors or suspicion of EOS	**PIP+placebo** (200) vs **AMP+AMK** (196); AG level monitored;	13 (3.3)	*S. epidermidis* 4; GBS 3; *E. coli* 2; *H. Influenza* 1; *Acinetobacter spp* 1; *Str mitior 1*; *Gardnerella vaginalis* 1	?	PIP vs AMP+AMK; n= **Death in 1 week of AB treatment** 17/200 vs 27/196 (p=0.11); **death from infection** 3/200 vs 2/196; **resistance**: none to either regimen; **side effects: renal** (% with creatinine > 100 µmol/L) 25 vs 22; **liver** (% with total bilirubin > 20 µmol/L) 58 vs 55
Outcomes for neonates with mixed outcomes for EOS and LOS						
De Louvois 1992 **Open-label RCT, 15 study centres/ ITT analysis; 172 (13%) protocol violations**	**1316** neonates/ **<29d**/ suspected infection requiring AB therapy	**CTZ** (281) or **CTZ + AMP** (378) vs **AMP + AG** incl **GEN/ TBR or AMK** (657)	176 (13.4); variation 6–39%; (150 evaluable)	102 (7.8) Gram-pos 75, G-neg 27	489 (37.1); 458 evaluable	**Overall treatment failure** rate 18.7%; **overall mortality** 4.9%; 15 deaths, 10 culture proven CTZ+AMP vs AG+AMP; n=: **Cure (proven sepsis)**: 30/31 vs 26/39; **p=0.002; cure (clinical sepsis)**: 139/142 vs 129/137; **infect. related mortality:** 3/43 vs 3/37 (GBS 60%)! CTZ vs AG+AMP; n=: Cure **(proven sepsis)**: 34/43 vs 32/37; **cure (clin. sepsis)**: 85/88 vs 91/91; **infect. Rel. mortality:** 2/39 vs 2/31; CTZ containing regimen vs AMP+AG; n= : **Resistance:** (to empiric AB) G-pos 51/225 vs 59/242; G-neg 7/152 vs 6/157, for AMP alone 48/78; **side effects:** 1 treatment stopped AMP+AG for renal failure

Study Study design	**Population** **N/ age/ inclusion criteria**	**Regimens (n)**	proven sepsis N (%)	Bacterial aetiology	Clinical sepsis n (%)	Main results
Adelman 1987 **Open label RCT**	**112** neonates/ **age not specified/** suspected infection	**AMP+AMK** (n=56) vs **MEZ** (n=56) for staphylococci +OXA	25 (22.3)			No difference in **clinical or bacteriologic response** MEZ vs AMP+AMK: **Side effects:** renal as decline of creatinine clearance more than **25%** 19% vs 40% ($p<0.005$); no difference in FeNa, FeP, urinary activity of NAG and AAP
Adelman 1987 **Open label RCT**	**90** neonates/ **age not specified/** suspected or proven sepsis	**AMP+GEN** (n=45) vs **MEZ** (n=45) for staphylococci +OXA	9 (10.0)			MEZ vs AMP+GEN **Side effects:** renal as decline of creatinine clearance more than 25% 19% vs 40%; decrease of creatinine clearance if present 44% vs 20%, $p<0.01$
Fogel 1983 **RCT**	**58** neonates (7ELBW) / /clinical + lab sign of sepsis	**AMP+GEN** (n=30) vs **AZL+GEN** (n=28)	25 (43.1) (15 EOS 7d)	*E. coli* 8; *K. pneumoniae* 7; *Pseudomonas* 6; *S. aureus* 4; *Str viridians* 3		AMP+GEN vs AZL+GEN; n= **Change of AB:** 6 vs 1; **Death:** 1 vs 1 (both VLBW, overwhelming sepsis); **Resistance:** in vitro to AMP+GEN higher
Marks 1987 **Open label RCT**	**60** neonates/ **6 h to 28 d/** pneumonia, sepsis or bacteraemia	**AMP+GEN** (20) vs **TOB+cephalothin** (20) vs **AMP+AMK** (20)	12 (20.0)		33 (55.0)	AMP + GEN vs TOB + cephalothin vs AMP + AMK; n=**Resistance:** 1 vs 1 vs 1, all died **Side effects:** liver – no bilirubin displacement from albumin for any AB; renal – no significant difference in nephrotoxicity
Gokalp 1990 **Open label RCT?**	**73** neonates/ median age 7.4 vs 6.9 d/ sepsis or suspected sepsis	**AMP + CTX** (30) or **CTR** (10) (n=40) vs **PEN + TOB** (5)/ **GEN** (22)/ **NTL** (6) (33)	48 (65.8)	*S aureus* 17; *S. epidermidis* 11; *E. coli* 13; GBS 3; enterococci 3; *Salmonella* 1		AMP+CTX vs PEN+AG; n= **Clinical cure** satisfactory 35 vs 18; **death:** 5 vs 15; **side effects:** no significant adverse effects
Miall-Allen 1988 **RCT**	**28** neonates/ **age >48 h/** LOS	**TICCA** (n=14) vs **Flucloxacillin +GEN** (n=14)	3 (10.7)	Group D strep 1; *Serratia spp.* 1; CONS 1		TICCA vs PIP+GEN; n= **Treatment failure:** 0 (1 excluded, cured) vs 2 (both died); **death:** 0 vs 2; **dose adjustment:** 1 GEN; **side effects:** no local or systemic with TICCA

Study Study design	Population N/ age/ inclusion criteria	**Regimens (n)**	proven sepsis N (%)	Bacterial aetiology	Clinical sepsis n (%)	Main results
Hall 1988 **Open label RCT 2 centres (99+123); BW stratified <1500g**	236 neonates/ 0 to 28 days/ suspected infection (**222** analysed)	**CTX** (n=111) vs **NTL+PEN** (n=111)	5 (2.3)	GBS 1; *S. epidermidis* 2; *H. parainfluenzae* 1; *K. oxytoca* 1 All isolated sensitive to CTX and NTL	34 (15.3)	CTX vs PEN+NET n= : **Clinical cure**: all **proven sepsis** cured, no **deaths**; **clinical cure:** 98/111 vs 102/111; **AB change** 9/111 vs 3/111**; Deaths**: 6/111 vs 6/111 (all deaths BW <1500 g); **resistance: R to CTX day 2–3:** G-neg rods 3/12 vs 0/30; staphylococci 0/4 vs 0/5; **day 4–10:** G-neg rods 7/12 vs 8/28; staphylococci 1/5 vs 3/5; **side effects:** none in either regimen
Wiese 1988 **RCT**	49 term and preterm neonates/ age not specified/ clinical sepsis (**sepsis score** 10 p)	**CTR+GEN** (n=25) vs **AZL+GEN** (n=24) on Day 1 and 2 IVIG 2 ml/kg in 20 vs 19	Not reported		49 (100)	No difference in **sepsis score** on days 1, 3, 5, 7 and 2 days after completion of therapy; CTR+GEN vs AZL+GEN; n= : **AB change:** 5/25 vs 5/24 (all cured with the other regimen); **Death during AB therapy** 2/25 vs 2/24 (all preterm+sepsis); CTR+GEN vs AZL+GEN; days; mean (SD): **Duration of AB therapy:** 8.7 (2.6) vs 8.7 (2.3); **Side effects: liver** – bilirubin within expected range in all; r**enal** – creatinine mg/dl 0.58 (0.15) vs 0.52 (0.20)
Umana 1990 **Open label RCT**	147 neonates/ age not specified/ suspected sepsis (60 analysed)	**ATM+AMP** (n=75) vs **AMK+AMP** (n=72)	48 (80)	*P. aeruginosa* 17; *K. pneumoniae* 12; *E. coli* 12; *P. cepacia* 1; *S. marcescens* 2; other G-neg 12	pneumonia 42; NEC 12; soft tissue 2	More pneumonia and *Pseudomonas* infections in AMK+AMP; AZT+AMP vs AMK+AMP; n= **Deaths** 2/28 vs 7/32 (p=0.011); **Treatment failure** 2/28 vs 9/32 (p=0.036); **Side effects:** no adverse reactions in either group; *Candida* superinfection 7/28 vs 6/32; **Resistance:** none R to AZT, 18/67 R to AMK, 43/67 R to AMP
Tessin 1989 **RCT**	**35 neonates/** /suspected sepsis or meningitis; (33 analysed)	**TOB +AMP** (n=17) vs **CTZ+AMP** (n=16)	4 (12.1) urine, blood, CSF	GBS, *E. coli, Klebsiella spp*		CTZ vs TOB vs AMP; median (range) □g/L **Trough serum levels** (8.5 (<3–33) vs 1.5 (<0.5–3.3) vs 13 (1.0–100.0); **peak serum levels** 100 (60–150) vs 5.0 (1.0–10) vs 175 (6.2–300); **CSF levels** 7.0 vs <0.5 vs 5.4

Study Study design	Population N/ age/ inclusion criteria	**Regimens (n)**	proven sepsis N (%)	Bacterial aetiology	Clinical sepsis n (%)	**Main results**
Bingen 1987 **?**	22 neonates/ /E. coli K1 sepsis	**CTX+NTL** (14) vs **AMXCL+NTL** (8)	22 (100)			**Clinical cure:** all patients cured; median SBA 1/128 vs 1/64; mean MTBS (h) 1.2 +/- 0.8 vs 3.9 +/- 1.4 ($p<0.01$)
Tessin 1988 **Open label RCT**	Neonates/ /suspected sepsis	**CTZ** vs **TOB**				Mean serum levels for TOB and CTZ 85 (+/- 4.4 SE) vs 5.8 (+/- 0.3 SE); TOB 9/33 toxic trough serum levels and 9/33 subtherapeutic peak level; CTZ 2/29 subtherapeutic peak levels
Odio 1987 **RCT**	66 neonates/ /proven invasive infection; (59 analysed)	**CTZ** vs **CARB+AMK**	59 (100)	*E. coli* 31%; *P. aeruginosa* 25%; *Klebsiella* sp. 13%; other Gram-negative enteric bacilli 17%; *S. aureus* 20%; CoNS 8%		CTZ vs CARB+AMK; n= : **Deaths:** 2/31 vs 6/28; **treatment failure:** 2/31 vs 8/28; $p<0.05$; **resistance:** G-neg coliforms none R to CTZ; 10, 56 and 77% R to AMK, CARB and AMP, respectively; **side effects:** invasive *Candida* superinfection 5/31 vs 1/28
Studies incorporating paediatric patients and neonates						
Haffejee 1984 **Blind RCT (meningitis over 3 mo age excluded)**	68 children incl **31 neonates/** /severe infection (n=72)	Neonates **CTX** (n=9) vs **PEN+GEN** (n=22)	9?	*E. coli* 2; *Salmonella* 1; *S. aureus* 1; *S. epidermidis* 1; *Str pneumoniae* 1; GBS 1; G-neg cocobacilli 1; *Proteus spp.* 1	63?	CTX vs PEN+GEN; n= : **Clinical cure** 34/36 vs 26/36 ($p<0.01$); **deaths** 1 vs 5 ($p<0.05$) (+ 3 changes of treatment regimen); **CTX level** 2h after i/v dose(mean (range); mg/l) plasma: 8.7 (5.7–12.3); CSF: 2.7 (1.7–4.6); **side effects:** no local or gastrointestinal, signs of bone marrow, renal, liver toxicity or dose adjustments
Begue 1997 **RCT 33 centres (86 eligible pts excluded)**	233 children incl. **22 neonates**/ 0d to 15y/ serious bacterial infections;	CRO vs CTX	202	Not reported separately for neonates	31	Not reported separately for neonates; **Clinical cure:** (defervence) CRO 114/117 vs CTX 103/109 **Bacterial eradication:** CRO 101/104 vs CTX 93/98

AG – aminoglycoside; AMX – amoxicillin; AMXCL – amoxicillin-clavulanic acid; AMP – ampicillin, AMPSB – ampicillin-sulbactam; AMK – amikacin; ATM – aztreonam; AZL – azlocillin; CTZ – ceftazidime; CTR – ceftriaxone; CTX – cefotaxime; GEN – gentamicin; IVIG – intravenous immunoglobulin; MEZ – mezlocillin; NTL – netilmicin; PIP – piperacillin; PIPTZ – piperacillin-tazobactam; TOB – tobramycin; AB – antibiotic; CSF – cerebrospinal fluid; LBW – low birth weight (<2.5 kg); R – resistant/ resistance; TBC – total blood count; LFT – liver function tests (SGOT and SGPT); d – days; MTBS – minimal bactericidal time of serum; SBA – serum bactericidal activity

Several **prospective observational studies** have looked at the treatment results of different antibiotic regimens in neonatal sepsis (de Louvois *et al.* 1981, Snelling *et al.* 1983, Nalin *et al.* 1987, Sklavunu-Tsurutsoglu *et al.* 1991) and clinical efficacy has been reported also in some PK studies (Motohiro *et al.* 1988a, Motohiro *et al.* 1988b, Fuji *et al.* 1990). Studied treatment regimens include imipenem cilastatin, mecillinam and penicillin combination, ceftazidime, ceftizoxime, ceftriaxone and aztreonam with or without penicillin G. Only one study performed as a substudy of a larger RCT of neonatal sepsis reports outcome for a non-randomised subgroup of LOS alone (Snelling *et al.* 1983), while none of the others, some including infants from 0 days to two or even six months of age (Nalin *et al.* 1987, Motohiro *et al.* 1988b), allows distinguishing between EOS and LOS outcomes. Most have concluded adequate therapeutic effect for the treatment studied with reported clinical cure rates ranging from 81% for imipenem-cilastatin in a population with high rate of *P. aeruginosa* and *K. pneumoniae* infection (Nalin *et al.* 1987) to 98% for aztreonam (Fuji *et al.* 1990). Relatively unfavourable side effect profile has been reported for imipenem-cilastatin with serious adverse events, most often seizures occurring in 5–9% of treated infants (Nalin *et al.* 1987, Boswald *et al.* 1999) and *Candida* superinfection seen in up to 20% (Oral *et al.* 1998). High treatment failure rate in CoNS infection with ceftazidime therapy has been outlined (Snelling *et al.* 1983).

Kalenic *et al.* in a prospective study with a historical control group showed, that cefuroxime plus gentamicin regimen was associated with lower antibiotic resistance among colonising strains and lower incidence of LOS caused by Gram-negative rods, compared to ampicillin plus gentamicin regimen (Kalenic *et al.* 1993). Unfortunately they did no data on treatment efficacy were presented.

While **retrospective review of clinical practice** allows including bacteriology and antibiotic susceptibility data, a meaningful comparison of different treatment regimens becomes at least questionable if not impossible. In a retrospective analysis of 341 episodes of neonatal sepsis in 338 neonates treated over 1975 to 1986 Tessin *et al.* found a failure rate of 20%, although 91% of isolates were sensitive to ampicillin or aminoglycoside or both (Tessin *et al.* 1991). More recently Clark *et al.* by reviewing large number (n=128,914) of hospital records of neonates receiving antibiotic therapy within the first three days of life found, that primary use of third generation cephalosporins was associated with increased mortality risk compared to ampicillin plus gentamicin (Clark *et al.* 2006). Still, due to the retrospective design in this study several confounding factors like higher rate of organ dysfunction and other serious complications in the cefotaxime group were not included in the multivariate analysis (Lee 2006).

Maayan-Metzger *et al.* reviewed the cases of 73 term and near-term and 30 preterm neonates developing positive blood cultures within the first 72h of life over a period of 10.5 years (1997–2007) and concluded that no increase in antibiotic resistance could be followed and the applied regimen of ampicillin plus gentamicin should be adequate in the empiric treatment of EOS (Maayan-

Metzger *et al.* 2009). In this series GBS was the most frequent isolate among term and near-term infants. However, among preterm infants Gram-negative bacteria predominated (23/30) with *E. coli* isolated in 19/30 neonates. Only 6/19 proved susceptible to both ampicillin and gentamicin, while four strains were resistant to both empiric antibiotics and the remaining nine to ampicillin alone. Neonates born to mothers with longer hospital admission and prior antibiotic treatment were more likely to have infections due to resistant pathogens. In a recent study from Mexico EOS therapeutic failure rate as high as 70% was reported with ampicillin plus gentamicin regimen (Reyna *et al.* 2008). Such high failure rate can be explained by the bacterial aetiology of EOS in the study centre, including only 5.8% of GBS, 42.2% of CONS and 15.6% of *Enterobacteriaceae*. The relatively liberal criteria of treatment failure, defined in this study as positive signs and symptoms of sepsis according to the International Consensus Conference criteria (Goldstein *et al.* 2005) and CRP >= 10 mg/dL after at least 72 hours of antibiotic therapy, may also have contributed.

Earlier retrospective studies generally suggest the adequacy of a penicillin and aminoglycoside combination, while more recent data point to higher resistance rates especially in preterm neonates with prevailing Gram-negative aetiology of EOS.

2.6.4. Duration of antibiotic treatment

The optimal duration of antibiotic therapy is an even more tangling issue with the present practice varying significantly independent of maternal risk factors and the infant's clinical condition beyond the first day of life (Cordero *et al.* 2003, Spitzer *et al.* 2005).

Discontinuation of antibiotic treatment after 24–48 hours has proven safe in healthy term infants who do not develop clinical and laboratory signs of serious bacterial infection (Escobar *et al.* 1994, Singhal *et al.* 1996, Philip *et al.* 2000).

In neonates with EOS (proven or clinical) the criteria for discontinuation of antibiotic therapy are much less straightforward. Short courses may hold the risk of relapse (Chowdhary *et al.* 2006), while excessive treatment duration may increase the risk of LOS (Labenne *et al.* 2007) and induce antibiotic resistance (Burman *et al.* 1993, Yurdakok 1998, Gordon *et al.* 2004, Choi *et al.* 2008). Studies in adults do not support the role of antibiotic duration as a risk factor of relapse, at least not with the presently applied courses exceeding 7 days for most serious infections; inappropriate initial antibacterial therapy has been identified as the only risk factor of relapse in patients with ventilator associated pneumonia (Nseir *et al.* 2008).

The routinely recommended course of antibiotic therapy is 7–10 days for Gram-positive and 14–21 days for Gram-negative EOS (WHO 2009). However, the rationale and safety of these recommendations have not been evaluated by adequately powered, randomized clinical trials (Schelonka 2007). For neonates receiving a single intramuscular dose of penicillin at birth and developing RDS

without positive blood cultures after 48h, four days of antibiotic therapy with an additional 24h follow-up period has been found as effective as a 7-day course, with none of the 35 infants (vs 0/38 with 7-day treatment) readmitted for sepsis or pneumonia (Engle *et al.* 2000). However, further shortening of the antibiotic treatment to 2 days has proven inadequate with recurrence of respiratory symptoms in 3/14 treated neonates (Engle *et al.* 2003). In a small group of 67 term and near-term infants with EOS due to GBS (10 proven and 57 probable infection) a 6 day course of ampicillin and cefotaxime appeared sufficient to avoid relapse within 4 weeks (Poschl *et al.* 2003). Another small blind RCT (n=70) comparing 7 vs 14 days of antibiotic treatment in neonates with culture proven sepsis, found a trend towards increased treatment failure rate with the 7-day course, which was significant in a subgroup of *S. aureus* infection (4/7 vs 0/7, p=0.022) (Chowdhary *et al.* 2006). Thus the optimal duration of antibiotic therapy in EOS probably depends on the choice of drug as well as the pathogen involved.

A reasonable approach seems discontinuation of antibiotic therapy based on normalisation of clinical signs and laboratory markers of infection. In adults this approach has proven useful in shortening antibiotic therapy for ventilator associated pneumonia (Micek *et al.* 2004). Serial measurements of CRP either alone (Bomela *et al.* 2000, Philip *et al.* 2000, Couto *et al.* 2007a) or in combination with IL-8 (Franz *et al.* 1999b) have been used effectively to limit the duration of antibiotic therapy and lower antibiotic exposure in neonates. Couto *et al.* have demonstrated that discontinuing antibiotic when CRP is <= 12 mg/L will result in similar overall mortality and relapse rate but shorter duration of therapy (9 vs 16 days, p>0.001) in neonates with culture proven LOS (Couto *et al.* 2007a). More recently serial PCT measurement has been successfully used to limit the duration of antibiotic therapy in adult intensive care (Nobre *et al.* 2008, Hochreiter *et al.* 2009, Tang *et al.* 2009) and has proven useful in identifying neonates at risk of EOS in whom empiric antibiotic therapy can be limited to 72h (Stocker *et al.* 2009).

2.7. Criteria for change of antibacterial therapy

About 10–20% of neonates with suspected sepsis fail on the conventional antibiotic regimen of ampicillin or penicillin G and gentamicin (Tessin *et al.* 1991, Metsvaht *et al.* 2006). In most neonatal units a switch from a penicillin and aminoglycoside combination is prompted by isolation of a resistant micro-organism or on clinical grounds by suspicion of meningitis, abdominal infection or renal failure (Clark *et al.* 2006). As discussed above bacteriological diagnosis is often delayed with present bacteriological methods, thus well defined clinical and laboratory criteria predicting failure of antibiotic therapy might have the potential to ensure earlier adequate antibiotic therapy. Pathogen-specific platelet response has been described with sustained thrombocytopenia occurring more frequently with Gram-negative and fungal infections (Bhat *et al.* 2009).

Only a few studies have looked at the prognostic factors of neonatal sepsis. Kermorvant *et al.* have identified lower weight at the onset of sepsis and Gram-negative infection as independent predictors of adverse outcome in neonatal septic shock (Kermorvant-Duchemin *et al.* 2008). An earlier study of 246 neonates with infections found higher rate of VLBW, GA < 28 weeks, RDS, total parenteral nutrition (TPN), cerclage in the mother, ear swab culture positive for a pathogen, meningitis and positive deep site culture but not the species of the pathogen isolated, to be associated with poor outcome in univariate regression analysis (Hafed *et al.* 2003). Neonates suffering adverse outcome, defined as death due to or attributable to infection or survival with neurological sequelae, had also higher PCT level and sepsis score. Unfortunately no additional analysis was undertaken, to identify factors independently associated with adverse outcome in neonatal infection. Lower plasma protein C level has been found to correlate with mortality in neonates with LOS (Lauterbach *et al.* 2006).

To our best knowledge no studies looking at clinical and laboratory markers of antibiotic treatment failure have been performed.

2.8. Summary of the literature

No single marker has solved the question of timely and accurate diagnosis of neonatal sepsis so far. The presently applied diagnostic criteria include a combination of clinical and haematological markers and acute phase reactants with or without interleukins. However, apart from the number of criteria used, the identified population depends on the actual cut-off values applied, as for most no uniform standard exists. As early antibacterial treatment reduces mortality antibiotics are prescribed to many neonates based on perinatal risk factors and/or non-specific signs or symptoms alone.

At the same time there is a lack of high-quality data on the efficacy and tolerance of different antibiotic regimens in neonates. A combination of a B-lactam with aminoglycoside has been the most widely used empiric regimen in the treatment of neonatal sepsis. As third generation cephalosporins, although having demonstrated high efficacy in clinical studies, have been associated with extensive spread of resistance and probably even with increased mortality , penicillin G and ampicillin have remained the first-line β-lactams in these combinations.

Recent changes in the bacterial aetiology of EOS, with decreasing rates of group B streptococci (GBS) and increasing *E. coli* raise the issue of potential differences between the two regimens. The predominance of Gram-negative rods in the bacterial aetiology of EOS among VLBW neonates, suggests higher potential efficacy of ampicillin at least in this subpopulation. Still, increasing ampicillin resistance may have abolished the advantage of broader spectrum in antibacterial activity compared to penicillin G. On the other hand ampicillin use

may be associated with the spread of extended spectrum β-lactamase (ESBL) producing Gram-negative rods like *K. pneumoniae* and induction of β-lactam resistance in neonatal strains of *E. cloacae*. Penicillin G at the same time has the least impact on neonatal bowel colonisation with resistant microorganisms – an issue to be considered, bearing in mind the large population of neonates receiving antibiotics according to the present best practice guidelines and the high probability of translocation, especially from the immature bowel.

The actual relation between the advantages and disadvantages of ampicillin vs penicillin G combinations with gentamicin is not clear as they have never been compared head to head in the treatment of neonates at risk of EOS.

The dosing of antibiotics needs to be based on a balance between maximal efficacy and minimal toxicity, as well as minimal induction of resistance, the latter often requiring higher therapeutic targets compared to just bactericidal effect. Although PK information in neonates is available for many frequently used antibiotics, the coverage of specific populations, like VLBW/ELBW neonates, is often inadequate. The penicillin G dosing regimen suggested for neonates is based on PK data from term or near-term infants; no data on penicillin G PK in VLBW neonates are available.

Given that at least 10–20% of neonates with suspected sepsis are going to fail on the presently most widely used antibiotic regimens of ampicillin or penicillin G and gentamicin and the persistently high mortality of EOS, especially among VLBW neonates, there is an urgent need for additional criteria identifying neonates requiring broader spectrum antibacterial coverage. Such approach would allow limiting the use of broad spectrum antibiotics to those who really need it thus avoiding further unnecessary spread of resistance and the risk of adverse side effects.

3. AIMS OF THE RESEARCH

The general aim of the research was to identify optimal antibiotic regimen for the empirical treatment of neonates at risk of EOS. We hypothesised that ampicillin plus gentamicin and penicillin G plus gentamicin combinations are equivalent in the early empiric treatment of neonates at risk of EOS.

The specific aims were as follows:

1. To identify the PK profile of penicillin G as one of the most widely used antibiotics in the empirical treatment of EOS, in premature VLBW neonates
2. To compare the clinical effectiveness of two first-line antibiotic regimens (penicillin G plus gentamicin and ampicillin plus gentamicin) in the empiric treatment of neonates at risk of EOS
3. To describe the clinical effectiveness of the studied regimens in the sub-population of ELBW neonates
4. To describe the safety and tolerance of early empirical antibiotic therapy in neonates at risk of EOS
5. To find early clinical and laboratory risk factors of antibiotic treatment failure in order to identify neonates who might benefit from early switch from the conventional empiric regimen to agents with broader spectrum of antimicrobial activity

4. PATIENTS AND METHODS

This thesis is based on the results of two clinical studies and an additional post-hoc analysis of data collected during the comparative efficacy study (Table 2. Description of studies and analyses of the thesis.

Table 2. Description of studies and analyses of the thesis.

Study characteristic	Timing	Population	Primary aim	publi-cation
PK study	01.10.2005 – 30.04.2006	18 neonates with BW < 1200g	PK characteristics of two doses of PEN To find the dose for the efficacy study	I
Comparative efficacy study	02.08.2006 – 30.11.2007	283 neonates	To compare the efficacy of AMP plus GEN and PEN plus GEN in empirical therapy of neonates at risk of EOS	II
ELBW subgroup analysis	02.08.2006 – 30.11.2007	75 ELBW neonates	To compare the efficacy of AMP plus GEN and PEN plus GEN in empirical therapy of ELBW neonates at risk of EOS	IV
Risk factor analysis	Post-hoc analysis	283 neonates recruited into comparative efficacy study	To identify risk factors of treatment failure on empirical AMP plus GEN and PEN plus GEN therapy	III

PK – pharmacokinetic; AMP – ampicillin; PEN – penicillin G; GEN – gentamicin; ELBW – extremely low birth weight (below 1000 g); BW – birth weight

4.1. Ethics

The studies were approved by the Ethics Committee of the University of Tartu. For participation in the PK study informed consent of the parents or legal guardian was obtained. In comparative efficacy study informed consent was considered not necessary for the following reasons: (1) there were no interventions that were conducted for study purposes only; (2) before initiating the study in both units the studied antibiotic regimens were cycled as empiric therapy for EOS; (3) prospective consent was considered unfeasible as the delay in initiating antibacterial therapy may have significant consequences and retrospective consent would have carried a high risk of introducing systematic bias; (4) the secondary aim of the study was to assess the influence of different

antibiotic regimens on the circulation of resistant microorganisms in NICU; the prerequisite of meaningful analysis being inclusion of all affected patients.

The comparative efficacy study was registered at ClinicalTrials.gov identifier: NCT00487019.

4.2. Design of the studies

The studies were conducted in the NICUs of Tartu University Hospital and Tallinn Children's Hospital.

First the PK of penicillin G was investigated in a prospective two-centre study enrolling 18 consecutive neonates in two dosing groups. The first nine infants received penicillin G at a dose of 50,000 IU (30 mg)/kg q12h (group 1). An interim analysis was conducted. The dose of penicillin G for the next nine subjects (group 2) was expected to be 25,000 IU (15 mg) q12h if the values of the PK parameters obtained after use of the initial dose appeared to be significantly higher than those achieved in adults with the currently recommended dose of 1,000,000 IU (600 mg) and trough levels were more than 10 times greater than the MIC_{90} for GBS.

After the dose of penicillin G was identified the efficacy of ampicillin plus gentamicin and penicillin G plus gentamicin regimens in the treatment of neonates at risk of EOS was compared in a prospective, open label cluster-randomized study (Table 2). The order of the antibiotic regimens in the two participating units was assigned randomly by flipping the coin. During the first phase of the study (from August 2, 2006 to March 20, 2007) gentamicin was combined with ampicillin in unit A and with penicillin G in unit B. In the second phase (from March 21, 2007 to November 30, 2007), after half of the pre-calculated sample size was recruited, β-lactam antibiotics were switched between the study centres.

After the recruitment of each quarter of the study population an interim safety analysis involving two paediatricians not associated with the study, assessed mortality and the rate of major neonatal complications (see secondary endpoints). If significant differences between the two treatment regimens were observed, the continuation of the study would have been decided by the expert committee.

A separate subgroup analysis of recruited ELBW neonates was pre-planned.

Finally a post-hoc analysis of the prospectively collected data was performed to identify risk factors of empirical antibiotic treatment failure. As no difference between the clinical efficacies of the empirical antibiotic regimens was detected, the data of patients recruited into both arms of the comparative efficacy study were pooled for this analysis.

4.3. Study patients

4.3.1. Pharmacokinetic study

Neonates were eligible if they fulfilled the following criteria: (1) GA ≤ 28 weeks and BW ≤ 1,200 g; (2) PNA ≤ 72 h; (3) hemodynamic stability, defined as mean arterial blood pressure equal to or above that appropriate for the GA, no signs of circulatory compromise (metabolic acidosis, poor perfusion, oliguria, food intolerance), and no need for inotropic/vasoactive support (Cunningham *et al.* 1999, LeFlore *et al.* 2000); (4) normal renal function, defined as urine output of >1 ml/kg/h (assessed within 6 h prior to inclusion), serum creatinine concentration within the normal range for age (69 to 141 μmol/L) (Sonntag *et al.* 1996), and no signs or suspicion of renal disease; (5) the need for early empirical antibiotic therapy, based on the CDC guidelines for the prevention of perinatal GBS disease (AAP 1997); (6) a clinical need for an arterial or central venous catheter; (7) expectancy of survival for more than 3 days; and (8) written informed consent signed by the parents or guardian. Subjects with major malformations and those who had received drugs that may significantly affect renal function, with the exception of gentamicin, were excluded.

4.3.2. Comparative efficacy study and risk factor analysis

Neonates were eligible if they were (1) admitted to NICU within 72 hours of life; (2) needed early empiric antibiotic treatment for EOS or risk factors of infection according to the CDC revised guidelines (e.g. maternal chorionamnionitis and/or maternal risk factors of infection and/or preterm labour in <35 weeks of gestation) (Schrag *et al.* 2002) and (3) were expected not to be transferred within the following 24 hours. Patients, who (1) had received a different antibiotic regimen for more than 24 hours or (2) had suspected or proven meningitis, necrotising enterocolitis (NEC), peritonitis, severe sepsis or septic shock with isolation of microorganisms resistant to the study regimen in maternal urinary tract or birth canal or (3) had other situations that required different antibacterial treatment, were excluded.

ELBW neonates meeting the above mentioned criteria and recruited into the comparative efficacy study constituted the population for ELBW subgroup analysis.

4.4. Study treatments

Study drugs together with dosing regimens and route of administration are presented in Table 3. As all regimens were applied within the first week of life, initial dosing interval of β-lactam antibiotics was similar for all GA groups. After seven days β-lactam dosing interval was decreased to 8h in term neonates. Gentamicin was administered q48h in preterm and q24h in term neonates.

Table 3. Study drugs – dosing regimens and comparators

Study	Study drug; route of administration	Dose (mg/kg); interval	Comparator drug; route of administration	dose (mg/kg); interval
PK study	PEN; iv. slow bolus injection (about 2 min)	15; q12h	PEN; iv. bolus injection	30; q12h
	GEN; iv. 1-hour infusion	5; q48h	GEN; iv. 1-hour infusion	5; q48h
Comparative efficacy study	AMP iv. bolus injection	25; q12h [a]	PEN; iv. bolus injection	15; q12h[a]
	GEN; iv. 1-hour infusion		GEN; iv. 1-hour infusion	

[a] – if meningitis was suspected the dose was doubled and dosing interval reduced to q6h
AMP – ampicillin; PEN – penicillin G; GEN – gentamicin; PK – pharmacokinetic; iv – intravenous

4.5. Data and sample collection

4.5.1. Pharmacokinetic study drug administration and sample collection

Penicillin G (Biochemie GmbH, Austria) was diluted in normal saline for no more than 30 min prior to administration to a final concentration of 50,000 IU (30 mg)/ml and administered via a peripheral or central venous catheter. Gentamicin was administered to all subjects by intravenous infusion over 1 h.

Blood samples for PK analysis were collected at steady state after administration of the third to eighth dose of penicillin G. To minimize the possible effect of gentamicin on renal function, all PK samplings were performed at least 12 h after administration of the last dose of gentamicin. A maximum of 0.3 ml of blood was drawn from an arterial or central venous catheter before and at 2, 30 min and 1.5, 4, 8, and 12 h after the end of the injection of penicillin G. Samples were immediately centrifuged at 2,000 × g for 5 min, and plasma was stored at −20°C for up to one week, when it was transferred to −80°C until analyzed. The actual sampling times were recorded and used for PK calculations if deviations from pre-specified sampling times were more than 1 min for the peak sample and more than 10 min for all other samples.

The penicillin G concentrations in plasma and urine were determined by high-performance liquid chromatography with UV detection, as described previously (Giachetto *et al.* 2004) and detailed in paper I.

Urine samples were collected into plastic urine bags within 12 h after penicillin G study dose administration at 4-h intervals. Simultaneously, possible losses were estimated by weighing the diapers. The data were used for PK calculations only when at least 90% of the total estimated urine output for the

given 4-h period was retrieved. To calculate CL_{CR}, the creatinine concentration was measured in the blood sample collected 12 h after administration of the study dose.

4.5.2. Data collection in comparative efficacy study

The following maternal and perinatal characteristics were registered: maternal age and parity; history of spontaneous and artificial abortions; history of a neonate with GBS disease; maternal chronic diseases, with special attention to diabetes, autoimmune diseases and malignancies; therapies used during pregnancy; drug and alcohol abuse; smoking; number of foetuses; invasive procedures during pregnancy (amniocentesis, foetal transfusions, cervical cerclage); date of maternal bacterial infections during pregnancy, including pathogens isolated from urinary tract and birth canal; type and time of antibiotic treatment during pregnancy and delivery; presence of premature rupture of membranes for more than 18 h; prenatal glycocorticoid prophylaxis, divided as partial (delivery occurred in less than 24 h after the administration of the first dose) or full course (delivery occurred more than 24 h after the administration of the first dose); mode of delivery, including reasons for caesarean section. The latter were further grouped as foetal if signs of acute foetal distress or medically indicated early caesarean section for signs of foetal compromise were present or maternal in all other cases (Liu *et al.* 2004, Joseph 2007).

Early neonatal parameters included demographic characteristics and need of intensive care interventions as follows: BW and GA; first and fifth minute Apgar score; need for respiratory support in the delivery room, age at intubation and surfactant administration, need and duration of sustained respiratory support; age on admission to NICU, time and type of initial and subsequent antibiotic regimens, need for vasoactive therapy with number of agents used. Mean arterial blood pressure below the value of GA in weeks and/or signs of inadequate tissue perfusion reflected by metabolic acidosis and elevated serum lactate level served as an indication for vasoactive therapy. Feeding regimen was documented on Days 1, 3, 7 and weekly thereafter with patients categorized into the following groups based on the route of nutrition and the character of enteral feeds: (1) TPN (intolerance of enteral feeds) when enteral calories constituted less than 10% of total daily calories; (2) breastfeeding when breast milk constituted more than 10% of enteral feeds and (3) formula feeding when formula constituted more than 89% of enteral feeds. Additional parenteral nutrition supplying up to 89% of daily caloric intake was accepted in the two latter groups.

For study purposes the most abnormal values for the following laboratory parameters were registered on the first, 2^{nd} to 3^{rd}; 4^{th} to 5^{th}; 6^{th} to 7^{th} and 10^{th} to 14^{th} day of life: total blood count including WBC, differential and I/T ratio, CRP; serum glucose and total bilirubin. The values of serum albumin, creatinine, urea and liver function tests (LFT) were registered for Days 2–3 and twice

a week thereafter, as the Day 1 values were considered to reflect the condition of the mother rather than that of the neonate.

The occurrence of major neonatal complications – NEC stage II–III (Bell *et al.* 1978), patent arterial duct requiring surgery, threshold retinopathy of prematurity (ROP) requiring laser-therapy, severe intraventricular haemorrhage (IVH) stage III-IV (Papile *et al.* 1978) and severe bronchopulmonary dysplasia (BPD) (Jobe *et al.* 2001, Ehrenkranz *et al.* 2005) was registered. Final outcome, including weight and clinical diagnosis at discharge, major neonatal complications and autopsy results, if applicable, were also recorded.

4.6. Monitoring and follow-up

All patients had basic hemodynamic variables, including heart rate, arterial blood pressure and transcutaneous oxygen saturation monitored continuously by attending staff and registered according to the clinical routine throughout NICU admission.

In the PK study from the study dose administration blood pressure, heart rate, and transcutaneous oxygen saturation were recorded every 30 min for 12 h by the attending nurse. For safety monitoring, serum sodium, potassium, calcium, glucose, albumin, creatinine, and urea levels were measured within 24 h before and 24 h after study drug administration. All infants were followed for the occurrence of adverse events for at least 7 days after the study. In order to identify possible adverse effects from repeated blood sampling, the need for blood component transfusions throughout the intensive care period was registered.

In comparative efficacy study neonates were followed up until discharge from NICU or until Day 60 of life, whichever occurred first. Laboratory tests were run as clinically indicated but not less than as described in chapter 0.

4.7. Definitions

Neonatal sepsis (proven or clinical) was diagnosed in the presence of at least two clinical (hyper- or hypothermia, apnoea or bradycardia spells, increased oxygen requirement, feeding intolerance, abdominal distension, lethargy and hypotonia, hypotension, skin and subcutaneous lesions such as petechial rash, abscesses, sclerema) and two laboratory criteria (WBC count <5000 or >20,000 x 10^9 cells/L; I/T ratio > 0.2; platelet count <100,000 x 10^9/L; CRP >10 mg/L).

Proven sepsis included cases where in addition to clinical and laboratory signs, a pathogen was isolated from a normally sterile body fluid (except CoNS that had to be isolated from at least two different specimens or with only one positive culture adequate antibiotic treatment had to be given for more than 72 hours). All other cases were termed **clinical sepsis.**

EOS was defined as sepsis occurring within the first 72h of life; all other cases were considered **LOS** (Stoll *et al.* 2002b).

Treatment failure (used in comparative efficacy study**)** was defined as the need for a change in the initial empiric antibiotic regimen within 72 hours and/or any death within seven days. The following situations were pre-specified to require change of antibiotic regimen: (1) suspicion of meningitis or abdominal infection/NEC; (2) isolation of bacteria resistant to the empiric antibiotic regimen from a neonate with sepsis; (3) isolation of bacteria resistant to the empiric antibiotic regimen from maternal urinary tract/birth canal of a neonate with sepsis; (4) no improvement or deterioration of clinical status; (5) change of antibiotic after 72h of empiric therapy; (6) other situations where the treating physician considered change of antibiotic regimen necessary – detailed reasons were documented.

Antibiotic treatment failure (used in risk factor analysis) included only treatment failure cases with clear evidence of infection, based on at least one of the following criteria: (1) isolation of EOS etiologic pathogen(s), resistant to initial therapy, from normally sterile body fluids; (2) diagnosis of clinical EOS and death with autopsy confirming congenital infection (neutrophil infiltration of multiple tissues with or without bacteria on Gram-stain and microbiological cultures positive for a known neonatal pathogen) or clinical EOS and major sepsis-related complication (i.e. IVH grade III-IV (Papile *et al.* 1978) within the first week of life; (3) change of empirical antibiotic therapy within 72 h due to deterioration of an EOS patient with the new regimen continued for more than 72 h.

4.8. Analysis of the data

4.8.1. Pharmacokinetic calculations

PK analysis was performed with WinNonlin software (version 5.0.1; Pharsight Corporation, CA) by applying a non-compartmental model that assumed the use of a bolus iv injection. The $AUC_{0–12}$ was calculated by use of the log-linear trapezoidal rule. The $AUC_{0–12}$ was used to calculate the total body CL; the clearance at steady state. The apparent V_{ss} was determined by calculating the mean residence time extrapolated to infinity. CL_R of penicillin G was calculated as follows: $CL_R = A_e\tau/AUC_{0–12}$, where $A_e\tau$ is the amount of unchanged drug excreted into urine (A_e) during the dosing interval (τ). CL_{CR} was calculated directly from the urine and serum creatinine concentrations at 12 h. Multiple linear regression analysis (StatsDirect software, version 2.3.4; Cheshire, United Kingdom) was used to estimate the influence of GA, gender, CL_{CR} and weight on $AUC_{0–12}$, CL_R, CL at steady state, and V_{ss}.

4.8.2. Comparative efficacy study

In comparative efficacy and risk factor studies statistical analysis was performed using statistical software R 2.4.0 and R 2.7.2 http://www.r-project.org/.

The sample size calculation in the comparative efficacy study was based on a retrospective analysis of medical records in 2003–2004 showing that about 10% of patients with suspected EOS need change of early empiric antibiotic regimen (Metsvaht *et al.* 2006). The study was planned as an equivalence trial assuming that the lower and upper boundary of the two-sided 95% CI for the difference in the treatment failure between the study arms will not exceed +/-10%. Accepting a two-sided type I error rate of ≤ 0.05 (equivalence trial in binary data) and a power of 80% both treatment arms were to enrol at least 140 patients (Pocock 1983, Hayes *et al.* 1999).

Primary endpoint of the comparative efficacy study was treatment failure (see definitions 0).

Secondary end-points were 28 day- and NICU mortality, NICU and hospital stay, duration of early empiric antibiotic treatment, duration of respiratory support and vasoactive treatment, rate of LOS and use of additional antibacterial therapy, the presence of NEC stage II–III, patent arterial duct requiring surgery, threshold ROP requiring laser-therapy, severe IVH stage III–IV and severe BPD.

Reverse treatment failure rate (treatment success) in comparative efficacy study was assessed by **Kaplan-Meyer curves**. For comparisons between groups **hierarchical (mixed effect) models** appropriate for cluster randomised design incorporating the influence of study centre and treatment period were used (Murray *et al.* 2004). A subgroup analysis of recruited ELBW neonates was performed the same way. To assess the impact of empiric antibiotic regimen on LOS due to *S. epidermidis* and ELBW mortality multivariate hierarchical models adjusted for previously described risk factors, identified from literature (Holmsgaard *et al.* 1996, Hoekstra *et al.* 2004, Perlman *et al.* 2007) was applied.

4.8.3. Risk factor analysis

In risk factor analysis logistic regression and classification and regression tree analysis was applied.

Univariate logistic regression analysis was used to identify predictors of antibiotic treatment failure from maternal and neonatal characteristics and laboratory tests. If both high and low levels of a laboratory test would be considered abnormal (i.e. WBC, blood glucose), generally accepted normal limits were applied to test possible predictive potential of a given parameter. For laboratory values indicative of sepsis the following cut-off levels used as the diagnostic criteria of neonatal sepsis were applied (see chapter 0) (Auriti *et al.* 2005). For

blood glucose values between 3.0 and 5.5 mmol/L were used as normal limits (Mehta 1994).

Multiple logistic regression analysis with backward stepwise removal in order of insignificance, including all parameters significant at a p value of ≤ 0.05 in univariate logistic regression analysis, was applied to find the best combination of predictors for antibiotic treatment failure. To ensure the earliest possible timing of decisions separate multiple logistic regression analysis of data available by 24 and 72 h of age was performed. In addition to sensitivity, specificity, PPV and NPV the prognostic value of the models was assessed by calculating the probability of antibiotic treatment failure for each possible number and combination of "positive" risk factors.

Classification and regression tree analysis was performed with the function ***rpart*** of package R (Maindonald *et al.* 2007). Recursive partitioning with binary cut of entered variables was used for the decision tree development and the best separator of treatment failure and success was chosen for tree root. The same process was repeated until the best discrimination between treatment success and failure was achieved or until the minimum allowed number of seven patients per node was met. For tree pruning the k-fold cross-validation was applied. Surrogate splitting was used to handle missing values. Like in multiple regression analysis, separate classification and regression trees for data available by 24 and 72 h of age were constructed.

For laboratory tests the highest and lowest values within 72 h were incorporated as continuous variables to allow generation of splitting values with maximum information gain. In order to avoid any investigator induced bias all maternal, early neonatal and laboratory parameters identified by univariate regression analysis as significant predictors of antibiotic treatment failure, were included. Specificity, sensitivity, PPV and NPV with 95% CI was calculated for each prediction.

5. RESULTS AND DISCUSSION

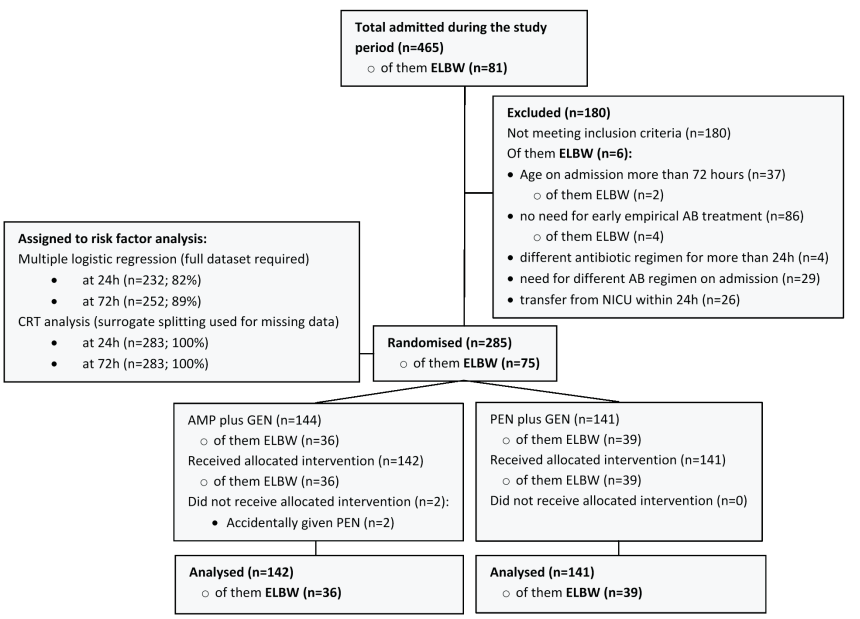
5.1. Demographics of the study populations

5.1.1. Pharmacokinetic study

During the study period a total of 24 neonates eligible by GA and BW were admitted. Six of them were excluded (three in each period) for the following reasons: major congenital malformation (n = 1), parental refusal (n = 1), and hemodynamic instability (n = 4). The study included nine patients in each dosing group; their baseline demographics were similar (paper I, Table 1). None of the study subjects had a positive blood culture prior to or during the study.

5.1.2. Comparative efficacy study

Patient flow and reasons for exclusion in comparative efficacy study, ELBW subgroup analysis and risk factor analysis of antibiotic treatment failure are shown on Figure 1. Interim safety analysis revealed no significant differences in mortality or any of the secondary endpoints and the study was completed as planned.



ELBW – extremely low birth weight; AB – antibiotic; NICU – neonatal intensive care unit; CRT – classification and regression tree analysis; AMP – ampicillin; PEN – penicillin G; GEN – gentamicin

Figure 1. Study outline and patient flow in comparative efficacy study and risk factor analysis.

A total of 465 neonates, among them 81 ELBW infants, were admitted to units A and B throughout the study period. Antibiotic treatment other than the study regimen was required in 29 cases (see paper II for details). The exclusion rate in unit A (43%) and B (34%) was similar. Absence of need for early empiric antibiotic treatment was more common in unit A than in unit B (OR 3.78; 95% CI 2.18–6.53), likely reflecting a difference in admitted population. All four ELBW neonates not needing empirical antibiotic treatment were small for gestational age infants born near or at term.

A total of 283 neonates (60.9% of admissions); 142 in ampicillin and 141 in penicillin G group constituted the study population. The subgroup analysis of ELBW neonates included 75 neonates, 36 in ampicillin and 39 in penicillin G arm, respectively (Figure 1). With regards to demographic characteristics both treatment arms were well balanced except for ventilatory support that was less commonly used in the ampicillin group (OR 0.44; 95% CI 0.24–0.81). There were no differences between the demographic characteristics of the ELBW subgroups (Table 4).

Table 4. Demographic data of the study groups

	Comparative efficacy study		ELBW subgroup analysis	
	AMP (n=142)	PEN (n=141)	AMP (n=36)	PEN (n=39)
GA – weeks; median (IQR)	31 (27–34)	31 (28–35)	25 (24–26)	25 (25–27)
>36 weeks – n (%)	29 (20)	28 (20)	NA	NA
<28 weeks – n (%)	41 (29)	34 (24)	33 (92)	29 (74)
<26 weeks – n (%)	24 (17)	21 (15)	24 (67)	21 (54)
BW – g; median (IQR)	1467 (920–2553)	1500 (960–2343)	770 (694–876)	810 (693–866)
<1501g – n (%)	73 (51.4)	72 (51.1)	NA	NA
<1001g – n (%)	36 (25.4)	39 (27.7)	36	39
<751g – n (%)	19 (13)	15 (11)	19 (53)	15 (38)
M/F sex – n	78/64	85/56	19/17	22/17
Mean Apgar score at 5 min	6.6 ± 1.5	6.3 ± 1.6	6.2 ± 1.7	5.9 ± 1.4
Ventilated – n (%)	99 (70)	116 (82)	36 (100)	39 (100)
Surfactant – n (%)	81 (56)	88 (62)	36 (100)	38 (97)
Caesarean section – n (%)	77 (54)	80 (57)	13 (36)	20 (51)
Multiple pregnancies – n (%)	34 (24)	23 (17)	5 (14)	7 (18)
Chorionamnionitis – n (%)	21 (15)	30 (21)	12 (33)	19 (49)
PROM >18 h – n (%)	25 (18)	28 (20)	9 (25)	13 (33)
Prenatal glycocorticoids	86 (61)	71 (50)	30 (83)	32 (82)
Maternal antibiotic therapy – n (%)				
during pregnancy	38 (27)	27 (19)	7 (19)	10 (26)
during delivery	51 (36)	46 (33)	19 (53)	20 (56)

AMP – ampicillin; PEN – penicillin G; IQR – interquartile range; GA – gestational age; BW – birth weight; PROM – premature rupture of membranes

5.1.3. Risk factor analysis of antibiotic treatment failure

A total of 32/283 neonates fulfilled the criteria of antibiotic treatment failure, 21 of whom had culture and/or histologically proven sepsis (Table 5). Another 11 neonates with the diagnosis of clinical EOS had antibiotic treatment changed within the first 72 h due to deteriorating clinical condition (n = 4) or suspected meningitis (n = 7); with the new treatment regimen started at a median age of 46 h (range 19–62). Neonates, who failed on empiric antibiotic regimen had significantly lower GA and BW, were more often born to mothers with chorionamnionitis, had lower first and fifth minute Apgar scores, required more often surfactant therapy, were more likely to have proven EOS and to die within 7 days than those with antibiotic treatment success (Paper III, Table 1).

Table 5. Distribution of antibiotic treatment success and failure cases in risk factor analysis.

Antibiotic treatment success	251
Failure of early empirical antibiotic regimen – N (% of all study patients)	32 (11.6)
1. Proven EOS with etiologic pathogen(s) resistant to initial empirical antibiotic therapy – n (% of treatment failure)	10 (31.3)
died within 7 days	4
2. clinical EOS and death (with autopsy confirming the diagnosis of congenital infection) and/or major sepsis-related complication (IVH III–IV) within the first week of life – n (% of treatment failure)	11 (34.4)[a]
died within 7 days	10
3. clinical EOS and change of initial empirical antibiotic therapy within 72h with new regimen continued for more than 72 h – n (% of treatment failure)	11 (34.4)

All cases are represented once – in the "highest" applicable category, i.e. a neonate with culture positive sepsis with a pathogen, resistant to empiric regimen is included only in proven EOS category independent of whether he died within 7 days or not.
[a] includes an ELBW neonate with clinical EOS and IVH IV by the third day of life who died on Day 8, autopsy confirmed the diagnosis of EOS
EOS – early onset sepsis; IVH – intraventricular haemorrhage

Complete dataset for multiple logistic regression analysis of risk factors predicting failure of empirical antibiotic therapy was available for 232/283 (82%) at 24h and for 252/283 (89%) neonates at 72h (Figure 1). While 31/32 (97%) treatment failure and 201/251 (80%) treatment success cases could be included in multiple logistic regression at 24h; a more equal distribution of missing values was seen at 72h with 30/32 (94%) treatment failure and 222/251 (88%) treatment success cases included. All patients were included into classification and regression tree analysis as surrogate splitting was used for missing values.

5.2. Pharmacokinetic profile of penicillin G in VLBW neonates

5.2.1. Comparison of two doses

Penicillin G PK parameters for the two dosing regimens in VLBW neonates in comparison to previously published data in term neonates and adults are presented in Tabel 6 and individual time-concentration curves in Figure 2.

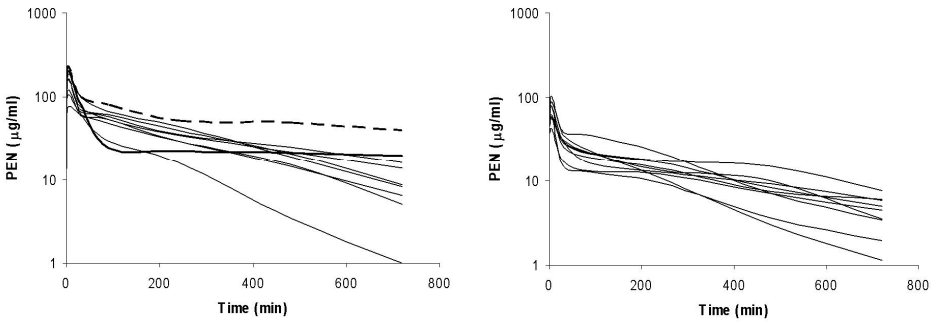


Figure 2. Individual time concentration curves of intravenous penicillin g in VLBW infants receiving the dose of 50,000 IU/kg (left) and 25,000 IU/kg (right).

Dotted line – the patient accidentally received penicillin G dose of 83,000 IU/kg; data were excluded from the PK calculations for group 1.

Bold line – an infant born from 26th week of gestation with birth weight of 700 g had clinical oedema and penicillin G elimination half-life of 35.1 h; data were included in the PK calculations for group 1.

PEN – penicillin G

In the first group with the dose of 50,000 IU (30 mg)/kg q12h, the values of C_{max}, C_{min}, and $AUC_{0–12}$, in neonates were much greater than the respective values in adults after the administration of the doses recommended in most textbooks (Chambers 2005) and the median C_{min} of 7.4 µg/ml exceeded the MIC_{90} for GBS by about 100 times. The values observed by us are in concordance with those, measured in a later study by Muller *et al* in a similar population of VLBW neonates (Muller *et al.* 2007). Therefore, for the second group, the penicillin G dose was reduced by half, to 25,000 IU (15 mg)/kg q12h. As expected, the dose reduction resulted in proportionally lower C_{max} and C_{min} and lower $AUC_{0–12}$ values (Tabel 6), however, it ensured C_{min} above the MIC_{90} for GBS in all patients (Jokipii *et al.* 1985, Figueira-Coelho *et al.* 2004, EUCAST 2010). With both dosing regimens a significantly longer $T_{1/2}$ compared to that in adults (Chambers 2005) and term infants (McCracken *et al.* 1973) was observed, but there was no difference between the two study groups (Table 6).

Table 6. Median (IQR) PK parameters for Penicillin G in VLBW neonates with two different dosing regimens in comparison to term neonates (McCracken *et al.* 1973) and adults (Chambers 2005)

Group	PEN dose (IU/kg) /route	PEN dose (mg(kg)	$t_{1/2}$ (h)	C_{max} (µg/ml)	C_{min} (µg/ml)	CL (ml/ min/kg)	V_{ss} (liter/kg)	$AUC_{0–12}$ (min·µg/ml)
Preterm VLBW neonates – present study results:								
Group 1 (*n* = 8 [a])	46.9 (46.4–48.1)/i.v	28 (27.9–28.9)	3.8 (3.3–7.0)	145.5 (108.6–157.3)	7.1 (5.2–12.9)	1.2 (1.1–1.4)	0.41 (0.33–0.57)	23,360.2 (20,480.6–26,174.1)
Group 2 (*n* = 9)	23,9 (22.9–24.1)/i.v	14.3 (13.8–14.5)	4.6 (3.8–5.6)	58.90 (52.9–77.5)	3.4 (2.9–3.6)	1.5 (1.3–1.8)	0.64 (0.50–0.71)	9,671.0 (8,180.0–10,180.0)
Term neonates – data from literature:								
age <7 d (*n* = 23)	25,000/i.m	15	3.4	22.0	2.3	ND	0.51	ND
Adults – data from literature:								
(*n* = 6)	1,000,000 dose/i.v	600	0.5	45	ND	ND	0.5	ND

i.v. – intravenous; i.m. – intramuscular; ND – no data; data in parentheses represent lower-upper quartiles

[a] one patient in group 1 accidentally received almost a double dose of penicillin G (83,300 IU/kg, equivalent to 50 mg/kg; shown as a dashed line in Figure 2; the data for this patient were excluded from the PK calculations for the first dosing regimen

IQR – interquartile range; PEN – penicillin G; VLBW – very low birth weight; $t_{1/2}$ – half-life; C_{max} – serum peak concentration; C_{min} – serum trough concentration; CL – body clearance; V_{ss} – volume of distribution at steady state; $AUC_{0–12}$ – area under time-concentration curve 0–12h

The longer $T_{1/2}$ and higher C_{max} and C_{min} of penicillin G in VLBW infants compared to adults and even term neonates, are well consistent with the results of previous PK studies on β-lactam antibiotics in VLBW infants and are most likely the result of the higher body water content and different compartmental distribution of the body water in VLBW infants, as well as their immature renal function (McCracken *et al.* 1973, Alcorn *et al.* 2003, Muller *et al.* 2007, Allegaert *et al.* 2008, Pacifici 2009). Lower dose, longer interval administration regimens for VLBW infants have been recommended for several β-lactam antibiotics, like amoxicillin, ampicillin, carbenicillin, and meropenem (Morehead *et al.* 1972, Kaplan *et al.* 1974, Huisman-de Boer *et al.* 1995, van Enk *et al.* 2001) and also for penicillin G in an recently conducted PK study (Muller *et al.* 2007).

5.2.2. Elimination of penicillin G

The CL_{CR} and 24-h creatinine excretion values were similar for both dosing regimens (paper I, Table 3), with a wide range of variation, despite the relatively narrow range of BWs and GAs of the infants in our study. There was a significant correlation between penicillin G CL_R and CL_{CR} (penicillin G CL_R (ml/min) = 0.221987 CL_{CR} (ml/min) + 0.233152; R^2 = 0.309596; P = 0.038), suggesting that GFR, not tubular secretion as in adults (Chambers 2005), is the predominant renal excretion mechanism of penicillin G in VLBW neonates. In preterm neonates with immature renal function and especially immature tubular function, the renal elimination of other β-lactam antibiotics within the first week(s) of life has been shown to be proportional to the GFR (Morehead *et al.* 1972, Huisman-de Boer *et al.* 1995, Capparelli *et al.* 2005, Bradley *et al.* 2008, van den Anker *et al.* 2009a). Total CL increases with age (Bertels *et al.* 2008) but CL_R exceeds the GFR only after several weeks, when significant maturation of tubular function has been achieved (Huisman-de Boer *et al.* 1995). A significant correlation between the amount of penicillin G excreted into urine and CL_{CR} in more mature neonates has been demonstrated by McCracken *et al.* (McCracken *et al.* 1973).

5.2.3. Dose recommendations for penicillin G

Our results suggest that penicillin G dose of 25,000 IU/kg q12h is well tolerated and adequate for the treatment of neonatal infections caused by penicillin-susceptible pathogens.

In general, paediatric PK studies aim to determine doses that approximate the exposure with standard dosing in adults, enabling the extrapolation of efficacy data from the adult studies (Lutsar *et al.* 2010a). More specifically than comparing the drug exposure with paediatric and adult patients, neonatal PK studies should target appropriate PK/PD parameters, like peak/MIC and AUC/MIC ratio or fT > MIC, which have been shown to be major determinants

of in vivo efficacy (Drusano 2004, van den Anker *et al.* 2009b, Lutsar *et al.* 2010a). However, the target values for such PK/PD correlates are based on expert opinion rather than scientific evidence (Lutsar *et al.* 2010a).

Both, severe infections and immunocompromised hosts (i.e. sepsis in pre-term infant) require higher PK/PD targets compared with mild to moderate infections in immune-competent host (Czock *et al.* 2007, Lutsar *et al.* 2010b). For β-lactam antibiotics fT>MIC of 100% has been suggested (McKinnon *et al.* 2008, Scaglione *et al.* 2008, Lutsar *et al.* 2010b). Due to the immaturity of the immune system, neonates are considered to be immune-compromised hosts (Strunk *et al.* 2004, Wynn *et al.* 2009). Bearing in mind the relatively high prevalence of meningeal involvement in EOS (Persson *et al.* 2002, Schrag *et al.* 2006b) and the nonspecificity of the clinical symptoms of meningitis in VLBW infants, the recommended penicillin G dosing regimen should also ensure adequate antibiotic concentrations in CSF.

Assuming that the penetration of penicillin G through the inflamed BBB is about 10% and that the principal determinant of antibiotic effectiveness is the relation between the antibiotic concentration in CSF and the MBC for the infecting organism, with the required time above the MBC of 100%, the penicillin G dose of 25,000 IU (15 mg)/kg q12h is likely to be adequate in most cases (Hieber *et al.* 1977, Lutsar *et al.* 1998, Trijbels-Smeulders *et al.* 2006). Assuming a 50% protein binding of penicillin G (McNamara *et al.* 2002) and an MBC of two times the MIC (0.12 to 0.18 μg/ml) (Jokipii *et al.* 1985, Ruess *et al.* 2000), the active non-protein-bound concentration of penicillin G would still exceed the MBC two times. However, such calculations should be interpreted with caution, as the true PK parameters of penicillin G in CSF may be influenced by a variety of other factors (Lutsar *et al.* 1998). In addition inoculum size dependent MIC has been demonstrated for GBS (Feldman 1976, Weeks *et al.* 1981).

Another priority should be avoiding unnecessary potential for toxicity, most likely associated with high concentrations rather than hypersensitivity in neonates. Based on these considerations and the PK of penicillin G we recommend the dose of 25,000 IU/kg q12h for VLBW neonates at risk of EOS, with the dose doubled for those with meningitis. This suggestion is further supported by the Monte Carlo Simulation data from the study by Muller *et al*., showing that the dose of 50,000 IU/kg q12h would ensure the fT>MIC of 100% up to the MIC values of 4 μg/ml (Muller *et al.* 2007). In the context of EOS penicillin G is active against streptococci (and probably *Listeria*) only, hardly ever showing such high MIC values (EUCAST 2010). Based on the above the dose of 25,000 IU/kg q12h was taken forward into the clinical trial.

5.3. Comparative efficacy of ampicillin vs penicillin G

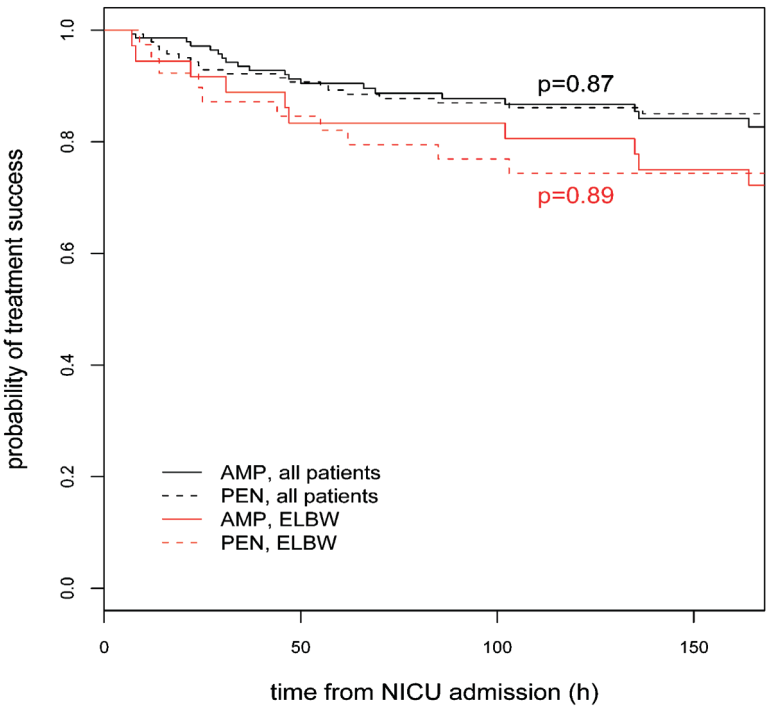
5.3.1. Primary endpoint

In the comparative efficacy study the overall treatment failure rates in neonates at risk of EOS treated with ampicillin plus gentamicin (14.1%) and penicillin G plus gentamicin (14.2%) as well as the proportions of the individual components of the primary endpoint were similar with the 95% CI remaining within the pre-specified range of +/- 10% (Table 7). The reasons for change of antibiotic therapy within 72 h were also similar in both treatment arms, with antibiotic changed for deteriorating condition in five and four; suspected meningitis in three and four and for NEC or other suspected abdominal infection in two and two neonates in the ampicillin and penicillin G groups respectively. In no case antibiotic therapy was changed due to isolation of resistant bacteria from the neonate or maternal birth canal.

Table 7. Primary endpoint– odds ratios (OR) and 95% confidence intervals (95% CI) from hierarchical model corrected for participating unit and treatment period.

	AMP (n=142)	PEN (n=141)	OR (95% CI)	Treatment difference (%; 95% CI)
Treatment failure (composite) – n (%)	20 (14.1)	20 (14.2)	1.01 (0.52–1.97)	0.1 (–8.1; 8.3)
Components of the composite endpoint:				
Antibiotic change in 72h – n /died	10/ 1	10/ 4	1.02 (0.40–2.59)	0.05 (–6.3; 6.4)
Death in 7 days – n (%)	11 (7.7)	14 (9.9)	0.76 (0.33–1.75)	2.2 (–4.7; 9.1)
Subgroup analysis of ELBW neonates – n	**36**	**39**		
Treatment failure in ELBW neonates – n (%)	10 (28)	10 (24)	1.1 (0.4–3.1)	
Antibiotic change in 72h in ELBW neonates – n /died	4/1	3/1	1.5 (0.3–9.3)	
Death in 7 days in ELBW neonates – n (%)	7 (22)	8 (21)	0.9 (0.3–2.9)	

Kaplan-Meier analysis revealed almost identical treatment success rate for both treatment regimens in the whole study group as well as among ELBW neonates (Figure 3).



AMP – ampicillin; PEN – penicillin G

Figure 3. Kaplan-Meier curves of cumulative treatment success by treatment arm in the whole study population (black) and in ELBW neonates (red).

The equivalence of ampicillin plus gentamicin vs penicillin G plus gentamicin in the treatment of neonates at risk of EOS is not surprising. Penicillin G is an antibiotic with narrow spectrum of activity, in the context of EOS limited mostly to streptococci (Ronnestad *et al.* 2005, EUCAST 2010), still prevailing in the bacterial aetiology of EOS among term and near-term infants (Cohen-Wolkowiez *et al.* 2009, Wu *et al.* 2009), and maybe *Listeria monocytogenes*. The high ampicillin resistance of most Gram-negative pathogens involved in EOS is likely to abolish the former advantage of broader spectrum coverage with ampicillin. In a recent study in Estonian ICUs 45% of *E. coli,* 82% of other *Enterobacteriaceae* and 93% of *K. pneumoniae* were ampicillin resistant (Mitt *et al.* 2009). In other countries even higher resistance rates have recently been reported for neonatal pathogens (Bizzarro *et al.* 2008, Litzow *et al.* 2009, Maayan-Metzger *et al.* 2009).

Caution should be taken when interpreting the results in ELBW neonates, as the study was not adequately powered for subgroup analyses. Also significant differences between the two antibiotic regimens appeared in secondary outcomes among ELBW neonates (see below chapters 0 and 0).

5.3.2. Bacterial aetiology of EOS and sensitivity to empiric antibiotic regimens

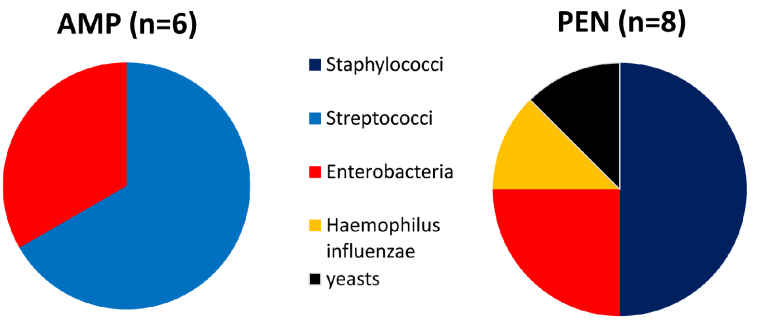
While the overall incidence of clinical and proven EOS in both treatment arms was similar (Table 8), among ELBW infants proven EOS was significantly more frequent in penicillin G than in ampicillin arm (6/39 vs 0/36; Table 8). There was no difference in proven plus clinical EOS related early neonatal mortality overall (6/34 vs 8/33 in the ampicillin and penicillin G group, respectively) or among ELBW neonates (4/15 vs 5/16 in the ampicillin and penicillin G group, respectively). Although the higher incidence of proven EOS among ELBW neonates in the penicillin G arm may just reflect the unequal distribution in an underpowered subgroup analysis, the different bacterial aetiology among ELBW neonates compared to term and near-term infants suggests the impact of empiric antibiotic regimen. A recent study of 140 preterm neonates found higher rate of proven EOS among infants not treated with antibiotics vs those treated with amoxicillin-clavulanic acid plus amikacin but no difference in the prevalence of EOS overall (Tagare *et al.* 2009) suggesting similar to us the role of early empiric antibiotic therapy in the prevention of culture positivity rather than EOS.

Table 8. Early onset sepsis – odds ratios (OR) and 95% confidence intervals (95% CI) from hierarchical model corrected for participating unit and treatment period

	AMP (n=142)	PEN (n=141)	OR (95% CI)
EOS (proven+clinical) – n (%)	34 (24)	33 (23)	1.03 (0.6–1.8)
Died – n (% EOS related early neonatal mortality)	6 (18)	8 (24)	NA
Proven EOS – n (%)/ died in 7 days	6 (4.2)/ 1	8 (5.7)/ 3	0.73 (0.3–2.2)
Clinical EOS – n (%)/ died in 7 days	28 (20)/ 5	25 (18)/ 5	1.14 (0.6–2.1)
ELBW subgroup analysis – n	**36**	**39**	
EOS (proven+clinical) in ELBW neonates – n (%)	15 (42)	16 (41)	1.1 (0.5–2.9)
Died – n (% EOS related early neonatal mortality)	4 (27)	5 (31)	NA
Proven EOS in ELBW neonates – n (%)/ died in 7 days	0	6 (15)/4	0.1 (0.01–0.6)

AMP – ampicillin; PEN – penicillin G; EOS – early onset sepsis; ELBW – extremely low birth weight; NA – not applicable

A total of 14 microorganisms were isolated from patients with EOS (Figure 4; detailed in paper II, Table 3). Gram-positive cocci were isolated in 8/14 and Gram-negative bacteria in 5/14 EOS cases; in an ELBW neonate *Candida albicans* was identified. While all four GBS sepsis occurred in term or near-term infants, all three cases of *S. epidermidis* EOS were diagnosed in preterm neonates with GA <= 28 weeks and the diagnosis was based on at least two positive blood cultures not more than 72h apart. Three of the six proven EOS cases among ELBW neonates were caused by *Enterobacteriaceae*.



AMP – ampicillin; PEN – penicillin G

Figure 4. Bacterial aetiology of early onset sepsis in the ampicillin (n=6) and penicillin G (n=8) treatment arm.

Thus our study, although relatively small, supports recent data showing the predominance of *Enterobacteriaceae* and CoNS in the aetiology of EOS in VLBW/ELBW neonates (Stoll *et al.* 2002a, Ronnestad *et al.* 2005, Stoll *et al.* 2005, Klinger *et al.* 2009).

In the ampicillin group all six isolates were susceptible to at least one component of the empiric antibiotic regimen compared to 3/8 in the penicillin G group (OR; 0.080; 95% CI 0.0095–0.67). Difference between the two groups was accounted for by the resistance of all four CoNS strains and *C. albicans* to both empiric antibiotics in the penicillin G group. All *E. cloacae* isolates and the *E. coli* strain in the ampicillin arm were resistant to ampicillin, but none of the Gram-negative isolates were resistant to gentamicin.

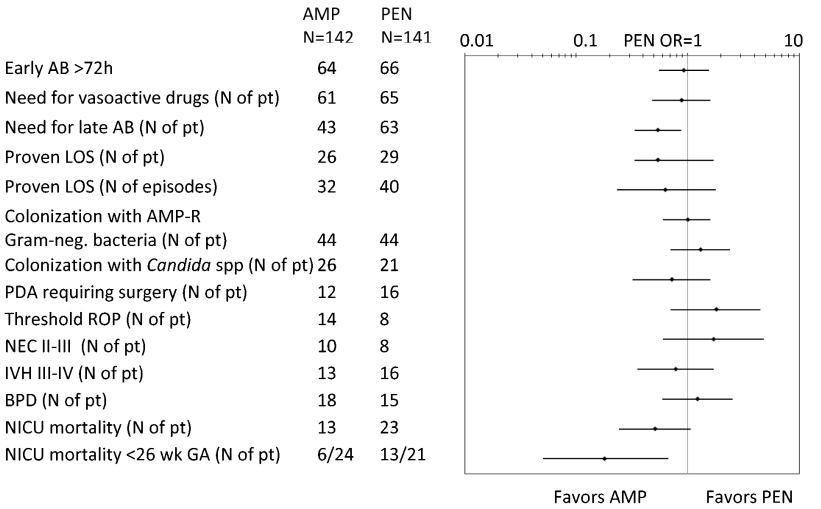
In the light of the high β-lactam resistance rates, the predominance of Gram-negative rods and CoNS in the aetiology (Stoll *et al.* 2002a, Ronnestad *et al.* 2005, Stoll *et al.* 2005, Klinger *et al.* 2009), differences in host response to infection (Schultz *et al.* 2004, Strunk *et al.* 2004, Wynn *et al.* 2009) and the persistently high mortality rate of EOS in VLBW/ELBW infants, reaching 40% in EOS with resistant *E. coli* (Schuchat *et al.* 2000), the adequacy of both studied and presently most widely applied empiric antibiotic regimens in the subpopulation of ELBW neonates should be questioned.

In part the issue of spreading β-lactam resistance has been addressed with concomitant use of gentamicin which has preserved high activity against most EOS causative organisms (Jones *et al.* 2004, Larson *et al.* 2005) as seen also in this study. However, aminoglycosides alone are generally believed not to be overly effective for treating many types of infections (Drusano *et al.* 2007). In an early study comparing ceftazidime with carbenicillin and amikacin combination in the treatment of proven invasive infections in neonates significantly higher case fatality and total failure rates were seen in the latter arm, despite that 90% of the isolates tested susceptible to amikacin (Odio *et al.* 1987). Some recent data suggest that the relatively low efficacy may have risen from inappropriate dosing regimens (i.e. lower doses and multiple daily dosing) as well as inappropriately high resistance breakpoints (Drusano *et al.* 2007). The latter has promoted aminoglycoside use in situations were low efficacy can be presumed.

Still, the empiric and widespread use of aminoglycosides in neonates is not problem free either. Renal toxicity has been pointed out in early studies (Adelman *et al.* 1987b; a, Tessin *et al.* 1988). More recently the safety in terms of potential influence on hearing function has been questioned. Among the carriers of a mitochondrial DNA mutation 1555A—G, the prevalence of which in populations of European descent is about 0.2%, permanent profound hearing loss develops after aminoglycoside exposure, even when drug levels are within therapeutic range and the duration of treatment is short (Hutchin *et al.* 1993, Bitner-Glindzicz *et al.* 2009, Vandebona *et al.* 2009). As elective genetic screening before aminoglycoside administration is not feasible in NICU and maternal screening may not detect low levels of heteroplasmy (Bitner-Glindzicz *et al.* 2009), a safer choice of antibiotic might be an alternative. At least there is an urgent need to identify neonates likely failing on the presently applied empiric antibiotic regimen.

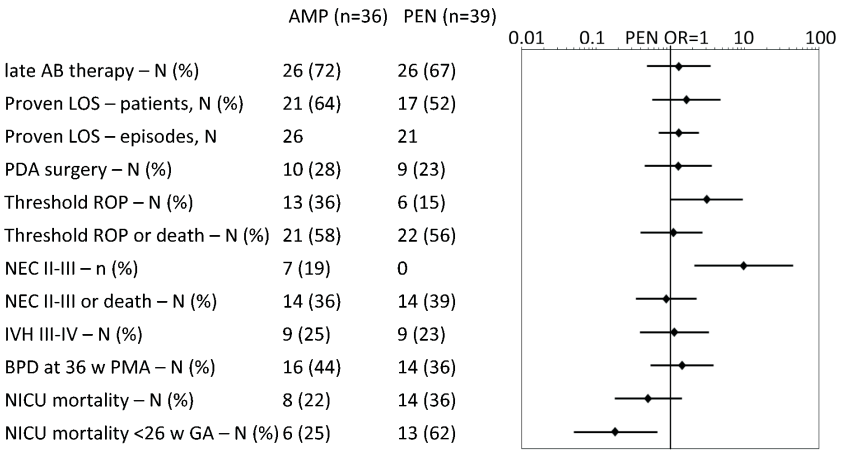
5.3.3. Other secondary endpoints

With regards to secondary endpoints both treatment arms were equal except for NICU mortality of neonates born before 26th week of gestation, *S. epidermidis* caused LOS rate and use of additional antibiotic therapy (Figure 5), all favouring ampicillin regimen. However, in ELBW neonates higher incidence of NEC II-III and ROP was observed in the ampicillin arm (Figure 6).



AB – antibiotic; AMP – ampicillin; PEN – penicillin G; LOS – late onset sepsis; PDA – patent arterial duct; ROP – retinopathy of prematurity; NEC – necrotizing enterocolitis; IVH – intraventricular haemorrhage; BPD – bronchopulmonary dysplasia; NICU – neonatal intensive care unit

Figure 5. Forest plot of secondary end-points – data are presented as point estimates of odds ratios (OR; indicated by diamonds) with the 95% confidence intervals (indicated by lines)



AB – antibiotic; AMP – ampicillin; PEN – penicillin G; LOS – late onset sepsis; PDA – patent arterial duct; ROP – retinopathy of prematurity; NEC – necrotizing enterocolitis; IVH – intraventricular haemorrhage; BPD – bronchopulmonary dysplasia; NICU – neonatal intensive care unit

Figure 6. Forest plot of secondary endpoints in ELBW neonates – data are presented as point estimates of odds ratios (OR; indicated by diamonds) with the 95% confidence intervals (indicated by lines)

5.3.3.1. NICU mortality

In NICU mortality a trend favouring ampicillin treatment (13/142 vs 23/141 in the ampicillin and penicillin G arm, respectively; OR 0.5; 95% CI 0.24–1.06) was seen, which was significant for infants born before 26th week of gestation (6/24 vs 13/21 in the ampicillin and penicillin G arm, respectively; OR 0.2; 95% CI 0.05–0.7) (Figure 5). This finding is further supported by the fact that the proportion of neonates with the highest risk of death in the ELBW group, i.e. with GA below 26 weeks, was even larger in the ampicillin than in the penicillin G group (67% vs 54%, respectively). Multivariate mixed model analysis of ELBW NICU mortality, corrected for study centre and treatment period, found greater GA, full course of steroid prophylaxis and singleton pregnancy to be associated with improved survival, while early antibiotic treatment with ampicillin regimen remained of borderline significance (Table 9).

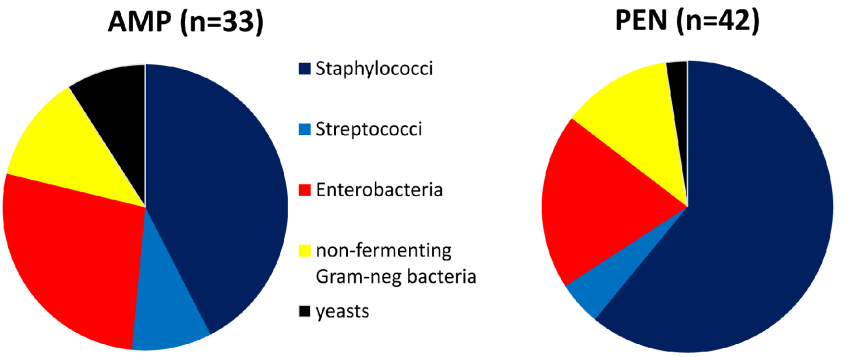
Table 9. Independent risk factors of ELBW NICU mortality – adjusted odds ratios (OR) from multivariate mixed model analysis

	OR (95% CI)	P-value
Glycocorticoid prophylaxis: full course	0.08 (0.01–0.5)	0.0049
partial course	0.4 (0.06–2.2)	0.276
Gestation (per w increase)	0.6 (0.4–0.9)	0.0252
Singleton pregnancy	0.1 (0.02–0.9)	0.0356
Treated with AMP (vs PEN)	0.3 (0.07–1.0)	0.0554
Sex (M vs F)	1.5 (0.4–5.7)	0.547

Although our study does not have the power to draw firm conclusions in subgroup analysis, the problem warrants further clarification. Similarly higher case fatality rate for penicillin G plus tobramycin regimen compared to amoxicillin plus cefotaxime was observed in a previous study of empiric antibiotic treatment of EOS in two Dutch NICUs (de Man *et al.* 2000). Possible reasons are most likely complex and not related solely to the lower antibacterial susceptibility of EOS causative microorganisms in the penicillin G arm, as CoNS strains with no mortality accounted for the majority of this difference in our study. Detailed analyses of reasons for death revealed that among neonates with RDS mortality was lower in the ampicillin than in penicillin G arm (3/72 vs 12/63; OR 0.2; 95% CI 0.05–0.70). Still based on the sample size this study cannot be conclusive; further adequately powered studies are needed to answer these questions.

5.3.3.2. Late onset sepsis

A total of 33 (23%) and 42 (30%) LOS cases occurred in the ampicillin and penicillin G arm, respectively. In the ampicillin group 51% and in the penicillin G group 67% of them were caused by Gram-positive microorganisms; the overall incidence of Gram-negative LOS was similar with 13 cases in both treatment arms (Figure 7; paper II, Table 3). All three episodes of invasive *Candida* infection occurred in the ampicillin arm. In univariate analysis there was a trend towards lower incidence rate of proven LOS caused by Gram-positive microorganisms per 1000 patient days in the ampicillin arm (9.0 vs 15.2; RR 0.60; 95% CI 0.33–1.10) and a significant difference in the rate of *S. epidermidis* sepsis (2.7 vs 7.6 in the ampicillin and penicillin G arm, respectively, RR 0.32; 95% CI 0.19–0.55).



AMP – ampicillin; PEN – penicillin G

Figure 7. Bacterial aetiology of late onset sepsis in the ampicillin and penicillin G treatment arm.

As development of LOS in a neonate is affected by a large variety of additional factors (Stoll *et al.* 2002b, Ronnestad *et al.* 2005, Benjamin *et al.* 2006, Klingenberg *et al.* 2007) the association was tested in a multivariate mixed effect model adjusted for peri- and neonatal risk factors (GA, route of delivery, chorionamnionitis, maternal antibiotic and prophylactic steroid use, route of feeding, character of enteral feeds, use of central venous and arterial lines and artificial ventilation). In this penicillin G regimen did not remain associated with *S. epidermidis* LOS at a p value of <0.05 (z-value –1.742; p = 0.0815).

The interpretation of a deep site culture result positive for CoNS is always an issue of debate, as this might represent either genuine infection or contamination (Modi *et al.* 2009). However, it has been recognised as a true pathogen in immunocompromised populations, including VLBW neonates (Costa *et al.* 2004, Modi *et al.* 2009). In our study all but two cases of LOS due to CoNS

were diagnosed in VLBW neonates with GA below 30 weeks. The two larger infants with *S. epidermidis* LOS were born at 36th and 38th week of gestation, required long-term parenteral nutrition for gastroschisis and small bowel atresia, respectively, and had repeated positive blood cultures of *S.epidermidis*. In concordance with Modi *et al*. (Modi *et al.* 2009) suggesting the best predictive accuracy for a positive blood culture with at least three concomitant clinical signs present, we applied relatively strict criteria for the diagnosis of sepsis, with at least two clinical and two laboratory signs required. In 68% of cases (in 3/5 *S. epidermidis* episodes in the penicillin G and 10/14 in the ampicillin group) the diagnosis was based on multiple positive blood cultures. Thus a diagnostic error would be most unlikely explanation for the between group difference.

We cannot exclude the clonal spread of more virulent *S. epidermidis* strain(s) during the penicillin G treatment period, like described in other settings (Klingenberg *et al.* 2007) and supported in our study by the fact that the difference between the two regimens originated predominantly from one of the participating units (2 vs 10 cases in the ampicillin and penicillin G groups, respectively, in centre A compared to 3 vs 4 in centre B).

Another possible explanation is the impact of the studied antibiotic regimens. It is generally believed that due to production of β-lactamases *S.epidermidis* is resistant to penicillin G and ampicillin. However, in vitro studies have demonstrated that CoNS, including *S. epidermidis,* MIC values of ampicillin are relatively low (Winter *et al.* 1999). We tested 100 randomly chosen colonizing strains of *S. epidermidis* and although all proved to be resistant to ampicillin at a MIC of 2 mg/L, the median MIC for these strains was 4 mg/L remaining well below the clinically achievable serum concentration in neonates (Kaplan *et al.* 1974). The two study antibiotics differ also in PK properties – ampicillin partly undergoes enterohepatic circulation and significant amounts of the drug can be found in stool while penicillin G is predominantly eliminated via the kidneys in neonates (Duchon *et al.* 2008). In animal experiments the biliary excretion of ampicillin and not the i.v. administered dose itself, has been shown to affect gut microflora with lower loads of staphylococci, streptococci, *Enterobacteriaceae* and *Bacteroidaceae* but not *Lactobacilli* or anaerobic bacteria recovered from caecal microflora 9h after an i.v. administered dose (Murakami *et al.* 1984). Mucosal and skin colonisation have been suggested as the predominant sources of invasive CoNS infections (Costa *et al.* 2004). Ampicillin efficacy against CoNS has been suggested before (Auriti *et al.* 2005) and in our study is further supported by the lower proportion of colonising days per 100 NICU days observed in the ampicillin compared to penicillin G arm (difference –8.5; p=0.039).

5.3.3.3. Additional antibiotic treatment

Additional antibiotic therapy was based on regimens pre-specified in the study protocol and was similar in both treatment arms (Table 10). After 72 hours empiric antibiotic regimen was changed in six neonates in the ampicillin and 18 in the penicillin G group (OR 0.30; 95% CI 0.12–0.78). Clinical or proven LOS and/or NEC as a reason for antibiotic change accounted for the majority of this between-group difference (4 vs 14 in the ampicillin and penicillin G groups, respectively, OR 0.26; 95% CI 0.084–0.82). Ampicillin regimen was also associated with lower rate of additional antibiotic use (43/142 vs 63/141; OR 0.54; 95% CI 0.33–0.87) and lower proportion of days on additional antibiotic therapy compared to penicillin G (31 vs 42 per 100 patient days; OR 0.63; 95% CI; 0.55–0.71).

Table 10. Additional broad spectrum antibiotic therapy

Antibiotic class	AMP n=43[a]	PEN n=63[a]
cephalosporin [b]	7	12
Carbapenem [c]	13	18
β-lactamase resistant penicillin [d]	23	25
Vancomycin, linezolid	27	28

[a] numbers in table do not add up to the total, as several neonates received more than one drug

[b] cefuroxime, cefotaxime, cefepime

[c] meropenem, imipenem

[d] ampicillin-sulbactam, amoxicillin, clavulanic acid, piperacillin-tazobactam

These findings further support the possible impact of early empiric antibiotic regimen on the incidence and aetiology of LOS. Although ampicillin use has been associated with the spread of ESBL producing *K. pneumoniae* (Crivaro *et al.* 2007, Kuo *et al.* 2007) and induction of β-lactam resistance in *E. cloacae* (Burman *et al.* 1993), these disadvantages may be outweighed by the lower need for additional antibiotics, as the latter has similarly been associated with the spread of resistance as well as with *Candida* colonisation (Benjamin *et al.* 2006, Cotten *et al.* 2006). In our study ampicillin use was not associated with higher incidence rate of Gram-negative sepsis, although an episode of clonal spread of *K. pneumoniae* with 5 invasive infections was seen in one of the study centres during the ampicillin period.

5.3.3.4. Complications of prematurity

The incidence of NEC stage II-III and threshold ROP among ELBW neonates was higher in the ampicillin as compared with penicillin G group. An as-

sociation between antenatal exposure to amoxicillin-clavulanic acid and neonatal NEC has been reported (Kenyon *et al.* 2001b; a), but not proven in later case-control studies (Al-Sabbagh *et al.* 2004). Similarly, in our study there was no difference in the combined outcomes of death and NEC II-III or death and ROP between the two study regimens, suggesting an impact of improved survival in ampicillin group in these findings.

5.3.4. Safety and tolerability of ampicillin and penicillin G combined with gentamicin in neonates at risk of early onset sepsis

We did not observe any drug-related adverse events throughout the PK and comparative efficacy study. Both regimens were equally well tolerated with no major differences in adverse events or laboratory abnormalities (including those potentially associated with antibiotic toxicity like creatinine and LFT) registered in either group at any time point studied.

Both, penicillin G and ampicillin are generally safe and well tolerated drugs in neonates. Drug hypersensitivity reactions are rare in neonates and children (Heckbert *et al.* 1990, Karabus *et al.* 2009); we were not able to identify any published case description of penicillin or ampicillin allergy in neonates. Still, serious dose-related organ toxicities have been reported, including central nervous system toxicity in the form of myoclonic seizures with a serum penicillin G concentration of 100 µg/ml, similar to the peak concentrations seen by us after administration of the dose of 50,000 IU (30 mg)/kg (Raichle *et al.* 1971, Barrons *et al.* 1992, Chambers 2005). With their large central nervous system volume, higher blood brain barrier permeation, and immature renal function, preterm infants are likely to be especially vulnerable (Paap *et al.* 1990, Alcorn *et al.* 2003). On the other hand the clinical manifestations of toxicity may not be easy to recognize in preterm neonates.

High doses of ampicillin and penicillin G and/or impaired renal function have been associated with prolonged bleeding time (Andrassy *et al.* 1976, Wisloff *et al.* 1983). More recently this effect has been proven for ampicillin in NICU patients (Sheffield *et al.* 2009). However, the clinical relevance of these findings, although supported by single case reports (Roberts 1974), has not been established (Pillgram-Larsen *et al.* 1985).

For the reasons, discussed above and as the lower penicillin G dose of 25,000 IU/kg showed adequate PK/PD profile in the PK study this was chosen for the comparative efficacy study. The inoculum size dependent MIC of GBS might have been of concern, but the absence of proven GBS cases in the penicillin G arm in our comparative efficacy study points to the contrary.

5.4. Factors predicting empiric antibiotic treatment failure in neonates at risk of EOS

The empiric antibiotic treatment failure rate of 11% (32/283) among neonates at risk of EOS in our study was similar to the 10% need of antibiotic change, seen in our pilot study (Metsvaht *et al.* 2006) but lower, than the 20% reported in a previous study by Tessin *et al.* for a mixed population of EOS and LOS patients (Tessin *et al.* 1991) and the 70% reported recently in neonates with clinical signs of sepsis (Reyna *et al.* 2008). Univariate logistic regression analysis identified a total of 23 factors (3 maternal, 6 neonatal and 14 laboratory parameters) as predictors of treatment failure (paper III, Table 3).

5.4.1. Risk factors of antibiotic treatment failure identified by multiple logistic regression analysis

A variety of maternal risk factors, like duration of premature rupture of membranes, chorionamnionitis and maternal antibiotic therapy (Yancey *et al.* 1996, Garland *et al.* 2002, Mishra *et al.* 2006, Klinger *et al.* 2009, Maayan-Metzger *et al.* 2009) have been associated with development of EOS. In this study chorionamnionitis and isolation of a pathogen from the birth canal during delivery appeared predictors of antibiotic treatment failure in univariate logistic regression analysis but none remained significant in multiple logistic regression. The factors, independently associated with antibiotic treatment failure are presented in Table 11.

Table 11. Multiple logistic regression analysis of clinical and laboratory variables predicting failure of empiric antibiotic regimen at 24 and 72 hours of age – data are presented as odds ratios (OR) and 95% confidence intervals (95% CI)

	OR (95% CI)	p-value
24 hour model (n=232)		
Platelet count on Day 1 (per 10×10^9 /L increase)	0.92 (0.86–0.98)	0.0124
Need for vasoactive treatment	2.83 (1.21–6.66)	0.0167
WBC $<5 \times 10^9$ or $>20 \times 10^9$ /L on Day 1	2.51 (1.09–5.81)	0.0308
I/T ratio >0.2 on Day 1	2.79 (1.10–7.11)	0.0312
72 hour model (n=252)		
Serum albumin on Day 2–3 (per 1 g/L increase)	0.87 (0.80–0.95)	0.0029
Need for vasoactive treatment	4.43 (1.55–12.68)	0.0055
Platelet count on Day 2–3 (per 10×10^9 /L increase)	0.92 (0.86–0.99)	0.0331
C-reactive protein (per 1 mg/L increase) on Day 1	1.02 (1.00–1.03)	0.0359

WBC – white blood count; I/T ratio – immature to total neutrophil count ratio

Factors identified at 24 hours predicted treatment failure and success correctly in 19/31 and 180/201 cases, respectively. The relatively low sensitivity of this

prediction is most probably caused by the nonspecificity of early signs of neonatal sepsis (Table 12). But one must also bear in mind that although in more than 90% of GBS cases clinical picture develops within 24 h from birth, it may be more delayed in EOS caused by *E. coli*, with only about 40% developing signs and symptoms by 24 h and 50% by 48 h of age (Schuchat *et al.* 2000).

Table 12. Diagnostic value of models for prediction of initial antibiotic treatment failure in neonates at risk of EOS.

	MLR analysis		CRT analysis	
	24 h model	72 h model	24 h model	72 h model
Sensitivity – %(CI)	61 (42–78)	80 (61–92)	75 (56–88)	81 (63–92)
Specificity – %(CI)	90 (84–93)	78 (72–83)	89 (84–92)	88 (83–92)[a]
PPV – %(CI)	48 (32–64)	33 (23–45)	46 (32–60)	46 (33–60)
NPV – %(CI)	94 (89–97)	97 (93–99)	97 (93–98)	97 (94–99)

[a] difference from multiple logistic regression model of the same time point significant at $p<0.05$

MLR – multiple logistic regression; CRT – classification and regression tree; PPV – positive predictive value; NPV – negative predictive value

Among predictors of treatment failure at 24 hours only need for vasoactive treatment and platelet count, remained significant also at 72 hours (Table 11). By 72 h the sensitivity of the prediction significantly improved (treatment failure was predicted correctly in 24/30; Table 12), however at the cost of lower specificity (treatment success 173/222; Table 12).

The presence of higher number of risk factors in multiple logistic regression models was accompanied by significantly increased likelihood of antibiotic treatment failure (Figure 8). The presence of platelet count $< 100 \times 10^9$ /L and need for vasoactive support ensured the highest probability both at 24 h and 72 h. Concomitant occurrence of these two and two additional predictors of treatment failure (four risk factors altogether) was associated with more than 0.5 probability of treatment failure at both time points.

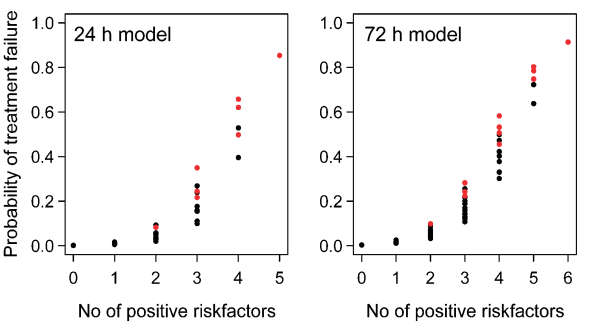


Figure 8. Probability of antibiotic treatment failure in relation to number of "positive" risk factors at 24 h (left) and 72 h (right); combinations of risk factors including thrombocytopenia and vasoactive treatment are shown in red.

5.4.2. Classification and regression tree analysis in predicting risk factors

Classification and regression tree analysis was chosen due to its proven ability of outcome prediction (Reibnegger *et al.* 1991) and because more traditional methods, like multiple logistic regression are often cumbersome or of limited utility for bedside application (Breiman *et al.* 1984, Lewis 2000).

The classification and regression model of parameters available by 24 h of age and the distribution of patients through the model are shown in paper III, Figure 1. The principal discriminator at 24 h of age was hypoglycaemia ≤ 2.55 mmol/L; followed by CRP values > 1.35 mg/L in infants with hypoglycaemia and by BW $\leq$ 678 g in those without. In infants without hypoglycaemia and with BW > 678 g further partitioning was based on GA ≤ 27 weeks and WBC $\leq 8.25 \times 10^9$ /L together with platelet count $\leq 143 \times 10^9$ /L in those with GA > 27 weeks. With antibiotic failure probability of 0.3 as a cut-off limit the algorithm allowed capture of 75% of treatment failure cases (24/32) with relatively high specificity within 24 h (Table 12).

The model using data available by 72 h of age together with the distribution of patients is shown in paper III, Figure 2. Compared to data available by 24 h, platelet count $\leq 94.5 \times 10^9$ /L was identified as the principal discriminator; together with need for vasoactive treatment a probability of antibiotic treatment failure of 0.7 was observed. In those without thrombocytopenia, WBC $< 3.5 \times 10^9$ /L was associated with a 0.4 probability of treatment failure. Without leucopaenia, WBC $> 39.8 \times 10^9$ /L and if leucocytosis was not present, hypoglycaemia with blood glucose ≤ 1.65 mmol/L were both associated with antibiotic treatment failure probability of more than 0.27. Incorporating 72 h data to classification and regression tree improved the sensitivity (Table 12). A total of 36 cases were misclassified (6 antibiotic failure and 30 success cases), resulting in an overall accuracy of 87%.

Not surprisingly, all parameters identified for the decision algorithms have been used as diagnostic markers of EOS earlier (Mishra *et al.* 2006) and hypoglycaemia as well as thrombocytopenia with septic shock have also been associated with adverse outcome in neonates (Kermorvant-Duchemin *et al.* 2008). In consistency with the time course of disease the model used slightly different choice of parameters and cut-off values depending on the evaluated time point; a more profound deviation from normal values was detected by 72 h, compared to 24 h of age.

Similar to Bachur *et al.* who used classification and regression tree analysis in febrile infants for identification of serious bacterial infections (Bachur *et al.* 2001) we found that not neutrophil count or I/T ratio but the total WBC has the best discriminative power among WBC indices in prediction of antibiotic treatment failure in neonates with risk of EOS. The 72 h blood glucose level of less than 1.65 mmol/L identified in this study is somewhat lower compared to 2.4 mmol/L shown to be associated with adverse outcome of neonatal septic shock in a recent study by Kermorvant-Duchemin (Kermorvant-Duchemin *et al.*

2008). In concordance with the latter study (Kermorvant-Duchemin *et al.* 2008) our results show that thrombocytopenia $\leq 94.5 \times 10^9$ /L has excellent discriminative power in infants needing concomitant vasoactive support by 72 h of age, i.e. those in shock. However, both are relatively late signs of sepsis. A significantly higher platelet count of $\leq 143 \times 10^9$ /L, when present concomitantly with WBC $<8.25 \times 10^9$ /L was found to be predictive of treatment failure in suspected EOS early in the course of disease, within 24 h of age.

Although based on slightly different choice of parameters, the performance of both, the multiple logistic regression and classification and regression tree models is comparable to those, described for identification of blood stream infections in febrile patients (Bachur *et al.* 2001, Peters *et al.* 2006). Compared with multiple logistic regression, classification and regression tree analysis achieved higher sensitivity in identification of patients who fail on initial antibiotic regimen at 24 h and higher specificity by 72 h of age, even with the relatively low cut-off probability of treatment failure (0.27–0.30). The latter ensured higher sensitivity, as inadequate antibiotic therapy carries high risk for the patient. Nevertheless, a high negative predictive value was preserved for both analyses, which is of equal importance in order to avoid unnecessary antibiotic exposure. Based on the relatively low PPV of the presently identified early markers vigilance in de-escalation where appropriate should be kept in mind. Increasing the number of risk factors present would allow increasing the PPV, when prioritising further limitation of broad spectrum antibiotic exposure.

5.4.3. Methodological issues of risk factor analysis

To our knowledge this is the first attempt to predict need for antibiotic change in neonates at risk of EOS based on scientific data. One of the main limitations of this study was the use of a surrogate outcome measure of antibiotic treatment failure, chosen as “hard” outcomes like antibiotic resistance in proven EOS or proven EOS related mortality are extremely rare and their use would probably exclude a number of clinically meaningful situations. The reasons for this choice are discussed in detail in chapter 0.

To ensure the highest possible specificity we included autopsy data as still the "golden standard" of proving clinical infection when bacteriological cultures remain negative. With this approach 2/3 of treatment failure cases were based either on positive culture or autopsy findings and the remaining were those that required wide-spectrum antibiotic therapy for more than 72 hours. We believe that the three clinically relevant situations selected by us serve as an adequate marker of ineffective antibiotic treatment.

Partial overlap between the study exclusion and antibiotic treatment change criteria is not surprising, as the clinical conditions (e.g. meningitis, NEC, septic shock caused by Gram-negative bacteria resistant to ampicillin etc) prompting the decisions are likely similar. The difference however, is that the study entry criteria were evaluated early in life, before any antibiotics had been given, whereas treatment failure criteria applied only when antibiotic treatment had been initiated.

6. GENERAL DISCUSSION

Despite major developments in the field of neonatology over the last decades many questions related to neonatal sepsis still remain unanswered. The present approach of wide-spread risk factor based use of empiric antibiotic therapy with relatively narrow spectrum agents, although having reduced EOS related mortality (Lannering *et al.* 1983, Mtitimila *et al.* 2004), is yet far from the goal, as set by Schelonka in 2007: "Treat only those who need it and treat them effectively; reduce the need to treat" (Schelonka 2007). The reasons are likely multiple and include the complex methodological and ethical issues of clinical trials in neonates as well as the diversity of the disease entity. Only very few prospective clinical trials have addressed antibiotic treatment in neonates over the last two decades (Hammerberg *et al.* 1989, Gokalp *et al.* 1990, Umana *et al.* 1990, de Louvois *et al.* 1992); even then most of them are still underpowered.

6.1. Complexities of conducting studies on empirical therapy of early onset sepsis

6.1.1. Study population

One of the first issues in these studies including the present one is the adequacy of study population. Diagnostic criteria of neonatal sepsis (suspected or proven) applied in different studies vary largely with some incorporating all neonates requiring systemic antibiotic therapy based on risk factors (Hall *et al.* 1988, Hammerberg *et al.* 1989) and others limiting recruitment only to those with at least some signs and symptoms (Marks *et al.* 1978, Fogel *et al.* 1983, Wiese 1988) or even proven sepsis (Bingen *et al.* 1987, Odio *et al.* 1987). Both approaches have their advantages and disadvantages. A study applying strict diagnostic criteria of EOS for inclusion and excluding the population with risk factor based antibiotic therapy has the potential to recruit a better targeted population and probably report valid outcomes with smaller sample size. However, due to the low specificity of the present diagnostic criteria (Mtitimila *et al.* 2004, Schelonka 2007) such approach is still likely to recruit neonates with a diverse range of problems, from RDS to true sepsis. On the other hand, as the severity of clinical picture in neonatal sepsis varies significantly, strict inclusion criteria carry high risk of missing several cases and thus introducing a potential bias.

We believe that the population selection might depend on the antibiotic regimens studied and should resemble the patient group, targeted in clinical practice. Studies looking at the efficacy of the widely used relatively narrow spectrum empiric antibacterial regimens should recruit all neonates treated based on the presence of risk factors, thus reporting outcomes for the entire population involved. The use of antibiotics with broader antibacterial spectrum

on the other hand should be limited to the sickest babies most likely having infection, caused by resistant microorganisms, meaning that such studies need to apply more strict inclusion criteria.

The population selected by us corresponds well to the one treated with empiric antibiotics in clinical practice today and we believe it reflects adequately the overall treatment efficacy of neonates at risk of EOS. Still, the high number of culture negative cases (about 95% in both arms) among those included based on the risk factors of infection is one of the main limitations of this, but also of most previous studies conducted in the field (Snelling *et al.* 1983, Miall-Allen *et al.* 1988). This may have introduced a significant bias as patients without bacterial infection would respond similarly to all antibiotics provided that these agents are equally well tolerated. Therefore the results of this study are applicable only to the population studied by us and should not be transferred to other settings, like treatment of proven EOS.

Furthermore, the population of neonates, even and especially those with EOS is not a homogenous one. As shown in this study and by others, EOS in ELBW neonates has several features unique to this age group, like different bacterial aetiology and host response, making study results obtained in ELBW neonates not transferable to term infants and *vice versa*. The results of our ELBW subgroup analysis should still be treated as preliminary and underline a desperate need for adequately powered studies in this specific subpopulation of neonates. To our best knowledge no such studies have been conducted yet.

6.1.2. Outcome measures

The next and the most important issue of every clinical trial are valid outcome measures. To describe the outcomes for sepsis outcome of proven EOS, characterised by EOS related mortality, complication rate and/or susceptibility of EOS strains to the empiric regimen would probably be the soundest measure of efficacy. However, proven sepsis is rare and found in only about 1.5–5% of neonates receiving antibiotic therapy for suspicion of EOS (Stoll, 1996). The case fatality of EOS is even more rare; mortality rates of 2 to 15% for GBS (Sjoberg *et al.* 1990, Tessin *et al.* 1990, Kalliola *et al.* 1999, Grimwood *et al.* 2002, Dahl *et al.* 2003) and 14–36 for all aetiologies in premature infants (Stoll *et al.* 1996, Lopez Sastre *et al.* 2000, Lopez Sastre *et al.* 2005, Ronnestad *et al.* 2005, Stoll *et al.* 2005) have been reported. Assuming a proven EOS related mortality of 2–5% (1.4% in this study) in a study including all neonates requiring early empiric antibiotics and accepting that the difference in the treatment failure between the study arms will not exceed +/-10% (a clinically acceptable difference), based on a two-sided sample size calculation for binary data (Pocock 1983) such study would need to enrol at least 7300 to 18824 neonates to prove equivalence of the two tested regimens. Such study would likely be extremely complicated to conduct even in multicenter multinational settings. Moreover, as a large number of neonates with clinical and laboratory

signs of sepsis never develop positive blood cultures, especially in the era of wide spread IAP (Heimler *et al.* 1995, Lopez Sastre *et al.* 2005), a study describing outcomes for proven EOS alone would have only a limited clinical application. Using clinical sepsis as an outcome measure as done recently by Tagare *et al.* (Tagare *et al.* 2009) is not ideal either as none of the clinical or laboratory markers of neonatal sepsis are pathognomic. There is a risk that a significant amount of patients treated in such study have other conditions the outcome of which does not depend on antibiotic treatment and thus the results will be biased. New diagnostic tests with better sensitivity and specificity and possibly a potential to discriminate between bacterial aetiologies are still under development (Kingsmore *et al.* 2008, Ohlin *et al.* 2008) and hopefully will change the design of future clinical trials.

Another possible approach for antibiotic efficacy assessment would be looking at meeting target PK/PD indices based on actual PK measurements and MIC values of neonatal isolates (Lutsar *et al.* 2010a). However, with a few exceptions (Sherwin *et al.* 2009b) there are no published data on validation of such outcome measures in neonates (Lutsar *et al.* 2010b). The majority of PK/PD targets recommended for neonates have been extrapolated from studies in adults. Furthermore, the antibiotic PK data in specific neonatal populations are scarce (White 1999, Giacoia *et al.* 2006), which made us to undertake the PK study of penicillin G in VLBW neonates. Such study would also require significant additional blood sampling, carrying extra risk especially in VLBW/ELBW neonates.

Therefore surrogate outcome measures, like mortality in the first 28 days of life; in-hospital mortality; treatment failure defined as the need to change empirical antibiotic therapy and bacteriological resistance defined as isolation of organisms resistant to assigned empirical treatment have been suggested (Mtitimila *et al.* 2004). Secondary outcome measures of interest could be the rate of adverse effects; superinfection; colonisation with bacteria resistant to allocated empirical antibiotic regimen during follow-up and incidence of NEC, as suggested by (Mtitimila *et al.* 2004).

The selection of an unvalidated surrogate primary endpoint, prompted by the reasons discussed above, was one of the limitations of our comparative efficacy study. However, we do believe, that both components of the primary endpoint in our study adequately described the efficacy of the antibiotic regimen, since clinical status of septic neonates deteriorates rapidly with inadequate treatment and the all cause mortality within the first seven days avoids skipping any unrecognised cases. Inclusion of all neonates, assigned to early empiric antibiotic treatment with ampicillin or penicillin G and gentamicin, to our mind, allows drawing meaningful conclusions for the entire population, recruited according to the present clinical approach.

6.1.3. Study design

Equivalence trials are relevant if the new treatment, though not expected to have a larger therapeutic effect, is associated with fewer side effects, is less expensive or simpler to apply (Christensen 2007). Based on the present practice no assumption on the superiority of either of the comparative efficacy study regimens seemed appropriate, although a favourable gut colonisation profile for penicillin G was expected; thus equivalence trial design was chosen. Major methodological challenges in conducting an equivalence or non-inferiority study include (a) choice of an active control treatment, (b) choice of an equivalence margin (range), i.e. the "irrelevant difference" (c) sample size estimation, and (d) statistical analysis (Lange *et al.* 2005, Greene *et al.* 2008). No golden standard exists for determining an appropriate "irrelevant difference", except that the margin should be determined in advance and that it should not be greater than the smallest effect size the active drug would be reliably expected to have compared with a placebo (Greene *et al.* 2008). Our predetermined equivalence margin of no more than +/-10% difference in treatment failure assumed to occur in 10% of study subjects (based on pilot study) certainly does not exceed the clinically meaningful difference. The sample size was based on a two-tailed calculation in binary data as appropriate for equivalence trials (Pocock 1983, Julious 2004)

We believe that the cluster randomised design is optimal for such studies as this allows to evaluate the possible effects of the study regimens on the entire NICU environment (e.g. circulating bacterial strains) and to look at associated bowel colonisation patterns. This would have been impossible in individual randomisation with the two regimens running at the same time in both wards. It also enabled to handle better the open label design, chosen due to logistic reasons. We believe that the final results were not influenced by the open label design as throughout the study the number of patients, considered to require antibiotics other than the study regimen, was similar, suggesting that physicians did not have any preferences in selecting empiric therapy. In addition, prior to the study both empiric regimens were equally used in both participating units (Metsvaht *et al.* 2006). In order to avoid misinterpretation of the clustering effect, we have applied hierarchical models, stratifying the results for random effects of treatment period and study centre (Murray *et al.* 2004).

6.2. Filling the gap in pharmacokinetic data in very low birth weight neonates

A crucial issue of any clinical study is the need for appropriate dosing regimen of studied agents. More than 90% of VLBW neonates receive antibiotics during hospitalization in NICU (Shani *et al.* 2008, Neubert *et al.* 2009). However, the

PK parameters of the majority of medicines, antibiotics among others, have not been properly studied in this population. Therefore, age-appropriate dosing schedules as well as safety and efficacy profiles have not been conclusively established (White 1999, Di Paolo *et al.* 2006, Giacoia *et al.* 2006, Hsien *et al.* 2008). Thus PK and PD studies in the field are urgently needed to optimise drug exposure and reduce the potential of adverse drug effects, especially in specific populations like preterm neonates to whom data from adult or even paediatric studies cannot be readily extrapolated.

Penicillins are a key component of drug therapy in neonatal intensive care units (Warrier *et al.* 2006) with up to half of the drugs, used in neonates within the first week belonging into this class (Group 1988). While the PK profile of several new β-lactams had been established (Morehead *et al.* 1972, Huisman-de Boer *et al.* 1995, Capparelli *et al.* 2005) in VLBW neonates, no data were available for penicillin G.

Our study was one of the two studies, both published in 2007, that have filled this gap (Muller *et al.* 2007). Both studies included extremely premature neonates with GA <28 (Metsvaht *et al.* 2007) and <32 weeks (Muller *et al.* 2007) within the first week of life (PNA 0–3 days) and showed further elongation of $T_{1/2}$ compared to term neonates and adults. Muller et al. have suggested the dose of 50,000 IU/kg (30 mg/kg) q12h as optimal for VLBW neonates, ensuring adequate PK profile for pathogens with MIC values up to 4 μg/L (Muller *et al.* 2007). However, in EOS penicillin G is effective against streptococci only, with such high MIC values hardly ever encountered in susceptible strains (EUCAST 2010). According to our results this regimen resulted in peak concentrations, close to those associated with CNS toxicity in adults. The lower dose of 25,000 IU/kg (15 mg/kg) q12h ensured serum concentrations exceeding the MIC of GBS for 90–100% of time – a PD surrogate value associated with excellent clinical efficacy in time-dependant antibacterial drugs (McKinnon *et al.* 2008). Therefore we suggest that in suspected EOS the dose of 15 mg/kg q12h doubled in neonates with suspected meningitis should be adequate in most situations.

6.3. Which antibiotic regimen should be preferred?

In 2007 Robert Schelonka wrote: "We have only recently begun to appreciate the "collateral damage" of this warfare (antibiotics, T.M.) not only to an individual caught in the fray, but to entire populations. This collateral damage includes alteration, perhaps irrevocably, in the composition of endogenous flora, infection with "tougher" multiple drug resistant bugs, longer hospital stays, and higher costs. In the infant and young child, alterations in the microflora may lead to opportunistic infection and importantly impact subsequent immune development". Until reliable and rapid diagnostic methods of EOS are available, many neonates will be unnecessarily exposed to antibiotics they may not need, which in turn prioritizes the safety and narrow antibacterial spectrum

of possible choices. Based on the results of our comparative efficacy study ampicillin or penicillin G combined with gentamicin appear a relatively safe choice ensuring equal clinical efficacy in the population of neonates at risk of EOS.

In our study ampicillin regimen was associated with lower need for late antibiotic therapy. As broad spectrum antibiotics have similar to ampicillin a higher potential of out-selecting or inducing resistance compared to penicillin G this difference may well outweigh the risks of early empiric ampicillin therapy. In our study ampicillin regimen was not associated with higher incidence rate of Gram-negative LOS, however the number of cases was relatively small in both treatment arms.

Although the studied antibiotic regimens appeared equivalent in terms of overall treatment failure, the role of ampicillin vs penicillin G regimen in the prevention of proven EOS remains controversial. Occurrence of culture proven EOS in ELBW neonates only in the penicillin G group in our study seems to support the idea that in the prevention of culture proven EOS the efficacy of penicillin G treatment might be inferior in this group of neonates. Still, prior antibiotic therapy may just lower the efficacy of microorganisms' isolation from normally sterile body fluids (Cartwright *et al.* 1992, Lopez Sastre *et al.* 2005) without altering the incidence rate of clinical sepsis, as suggested by Tagare *et al.* (Tagare *et al.* 2009).

Based on the above discussed findings and the lower mortality of neonates born before 26th week of gestation seen in the ampicillin arm in our comparative efficacy study, the best antibiotic choice in the subpopulation of ELBW neonates remains to be determined by future adequately powered clinical trials.

6.4. Who needs broader spectrum antibiotic coverage?

As long as clinically applicable diagnostic measures of neonatal sepsis do not include timely information on the bacterial aetiology or even better, on antibiotic susceptibility, clinical criteria for better targeted antibiotic therapy are needed. According to our results in 65% of cases change of empirical antibiotic therapy is a clinical decision, as clinical condition may deteriorate fast but culture results are often delayed for 36–72 h (Jardine *et al.* 2006) and many cases of neonatal sepsis remain culture negative with present microbiological methods (Heimler *et al.* 1995, Kellogg *et al.* 1997, Kellogg *et al.* 2000). There is a large body of data from adult intensive care supporting the crucial role of adequate initial antibiotic therapy in the outcome of severe infections (Kollef 2000, Iregui *et al.* 2002, Lodise *et al.* 2003, Garnacho-Montero *et al.* 2006).

To our knowledge this is the first attempt to predict the need for antibiotic change in neonates at risk of EOS based on scientific data. Our results reflect the difficulties in achieving accurate predictions based on the non-specific clinical signs and symptoms of neonatal sepsis as well as the advantages of new

statistical method like classification and regression tree analysis in terms of clinical applicability.

Neonates at risk of EOS developing low platelet count and requiring vasoactive therapy, both considered relatively late signs though, have the highest probability of antibiotic treatment failure. However, the presence of both these risk factors is still associated with the probability of 0.1 only in multiple logistic regression analysis. Addition of another risk factor (WBC $<5 \times 10^9$ or $>20 \times 10^9$ /L or I/T ratio >0.2 within 24 h or elevated CRP or hypoalbuminemia within 72 h of life) increases the probability of treatment failure to 0.3–0.4 and the presence of two more to 0.6. The cut-off values of suggested parameters as well as the treatment failure probability appropriate to prompt switch to broader spectrum coverage, however, remain to be decided by the clinician.

In classification and regression tree analysis antibiotic treatment failure probability of 0.27–0.3 was identified as a cut-off allowing the best discrimination between treatment failure and success cases without compromising sensitivity. Given the high risk of adverse outcome associated with inadequate antibiotic therapy targeting higher sensitivity seems reasonable. We suggest that the best approach in the empirical antibiotic therapy of neonates with high risk of EOS could be a tailored choice of the presently recommended regimen for most cases with early implementation of broader spectrum antibiotics reserved for high risk infants developing thrombocytopenia $<95 \times 10^9$ /L with concomitant need for vasoactive treatment; or WBC below 3.5×10^9 /L or above 40.0×10^9 /L; or blood glucose below 1.7 mmol/L within 72 h of life. Based on the relatively low specificity of the presently identified early markers vigilance in de-escalation where appropriate should be kept in mind. The clinical relevance of such approach remains to be established in future clinical trials.

7. CONCLUSIONS

I The values of the PK parameters of drugs in extremely immature infants are different from those in adults, older children, and even term infants, as shown in our PK study in the example of penicillin G. These differences most likely apply to other renally eliminated drugs. Therefore there is an urgent need for more extensive PK studies of drugs in this population to avoid unnecessary adverse effects and insufficient efficacy.

In contrast to adults the predominant renal elimination mechanism of penicillin G in VLBW neonates is GFR instead of tubular secretion and the $T_{1/2}$ of penicillin G is about seven-fold longer, resulting in need for less frequent dosing.

In VLBW infants penicillin G dose of 25,000 IU (15 mg)/kg q12h is safe and sufficient to achieve concentrations in serum and most likely CSF above the MIC_{90} for GBS and other streptococci for the entire dosing interval.

II Ampicillin or penicillin G combined with gentamicin are equally effective and safe agents in the early initial empiric treatment of neonates with risk factors of EOS. Which of these two regimens is to be preferred should depend on the local distribution of EOS causing microorganisms together with their antibacterial susceptibility.

Ampicillin regimen is associated with lower use of additional broad spectrum antibiotic therapy, probably out-weighing the higher potential for resistance selection and induction; with lower incidence rate of *S. epidermidis* LOS and a trend towards lower incidence rate of LOS due to Gram-positive microorganisms. The different elimination of ampicillin compared to penicillin G with significant amounts excreted with bile may affect host microbial ecology and thus the bacterial aetiology of LOS, often originating from gut, in neonates.

III Compared to the combination of ampicillin, penicillin G containing initial empiric antibiotic regimen may be associated with higher NICU mortality, especially among ELBW neonates born before 26^{th} week of gestation. The suitability of ampicillin and penicillin G combinations with gentamicin in ELBW neonates at risk of EOS needs further confirmation in adequately powered clinical trials.

IV The combinations of ampicillin and penicillin G with gentamicin are safe and well tolerated in neonates at risk of EOS, requiring intensive care.

V Neonates at risk of EOS admitted to NICU and developing thrombocytopenia (platelet count $\leq 95 \times 10^9$ /L) with concomitant need for vasoactive treatment; or leucopaenia (WBC $< 3.5 \times 10^9$ /L) or leucocytosis (WBC $> 39 \times 10^9$ /L) or hypoglycaemia (blood glucose ≤ 1.7 mmol/L) within 72 h of life have a high risk of empiric ampicillin plus gentamicin or penicillin G plus gentamicin treatment failure. Whether implementation of broader spectrum antibiotics, based on these criteria will have any

influence on the outcome of EOS remains to be answered in prospective clinical studies.

Classification and regression tree analysis has proven a valuable tool in construction of decision algorithms easily applicable in clinical practice, often ensuring higher sensitivity and/or specificity compared to the more traditional approach of multiple logistic regression analysis.

8. REFERENCES

AAP 1997. 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;99(3):489–96

Abramowicz, M., J. O. Klein, D. Ingall and M. Finland 1966. Levels of penicillin in serum of newborn infants. Am J Dis Child;111(3):267–71

Acocella, G., R. Mattiussi, F. B. Nicolis, R. Pallanza and L. T. Tenconi 1968. Biliary excretion of antibiotics in man. Gut;9(5):536–45

Adelman, R. D., F. Wirth and T. Rubio 1987a. A controlled study of the nephrotoxicity of mezlocillin and amikacin in the neonate. Am J Dis Child;141(11):1175–8

Adelman, R. D., F. Wirth and T. Rubio 1987b. A controlled study of the nephrotoxicity of mezlocillin and gentamicin plus ampicillin in the neonate. J Pediatr;111(6 Pt 1):888–93

Agunod, M., N. Yamaguchi, R. Lopez, A. L. Luhby and G. B. Glass 1969. Correlative study of hydrochloric acid, pepsin, and intrinsic factor secretion in newborns and infants. Am J Dig Dis;14(6):400–14

Ainbender, E., E. E. Cabatu, D. M. Guzman and A. Y. Sweet 1982. Serum C-reactive protein and problems of newborn infants. J Pediatr;101(3):438–40

Al-Sabbagh, A., S. Moss and N. Subhedar 2004. Neonatal necrotising enterocolitis and perinatal exposure to co-amoxyclav. Arch Dis Child Fetal Neonatal Ed;89(2):F187

Al-Zwaini, E. J. 2002. Neonatal septicaemia in the neonatal care unit, Al-Anbar governorate, Iraq. East Mediterr Health J;8(4–5):509–14

Alcorn, J. and P. J. McNamara 2002a. Ontogeny of hepatic and renal systemic clearance pathways in infants: part I. Clin Pharmacokinet;41(12):959–98

Alcorn, J. and P. J. McNamara 2002b. Ontogeny of hepatic and renal systemic clearance pathways in infants: part II. Clin Pharmacokinet;41(13):1077–94

Alcorn, J. and P. J. McNamara 2003. Pharmacokinetics in the newborn. Adv Drug Deliv Rev;55(5):667–86

Allegaert, K., R. Verbesselt, G. Naulaers, J. N. van den Anker, M. Rayyan, A. Debeer and J. de Hoon 2008. Developmental pharmacology: neonates are not just small adults. Acta Clin Belg;63(1):16–24

Andrassy, K., E. Ritz, B. Hasper, M. Scherz, E. Walter and H. Storch 1976. Penicillin-induced coagulation disorder. Lancet;2(7994):1039–41

Ansong, A. K., P. B. Smith, D. K. Benjamin, R. H. Clark, J. S. Li, C. M. Cotten, B. Mangum, H. P. Garges and D. K. Benjamin, Jr. 2009. Group B streptococcal meningitis: cerebrospinal fluid parameters in the era of intrapartum antibiotic prophylaxis. Early Hum Dev;85(10 Suppl):S5–7

Anthony, B. F. and N. F. Concepcion 1975. Group B streptococcus in a general hospital. J Infect Dis;132(5):561–7

Arbeter, A. M., C. L. Saccar, S. Eisner, E. Sarni and S. J. Yaffe 1983. Tobramycin sulfate elimination in premature infants. J Pediatr;103(1):131–5

Ascher, D. P., S. Wilson and G. W. Fischer 1991a. Comparison of commercially available group B streptococcal latex agglutination assays. J Clin Microbiol; 29(12):2895–6

Ascher, D. P., S. Wilson, J. Mendiola and G. W. Fischer 1991b. Group B streptococcal latex agglutination testing in neonates. J Pediatr;119(3):458–61

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

August Fuhr, N., C. Becker, A. van Baalen, K. Bauer and H. Hopp 2006. Antibiotic therapy for preterm premature rupture of membranes - results of a multicenter study. J Perinat Med;34(3):203–6

Auriti, C., L. Rava, V. Di Ciommo, M. P. Ronchetti and M. Orzalesi 2005. Short antibiotic prophylaxis for bacterial infections in a neonatal intensive care unit: a randomized controlled trial. J Hosp Infect;59(4):292–8

Axline, S. G., S. J. Yaffe and H. J. Simon 1967. Clinical pharmacology of antimicrobials in premature infants. II. Ampicillin, methicillin, oxacillin, neomycin, and colistin. Pediatrics;39(1):97–107

Bachur, R. G. and M. B. Harper 2001. Predictive model for serious bacterial infections among infants younger than 3 months of age. Pediatrics;108(2):311–6

Baker, C. and M. Edwards 1990. Group B Streptococcal Infections. J. Remington and J. Klein. Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W.B. Saunders. pp

Baker, C. J., B. J. Webb and F. F. Barrett 1976. Antimicrobial susceptibility of group B streptococci isolated from a variety of clinical sources. Antimicrob Agents Chemother;10(1):128–31

Baltimore, R. S., S. M. Huie, J. I. Meek, A. Schuchat and K. L. O'Brien 2001. Early-onset neonatal sepsis in the era of group B streptococcal prevention. Pediatrics; 108(5):1094–8

Bang, A. T., R. A. Bang, S. B. Baitule, M. H. Reddy and M. D. Deshmukh 1999. Effect of home-based neonatal care and management of sepsis on neonatal mortality: field trial in rural India. Lancet;354(9194):1955–61

Bang, A. T., R. A. Bang, B. J. Stoll, S. B. Baitule, H. M. Reddy and M. D. Deshmukh 2005a. Is home-based diagnosis and treatment of neonatal sepsis feasible and effective? Seven years of intervention in the Gadchiroli field trial (1996 to 2003). J Perinatol;25 Suppl 1(S62–71

Bang, A. T., H. M. Reddy, M. D. Deshmukh, S. B. Baitule and R. A. Bang 2005b. Neonatal and infant mortality in the ten years (1993 to 2003) of the Gadchiroli field trial: effect of home-based neonatal care. J Perinatol;25 Suppl 1(S92–107

Barnett, H. L., N. H. Mc and et al. 1949. Renal clearances of sodium penicillin G, procaine penicillin G, and inulin in infants and children. Pediatrics;3(4):418–22

Barrons, R. W., K. M. Murray and R. M. Richey 1992. Populations at risk for penicillin-induced seizures. Ann Pharmacother;26(1):26–9

Bassler, D., B. J. Stoll, B. Schmidt, E. V. Asztalos, R. S. Roberts, C. M. Robertson and R. S. Sauve 2009. Using a count of neonatal morbidities to predict poor outcome in extremely low birth weight infants: added role of neonatal infection. Pediatrics; 123(1):313–8

Becker, K. L., R. Snider and E. S. Nylen 2009. Procalcitonin in sepsis and systemic inflammation: a harmful biomarker and a therapeutic target. Br J Pharmacol;

Begg, E. J., J. W. Vella-Brincat, B. Robertshawe, M. J. McMurtrie, C. M. Kirkpatrick and B. Darlow 2009. Eight years' experience of an extended-interval dosing protocol for gentamicin in neonates. J Antimicrob Chemother;63(5):1043–9

Begue, P., J. Astruc, P. Francois and D. Floret 1997. Evaluation de la ceftraixone et du cefotaxime dans l'infection bacterienne severe en pediatrie: etude multicentrique. Med Mal Infect 1997;27(300–306

Bell, M. J., J. L. Ternberg, R. D. Feigin, J. P. Keating, R. Marshall, L. Barton and T. Brotherton 1978. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg;187(1):1–7

Bender, L., J. Thaarup, K. Varming, H. Krarup, S. Ellermann-Eriksen and F. Ebbesen 2008. Early and late markers for the detection of early-onset neonatal sepsis. Dan Med Bull;55(4):219–23

Benjamin, D. K., Jr., B. J. Stoll, A. A. Fanaroff, S. A. McDonald, W. Oh, R. D. Higgins, S. Duara, K. Poole, A. Laptook and R. Goldberg 2006. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics;117(1):84–92

Berkus, M. D., O. Langer, A. Samueloff, E. M. Xenakis, N. T. Field and L. E. Ridgway 1994. Meconium-stained amniotic fluid: increased risk for adverse neonatal outcome. Obstet Gynecol;84(1):115–20

Berseth, C. L. 1989. Gestational evolution of small intestine motility in preterm and term infants. J Pediatr;115(4):646–51

Bertels, R. A., B. A. Semmekrot, G. P. Gerrits and J. W. Mouton 2008. Serum concentrations of cefotaxime and its metabolite desacetyl-cefotaxime in infants and children during continuous infusion. Infection;36(5):415–20

Besunder, J. B., M. D. Reed and J. L. Blumer 1988. Principles of drug biodisposition in the neonate. A critical evaluation of the pharmacokinetic-pharmacodynamic interface (Part I). Clin Pharmacokinet;14(4):189–216

Bhat, M. A., J. I. Bhat, M. S. Kawoosa, S. M. Ahmad and S. W. Ali 2009. Organism-specific platelet response and factors affecting survival in thrombocytopenic very low birth weight babies with sepsis. J Perinatol;29(10):702–8

Bhutta, Z. A., A. K. Zaidi, D. Thaver, Q. Humayun, S. Ali and G. L. Darmstadt 2009. Management of newborn infections in primary care settings: a review of the evidence and implications for policy? Pediatr Infect Dis J;28(1 Suppl):S22–30

Biedenbach, D. J. and R. N. Jones 2009. Multicenter evaluation of the in vitro activity of dalbavancin tested against staphylococci and streptococci in 5 European countries: results from the DECIDE Surveillance Program (2007). Diagn Microbiol Infect Dis;64(2):177–84

Bingen, E., N. Lambert-Zechovsky, E. Guihaire, P. Mariani, M. Coache, E. Jacqz, Y. Aujard and H. Mathieu 1987. [Importance of the study of the minimal bactericidal time of serum in the choice of optimal treatment of neonatal septicemias]. Pathol Biol (Paris);35(5):599–602

Bizzarro, M. J., L. M. Dembry, R. S. Baltimore and P. G. Gallagher 2008. Changing patterns in neonatal Escherichia coli sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. Pediatrics;121(4):689–96

Bizzarro, M. J., C. Raskind, R. S. Baltimore and P. G. Gallagher 2005. Seventy-five years of neonatal sepsis at Yale: 1928–2003. Pediatrics;116(3):595–602

Bitner-Glindzicz, M., M. Pembrey, A. Duncan, J. Heron, S. M. Ring, A. Hall and S. Rahman 2009. Prevalence of mitochondrial 1555A-->G mutation in European children. N Engl J Med;360(6):640–2

Bjorksten, B., E. Sepp, K. Julge, T. Voor and M. Mikelsaar 2001. Allergy development and the intestinal microflora during the first year of life. J Allergy Clin Immunol;108(4):516–20

Bloos, F., F. Hinder, K. Becker, S. Sachse, A. Mekontso Dessap, E. Straube, V. Cattoir, C. Brun-Buisson, K. Reinhart, G. Peters and M. Bauer 2009. A multicenter trial to compare blood culture with polymerase chain reaction in severe human sepsis. Intensive Care Med;

Boe, R. W., C. P. Williams, J. V. Bennett and T. K. Oliver, Jr. 1967. Serum levels of methicillin and ampicillin in newborn and premature infants in relation to postnatal age. Pediatrics;39(2):194–201

Bomela, H. N., D. E. Ballot, B. J. Cory and P. A. Cooper 2000. Use of C-reactive protein to guide duration of empiric antibiotic therapy in suspected early neonatal sepsis. Pediatr Infect Dis J;19(6):531–5

Bone, R. C., W. J. Sibbald and C. L. Sprung 1992a. The ACCP-SCCM consensus conference on sepsis and organ failure. Chest;101(6):1481–3

Bone, R. C., C. L. Sprung and W. J. Sibbald 1992b. Definitions for sepsis and organ failure. Crit Care Med;20(6):724–6

Boswald, M., C. Dobig, C. Kandler, C. Kruger, J. Scharf, F. Soergel, S. Zink and J. P. Guggenbichler 1999. Pharmacokinetic and clinical evaluation of serious infections in premature and newborn infants under therapy with imipenem/cilastatin. Infection;27(4–5):299–304

Boyer, K. M., C. A. Gadzala, L. I. Burd, D. E. Fisher, J. B. Paton and S. P. Gotoff 1983a. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. I. Epidemiologic rationale. J Infect Dis;148(5):795–801

Boyer, K. M., C. A. Gadzala, P. D. Kelly, L. I. Burd and S. P. Gotoff 1983b. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. II. Predictive value of prenatal cultures. J Infect Dis;148(5):802–9

Boyer, K. M., C. A. Gadzala, P. D. Kelly and S. P. Gotoff 1983c. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. III. Interruption of mother-to-infant transmission. J Infect Dis;148(5):810–6

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

Bradley, J. S., J. B. Sauberan, P. G. Ambrose, S. M. Bhavnani, M. R. Rasmussen and E. V. Capparelli 2008. Meropenem pharmacokinetics, pharmacodynamics, and Monte Carlo simulation in the neonate. Pediatr Infect Dis J;27(9):794–9

Bratlid, D. and T. Lindback 1978. Bacteriolytic activity of amniotic fluid. Obstet Gynecol;51(1):63–6

Bray, R. E., R. W. Boe and W. L. Johnson 1966. Transfer of ampicillin into fetus and amniotic fluid from maternal plasma in late pregnancy. Am J Obstet Gynecol; 96(7):938–42

Breiman, L., J. Friedman, R. Olshen and C. Stone 1984. Classification and Regression Trees. New York

Brogard, J. M., J. P. Arnaud, J. Dauchel, M. Pinget and J. Lavillaureix 1979. [Evaluation of the biliary excretion of penicillin G. (author's transl)]. Pathol Biol (Paris);27(7):403–10

Bromberger, P., J. M. Lawrence, D. Braun, B. Saunders, R. Contreras and D. B. Petitti 2000. The influence of intrapartum antibiotics on the clinical spectrum of early-onset group B streptococcal infection in term infants. Pediatrics;106(2 Pt 1):244–50

Brown, D. R., D. Kutler, B. Rai, T. Chan and M. Cohen 1995. Bacterial concentration and blood volume required for a positive blood culture. J Perinatol;15(2):157–9

Brown, W. J., N. R. Buist, H. T. Gipson, R. K. Huston and N. G. Kennaway 1982. Fatal benzyl alcohol poisoning in a neonatal intensive care unit. Lancet;1(8283):1250

Buck, C., J. Bundschu, H. Gallati, P. Bartmann and F. Pohlandt 1994. Interleukin-6: a sensitive parameter for the early diagnosis of neonatal bacterial infection. Pediatrics; 93(1):54–8

Burman, L. G., B. Berglund, P. Huovinen and K. Tullus 1993. Effect of ampicillin versus cefuroxime on the emergence of beta-lactam resistance in faecal Enterobacter cloacae isolates from neonates. J Antimicrob Chemother;31(1):111–6

Buttery, J. P. 2002. Blood cultures in newborns and children: optimising an everyday test. Arch Dis Child Fetal Neonatal Ed;87(1):F25–8

Cao, H., D. E. Lake, M. P. Griffin and J. R. Moorman 2004. Increased nonstationarity of neonatal heart rate before the clinical diagnosis of sepsis. Ann Biomed Eng;32(2):233–44

Capparelli, E., C. Hochwald, M. Rasmussen, A. Parham, J. Bradley and F. Moya 2005. Population pharmacokinetics of cefepime in the neonate. Antimicrob Agents Chemother;49(7):2760–6

Carbonell-Estrany, X., J. Figueras-Aloy, S. Salcedo-Abizanda and M. de la Rosa-Fraile 2008. Probable early-onset group B streptococcal neonatal sepsis: a serious clinical condition related to intrauterine infection. Arch Dis Child Fetal Neonatal Ed;93(2):F85–9

Cartwright, K., S. Reilly, D. White and J. Stuart 1992. Early treatment with parenteral penicillin in meningococcal disease. Bmj;305(6846):143–7

CDC 1982. Neonatal Deaths Associated With Use Of Benzyl Alcohol -- United States MMWR;31(22):290–291

Chambers, H. 2005. Penicillins. Philadelphia

Charles, D. 1977. Dynamics of antibiotic transfer from mother to fetus. Semin Perinatol;1(1):89–100

Chen, K. T., K. M. Puopolo, E. C. Eichenwald, A. B. Onderdonk and E. Lieberman 2005. No increase in rates of early-onset neonatal sepsis by antibiotic-resistant group B Streptococcus in the era of intrapartum antibiotic prophylaxis. Am J Obstet Gynecol;192(4):1167–71

Chen, K. T., R. E. Tuomala, A. P. Cohen, E. C. Eichenwald and E. Lieberman 2001. No increase in rates of early-onset neonatal sepsis by non-group B Streptococcus or ampicillin-resistant organisms. Am J Obstet Gynecol;185(4):854–8

Chiesa, C., A. Panero, N. Rossi, M. Stegagno, M. De Giusti, J. F. Osborn and L. Pacifico 1998. Reliability of procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. Clin Infect Dis;26(3):664–72

Chiesa, C., G. Pellegrini, A. Panero, T. De Luca, M. Assumma, F. Signore and L. Pacifico 2003a. Umbilical cord interleukin-6 levels are elevated in term neonates with perinatal asphyxia. Eur J Clin Invest;33(4):352–8

Chiesa, C., G. Pellegrini, A. Panero, J. F. Osborn, F. Signore, M. Assumma and L. Pacifico 2003b. C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. Clin Chem;49(1):60–8

Choi, S. H., J. E. Lee, S. J. Park, S. H. Choi, S. O. Lee, J. Y. Jeong, M. N. Kim, J. H. Woo and Y. S. Kim 2008. Emergence of antibiotic resistance during therapy for infections caused by Enterobacteriaceae producing AmpC beta-lactamase: implications for antibiotic use. Antimicrob Agents Chemother;52(3):995–1000

Chowdhary, G., S. Dutta and A. Narang 2006. Randomized controlled trial of 7-Day vs. 14-Day antibiotics for neonatal sepsis. J Trop Pediatr;52(6):427–32

Christensen, E. 2007. Methodology of superiority vs. equivalence trials and non-inferiority trials. J Hepatol;46(5):947–54

Clark, R. H., B. T. Bloom, A. R. Spitzer and D. R. Gerstmann 2006. Empiric use of ampicillin and cefotaxime, compared with ampicillin and gentamicin, for neonates at

risk for sepsis is associated with an increased risk of neonatal death. Pediatrics; 117(1):67–74

Cohen-Wolkowiez, M., C. Moran, D. K. Benjamin, C. M. Cotten, R. H. Clark, D. K. Benjamin, Jr. and P. B. Smith 2009. Early and late onset sepsis in late preterm infants. Pediatr Infect Dis J;28(12):1052–6

Connell, T. G., M. Rele, D. Cowley, J. P. Buttery and N. Curtis 2007. How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. Pediatrics;119(5):891–6

Contopoulos-Ioannidis, D. G., N. D. Giotis, D. V. Baliatsa and J. P. Ioannidis 2004. Extended-interval aminoglycoside administration for children: a meta-analysis. Pediatrics;114(1):e111–8

Cordero, L. and L. W. Ayers 2003. Duration of empiric antibiotics for suspected early-onset sepsis in extremely low birth weight infants. Infect Control Hosp Epidemiol;24(9):662–6

Costa, S. F., M. H. Miceli and E. J. Anaissie 2004. Mucosa or skin as source of coagulase-negative staphylococcal bacteraemia? Lancet Infect Dis;4(5):278–86

Cotten, C. M., S. McDonald, B. Stoll, R. N. Goldberg, K. Poole and D. K. Benjamin, Jr. 2006. The association of third-generation cephalosporin use and invasive candidiasis in extremely low birth-weight infants. Pediatrics;118(2):717–22

Couto, R. C., J. A. Barbosa, T. M. Pedrosa and F. M. Biscione 2007a. C-reactive protein-guided approach may shorten length of antimicrobial treatment of culture-proven late-onset sepsis: an intervention study. Braz J Infect Dis;11(2):240–5

Couto, R. C., E. A. Carvalho, T. M. Pedrosa, E. R. Pedroso, M. C. Neto and F. M. Biscione 2007b. A 10-year prospective surveillance of nosocomial infections in neonatal intensive care units. Am J Infect Control;35(3):183–9

Crivaro, V., M. Bagattini, M. F. Salza, F. Raimondi, F. Rossano, M. Triassi and R. Zarrilli 2007. Risk factors for extended-spectrum beta-lactamase-producing Serratia marcescens and Klebsiella pneumoniae acquisition in a neonatal intensive care unit. J Hosp Infect;67(2):135–41

Czock, D. and F. Keller 2007. Mechanism-based pharmacokinetic-pharmacodynamic modeling of antimicrobial drug effects. J Pharmacokinet Pharmacodyn;34(6):727–51

Cunningham, S., A. G. Symon, R. A. Elton, C. Zhu and N. McIntosh 1999. Intra-arterial blood pressure reference ranges, death and morbidity in very low birthweight infants during the first seven days of life. Early Hum Dev;56(2–3):151–65

Da Silva, O., A. Ohlsson and C. Kenyon 1995. Accuracy of leukocyte indices and C-reactive protein for diagnosis of neonatal sepsis: a critical review. Pediatr Infect Dis J;14(5):362–6

Dahesh, S., M. E. Hensler, N. M. Van Sorge, R. E. Gertz, Jr., S. Schrag, V. Nizet and B. W. Beall 2008. Point mutation in the group B streptococcal pbp2x gene conferring decreased susceptibility to beta-lactam antibiotics. Antimicrob Agents Chemother; 52(8):2915–8

Dahl, L. B., K. Melby, T. J. Gutteberg and G. Storvold 1986. Serum levels of ampicillin and gentamycin in neonates of varying gestational age. Eur J Pediatr;145(3):218–21

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

Daley, A. J. and D. Isaacs 2004. Ten-year study on the effect of intrapartum antibiotic prophylaxis on early onset group B streptococcal and Escherichia coli neonatal sepsis in Australasia. Pediatr Infect Dis J;23(7):630–4

Dandona, P., D. Nix, M. F. Wilson, A. Aljada, J. Love, M. Assicot and C. Bohuon 1994. Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab;79(6):1605–8

Dark, P. M., P. Dean and G. Warhurst 2009. Bench-to-bedside review: the promise of rapid infection diagnosis during sepsis using polymerase chain reaction-based pathogen detection. Crit Care;13(4):217

Darmstadt, G. L., M. Batra and A. K. Zaidi 2009. Oral antibiotics in the management of serious neonatal bacterial infections in developing country communities. Pediatr Infect Dis J;28(1 Suppl):S31–6

Dawodu, A. H. and C. E. Effiong 1985. Neonatal mortality: effects of selective pediatric interventions. Pediatrics;75(1):51–7

de Hoog, M., J. W. Mouton and J. N. van den Anker 2005. New dosing strategies for antibacterial agents in the neonate. Semin Fetal Neonatal Med;10(2):185–94

de Louvois, J., R. Dagan and I. Tessin 1992. A comparison of ceftazidime and aminoglycoside based regimens as empirical treatment in 1316 cases of suspected sepsis in the newborn. European Society for Paediatric Infectious Diseases – Neonatal Sepsis Study Group. Eur J Pediatr;151(12):876–84

de Louvois, J., A. Mulhall and R. Hurley 1981. Mecillinam (Selexidin) in the treatment of neonates. J Perinat Med;9(2):87–95

de Man, P., B. A. Verhoeven, H. A. Verbrugh, M. C. Vos and J. N. van den Anker 2000. An antibiotic policy to prevent emergence of resistant bacilli. Lancet;-355(9208):973–8

de Wildt, S. N., G. L. Kearns, J. S. Leeder and J. N. van den Anker 1999a. Cytochrome P450 3A: ontogeny and drug disposition. Clin Pharmacokinet;37(6):485–505

de Wildt, S. N., G. L. Kearns, J. S. Leeder and J. N. van den Anker 1999b. Glucuronidation in humans. Pharmacogenetic and developmental aspects. Clin Pharmacokinet; 36(6):439–52

Di Paolo, E. R., H. Stoetter, J. Cotting, P. Frey, M. Gehri, M. Beck-Popovic, J. F. Tolsa, S. Fanconi and A. Pannatier 2006. Unlicensed and off-label drug use in a Swiss paediatric university hospital. Swiss Med Wkly;136(13–14):218–22

Dollner, H., L. Vatten, I. Linnebo, G. F. Zanussi, A. Laerdal and R. Austgulen 2001. Inflammatory mediators in umbilical plasma from neonates who develop early-onset sepsis. Biol Neonate;80(1):41–7

Drusano, G. L. 2004. Antimicrobial pharmacodynamics: critical interactions of 'bug and drug'. Nat Rev Microbiol;2(4):289–300

Drusano, G. L., P. G. Ambrose, S. M. Bhavnani, J. S. Bertino, A. N. Nafziger and A. Louie 2007. Back to the future: using aminoglycosides again and how to dose them optimally. Clin Infect Dis;45(6):753–60

Duchon, J., P. Graham Iii, P. Della-Latta, S. Whittier, D. Carp, D. Bateman and L. Saiman 2008. Epidemiology of enterococci in a neonatal intensive care unit. Infect Control Hosp Epidemiol;29(4):374–6

Dunham, E. 1933. Septicemia in the newborn. American Journal of Diseases of Children; 45(2):229–253

Dutta, S., A. Narang, A. Chakraborty and P. Ray 2009. Diagnosis of neonatal sepsis using universal primer polymerase chain reaction before and after starting antibiotic drug therapy. Arch Pediatr Adolesc Med;163(1):6–11

Ehrenkranz, R. A., M. C. Walsh, B. R. Vohr, A. H. Jobe, L. L. Wright, A. A. Fanaroff, L. A. Wrage and K. Poole 2005. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics;116(6):1353–60

Eickhoff, T. C., J. O. Klein, A. K. Daly, D. Ingall and M. Finland 1964. Neonatal Sepsis and Other Infections Due to Group B Beta-Hemolytic Streptococci. N Engl J Med;271(1221–8

Engle, W. D., G. L. Jackson, D. Sendelbach, D. Ford, B. Olesen, K. M. Burton, M. A. Pritchard and W. H. Frawley 2000. Neonatal pneumonia: comparison of 4 vs 7 days of antibiotic therapy in term and near-term infants. J Perinatol;20(7):421–6

Engle, W. D., G. L. Jackson, D. M. Sendelbach, E. K. Stehel, D. M. Ford, K. M. McHugh, M. R. Norris, D. A. Vedro, S. Velaphi, I. C. Michelow and K. D. Olsen 2003. Pneumonia in term neonates: laboratory studies and duration of antibiotic therapy. J Perinatol;23(5):372–7

Engle, W. D. and C. R. Rosenfeld 1984. Neutropenia in high-risk neonates. J Pediatr; 105(6):982–6

Escobar, G. J., T. Zukin, M. S. Usatin, J. Lemesurier, D. Honeychurch, M. A. Armstrong and B. Folck 1994. Early discontinuation of antibiotic treatment in newborns admitted to rule out sepsis: a decision rule. Pediatr Infect Dis J;13(10):860–6

EUCAST 2010. Antimicrobial wild type distributions of microorganisms.

Euler, A. R., W. J. Byrne, L. M. Cousins, M. E. Ament and J. H. Walsh 1977. Increased serum gastrin concentrations and gastric acid hyposecretion in the immediate newborn period. Gastroenterology;72(6):1271–3

Fanaroff, A. A., B. J. Stoll, L. L. Wright, W. A. Carlo, R. A. Ehrenkranz, A. R. Stark, C. R. Bauer, E. F. Donovan, S. B. Korones, A. R. Laptook, J. A. Lemons, W. Oh, L. A. Papile, S. Shankaran, D. K. Stevenson, J. E. Tyson and W. K. Poole 2007. Trends in neonatal morbidity and mortality for very low birthweight infants. Am J Obstet Gynecol;196(2):147 e1–8

Feldman, W. E. 1976. Concentrations of bacteria in cerebrospinal fluid of patients with bacterial meningitis. J Pediatr;88(4 Pt. 1):549–52

Figueira-Coelho, J., M. Ramirez, M. J. Salgado and J. Melo-Cristino 2004. Streptococcus agalactiae in a large Portuguese teaching hospital: antimicrobial susceptibility, serotype distribution, and clonal analysis of macrolide-resistant isolates. Microb Drug Resist;10(1):31–6

Fisher, R. S. 1989. Animal models of the epilepsies. Brain Res Brain Res Rev;14(3): 245–78

Fogel, D., L. Farfel, A. Miskin and B. M. Mogilner 1983. Comparison between the combination of azlocillin-gentamicin and ampicillin-gentamicin in the treatment of a nursery population. Isr J Med Sci;19(11):1009–15

Forest, J. C., F. Lariviere, P. Dolce, M. Masson and L. Nadeau 1986. C-reactive protein as biochemical indicator of bacterial infection in neonates. Clin Biochem;19(3):192–4

Fowlie, P. W. and B. Schmidt 1998. Diagnostic tests for bacterial infection from birth to 90 days – a systematic review. Arch Dis Child Fetal Neonatal Ed;78(2):F92–8

Franz, A. R., M. Kron, F. Pohlandt and G. Steinbach 1999a. Comparison of procalcitonin with interleukin 8, C-reactive protein and differential white blood cell count for the early diagnosis of bacterial infections in newborn infants. Pediatr Infect Dis J;18(8):666–71

Franz, A. R., G. Steinbach, M. Kron and F. Pohlandt 1999b. Reduction of unnecessary antibiotic therapy in newborn infants using interleukin-8 and C-reactive protein as markers of bacterial infections. Pediatrics;104(3 Pt 1):447–53

Franz, A. R., G. Steinbach, M. Kron and F. Pohlandt 2001. Interleukin-8: a valuable tool to restrict antibiotic therapy in newborn infants. Acta Paediatr;90(9):1025–32

Freedman, R. M., D. L. Ingram, I. Gross, R. A. Ehrenkranz, J. B. Warshaw and R. S. Baltimore 1981. A half century of neonatal sepsis at Yale: 1928 to 1978. Am J Dis Child;135(2):140–4

Fuji, R., S. Hashira, T. Tajima, H. Meguro, T. Abe, H. Sakata, H. Kakehashi, K. Fujita, K. Murono, M. Kaeriyama and et al. 1990. [Pharmacokinetics and clinical studies on aztreonam in neonates and premature infants (the first report). Study on effectiveness and safety in mono-therapy with aztreonam. A study of aztreonam in the Perinatal Co-research Group]. Jpn J Antibiot;43(3):543–62

Fujita, K. and H. Yoshioka 1977. Relevance of concentration of pathogenic bacteria in cerebrospinal fluid to antibiotic therapy. J Pediatr;90(2):328–9

Furrie, E. 2005. Probiotics and allergy. Proc Nutr Soc;64(4):465–9

Garland, S. M., F. Ni Chuileannain, C. Satzke and R. Robins-Browne 2002. Mechanisms, organisms and markers of infection in pregnancy. J Reprod Immunol;57(1–2):169–83

Garnacho-Montero, J., T. Aldabo-Pallas, C. Garnacho-Montero, A. Cayuela, R. Jimenez, S. Barroso and C. Ortiz-Leyba 2006. Timing of adequate antibiotic therapy is a greater determinant of outcome than are TNF and IL-10 polymorphisms in patients with sepsis. Crit Care;10(4):R111

Gerard, P., M. Verghote-D'Hulst, A. Bachy and G. Duhaut 1979. Group B streptococcal colonization of pregnant women and their neonates. Epidemiological study and controlled trial of prophylactic treatment of the newborn. Acta Paediatr Scand; 68(6):819–23

Gerberding, K. M., C. C. Eisenhut, W. A. Engle and M. D. Cohen 1989. Congenital candida pneumonia and sepsis: a case report and review of the literature. J Perinatol; 9(2):159–61

Gerdes, J. S. 1991. Clinicopathologic approach to the diagnosis of neonatal sepsis. Clin Perinatol;18(2):361–81

Gerdes, J. S. and R. A. Polin 1987. Sepsis screen in neonates with evaluation of plasma fibronectin. Pediatr Infect Dis J;6(5):443–6

Gerdes, J. S., M. C. Yoder, S. D. Douglas and R. A. Polin 1983. Decreased plasma fibronectin in neonatal sepsis. Pediatrics;72(6):877–81

Gessler, P., N. Kirchmann, R. Kientsch-Engel, N. Haas, P. Lasch and W. Kachel 1993. Serum concentrations of granulocyte colony-stimulating factor in healthy term and preterm neonates and in those with various diseases including bacterial infections. Blood;82(10):3177–82

Giachetto, G., M. C. Pirez, L. Nanni, A. Martinez, A. Montano, G. Algorta, S. L. Kaplan and A. M. Ferrari 2004. Ampicillin and penicillin concentration in serum and pleural fluid of hospitalized children with community-acquired pneumonia. Pediatr Infect Dis J;23(7):625–9

Giacoia, G. P., D. L. Birenbaum, H. C. Sachs and D. R. Mattison 2006. The newborn drug development initiative. Pediatrics;117(3 Pt 2):S1–8

Gibbs, R. 1990. Obstetric factors Associated with Infections of the Fetus and Newborn Infants. J. Remington and J. Klein. Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W.B. Saunders. pp 981–999

Gibbs, R. S., M. S. Castillo and P. J. Rodgers 1980. Management of acute chorioamnionitis. Am J Obstet Gynecol;136(6):709–13

Giles, M., S. Garland and J. J. Oats 2005. Management of preterm prelabour rupture of membranes: an audit. How do the results compare with clinical practice guidelines? Aust N Z J Obstet Gynaecol;45(3):201–6

Gladstone, I. M., R. A. Ehrenkranz, S. C. Edberg and R. S. Baltimore 1990. A ten-year review of neonatal sepsis and comparison with the previous fifty-year experience. Pediatr Infect Dis J;9(11):819–25

Gokalp, A. S. and A. Oguz 1990. Neonatal sepsis in Turkey: the comparison between penicillin plus aminoglycoside and ampicillin plus third-generation cephalosporin chemotherapies. J Trop Pediatr;36(4):200

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

Gordon, A. and D. Isaacs 2004. Late-onset infection and the role of antibiotic prescribing policies. Curr Opin Infect Dis;17(3):231–6

Gordon, A. and H. E. Jeffery 2005. Antibiotic regimens for suspected late onset sepsis in newborn infants. Cochrane Database Syst Rev;3):CD004501

Gras-Le Guen, C., C. Boscher, N. Godon, J. Caillon, C. Denis, J. M. Nguyen, M. F. Kergueris and J. C. Roze 2007. Therapeutic amoxicillin levels achieved with oral administration in term neonates. Eur J Clin Pharmacol;63(7):657–62

Greenberg, D. N., D. P. Ascher, B. A. Yoder, D. M. Hensley, H. S. Heiman and J. F. Keith, 3rd 1995. Sensitivity and specificity of rapid diagnostic tests for detection of group B streptococcal antigen in bacteremic neonates. J Clin Microbiol;33(1):193–8

Greene, C. J., L. A. Morland, V. L. Durkalski and B. C. Frueh 2008. Noninferiority and equivalence designs: issues and implications for mental health research. J Trauma Stress;21(5):433–9

Griffin, M. P., D. E. Lake and J. R. Moorman 2005. Heart rate characteristics and laboratory tests in neonatal sepsis. Pediatrics;115(4):937–41

Griffin, M. P. and J. R. Moorman 2001. Toward the early diagnosis of neonatal sepsis and sepsis-like illness using novel heart rate analysis. Pediatrics;107(1):97–104

Griffin, M. P., T. M. O'Shea, E. A. Bissonette, F. E. Harrell, Jr., D. E. Lake and J. R. Moorman 2003. Abnormal heart rate characteristics preceding neonatal sepsis and sepsis-like illness. Pediatr Res;53(6):920–6

Grimwood, K., B. A. Darlow, I. A. Gosling, R. Green, D. R. Lennon, D. R. Martin and P. R. Stone 2002. Early-onset neonatal group B streptococcal infections in New Zealand 1998–1999. J Paediatr Child Health;38(3):272–7

Grossman, M. and W. Ticknor 1965. Serum levels of ampicillin, cephalothin, cloxacillin, and nafcillin in the newborn infant. Antimicrob Agents Chemother (Bethesda);5(214–9

Group, I. C. 1988. Early neonatal drug utilization in preterm newborns in neonatal intensive care units. Italian Collaborative Group on Preterm Delivery. Dev Pharmacol Ther. 1988;11(1):1–7;11(1):1–7

Gupta, M. and Y. W. Brans 1978. Gastric retention in neonates. Pediatrics;62(1):26–9

Hafed, B. M., E. Bilikova, G. Kovacicova, J. Koprnova, I. Svetlansky, D. Chovancova, M. Huttova and V. Krcmery 2003. Prognostic factors for 246 neonates with infections. Clin Infect Dis;36(9):1203–5

Haffejee, I. E. 1984. A therapeutic trial of cefotaxime versus penicillin-gentamicin for severe infections in children. J Antimicrob Chemother;14 Suppl B(147–52

Hall, M. A., D. A. Ducker, J. A. Lowes, J. McMichael, P. Clarke, D. Rowe, A. Gordon and D. S. Cole 1988. A randomised prospective comparison of cefotaxime versus netilmicin/penicillin for treatment of suspected neonatal sepsis. Drugs;35 Suppl 2(169–77

Halliday, H. L. and T. Hirata 1979. Perinatal listeriosis – a review of twelve patients. Am J Obstet Gynecol;133(4):405–10

Hammerberg, O., C. Kurnitzki, J. Watts and D. Rosenbloom 1989. Randomized trial using piperacillin versus ampicillin and amikacin for treatment of premature neonates with risk factors for sepsis. Eur J Clin Microbiol Infect Dis;8(3):241–4

Harris, M. C., C. Deuber, R. A. Polin and I. Nachamkin 1989. Investigation of apparent false-positive urine latex particle agglutination tests for the detection of group B streptococcus antigen. J Clin Microbiol;27(10):2214–7

Hatzidaki, E., D. Gourgiotis, A. Manoura, E. Korakaki, A. Bossios, E. Galanakis and C. Giannakopoulou 2005. Interleukin-6 in preterm premature rupture of membranes as an indicator of neonatal outcome. Acta Obstet Gynecol Scand;84(7):632–8

Hayes, R. J. and S. Bennett 1999. Simple sample size calculation for cluster-randomized trials. Int J Epidemiol;28(2):319–26

Heckbert, S. R., W. S. Stryker, K. L. Coltin, J. E. Manson and R. Platt 1990. Serum sickness in children after antibiotic exposure: estimates of occurrence and morbidity in a health maintenance organization population. Am J Epidemiol;132(2):336–42

Heimler, R., L. D. Nelin, D. O. Billman and P. Sasidharan 1995. Identification of sepsis in neonates following maternal antibiotic therapy. Clin Pediatr (Phila);34(3):133–7

Hieber, J. P. and J. D. Nelson 1977. A pharmacologic evaluation of penicillin in children with purulent meningitis. N Engl J Med;297(8):410–3

Hira, V., M. Sluijter, S. Estevao, D. Horst-Kreft, A. Ott, R. de Groot, P. W. Hermans and R. F. Kornelisse 2007. Clinical and molecular epidemiologic characteristics of coagulase-negative staphylococcal bloodstream infections in intensive care neonates. Pediatr Infect Dis J;26(7):607–12

Hochreiter, M., T. Kohler, A. M. Schweiger, F. S. Keck, B. Bein, T. von Spiegel and S. Schroeder 2009. Procalcitonin to guide duration of antibiotic therapy in intensive care patients: a randomized prospective controlled trial. Crit Care;13(3):R83

Hoekstra, R. E., T. B. Ferrara, R. J. Couser, N. R. Payne and J. E. Connett 2004. Survival and long-term neurodevelopmental outcome of extremely premature infants born at 23–26 weeks' gestational age at a tertiary center. Pediatrics;113(1 Pt 1):e1–6

Holmsgaard, K. W. and S. Petersen 1996. Infants with gestational age 28 weeks or less. Dan Med Bull;43(1):86–91

Horspool, L. J. and Q. A. McKellar 1995. Disposition of penicillin G sodium following intravenous and oral administration to Equidae. Br Vet J;151(4):401–12

Hsien, L., A. Breddemann, A. K. Frobel, A. Heusch, K. G. Schmidt and S. Laer 2008. Off-label drug use among hospitalised children: identifying areas with the highest need for research. Pharm World Sci;30(5):497–502

Huang, N. N. and R. H. High 1953. Comparison of serum levels following the administration of oral and parenteral preparations of penicillin to infants and children of various age groups. J Pediatr;42(6):657–8

Huisman-de Boer, J. J., J. N. van den Anker, M. Vogel, W. H. Goessens, R. C. Schoemaker and R. de Groot 1995. Amoxicillin pharmacokinetics in preterm infants with gestational ages of less than 32 weeks. Antimicrob Agents Chemother; 39(2):431–4

Hutchin, T., I. Haworth, K. Higashi, N. Fischel-Ghodsian, M. Stoneking, N. Saha, C. Arnos and G. Cortopassi 1993. A molecular basis for human hypersensitivity to aminoglycoside antibiotics. Nucleic Acids Res;21(18):4174–9

Hyde, T. B., T. M. Hilger, A. Reingold, M. M. Farley, K. L. O'Brien and A. Schuchat 2002. Trends in incidence and antimicrobial resistance of early-onset sepsis: population-based surveillance in San Francisco and Atlanta. Pediatrics;110(4):690–5

Ingram, D. L., D. M. Suggs and A. W. Pearson 1982. Detection of group B streptococcal antigen in early-onset and late-onset group B streptococcal disease with the Wellcogen Strep B latex agglutination test. J Clin Microbiol;16(4):656–8

Iregui, M., S. Ward, G. Sherman, V. J. Fraser and M. H. Kollef 2002. Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. Chest;122(1):262–8

Isaacs, D. and J. A. Royle 1999. Intrapartum antibiotics and early onset neonatal sepsis caused by group B Streptococcus and by other organisms in Australia. Australasian Study Group for Neonatal Infections. Pediatr Infect Dis J;18(6):524–8

Jardine, L., M. W. Davies and J. Faoagali 2006. Incubation time required for neonatal blood cultures to become positive. J Paediatr Child Health;42(12):797–802

Jobe, A. H. and E. Bancalari 2001. Bronchopulmonary dysplasia. Am J Respir Crit Care Med;163(7):1723–9

Johnson, T. N., M. S. Tanner, C. J. Taylor and G. T. Tucker 2001. Enterocytic CYP3A4 in a paediatric population: developmental changes and the effect of coeliac disease and cystic fibrosis. Br J Clin Pharmacol;51(5):451–60

Jokipii, L., P. Brander and A. M. Jokipii 1985. Reverse inoculum effect in bactericidal activity and other variables affecting killing of group B streptococci by penicillin. Antimicrob Agents Chemother;27(6):948–52

Jones, B., K. Peake, A. J. Morris, L. M. McCowan and M. R. Battin 2004. Escherichia coli: a growing problem in early onset neonatal sepsis. Aust N Z J Obstet Gynaecol;44(6):558–61

Jones, W. F., Jr., H. A. Feldman and M. Finland 1957. Susceptibility of hemolytic streptococci, other than those of group D, to eleven antibiotics in vitro. Am J Clin Pathol;27(2):159–69

Jordan, J. A., M. B. Durso, A. R. Butchko, J. G. Jones and B. S. Brozanski 2006. Evaluating the near-term infant for early onset sepsis: progress and challenges to consider with 16S rDNA polymerase chain reaction testing. J Mol Diagn;8(3):357–63

Joseph, K. S. 2007. Theory of obstetrics: an epidemiologic framework for justifying medically indicated early delivery. BMC Pregnancy Childbirth;7(4

Joseph, T. A., S. P. Pyati and N. Jacobs 1998. Neonatal early-onset Escherichia coli disease. The effect of intrapartum ampicillin. Arch Pediatr Adolesc Med;152(1):35–40

Julious, S. A. 2004. Sample sizes for clinical trials with normal data. Stat Med; 23(12):1921–86

Jurges, E. S. and D. C. Henderson 1996. Inflammatory and immunological markers in preterm infants: correlation with disease. Clin Exp Immunol;105(3):551–5

Kalenic, S., I. Francetic, J. Polak, L. Zele-Starcevic and Z. Bencic 1993. Impact of ampicillin and cefuroxime on bacterial colonization and infection in patients on a neonatal intensive care unit. J Hosp Infect;23(1):35–41

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

Kaplan, J. M., G. H. McCracken, Jr., L. J. Horton, M. L. Thomas and N. Davis 1974. Pharmacologic studies in neonates given large dosages of ampicillin. J Pediatr; 84(4):571–7

Kapoor, L., V. S. Randhawa and M. Deb 2005. Microbiological profile of neonatal septicemia in a pediatric care hospital in Delhi. J Commun Dis;37(3):227–32

Karabus, S. and C. Motala 2009. Penicillin Allergy in Children. Current Allergy & Clinical Immunology;22(2):64–66

Kellogg, J. A., F. L. Ferrentino, M. H. Goodstein, J. Liss, S. L. Shapiro and D. A. Bankert 1997. Frequency of low level bacteremia in infants from birth to two months of age. Pediatr Infect Dis J;16(4):381–5

Kellogg, J. A., J. P. Manzella and D. A. Bankert 2000. Frequency of low-level bacteremia in children from birth to fifteen years of age. J Clin Microbiol; 38(6):2181–5

Kennon, C., G. Overturf, S. Bessman, E. Sierra, K. J. Smith and B. Brann 1996. Granulocyte colony-stimulating factor as a marker for bacterial infection in neonates. J Pediatr;128(6):765–9

Kenyon, S. L., D. J. Taylor and W. Tarnow-Mordi 2001a. Broad-spectrum antibiotics for preterm, prelabour rupture of fetal membranes: the ORACLE I randomised trial. ORACLE Collaborative Group. Lancet;357(9261):979–88

Kenyon, S. L., D. J. Taylor and W. Tarnow-Mordi 2001b. Broad-spectrum antibiotics for spontaneous preterm labour: the ORACLE II randomised trial. ORACLE Collaborative Group. Lancet;357(9261):989–94

Kermorvant-Duchemin, E., S. Laborie, M. Rabilloud, A. Lapillonne and O. Claris 2008. Outcome and prognostic factors in neonates with septic shock. Pediatr Crit Care Med;9(2):186–91

Kerr, J. R. 1994. Penicillin allergy: a study of incidence as reported by patients. Br J Clin Pract;48(1):5–7

Kildoo, C., H. D. Modanlou, G. Komatsu, A. Harralson and J. Hodding 1984. Developmental pattern of gentamicin kinetics in very low birth weight (VLBW) sick infants. Dev Pharmacol Ther;7(6):345–56

Kimura, K., S. Suzuki, J. Wachino, H. Kurokawa, K. Yamane, N. Shibata, N. Nagano, H. Kato, K. Shibayama and Y. Arakawa 2008. First molecular characterization of group B streptococci with reduced penicillin susceptibility. Antimicrob Agents Chemother;52(8):2890–7

Kingsmore, S. F., N. Kennedy, H. L. Halliday, J. C. Van Velkinburgh, S. Zhong, V. Gabriel, J. Grant, W. D. Beavis, V. T. Tchernev, L. Perlee, S. Lejnine, B. Grimwade, M. Sorrette and J. D. Edgar 2008. Identification of diagnostic biomarkers for infection in premature neonates. Mol Cell Proteomics;

Klein, J. and S. Marcy 1990. Bacterial Sepsis and Meningitis. J. Reminggton and J. Klein. Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W:B: Saunders. pp

Klein, J. O. 1990. Bacteriology of neonatal sepsis. Pediatr Infect Dis J;9(10):778

Klingenberg, C., A. Ronnestad, A. S. Anderson, T. G. Abrahamsen, J. Zorman, A. Villaruz, T. Flaegstad, M. Otto and J. E. Sollid 2007. Persistent strains of coagulase-negative staphylococci in a neonatal intensive care unit: virulence factors and invasiveness. Clin Microbiol Infect;13(11):1100–11

Klingenberg, C., A. Sundsfjord, A. Ronnestad, J. Mikalsen, P. Gaustad and T. Flaegstad 2004. Phenotypic and genotypic aminoglycoside resistance in blood culture isolates of coagulase-negative staphylococci from a single neonatal intensive care unit, 1989–2000. J Antimicrob Chemother;54(5):889–96

Klinger, G., I. Levy, L. Sirota, V. Boyko, B. Reichman and L. Lerner-Geva 2009. Epidemiology and risk factors for early onset sepsis among very-low-birthweight infants. Am J Obstet Gynecol;201(1):38 e1–6

Koh, K. S., F. H. Chan, A. H. Monfared, W. J. Ledger and R. H. Paul 1979. The changing perinatal and maternal outcome in chorioamnionitis. Obstet Gynecol; 53(6):730–4

Kollef, M. H. 2000. Inadequate antimicrobial treatment: an important determinant of outcome for hospitalized patients. Clin Infect Dis;31 Suppl 4(S131–8

Koren, G., S. Leeder, E. Harding, D. Jacques and S. M. MacLeod 1985. Optimization of gentamicin therapy in very low birth weight infants. Pediatr Pharmacol (New York);5(1):79–87

Krueger, M., M. S. Nauck, S. Sang, R. Hentschel, H. Wieland and R. Berner 2001. Cord blood levels of interleukin-6 and interleukin-8 for the immediate diagnosis of early-onset infection in premature infants. Biol Neonate;80(2):118–23

Kumhar, G. D., V. G. Ramachandran and P. Gupta 2002. Bacteriological analysis of blood culture isolates from neonates in a tertiary care hospital in India. J Health Popul Nutr;20(4):343–7

Kuo, K. C., Y. H. Shen and K. P. Hwang 2007. Clinical implications and risk factors of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae infection in children: a case-control retrospective study in a medical center in southern Taiwan. J Microbiol Immunol Infect;40(3):248–54

Kushner, I. and G. Feldmann 1978. Control of the acute phase response. Demonstration of C-reactive protein synthesis and secretion by hepatocytes during acute inflammation in the rabbit. J Exp Med;148(2):466–77

Kuster, H., M. Weiss, A. E. Willeitner, S. Detlefsen, I. Jeremias, J. Zbojan, R. Geiger, G. Lipowsky and G. Simbruner 1998. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. Lancet;352(9136):1271–7

Labenne, M., F. Michaut, B. Gouyon, C. Ferdynus and J. B. Gouyon 2007. A population-based observational study of restrictive guidelines for antibiotic therapy in early-onset neonatal infections. Pediatr Infect Dis J;26(7):593–9

Lahra, M. M., P. J. Beeby and H. E. Jeffery 2009. Intrauterine inflammation, neonatal sepsis, and chronic lung disease: a 13-year hospital cohort study. Pediatrics; 123(5):1314–9

Lake, D. E., J. S. Richman, M. P. Griffin and J. R. Moorman 2002. Sample entropy analysis of neonatal heart rate variability. Am J Physiol Regul Integr Comp Physiol;283(3):R789–97

Landers, S., P. L. Berry, G. L. Kearns, S. L. Kaplan and A. J. Rudolph 1984. Gentamicin disposition and effect on development of renal function in the very low birth weight infant. Dev Pharmacol Ther;7(5):285–302

Lange, S. and G. Freitag 2005. Choice of delta: requirements and reality – results of a systematic review. Biom J;47(1):12–27; discussion 99–107

Lannering, B., L. E. Larsson, J. Rojas and M. T. Stahlman 1983. Early onset group B streptococcal disease. Seven year experience and clinical scoring system. Acta Paediatr Scand;72(4):597–602

Lapillonne, A., E. Basson, G. Monneret, J. Bienvenu and B. L. Salle 1998. Lack of specificity of procalcitonin for sepsis diagnosis in premature infants. Lancet;351(9110):1211–2

Larson, E. L., J. P. Cimiotti, J. Haas, M. Nesin, A. Allen, P. Della-Latta and L. Saiman 2005. Gram-negative bacilli associated with catheter-associated and non-catheter-associated bloodstream infections and hand carriage by healthcare workers in neonatal intensive care units. Pediatr Crit Care Med;6(4):457–61

Laugel, V., P. Kuhn, J. Beladdale, L. Donato, B. Escande, D. Astruc and J. Messer 2003. Effects of antenatal antibiotics on the incidence and bacteriological profile of early-onset neonatal sepsis. A retrospective study over five years. Biol Neonate; 84(1):24–30

Lauterbach, R., D. Pawlik, R. Radziszewska, J. Wozniak and K. Rytlewski 2006. Plasma antithrombin III and protein C levels in early recognition of late-onset sepsis in newborns. Eur J Pediatr;165(9):585–9

Le, J., T. Nguyen, A. V. Law and J. Hodding 2006. Adverse drug reactions among children over a 10-year period. Pediatrics;118(2):555–62

Lee, P. I. 2006. Ampicillin and cefotaxime as a risk factor of neonatal death. Pediatrics;117(5):1857; author reply 1857–8

LeFlore, J. L., W. D. Engle and C. R. Rosenfeld 2000. Determinants of blood pressure in very low birth weight neonates: lack of effect of antenatal steroids. Early Hum Dev;59(1):37–50

Lehrnbecher, T., L. Schrod, D. Kraus, T. Roos, J. Martius and H. B. von Stockhausen 1995. Interleukin-6 and soluble interleukin-6 receptor in cord blood in the diagnosis of early onset sepsis in neonates. Acta Paediatr;84(7):806–8

Lehrnbecher, T., L. Schrod, P. Rutsch, T. Roos, J. Martius and H. B. von Stockhausen 1996. Immunologic parameters in cord blood indicating early-onset sepsis. Biol Neonate;70(4):206–12

Leibovitz, E., A. Iuster-Reicher, M. Amitai and B. Mogilner 1992. Systemic candidal infections associated with use of peripheral venous catheters in neonates: a 9-year experience. Clin Infect Dis;14(2):485–91

Lewis, R. 2000. An Introduction to Classification and Regression Tree (CART) Analysis. San Francisco, California

Lingvall, M., D. Reith and R. Broadbent 2005. The effect of sepsis upon gentamicin pharmacokinetics in neonates. Br J Clin Pharmacol;59(1):54–61

Litzow, J. M., C. J. Gill, J. B. Mantaring, M. P. Fox, W. B. MacLeod, M. Mendoza, S. Mendoza, R. Scobie, C. W. Huskins, D. A. Goldman and D. H. Hamer 2009. High frequency of multidrug-resistant gram-negative rods in 2 neonatal intensive care units in the Philippines. Infect Control Hosp Epidemiol;30(6):543–9

Liu, S., I. D. Rusen, K. S. Joseph, R. Liston, M. S. Kramer, S. W. Wen and R. Kinch 2004. Recent trends in caesarean delivery rates and indications for caesarean delivery in Canada. J Obstet Gynaecol Can;26(8):735–42

Lodise, T. P., P. S. McKinnon, L. Swiderski and M. J. Rybak 2003. Outcomes analysis of delayed antibiotic treatment for hospital-acquired Staphylococcus aureus bacteremia. Clin Infect Dis;36(11):1418–23

Lopez Sastre, J. B., G. D. Coto Cotallo and B. Fernandez Colomer 2000. Neonatal sepsis of vertical transmission: an epidemiological study from the "Grupo de Hospitales Castrillo". J Perinat Med;28(4):309–15

Lopez Sastre, J. B., B. Fernandez Colomer, G. D. Coto Cotallo and A. Ramos Aparicio 2005. Trends in the epidemiology of neonatal sepsis of vertical transmission in the era of group B streptococcal prevention. Acta Paediatr;94(4):451–7

Luce, W. A., T. M. Hoffman and J. A. Bauer 2007. Bench-to-bedside review: Developmental influences on the mechanisms, treatment and outcomes of cardiovascular dysfunction in neonatal versus adult sepsis. Crit Care;11(5):228

Lukacs, S. L., K. C. Schoendorf and A. Schuchat 2004. Trends in sepsis-related neonatal mortality in the United States, 1985–1998. Pediatr Infect Dis J;23(7):599–603

Lutsar, I. and I. Friedland 2010a. Clinical trials of anti-infective agents in paediatrics. K. Rose and J. van den Anker. Guide to Paeditric Drug Development and Clinical Research. Basel, Karger. pp 170–177

Lutsar, I., G. H. McCracken, Jr. and I. R. Friedland 1998. Antibiotic pharmacodynamics in cerebrospinal fluid. Clin Infect Dis;27(5):1117–27, quiz 1128–9

Lutsar, I. and T. Metsvaht 2010b. Understanding pharmacokinetic/pharmacodynamic in managing neonatal sepsis. Current Opinion in Infectious Disease;23(in press

Maayan-Metzger, A., A. Barzilai, N. Keller and J. Kuint 2009. Are the "good old" antibiotics still appropriate for early-onset neonatal sepsis? A 10 year survey. Isr Med Assoc J;11(3):138–42

Magwali, T. L., T. Chipato, F. Majoko, S. Rusakaniko and C. Mujaji 1999. Prophylactic augmentin in prelabor preterm rupture of the membranes. Int J Gynaecol Obstet;65(3):261–5

Maindonald, J. and J. Braun 2007. Chapter 11. Data Analysis and Graphics Using R – an Example-Based Approach. Cambridge University Press. pp 350–375

Mandar, R., L. i. K, A. Ehrenberg, I. Smidt, E. Raukas, V. Kask and M. Mikelsaar 2001. Amniotic fluid microflora in asymptomatic women at mid-gestation. Scand J Infect Dis;33(1):60–2

Mandar, R. and M. Mikelsaar 1996. Transmission of mother's microflora to the newborn at birth. Biol Neonate;69(1):30–5

Manroe, B. L., C. R. Rosenfeld, A. G. Weinberg and R. Browne 1977. The differential leukocyte count in the assessment and outcome of early-onset neonatal group B streptococcal disease. J Pediatr;91(4):632–7

Manroe, B. L., A. G. Weinberg, C. R. Rosenfeld and R. Browne 1979. The neonatal blood count in health and disease. I. Reference values for neutrophilic cells. J Pediatr;95(1):89–98

Manzoni, P., D. Farina, M. Leonessa, E. A. d'Oulx, P. Galletto, M. Mostert, R. Miniero and G. Gomirato 2006. Risk factors for progression to invasive fungal infection in preterm neonates with fungal colonization. Pediatrics;118(6):2359–64

Marks, S., M. I. Marks, C. Dupont and S. Hammerberg 1978. Evaluation of three antibiotic programs in newborn infants. Can Med Assoc J;118(6):659–62

Mathers, N. J. and F. Pohlandt 1987. Diagnostic audit of C-reactive protein in neonatal infection. Eur J Pediatr;146(2):147–51

Mautone, A., P. Giordano, O. Montagna, M. Quercia, M. Altomare and D. De Mattia 1997. Coagulation and fibrinolytic systems in the ill preterm newborn. Acta Paediatr;86(10):1100–4

Mayor-Lynn, K., V. H. Gonzalez-Quintero, M. J. O'Sullivan, A. I. Hartstein, S. Roger and M. Tamayo 2005. Comparison of early-onset neonatal sepsis caused by Escherichia coli and group B Streptococcus. Am J Obstet Gynecol;192(5):1437–9

McCracken, G. H., Jr. and J. F. Bishara 1990. Clinical pharmacology of antimicrobial agents. J. O. Klein and J. S. Remington. Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, W. B. Saunders. pp

McCracken, G. H., Jr., C. Ginsberg, D. F. Chrane, M. L. Thomas and L. J. Horton 1973. Clinical pharmacology of penicillin in newborn infants. J Pediatr;82(4):692–8

McCracken, G. H., Jr., S. G. Mize and N. Threlkeld 1980. Intraventricular gentamicin therapy in gram-negative bacillary meningitis of infancy. Report of the Second Neonatal Meningitis Cooperative Study Group. Lancet;1(8172):787–91

McGuire, W., L. Clerihew and P. W. Fowlie 2004. Infection in the preterm infant. Bmj;329(7477):1277–80

McIntosh, E. D. and H. E. Jeffery 1992. Clinical application of urine antigen detection in early onset group B streptococcal disease. Arch Dis Child;67(10 Spec No):1198–200

McKiernan, C. A. and S. A. Lieberman 2005. Circulatory shock in children: an overview. Pediatr Rev;26(12):451–60

McKinnon, P. S., J. A. Paladino and J. J. Schentag 2008. Evaluation of area under the inhibitory curve (AUIC) and time above the minimum inhibitory concentration (T>MIC) as predictors of outcome for cefepime and ceftazidime in serious bacterial infections. Int J Antimicrob Agents;31(4):345–51

McNamara, P. J. and J. Alcorn 2002. Protein binding predictions in infants. AAPS PharmSci;4(1):E4

Mehta, A. 1994. Prevention and management of neonatal hypoglycaemia. Arch Dis Child Fetal Neonatal Ed;70(1):F54–9; discussion F59–60

Metsvaht, T., M. L. Ilmoja, U. Parm, L. Maipuu, M. Merila and I. Lutsar Comparison of ampicillin plus gentamicin vs. penicillin plus gentamicin in empiric treatment of neonates at risk of early onset sepsis. Acta Paediatr;

Metsvaht, T., K. Oselin, M. L. Ilmoja, K. Anier and I. Lutsar 2007. Pharmacokinetics of penicillin g in very-low-birth-weight neonates. Antimicrob Agents Chemother; 51(6):1995–2000

Metsvaht , T., H. Padari, K. Lang and I. Lutsar 2006. Penicillin + gentamicin versus ampicillin + gentamicin in the empiric treatment of early neonatal sepsis. 16th European Congress of Clinical Microbiology and Infectious Diseases, abstract number: r2260

Clinical Microbiology and Infection;12(Supplement 4, Part 6):129

Miall-Allen, V. M., A. G. Whitelaw and J. H. Darrell 1988. Ticarcillin plus clavulanic acid (Timentin) compared with standard antibiotic regimes in the treatment of early and late neonatal infections. Br J Clin Pract;42(7):273–9

Micek, S. T., S. Ward, V. J. Fraser and M. H. Kollef 2004. A randomized controlled trial of an antibiotic discontinuation policy for clinically suspected ventilator-associated pneumonia. Chest;125(5):1791–9

Millar, M., A. Philpott, M. Wilks, A. Whiley, S. Warwick, E. Hennessy, P. Coen, S. Kempley, F. Stacey and K. Costeloe 2008. Colonization and persistence of antibiotic-resistant Enterobacteriaceae strains in infants nursed in two neonatal intensive care units in East London, United Kingdom. J Clin Microbiol;46(2):560–7

Miranda, J. C., M. M. Schimmel, L. S. James, W. Spinelli and T. S. Rosen 1985. Gentamicin kinetics in the neonate. Pediatr Pharmacol (New York);5(1):57–61

Mishra, U. K., S. E. Jacobs, L. W. Doyle and S. M. Garland 2006. Newer approaches to the diagnosis of early onset neonatal sepsis. Arch Dis Child Fetal Neonatal Ed;91(3):F208–12

Mitt, P., V. Adamson, K. Loivukene, K. Lang, K. Telling, K. Paro, A. Room, P. Naaber and M. Maimets 2009. Epidemiology of nosocomial bloodstream infections in Estonia. J Hosp Infect;71(4):365–70

Modi, N., C. J. Dore, A. Saraswatula, M. Richards, K. B. Bamford, R. Coello and A. Holmes 2009. A case definition for national and international neonatal bloodstream infection surveillance. Arch Dis Child Fetal Neonatal Ed;94(1):F8–12

Moore, M. R., S. J. Schrag and A. Schuchat 2003. Effects of intrapartum antimicrobial prophylaxis for prevention of group-B-streptococcal disease on the incidence and ecology of early-onset neonatal sepsis. Lancet Infect Dis;3(4):201–13

Morantz, C. A. 2003. CDC updates guidelines for prevention of perinatal group B streptococcal disease. Am Fam Physician;67(5):1121, 1125–6, 1129–30

Morehead, C. D., S. Shelton, H. Kusmiesz and J. D. Nelson 1972. Pharmacokinetics of carbenicillin in neonates of normal and low birth weight. Antimicrob Agents Chemother;2(4):267–71

Morris, D. L., C. S. Ubhi, C. S. Robertson and K. W. Brammer 1986. Biliary pharmacokinetics of sulbactam plus ampicillin in humans. Rev Infect Dis;8 Suppl 5(S589–92

Motohiro, T., K. Oda, M. Aramaki, K. Tanaka, A. Kawakami, Y. Shimada, T. Koga, S. Tomita, Y. Sakata, T. Fujimoto and et al. 1988a. [Pharmacokinetic and clinical evaluations of ceftriaxone in neonates and premature infants]. Jpn J Antibiot; 41(3):276–305

Motohiro, T., Y. Sakata, K. Tominaga, K. Oda, M. Aramaki, K. Tanaka, A. Kawakami, Y. Shimada, T. Koga, S. Tomita and et al. 1988b. [Pharmacokinetic, bacteriological and clinical studies of ceftizoxime in neonates and low birth weight infants]. Jpn J Antibiot;41(8):1116–28

Mtitimila, E. I. and R. W. Cooke 2004. Antibiotic regimens for suspected early neonatal sepsis. Cochrane Database Syst Rev;4):CD004495

Mulhall, A., J. de Louvois and R. Hurley 1983. Chloramphenicol toxicity in neonates: its incidence and prevention. Br Med J (Clin Res Ed);287(6403):1424–7

Muller, A. E., J. DeJongh, Y. Bult, W. H. Goessens, J. W. Mouton, M. Danhof and J. N. van den Anker 2007. Pharmacokinetics of penicillin G in infants with a gestational age of less than 32 weeks. Antimicrob Agents Chemother;51(10):3720–5

Murakami, T., S. Karino, N. Nakamura and N. Yata 1984. Influence of administration routes of sodium ampicillin on the cecal flora in rats: role of biliary excretion. Chem Pharm Bull (Tokyo);32(10):4175–8

Murray, D. M., S. P. Varnell and J. L. Blitstein 2004. Design and analysis of group-randomized trials: a review of recent methodological developments. Am J Public Health;94(3):423–32

Nagano, N., Y. Nagano, K. Kimura, K. Tamai, H. Yanagisawa and Y. Arakawa 2008. Genetic heterogeneity in pbp genes among clinically isolated group B Streptococci with reduced penicillin susceptibility. Antimicrob Agents Chemother;52(12):4258–67

Nahata, M. C., D. A. Powell, D. E. Durrell and M. A. Miller 1986. Tobramycin pharmacokinetics in very low birth weight infants. Br J Clin Pharmacol;21(3):325–7

Nakae, S., M. Yamada, T. Ito, Y. Chiba, E. Sasaki, M. Sakamoto, K. Tada, T. Yamada and S. Mori 1988. Gentamicin dosing and pharmacokinetics in low birth weight infants. Tohoku J Exp Med;155(3):213–23

Nalin, D. R. and C. A. Jacobsen 1987. Imipenem/cilastatin therapy for serious infections in neonates and infants. Scand J Infect Dis Suppl;52(46–55

Nambiar, S. and N. Singh 2002. Change in epidemiology of health care-associated infections in a neonatal intensive care unit. Pediatr Infect Dis J;21(9):839–42

Nau, H. 1987. Clinical pharmacokinetics in pregnancy and perinatology. II. Penicillins. Dev Pharmacol Ther;10(3):174–98

Nelson, J. D. and G. H. McCracken, Jr. 1973. Clinical pharmacology of carbenicillin and gentamicin in the neonate and comparative efficacy with ampicillin and gentamicin. Pediatrics;52(6):801–12

Neubert, A., K. Lukas, T. Leis, H. Dormann, K. Brune and W. Rascher 2009. Drug utilisation on a preterm and neonatal intensive care unit in Germany: a prospective, cohort-based analysis. Eur J Clin Pharmacol;

Ng, P. C. 2004. Diagnostic markers of infection in neonates. Arch Dis Child Fetal Neonatal Ed;89(3):F229–35

Ng, P. C., S. H. Cheng, K. M. Chui, T. F. Fok, M. Y. Wong, W. Wong, R. P. Wong and K. L. Cheung 1997. 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;77(3):F221–7

Nobre, V., S. Harbarth, J. D. Graf, P. Rohner and J. Pugin 2008. Use of procalcitonin to shorten antibiotic treatment duration in septic patients: a randomized trial. Am J Respir Crit Care Med;177(5):498–505

Nseir, S., X. Deplanque, C. Di Pompeo, M. Diarra, M. Roussel-Delvallez and A. Durocher 2008. Risk factors for relapse of ventilator-associated pneumonia related to nonfermenting Gram negative bacilli: a case-control study. J Infect;56(5):319–25

Nupponen, I., S. Andersson, A. L. Jarvenpaa, H. Kautiainen and H. Repo 2001. Neutrophil CD11b expression and circulating interleukin-8 as diagnostic markers for early-onset neonatal sepsis. Pediatrics;108(1):E12

Nyhan, W. L. and M. D. Fousek 1958. Septicemia of the newborn. Pediatrics; 22(2):268–78

Odio, C. M., M. A. Umana, A. Saenz, J. L. Salas and G. H. McCracken, Jr. 1987. Comparative efficacy of ceftazidime vs. carbenicillin and amikacin for treatment of neonatal septicemia. Pediatr Infect Dis J;6(4):371–7

Ohlin, A., A. Backman, M. Bjorkqvist, P. Molling, M. Jurstrand and J. Schollin 2008. Real-time PCR of the 16S-rRNA gene in the diagnosis of neonatal bacteraemia. Acta Paediatr;97(10):1376–80

Ojukwu, J. U., L. E. Abonyi, J. Ugwu and I. K. Orji 2006. Neonatal septicemia in high risk babies in South-Eastern Nigeria. J Perinat Med;34(2):166–72

Oral, R., M. Akisu, N. Kultursay, F. Vardar and N. Tansug 1998. Neonatal Klebsiella pneumonia sepsis and imipenem/cilastatin. Indian J Pediatr;65(1):121–9

Osrin, D., S. Vergnano and A. Costello 2004. Serious bacterial infections in newborn infants in developing countries. Curr Opin Infect Dis;17(3):217–24

Ottolini, M. C., K. Lundgren, L. J. Mirkinson, S. Cason and M. G. Ottolini 2003. Utility of complete blood count and blood culture screening to diagnose neonatal sepsis in the asymptomatic at risk newborn. Pediatr Infect Dis J;22(5):430–4

Paap, C. M. and M. C. Nahata 1990. Clinical pharmacokinetics of antibacterial drugs in neonates. Clin Pharmacokinet;19(4):280–318

Pacifici, G. M. 2009. Clinical pharmacokinetics of aminoglycosides in the neonate: a review. Eur J Clin Pharmacol;65(4):419–27

Pacifici, G. M., J. Labatia, H. Mulla and I. Choonara 2009. Clinical pharmacokinetics of penicillins in the neonate: a review of the literature. Eur J Clin Pharmacol;65(2): 191–8

Panda, B., I. Iruretagoyena, R. Stiller and A. Panda 2009. Antibiotic resistance and penicillin tolerance in ano-vaginal group B streptococci. J Matern Fetal Neonatal Med;22(2):111–4

Papile, L. A., J. Burstein, R. Burstein and H. Koffler 1978. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr;92(4):529–34

Park, K. H., B. H. Yoon, S. S. Shim, J. K. Jun and H. C. Syn 2004. Amniotic fluid tumor necrosis factor-alpha is a marker for the prediction of early-onset neonatal sepsis in preterm labor. Gynecol Obstet Invest;58(2):84–90

Patel, D. M., P. G. Rhodes, M. H. LeBlanc, G. R. Graves, C. Glick and J. Morrison 1999. Role of postnatal penicillin prophylaxis in prevention of neonatal group B streptococcus infection. Acta Paediatr;88(8):874–9

Perlman, S. E., L. Saiman and E. L. Larson 2007. Risk factors for late-onset health care-associated bloodstream infections in patients in neonatal intensive care units. Am J Infect Control;35(3):177–82

Persson, E., S. Berg, H. Bergseng, K. Bergh, R. Valso-Lyng and B. Trollfors 2008. Antimicrobial susceptibility of invasive group B streptococcal isolates from south-west Sweden 1988–2001. Scand J Infect Dis;40(4):308–13

Persson, E., B. Trollfors, L. L. Brandberg and I. Tessin 2002. Septicaemia and meningitis in neonates and during early infancy in the Goteborg area of Sweden. Acta Paediatr;91(10):1087–92

Peters, R. P., J. W. Twisk, M. A. van Agtmael and A. B. Groeneveld 2006. The role of procalcitonin in a decision tree for prediction of bloodstream infection in febrile patients. Clin Microbiol Infect;12(12):1207–13

Philip, A. G. 1982. Detection of neonatal sepsis of late onset. Jama;247(4):489–92

Philip, A. G. and J. R. Hewitt 1980. Early diagnosis of neonatal sepsis. Pediatrics; 65(5):1036–41

Philip, A. G. and P. C. Mills 2000. Use of C-reactive protein in minimizing antibiotic exposure: experience with infants initially admitted to a well-baby nursery. Pediatrics;106(1):E4

Pillgram-Larsen, J., F. Wisloff, J. J. Jorgensen, H. C. Godal and G. Semb 1985. Effect of high-dose ampicillin and cloxacillin on bleeding time and bleeding in open-heart surgery. Scand J Thorac Cardiovasc Surg;19(1):45–8

Pinget, M., J. M. Brogard, J. Dauchel and J. Lavillaureix 1976. Biliary excretion of ampicillin, metampicillin and carbenicillin. J Antimicrob Chemother;2(2):195–201

Pinto, N. M., E. I. Soskolne, M. D. Pearlman and R. G. Faix 2003. Neonatal early-onset group B streptococcal disease in the era of intrapartum chemoprophylaxis: residual problems. J Perinatol;23(4):265–71

Pocock, S. 1983. Clinical Trials: A Practical Approach.

Pons, G., P. d'Athis, E. Rey, D. de Lauture, M. O. Richard, J. Badoual and G. Olive 1988. Gentamicin monitoring in neonates. Ther Drug Monit;10(4):421–7

Poschl, J. M., G. Hellstern, N. Dertlioglou, P. Ruef, J. Meyburg, B. Beedgen and O. Linderkamp 2003. Six day antimicrobial therapy for early-onset group B streptococcal infection in near-term and term neonates. Scand J Infect Dis;35(5): 302–5

Potter, J. L., A. G. Weinberg and R. West 1971. Ampicillinuria and ampicillin crystalluria. Pediatrics;48(4):636–9

Pryse-Davies, J. and R. Hurley 1979. Infections and perinatal mortality. J Antimicrob Chemother;5 Suppl A(59–70

Pyati, S. P., R. S. Pildes, N. M. Jacobs, R. S. Ramamurthy, T. F. Yeh, D. S. Raval, L. D. Lilien, P. Amma and W. I. Metzger 1983. Penicillin in infants weighing two kilograms or less with early-onset Group B streptococcal disease. N Engl J Med;308(23):1383–9

Raichle, M. E., H. Kutt, S. Louis and F. McDowell 1971. Neurotoxicity of intravenously administered penicillin G. Arch Neurol;25(3):232–9

Rao, S., Z. Pavlova, M. H. Incerpi and R. Ramanathan 2001. Meconium-stained amniotic fluid and neonatal morbidity in near-term and term deliveries with acute histologic chorioamnionitis and/or funisitis. J Perinatol;21(8):537–40

Rao, S. C., M. Ahmed and R. Hagan 2006. One dose per day compared to multiple doses per day of gentamicin for treatment of suspected or proven sepsis in neonates. Cochrane Database Syst Rev;1):CD005091

Reibnegger, G., G. Weiss, G. Werner-Felmayer, G. Judmaier and H. Wachter 1991. Neural networks as a tool for utilizing laboratory information: comparison with linear discriminant analysis and with classification and regression trees. Proc Natl Acad Sci U S A;88(24):11426–30

Reier-Nilsen, T., T. Farstad, B. Nakstad, V. Lauvrak and M. Steinbakk 2009. Comparison of broad range 16S rDNA PCR and conventional blood culture for diagnosis of sepsis in the newborn: a case control study. BMC Pediatr;9(5

Reyna, J. and F. Ortiz 2008. Therapeutic failure of the ampicillin plus aminoglycoside scheme in the treatment of early neonatal sepsis. Arch Med Res;39(5):546–7

Roberts, P. L. 1974. Letter: High-dose penicillin and bleeding. Ann Intern Med;81(2): 267–8

Rodwell, R. L., A. L. Leslie and D. I. Tudehope 1988. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr;112(5):761–7

Ronnestad, A., T. G. Abrahamsen, P. Gaustad and P. H. Finne 1999. Antibiotic susceptibility of blood culture isolates after nearly two decades with netilmicin and ampicillin in neonatal septicaemia. Apmis;107(2):257–62

Ronnestad, A., T. G. Abrahamsen, S. Medbo, H. Reigstad, K. Lossius, P. I. Kaaresen, I. E. Engelund, L. M. Irgens and T. Markestad 2005. Septicemia in the first week of life in a Norwegian national cohort of extremely premature infants. Pediatrics; 115(3):e262–8

Ruess, M., U. Muller, A. Sander and R. Berner 2000. Antimicrobial susceptibility patterns of Streptococcus agalactiae in a German university hospital. Scand J Infect Dis;32(6):623–6

Ruimy, R., M. Dos-Santos, L. Raskine, F. Bert, R. Masson, S. Elbaz, C. Bonnal, J. C. Lucet, A. Lefort, B. Fantin, M. Wolff, M. Hornstein and A. Andremont 2008. Accuracy and potential usefulness of triplex real-time PCR for improving antibiotic treatment of patients with blood cultures showing clustered gram-positive cocci on direct smears. J Clin Microbiol;46(6):2045–51

Saiman, L., E. Ludington, J. D. Dawson, J. E. Patterson, S. Rangel-Frausto, R. T. Wiblin, H. M. Blumberg, M. Pfaller, M. Rinaldi, J. E. Edwards, R. P. Wenzel and W. Jarvis 2001. Risk factors for Candida species colonization of neonatal intensive care unit patients. Pediatr Infect Dis J;20(12):1119–24

Salmi, T. T. 1973. Haptoglobin levels in the plasma of newborn infants with special reference to infections. Acta Paediatr Scand Suppl;241(1–55

Sanchez, P. J., J. D. Siegel, N. B. Cushion and N. Threlkeld 1990. Significance of a positive urine group B streptococcal latex agglutination test in neonates. J Pediatr;116(4):601–6

Sann, L., F. Bienvenu, J. Bienvenu, J. Bourgeois and M. Bethenod 1984. Evolution of serum prealbumin, C-reactive protein, and orosomucoid in neonates with bacterial infection. J Pediatr;105(6):977–81

Santana, C., M. C. Guindeo, G. Gonzalez, F. Garcia-Munoz, P. Saavedra and E. Domenech 2001. Cord blood levels of cytokines as predictors of early neonatal sepsis. Acta Paediatr;90(10):1176–81

Sarkar, S., I. Bhagat, J. D. DeCristofaro, T. E. Wiswell and A. R. Spitzer 2006. A study of the role of multiple site blood cultures in the evaluation of neonatal sepsis. J Perinatol;26(1):18–22

Sarkar, S., I. Bhagat, T. E. Wiswell and A. R. Spitzer 2007. Role of multiple site blood cultures to document the clearance of bacteremia in neonates. J Perinatol;27(2):101–2

Scaglione, F. and L. Paraboni 2008. Pharmacokinetics/pharmacodynamics of antibacterials in the Intensive Care Unit: setting appropriate dosing regimens. Int J Antimicrob Agents;32(4):294–301
Schelonka, R. L. 2007. Detente in the war against bugs. Pediatr Infect Dis J;26(7):600–1
Schelonka, R. L., M. K. Chai, B. A. Yoder, D. Hensley, R. M. Brockett and D. P. Ascher 1996. Volume of blood required to detect common neonatal pathogens. J Pediatr;129(2):275–8
Schelonka, R. L., B. A. Yoder, R. B. Hall, T. M. Trippett, D. S. Louder, J. R. Hickman and C. G. Guerra 1995. Differentiation of segmented and band neutrophils during the early newborn period. J Pediatr;127(2):298–300
Schiano, M. A., J. C. Hauth and L. C. Gilstrap, 3rd 1984. Second-stage fetal tachycardia and neonatal infection. Am J Obstet Gynecol;148(6):779–81
Schouten-Van Meeteren, N. Y., A. Rietveld, A. J. Moolenaar and F. Van Bel 1992. Influence of perinatal conditions on C-reactive protein production. J Pediatr;120(4 Pt 1):621–4
Schrag, S., R. Gorwitz, K. Fultz-Butts and A. Schuchat 2002. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recomm Rep;51(RR-11):1–22
Schrag, S. J., J. L. Hadler, K. E. Arnold, P. Martell-Cleary, A. Reingold and A. Schuchat 2006a. Risk factors for invasive, early-onset Escherichia coli infections in the era of widespread intrapartum antibiotic use. Pediatrics;118(2):570–6
Schrag, S. J. and B. J. Stoll 2006b. Early-onset neonatal sepsis in the era of widespread intrapartum chemoprophylaxis. Pediatr Infect Dis J;25(10):939–40
Schuchat, A., S. S. Zywicki, M. J. Dinsmoor, B. Mercer, J. Romaguera, M. J. O'Sullivan, D. Patel, M. T. Peters, B. Stoll and O. S. Levine 2000. Risk factors and opportunities for prevention of early-onset neonatal sepsis: a multicenter case-control study. Pediatrics;105(1 Pt 1):21–6
Schultz, C., P. Temming, P. Bucsky, W. Gopel, T. Strunk and C. Hartel 2004. Immature anti-inflammatory response in neonates. Clin Exp Immunol;135(1):130–6
Scott, P. H. 1989. Plasma lactoferrin levels in newborn preterm infants: effect of infection. Ann Clin Biochem;26 (Pt 5)(412–5
Sehgal, R., R. Gaind, H. Chellani and P. Agarwal 2007. Extended-spectrum beta lactamase-producing gram-negative bacteria: clinical profile and outcome in a neonatal intensive care unit. Ann Trop Paediatr;27(1):45–54
Sepp, E., K. Julge, M. Vasar, P. Naaber, B. Bjorksten and M. Mikelsaar 1997a. Intestinal microflora of Estonian and Swedish infants. Acta Paediatr;86(9):956–61
Sepp, E., S. Kõljalg, P. Naaber, K. Truusalu, L. Štšepetova, T. Metsvaht, L. Sau, M. Jürna, H. Mägi, M. Allik and M. Mikelsaar 2003. Staphylococcus epidermidis ja Klebsiella pneumoniae – võimalikud hospitaalinfektsioonide tekitajad Eesti lasteintensiivravi osakondades. Eesti Arst;4(239–248
Sepp, E. and T. Metsvaht 1997b. Vastsündinute bakterieemiate etioloogiline struktuur ja laboratoorne diagnoosimine Tartu Lastekliiniku intensiivravi osakonna andmeil. Eesti Arst;6(513–519
Shah, G. S., S. Budhathoki, B. K. Das and R. N. Mandal 2006. Risk factors in early neonatal sepsis. Kathmandu Univ Med J (KUMJ);4(2):187–91
Shani, L., D. Weitzman, R. Melamed, E. Zmora and K. Marks 2008. Risk factors for early sepsis in very low birth weight neonates with respiratory distress syndrome. Acta Paediatr;97(1):12–5

Sheffield, M. J., D. K. Lambert, E. Henry and R. D. Christensen 2009. Effect of ampicillin on the bleeding time of neonatal intensive care unit patients. J Perinatol;
Sherwin, C. M., R. S. Broadbent, N. J. Medlicott and D. M. Reith 2008. Individualising netilmicin dosing in neonates. Eur J Clin Pharmacol;64(12):1201–8
Sherwin, C. M., E. Kostan, R. S. Broadbent, N. J. Medlicott and D. M. Reith 2009a. Evaluation of the effect of intravenous volume expanders upon the volume of distribution of gentamicin in septic neonates. Biopharm Drug Dispos;30(5):276–80
Sherwin, C. M., S. Svahn, A. Van der Linden, R. S. Broadbent, N. J. Medlicott and D. M. Reith 2009b. Individualised dosing of amikacin in neonates: a pharmacokinetic/ pharmacodynamic analysis. Eur J Clin Pharmacol;65(7):705–13
Siegel, J. D., G. H. McCracken, Jr., N. Threlkeld, B. Milvenan and C. R. Rosenfeld 1980. Single-dose penicillin prophylaxis against neonatal group B streptococcal infections. A controlled trial in 18,738 newborn infants. N Engl J Med;303(14):769–75
Silveira, R. C., R. S. Procianoy, J. C. Dill and C. S. da Costa 2008. Periventricular leukomalacia in very low birth weight preterm neonates with high risk for neonatal sepsis. J Pediatr (Rio J);84(3):211–6
Singhal, K. K. and E. F. La Gamma 1996. Management of 168 neonates weighing more than 2000 g receiving intrapartum chemoprophylaxis for chorioamnionitis. Evaluation of an early discharge strategy. Arch Pediatr Adolesc Med;150(2):158–63
Sjoberg, I., S. Hakansson, A. Eriksson, J. Schollin, B. Stjernstedt and I. Tessin 1990. Incidence of early onset group B streptococcal septicemia in Sweden 1973 to 1985. Eur J Clin Microbiol Infect Dis;9(4):276–8
Sklavunu-Tsurutsoglu, S., M. Gatzola-Karaveli, K. Hatziioannidis and G. Tsurutsoglu 1991. Efficacy of aztreonam in the treatment of neonatal sepsis. Rev Infect Dis;13 Suppl 7(S591–3
Smulian, J. C., V. Bhandari, W. A. Campbell, J. F. Rodis and A. M. Vintzileos 1997. Value of umbilical artery and vein levels of interleukin-6 and soluble intracellular adhesion molecule-1 as predictors of neonatal hematologic indices and suspected early sepsis. J Matern Fetal Med;6(5):254–9
Snelling, S., C. A. Hart and R. W. Cooke 1983. Ceftazidime or gentamicin plus benzylpenicillin in neonates less than forty-eight hours old. J Antimicrob Chemother;12 Suppl A(353–6
Society, C. P. 2007. Management of the infant at increased risk for sepsis. Canadian Paediatric Society guideline. Paediatr Child Health;12(10):893–898
Sonntag, J., B. Prankel and S. Waltz 1996. Serum creatinine concentration, urinary creatinine excretion and creatinine clearance during the first 9 weeks in preterm infants with a birth weight below 1500 g. Eur J Pediatr;155(9):815–9
Spada, S., A. Cuccu, M. Mussap, M. Testa, M. Puddu, C. Pisu, P. Burrai and V. Fanos 2009. Reliability of procalcitonin in neonatology. Experience in 59 preterm newborns. J Matern Fetal Neonatal Med;22 Suppl 3(96–101
Speer, C., A. Bruns and M. Gahr 1983. Sequential determination of CRP, alpha 1-antitrypsin and haptoglobin in neonatal septicaemia. Acta Paediatr Scand;72(5):679–83
Sperling, R. S., R. S. Ramamurthy and R. S. Gibbs 1987. A comparison of intrapartum versus immediate postpartum treatment of intra-amniotic infection. Obstet Gynecol; 70(6):861–5
Spitzer, A. R., S. Kirkby and M. Kornhauser 2005. Practice variation in suspected neonatal sepsis: a costly problem in neonatal intensive care. J Perinatol;25(4):265–9

Szefler, S. J., R. J. Wynn, D. F. Clarke, S. Buckwald, D. Shen and J. J. Schentag 1980. Relationship of gentamicin serum concentrations to gestational age in preterm and term neonates. J Pediatr;97(2):312–5

Stickland, M. D., C. M. Kirkpatrick, E. J. Begg, S. B. Duffull, S. J. Oddie and B. A. Darlow 2001. An extended interval dosing method for gentamicin in neonates. J Antimicrob Chemother;48(6):887–93

Stocker, M., M. Fontana, S. El Helou, K. Wegscheider and T. M. Berger 2009. Use of Procalcitonin-Guided Decision-Making to Shorten Antibiotic Therapy in Suspected Neonatal Early-Onset Sepsis: Prospective Randomized Intervention Trial. Neonatology;97(2):165–174

Stoll, B. J., T. Gordon, S. B. Korones, S. Shankaran, J. E. Tyson, C. R. Bauer, A. A. Fanaroff, J. A. Lemons, E. F. Donovan, W. Oh, D. K. Stevenson, R. A. Ehrenkranz, L. A. Papile, J. Verter and L. L. Wright 1996. Early-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. J Pediatr;129(1):72–80

Stoll, B. J., N. Hansen, A. A. Fanaroff, L. L. Wright, W. A. Carlo, R. A. Ehrenkranz, J. A. Lemons, E. F. Donovan, A. R. Stark, J. E. Tyson, W. Oh, C. R. Bauer, S. B. Korones, S. Shankaran, A. R. Laptook, D. K. Stevenson, L. A. Papile and W. K. Poole 2002a. Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. N Engl J Med;347(4):240–7

Stoll, B. J., N. Hansen, A. A. Fanaroff, L. L. Wright, W. A. Carlo, R. A. Ehrenkranz, J. A. Lemons, E. F. Donovan, A. R. Stark, J. E. Tyson, W. Oh, C. R. Bauer, S. B. Korones, S. Shankaran, A. R. Laptook, D. K. Stevenson, L. A. Papile and W. K. Poole 2002b. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics;110(2 Pt 1):285–91

Stoll, B. J., N. I. Hansen, I. Adams-Chapman, A. A. Fanaroff, S. R. Hintz, B. Vohr and R. D. Higgins 2004. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. Jama;292(19):2357–65

Stoll, B. J., N. I. Hansen, R. D. Higgins, A. A. Fanaroff, S. Duara, R. Goldberg, A. Laptook, M. Walsh, W. Oh and E. Hale 2005. 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;24(7):635–9

Stoll, B. J., R. C. Holman and A. Schuchat 1998. Decline in sepsis-associated neonatal and infant deaths in the United States, 1979 through 1994. Pediatrics;102(2):e18

Struelens, M. J. 2009. Detection of microbial DNAemia: does it matter for sepsis management? Intensive Care Med;

Strunk, T., P. Temming, U. Gembruch, I. Reiss, P. Bucsky and C. Schultz 2004. Differential maturation of the innate immune response in human fetuses. Pediatr Res;56(2):219–26

Suri, M., S. Thirupuram and V. K. Sharma 1991. Diagnostic and prognostic utility of C-reactive protein, alpha-1-antitrypsin and alpha-2-macroglobulin in neonatal sepsis: a comparative account. Indian Pediatr;28(10):1159–64

Sutkin, G., M. A. Krohn, R. P. Heine and R. L. Sweet 2005. Antibiotic prophylaxis and non-group B streptococcal neonatal sepsis. Obstet Gynecol;105(3):581–6

Zaidi, A., S. Sundar and G. Darmstadt 2006. Antibiotic therapy for serious bacterial infections in young infants: randomized community-based trial. San Francisco, CA

Zaidi, A. K., D. Thaver, S. A. Ali and T. A. Khan 2009. Pathogens associated with sepsis in newborns and young infants in developing countries. Pediatr Infect Dis J;28(1 Suppl):S10–8

Zeitoun, A. A., S. S. Gad, F. M. Attia, A. S. Abu Maziad and E. F. Bell Evaluation of neutrophilic CD64, interleukin 10 and procalcitonin as diagnostic markers of early- and late-onset neonatal sepsis. Scand J Infect Dis;

Zygmunt, W. A., H. P. Browder and P. A. Tavormina 1968. Susceptibility of coagulase-negative staphylococci to lysostaphin and other antibiotics. Appl Microbiol; 16(8):1168–73

Tagare, A., S. Kadam, U. Vaidya and A. Pandit 2009. Routine antibiotic use in preterm neonates: a randomised controlled trial. J Hosp Infect;

Tang, H., T. Huang, J. Jing, H. Shen and W. Cui 2009. Effect of procalcitonin-guided treatment in patients with infections: a systematic review and meta-analysis. Infection;37(6):497–507

Tessin, I., K. Thiringer, B. Trollfors and J. E. Brorson 1988. Comparison of serum concentrations of ceftazidime and tobramycin in newborn infants. Eur J Pediatr; 147(4):405–7

Tessin, I., B. Trollfors and K. Thiringer 1990. Incidence and etiology of neonatal septicaemia and meningitis in western Sweden 1975–1986. Acta Paediatr Scand; 79(11):1023–30

Tessin, I., B. Trollfors, K. Thiringer and P. Larsson 1991. Ampicillin-aminoglycoside combinations as initial treatment for neonatal septicaemia or meningitis. A retrospective evaluation of 12 years' experience. Acta Paediatr Scand;80(10):911–6

Tessin, I., B. Trollfors, K. Thiringer, Z. Thorn and P. Larsson 1989. Concentrations of ceftazidime, tobramycin and ampicillin in the cerebrospinal fluid of newborn infants. Eur J Pediatr;148(7):679–81

Thaver, D., S. A. Ali and A. K. Zaidi 2009. Antimicrobial resistance among neonatal pathogens in developing countries. Pediatr Infect Dis J;28(1 Suppl):S19–21

Thomas, N. J., J. A. Carcillo, L. A. Doughty, H. Sasser and R. P. Heine 2002. Plasma concentrations of defensins and lactoferrin in children with severe sepsis. Pediatr Infect Dis J;21(1):34–8

Thomson, A. H., S. Way, S. M. Bryson, E. M. McGovern, A. W. Kelman and B. Whiting 1988. Population pharmacokinetics of gentamicin in neonates. Dev Pharmacol Ther;11(3):173–9

Thureen, P. J., P. D. Reiter, A. Gresores, N. M. Stolpman, K. Kawato and D. M. Hall 1999. Once- versus twice-daily gentamicin dosing in neonates >/=34 Weeks' gestation: cost-effectiveness analyses. Pediatrics;103(3):594–8

Tiskumara, R., S. H. Fakharee, C. Q. Liu, P. Nuntnarumit, K. M. Lui, M. Hammoud, J. K. Lee, C. B. Chow, A. Shenoi, R. Halliday and D. Isaacs 2009. Neonatal infections in Asia. Arch Dis Child Fetal Neonatal Ed;94(2):F144–8

Tollner, U. and F. Pohlandt 1976. Septicemia in the newborn due to gram-negative bacilli. Risk factors, clinical symptoms, and hematologic changes. Eur J Pediatr;123(4):243–54

Toltzis, P. 2003. Colonization with antibiotic-resistant Gram-negative bacilli in the neonatal intensive care unit. Minerva Pediatr;55(5):385–93

Trijbels-Smeulders, M., G. A. de Jonge, P. C. Pasker-de Jong, L. J. Gerards, A. H. Adriaanse, R. A. van Lingen and L. A. Kollee 2007. Epidemiology of neonatal group B streptococcal disease in the Netherlands before and after introduction of guidelines for prevention. Arch Dis Child Fetal Neonatal Ed;92(4):F271–6

Trijbels-Smeulders, M. A., J. L. Kimpen, L. A. Kollee, J. Bakkers, W. Melchers, L. Spanjaard, W. J. Wannet and M. A. Hoogkamp-Korstanje 2006. Serotypes, genotypes, and antibiotic susceptibility profiles of group B streptococci causing

neonatal sepsis and meningitis before and after introduction of antibiotic prophylaxis. Pediatr Infect Dis J;25(10):945–8
Trotman, H. and Y. Bell 2006. Neonatal sepsis in very low birthweight infants at the University Hospital of the West indies. West Indian Med J;55(3):165–9
Turner, D., C. Hammerman, B. Rudensky, Y. Schlesinger, C. Goia and M. S. Schimmel 2006a. Procalcitonin in preterm infants during the first few days of life: introducing an age related nomogram. Arch Dis Child Fetal Neonatal Ed;91(4):F283–6
Turner, D., C. Hammerman, B. Rudensky, Y. Schlesinger and M. S. Schimmel 2006b. The role of procalcitonin as a predictor of nosocomial sepsis in preterm infants. Acta Paediatr;95(12):1571–6
Umana, M. A., C. M. Odio, E. Castro, J. L. Salas and G. H. McCracken, Jr. 1990. Evaluation of aztreonam and ampicillin vs. amikacin and ampicillin for treatment of neonatal bacterial infections. Pediatr Infect Dis J;9(3):175–80
Ungerer, R. L., O. Lincetto, W. McGuire, H. Saloojee and A. M. Gulmezoglu 2004. Prophylactic versus selective antibiotics for term newborn infants of mothers with risk factors for neonatal infection. Cochrane Database Syst Rev;4):CD003957
Vael, C., V. Nelen, S. L. Verhulst, H. Goossens and K. N. Desager 2008. Early intestinal Bacteroides fragilis colonisation and development of asthma. BMC Pulm Med;8(19
Waheed, M., A. Laeeq and S. Maqbool 2003. The etiology of neonatal sepsis and patterns of antibiotic resistance. J Coll Physicians Surg Pak;13(8):449–52
van den Anker, J. N., P. Pokorna, M. Kinzig-Schippers, J. Martinkova, R. de Groot, G. L. Drusano and F. Sorgel 2009a. Meropenem pharmacokinetics in the newborn. Antimicrob Agents Chemother;53(9):3871–9
van den Anker, J. N., P. Pokorna, M. Kinzig-Schippers, J. Martinkova, R. de Groot, G. L. Drusano and F. Sorgel 2009b. Meropenem Pharmacokinetics in the Newborn. Antimicrob Agents Chemother;
van Enk, J. G., D. J. Touw and H. N. Lafeber 2001. Pharmacokinetics of meropenem in preterm neonates. Ther Drug Monit;23(3):198–201
Vandebona, H., P. Mitchell, N. Manwaring, K. Griffiths, B. Gopinath, J. J. Wang and C. M. Sue 2009. Prevalence of mitochondrial 1555A→G mutation in adults of European descent. N Engl J Med;360(6):642–4
Warrier, I., W. Du, G. Natarajan, V. Salari and J. Aranda 2006. Patterns of drug utilization in a neonatal intensive care unit. J Clin Pharmacol;46(4):449–55
Wasz-Hockert, O., S. Nummi, S. Vuopala and P. A. Jarvinen 1970. Transplacental passage of azidocillin, ampicillin and penicillin G during early and late pregnancy. Acta Paediatr Scand Suppl;206(Suppl 206:109+
Vazzalwar, R., E. Pina-Rodrigues, B. L. Puppala, D. B. Angst and L. Schweig 2005. Procalcitonin as a screening test for late-onset sepsis in preterm very low birth weight infants. J Perinatol;25(6):397–402
Watterberg, K. L., H. W. Kelly, J. D. Johnson, M. Aldrich and P. Angelus 1987. Effect of patent ductus arteriosus on gentamicin pharmacokinetics in very low birth weight (less than 1,500 g) babies. Dev Pharmacol Ther;10(2):107–17
Weber, M. W., J. B. Carlin, S. Gatchalian, D. Lehmann, L. Muhe and E. K. Mulholland 2003. Predictors of neonatal sepsis in developing countries. Pediatr Infect Dis J;22(8):711–7
Weeks, J. L., E. O. Mason, Jr. and C. J. Baker 1981. Antagonism of ampicillin and chloramphenicol for meningeal isolates of group B streptococci. Antimicrob Agents Chemother;20(3):281–5

Weinstein, A. J., R. S. Gibbs and M. Gallagher 1976. Placental transfer of clindamycin and gentamicin in term pregnancy. Am J Obstet Gynecol;124(7):688–91
Veldhoen, E. S., T. F. Wolfs and A. J. van Vught 2009. Changes in infectious disease mortality among children in the Netherlands. Eur J Pediatr;168(4):465–8
Vergnano, S., M. Sharland, P. Kazembe, C. Mwansambo and P. T. Heath 2005. Neonatal sepsis: an international perspective. Arch Dis Child Fetal Neonatal Ed; 90(3):F220–4
Vesikari, T., M. Janas, P. Gronroos, N. Tuppurainen, M. Renlund, P. Kero, M. Koivisto, M. Kunnas, K. Heinonen, R. Nyman and et al. 1985. Neonatal septicaemia. Arch Dis Child;60(6):542–6
White, C. 1999. Newborns prescribed drugs only tested in adults. Bmj;318(7183):554B
WHO 2003. Explore simplified antimicrobial regimens for the treatment of neonatal sepsis. Geneva
WHO 2009. Neonatal sepsis – a major killer to be tackled in communities.
Wiese, G. 1988. Treatment of neonatal sepsis with ceftriaxone/gentamicin and with azlocillin/gentamicin: a clinical comparison of efficacy and tolerability. Chemotherapy;34(2):158–63
Williamson, M., S. H. Fraser and M. Tilse 1995. Failure of the urinary group B streptococcal antigen test as a screen for neonatal sepsis. Arch Dis Child Fetal Neonatal Ed;73(2):F109–11
Winter, P., A. Hoflechner and W. Baumgartner 1999. [Resistance patterns of ovine mastitis pathogens]. Berl Munch Tierarztl Wochenschr;112(6–7):216–22
Wisloff, F., J. P. Larsen, A. Dahle, M. Lie and H. C. Godal 1983. Effect of prophylactic high-dose treatment with ampicillin and cloxacillin on bleeding time and bleeding in patients undergoing elective vascular surgery. Scand J Haematol;31(2):97–101
Woodgate, P., V. Flenady and P. Steer 2004. Intramuscular penicillin for the prevention of early onset group B streptococcal infection in newborn infants. Cochrane Database Syst Rev;3):CD003667
Wu, J. H., C. Y. Chen, P. N. Tsao, W. S. Hsieh and H. C. Chou 2009. Neonatal sepsis: a 6-year analysis in a neonatal care unit in Taiwan. Pediatr Neonatol;50(3):88–95
Wynn, J. L., J. Neu, L. L. Moldawer and O. Levy 2009. Potential of immunomodulatory agents for prevention and treatment of neonatal sepsis. J Perinatol;29(2):79–88
Yancey, M. K., P. Duff, P. Kubilis, P. Clark and B. H. Frentzen 1996. Risk factors for neonatal sepsis. Obstet Gynecol;87(2):188–94
Yurdakok, M. 1998. Antibiotic use in neonatal sepsis. Turk J Pediatr;40(1):17–33

9. SUMMARY IN ESTONIAN

Optimaalne antibakteriaalne ravi varase sepsise riskiga vastsündinutel

Vastsündinu sepsis on jätkuvalt kõrge surevuse ja tõsiste kaugtagajärgedega lõppev kliiniline probleem. Kuna alati ei ole võimalik kindlaks teha haigustekitajate täpset levikuteed, eristatakse tõenäolist nakatumismehhanismi haigestumise aja alusel. Vastsündinu varase sepsise puhul on enamasti tegemist ema sünniteedest sünni eel või ajal omandatud haigustekitajatest põhjustatud infektsiooniga; kliiniline pilt tekib 48–72 elutunni jooksul. Hilise vastsündinu sepsise korral on haigustekitaja(d) suurema tõenäosusega omandatud väliskeskkonnast; diagnoosi kriteeriumiks on kliinilise pildi avaldumine pärast 72 elutundi.

Alates 1980-ndatest aastatest on B-grupi streptokokid olnud kõige sagedasemaks varase vastsündinu sepsise tekitajaks (Bizzarro *et al.* 2005, Schrag *et al.* 2006b). Viimasel paaril aastakümnel on laialdase sünnitusaegse antibiootikumprofülaktika rakendamisega B-grupi streptokokkide osakaal vähenenud ja Gram-negatiivsete bakterite, s.h. *Escherichia coli* osakaal suurenenud. Väga väikese sünnikaaluga (s.o. sünnikaal < 1500g) enneaegsetel vastsündinutel on *E. coli* muutunud kõige sagedasemaks varase sepsise tekitajaks (Stoll *et al.* 1996, Stoll *et al.* 2002a, Ronnestad *et al.* 2005, Stoll *et al.* 2005, Klinger *et al.* 2009, Maayan-Metzger *et al.* 2009).

Vastsündinute sepsise kliinilised tunnused on mittespetsiifilised, samas on uuringud näidanud, et kohene antibakteriaalne ravi vähendab surevust (Lannering *et al.* 1983, Mtitimila *et al.* 2004). Seetõttu on igapäevases praktikas laialdaselt kasutusel antibiootikumravi rakendamine riskifaktorite ja mittespetsiifiliste haigussümptoomide esinemisel.

Kuigi enam kui 90% intensiivravi vajavatest vastsündinutest saavad antibiootikume (Shani *et al.* 2008, Neubert *et al.* 2009), ei ole nende ravimite farmakokineetikat vastsündinutel sageli piisavalt uuritud (Di Paolo *et al.* 2006, Hsien *et al.* 2008). Seetõttu kasutatakse ravimeid pigem ekspertarvamuse kui spetsiifiliste kliiniliste uuringute alusel. Nii põhinevad sügavalt enneaegsetel vastsündinutel kasutatud penitsilliini annused ajalistel lastel läbi viidud uuringu tulemustel (McCracken *et al.* 1973). Sügavalt enneaegsete vastsündinute füsioloogilised eripärad, nagu suurem keha vedelikusisaldus, madalam glomerulaarfiltratsioon ja tubulaarne sekretsioon neerudes, lubavad aga oletada olulisi erinevusi farmakokineetikas (Paap *et al.* 1990, Allegaert *et al.* 2008).

Siiani pole päris selge missugused antibiootikumid on kõige efektiivsemad vastsündinu varase sepsise empiirilises ravis (Mtitimila *et al.* 2004). Hiljutine Cochrane'i meta-analüüs leidis ainult kaks uuringut, mis võrdlesid erinevaid antibakteriaalse ravi režiime antud näidustuse korral (Snelling *et al.* 1983, Miall-Allen *et al.* 1988). Mõlemad uuringud on läbi viidud enam kui 20 aastat tagasi ja antibiootikumidega, mis ei ole tänapäeval kasutusel. Hilisem 128 914 vastsündinut hõlmav retrospektiivne uuring on leidnud, et tsefotaksiim-ravi oli seotud kõrgema surma riskiga võrreldes ampitsilliin-gentamütsiini kombinat-

siooniga (Clark *et al.* 2006). Vaatamata tõenduspõhiste andmete puudumisele, on enamuses vastsündinute osakondades aminoglükosiidi kombinatsioon ampitsilliini või penitsilliiniga valikraviks vastsündinu varase sepsise korral (Clark *et al.* 2006, Maayan-Metzger *et al.* 2009). *In vitro* on mõlemad beeta-laktaam antibiootikumid sarnaselt efektiivsed sagedasemate Gram-positiivsete vastsündinu varase sepsise tekitajate, nagu B-grupi streptokokid ja *Listeria monocytogenes*, suhtes. Samas on ampitsilliinil oluliselt laiem toimespekter Gram-negatiivsete mikroorganismide suhtes. Samas on viimasel aastakümnel oluliselt suurenenud Gram-negatiivsete mikroorganismide, s.h. *E. coli* resistentsus ampitsilliini suhtes, ulatudes mõnedes keskustes kuni 80%-ni (Bizzarro *et al.* 2008), mis tõenäoliselt vähendab eeldatavat erinevust kliinilises efektiivsuses. Samuti suhteliselt kõrgena püsinud Gram-negatiivsete tekitajate tundlikkus aminoglükosiidide suhtes (Millar *et al.* 2008). Samas seostatakse ampitsilliini laispektrilist beeta-laktamaasi produtseerivate *Klebsiella pneumoniae* tüvede leviku (Couto *et al.* 2007b, Crivaro *et al.* 2007) ja *Enterobacter cloacae* beeta-laktaam resistentsuse induktsiooniga laste ja vastsündinute intensiivravi osakondades (Burman *et al.* 1993). Nimetatud mikroobidest põhjustatud infektsioonid vastsündinutel on seotud kõrge surevusega (Larson *et al.* 2005, Schrag *et al.* 2006a).

Umbes 10–20% varase vastsündinu sepsise riski tõttu penitsilliini või ampitsilliini ja gentamütsiini kombinatsiooniga ravitud vastsündinutest vajab antibiootikumravi vahetamist (Tessin *et al.* 1991, Metsvaht *et al.* 2006). Kuna tänased diagnostikameetodid ei võimalda piisavalt kiiret haigustekitaja isoleerimist, tuleb sellised otsused teha tavaliselt haige seisundi ja teiste kliiniliste ning laboratoorsete näitajate alusel. Samas ei ole kunagi püütud teaduslike meetoditega kindlaks teha riskifaktoreid, mis võimaldaksid ennustada varase empiirilise antibakteriaalse ravi ebaõnnestumist.

Uurimistöö eesmärgid

Uurimistöö peamiseks eesmärgiks oli teha kindlaks optimaalne antibiootikumravi režiim varase sepsise riskiga vastsündinutel. Uuringu hüpoteesiks oli, et ampitsilliini ja gentamütsiini ning penitsilliini ja gentamütsiini kombinatsioonid on sarnase efektiivsusega vastsündinu varase sepsise empiirilises ravis.
Konkreetsed eesmärgid:

1. Iseloomustada bensüülpenitsilliini farmakokineetikat väga madala sünnikaaluga enneaegsetel vastsündinutel, kellel on oht varase vastsündinu sepsise tekkeks
2. Võrrelda amptsilliini ja gentamütsiini ning penitsilliini ja gentamütsiini kombinatsioonide efektiivsust varase sepsise riskiga vastsündinute ravis
3. Kirjeldada uuritavate antibiootikumravi režiimide efektiivsust äärmiselt madala sünnikaaluga (sünnikaal < 1000 g) enneaegsete vastsündinute empiirilises ravis
4. Kirjeldada nimetatud ravirežiimide ohutust ja talutavust vastsündinutel

5. Teha kindlaks varased kliinilised ja laboratoorsed riskifaktorid, mis võimaldaksid ennustada ampitsilliin-gentamütsiin- ja bensüülpenitsilliin-gentamütsiin-ravi ebaõnnestumist

Patsiendid ja metoodika

Käesolev uurimus põhineb kahe SA Tallinna Lastehaigla ja SA Tartu Ülikooli Kliinikumi lasteintensiivravi osakondades vastsündinutel läbi viidud kliinilisel uuringul.

Farmakokineetika uuring viidi läbi 01.10.2005–30.04.2006. Uuringusse haarati enneaegsed vastsündinud sünnikaaluga kuni 1200 g ja gestatsioonivanusega kuni 28 rasedusnädalat, kes vajasid varast empiirilist antibakteriaalset ravi bensüülpenitsilliiniga, kellel ei olnud vereringe ega neerude puudulikkuse nähtusid ja kelle vanem või hooldaja oli andnud nõusoleku uuringus osalemiseks. Esimesse uuringugruppi kuulus 9 vastsündinut, kellele manustati bensüülpenitsilliini annuses 50 000 IU/kg (30 mg/kg). Enne kolmanda kuni kaheksanda penitsilliini G annuse manustamist (s.o. stabiilses faasis) ning 30 min; 1,5; 4; 8 ja 12 tundi pärast seda võeti vereproovid penitsilliini kontsentratsiooni määramiseks. Eelnevalt oli planeeritud, et kui bensüülpenitsilliini kontsentratsioonid vastsündinu veres ületavad tunduvalt täiskasvanutel kasutatava tavapärase annusega saavutatuid, siis manustatakse teises uuringugrupis 9-le vastsündinule penitsilliini poole võrra väiksemas annuses, s.o. 25 000 IU/kg (15 mg/kg). Lisaks arvutati kreatiniini kliirens ja koguti 12 tunni jooksul uuritava penitsilliini annuse manustamisest uriin penitsilliini eritumise hindamiseks.

Olles välja selgitanud penitsilliini optimaalse annuse vastsündinutel, võrdlesime prospektiivses kahekeskuselises klaster-randomiseeritud uuringus amptsilliini ja gentamütsiini ning penitsilliini ja gentamütsiini kombinatsioonide kliinilist efektiivsust varase sepsise riskiga vastsündinutel. Uuring planeeriti ekvivalentsusuuringuna, kus ravirežiimide erinevus ei tohtinud ületada +/- 10%. Uuringu esimesel perioodil kasutati keskuses A ampitsilliini kombinatsiooni gentamütsiiniga ning keskuses B penitsilliini gentamütsiiniga. Kui pool võimsusanalüüsi alusel kalkuleeritud uuritavate arvust oli uuringusse kaasatud, toimus beeta-laktaam antibiootikumide vahetus, nii et keskuses A kasutati penitsilliini ja keskuses B ampitsilliini kombinatsiooni gentamütsiiniga. Uuringusse kaasati esimese 72 elutunni jooksul intensiivravi osakondadesse hospitaliseeritud vastsündinud, kes sepsise kahtluse või riskifaktorite olemasolu tõttu vajasid eelnimetatud antibakteriaalset ravi ning keda järgneva 24 tunni jooksul ei viidud üle teistesse osakondadesse. Ravimikombinatsioonide kliinilist efektiivsust hinnati kombineeritud lõpptulemi 72 tunni jooksul toimunud antibiootikumravi vahetuse ja/või surevus esimese seitsme elupäeva jooksul alusel. Teisese lõpptulemina hinnati intensiivravi, kopsude kunstliku ventilatsiooni, vasoaktiivse ravi ja antibakteriaalse ravi kestust, hilise vastsündinu sepsise esinemissagedust ja tekitajate struktuuri ning vastsündinutel esinevate tüsistuste, nagu vastsündinute krooniline kopsuhaigus, nekrootiline enterokoliit,

raske aju vatsakestesisene verevalum ja enneaegsete retinopaatia esinemissagedust. Statistilises analüüsis kasutati hierarhilisi mudeleid, mis olid tasakaalustatud uuringukeskuse ja –perioodi suhtes. Eraldi viidi läbi äärmiselt madala sünnikaaluga (sünnikaal < 1001 g) vastsündinute alagrupi analüüs.

Antibakteriaalse ravi efektiivsuse võrdlevas kliinilises uuringus kogutud andmete põhjal selgitati hiljem mitmest logistilist regressiooni ja klassifikatsiooni ja regressiooni puu analüüsi kasutades kliinilised ja laboratoorsed riskifaktorid, mis võimaldava ennustada antibakteriaalse ravi vahetamise vajadust sepsise riskiga vastsündinutel.

Peamised tulemused

Sügavalt enneaegsetel vastsündinutel penitsilliini annusega 50 000 IU/kg (30 mg/kg) saavutatud plasmakontsentratsioonid (C_{max} mediaan 146 mg/L; kvartiilid 109–157 mg/L; C_{min} 7; 5–13 mg/L) ületasid oluliselt täiskasvanutel tavapäraselt kasutatavaid ning minimaalse kontsentratsiooni mediaan uuritaval ajavahemikul oli enam kui 100 korda kõrgem B-grupi streptokokkide minimaalsest inhibeerivast kontsentratsioonist. Seetõttu vähendati teises grupis penitsilliini annust poole võrra (25 000 IU/kg e. 15 mg/kg), mille tulemusena vähenes proportsionaalselt nii maksimaalne kui minimaalne plasmakontsentratsioon ja kontsentratsiooni-aja kõvera alune pindala. Samas jäi plasmakontsentratsioon kõigil uuritavatel kogu annustamise intervalli (12 tunni) vältel kõrgemaks B-grupi streptokokkide minimaalsest inhibeerivast kontsentratsioonist. Penitsilliini poolväärtusaeg enneaegsetel vastsündinutel oli 3,8–4,6 tundi, mis on oluliselt pikem kui ajalistel vastsündinutel (3,4 tundi) ja täiskasvanutel (0,5 tundi). Penitsilliini kliirens korreleerus kreatiniini kliirensiga, viidates, et vastsündinutel on penitsilliini peamiseks renaalse eritumise mehhanismiks glomerulaarfiltratsioon, mitte tubulaarne sekretsioon nagu täiskasvanul. Bensüülpenitsilliini annusega 25 000 IU/kg (15 mg/kg) saavutatud seerumikontsentratsioon osutus piisavaks. Annusega 50 000 IU/kg (30 mg/kg) lähenesid plasmakontsentratsioonid sügavalt enneaegsetel vastsündinutel väärtustele, millega täiskasvanutel on kirjeldatud ägedat entsefalopaatiat krampidega. Seetõttu kasutati kliinilise efektiivsuse uuringus nimetatud väiksemat doosi.

Ampitsilliini-gentamütsiini ja bensüülpenitsilliin-gentamütsiini kombinatsioonide võrdlevasse uuringusse hõlmati 283 vastsündinut, neist 142 sai ampitsilliin- ja 141 penitsilliinravi. Uuritavad ravirežiimid osutusid võrdseks esmase kombineeritud lõpptulemi suhtes (20/142 ampitsilliini ja 20/141 penitsilliini grupis; OR 1,01; 95% CI 0,52–1,97) ning samuti mõlema komponendi (antibakteriaalse ravi vahetuse vajadus 10/142 ja 10/141; OR 1,02; 95% CI 0,40–2,59; varane neonataalne surm 11/142 vs 14/141; OR 0,76; 95% CI 0,33–1,75 ampitsilliini ja penitsilliini grupis, vastavalt) esinemissageduse osas. Bakterioloogiliselt tõestatud varast sepsist esines mõlemas uuringugrupis võrdselt harva – 6 juhtu ampitsilliini ja 8 juhtu penitsilliini grupis. Haigustekitajate tundlikkus võrreldud ravirežiimide suhtes oli parem ampitsilliini

grupis – 6/8 isoleeritud mikroobitüvest olid tundlikud vähemalt ühele ravirežiimi komponendile vs 3/8 penitsilliini grupis (OR 0,080; 95% CI 0,0095–0,67). Erinevuse põhjuseks olid penitsilliini grupis isoleeritud koagulaas-negatiivsed stafülokokid (n=4) ja *Candida albicans* (n=1). Hilist vastsündinu sepsist esines mõlemas grupis võrdse sagedusega (33/142 vs 42/141 ampitsilliini ja penitsilliini grupis, vastavalt), kuid haigustekitajatest esines *S. epidermidis*'e põhjustatud sepsist sagedamini penitsilliini grupis (vastavalt 2,7 vs 7,6 juhtu 1000 haige-päeva kohta ampitsilliini ja penitsilliini grupis; RR 0,32; 95% CI 0,19–0,55). Samuti kasutati täiendavat antibakteriaalset ravi ampitsilliini grupis harvemini kui penitsilliini grupis (43/142 vs 63/141; OR 0.63; 95% CI; 0.55–0.71).

Ka äärmiselt madala sünnikaaluga enneaegsetel vastsündinutel esines kombineeritud esmast lõpptulemit võrdse sagedusega mõlema ravirežiimiga (10/36 ampitsilliini vs 10/39penitsilliini grupis; OR 1.01; 95% CI 0.52–1.97). Bakterioloogiliselt tõestatud varast vastsündinu sepsist esines oluliselt sagedamini bensüülpenitsilliini grupis (0/36 vs 6/39; OR 0.1; 95% CI 0.01–0.6). Samuti oli penitsilliini grupis oluliselt kõrgem enne 26-ndat rasedusnädalat sündinud enneaegsete vastsündinute surevus (6/24 vs 13/21 ampitsilliini ja penitsilliini grupis vastavalt; OR 0.2; 95% CI 0.05–0.7). Rasket nekrootilist enterokoliiti ja enneaegsete retinopaatiat esines sagedamini ampitsilliini grupis, kuid erinevused kadusid kui arvesse võeti suuremat surevust penitsilliini grupis.

Mitmese logistilise regressiooni alusel leiti, et antibakteriaalse ravi ebaõnnestumise riskifaktoriteks esimese 24 elutunni jooksul on vasoaktiivse ravi vajadus, leukotsüütide üldarv $< 5000 \times 10^9$ /L või üle $20\,000 \times 10^9$ /L, ebaküpsete neutrofiilsete leukotsüütide suhe nende üldarvusse >0,2 ja trombotsüütide üldarv $< 100\,000 \times 10^9$ /L. 72 elutunni vanuses ennustas antibakteriaalse ravi ebaõnnestumist kõige täpsemalt vasoaktiivse ravi vajadus, trombotsüütide üldarv $< 100\,000 \times 10^9$ /L, C-reaktiivse valgu kontsentratsiooni suurenemine ja hüpoalbumineemia. Klassifikatsiooni ja regressiooni puu alusel olid olulisimateks antibakteriaalse ravi ebaõnnestumise riskifaktoriteks esimese 24 elutunni jooksul hüpoglükeemia ≤ 2.55 mmol/L koos C-reaktiivse valgu kontsentratsiooniga > 1.35 mg/L; normoglükeemilistel vastsündinutel oli kõrge risk seotud sünnikaaluga ≤ 678 g või suurema sünnikaalu korral gestatsioonivanusega ≤ 27 rasedusnädala või leukotsüütide üldarvuga $\leq 8.25 \times 10^9$ /L koos trombotsüütide arvuga $\leq 143 \times 10^9$ /L. Esimese 72 elutunni jooksul osutusid parimateks antibakteriaalse ravi ebaõnnestumise riskifaktoriteks trombotsütopeenia $\leq 94.5 \times 10^9$ /L koos vasoaktiivse ravi vajadusega ja leukotsüütide arv $< 3.5 \times 10^9$ /L või $> 39.8 \times 10^9$ /L kui samaaegselt esines ka hüpoglükeemia ≤ 1.65 mmol/l. Võrreldes mitmese logistilise regressiooniga võimaldasid klassifikatsiooni ja regressiooni puu alusel koostatud algoritmid ennustada antibakteriaalse ravi ebaõnnestumist suurema tundlikkusega 24 tunni ja suurema spetsiifilisusega 72 tunni vanuses.

Järeldused

I Ravimite farmakokineetika sügavalt enneaegsetel vastsündinutel erineb oluliselt täiskasvanutest, vanematest lastest ja isegi ajalistest vastsündinutest, nagu on näha bensüülpenitsilliini näitel. Võib eeldada, et sarnased erinevused esinevad ka teiste neerude kaudu erituvate ravimite farmakokineetikas, seetõttu on ravimite kõrvaltoimete vältimiseks ja optimaalse efektiivsuse tagamiseks vaja täiendavaid farmakokineetilisi uuringuid selles haigete grupis.

Erinevalt täiskasvanutest on bensüülpenitsilliini peamiseks renaalse eliminatsiooni mehhanismiks väga madala sünnikaaluga enneaegsetel vastsündinutel glomerulaarfiltratsioon, mitte tubulaarne sekretsioon ja bensüülpenitsilliini poolestusaeg on ligikaudu seitse korda pikem, mistõttu ravimit tuleb manustada oluliselt pikema intervalliga.

Väga madala sünnikaaluga enneaegsetel vastsündinutel on bensüülpenitsilliini annus 25 000 IU/kg (15 mg/kg) 12 tunni järel ohutu ja piisav, et tagada B-grupi streptokokkide minimaalsest inhibeerivast kontsentratsioonist kõrgem seerumi ja tõenäoliselt ka liikvori kontsentratsioon kogu doseerimisintervalli vältel.

II Ampitsilliini või bensüülpenitsilliini kombinatsioonid gentamütsiiniga on sarnase efektiivsuse ja ohutusega intensiivravi vajavate varase sepsise riskiga vastsündinute empiirilises ravis. Konkreetne valik ühe või teise kasuks tuleks teha lähtudes kohalikust vastsündinu sepsise tekitajate struktuurist ja antibiootikum-tundlikkusest.

Ampitsilliin-gentamütsiini kombinatsioon on seotud väiksema täiendava laiaspektrilise antibakteriaalse ravi vajadusega, mis võib tasakaalustada ampitsilliini suuremat resistentsust selekteerivat ja indutseerivat toimet. Võrreldes bensüülpenitsilliin-gentamütsiini kombinatsiooniga esineb ampitsilliinraviga vähem *S. epidermidis*-est põhjustatud hilist vastsündinu sepsist. Ampitsilliini osaline eliminatsioon sapiga võib võrreldes bensüülpenitsilliiniga enam mõjutada mikroobiökoloogiat ja seega vastsündinute hilise sepsise bakteriaalset etioloogiat.

III Võrreldes ampitsilliin-gentamütsiiniga võib bensüülpenitsilliin-gentamütsiini kombinatsioon olla seotud suurema intensiivravi surevusega, eriti enne 26-ndat rasedusnädalat sündinud enneaegsetel vastsündinutel. Ampitsilliini või bensüülpenitsilliini ja gentamütsiini kombinatsioonide sobivuse selgitamiseks äärmiselt madala sünnikaaluga enneaegsetel, aga ka kõrge beeta-laktaam resistentsusega populatsioonides on vaja täiendavaid piisava võimsusega kliinilisi uuringuid.

IV Ampitsilliin-gentamütsiini ja bensüülpenitsilliin-gentamütsiini kombinatsioonid on hästi talutavad ja ohutud alternatiivid varase sepsise riskiga vastsündinute empiirilises ravis.

V Ampitsilliin- või bensüülpenitsilliin- ja gentamütsiinravi ebaõnnestumise riskifaktoriteks varase sepsise riskiga vastsündinutel on esimese 72 elutunni jooksul kujunenud trombotsütopeenia (trombotsüütide arv $\leq 95 \times 10^9$ /L)

koos vasoaktiivse ravi vajadusega või leukopeenia (leukotsüütide arv $<3,5 \times 10^9$ /L) või leukotsütoos (leukotsüütide arv $> 40,0 \times 10^9$ /L) koos hüpoglükeemiaga (veresuhkur $\leq$ 1,7 mmol/L). Edasised kliinilised uuringud peavad näitama, kas laiema toimespektriga antibiootikumide varane rakendamine nendel haigetel mõjutab vastsündinu varase sepsise ravitulemusi.
Klassifikatsiooni regressiooni puu analüüs on efektiivne võimalus kliinilises töös kergesti rakendatavate algoritmide välja töötamiseks, mis võrreldes traditsioonilisema mitmese regressioonanalüüsiga võimaldab sageli saavutada suurema tundlikkuse ja/või spetsiifilisuse.

10. ACKNOWLEDGEMENTS

This work was carried out in the Paediatric Intensive Care Unit of Tallinn Children's Hospital and Tartu University Hospitals and supported by the Estonian Science Foundation grant No 6984; Estonian Target Financing No 2726 and ESPID Small Grant Award.

The studies presented in the thesis are based on teamwork with the contribution of many persons whom I wish to express my deepest gratitude. In particular I would like to acknowledge the following persons:

- Professor Irja Lutsar, my dissertation supervisor, for her friendship and true mentorship throughout the process. She introduced me with the details in the fascinating field of clinical research and provided the working facilities at my disposal. I highly appreciate her constructive criticism and her way of always setting the highest targets. I am very grateful for the incredible opportunity she gave me, to learn so much.
- Professor emeritus Raul Talvik, the supervisor of this thesis as well as my first steps in research, who despite my many other interests at the early steps gave me the understanding that brought me back to the issue over several years. I highly appreciate his way of never forgetting the forest behind the trees, so essential to avoid drowning in details.
- Professor Joel Starkopf for his help and support as a tutor and a friend. I am very grateful for the many fruitful discussions and bright ideas he has provided for this thesis as well as for the review that certainly helped to further improve it.
- Professor Vallo Tillmann for reviewing the dissertation and for all the useful remarks. I appreciate the time he took with my work.
- Mari-Liis Ilmoja, a dear friend and my closest co-operator in the clinical studies, who carried the responsibility for performing all the studies in Tallinn Children's Hospital.
- Heti Pisarev for her never-ending enthusiasm in the probably hard work of introducing me into the field of medical statistics. I have highly appreciated her ability of finding the right answers with just a word to the wise.
- Ülle Parm, a co-student in this research who undertook the enormous work of colonisation studies without any complaint and has stood with me the test of all the ups and downs this work has brought along.
- My colleagues Lea Maipuu, Mirjam Merila, Piia Müürsepp and Kadri Julge for their cooperation and support in data collection in the clinical efficacy study.
- Head nurse Tiina Kilg who managed to keep a supportive atmosphere in our intensive care unit throughout the clinical studies and took care of the colonisation sample collection in a most systematic way.
- Nurses Raili Sommer, Pille Org, Meta Kornejeva, Kristel Sillaste, Irina Bljudz and Inna Jegorova, who managed the demanding sampling schedule in the PK study with exquisite accuracy.

– All my colleagues in the Paediatric Intensive Care Units of Tartu University Hospitals and Tallinn Children's Hospital for their help and support.
– All patients and their families, who participated in the study for their cooperation.
– I would like to cherish the people who have afforded me the personal background that has supported me throughout this work:
– Professor emeritus Tiina Talvik, Inga and Katrin for including me in their extended family and thus setting the most supportive but also highly professional background for my aspirations and keeping a watchful eye over all my professional and personal commitments and progress.
– My dear friend Inga Vainumäe for sharing the ups and downs and becoming a true specialist in bringing me through the deepest frustration at times.

My greatest gratitude goes to my family – my mother and my father, who have given me the endeavour and the means; my sister Kadri for her total support in everything I've ever done and her husband Toomas and children Kärt Maria and Paul Johan for following the same line without undue questions; last but not least my husband Raivo and our children Hanna Kadri and Laura Liis for the inspiration but also for the understanding and patience available in well defined doses, not letting me to detach from reality.

PUBLICATIONS

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1. Kokk T, Talvik R, Zilmer M, Kütt E, Talvik T. Markers of oxidative stress before and after exchange transfusion for neonatal sepsis. Acta Paediatr 1996; 85:1244–6
2. Talvik R, Liigant A, Tapfer H, Tamme K, Metsvaht T. Septic shock with disseminated microfoci in multiple organs in man. Intensive Care Medicine 1998; 24:73–76
3. T. Metsvaht, P. Ilves, T. Talvik, K. Zilmer, M. Zilmer, R. Talvik. Possible oxidative stress in healthy term newborns. Acta Paediatr 1999; 88:1299–1300
4. T. Metsvaht, I. Talvik, P. Sillapere, M. Paluste, E. Kütt, L. Sau, T. Talvik, R. Talvik. IgM-enriched intravenous immunoglobulin for the prevention of infections in low birth weight neonates. Focus Medicinae 2001; 4:17–27
5. P. Ilves, M. Lintrop, T. Metsvaht, U. Vaher, T. Talvik. Cerebral blood-flow velocities in predicting outcome of asphyxiated newborn infants. Acta Paediatr 2004; 93:523–528
6. V. Tillmann, M.-L. Mägi, T. Metsvaht. Prenatal Cushing's syndrome due to nodular adrenocortical hyperplasia with unsuppressed plasma ACTH levels. Journal of Pediatric Endocrinology & Metabolism 2005; 18:1027–1031
7. Metsvaht, T.; Hermlin, T.; Kern, H.; Kahre, T.; Starkopf, J. Aortic arch thrombosis in a neonate with heterozygous carrier status of factor V Leiden mutation. Congenital Heart Disease 2006; 1:40–45
8. Talvik, I.; Metsvaht, T.; Leito, K.; Põder, H.; Kool, P.; Väli, M.; Lintrop, M.; Kolk, A.; Talvik, T. Inflicted traumatic brain injury (ITBI) or shaken baby syndrome (SBS) in Estonia. Acta Paediatrica 2006; 95:799–8
9. Vaidla, E.; Talvik, I.; Kulla, A.; Kahre, T.; Hämarik, M.; Napa, A.; Metsvaht, T.; Piirsoo, A.; Talvik, T. Descriptive epidemiology of spinal muscular atrophy type I in Estonia. Neuroepidemiology 2006; 27:164–168
10. E. Vaidla, I. Talvik, A. Kulla, H. Sibul, K. Maasalu, T. Metsvaht, A. Piirsoo, T. Talvik. Neonatal spinal muscular atrophy type I with bone fractures and heart defect. Journal of Child Neurology 2007; 22:67–70
11. T. Metsvaht, K. Oselin, M-L. Ilmoja, K. Anier, I. Lutsar. Pharmacokinetics of Penicillin G in very-low-birth-weight neonates. Antimicrobial Agents and Chemotherapy 2007; 51:1995–2000

12. R. Laugesaar, A. Kolk, T Tomberg, T Metsvaht, M Lintrop, H Varendi, T Talvik. Acutely and retrospectively diagnosed perinatal stroke: a population-based study. Stroke 2007; 38:2234–2240
13. T. Metsvaht, R. Laugesaar, A. Kolk, T. Talvik. Response to Letter by Temesvári. Stroke 2008; 39:e36–e37
14. P. Ilves, M. Lintrop, I. Talvik, K. Muug, L. Maipuu, T. Metsvaht. Low cerebral blood flow velocity and head circumference in asphyxiated infants with severe hypoxic ischemic encephalopathy and poor outcome. Acta Paediatrica 2009; 98:459–65
15. T. Metsvaht, H. Pisarev, M-L. Ilmoja, Ü. Parm, L. Maipuu, M. Merila, P. Muursepp, I. Lutsar. Clinical parameters predicting failure of empirical antibacterial therapy in early onset neonatal sepsis, identified by classification and regression tree analysis. *BMC* Pediatrics 2009, 9:72
16. Metsvaht T, Ilmoja ML, Parm U, Maipuu L, Merila M, Lutsar I. Comparison of ampicillin plus gentamicin vs. penicillin plus gentamicin in empiric treatment of neonates at risk of early onset sepsis. Acta Paediatrica 2010; Jan 20 (Epub ahead of print)
17. Lutsar I, Metsvaht T. Understanding pharmacokinetic/pharmacodynamic in managing neonatal sepsis. Current Opinion in Infect Dis 2010; 23:in press

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Publikatsioonid

1. Kokk T, Talvik R, Zilmer M, Kütt E, Talvik T. Markers of oxidative stress before and after exchange transfusion for neonatal sepsis. Acta Paediatr 1996; 85:1244–6
2. Talvik R, Liigant A, Tapfer H, Tamme K, Metsvaht T. Septic shock with disseminated microfoci in multiple organs in man. Intensive Care Medicine 1998; 24:73–76
3. T. Metsvaht, P. Ilves, T. Talvik, K. Zilmer, M. Zilmer, R. Talvik. Possible oxidative stress in healthy term newborns. Acta Paediatr 1999; 88:1299–1300
4. T. Metsvaht, I. Talvik, P. Sillapere, M. Paluste, E. Kütt, L. Sau, T. Talvik, R. Talvik. IgM-enriched intravenous immunoglobulin for the prevention of infections in low birth weight neonates. Focus Medicinae 2001; 4:17–27
5. P. Ilves, M. Lintrop, T. Metsvaht, U. Vaher, T. Talvik. Cerebral blood-flow velocities in predicting outcome of asphyxiated newborn infants. Acta Paediatr 2004; 93:523–528
6. V. Tillmann, M.-L. Mägi, T. Metsvaht. Prenatal Cushing's syndrome due to nodular adrenocortical hyperplasia with unsuppressed plasma ACTH levels. Journal of Pediatric Endocrinology & Metabolism 2005; 18:1027–1031
7. Metsvaht, T.; Hermlin, T.; Kern, H.; Kahre, T.; Starkopf, J. Aortic arch thrombosis in a neonate with heterozygous carrier status of factor V Leiden mutation. Congenital Heart Disease 2006; 1:40–45
8. Talvik, I.; Metsvaht, T.; Leito, K.; Põder, H.; Kool, P.; Väli, M.; Lintrop, M.; Kolk, A.; Talvik, T. Inflicted traumatic brain injury (ITBI) or shaken baby syndrome (SBS) in Estonia. Acta Paediatrica 2006; 95:799–8
9. Vaidla, E.; Talvik, I.; Kulla, A.; Kahre, T.; Hämarik, M.; Napa, A.; Metsvaht, T.; Piirsoo, A.; Talvik, T. Descriptive epidemiology of spinal muscular atropy type I in Estonia. Neuroepidemiology 2006; 27:164–168
10. E. Vaidla, I. Talvik, A. Kulla, H. Sibul, K. Maasalu, T. Metsvaht, A. Piirsoo, T. Talvik. Neonatal spinal muscular atrophy type I with bone fractures and heart defect. Journal of Child Neurology 2007; 22:67–70
11. T. Metsvaht, K. Oselin, M-L. Ilmoja, K. Anier, I. Lutsar. Pharmacokinetics of Penicillin G in very-low-birth-weight neonates. Antimicrobial Agents and Chemotherapy 2007; 51:1995–2000

12. R. Laugesaar, A. Kolk, T Tomberg, T Metsvaht, M Lintrop, H Varendi, T Talvik. Acutely and retrospectively diagnosed perinatal stroke: a population-based study. Stroke 2007; 38:2234–2240
13. T. Metsvaht, R. Laugesaar, A. Kolk, T. Talvik. Response to Letter by Temesvári. Stroke 2008; 39:e36–e37
14. P. Ilves, M. Lintrop, I. Talvik, K. Muug, L. Maipuu, T. Metsvaht. Low cerebral blood flow velocity and head circumference in asphyxiated infants with severe hypoxic ischemic encephalopathy and poor outcome. Acta Paediatrica 2009; 98:459–65
15. T. Metsvaht, H. Pisarev, M-L. Ilmoja, Ü. Parm, L. Maipuu, M. Merila, P. Muursepp, I. Lutsar. Clinical parameters predicting failure of empirical antibacterial therapy in early onset neonatal sepsis, identified by classification and regression tree analysis. *BMC* Pediatrics 2009, 9:72
16. Metsvaht T, Ilmoja ML, Parm U, Maipuu L, Merila M, Lutsar I. Comparison of ampicillin plus gentamicin vs. penicillin plus gentamicin in empiric treatment of neonates at risk of early onset sepsis. Acta Paediatrica 2010; Jan 20 (Epub ahead of print)
17. Lutsar I, Metsvaht T. Understanding pharmacokinetic/pharmacodynamic in managing neonatal sepsis. Current Opinion in Infect Dis 2010; 23:in press

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