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Summary in English

**Background and objective** Neonatal sepsis is a severe disease in preterm infants that can result in significant morbidity in this vulnerable patient group. Our aim was to identify and assess the risk factors and incidence of neonatal sepsis in two different groups of preterm infants (very preterm-and moderate to late preterm infants) in a level 2 neonatal ward.

**Methods** We conducted a retrospective cohort study in preterm infants with a gestational age (GA) of <36 weeks transferred to or born in our centre from 2009 to 2012. Sepsis was defined as a positive blood culture and clinical symptoms, with early- and late onset sepsis defined as onset of symptoms before 72 hours and after 72 hours of life, respectively. Univariate and multivariate logistic analyses were performed.

**Results** We included 163 very preterm infants (GA <32 weeks) and 434 moderate-to-late preterm infants (GA 32-36 weeks). Neonatal sepsis was found in 6% (36/597) of preterm infants. There was a significant association between the type of group a neonate belonged to (according to GA) and whether or not sepsis was diagnosed (p < 0.002). The odds of sepsis for neonates were 2.7 times higher if they were born with a GA <32 weeks (group A) than if they were born with a GA 32-36 weeks (group B). Early-onset sepsis (EOS) with group B streptococcus occurred in one late preterm infant (0.7%) of 146 suspected episodes. Late-onset sepsis (LOS) occurred in 35 preterm infants of a total of 90 suspected episodes (39%). Coagulase-negative staphylococci was the most frequently pathogen isolated in LOS. Total parenteral nutrition administration, vaginal spontaneous birth and gestational age were significantly associated with LOS.

**Conclusion** This study shows a very low prevalence of EOS and LOS in both very preterm and moderate preterm infants. Vaginal spontaneous birth was found to be a significant protective factor for LOS. TPN administration (by central venous catheter, peripheral inserted catheter), vaginal spontaneous birth and lower GA were found to be significant risk factors for development of LOS.
**Summary in Dutch**

**Inleiding** Neonatale sepsis is een ernstig ziektebeeld bij prematuren en kan leiden tot significante morbiditeit met mogelijke negatieve lange termijn effecten.

**Doel** Deze retrospectieve studie is verricht om de risicofactoren en de incidentie van neonatale sepsis bij 2 verschillende groepen prematuren te onderzoeken in een level 2 ziekenhuis.

**Methode** We hebben sepsis data verzameld van 2 verschillende groepen prematuren: groep A zwangerschapsduur <32 weken, en groep B zwangerschapsduur 32-36 weken gedurende de periode 2009-2012. Sepsis was gedefinieerd als een positieve bloedkweek met een klinische verdenking, waarbij early onset sepsis (EOS) de eerste 72 uur postpartum optreedt en late onset sepsis (LOS) 72 uur postpartum. Univariate and multivariate logistische analyse werden toegepast.

**Resultaten** Groep A bestond uit 163 prematuren en groep B uit 434 prematuren. Van de totale groep ontwikkelde 36/597 (6%) een episode van neonatale sepsis. Er was een significant verschil tussen de groepen voor wat betreft zwangerschapsduur en bewezen sepsis (p < .002). Het risico op het krijgen van sepsis voor neonaten met een zwangerschapsduur <32 weken was 2,7 keer zo groot als in neonaten met een zwangerschapsduur van 32-36 weken (groep B). Er was 1 EOS met groep B streptokokken in groep B van 146 klinische verdenkingen (0,7%). LOS werd bij 35 neonaten (39%) gediagnosticeerd bij de 90 verdachte gevallen, 18 in groep A en 17 in groep B. Coagulase negatieve stafylococcus was de meest voorkomende pathogene geïsoleerd in de LOS groep. Spontane vaginale baring, toediening van totale parenterale voeding (TPV) en zwangerschapsduur waren significant geassocieerd met LOS.

**Conclusie** De incidentie van zowel vroege als late sepsis is zeer laag in zowel extreme als late prematuren in een level 2 ziekenhuis. Spontane vaginale baring bleek een significante beschermende factor tegen LOS. Verder, bleek dat toediening van TPV en zwangerschapsduur significante determinanten waren voor het krijgen van LOS.
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1. Introduction

1.1 Neonatal sepsis

Neonatal sepsis is a severe disease in preterm infants and can result in significant morbidity. It is important to stress that Stoll et al. showed that infection in the neonatal period is associated with poor neurodevelopmental and growth outcomes in early childhood. Neonatal sepsis is often defined as a disease in an infant 28 days of life or younger characterized by systemic signs of infection and isolation of a bacterial pathogen from the bloodstream. The incidence of neonatal sepsis varies from less than 1 to 3 cases per 1000 live births. This variance in incidence rate can be explained by different factors: gestational age, birth weight and a higher incidence rate found in developing countries. Neonatal sepsis is often divided into two variants, based on the onset of the symptoms, early- and late onset sepsis, respectively. Early-onset sepsis (EOS) is variably defined in the literature. Some studies define EOS as sepsis that occurs in the first 3 days of life. In other studies it is classified as sepsis that occurs in the first week of life. Late onset sepsis (LOS) is also inconstantly defined according to the literature. For epidemiologic purposes it has been defined as presentation of sepsis after 72 hours to 7 days of life. The definition of EOS and LOS is therefore lacking consensus.

The symptoms of neonatal sepsis can be diverse, ranging from subtle signs such as feeding problems, fatigue and respiratory incidents to a state of full-blown septic shock. Due to a diverse clinical presentation it remains difficult to diagnose, even in a level 3 setting. Neonatal sepsis has to be distinguished from other diagnoses; like respiratory distress syndrome, hypoglycaemia, meningitis and necrotizing enterocolitis. Early diagnosis and prompt therapy optimize patient outcomes. Hence, knowledge and awareness of the wide range of symptoms, neonates at risk and the local epidemiology pattern are therefore pivotal information in a neonatal ward. Blood culture results are mostly obtained first after 48 hours to either confirm or reject the diagnosis. Other diagnostic parameters (CRP and total number of neutrophils) have also been associated with the diagnosis, but are inconclusive and less sensitive and thus can only be used as indication of the infection status. Despite a negative blood culture, some infants may show clinical and laboratory signs of infection, which requires antibiotic treatment and respiratory support. Some of these blood cultures may have been false negative, and further complicate the diagnosis of proven sepsis.

Neonatal sepsis is initially treated with antibiotics, which is dependent on multiple factors, among others, likely pathogen and its susceptibility, local epidemiology pattern, community acquired or hospital-acquired pathogens. Obstetric risk factors are most commonly associated with EOS, including prolonged rupture of the membranes, maternal fever, maternal antibiotic use and mothers that are infected with Group B positive streptococcus (GBS). The most common pathogen associated with EOS is GBS. The incidence has decreased due to elaborate use of maternal antibiotics to prevent GBS infection in the neonate. Pathogens associated with LOS are most commonly acquired from the environment. Thus, proper hygienic measures are important for both caregivers and family. Risk factors commonly associated with LOS are central venous inserted catheters and mechanical ventilation. The most common pathogen associated with LOS is coagulase-negative staphylococci.

Preterm infants are at increased risk of developing sepsis compared to term infants and it is inversely related to the degree of maturity. First of all, preterm infants have suboptimal immune systems, which makes them more prone to infection. Secondly, they have immature epithelial barriers, which makes it easier for pathogens to enter. Lastly, premature infants are
more likely to undergo invasive procedures such as central venous catheters or mechanical ventilation, which furthermore enhances the risk of infection. The most important risk factors of neonatal sepsis are very low birth weight and preterm birth. In addition studies have found that male gender and black race increase the risk of neonatal sepsis. Furthermore, a previous episode of neonatal sepsis increases the risk of recurrent sepsis.

Although preterm birth is defined at less than 37 completed gestational weeks, most studies on neonatal sepsis have focused on very preterm infants (GA <32 weeks) because of their high risk of mortality and serious morbidity. The incidence of neonatal sepsis in post-intensive care institutions (level 2 hospital) has not been investigated yet. In 2013, 80% of the newborn preterm infants in the Netherlands were born moderate to late preterm (GA 32-36 weeks), in contrast to 20% who were born very preterm (GA 24-31 weeks). Despite the majority of moderate to late preterm infants there is a paucity of information on the incidence and neonatal outcomes in this group. Especially the potential of serious adverse outcomes is of great consequence so it needs proper attention while caring for the preterm infant.

1.2 Post-Intensive care / High Care centre

In the Netherlands, preterm infants with gestational age (GA) <32 weeks are referred and born in a NICU. Often these infants are transferred to a post-Intensive Care/High Care (post-IC/HC) centre around the post-conceptional age of 32 weeks, to be treated until discharge. Preterm infants with a gestational age >32 weeks are normally born in a post-IC/HC centre, without the need of an intensive care treatment. OLVG East is a post-IC/HC centre.

1.3 Standard open-bay - versus single room family integrated care.

The current standard for neonatal care is a multi-patient, open-bay ward. In most level-2 hospitals the premature infant is cared for in an open-bay setting and not in single rooms. In this setting nurses care for the preterm infants. In the technological environment of the modern neonatal ward infants are physically, psychologically and emotionally separated from their parents. To address this issue, many programs, such as kangaroo care, skin-to-skin care and family-integrated care (FIC), encourage greater parent involvement. Family integrated care is performed in single rooms and might improve certain neonatal outcomes, including reducing sepsis rates. A study done in NICU preterm infants taken care with FIC concept showed in shortening of hospital stay and higher breastfeeding rates and growth rates. In OLVG East we introduced FIC in 2014.

1.4 Aim of study

This study will examine neonatal outcomes, especially the rate of early and late onset sepsis and also the risk factors of sepsis in very preterm (GA <32 weeks) and moderate to late preterm infants (GA 32-<36 weeks) admitted to the Neonatology Department in the OLVG East between 2009 and 2012. These infants were treated with standard neonatal care in an open bay. Since very preterm infants are more prone and vulnerable to diseases we expect that this group will have a higher incidence of neonatal sepsis than the moderately preterm infants (GA 32-<36 week). In future perspective, this study is important to evaluate the open bay ward treatment and its effect on neonatal outcomes in relation to single room family integrated care.
1.5 Research questions & hypothesis

1. What is the difference in incidence on sepsis rates between very preterm (GA <32 weeks, group A) and moderate - to late preterm infants (GA 32 - <36 weeks, group B) cared with standard neonatal care from admission till discharge?

2. What are other differences in in-hospital outcomes such as: readmission to the NICU, breastfeeding rates, total parenteral nutrition rates, central venous line, peripherally inserted catheter, respiratory support (mechanical ventilation/CPAP/ nasal prongs), NEC rates and length of hospital stay?

The following null hypothesis was formulated: there is no difference in sepsis rates between the very preterm neonates in group A (GA < 32 weeks, post-IC/HC) and the moderate-to late preterm infants in group B (GA 32- < 36 weeks).
2. Methods

2.1 Study design
This study was conducted at the OLVG East in Amsterdam, the Netherlands, in the time period from February 5th till June 25th 2016. Clinical and microbiological data were obtained retrospectively from existing digital and written obstetric and paediatric medical files. All preterm infants born at a gestational age <36 weeks admitted to the Neonatology Ward of the OLVG East in the time period 2009-2012 were included in the study.

2.2 Study setting
The OLVG East in Amsterdam provides post IC/HC neonatal care to preterm infants. Some of these infants were less than 32 weeks GA and were born in a neonatal intensive care unit (NICU). Preterm infants born after 32 weeks are treated in the OLVG East from birth onwards, mostly without the need of an intensive care treatment. Every year approximately 60 post-IC/HC preterm infants are admitted to the neonatal ward in the OLVG East from the NICU. Above this number, approximately 200 preterm infants per year are treated in OLVG East. Since October 2014 neonates are treated with the FIC concept. Before October 2014 all neonates were treated in an open bay ward. In the period of this current study, all neonates included were treated in a routine standard open bay setting. This study is a part of a larger trial to assess the effect of FIC on neonatal outcomes in relation to open bay ward treatment.

2.3 Study population
Very preterm infants were defined as infants born at a gestational age less than 32 0/7 weeks, and moderate to –late preterm infants were defined as infants born between 32 0/7 and 36 0/7 weeks gestation. The study consisted of these two groups of preterm infants: Group A (very preterm infants, post-IC/HC, GA<32 weeks) from admission until discharge and Group B (moderate - to late preterm infants, GA >32 to <36 weeks) from admission until discharge.

2.3.1 Inclusion and exclusion criteria
The following inclusion criteria were applied: all preterm infants born at a gestational age <36 weeks admitted to the Neonatology Ward of the OLVG East in the time period 2009-2012 were included in the study. Neonates born in OLVG and transferred to a NICU on their day of birth and later on re-admitted to OLVG, the second time of admission was defined as the first admission day. 
Exclusion criteria: preterm infants with a gestational age >36 weeks were not included in the study. Preterm neonates with multiple transfers between a NICU and OLVG were excluded from the study population (n=19).

2.3.2 Demographic characteristics
The patient characteristics described of both groups are: gender, gestational age in days, congenital disorders, birth weight, head circumference (HC) at birth, length at birth, place of birth (OLVG East, tertiary hospital, other level 2 hospitals than OLVG east or birth at home), type of birth (spontaneous vaginal, vaginal with an intervention, primary caesarean section and secondary caesarean section), primipara, multiparous, Apgar scores and antenatal corticosteroid therapy.

2.4 Infection protocol in OLVG east
In our centre all neonates with a suspected perinatal infection, a blood culture sample of minimum 1ml is collected before initiating antibiotic therapy. In Table 1 the antibiotic treatment regimen used in our centre is presented20.
Table 1. Protocol in our centre of antibiotic therapy in case of suspected neonatal sepsis.

<table>
<thead>
<tr>
<th>Probable diagnosis</th>
<th>Patient</th>
<th>Empirical therapy</th>
<th>Duration of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sepsis</td>
<td>≤ 48 hours p.p.</td>
<td>Penicillin + Gentamicin *</td>
<td>Negative blood culture &amp; no clinical symptoms: 48-72 hours</td>
</tr>
<tr>
<td></td>
<td>≥ 3 days p.p.</td>
<td>Cefuroxime</td>
<td>Negative blood culture &amp; sick child: 7 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive blood culture: 7-14 days**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• GBS: 10 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• E. Coli: 14 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• S. Epidermidis: 7 days</td>
</tr>
</tbody>
</table>

* in case of a negative blood culture: switch to cefuroxime after 48-72 hours if the antibiotic is continued
** depending on the causative organism

2.5 Diagnosis of sepsis

In case of clinical suspicion of neonatal sepsis, the attending paediatric resident or paediatrician in our hospital defined the diagnosis. Both EOS and LOS were defined as a combination of a positive blood culture and systemic signs of infection. Episodes of sepsis with organisms considered contaminants were not defined as a positive blood culture. High infection parameters in the blood supported the diagnosis, but were not crucial to the diagnosis due to the lack of diagnostic sensitivity.

2.5.1 Classification of EOS

Clinical EOS was defined as a clinical suspicion of infection without a proven blood culture. If the suspicion of EOS was high, for example in the presence of risk factors, high CRP value or persistent clinical instability, antibiotics were often continued for 7 days or more.

Not classified as EOS was defined as the cases in which there was a primary suspicion of infection with consequently collection of a blood culture sample and a directly initiation of antimicrobial treatment. If the blood culture was negative after 48 hours and the infant had an asymptomatic presentation there was a cessation of the antibiotic therapy. Also the cases in which no blood culture was drawn, but antibiotic treatment was given for 48 hours, counted as a non-classified sepsis.

A rejected diagnosis of EOS was defined as cases with a low suspicion of infection, with infection parameters not or mildly raised. Sometimes a blood culture was collected, but without initiating directly antibiotic treatment. Thus, the diagnosis of EOS was rejected and antibiotic treatment was not initiated.

2.5.2 Classification of LOS

The diagnosis of catheter related sepsis was also defined as proven LOS. When the blood culture was negative and the culture of the catheter was positive with a pathogen which required antibiotic treatment (e.g. staphylococcus Aureus) then we also registered this as bacteraemia. In suspicion of catheter related sepsis in proven LOS cases, removal of the line was done next to the standard treatment with antibiotics.
Clinical LOS was defined as a clinical suspicion of infection with a negative or contaminated (likely) blood culture. If the suspicion of LOS was high, for example in the presence of high CRP value, prematurity (eci) or persistent clinical instability (respiratory support, fever), antibiotics were often continued for 7 days or more despite negative blood culture results.

Not classified as LOS was defined as the cases in which there was a primary suspicion of infection with consequently collection of a blood culture sample and a directly initiation of antimicrobial treatment. If the result of the blood culture was negative after 48 hours combined with an asymptomatic presentation, this was the time of cessation of the antibiotic. Also the cases in which no blood culture was drawn, but antibiotic treatment was given for 48 hours, was registered as a non-classified sepsis. In some cases there was a suspicion of both sepsis and NEC, if the blood culture showed to be negative with a persistent suspicion of LOS this was not classified as LOS.

A rejected diagnosis of LOS was defined as the cases with suspicion of infection, with no or minimal raised infection parameters. In some of these cases a blood culture was drawn, without starting antibiotics. With no clinical symptoms, changes in the laboratory values and/or a negative blood culture after 48 hours, the diagnosis of LOS was rejected and antibiotic treatment was not initiated.

2.6 Classification of risk factors

In the literature a wide range of covariates are mentioned as possible risk factors for neonatal sepsis. These determinants are presented in Table 2 with their corresponding references from the literature.

<table>
<thead>
<tr>
<th>Table 2. Covariates of the outcome measure neonatal sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent variable</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Neonatal sepsis</td>
</tr>
<tr>
<td>EOS</td>
</tr>
<tr>
<td>LOS</td>
</tr>
</tbody>
</table>

Risk factors defined for neonatal sepsis were low birth weight, low gestational age, male gender and congenital disorder. Risk factors for EOS were defined as prolonged rupture of the membranes $>24$ hours (based on the current definition from the time period investigated), maternal fever of 38 degrees C or more ($\geq 100.4$ degrees F)$^{30}$, maternal positive Group B Streptococcal status and maternal antibiotic use (not antibiotics used for GBS infection). Risk factors for LOS were defined as days of central venous catheter (in place at time of infection or in situ $<2$ days prior to start infection), peripheral inserted catheter days (in place at time of infection or in situ $<2$ days prior to start infection) and a prolonged duration of parenteral nutrition.

It is important to mention that mechanical ventilation could not be included as a possible risk factor for LOS in our study because none of the preterm infants had mechanical ventilation.
prior to, or at time of infection during their stay in OLVG East.

2.7 Outcome parameters during stay
The following parameters were measured during the stay in OLVG:

2.7.1 Main study endpoints
The primary outcome was the percentage of neonatal sepsis diagnosed in the two studied groups. Rates of EOS (onset <72hours) and LOS (onset >72 hours) were registered.

2.7.2 Secondary study parameters
Other neonatal in-hospital outcomes we included were: antenatal corticosteroid therapy, Apgar scores, use of surfactant, total parental nutrition rates during admission, use of respiratory support >12 hours (Continuous Positive Airway Pressure (CPAP) or nasal prongs), central venous catheter use, intravenous catheter, diagnosis of bronchopulmonary dysplasia, intraventricular haemorrhage/periventricular leukomalacia, congenital heart defects, Down syndrome, length of hospital stay, breastfeeding versus formula feeding at discharge (fully breast milk, more than 75% of breast milk, any breast milk (<75%) and fully formula), readmission to a NICU during hospital stay, discharge to a NICU during hospital stay and any readmission within 9 months with recurrent late onset sepsis.

2.8 Data collection
Electronic and paper medical record database were conducted. Data used for this study was extracted from the doctor and nurse record. Published data from this study cannot be traced to a specific infant. The Medical Ethical committee of OLVG East approved this study.

2.9 Statistical analysis
The data were tabulated in Microsoft Excel and later transferred to SPSS 24.0. Statistical analysis was performed with SPSS 24.0 statistical software package (SPSS Inc., Chicago, IL). We calculated a power analysis before the study started with an alpha 0.05 and a power of 80%. Based on percentages from the literature the expected percentages were the following: group A (29.6% - 36.3%) and group B (17.5%). In Group A 29.6% is used as the reference to be able to do the power calculation on the smallest difference between Group A and Group B. Based on this power analysis, there should be at least 195 patients included in each group. However, this power analysis is based on an article from the NICU and not on numbers from level 2 hospitals.

We treated EOS and LOS as dichotomous variables. Variables were summarized as frequencies and percentages, medians or means and standard errors. Baseline variables and outcome variables between the two groups were tested with the chi square test, independent T-test and Mann-Whitney U test. A p-value of < 0.05 was considered statistical significant. All tests were two-sided unless otherwise stated. Continuous variables were presented as ‘mean’ SD. Categorical variables were presented as frequencies and percentages. To examine differences between continuous variables with a normal distribution, the student T test was used. We used the Chi square test to examine if there was a significant relation between gestational age and sepsis, EOS and LOS respectively. All tests were two-sided unless otherwise stated. Univariate analyses were performed to identify possible risk factors for neonatal sepsis. We assessed the effect of maternal and infant risk factors for LOS by multivariable binary logistic regression. It should be noted that the analyses were based on the first episode of LOS if an infant had more than one episode during the stay.
In order to determine a significant difference in neonatal sepsis between the two groups a chi square test was utilized. Statistical significance was set at the level $\alpha < 0.05$. To test the statement defined in the introduction, the following hypothesis was statistically tested:

$H_0$: there is no difference in sepsis rates between group A and group B.

$H_1$: there are higher sepsis rates in the post-IC/HC group (group A) than in group B (GA>32 -36weeks).
3 Results

3.1 Patient characteristics

During the 4-year period data of 597 preterm infants, which met our criteria for inclusion were analysed. There were 60.2% (n=357) admissions from preterm infants born in our hospital and 38.1% (n=226) infants admitted to our centre from the NICU. The median GA of the preterm infants included was 235 days. The median birth weight for all the neonates was 1910 grams. In group A, the median of GA was 206 days and in group B it was 240 days. The overall median length of stay in our centre was 18 days. The median for length of stay in group A was 34 days and in group B 16 days. Further, baseline characteristics of the two groups are shown in Table 3 below.

Table 3. Neonatal baseline characteristics from Group A and Group B

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group A</th>
<th>Group B</th>
<th>Total patient population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n (%)</td>
<td>163 (27.3%)</td>
<td>434 (72.7%)</td>
<td>597 (100%)</td>
</tr>
<tr>
<td>Median GA, days</td>
<td>206 (171-223)</td>
<td>240 (224-251)</td>
<td>235 (171-251)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>85 (52.1%)</td>
<td>195 (44.9%)</td>
<td>280 (46.9%)</td>
</tr>
<tr>
<td>M</td>
<td>78 (47.9%)</td>
<td>239 (55.1%)</td>
<td>317 (53.1%)</td>
</tr>
<tr>
<td>Median birth weight, g</td>
<td>1230 (610-2300)</td>
<td>2073 (890-3590)</td>
<td>1910 (610-3590)</td>
</tr>
<tr>
<td>Median HC at birth, cm</td>
<td>27 (17.5-22.5)</td>
<td>31 (27.0-35.3)</td>
<td>30 (21.5-35.3)</td>
</tr>
<tr>
<td>Median length at birth, cm</td>
<td>37 (31-45)</td>
<td>43 (27-50)</td>
<td>40 (27-50)</td>
</tr>
<tr>
<td>Birthplace</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 3</td>
<td>152 (93.8%)</td>
<td>74 (17.2%)</td>
<td>226 (38.1%)</td>
</tr>
<tr>
<td>OLVG East</td>
<td>8 (4.9%)</td>
<td>349 (81%)</td>
<td>357 (60.2%)</td>
</tr>
<tr>
<td>Other level 2 hospital</td>
<td>0 (0%)</td>
<td>5 (1.2%)</td>
<td>5 (0.8%)</td>
</tr>
<tr>
<td>Born at home</td>
<td>2 (1.2%)</td>
<td>3 (0.7%)</td>
<td>5 (0.8%)</td>
</tr>
<tr>
<td>Apgar score of &lt;6 at 5 min</td>
<td>30 (19%)</td>
<td>27 (6.2%)</td>
<td>57 (9.6%)</td>
</tr>
<tr>
<td>Antenatal steroids</td>
<td>113 (71.1%)</td>
<td>162 (37.5%)</td>
<td>275/591 (46.5%)</td>
</tr>
<tr>
<td>Congenital disorder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>40 (24.7%)</td>
<td>10 (2.3%)</td>
<td>50 (8.4%)</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>0 (0.0%)</td>
<td>2 (0.5%)</td>
<td>2 (0.3%)</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>0 (0.0%)</td>
<td>2 (0.5%)</td>
<td>2 (0.3%)</td>
</tr>
<tr>
<td>Mother primipara</td>
<td>80 (50%)</td>
<td>256 (59.7%)</td>
<td>336/589 (57%)</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal spontaneous</td>
<td>78 (48.1%)</td>
<td>227 (52.9%)</td>
<td>305 (51.6%)</td>
</tr>
<tr>
<td>Vaginal w/intervention</td>
<td>3 (1.9%)</td>
<td>34 (7.9%)</td>
<td>37 (6.3%)</td>
</tr>
<tr>
<td>Prim. C-section</td>
<td>20 (12.3%)</td>
<td>78 (18.2%)</td>
<td>98 (16.6%)</td>
</tr>
<tr>
<td>Sec. C-section</td>
<td>61 (37.7%)</td>
<td>90 (21%)</td>
<td>151 (25.5%)</td>
</tr>
<tr>
<td>Median length of stay, days</td>
<td>34 (0-76)</td>
<td>16 (0-127)</td>
<td>18 (0-127)</td>
</tr>
</tbody>
</table>

*Information was missing on HC at birth for 168 neonates, length at birth for 477, birthplace for 4, apgar score of <6 at 5 min for 6, antenatal steroids for 6, congenital disorder for 3, primipara for 8, mode of delivery for 6.

**Chi-square post hoc, significant adjusted p-value set at <0.00625.
3.2 Neonatal sepsis (both EOS & LOS) in Group A versus Group B

Table 4 depicts that 6% (36/597) of the preterm infants born at or admitted to OLVG were diagnosed with neonatal sepsis in the period from 2009 till 2012. The distribution of neonatal sepsis per year of admissions was the following: 11 infants with a diagnosis of sepsis in 2009, followed by 12 in 2010, 5 in 2011 and 8 in 2012.

3.2.1 Hypothesis

It was hypothesized that there would be more neonates diagnosed with neonatal sepsis in Group A than in Group B. In doing so, the Pearson chi square test was used with an alpha of .05 to determine the difference between sepsis rates in the two groups. There was a significant association between the type of group a neonate belonged to (according to GA) and whether or not sepsis was diagnosed \( x^2 (1) = 9.943, p < .002 \). This seems to represent the fact that, based on the odds ratio, the odds of neonates with sepsis were 2.7 times higher if they born with a gestational age <32 weeks (group A) than if they belonged to group B. Thus, the null hypothesis can be rejected.

Table 4 gives an overview of the different sepsis rates in group A and group B.

Table 4. Overview Sepsis rate in Group A and Group B

<table>
<thead>
<tr>
<th>Sepsis definition</th>
<th>Group A</th>
<th>Group B</th>
<th>Total patient population</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n (%)</td>
<td>163 (27.3%)</td>
<td>434 (72.7%)</td>
<td>597 (100%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Neonatal sepsis Patients with proven EOS</td>
<td>18 (11%)</td>
<td>18 (4.1%)</td>
<td>36 (6%)</td>
<td></td>
</tr>
<tr>
<td>Patients with proven LOS Episodes of LOS</td>
<td>0 (0%)</td>
<td>1 (0.3%)</td>
<td>1 (0.7%)</td>
<td></td>
</tr>
<tr>
<td>Clinical sepsis Clinical EOS</td>
<td>18 (11.2%)</td>
<td>17 (4%)</td>
<td>35 (5.9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Clinical LOS Underwent EOS evaluation</td>
<td>20 (12.3%)</td>
<td>17 (3.9%)</td>
<td>37 (6.2%)</td>
<td></td>
</tr>
<tr>
<td>Clinical EOS Underwent LOS evaluation</td>
<td>9 (5.5%)</td>
<td>38 (8.8%)</td>
<td>47 (7.9%)</td>
<td>0.191</td>
</tr>
<tr>
<td>Clinical LOS Underwent EOS evaluation</td>
<td>0</td>
<td>22</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Underwent LOS evaluation</td>
<td>9 (5.6%)</td>
<td>16 (3.7%)</td>
<td>25/589 (4.2%)</td>
<td>0.320</td>
</tr>
<tr>
<td>Underwent EOS evaluation</td>
<td>5 (3.1%)</td>
<td>141 (32.5%)</td>
<td>146 (24.5%)</td>
<td>0.000</td>
</tr>
<tr>
<td>Underwent LOS evaluation</td>
<td>41 (15.6%)</td>
<td>52 (12%)</td>
<td>93 (15.5%)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Neonatal sepsis: both EOS and LOS, clinical sepsis (both EOS and LOS)

Furthermore, Table 4 describes that 38.7% \((n= 231)\) of all the infants admitted underwent sepsis evaluation during their stay in our centre. Of these, 36 infants (15.6 %) of the infants were diagnosed with neonatal sepsis. In contrast to the evaluation for EOS, some of the infants evaluated for LOS, had more than one suspected episode of LOS during stay. There were a total of 90 suspected episodes of LOS. Of these, 40 (44.4%) episodes proved to be real LOS episodes. In addition, there were 13 episodes of suspected NEC during stay.

Moreover, a descriptive analysis of data was performed to verify the characteristics of neonates with and without sepsis.
Furthermore, data on early and late onset sepsis are presented, respectively.

### 3.3 Early onset sepsis

There were 400 eligible patients for an EOS. Eighteen patients belonged to group A and 382 patients belonged to group B. Of the eligible infants, 33% (n=132) had risk factors present for EOS; 3.3% of the infants had a mother with maternal fever (n=13, unknown=1), 7.3%...
(n=29, unknown=18) had a mother with a positive GBS status, 24% (n=96, unknown=2) had a mother with PROM > 24 hours and 15% (n=60, unknown=2) of the infants had mother who had used antibiotics that was not related to GBS status. Only one infant (male, GA 34 weeks) had a positive blood culture combined with clinical signs and what was considered to be a true EOS, of a total of 146 suspected episodes of EOS. The pathogen found was a group B streptococcus and was treated with antibiotic therapy for 10 days. In this case the following risk factors were present; male, maternal fever, PROM >24hrs, maternal antibiotic use and unknown maternal GBS status. The infant had a birth weight of 2800 gram. One infant had a proven blood culture with coagulase negative staphylococcus but was not considered to be at true EOS due to lack of clinical symptoms and antibiotic treatment. This infant was also male, with a gestational age of 34 6/7 weeks and a birth weight of 2600 gram. For this infant there were no risk factors for EOS present. Both cases were found in Group B. It is notable that there were 144 patients treated with antibiotics with a negative blood culture. The median duration of antibiotic use was 2 days.

In our study there were 23 neonates with suspected EOS and negative blood culture that received antibiotic treatment ≥ 5 days.

3.3.1 Difference of sepsis rate in very preterm infants and moderate-to late preterm infants
Because only one infant was diagnosed with a proven EOS in our study, testing the significance between different groups is not relevant and not to mention would compute an unrelible result. Although we can not conclude that one of the groups has a significant higher sepsis rate than the other, it is important to notice that group A had a zero event-rate.

3.3.2 Clinical EOS
Clinical EOS was diagnosed in 5.5% (22/400) of the eligible neonates for EOS. To assess the relation between clinical EOS and risk factors a Fisher exact test was used. We explored if there was a significant association between EOS and the presence of one or more of the following risk factors: positive GBS mother, PROM >24 hours, maternal fever, maternal antibiotic use (not related to GBS status). Our Fisher's exact test revealed that the percentage of clinical sepsis significantly differed by the presence of EOS risk factors (p= 0.023).

3.4 Late onset sepsis
There was a significant association between the two types of groups according to gestational age and whether or not LOS was diagnosed $x^2 (1) = 10.734, p < .001, OR=3.1$. This seems to represent the fact that, based on the odds ratio, the odds of neonates with LOS were 3.1 times higher if they belonged to group A than if they belonged to group B.

There were 590 eligible neonates with a possible risk of LOS. Of the admitted eligible patients, 14.5% (n=85) (14.4%) had a suspicion of LOS during their stay. Thirty-eight patients belonged to group A and 47 patients belonged to group B. In total there were 90 episodes of suspected LOS. Of these there were 42 suspected episodes in Group A and 48 in group B. A positive blood culture was found in 43.3% of the 90 suspected episodes of LOS. Thirty-five (38.9%) of the suspected episodes of LOS were defined as an episode of proven LOS. The median age of onset of the first episode of LOS was 9 days (range 4-48).

The median duration of antibiotic use in the neonates who underwent LOS evaluation was $X$ days. Some of the infants diagnosed with LOS progressively worsened and required intubation and invasive ventilation. These infants were transferred to NICU as fast as possible and may only have received one day of supportive treatment in our centre, but where also
included in the definition of proven LOS if the diagnosis sepsis was confirmed in the NICU. None of the infants with LOS admitted in our centre presented themselves with a recurrent LOS episode after discharge from our centre within 9 months. There were some infants (with no prior history of sepsis) who where re-admitted to our centre within 9 months presenting with the following: one infant with suspicion of LOS (negative blood culture, received antibiotic treatment for 7 days due to the clinical presentation), one infant with LOS shortly after transferred to a NICU, one infant with GBS sepsis, one infant suspected for bacteraemia, one infant with a recent history of meningococcal sepsis and one infant with urosepsis. There were also some infants who were suspected of having an infection, in which antibiotic treatment was given for 48 hours.

3.4.1 Distribution of pathogens found in proven blood cultures for LOS
It is notable that there were 84 patients who underwent late onset sepsis evaluation and were treated with antibiotics. In total there were 90 suspected episodes of late onset sepsis. Coagulase-negative staphylococci was the most commonly pathogen isolated (67.5%) in LOS, followed by enterococcus faecalis (7.5%).

In Figure 1 the other pathogens are described. Of the blood cultures, 5 were suspected of being contaminated, these are not shown in Figure 1.

Figure 1: Distribution of pathogens (n) in all episodes of LOS

Of all patients, 17.8% (n=106) neonates had a central venous catheter during stay. The total CVC days was 670. Furthermore, 65.7% (n=392) of the neonates had a PIC during stay, with an overall of 2065 days. Of all patients, 42.2% (n=252) received TPN during stay, with 1383 TPN days in total.

<table>
<thead>
<tr>
<th>Therapeutic intervention</th>
<th>Total interventions in all patients N (%)</th>
<th>Interventions in patients with LOS, n (%)</th>
<th>Interventions in patients without LOS, n=562 (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVC</td>
<td>106 (17.8%)</td>
<td>19 (54.3%)</td>
<td>87 (15.5%)</td>
<td>0.000</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>16 (2.7%)</td>
<td>5 (14.3%)</td>
<td>11 (2%)</td>
<td>0.000</td>
</tr>
<tr>
<td>TPN</td>
<td>252 (42.2%)</td>
<td>34 (97.1%)</td>
<td>217 (97.1%)</td>
<td>0.000</td>
</tr>
<tr>
<td>PIC</td>
<td>392 (65.7%)</td>
<td>35 (100%)</td>
<td>357 (63.5%)</td>
<td>0.775</td>
</tr>
<tr>
<td>O2</td>
<td>62 (10.4%)</td>
<td>4 (11.4%)</td>
<td>58 (10.3%)</td>
<td>0.781</td>
</tr>
<tr>
<td>CPAP</td>
<td>141 (23.6%)</td>
<td>21 (60%)</td>
<td>120 (21.4%)</td>
<td>0.000</td>
</tr>
<tr>
<td>FS</td>
<td>265 (44.4%)</td>
<td>30 (85.7%)</td>
<td>235 (41.8%)</td>
<td>0.000</td>
</tr>
<tr>
<td>Breast milk at discharge</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
No breast milk at discharge 100 (16.8%) 4 (11.4%) 96 (17.1%) 0.390
Breast milk yes any 158 (26.5%) 9 (25.7%) 149 (26.5%) 0.321
Breast milk >75% 91 (15.2%) 8 (22.9%) 83 (14.8%) 0.360
Fully breast milk at discharge NICU transfer during stay 236 (39.5%) 15 (42.9%) 221 (39.3) 0.448
Median length of stay, days 28 (4.7%) 5 (14.3%) 23 (4.1%) 0.000

Information is missing for O2 for 10 neonates, IV for 5, cvl for 1, TPV for 2, mechanical ventilation for 1, PIC for 6
* In this table all therapeutic interventions for all patients were registered and was not related to, or distinguished from time of infection.

### 3.4.2 Univariate logistic regression, proven LOS population
First we performed a univariate logistic regression analysis to detect possible predicting risk factors for being diagnosed with LOS. The inclusion of different factors was based on extensive literature search.

**Table 7. Univariate logistic regression of possible risk factors for proven LOS**

<table>
<thead>
<tr>
<th>Diagnose of proven LOS</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA at birth, days</td>
<td>0.983 (0.966-1.000)</td>
<td>0.047</td>
</tr>
<tr>
<td>Birth weight</td>
<td>0.999 (0.998-1.000)</td>
<td>0.005</td>
</tr>
<tr>
<td>CVC (without TPN), days</td>
<td>0.954 (0.578-1.574)</td>
<td>0.855</td>
</tr>
<tr>
<td>PIC (without TPN), days</td>
<td>0.959 (0.833-1.103)</td>
<td>0.557</td>
</tr>
<tr>
<td>TPN via CVC, days</td>
<td>1.208 (1.089-1.339)</td>
<td>0.000</td>
</tr>
<tr>
<td>TPN via PIC, days</td>
<td>1.253 (1.117-1.407)</td>
<td>0.000</td>
</tr>
<tr>
<td>Gender</td>
<td>1.162 (0.583-2.317)</td>
<td>0.669</td>
</tr>
<tr>
<td>Apgar score ≤6 at 5 min</td>
<td>0.272 (0.037-2.029)</td>
<td>0.204</td>
</tr>
<tr>
<td>Apgar score ≤6 at 1 min</td>
<td>0.604 (0.259-1.411)</td>
<td>0.244</td>
</tr>
<tr>
<td>Primipara</td>
<td>1.412 (0.685-2.911)</td>
<td>0.350</td>
</tr>
<tr>
<td>Multiparous</td>
<td>0.708 (0.344-1.460)</td>
<td>0.350</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal spontaneous</td>
<td>0.837 (0.723-0.969)</td>
<td>0.017</td>
</tr>
<tr>
<td>Vaginal w/intervention</td>
<td>1.417 (0.413-4.865)</td>
<td>0.579</td>
</tr>
<tr>
<td>Prim. C-section</td>
<td>1.067 (0.430-2.645)</td>
<td>0.889</td>
</tr>
<tr>
<td>Sec. C-section</td>
<td>2.272 (1.132-4.561)</td>
<td>0.021</td>
</tr>
<tr>
<td>Congenital disorder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart- related</td>
<td>1.467 (0.495-4.345)</td>
<td>0.489</td>
</tr>
<tr>
<td>Down syndrome</td>
<td>0.000 (0.000-.)</td>
<td>0.999</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>0.000 (0.000-.)</td>
<td>0.999</td>
</tr>
<tr>
<td>Surfactant use</td>
<td>1.524 (0.610-3.807)</td>
<td>0.368</td>
</tr>
<tr>
<td>Celestone use</td>
<td>1.762 (0.878-3.537)</td>
<td>0.111</td>
</tr>
<tr>
<td>Invasive ventilation previously in NICU</td>
<td>1.566 (0.689-3.559)</td>
<td>0.285</td>
</tr>
<tr>
<td>Previous diagnose of Clinical EOS</td>
<td>0.000 (0.000-.)</td>
<td>0.998</td>
</tr>
<tr>
<td>Previous sepsis in NICU</td>
<td>1.001 (0.999-1.003)</td>
<td>0.443</td>
</tr>
<tr>
<td>Twins</td>
<td>1.405 (0.691-2.857)</td>
<td>0.347</td>
</tr>
</tbody>
</table>
Risk factors EOS 0.548 (0.244-1.230) 0.145

Information was missing on Apgar score <6 at five minutes for 6 neonates, surfactant use for 2, birthplace for 4, IVH grade 1-2 for 1, congenital disorder for 3, mode of delivery for 6, IVH grade 3 for 1, NEC for 1.

Abbreviations: CVC (central venous catheter), TPN (total parenteral nutrition), PIC (peripheral intravenous catheter), Heart related congenital disorder = ODB, ASD, pulm. stenose, VSD, OFO

If the covariates had a p-value of 0.10 or lower in the univariate analysis (Table 6) they were considered for inclusion in the multiple logistic regression. Table 6 depicts that both CVC (without TPN) and IV (without TPN) were not associated with sepsis. GA at birth (days), birth weight (g), TPN via CVC (days), TPN via PIC (days), vaginal spontaneous delivery and secondary C-section all showed somehow an association with late onset sepsis.

3.4.3 Binary logistic regression of the population with proven LOS
To predict which factors were associated with LOS we performed a multivariable logistic regression. In this section the binary logistic regression is explained, in which different models were tested (with different variables) to find the best predicting model for LOS. It should be mentioned that TPN is always given via a CVC or a PIC, but is never given via both routes at the same time. To make a clinical relevant model we created two logistic regression models to be able to compare these two factors independently (TPN according to route of administration). To create the best model to predict neonatal sepsis we used 3 variables in each model, with next to route of administration (PIC vs CVC) the two other variables (GA, birth weight and vaginal spontaneous birth) being the same in the two different models.

3.4.3.1 Model 1
In the first part of logistic regression model 1 we used the following risk factors (p < 0.10): TPN via PIC (days), GA at birth (days) and birth weight (g). In the final possible predictor model only birth weight (0.009) and TPN via PIC (0.000) were found to be significant predictors (Table 8). It should be mentioned that in the previous predictor model where gestational age was included as a variable, the Cox & Snell R Square and Nagelkerke R Square demonstrated that between 3.5% and 9.5% of the variability could be explained by this set of variables. By removing the variable gestational age the variability explained by the predictor model was exactly the same as before. In addition the -2log-likelihood of the model did not change noteworthy, and went from 244.563 to 244.565, indicating that the accuracy of the model stayed the same when the factor gestational age was removed.

Table 8: Alternative 1 model 1, Multiple logistic regression of predictive risk factors for LOS

<table>
<thead>
<tr>
<th>Variables in multiple logistic regression</th>
<th>B</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, g</td>
<td>-0.001</td>
<td>0.999 (0.998-1.000)</td>
<td>0.009</td>
</tr>
<tr>
<td>TPN via PIC, days</td>
<td>0.217</td>
<td>1.243 (1.105-1.398)</td>
<td>0.000</td>
</tr>
<tr>
<td>Constant</td>
<td>0.213</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In this model the qui-square value for the Hosmer Lemeshow test was 15.224 with a significance level of 0.055 (> .05), indicating a support of the predictor model.

In the other alternately model we put the following risk factors (p < 0.10) in the multiple logistic regression: TPN via CVC (days), GA at birth (days) and birth weight (g). In the final possible predictor model only birth weight (0.014) and CVC with TPN (0.001) were found to be significant predictors (Table 10). It should be mentioned that in the previous predictor
model gestational age was included as a variable, the Cox & Snell R Square and Nagelkerke R Square demonstrated that between 2.8% and 7.8% of the variability could be explained by this set of variables. By removing the variable gestational age the variability explained by the predictor model was the same as in the initial model. In addition the -2log-likelihood of the model went from 248.429 to 248.442, indicating that the accuracy of the model stayed the same when the factor gestational age was removed.

In this model the chi-square value for the Hosmer Lemeshow test was 14.642 with a significance level of 0.067 (>0.05), indicating a support of the predictor model.

The OR and -2 log likelihood are practically identical in both model 1 (TPN via CVC) and model 2 (TPN via PIC). Therefore we can conclude that there is no difference in route of administration (PIC vs CVC) of TPN associated with sepsis.

3.4.3.2 Model 2

A second logistic regression model was conducted to test whether mode of delivery (vaginal spontaneous, secondary C-section) predicted sepsis. Secondary C-section did not contribute to the model. The following model (model 2) was defined when adjusting for vaginal spontaneous birth and had a higher OR constant than in model 1 and 2, which indicates a better model. Birth weight was also included instead of GA but did not result in a better model.

Table 10: Alternative 1 model 2: Multiple logistic regression of predictive risk factors for LOS

<table>
<thead>
<tr>
<th>Variables in multiple logistic regression</th>
<th>B</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA at birth, days</td>
<td>-0.019</td>
<td>0.981 (0.963-0.999)</td>
<td>0.039</td>
</tr>
<tr>
<td>Vaginal spontaneous</td>
<td>-0.768</td>
<td>0.464 (0.220-0.978)</td>
<td>0.044</td>
</tr>
<tr>
<td>PIC with TPV, days</td>
<td>0.182</td>
<td>1.199 (1.079-1.333)</td>
<td>0.001</td>
</tr>
<tr>
<td>Constant</td>
<td>1.735</td>
<td>5.668</td>
<td>0.411</td>
</tr>
</tbody>
</table>

Model 2 has better predictive value for sepsis than model 1. Also this second model (alternative 1 & 2) did not differ that much, with an almost identical OR and -2 log likelihood (242 vs 245).

3.4.4 Choice of predicting model for LOS

Due to the minuscule difference between the two alternatives in model 2, we can suggest that there is no difference in route of administration of TPN associated with sepsis. We can
therefore conclude that TPN (administered via a CVC or PIC) is an independent risk factor for LOS, and that TPN administration by itself is a risk factor for neonatal sepsis.

Since the difference between alternative 1 and 2 for model 2 is not very large we chose the model with the most predictive value, thus we chose the most conservative model. As can be seen in Table 11 and Table 12 respectively, the OR of TPN via PIC was 1.253 in alternative 1, in contrast to 1.199 for TPN via CVC in alternative 2.

Therefore the model with the best predicting value, next to the most conservative of the two, is model 2- alternative 2 with GA at birth, vaginal spontaneous birth and TPN via CVC. This model is furthermore, most clinical relevant because the prolonged TPN is mostly given via a CVC and not via a PIC.

3.4.5 Goodness of Fit for the final predictor model of late onset sepsis

The Hosmer Lemeshow test in Table 13 indicates the Goodness of Fit. In small sample sizes as in ours, the Goodness of fit should be interpreted with caution. In our final prediction model for risk factors for LOS (gestational age, TPN via CVC and vaginal spontaneous birth) the chi-square value for the Hosmer Lemeshow test is 9.738 with a significance level of 0.284. The significance level is >0.05; indicating a good fit of the predictor model.

Table 12: Measure of Goodness of Fit, Alternative 2, Model 2

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Hosmer Lemeshow test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late onset sepsis</td>
<td>Chi-square: 9.738. Df: 8. P= 0.284</td>
</tr>
</tbody>
</table>

3.4.6 Results of the final predicting model

This final predicting multivariate logistic regression model (model 2 - alternative 2) had the following combination of predictive factors for sepsis: GA at birth (OR: 0.981, CI 0.963-0.999), vaginal spontaneous birth (OR: 0.464, CI: 0.220-0.978) and TPN via CVC (OR: 1.199, CI: 1.079-1.333). The Cox & Snell R Square and Nagelkerke R Square demonstrated that between 3.2% and 8.8% of the variability could be explained by this set of variables.

This result showed that the odds decrease by a factor of 0.464 when comparing vaginal spontaneous mode of delivery with not vaginal spontaneous mode of delivery. Thus, there is a lower probability of having sepsis for neonates born with vaginal spontaneous mode of delivery.

Furthermore, it depicts that the odds of sepsis for neonates increase with factor 1.2 per day receiving TPV via CVC. Furthermore, in the univariate analysis, birth weight (OR: 0.999, CI: 0.998-1.000) seemed to predispose to LOS, but was not included in the final multivariate regression analysis due to an underpowered sample size.

Additionally, in the univariate analyse, secondary C-section (OR: 2.272, CI: 1.132-4.561) seemed to predispose to LOS, but this result was not confirmed by multivariate regression analysis evaluation.

The regression equation (y=a+bx) for the final predicting model is the following:

\[ Y = 1.735 + 0.182 \times (n, \text{ mean days of TPN via CVC}) + 0.768 \times (n) - 0.019 = \text{total sum} \]
4. Discussion
Our study of moderate-to late preterm infants is the first study to our knowledge in the Netherlands to explore the incidence and risk factors of neonatal sepsis in a level II neonatal ward. The study provides an overview of a four-year period in a secondary care setting of 597 admitted neonates. As the main purpose of this study was to describe the incidence rate of neonatal sepsis we will discuss this first.
The cumulative incidence rate of neonatal sepsis was 6%. This is line with previous data from the literature. The hypothesis (H0) of this study assumed that there would be no difference in sepsis rates between group A and group B. This null hypothesis could be rejected. There was a significant association between the type of group a neonate belonged to (according to GA) and whether or not sepsis was diagnosed (p < 0.002). This seems to represent the fact that, based on the odds ratio, the odds of neonates with sepsis were 2.7 times higher if they were born with a gestational age <32 weeks (group A) than if they belonged to group B. Although, the neonatal sepsis incidence rate presented as expected in this study, most studies were performed in NICUs with even more extreme preterm infants.

4.1 Early-onset sepsis
Despite the fact that a third (33%) of the eligible infants for EOS had a risk factor present, the rate of EOS was extremely low in our study, with only one infant diagnosed with a proven EOS. It is important to notice that group A (GA <32 weeks) had a zero event-rate. This can probably partially be explained by the fact that the number of eligible neonates for EOS was much smaller in group A (n=18) than the number in group B (n=382). Many of the neonates not eligible for EOS (admission >3 days postpartum) were in fact first admitted to a NICU before subsequently being admitted to OLVG. A logical explanation is therefore that due to the history of NICU admission in the past, a selection bias was created for EOS. The very low prevalence of EOS could also be explained by increased and elaborate use of antibiotics to prevent GBS infection in the neonate in the recent decennia. Moreover, a possible explanation of this low rate of EOS can be attributed to our definition of EOS in the first 3 days of life unlike the definition used in some studies with EOS presenting in the first week of life. However, this minuscule incidence of EOS is lower or in line with existing reports from NICUs around the world.

4.2 Late-onset sepsis
In this study, the vast majority of neonatal sepsis was LOS. LOS was diagnosed in 35 infants (5.9%), with the highest rate among the most premature infants (GA<32 weeks). The finding that the LOS prevalence was low is in agreement with other reports. The median age of onset for the first episode of LOS was after 7 days of life. This study confirmed that CONS are the most common organisms associated with LOS. Despite the fact that knowledge of sepsis outcomes in the moderate to late preterm infants is lacking, this study showed that almost 50% of LOS occurred in moderate-to late preterm infants (GA 32 - <36 weeks). This emphasizes the need for further research in this group. This is also supported by other reports.

4.2.1 Risk factors for neonatal sepsis
We aimed to describe predictive risk factors of neonatal sepsis. Because there was only one infant diagnosed with EOS, risk factors were only explored for late onset sepsis. The multivariate logistic regression analysis of 35 LOS neonates showed that TPN administered via a CVC, gestational age and vaginal spontaneous birth were the predicting factors of LOS.
An important finding was that TPN (administered via CVC or PIC) was found to be a significant risk factor for LOS, independently of route of administration. Our analysis revealed that nor CVC without TPN or PIC without TPN was found to be associated with LOS. Therefore, we believe that TPN is an independent risk factor of LOS in our study population. Though the underlying mechanism of TPN and infection is not fully understood, a study performed in neonatal piglets suggests an impairment of the neonatal gut barrier function as measured by increased epithelial permeability. The intestines contain Peyer's Patches, which are key players of the mucosal immune host response toward gut antigens and bacteria. A consequence of giving TPN and little enteral feeding is intestinal atrophy and thus a higher chance of translocation and resulting sepsis. In contrary to other studies, we did not find CVC or PIC to be independent risk factors of LOS. Early enteral feeding has been shown to decrease the rate of infection. One explanation is that it allows decreased use of TPN.

Gestational age was also found to be a predictive risk factor. The inverse relationship between gestational age and sepsis is well known in the literature. Our analysis showed that the odds of sepsis for neonates decrease with factor 0.981 per day of GA. Although, it was a predictive risk factor it would probably be stronger associated if time between birth and infection would be closer.

Although a colonized birth canal has been associated with GBS in EOS, this current study suggests that a vaginal spontaneous birth is a significant protective factor for LOS. Bulkowstein et al. found that a significant risk factor for EOS was, among others, non-vaginal delivery. During vaginal delivery, the contact with the maternal vaginal and intestinal flora is an important source for the start of the infant’s colonization. In a study done by Grönlund et al. it was shown that the primary gut flora in infants born by caesarean delivery may be disturbed for up to 6 months after birth. The hygiene hypothesis suggests that an overly clean microbial environment at birth may contribute to the development of certain childhood diseases. Thus, in our study there was a lower probability of having sepsis for neonates born with vaginal spontaneous mode of delivery. A possible explanation can be that neonates are then colonized with the normal flora of the mother, with a resulting protecting effect. Further research on a greater scale is indicated to understand this fully.

4.3 Antibiotic treatment regimen
An important finding in our study was that almost 40% of the neonates admitted to our centre underwent sepsis evaluation and received antibiotic treatment. In other words, a suspicion of infection with collection of blood culture sample and thereupon initiating broad spectre antibiotic treatment. According to our findings 144 neonates with a suspicion of EOS received antibiotic treatment with a negative blood culture within 24 hours. As mentioned in the introduction early diagnosis and therapy optimize patient outcomes. The time to positivity of a blood culture remain a challenge with respect to unnecessary use of empirically started antibiotics. Furthermore, though promptly initiation of empirical therapy in a timely manner is evident, growing resistance against antibiotics should not be neglected and remains a significant problem in treatment decisions. Recent studies suggest an association with prolonged administration of antimicrobial agents (>5 days) in infants with suspected EOS (and negative blood cultures) with death and necrotizing enterocolitis. Of 144 infants who underwent evaluation of EOS and had negative blood cultures, 16% (n=23) received antibiotic treatment > 5 days. In our study a small sample size did not confirm the findings of this recent study with none of these 23 infants developing a NEC during stay. In the same population the mortality rate was 0. This could partially be explained by the fact that very sick
neonates are transferred to NICU and consequently being diagnosed with NEC at admission in NICU.

4.4 Post intensive care setting versus NICU
Neonatal outcomes are important markers for the efficacy and quality of the different levels of neonatal care. Moreover, complications from premature birth contribute to 35% of neonatal deaths globally; therefore, efforts to improve care of preterm infants are imperative. Because most studies on neonatal sepsis have been done in neonatal intensive care units (level 3 hospital), it is difficult to compare the findings in this study to other studies. Prompt diagnosis of neonatal sepsis results in early initiation of therapy. It is important to describe possible improvements in prevention and treatment of neonatal infection. Therefore studies performed in a secondary care setting are valuable for information to improve certain types of treatment options and mapping of possible risk factors.

Even though incidence is low in the moderate to late preterm infants, the potential for serious adverse outcomes after neonatal sepsis is of significant importance. This study is important to evaluate the open bay ward treatment and its effect on neonatal outcomes in a post-IC/HC setting. These centres treat very preterm infants with a history of neonatal intensive care and often these neonates already were diagnosed with a sepsis in the NICU before admitted to our centre. Although not proven in our study, it is known in the literature that a previous diagnosis of sepsis increases the risk of recurrent sepsis.

4.5 Open bay ward versus FIC
As mentioned earlier this study will be a part of a future study, which will evaluate the FIC concept with standard routine care. Investigators have found that increased skin to skin contact decrease rates of sepsis. In the current standard of neonatal care requires frequent contact with hospital personnel, which in the case of incomplete hygienic measures could also increase the risk of infection. Whether or not FIC improves neonatal outcome and is superior to standard care, is yet to be proven on a greater scale, also in the OLVG East.

4.6 Strengths and limitations
The strengths of this study were that it was a single centre study with the use of consistent definitions of neonatal sepsis and outcomes by the neonatologists, as well as the same standard of care for all infants in the study period. This study is the first to our knowledge to explore incidence and risk factors of neonatal sepsis in a post intensive care/high care institution.

This study has some limitations that need to be accounted for in the interpretation of the present findings. Its retrospective nature may limit it’s conclusions. Furthermore, the obstetric and paediatric records were not always complete documented. In some of the patient records referral letters from the NICU were missing. Furthermore, the rate of EOS was extremely low in this study, with only one infant diagnosed with a proven sepsis. A logical explanation is due to the selection bias of the study. The neonates born at a GA <32 weeks were mostly admitted to a NICU before being admitted to our centre. A rule of thumb in logistic regression is that the number of parameters in a multivariable model should be maximum 5-10% of the minimum of the number of people with and without the event of the analysis (LOS). Since our study is underpowered with only 35 neonates with LOS, we chose to include maximum 3 variables into the analysis. Though it is known in the literature that the rate of infection is inversely related to birth weight and gestational age this was less evident in our study. Because of the power, we could not correct for birth weight together with gestational age. It should be mentioned that when including
birth weight instead of gestational age, the predictive model was less good at predicting the risk factors for LOS.

4.7 Practical consequences of the findings/clinical and research implications

As mentioned in the introduction, it can be difficult to diagnose neonatal sepsis. Prevention is the key to lower neonatal sepsis rates and adherence to infection protocols in every neonatal ward is of utmost importance. Our study showed that TPN was a predictive risk factor for LOS. Avoiding unnecessary use of TPN, next to prolonged administration should be avoided. Fivez et al showed that early administration of total parenteral nutrition resulted in more infections.\textsuperscript{42} In future perspective, this study is important to evaluate the open bay ward treatment and its effect on neonatal outcomes in relation to single room family integrated care. The fact that vaginal spontaneous birth was a protecting factor suggests that implementation of treatment choices of mode of delivery may be revised. Implementation of FIC may further decrease the incidence of sepsis by means of single room effect. Further research is required to confirm this. In addition frequent mapping of epidemiology of neonatal sepsis is necessary to detect new trends and changes in pathogenic microbes, hygienic standards and infant population more at risk. A better understanding of the effect of TPN and mode of delivery on neonatal sepsis is also needed to create improved treatment policies and awareness of populations at risk.

5. Conclusion

In conclusion, this study shows a minuscule prevalence of EOS and a low prevalence of LOS in very preterm and moderate- to late preterm infants in a level 2 Neonatal Ward. The highest rate of neonatal sepsis was identified among neonates with a gestational age of less than 32 weeks. Vaginal spontaneous birth was found to be a significant protective factor for late onset sepsis. TPN administration (CVC, IV) and gestational age were found to be significant risk factors for development of late onset sepsis in preterm infants.
6. References
33. Li Z, Xiao Z, Li Z, Zhong Q, Zhang Y, Xu F. 116 cases of neonatal early-onset or late-


7. List of abbreviations

EOS: Early-onset sepsis
LOS: Late-onset sepsis
TPN: Total parenteral nutrition
CVC: Central venous catheter
PIC: Peripheral inserted catheter
OLVG East: Onze Lieve Vrouwe Gasthuis, locatie oost
METC: Medisch Ethische Toetsingscommissie
Post-IC/HC centre: post-intensive care/high care centre
FIC: Family Integrated Care
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