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Risk Factors for Late-Onset Sepsis in Preterm Infants: A Multicenter Case-Control Study

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Keywords
Risk factors · Parenteral feeding · Breast feeding · Late-onset sepsis · Coagulase-negative staphylococcus

Abstract
Background: Late-onset sepsis (LOS) in preterm infants is a leading cause of mortality and morbidity. Timely recognition and initiation of antibiotics are important factors for improved outcomes. Identification of risk factors could allow selection of infants at an increased risk for LOS. ***Objectives:*** The aim was to identify risk factors for LOS. ***Methods:*** In this multicenter case-control study, preterm infants born at ≤30 weeks of gestation were included at 9 neonatal intensive care units. Detailed demographical and clinical data were collected daily up to day 28 postnatally. Clinical and demographic risk factors were identified using univariate and multivariate regression analyses in a 1:1 matched case-control cohort. ***Results:*** In total, 755 infants were included, including 194 LOS cases (41 gram-negative cases, 152 gram-positive cases, and 1 fungus). In the case-control cohort, every additional day of parenteral feeding increased the risk for LOS

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(adjusted OR = 1.29; 95% CI 1.07–1.55; $p = 0.006$), whereas antibiotics administration decreased this risk (OR = 0.08; 95% CI 0.01–0.88; $p = 0.039$). These findings could largely be attributed to specific LOS-causative pathogens, since these predictive factors could be identified for gram-positive, but not for gram-negative, LOS cases. Specifically cephalosporins administration prior to clinical onset was inversely related to coagulase-negative staphylococcus LOS (CoNS-LOS) development. Formula feeding was an independent risk factor for development of CoNS-LOS (OR = 3.779; 95% CI 1.257–11.363; $p = 0.018$). ***Conclusion:*** The length of parenteral feeding was associated with LOS, whereas breastmilk administration was protective against CoNS-LOS. A rapid advancement of enteral feeding, preferably with breastmilk, may proportionally reduce the number of parenteral feeding days and consequently the risk for LOS.

Introduction

Late-onset sepsis (LOS), defined as sepsis onset after 72 h of life, is a leading cause of mortality in the neonatal intensive care unit (NICU) [1]. The incidence rates for LOS in preterm infants vary between 20 and 38% in the first 120 days of life, and mortality rates range from 13 to 19% [1–4]. Survivors are at risk for prolonged hospitalization, development of necrotizing enterocolitis (NEC), bronchopulmonary dysplasia, and neurodevelopmental impairment [1, 2, 5]. The diagnosis of LOS in daily clinical practice may be challenging, especially in preterm infants, as clinical symptoms have limited sensitivity and specificity. The gold standard for diagnosis is confirmation of a pathogen in the blood culture, which is limited by suboptimal sensitivity and delay of a definite diagnosis because of the turnaround time to become positive [6]. In addition, screening of bodily fluids (e.g., blood and urine) may also require an invasive procedure, increasing the risk for LOS independently [6]. Several studies have identified risk factors for LOS, including a lower birth weight, gestational age (GA), and the presence of central venous catheters [1, 3, 7]. In addition, breastmilk feeding within the first month of life has been shown to be protective against LOS development [8]. However, most of these studies are characterized by a small number of cases, retrospective and single-center study designs, and the absence of detailed (daily) clinical data, limiting the possibility of adequate matching with controls and thus the ability to draw firm conclusions. Identification of independent risk factors for LOS in preterm infants may allow selection of infants at an increased risk and the development of novel, personalized therapeutic strategies aimed at reducing the LOS incidence. Therefore, we aimed to identify independent risk factors contributing to the development of LOS in preterm born infants in a multicenter case-control study with an overview of the clinical characteristics of patients with LOS within the first month of life.

Materials and Methods

Patients and Data Collection

This case-control study was conducted between October 2014 and January 2017 at 2 level II and 7 level III NICU situated in The Netherlands and Belgium (online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000497781). None of the participating centers administered probiotics routinely. The current study was nested in an ongoing study on the identification of early diagnostic biomarkers for NEC and LOS [9]. In that study, fecal samples and clinical data were collected (if applicable on a daily base) from infants born at a GA ≤30 weeks, up to 28 days' postnatal age (Table 1). In order to identify factors associated with LOS development the variables were assessed prior to clinical onset in the matched case-control cohort. In case of transfer from the NICU to a referral hospital or death prior to 28 days' postnatal age, data collection was ceased.

Matching Procedure

From this original cohort, infants diagnosed with LOS were strictly matched to 1 healthy control infant based on GA (±3 days), birthweight (±10 g), and postnatal age at LOS onset (±0 days). Infants who developed LOS as defined below were included as cases, and infants who did not develop LOS were included as controls. Both cases and controls were excluded in case of early-onset sepsis (positive blood cultures <72 h postnatally) or in case of NEC (≥Bell's stage 2A) or SIP during the follow-up period and an incomplete or missing medical file.

Definitions

LOS cases were defined as infants with a pathogen isolated from the blood culture drawn ≥72 h postnatally and pathogen-based antibiotic treatment was continued for ≥5 days, according to Vermont Oxford criteria [10]. Only the first LOS episode was included in the analysis. Isolated pathogens from blood cultures were classified into 4 categories: gram-positive bacteria, gram-negative bacteria, fungi, and coagulase-negative staphylococci (CoNS). A CoNS-positive culture was considered a CoNS-LOS when a CRP level ≥10 mg/L was measured within 72 h of LOS onset. When ≥2 pathogens were isolated from the blood culture (one being CoNS), the presence of CoNS was considered as contamination. Remaining definitions of collected data are described in Table 1.

Statistical Analysis

Statistical Package for the Social Science (SPSS) version 22.0 (IBM, Armonk, NY, USA) was used for the statistical analysis. First, during the entire inclusion period of 28 days, collected demographic and clinical variables from all infants with LOS were compared with non-LOS cases. Potential associations between as-

Table 1. Collected variables and definitions

Perinatal variables	Postnatal variables
Delivery mode (i.e., vaginal or caesarian section)	Gestational age
Single or multiple births	Birthweight
Preterm premature rupture of membranes (i.e., ≥24 h before delivery)	Apgar score
Meconium-stained amniotic fluid	Patent ductus arteriosus
	Medication
	Antibiotics 1 Total duration of treatment (days) 2 Duration of antibiotic treatment initiated within 24 h postnatally (0, 1–3, or >3 days) 3 Antibiotic exposure (yes/no)
	Ventilation mode
	Diagnosis of necrotizing enterocolitis
	Diagnosis of spontaneous intestinal perforation
	Diagnosis of sepsis, including the causative pathogen: 1 Gram-positive bacteria (including CoNS) 2 Gram-negative bacteria 3 Fungi 4 CoNS
	Number of red blood cell transfusions
	Central (umbilical line and/or peripherally inserted central catheter) and peripheral venous catheter use: 1 Cumulative number of days a certain line was present 2 Presence of a line within 48 h prior to LOS onset
	Parenteral feeding practices (lipids or amino acids)
	Enteral feeding practices 1 Breast-fed, defined as the daily average enteral feeding volume consisting of >80% breastmilk, including donor milk 2 Formula-fed, defined as the daily average enteral feeding volume consisting of >50% formula 3 Combination of breastmilk and formula, that encompasses infants not meeting the criteria for (1) and (2)
	Time to full enteral feeding, defined as at least 2 consecutive days without additional parenteral feeding administration
	Radiologic results (i.e., abdominal radiography)
	Laboratory results (i.e., C-reactive protein and blood cultures)

sessed variables and the development of LOS were identified via univariate logistic regression analysis.

Secondly, in the strictly 1:1 matched case-control cohort univariate logistic regression analysis was performed on clinical and demographical variables in the period preceding the day of LOS onset. Subsequently, independent risk factors were identified via multivariable regression analysis. This model was constructed using the backward likelihood ratio method, ultimately including statistically significant variables (p value <0.05). Variables included in this model were selected based on their two-tailed p value calculated from the univariate regression analyses. Only variables with a p value ≤0.30 were included. For every 10 cases one variable was included in the multivariable regression analysis. Results were considered statistically significant for p values ≤0.05.

Thirdly, potential predictive factors were assessed for the 3 subgroups via univariate logistic regression. In addition, independent

Table 2. Characteristics of LOS infants and matched controls in the period preceding LOS diagnosis (T_0)

	LOS (n = 194)	Non-LOS (n = 194)	Univariate analysis[1]	p value	Multivariate analysis[1]	p value
Median gestational age (IQR), weeks+days	27+1 (25+5–28+5)	27+1 (25+5–28+5)	1.000 (0.984–1.017)	0.980		
Mean birth weight (±SD), g	965.85±280.21	966.64±273.71	1.000 (0.999–1.001)	0.978		
Gender, male, n (%)	106 (54.9)	97 (50.3)	1.206 (0.808–1.799)	0.359		
Vaginal delivery, n (%)	89 (46.1)	99 (51.3)	1.078 (0.722–1.609)	0.715		
Multiple births, n (%)	74 (38.3)	65 (33.7)	1.208 (0.796–1.833)	0.375		
PPROM, n (%)	46 (23.8)	47 (24.4)	0.999 (0.626–1.595)	0.998		
Meconium amniotic fluid, n (%)	4 (2.1)	4 (2.1)	1.011 (0.249–4.106)	0.988		
Median 1-min Apgar score (IQR)	5 (3–7)	6 (3–7)	0.991 (0.909–1.081)	0.839		
Median 5-min Apgar score (IQR)	7 (6–8)	7 (6–8)	0.982 (0.872–1.105)	0.759		
PDA, n (%)	76 (39.4)	62 (32.1)	1.430 (0.617–3.315)	0.404		
PDA treatment type, n (%)						
Ibuprofen	70 (36.3)	59 (30.6)	Reference	0.840		
Indomethacin	4 (5.4)	2 (3.2)	1.686 (0.298–9.531)	0.555		
Surgical	0	1 (1.6)	NA	1.000		
Central line exposure, n (%)	149 (77.2)	161 (83.4)	0.673 (0.405–1.117)	0.126		
Median central line time (IQR), days	8 (6–10)	7 (5–10)	1.028 (0.963–1.097)	0.403		
Central line exposure 48 h prior T_0, n (%)	111 (57.5)	118 (61.1)	0.860 (0.573–1.292)	0.468		
Peripheral line exposure, n (%)	186 (96.4)	187 (96.9)	0.853 (0.281–2.585)	0.778		
Median peripheral line time (IQR), days	7 (4–10)	7 (4–9)	1.006 (0.961–1.054)	0.790		
Peripheral line exposure 48 h prior T_0, n (%)	154 (79.8)	141 (73.1)	1.456 (0.907–2.339)	0.120	1.693 (0.943–3.043)	0.078
Median RBC transfusion time (IQR), days	2 (1–2)	2 (1–2)	1.097 (0.871–1.382)	0.432		
Invasive ventilation exposure, n (%)	103 (53.4)	107 (55.4)	0.920 (0.616–1.373)	0.683		
Median invasive ventilation time (IQR), days	4 (2–9)	5 (2–9)	1.000 (0.947–1.056)	0.996		
Noninvasive ventilation exposure, n (%)	175 (90.7)	166 (86.0)	1.581 (0.840–2.978)	0.156		
Median noninvasive ventilation time (IQR), days	7 (4–9)	6 (4–10)	0.996 (0.955–1.040)	0.870		
Enteral feeding type, n (%)						
Breast milk	80 (41.5)	77 (39.9)	Reference	0.716		
Formula milk	49 (25.4)	41 (21.2)	1.150 (0.684–1.934)	0.597		
Combination	56 (29.0)	59 (30.6)	0.914 (0.565–1.478)	0.713		
Achieved full enteral feeding, n (%)	27 (14)	36 (18.7)	0.854 (0.482–1.511)	0.587		
Median total of parental feeding time (IQR), days	9 (7–11)	8 (6–10)	1.095 (1.018–1.177)	*0.014**	1.125 (1.041–1.216)	*0.003**
Total time from birth (days), n (%)						
0–5	20 (10.4)	34 (17.6)	Reference	0.090		
5–10	53 (27.5)	68 (35.2)	1.325 (0.686–2.561)	0.402		
>10	55 (28.5)	46 (23.8)	2.033 (1.033–4.000)	*0.040**		
Medication, n (%)						
Inotropes	11 (5.7)	19 (9.8)	0.341 (0.116–1.001)	0.050		
Antimycotics	9 (4.7)	11 (5.7)	0.491 (0.158–1.527)	0.219		
Postpartum antibiotic administration (days), n (%)						
None	27 (14.0)	28 (14.5)	Reference	0.872		
1–3	111 (57.5)	106 (54.9)	1.086 (0.601–1.963)	0.785		
>3	55 (28.5)	59 (30.6)	0.967 (0.508–1.840)	0.918		
Antibiotic exposure (yes), n (%)	176 (91.2)	184 (95.3)	0.506 (0.220–1.166)	0.110		
Median antibiotic time (IQR), days	4 (3–6)	3 (2–6)	1.027 (0.956–1.103)	0.469		
Antibiotic exposure per group, n (%)						
Aminoglycoside	150 (77.7)	152 (78.8)	1.177 (0.667–2.076)	0.575		
Carbapenem	2 (1.0)	6 (3.1)	0.339 (0.068–1.703)	0.189	0.257 (0.048–1.386)	0.114
Cephalosporin	32 (16.6)	52 (26.9)	0.560 (0.340–0.923)	*0.023**	0.562 (0.320–0.987)	*0.045**
Glycopeptide	21 (10.9)	26 (13.5)	0.818 (0.442–1.515)	0.523		
Macrolides	2 (1.0)	4 (2.1)	0.514 (0.093–2.844)	0.446		
Oxazolidinones	0	0	NA	NA		
Penicillin (-clavulanic acid)	170 (88.1)	177 (91.7)	0.960 (0.304–3.036)	0.945		
Quinolones	0	0	NA	NA		
Rifampicin	0	0	NA	NA		
Trimethoprim-sulfamethoxazole	1 (0.5)	0	NA	NA		
Mortality, n (%)	13 (6.7)	3 (1.6)	4.574 (1.282–16.317)	*0.019**		
Median age at death (IQR), days	17 (10–21)	13 (10–13)	1.097 (0.869–1.383)	0.437		
Discharge before 28 days, n (%)	50 (25.9)	54 (28.0)	0.900 (0.574–1.411)	0.646		
Median age at discharge (IQR), days	18 (13–21)	18 (12–21)	0.996 (0.928–1.068)	0.905		

LOS, late-onset sepsis; NA, not applicable; T_0, clinical onset of sepsis; PPROM, preterm premature rupture of membranes; PDA, patent ductus arteriosus; RBC, red blood cells. [1] Values are presented as OR (95% CI). * Statistically significant difference.

risk factors were identified using multivariable logistic regression models. This model was constructed using the forward likelihood ratio method, considering the smaller sample size, ultimately including statistically significant variables (p value <0.10). Other statistical settings remained the same as described for the total matched case-control cohort.

All results are displayed as (unadjusted) OR with corresponding 95% CI and p values.

Results

In total, 755 infants were included in the analysis, constituting 194 LOS cases (23%). The demographic and clinical characteristics of the LOS cases and controls in the overall cohort are depicted in online supplementary Table 2. Further clarification of the LOS incidence, the causative pathogen distribution, and the median age

of onset are provided in online supplementary Tables 3 and 4.

Table 2 provides an overview of the demographics and characteristics of the LOS cases versus the matched controls (1:1) irrespectively of the causative pathogen. Duration of parenteral feeding was identified as an independent risk factor for LOS development irrespectively of the causative pathogen (OR = 1.125; 95% 1.041–1.216; p = 0.003). Third-generation cephalosporins administration was identified as an independent factor inversely associated with LOS development (OR = 0.562; 95% CI 0.320–0.987; p = 0.045). Remaining variables showed no significant differences.

Gram-Negative Bacteria

No differences in clinical and demographic characteristics were found between gram-negative LOS cases and controls prior to LOS onset. However, a higher mortality rate was observed in LOS cases (unadjusted OR = 11.400; 95% CI 1.367–95.043; p = 0.025) (Table 3).

Gram-Positive Bacteria

Duration of parenteral feeding (days) was identified as an independent risk factor for Gram-positive LOS (OR = 1.289; 95% CI 1.074–1.547; p = 0.006). Antibiotics exposure prior to clinical onset was inversely related to LOS development (OR = 0.078; 95% CI 0.007–0.879; p = 0.039). Remaining variables showed no significant differences (Table 4).

Coagulase-Negative Staphylococcus

The total number of days of peripheral line exposure (OR = 1.238; 95% CI 1.086–1.411; p = 0.001) and formula feeding (OR = 3.779; 95% CI 1.257–11.363, p = 0.018) preceding clinical onset were identified as independent risk factors for CoNS-LOS. Administration of third-generation cephalosporins was found to be an independent factor inversely associated with CoNS-LOS (OR = 0.229; 95% CI 0.086–0.612; p = 0.003). There were no significant differences regarding the remaining variables between the 2 subgroups (Table 5).

Discussion

This case-control study aimed at identifying demographic and clinical risk factors associated with the development of LOS in preterm infants in a multicenter setting. We demonstrated that every additional day of parenteral feeding was associated with an increased risk of LOS development. Third-generation cephalosporins administration was identified as an independent factor inversely associated with the development of CONS-LOS, whereas formula feeding was associated with an increased risk.

In a previous study, formula-fed infants showed increased odds for CoNS-LOS development compared to breast-fed infants [11], and this was confirmed in the current study. Breast milk might be protective due to its anti-infective, microbiome-modulating, and immune-stimulatory properties [12]. Several studies have demonstrated that infants who receive breastmilk are more likely to achieve full enteral feeding at an earlier stage compared to formula-fed infants, resulting in earlier cessation of parenteral feeding [1, 4, 13]. We demonstrated that exposure to parenteral feeding for more than 10 consecutive days was associated with an increased risk of LOS development. It could be debated whether clinicians should aim to limit the exposure to parenteral feeding to no longer than 10 days by a more rapid advancement of enteral feeding with preferably breastmilk to reduce the risk of LOS development. On the contrary, a rapid advancement of enteral feeding might increase the risk for NEC development. However, studies have shown that rapid advancement of the enteral feeding volume within the first week of life is not significantly associated with NEC in preterm and very low birthweight infants [14, 15].

In this study, exposure to antibiotics was associated with decreased odds for the development of gram-positive LOS, irrespectively of the type and duration of antibiotics. Cephalosporin exposure was associated with a decreased risk for CoNS-LOS, possibly due to the sensitivity of CoNS species to cephalosporins. Therefore, exposure to this agent could reduce the risk of invasion of CoNS from either the skin or the gut into the bloodstream [16, 17]. However, implementation of routine administration of cephalosporins in preterm infants remains a topic of debate mainly because of the increased risk for colonization with extended-spectrum β-lactamase producing bacteria [18]. The observed protective effects of early exposure to specific antibiotics against the development of LOS indicate that the microbiota may be involved in the pathophysiology of at least a selection of LOS cases. The influence of early microbiota colonization and alterations in microbiota composition in LOS pathophysiology has been considered in several studies [19, 20]. This phenomenon might lead to the development of strategies aimed at early manipulation of the microbiota to prevent LOS development, for example by administration of probiotics instead of antibiotic pro-

Table 3. Characteristics of LOS infants caused by gram-negative bacteria and matched controls in the period preceding LOS diagnosis (T_0)

Characteristic	LOS (n = 39)	Non LOS (n = 39)	Univariate analysis[1]	p value
Median gestational age (IQR), weeks+days	26+2 (25+2–28+1)	6+2 (25+2-28+1)	1.000 (0.964–1.038)	0.992
Median birth weight (IQR), g	930.0 (740.0–1170.0)	865 (760.0–1135.0)	1.000 (0.998–1.001)	0.985
Male gender, *n* (%)	19 (48.7)	22 (56.4)	0.734 (0.301–1.790)	0.497
Vaginal delivery, *n* (%)	24 (61.5)	25 (64.1)	1.116 (0.445–2.797)	0.815
Multiple births, *n* (%)	13 (33.3)	16 (41.0)	0.719 (0.286–1.807)	0.483
PPROM, *n* (%)	7 (17.9)	11 (28.2)	0.557 (0.190–1.631)	0.286
Meconium amniotic fluid, *n* (%)	1 (2.6)	1 (2.6)	1.000 (0.060–16.594)	1.000
Median 1-min Apgar score (IQR)	5 (3–6)	6 (4–7)	0.885 (0.721–1.086)	0.242
Median 5-min Apgar score (IQR)	7 (6–8)	7 (6–8)	0.901 (0.668–1.217)	0.497
PDA, *n* (%)	18 (46.2)	14 (35.9)	0.643 (0.053–7.832)	0.729
PDA treatment type, *n* (%)				
Ibuprofen	15 (38.5)	13 (33.3)	NA	NA
Indomethacin	0	0	NA	NA
Surgical	2 (5.1)	1 (2.6)	NA	NA
Central line exposure, *n* (%)	36 (92.3)	34 (87.2)	1.765 (0.391–7.958)	0.460
Median central line time (IQR), days	8 (5–12)	8 (5–11)	1.006 (0.899–1.125)	0.923
Central line exposure 48h prior T_0, *n* (%)	27 (69.2)	23 (59.0)	1.565 (0.616–3.977)	0.346
Peripheral line exposure, *n* (%)	36 (92.3)	38 (97.4)	0.316 (0.031–3.177)	0.328
Median peripheral line time (IQR), days	8 (5–10)	8 (4–10)	1.029 (0.923–1.148)	0.607
Peripheral line exposure 48h prior T_0, *n* (%)	32 (82.1)	31 (79.5)	1.180 (0.382–3.646)	0.774
Median RBC transfusions (IQR), *n*	2 (1–2)	2 (1–3)	1.094 (0.728–1.646)	0.665
Invasive ventilation exposure, *n* (%)	23 (59.0)	19 (48.7)	1.513 (0.618–3.704)	0.364
Median invasive ventilation time (IQR), days	6 (2–12)	11 (5–13)	0.925 (0.815–1.050)	0.228
Noninvasive ventilation exposure, *n* (%)	34 (87.2)	31 (79.5)	1.755 (0.519–5.937)	0.366
Median noninvasive ventilation time (IQR), days	8 (5–10)	8 (5–11)	0.962 (0.867–1.068)	0.471
Enteral feeding type, *n* (%)				
Breast milk	20 (51.3)	22 (56.4)	Reference	0.179
Formula milk	3 (7.7)	8 (20.5)	0.413 (0.096–1.774)	0.234
Combination	12 (30.8)	7 (17.9)	1.886 (0.620–5.731)	0.263
Achieved full enteral feeding, *n* (%)	5 (12.8)	8 (21.1)	0.595 (0.166–2.218)	0.595
Median total parental feeding time (IQR), days	9 (7–13)	9 (7–11)	1.079 (0.940–1.240)	0.280
Total time from birth (days), *n* (%)				
0–5	6 (15.4)	4 (10.3)	Reference	0.575
5–10	13 (33.3)	17 (43.6)	0.510 (0.119–2.188)	0.365
>10	12 (30.8)	10 (25.6)	0.800 (0.175–3.651)	0.773
Medication, *n* (%)				
Inotropes	5 (12.8)	3 (7.7)	1.000 (0.132–7.570)	1.000
Antimycotics	1 (2.6)	3 (7.7)	0.167 (0.012–2.368)	0.186
Postpartum antibiotic administration time (days), *n* (%)				
None	4 (10.3)	2 (5.1)	*Reference*	0.388
1–3	26 (66.7)	23 (59.0)	0.565 (0.095–3.378)	0.532
>3	9 (23.1)	14 (35.9)	0.321 (0.048–2.133)	0.240

phylaxis, reducing the risk for colonization with multiresistant pathogens [21]. It has been demonstrated that probiotic supplementation significantly reduced the risk of LOS in preterm infants (n = 9,416) [22]. However, additional studies are needed to evaluate the optimal dosage, duration, and identification of the best suitable bacterial strains for supplementation.

Previous studies have demonstrated an association between (the duration of) central line exposure and the development of LOS in preterm infants [1, 7, 23]. Line exposure significantly increased the risk of gram-positive bacteria-related LOS in preterm infants, especially CoNS-LOS. This increased risk may be caused by contaminated intravenous fluids or catheter hubs (intraluminal con-

Table 3 (continued)

Characteristic	LOS (n = 39)	Non LOS (n = 39)	Univariate analysis[1]	p value
Antibiotic exposure (yes), n (%)	36 (92.3)	39 (100)	NA	0.999
Median antibiotics time (IQR), days	3 (3–8)	3 (3–8)	1.025 (0.887–1.184)	0.740
Antibiotic exposure per group, n (%)				
Aminoglycosides	31 (79.5)	30 (76.9)	1.447 (0.413–5.063)	0.563
Carbapenems	1 (2.6)	3 (7.7)	0.324 (0.032–3.268)	0.339
Cephalosporins	9 (23.1)	13 (33.3)	0.615 (0.224–1.693)	0.347
Glycopeptides	3 (7.7)	6 (15.4)	0.470 (0.108–2.043)	0.314
Macrolides	0	4 (10.3)	NA	NA
Oxazolidinones	0	0	NA	NA
Penicillins (-clavulanic acid)	36 (92.3)	37 (94.9)	NA	1.000
Quinolones	0	0	NA	NA
Rifampicin	0	0	NA	NA
Trimethoprim-sulfamethoxazole	1 (2.6)	0	NA	NA
Mortality, n (%)	9 (23.1)	1 (2.6)	11.400 (1.367–95.043)	*0.025**
Median age at death (IQR), days	17 (12–19)	10 (NA)	NA	0.998
Discharge before 28 days, n (%)	13 (33.3)	8 (20.5)	1.937 (0.696–5.391)	0.205
Median age at discharge (IQR), days	18 (15–21)	15 (10–21)	1.093 (0.878–1.362)	0.425

LOS, late-onset sepsis; NA, not applicable; T_0, clinical onset of sepsis; PPROM, preterm premature rupture of membranes; PDA, patent ductus arteriosus; RBC, red blood cells. [1] Values are presented as OR (95% CI). * Statistically significant difference.

tamination) or by skin-colonizing organisms invading the bloodstream via the catheter track (extraluminal contamination) [24]. We observed that every additional day that a peripheral line was present the risk of CoNS-LOS increased, while central line exposure (presence/absence and duration) was not an independent risk factor for CoNS-LOS. The apparent discrepancy in the study results might be explained by differences in study design. The majority of preterm infants have either a central or a peripheral line during the first month of life, and a younger GA is associated with an increased risk for LOS [1, 7]. In the current study we matched study participants on GA to prevent bias by this age-related catheter exposure. A positive association between the dwell time of peripheral catheters and central venous lines and LOS development has been described in several studies, although the results are contradictory [23, 25, 26]. We found no association between the dwell time of both central and peripheral catheters and LOS. We hypothesized that an increased risk of LOS development is not merely influenced by the dwell time of either central or peripheral lines but predominantly by frequent replacement of central and/or peripheral catheters. This may increase the risk of insertion of potential causative pathogens by contaminated catheter hubs or by creation of new entrance sites [25, 26]. However, this variable was not taken into account in the present study.

This study has several strengths; detailed data collection in a multicenter design allowed for a strictly matched case-control comparison and the relatively large sample size allowed to determination of predictive factors per subgroup of causative pathogens. This study has also several limitations that need to be addressed. First, data collection was limited to the first 28 days postnatally, which might have resulted in a lower LOS incidence and mortality rate. Hypothetically, limiting data collection until a postnatal age of 28 days might also result in allocation of infants into the control group while they might have developed sepsis after the defined follow-up period, therefore possibly resulting in an underestimation of the potential risk factors. Secondly, this study contained limited obstetric data. This could hypothetically have influenced the outcome, since maternal factors may also include risk factors for LOS as they have been described to influence the neonatal immune system. Thirdly, center-specific effects could not be excluded from the analyses due to variating LOS incidences, limiting center-based matching. However, this could allow for identification of factors leading to an increased risk for LOS development as a result of local protocols used. Lastly, prolonged parenteral nutrition could also be seen as an early sign of LOS, particularly in less-fulminant CoNS-LOS, rather than a preonset risk factor. However, the relatively large number of LOS cases allowed us to focus on

Table 4. Characteristics of LOS infants caused by gram-positive bacteria and matched controls in the period preceding LOS diagnosis (T_0)

	LOS (*n* = 152)	Non-LOS (*n* = 152)	Univariate analysis[1]	*p* value	Multivariate analysis[1]	*p* value
Median gestational age (IQR), weeks+days	27+4 (25+6–28+5)	27+4 (25+6–28+5)	1.000 (0.981–1.020)	0.981		
Mean birth weight (±SD), g	965.8 (274.7)	968.0 (273.3)	1.000 (0.999–1.001)	0.944		
Male gender, *n* (%)	85 (55.9)	74 (48.7)	1.337 (0.852–2.100)	0.207		
Vaginal delivery, *n* (%)	64 (42.1)	84 (55.3)	1.075 (0.682–1.695)	0.755		
Multiple births, *n* (%)	60 (39.5)	48 (31.5)	1.413 (0.881–2.265)	0.151		
PPROM, *n* (%)	39 (25.7)	35 (23.0)	1.197 (0.707–2.026)	0.503		
Meconium amniotic fluid, *n* (%)	3 (2.0)	2 (1.3)	1.521 (0.250–9.242)	0.649		
Median 1-min Apgar score (IQR)	6 (3–7)	5 (3–7)	1.011 (0.917–1.114)	0.827		
Median 5-min Apgar score (IQR)	7 (6–9)	7 (7–8)	0.991 (0.870–1.129)	0.890		
PDA, *n* (%)	66 (43.4)	59 (38.8)	1.206 (0.740–1.966)	0.453		
PDA treatment type, *n* (%)						
Ibuprofen	54 (35.5)	45 (29.6)	Reference	0.919		
Indomethacin	0	1 (0.7)	NA	1.000		
Surgical	2 (1.3)	1 (0.7)	1.667 (0.146–18.985)	0.681		
Central line exposure, *n* (%)	111 (73)	125 (82.2)	0.585 (0.338–1.012)	0.055		
Median central line time (IQR), days	8 (6–9)	7 (5–10)	1.034 (0.953–1.121)	0.420		
Central line exposure 48h prior T_0, *n* (%)	83 (54.6)	94 (61.8)	0.742 (0.470–1.172)	0.201		
Peripheral line exposure, *n* (%)	148 (97.4)	147 (96.7)	1.259 (0.331–4.780)	0.736		
Median peripheral line time (IQR), days	7 (4–10)	7 (4–9)	1.006 (0.955–1.060)	0.812		
Peripheral line exposure 48 h prior T_0, *n* (%)	120 (78.9)	108 (71.1)	1.528 (0.904–2.581)	0.113		
Median RBC transfusions (IQR), *n*	2 (1–2)	2 (1–2)	1.091 (0.823–1.444)	0.545		
Invasive ventilation exposure, *n* (%)	26 (17.1)	38 (25.0)	2.098 (0.974–4.519)	0.058		
Median invasive ventilation time (IQR), days	4 (2–9)	5 (2–9)	1.011 (0.949–1.076)	0.740		
Noninvasive ventilation exposure, *n* (%)	139 (91.4)	133 (87.5)	1.527 (0.726–3.216)	0.265		
Median noninvasive ventilation time (IQR), days	6 (4–9)	6 (4–9)	1.003 (0.956–1.052)	0.912		
Enteral feeding type, *n* (%)						
Breast milk	58 (38.2)	55 (36.2)	Reference	0.227		
Formula milk	46 (30.3)	32 (21.1)	1.363 (0.761–2.441)	0.297		
Combination	44 (28.9)	52 (34.2)	0.802 (0.465–1.384)	0.429		
Achieved full enteral feeding, *n* (%)	22 (14.4)	28 (18.2)	0.938 (0.495–1.780)	0.846		
Median total parental feeding time (IQR), days	9 (7–11)	8 (5–10)	1.102 (1.010–1.202)	*0.029**	1.289 (1.074–1.547)	*0.006**
Total time from birth (days), *n* (%)						
0–5	14 (9.2)	30 (19.7)	Reference	0.066		
5–10	55 (36.2)	65 (42.8)	1.813 (0.875–3.759)	0.110		
>10	26 (17.1)	20 (13.2)	2.786 (1.177–6.593)	*0.020**		
Medication, *n* (%)						
Inotropes	6 (3.9)	16 (10.5)	0.188 (0.048–0.728)	*0.016**		
Antimycotics	8 (5.3)		0.791 (0.211–2.972)	0.729		
Postpartum antibiotics administration (days), *n* (%)						
None	22 (14.5)	26 (17.1)	Reference	0.820		
1–3	84 (55.3)	81 (53.3)	1.226 (0.643–2.335)	0.536		
>3	46 (30.3)	45 (29.6)	1.208 (0.599–2.435)	0.597		
Antibiotic exposure (yes), *n* (%)	138 (90.8)	143 (94.1)	0.620 (0.260–1.480)	0.282	0.078 (0.007–0.879)	*0.039**
Median antibiotics time (IQR), days	4 (3–6)	3 (2–6)	1.032 (0.950–1.121)	0.462		
Antibiotic exposure per group, *n* (%)						
Aminoglycosides	117 (77.0)	121 (79.6)	1.059 (0.556–2.016)	0.861		
Carbapenems	1 (0.7)	3 (2.0)	0.343 (0.035–3.338)	0.357		
Cephalosporins	23 (15.1)	38 (25.0)	0.558 (0.312–0.998)	*0.049**		
Glycopeptides	18 (11.8)	19 (12.5)	0.987 (0.494–1.971)	0.970		
Macrolides	2 (1.3)	0	NA	NA		
Oxazolidinones	0	0	NA	NA		
Penicillins (-clavulanic acid)	132 (86.8)	138 (90.8)	0.957 (0.301–3.040)	0.940		
Quinolones	0	0	NA	NA		
Rifampicin	0	0	NA	NA		
Trimethoprim-sulfamethoxazole	0	0	NA	NA		
Mortality, *n* (%)	4 (2.6)	2 (1.3)	2.027 (0.366–11.235)	0.419		
Median age at death (IQR), days	16 (6–25)	15 (NA)	1.018 (0.811–1.278)	0.878		
Discharge before 28 days, *n* (%)	55 (36.2)	50 (32.9)	1.157 (0.721–1.857)	0.547		
Median age at discharge (IQR), days	18 (13–22)	19 (12–22)	0.985 (0.913–1.062)	0.693		

LOS, late-onset sepsis; NA, not applicable; T_0, clinical onset of sepsis; PPROM, preterm premature rupture of membranes; PDA, patent ductus arteriosus; RBC, red blood cells. [1] Values are presented as OR (95% CI). * Statistically significant difference.

risk factors per pathogen. So, this possible limitation may only account for CoNS-LOS cases. In the case of other pathogens, the course of sepsis is considered to be more fulminant.

In conclusion, since in the current study parenteral feeding was strongly associated with LOS development, it could be hypothesized that reduction of the number of parenteral feeding days might reduce the risk of LOS, which may be achieved by advancement of enteral feeding, preferably with breastmilk. Protective effects of early exposure to specific antibiotics underline the increasing notion that a disturbed microbial colonization may be involved in the pathophysiology of at least a selection of LOS cases.

Table 5. Characteristics of infants with LOS caused by CoNS bacteria and matched controls in the period preceding LOS diagnosis (T_0)

	LOS (*n* = 111)	Non-LOS (*n* = 111)	Univariate analysis[1]	*p* value	Multivariate analysis[1]	*p* value
Median gestational age (IQR), weeks+days	27+4 (25+6–28+6)	27+4 (25+6–28+6)	1.000 (0.979–1.022)	0.991		
Median birth weight (IQR), g	930 (725–1,180)	900 (750–1,190)	1.000 (0.999–1.001)	0.940		
Male gender, *n* (%)	61 (55)	54 (48.6)	1.288 (0.760–2.183)	0.347		
Vaginal delivery, *n* (%)	46 (41.1)	50 (45.0)	1.105 (0.648–1.885)	0.715		
Multiple births, *n* (%)	42 (37.8)	31 (27.9)	1.571 (0.893–2.763)	0.117		
PPROM, *n* (%)	32 (28.8)	27 (24.3)	1.312 (0.720–2.390)	0.375		
Meconium-stained amniotic fluid, *n* (%)	2 (1.8)	1 (0.9)	2.020 (0.180–22.622)	0.569		
Median 1-min Apgar score (IQR)	5 (3–7)	5 (3–7)	1.018 (0.906–1.143)	0.767		
Median 5-min Apgar score (IQR)	7 (6–8)	7 (6–8)	0.986 (0.850–1.143)	0.850		
PDA, *n* (%)	39 (35.1)	31 (27.9)	1.677 (0.627–4.490)	0.303		
PDA treatment type, *n* (%)						
Ibuprofen	36 (32.4)	30 (27.0)	Reference	1.000		
Indomethacin	0	1 (0.9)	NA	1.000		
Surgical	2 (1.8)	0	NA	0.999		
Central line exposure, *n* (%)	77 (69.4)	94 (84.7)	0.410 (0.213–0.789)	*0.008**		
Median central line time (IQR), days	7 (6–9)	7 (5–9)	1.050 (0.937–1.176)	0.403		
Central line exposure 48h prior T_0, *n* (%)	62 (55.9)	78 (70.3)	0.535 (0.308–0.931)	*0.027**		
Peripheral line exposure, *n* (%)	107 (96.4)	107 (96.4)	1.000 (0.244–4.102)	1.000	1.238 (1.086–1.411)	*0.001**
Median peripheral line time (IQR), days	6 (4–9)	6 (4–8)	1.060 (0.981–1.146)	0.142		
Peripheral line exposure 48 h prior T_0, *n* (%)	89 (80.2)	77 (69.4)	1.786 (0.964–3.311)	0.065		
Median RBC transfusions (IQR), *n*	2 (1–3)	1 (1–2)	1.217 (0.836–1.770)	0.305		
Invasive ventilation exposure, *n* (%)	54 (48.6)	64 (57.7)	0.696 (0.410–1.181)	0.179		
Median invasive ventilation time (IQR), days	4 (3–8)	5 (2–9)	1.012 (0.918–1.117)	0.804		
Noninvasive ventilation exposure, *n* (%)	103 (92.8)	96 (86.5)	2.012 (0.816–4.958)	0.129		
Median noninvasive ventilation time (IQR), days	6 (4–9)	6 (4–8)	0.996 (0.928–1.070)	0.922		
Enteral feeding type, *n* (%)						
Breast milk	35 (31.5)	37 (33.3)	Reference	0.171	Reference	*0.019**
Formula milk	38 (34.2)	23 (20.7)	1.747 (0.873–3.496)	0.115	3.779 (1.257–11.363)	*0.018**
Combination	37 (33.3)	41 (36.9)	0.954 (0.502–1.811)	0.954	0.782 (0.328–1.865)	0.580
Achievement of full enteral feeding	22 (14.4)	28 (18.2)	0.938 (0.495–1.780)	0.846		
Median total parental feeding time (IQR), days	8 (7–10)	8 (5–9)	1.075 (0.967–1.193)	0.180		
Total time from birth (days), *n* (%)						
0–5	8 (7.2)	23 (20.7)	Reference	0.122		
5–10	34 (30.6)	44 (39.6)	2.222 (0.885–5.578)	0.089		
>10	18 (16.2)	18 (16.2)	2.875 (1.020–8.104)	*0.046**		
Medication, *n* (%)						
Inotropes	2 (1.8)	11 (9.9)	0.104 (0.015–0.726)	*0.022**		
Antimycotics	3 (2.7)	5 (4.5)	0.382 (0.069–2.125)	0.272		
Postpartum antibiotics administration (days), *n* (%)						
None	14 (12.6)	18 (16.2)	Reference	0.656		
1–3	66 (59.5)	60 (54.1)	1.414 (0.648–3.088)	0.384		
>3	31 (27.9)	33 (29.7)	1.208 (0.515–2.865)	0.665		
Antibiotic exposure (yes), *n* (%)	104 (93.7)	107 (93.7)	1.000 (0.339–2.952)	1.000	0.229 (0.086–0.612)	*0.003**
Median antibiotics time (IQR), days	4 (3–6)	3 (2–6)	1.043 (0.932–1.167)	0.460		
Antibiotic exposure per group, *n* (%)						
Aminoglycosides	92 (82.9)	89 (80.2)	1.504 (0.661–3.418)	0.330		
Carbapenems	0	3 (2.7)	NA	NA		
Cephalosporins	13 (11.7)	27 (24.3)	0.417 (0.202–0.864)	*0.019**		
Glycopeptides	9 (8.1)	15 (13.5)	0.574 (0.239–1.379)	0.215		
Macrolides	1 (0.9)	0	NA	NA		
Oxazolidinones	0	0	NA	NA		
Penicillins (-clavulanic acid)	100 (90.1)	100 (90.1)	1.667 (0.388–7.162)	0.492		
Quinolones	0	0	NA	NA		
Rifampicin	0	0	NA	NA		
Trimethoprim- sulfamethoxazole	0	0	NA	NA		
Mortality, *n* (%)	1 (0.9)	2 (1.8)	0.495 (0.044–5.544)	0.569		
Median age at death (IQR), days	5	15	NA	NA		
Discharge before 28 days, *n* (%)	41 (36.9)	40 (36.0)	1.040 (0.602–1.796)	0.889		
Median age at discharge (IQR), days	18 (13–21)	19 (12–22)	0.989 (0.909–1.077)	0.807		

LOS, late-onset sepsis; NA, not applicable; T_0, clinical onset of sepsis; PPROM, preterm premature rupture of membranes; PDA, patent ductus arteriosus; RBC, red blood cells. [1] Values are presented as OR (95% CI). * Statistically significant difference.

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Statement of Ethics

The local institutional review boards of all 9 participating centers granted approval (amendment A2016.363). The parents of all of the included infants gave written informed consent.

Disclosure Statement

The authors have no conflicts of interests to declare.

Author Contributions

Dr. el Manouni el Hassani conceptualized and designed this study, coordinated and supervised data collection, carried out the initial analyses, drafted the initial version of this paper, and reviewed and revised this paper.

Dr. Berkhout, Dr. de Boer, and Dr. de Meij conceptualized and designed this study, coordinated and supervised data collection, and critically reviewed this paper for important intellectual content.

Dr. Mann designed the data collection instruments, collected data, and carried out the initial analyses.

Dr. Niemarkt, Dr. de Boode, Prof. Dr. Cossey, Dr. Hulzebos, Prof. Dr. van Kaam, Prof. Dr. Kramer, Dr. van Lingen, Prof. Dr. van Goudoever, Dr. Vijlbrief, Prof. Dr. van Weissenbruch, and Prof. Dr. Benninga critically reviewed this paper for important intellectual content.

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