Late-Onset Sepsis in very Low Birth Weight Infants: A Brazilian Neonatal Research Network Study

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Summary

Background: Late-onset sepsis (LOS) is an important cause of morbidity and mortality in very low birth weight (VLBW) infants.

Aim: To determine the incidence, risk factors and etiology of LOS. Methods: LOS was investigated in a multicenter prospective cohort of infants at eight public university neonatal intensive care units (NICUs). Inclusion criteria included inborn, 23–33 weeks of gestational age, 400–1499 g birth weight, who survived >3 days.

Results: Of 1507 infants, 357 (24%) had proven LOS and 345 (23%) had clinical LOS. Infants with LOS were more likely to die. The majority of infections (76%) were caused by Gram-positive organisms. Independent risk factors for proven LOS were use of central venous catheter and mechanical ventilation, age at the first feeding and number of days on parenteral nutrition and on mechanical ventilation.

Conclusion: LOS incidence and mortality are high in Brazilian VLBW infants. Most risk factors are associated with routine practices at NICU.

Key words: Sepsis, infection, newborn infant, very low birth weight, premature.

Introduction

Late-onset sepsis (LOS) is an important cause of morbidity and mortality among preterm infants [1–3]. Numerous factors contribute to high incidence of infection including prematurity, very low birth weight (VLBW), prolonged intravascular access, parenteral nutrition, prolonged mechanical ventilation, hospitalization length, use of broad-spectrum antibiotics and nutritional practices [1, 2, 4, 5].

The causative organism in LOS is an important predictor of its outcomes. The majority of LOS episodes are caused by Gram-positive organisms, as shown by a multicenter study on the National Institute of Child and Human Development (NICHD) Neonatal Research Network. Coagulase-negative staphylococci (CoNS) accounted for 48% of infections; Gram-negative organisms were responsible for almost 18% and fungi for 12% infections. The mortality rate for Gram-positive organisms was 11%, for Gram-negative 36% and for fungi 32% [1, 2]. Similar results were found in a neonatal infection surveillance network in England (NeonIn) [6].

Neonatal infection surveillance networks have been established in several countries to provide a national overview and to compare results with other networks. On the Brazilian Neonatal Research Network, eight centers have contributed to create a database on VLBW infants. LOS is an investigation...
priority on this Network because national data on neonatal infection are very scarce. The objective of this study was to determine the incidence and mortality of LOS, its risk factors and the distribution of infecting pathogens in infants from the Brazilian Neonatal Research Network.

Methods

Study design
A multicenter study was conducted on a cohort of VLBW infants (birth weight <1500 g), inborn, in eight centers on the Brazilian Neonatal Research Network between 1 January 2006 and 31 December 2008. The study was approved by the ethics committee of all centers. Written informed consent was obtained from all participants. All of the eight neonatal intensive care units (NICUs) are located in university hospitals that are referral centers for high-risk pregnancies, providing care to patients on the Brazilian Public National Health Care Service.

Study population
Among the 2370 VLBW infants admitted to the network centers, 1507 (63.6%) met the study inclusion criteria: inborn preterm infants born at 23–33 complete gestational weeks, with birth weight from 400 to 1499 g who survived for >72 h after birth. These infants were observed until discharge, death or 120 days of hospitalization. Figure 1 shows a flowchart of the cohort.

Three study groups were composed: Proven LOS: clinical signs of sepsis (temperature instability, cardiorespiratory or gastrointestinal disturbances, lethargy or irritability) with laboratory evidence supporting infection (abnormalities in complete blood count and increase in acute phase serum proteins levels) and a positive blood culture; Clinical LOS: clinical and hematological manifestations of systemic infection and a negative blood culture; No LOS: patients without clinical-hematological manifestations of infection.

CoNS infection was defined by clinical and laboratory signs of sepsis, and two positive blood cultures wherever possible, associated with appropriate antimicrobial therapy. Organisms considered to be contaminants such as Micrococcus sp, Corynebacterium sp and Propionibacterium sp were excluded.

The first LOS episode was considered the end point for this analysis, and the variables studied were Maternal and gestational data—antenatal care, gestational morbidities, antenatal steroids use, single or multiple pregnancy and mode of delivery; Delivery data—gender, birth weight, gestational age (best obstetric estimate), appropriate or low weight for gestational age [7], Apgar score, resuscitation at birth; Procedures—mechanical ventilation, intravascular catheter, parenteral nutrition, use of antibiotics on the first 3 days of life, surgery; Neonatal outcome—illness severity and mortality risk score—SNAPPE II [8]; respiratory distress syndrome; early-onset sepsis (clinical signs and positive blood culture within 72 h of life); pneumonia: diagnosed according to the criteria used in the NICU; confirmed necrotizing enterocolitis according to Walsh & Kliegmann [9]; bronchopulmonary dysplasia: oxygen requirement at the postmenstrual age of 36 weeks [10]; patent ductus arteriosus: diagnosed by echocardiography; intraventricular hemorrhage classified according to Papile et al. [11]; retinopathy of prematurity stage ≥2; age at the first feeding; days to regain birth weight; length of hospital stay; and death.

Statistical methods
LOS incidence among the centers was compared by the two-tailed $\chi^2$ test. Associations between LOS and the variables studied were investigated by analysis of variance (ANOVA) followed by Tukey post hoc test and the $\chi^2$ test. Variables associated with LOS ($p \leq 0.05$) in the univariate analysis were included in multiple logistic regression models. Non-significant variables were removed from the model in a backward stepwise way. $p < 0.05$ was considered statistically significant.

Results
Of the 1507 infants who fulfilled the inclusion criteria, mean gestational age and birth weight were 29±2.2 weeks and 1071±267 g, respectively. A total of 357 infants (23.7%; CI 95% 21.6, 26.9) had
positive blood culture and were diagnosed with proven LOS. There was a wide variability of incidence rates between centers, ranging from 15.6 to 39%. Clinical LOS was diagnosed in 345 neonates (22.9%; CI 95% 20.8, 25.1), with rates ranging from 16.5 to 40%. The remaining 805 infants (53.4%; CI 95% 50.9, 55.9) comprised the No LOS group. The mortality rate was significantly higher in infants with proven LOS (26.6%, range: 13.6–43.9%) and clinical LOS (34.2%, 16.7–49.4%) as compared with No LOS (9.4%, 3.9–15.8%).

Among the 357 premature infants with proven LOS, 55 (15.4%) had two episodes, and 4 (1%) had three episodes. The majority (76%) of first LOS episodes were caused by Gram-positive organisms, and CoNS were the most frequent organisms isolated in 213 of 357 (60%) blood cultures, followed by Gram-negative organisms (15%), *Staphylococcus aureus* (12%) and fungi (9.5%) (Fig. 2).

The highest mortality rates were found for infants with fungal sepsis (52%), followed by those with infection caused by Gram-negative agents (35%), whereas for infants with CoNS sepsis, the mortality rate was 18%.

As shown in Table 1, with the exception of chorioamnionitis and single pregnancy, which were more frequent in mothers of infants with LOS (clinical and proven), other maternal gestational and delivery characteristics did not differ between the three groups. Preterm infants with clinical or proven LOS showed lower gestational age and birth weight, had lower Apgar scores and increased need for resuscitation in the delivery room. There were significant differences in all newborn data between the two LOS groups compared with No LOS group, except for gender. There were no differences between clinical and proven LOS (Table 1).

VLBW infants with clinical or proven LOS had higher SNAPPE II scores on the first day of life, higher neonatal morbidity, more frequent use of invasive procedures and longer length of hospitalization than infants without sepsis. Comparing LOS groups, bronchopulmonary dysplasia and use of percutaneous central line were more frequent in proven LOS. Moreover, these babies used parenteral nutrition and mechanical ventilation for a longer time, and they needed more days to regain birth weight and to discharge (Table 2).

All variables showing significant association with LOS were included in multivariate analysis using logistic regression models. After additional adjustment for center, the following variables were identified as independent risk factors for LOS (clinical or proven): degree of prematurity, number of antibiotics used in the first 72 h of life, use of mechanical ventilation and central venous catheter and number of days of mechanical ventilation and parenteral nutrition (Table 3).

Another multiple logistic regression model was fitted to identify which variables were risk factors for proven LOS. In this model, the risk factors were degree of prematurity, age at the first enteral feeding, days of mechanical ventilation and parenteral nutrition and use of mechanical ventilation and central venous catheter (Table 4).

**Discussion**

The neonatal infection surveillance networks established in several countries are important to know about LOS incidence and its variability, to compare results with those of other networks and to propose...
public health policies to improve neonatal care quality [12, 13].

The study on the Brazilian Neonatal Research Network showed that 24% of VLBW infants developed proven LOS, which is a similar result to that of other research networks [1, 14] However, in this study the finding that adds significantly to the knowledge regarding LOS was the high incidence of clinical LOS (23%), a serious problem in the daily practice of NICUs that is not addressed in the literature. Although the diagnosis of clinical sepsis may be questioned because there is no gold standard, in this

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No LOS (n = 805)</th>
<th>Proven LOS (n = 357)</th>
<th>Clinical LOS (n = 345)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antenatal care</td>
<td>761 (95)</td>
<td>326 (91)</td>
<td>318 (92)</td>
<td>0.071</td>
</tr>
<tr>
<td>Maternal hypertension</td>
<td>344 (43)</td>
<td>146 (41)</td>
<td>134 (39)</td>
<td>0.507</td>
</tr>
<tr>
<td>Maternal diabetes mellitus</td>
<td>46 (6)</td>
<td>18 (5)</td>
<td>15 (4)</td>
<td>0.628</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
<td>54 (7)</td>
<td>47 (13) b</td>
<td>36 (11) b</td>
<td>0.001</td>
</tr>
<tr>
<td>Antenatal steroids</td>
<td>550 (69)</td>
<td>224 (63)</td>
<td>217 (63)</td>
<td>0.068</td>
</tr>
<tr>
<td>Third trimester hemorrhage</td>
<td>58 (7)</td>
<td>29 (8)</td>
<td>30 (9)</td>
<td>0.659</td>
</tr>
<tr>
<td>Single pregnancy</td>
<td>590 (73) a</td>
<td>281 (79) ab</td>
<td>282 (82) b</td>
<td>0.017</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>604 (75)</td>
<td>253 (71)</td>
<td>241 (70)</td>
<td>0.122</td>
</tr>
<tr>
<td>Male gender</td>
<td>401 (50)</td>
<td>174 (49)</td>
<td>176 (51)</td>
<td>0.472</td>
</tr>
<tr>
<td>Resuscitation at birth</td>
<td>412 (51) a</td>
<td>252 (71) b</td>
<td>249 (72) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5-minute Apgar score</td>
<td>9 ± 2 a</td>
<td>8 ± 2 b</td>
<td>8 ± 2 b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>1161 ± 245 a</td>
<td>971 ± 254 b</td>
<td>967 ± 256 b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>30 ± 2 a</td>
<td>29 ± 2 b</td>
<td>29 ± 2 b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>409 (51) a</td>
<td>132 (37) b</td>
<td>140 (41) b</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values expressed as number and percentage, mean ± SD with ANOVA followed by Tukey post hoc test and χ² for difference in proportions. Letters “a” and “b” are used to show differences among the groups. Same letter indicates no significant difference; whereas groups with different letters are statistically different.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No LOS (n = 805)</th>
<th>Proven LOS (n = 357)</th>
<th>Clinical LOS (n = 345)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNAPPE II</td>
<td>16 ± 17 a</td>
<td>26 ± 21 b</td>
<td>25 ± 22 b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Respiratory distress syndrome &lt; 24h</td>
<td>385 (48) a</td>
<td>225 (63) b</td>
<td>236 (68) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>282 (35) a</td>
<td>210 (59) b</td>
<td>187 (54) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proven early-onset sepsis</td>
<td>14 (2)</td>
<td>12 (3)</td>
<td>10 (3)</td>
<td>0.193</td>
</tr>
<tr>
<td>Nosocomial pneumonia</td>
<td>30 (4)a</td>
<td>76 (21)b</td>
<td>90 (26)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td>16 (2) a</td>
<td>56 (16) b</td>
<td>60 (17) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intraventricular hemorrhage grades 3–4</td>
<td>36 (5) a</td>
<td>59 (17) b</td>
<td>67 (20) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia—O₂ 36 w</td>
<td>84 (10) a</td>
<td>119 (33) b</td>
<td>71 (21) c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Retinopathy of prematurity ≥ stage 2</td>
<td>13 (2) a</td>
<td>31 (10) b</td>
<td>21 (9) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>363 (45) a</td>
<td>294 (82) b</td>
<td>287 (83) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Umbilical catheter</td>
<td>300 (37) a</td>
<td>181 (51) b</td>
<td>210 (61) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Central venous catheter</td>
<td>27 (3) a</td>
<td>100 (28) b</td>
<td>57 (16) c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percutaneous central line</td>
<td>487 (60) a</td>
<td>282 (79) b</td>
<td>285 (83) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parenteral nutrition</td>
<td>705 (88) a</td>
<td>352 (99) b</td>
<td>337 (98) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Red blood cell transfusion</td>
<td>298 (37) a</td>
<td>292 (82) b</td>
<td>283 (82) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Surgery</td>
<td>15 (2) a</td>
<td>43 (12) b</td>
<td>26 (7) b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of antibiotics &lt;72 h</td>
<td>0.8 ± 1 a</td>
<td>1.4 ± 1 b</td>
<td>1.6 ± 1 b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at first enteral feeding (days)</td>
<td>3 ± 3 a</td>
<td>6 ± 7 b</td>
<td>5 ± 5 b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Days to regain birth weight</td>
<td>13 ± 6 a</td>
<td>16 ± 10 b</td>
<td>14 ± 7 c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parenteral nutrition (days)</td>
<td>9 ± 7 a</td>
<td>24 ± 18 b</td>
<td>18 ± 15 c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mechanical ventilation (days)</td>
<td>3 ± 6 a</td>
<td>20 ± 23 b</td>
<td>16 ± 24 c</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Length of hospital stay (days)</td>
<td>44 ± 22 a</td>
<td>68 ± 41 b</td>
<td>58 ± 42 c</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values expressed as number and percentage, mean ± SD with ANOVA followed by Tukey post hoc test and χ² for difference in proportions. Letters “a” and “b” are used to show differences among the groups. Same letter indicates no significant difference; whereas groups with different letters are statistically different.
Parenteral nutrition may be contaminated [2, 23]. Nutritional practices are strongly associated with LOS. Premature infants with LOS had higher mortality compared with no LOS infants. The mortality in clinical LOS could not be attributed to other morbidities other than infection. This finding highlights the magnitude of LOS even without positive blood culture.

In agreement with previous studies, CoNS were the most common pathogens isolated in our VLBW infants [1, 3, 6, 15, 16]. The common use of central venous lines may have been responsible for that finding. Gram-negative agents were identified in 15%, whereas the rates reported in the literature are >20% [6, 17–19]. However, there is great concern about these agents as well as fungi owing to high associated mortality [20].

The LOS rate varied widely between the centers, and such variation may be due to differences in clinical practices. Therefore, it may be amenable to interventions that alter local practice. Center-to-center variability was found by other multicenter studies, suggesting that the care site influences neonatal outcomes [1, 14, 21, 22].

Univariate analysis showed several factors to be associated with LOS. Premature infants with LOS (clinical and proven) had lower birth weight and gestational age, worse perinatal conditions and higher severity scores on the first day of life. During hospitalization, they had higher morbidity and more frequent use of invasive devices compared with those without infection.

Catheters are frequently needed in NICU patients to administer parenteral nutrition, medicines and antibiotics; however, they can be a source of infection [2, 23]. Nutritional practices are strongly associated with LOS. Parenteral nutrition may be contaminated during preparation, storage or administration [23].

Additionally, the delay to initiate enteral feeding and the prolonged time to reach full enteral feeding or to regain birth weight has been associated with increased risk for LOS. Efforts to initiate enteral feedings as early as possible reduce the use of central venous lines, may improve nutritional status and decrease the risk for infection [1, 2, 23].

Mechanical ventilation was more frequent and prolonged in infants who developed LOS. Ventilator use provides a portal of entry for pathogens, and prolonged mechanical ventilation is associated with high bronchopulmonary dysplasia incidence [1, 2, 23]. Prolonged hospitalization of VLBW infants is a major factor in the high cost of NICUs and can further compromise the prognosis of such premature infants, increasing their risk for developing nosocomial infection [14, 24].

Multivariate analysis indicated that several factors were independently associated with LOS. The greatest risk was observed for nutritional practices, invasive devices and number of antibiotics used in the first 72 h of life. We performed separated analyses on risk factors for proven LOS by adjusting for each center. The age at the first enteral feeding, days of mechanical ventilation and parenteral nutrition and use of mechanical ventilation and central venous catheter were independent risk factors for confirmed sepsis. We highlight the protective role of gestational age, as each additional week in gestational age was associated with 15% decrease in the risk of LOS.

This study confirms the importance of invasive devices as risk factors of LOS. The implementation of evidence-based practices, such as hand hygiene, the insertion and maintenance of central lines (barrier precautions, skin antisepsis, dedicated team) associated with efforts to initiate early enteral feeding, are important and may reduce the risk for infection [2, 25].

The number of antibiotics used in the first 72 h of life is an additional risk factor for LOS [26].
Empirical antibiotic therapy should be rigorously monitored and reevaluated promptly.

One limitation in this study is that information was obtained from a database with a limited temporal relationship between LOS and the other events. The great contribution was to show that most risk factors are associated with routine practices and procedures at NICUs, many of which could be improved, whereas others should be used with caution and could be potentially avoided.

In conclusion, this study on the Brazilian Neonatal Research Network showed that LOS is common in VLBW infants, and it is associated with significant mortality. Several risk factors were identified, and almost all of them were related to clinical practices. This can be an important step in providing information for health care investments and guiding to the best practices to improve neonatal care quality.

References


Appendix

The Brazilian Network on Neonatal Research members are as follows:

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Marisa Mussi-Pinhata and Francisco Eulogio Martinez.

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Instituto Fernandes Figueira, Fundacao Osvaldo Cruz (Rio de Janeiro, RJ 22.250-020): Jose Maria de Andrade Lopes and Olga Bonfim.


Hospital das Clinicas de Sao Paulo, Universidade de Sao Paulo (Sao Paulo, SP 05.403-000): Clea Rodrigues Leone, Lilian dos Santos Rodrigues Sadeck, Vera Lucia J. Krebs and Werther B. de Carvalho.