Published in final edited form as:
Pediatr Infect Dis J. 2014 February ; 33(2): 143–146. doi:10.1097/INF.0000000000000024.

Clinical and Laboratory Factors that Predict Death in Very Low Birth Weight Infants Presenting with Late-Onset Sepsis

Orly Levit, MD[1,*], **Vineet Bhandari, MD, DM**[1], **Fang-Yong Li, MPH**[2], **Veronika Shabanova, MPH**[2], **Patrick G. Gallagher, MD**[1], and **Matthew J. Bizzarro, MD**[1]

[1]Yale-University School of Medicine, Department of Pediatrics, Yale University School of Medicine, New Haven, CT

[2]Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT

Keywords

Infection; Newborn infant; Risk factors for death; Gram negative; Fungal; Late-onset sepsis

Introduction

Late-onset sepsis (LOS; laboratory confirmed bloodstream infection at >72 hours of life) remains a significant source of morbidity and mortality in very low birth weight (VLBW) infants. The majority of risk associated with LOS is directly or indirectly related to prematurity, including lower gestational age (GA) and birth weight (BW) [1], delivery by cesarian section[2], the need for central venous catheters, hyperalimentation, invasive procedures, mechanical ventilation [2-4] and the presence of comorbidities like patent ductus arteriosus (PDA), necrotizing eneterocolitis (NEC), bronchopulmonary dysplasia (BPD).[2]

Identification of these risk factors has facilitated the design and implementation of strategies for the prevention of LOS. However, once LOS is diagnosed the focus shifts toward effective and expedient intervention as well as prognostication. The risk of LOS-associated death may be significant [1] and is influenced by several factors including, but not limited to, the infecting organism(s). While prior investigations have compared mortality rates among infants with Gram-positive, Gram-negative, and fungal LOS [3], data on presenting clinical and laboratory signs of infection as independent predictors for sepsis-related death are limited.

We performed a 19-year, retrospective review of all cases of LOS in a single level IV neonatal intensive care unit (NICU). The primary aim of our investigation was to identify predictors of sepsis-associated mortality from a composite risk profile that included demographic data, clinical and laboratory data at the time of presentation, and preliminary blood culture results defined as isolation of a Gram-positive, Gram-negative, or fungal organism.

[*]Address for correspondence: Orly Levit, MD, Department of Pediatrics – Yale University, 333 Cedar Street, New Haven, CT 06510, Phone: 203- 688-2320, Fax: 203-688-5426, orly.levit@yale.edu.

Disclosures: The authors have no conflicts of interest to disclose.

Methods

Patient population and data collection

The Yale-New Haven Children's Hospital NICU has maintained an electronic database of all laboratory confirmed bloodstream infections (BSI) since 1979. Each positive blood culture is reviewed and specific criteria applied prior to data entry which includes demographics, timing of infection, specific organism(s), clinical and laboratory data related to the onset of infection, and outcome. For the purpose of this investigation, data from all VLBW infants admitted to the NICU, both inborn and outborn, from January 1, 1989 to December 31, 2007 with at least one episode of monomicrobial blood culture-proven LOS during their hospitalization were included. Episodes of polymicrobial sepsis were excluded and, in infants with multiple episodes of LOS, only the first episode was included for analysis (i.e. subsequent episodes of bacteremia were excluded). Age at the time of infection was considered the day of life the first positive blood culture was drawn. LOS was defined as a laboratory confirmed BSI, per criteria established by the Center for Disease Control and Prevention (CDC), occurring at >72 hours of life [5]. The CDC criteria, published in 1988 were eventually modified in 2008 [6] but, given the time period of this study, did not affect data reporting.

Once cases of LOS were identified they were further categorized as Gram-positive, Gram-negative and fungal for comparison. Additional data collection consisted of 1) demographic, antepartum, and intrapartum data including GA, BW, gender, 5-minute Apgar score, location of birth (*i.e.* inborn or outborn), method of delivery, and exposure to antenatal steroids; 2) potential clinical and laboratory risk factors for LOS including duration of antibiotic exposure prior to the onset of LOS, the use of a central venous catheter, total parenteral nutrition (TPN), intralipid solution (IL), H_2 blockers, and postnatal steroids at the time of diagnosis of LOS, the need for respiratory and cardiovascular support at the time of diagnosis, the presence of apnea, bradycardia, temperature and glucose instability in the 24 hours preceding sepsis evaluation, as well as neutrophil and platelet abnormalities at the time the blood culture was obtained; 3) outcomes and comorbidities including meningitis and/or urinary tract infection concurrent with BSI, respiratory distress syndrome, length of hospital stay, bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), and death.

Definitions

Respiratory distress syndrome was diagnosed according to the presence of respiratory distress and a characteristic chest radiograph. NEC was defined according to the modified Bell's criteria and included only those cases ≥ stage IIA [7]. BPD was defined as need for supplemental oxygen at 36 weeks corrected gestational age [8]. IVH was defined using Papile's classification and included all grades [9].

Hypoglycemia was defined as a serum glucose of <40 mg/dl and hyperglycemia as >140 mg/dl. Neutropenia was defined as an absolute neutrophil count (ANC) less than 1500/mm^3 and thrombocytopenia as a platelet count of less than 150,000. Leukocytosis was defined as a white blood cell count of greater than 30,000/mm^3.

Death was considered related to infection if it occurred within 7 days of the BSI or if clinical signs and symptoms of sepsis were believed to be the direct cause of death.

Statistical methods

Statistical analyses were performed only for the first episode of LOS in each infant. Demographics and risk factors were compared by type of infection using Chi-square test,

Fisher's exact test for categorical data, and t-test or Kruskal Wallis test for continuous data, where appropriate. Group pair-wise comparisons were conducted where the omnibus test was significant. For the purpose of multiple comparison adjustment, a p-value of 0.017 was considered significant according to the Bonferroni method. Type of infection was assessed as the primary risk factor of interest and other risk factors grouped into three domains: 1) known risk factors of LOS which included extremely low BW (< 1000 grams), central line use, endotracheal intubation and respiratory support, prior antibiotic exposure, and the use of TPN, IL, and H_2 blockers; 2) presenting clinical and laboratory signs at onset of LOS including apnea, bradycardia, temperature and glucose instability, and laboratory abnormalities, and 3) the domain of concurrent infection and comorbidities included urinary tract infection, meningitis, concurrent NEC, BPD, and other underlying conditions.

The bivariate logistic regression analysis was first employed to examine the crude association between sepsis-related death and risk factors. Further multivariate logistic regression analysis was performed to examine the effect of type of infection with covariates adjustment within each domain separately. We used a manual selection strategy using p = 0.05 as the cut-off for modeling. The unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CI) were reported. The significance cut-off value was p=0.05, two-sided unless otherwise specified. All the statistical analyses were performed using software SAS 9.2 (Cary, NC).

This study was approved by the Human Investigation Committee of the Yale University School of Medicine.

Results

There were a total of 541 sepsis episodes in 477 VLBW infants admitted to the Yale-New Haven Children's Hospital NICU from January 1, 1989 through December 31, 2007. After applying exclusion criteria, 424 monomicrobial BSI in 424 infants were included in the final analysis. 262 (61.8%) were categorized as Gram-positive, 126 (29.7%) as Gram-negative, and 36 (8.5%) as fungal LOS (Table 1). The predominant organisms cultured were coagulase-negative *staphylococci* (CONS), *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis* (Table 1).

When all three categories of LOS were compared, the timing of onset of infection was similar (Gram-positive: day of life 24.3 ± 17.3 *v*. Gram-negative: day of life 26.9 ± 18.5 *v*. Fungal: day of life 27.4 ± 10.6; p= 0.60). Mean GA and BW were lowest among infants with fungal LOS as compared to those with Gram-positive sepsis and Gram-negative sepsis (Table 2). Infants with fungal LOS were also most likely to be intubated, to have a central venous catheter in place, to be receiving IL and H_2 blockers, and to have been previously exposed to antimicrobials as compared to those with Gram-positive or Gram-negative LOS (Table 2).

Clinical and laboratory signs as well as comorbidities differed significantly across the three categories of infection (Table 3). Infants with fungal infection were most likely to present with leukocytosis and had the highest rates of concurrent urinary tract infection and meningitis, IVH, and BPD. VLBW infants with Gram-negative LOS had the highest rate of NEC (Table 3). No significant difference was found between the three categories of LOS with respect to temperature instability at the onset of infection, disturbances in glucose metabolism, and bradycardia.

There were a total of 98 deaths (23.1% of entire cohort) in our study population, with 45 (45.9% of all deaths; 10.6% of entire cohort) classified as sepsis-related. The majority of

sepsis-related deaths occurred in infants with Gram-negative LOS (60%; p<0.0001). When assessing death from any cause, infants with Gram-negative-LOS died at a median of 1 day after diagnosis of LOS (range: 0 to 181 days) compared to a median of 11 days after diagnosis for infants with fungal LOS (range: 0 to 140 days; p<0.0001) and 20 days for those with Gram-positive LOS (range: 0 to 194 days; p=0.004). For infants who survived sepsis, those with fungal LOS had the longest duration of hospital stay after the sepsis episode with a median 97 days (range: 58 to 155 days), compared with 54 days in those with Gram negative LOS (range: 3 to 351 days) and 63 days in those with Gram positive LOS (range: 4 to 273 days; p<0.0001).

Bivariate analyses were employed to assess factors associated with sepsis-related death including category of LOS, known risk factors for LOS, presenting laboratory and clinical signs of sepsis, and comorbidities. In unadjusted analyses, infection with a Gram-positive organism had a protective effect (see Table 1a, Supplemental Digital Content 1), whereas endotracheal intubation, prior antibiotic exposure, the need for pressors and H_2 blocker exposure, and NEC were all associated with a significantly higher risk of death. In addition, a higher risk of death was observed in infants presenting with hypoglycemia, neutropenia, thrombocytopenia, and leukocytosis.

When multivariable analyses were performed to assess factors associated with sepsis-related death, infants with Gram-positive LOS had significantly lower odds of death compared to those with Gram-negative or fungal LOS. After adjustment for several confounders, Gram-positive LOS was associated with a 78% lower likelihood of sepsis-related death compared to fungal LOS (OR: 0.22, 95% CI 0.07-0.64, p=0.006) and 83% less compared to Gram-negative LOS (OR: 0.17, 95% CI 0.08-0.36, p<.0001). There were no significant differences with respect to the risk of death between fungal and Gram-negative LOS (see Table 2a, Supplemental Digital Content 2). Several clinical and laboratory factors were associated with a significantly higher risk of death including the need for intubation and pressors, hypoglycemia, thrombocytopenia at presentation of illness, and concurrent NEC (see Table 2a, Supplemental Digital Content 2).

Discussion

LOS continues to be a relatively common occurrence in the NICU with substantial consequences. Prior investigations have identified risk factors for Gram-positive, Gram-negative, and fungal LOS [10-16]. When clinical and laboratory signs suggest LOS in this high risk population, an evaluation is performed, broad-spectrum antimicrobial therapy instituted and, if needed, cardiopulmonary support provided. The antimicrobial regimen selected is based on NICU-specific microbiologic data and local antimicrobial sensitivity patterns. Traditionally, the majority of LOS has traditionally been attributed to Gram-positive bacteria, followed by Gramnegatives, and with a small percentage attributed to fungi [3]. As a result, the standard empiric antimicrobial regimen in most NICUs excludes antifungals. The clinician must therefore be aware of risk factors and presenting clinical and laboratory signs of fungal LOS that may warrant empiric coverage and as recommended in a previous study [17], this coverage should be considered on an individual NICU basis. In comparing categories of LOS, we determined that VLBW infants with fungal infections were of lowest GA and BW, were most likely to be intubated, to have a central venous catheter in place and to be receiving IL and H_2 blockers at onset of LOS, to present with leukocytosis, and to have been previously exposed to antibiotics. No specific correlations were found between temperature instability or glucose disturbances and the type of pathogen.

Once LOS is diagnosed, the antimicrobial regimen is focused and discussions turn toward potential outcomes and prognosis. In our cohort of VLBW infants with LOS, sepsis-related death occurred in 10.6%, which is than that previously described [13]. We isolated these infants in an effort to identify clinical and laboratory factors that, when present at the onset of the disease, predict a fatal outcome. Prior investigations have determined that male gender, race, shock, need for mechanical ventilation, severe IVH, thrombocytopenia, and younger postconceptional age at time of infection have been associated with increased mortality in VLBW infants with LOS [18-22]. We determined that respiratory decompensation requiring endotracheal intubation and hypotension requiring initiation of pressors at the onset of the sepsis episode, hypoglycemia and thrombocytopenia as presenting laboratory signs of infection, and concurrent NEC all significantly and independently increased the likelihood of LOS-related death.

In our investigation, infants with Gram-positive LOS were significantly less likely to die from their infection as compared to those with Gram-negative or fungal LOS. Similar findings have been reported which suggest that Gram-positive organisms may be less virulent than Gramnegatives and fungi [2, 16]. However, these findings may also suggest a potential limitation of the investigation. Our retrospective data spanned the period of time from 1989 to 2007, when the CDC criteria for commensal species (i.e. CONS)-related BSI necessitated only one positive blood culture [5]. In 2008, the criteria were modified and two positive blood cultures required in an effort to limit over-reporting and inappropriate treatment of false positives [5]. In our investigation, CONS were the most common organisms cultured, representing 38% of all cases of LOS. It is especially difficult to deduce in a retrospective analysis whether a positive culture for CONS was a true or false positive. It is therefore highly likely that if we applied the current CDC criteria, the number of cases of LOS would have decreased substantially with a concomitant decrease in statistical power. In addition to this and the limitations inherent to a retrospective study that was done over such a long duration that includes changes in medical management protocols, another main limitation is that death was utilized as the primary endpoint in our analyses. Death is a multi-factorial endpoint, and despite best efforts, it is likely that we did not capture all contributing factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The project was partially supported by NIH - T32 HD007094

References

1. Stoll BJ, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics. 2010; 126(3):443–56. [PubMed: 20732945]
2. Makhoul IR, et al. Epidemiological, clinical, and microbiological characteristics of late-onset sepsis among very low birth weight infants in Israel: a national survey. Pediatrics. 2002; 109(1):34–9. [PubMed: 11773539]
3. Stoll BJ, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics. 2002; 110(2 Pt 1):285–91. [PubMed: 12165580]
4. Boghossian NS, et al. Late-Onset Sepsis in Very Low Birth Weight Infants from Singleton and Multiple-Gestation Births. J Pediatr. 2013
5. Garner JS, et al. CDC definitions for nosocomial infections, 1988. Am J Infect Control. 1988; 16(3): 128–40. [PubMed: 2841893]

6. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008; 36(5):309–32. [PubMed: 18538699]
7. Kliegman RM, Walsh MC. Neonatal necrotizing enterocolitis: pathogenesis, classification, and spectrum of illness. Curr Probl Pediatr. 1987; 17(4):213–88. [PubMed: 3556038]
8. Ehrenkranz RA, et al. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics. 2005; 116(6):1353–60. [PubMed: 16322158]
9. Papile LA, et al. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr. 1978; 92(4):529–34. [PubMed: 305471]
10. Benjamin DK Jr, et al. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics. 2006; 117(1):84–92. [PubMed: 16396864]
11. Saiman L, et al. Risk factors for candidemia in Neonatal Intensive Care Unit patients. The National Epidemiology of Mycosis Survey study group. Pediatr Infect Dis J. 2000; 19(4):319–24. [PubMed: 10783022]
12. Samanta S, et al. Risk factors for late onset gram-negative infections: a case-control study. Arch Dis Child Fetal Neonatal Ed. 2011; 96(1):F15–8. [PubMed: 20538712]
13. Hornik CP, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. Early Hum Dev. 2012; 88(2):S69–74. [PubMed: 22633519]
14. Graham PL 3rd, et al. Risk factors for late onset gram-negative sepsis in low birth weight infants hospitalized in the neonatal intensive care unit. Pediatr Infect Dis J. 2006; 25(2):113–7. [PubMed: 16462286]
15. Downey LC, Smith PB, Benjamin DK Jr. Risk factors and prevention of late-onset sepsis in premature infants. Early Hum Dev. 2010; 86(1):7–12. [PubMed: 20116186]
16. Shetty SS, et al. Determining risk factors for candidemia among newborn infants from population-based surveillance: Baltimore, Maryland, 1998-2000. Pediatr Infect Dis J. 2005; 24(7):601–4. [PubMed: 15999000]
17. Makhoul IR, et al. Review of 49 neonates with acquired fungal sepsis: further characterization. Pediatrics. 2001; 107(1):61–6. [PubMed: 11134435]
18. Benjamin DK, et al. Mortality following blood culture in premature infants: increased with Gram-negative bacteremia and candidemia, but not Gram-positive bacteremia. J Perinatol. 2004; 24(3): 175–80. [PubMed: 14985775]
19. Makhoul IR, et al. Pathogen-specific early mortality in very low birth weight infants with late-onset sepsis: a national survey. Clin Infect Dis. 2005; 40(2):218–24. [PubMed: 15655738]
20. Bhat MA, et al. Organism-specific platelet response and factors affecting survival in thrombocytopenic very low birth weight babies with sepsis. J Perinatol. 2009; 29(10):702–8. [PubMed: 19554015]
21. Zakariya BP, et al. Risk factors and predictors of mortality in culture proven neonatal sepsis. Indian J Pediatr. 2012; 79(3):358–61. [PubMed: 21997866]
22. Leal YA, et al. Risk factors and prognosis for neonatal sepsis in southeastern Mexico: analysis of a four-year historic cohort follow-up. BMC Pregnancy Childbirth. 2012; 12:48. [PubMed: 22691696]

Table 1

Category and specific organisms associated with late-onset sepsis, N=424

Type of Infection	Isolate Name	Frequency	Percent
Gram-positive bacteria		262	61.8
	Coagulase-negative *Staph*.	160	37.7
	Staphylococcus aureus	45	10.6
	Enterococcus faecalis	29	6.8
	Group B *streptococcus*	19	4.5
	Enterococcus faecium	2	0.5
	Other *streptococcus*	7	1.7
Gram-negative bacteria		126	29.7
	Escherichia coli	32	7.6
	Klebsiella pneumoniae	25	5.9
	Pseudomonas aeruginosa	24	5.7
	Klebsiella oxytoca	14	3.3
	Serratia marcescens	14	3.3
	Enterobacter cloacae	8	1.9
	Enterobacter aerogenes	2	0.5
	Other Gram-negative rods	7	1.7
Fungi		36	8.5
	Candida albicans	21	5.0
	Candida parapsilosis	8	1.9
	Other fungi	7	1.7

Table 2
Characteristics of VLBW neonates by type of infection

Characteristics	Gram positive N=262	Gram negative N=126	Fungal N=36	p-value
Gestational age, weeks (mean ± SD)	26.6 ± 2.3 [3]	26.8 ± 2.3 [3]	25.5 ± 2.2 [1,2]	0.01
Birth weight, grams (mean ± SD)	856 ± 248 [2]	929 ± 280 [1,3]	783 ± 214 [2]	0.003
ELBW, N (%)	199(76.0) [2]	79(62.7) [1,3]	31(86.1) [2]	0.004
Male gender, N (%)	128(48.9) [2]	77(61.6) 1	22(61.1)	0.04
Central line, N (%)	218(83.2) [3]	93(74.4) [3]	36(100) [1,2]	0.002
Any respiratory support, N (%)	198(75.6) [3]	93(73.8) [3]	34(9.4.4) [1,2]	0.03
Endotracheal intubation, N (%)	134(51.1) [3]	67(53.2) [3]	29(80.6) [1,2]	0.004
Prior antibiotic treatment, N (%)	41(15.7) [3]	23(18.3) [3]	13(36.1) [1,2]	0.01
Pressor treatment, N (%)	24(9.2) [3]	18(14.3)	8(22.2) [1]	0.05
TPN, N (%)	210(80.5) [2]	82(65.6) [1,3]	32(88.9) [2]	0.001
IL, N(%)	192(73.8) [2]	71(56.8) [1,3]	31(86.1) [2]	0.0002
H_2 Blockers, N(%)	103(40.9) [3]	50(41.3) [3]	25(71.4) [1,2]	0.002
Length of stay, days (mean ± SD)	87 ±47	78± 61 [3]	100 ±53 [2]	0.05

VLBW: very low birth weight; ELBW: extremely low birth weight; TPN: total parenteral nutrition; IL: intra lipid.
The superscripts indicate the significant pair-wise comparison of which column 1, 2 and 3 correspond to Gram positive, negative and fungi infection, respectively.

Table 3
Clinical signs and comorbidities at onset of LOS

Characteristics	Gram positive N=262	Gram negative N=126	Fungal N=36	p-value
Apnea	171 (65.5)[3]	74 (59.2)[3]	14 (38.9)[1,2]	0.007
Leukocytosis	53 (20.9)[2]	43 (35.0)[1]	12 (35.3)	0.007
UTI/Meningitis	12 (4.6)[3]	2 (1.6)[3]	8 (22.2)[1,2]	<0.0001
IVH	17 (6.5)	3 (2.4)[3]	5 (13.9)[2]	0.03
BPD	137 (57.6)	49 (46.7)[3]	22 (75.9)[2]	0.01
NEC	48 (18.6)[2]	40 (31.7)[1]	10 (27.8)	0.01

LOS: late-onset sepsis; UTI: urinary tract infection; IVH: intraventricular hemorrhage; BPD: bronchopulmonary dysplasia; NEC: necrotizing enterocolitis.

Data are presented as N (%). The superscripts indicate the significant pair-wise comparison of which column 1, 2 and 3 correspond to Gram positive, negative and fungi infection, respectively.