Published in final edited form as:
Acta Paediatr. 2012 April ; 101(4): 355–359. doi:10.1111/j.1651-2227.2011.02527.x.

Systemic responses of preterm newborns with presumed or documented bacteremia

Alan Leviton, MD[1], **T. Michael O'Shea, MD**[2], **Francis J. Bednarek, MD**[3], **Elizabeth N. Allred, MS**[1], **Raina N. Fichorova, MD, PhD**[4], and **Olaf Dammann, MD**[5] **for the ELGAN Study Investigators**

[1]Department of Neurology, Children's Hospital Boston, and Harvard Medical School, Boston MA 02115 USA

[2]Division of Neonatology, Department of Pediatrics, Wake Forest University School of Medicine, Winston-Salem NC 27157 USA

[3]Department of Pediatrics, University of Massachusetts Memorial Medical Center, Worcester MA 01605 USA

[4]Department of Obstetrics, Brigham and Women's Hospital and Harvard Medical School, Boston MA 02115 USA

[5]Floating Hospital for Children at Tufts Medical Center, Tufts University School of Medicine, Boston, MA, 02111 USA

Abstract

Aim—To compare the frequency of elevated concentrations of inflammation-related proteins in the blood of infants born before the 28th week of gestation who had documented bacteremia to those who had presumed (antibiotic-treated but culture-negative) bacteremia to those who neither.

Methods—The subjects of this study are the 868 infants born at 14 institutions for whom information about protein measurements on at least two of the three protocol days (days 1, 7, and 14) was available and who did not have Bell stage 3 necrotizing enterocolitis or isolated bowel perforation, which were strongly associated with bacteremia in this sample.

Results—Newborns with presumed early (week 1) bacteremia had elevated concentrations of only a few inflammation-related proteins, while those who had presumed late (weeks 2–4) bacteremia did not have any elevations. In contrast, newborns who had documented early bacteremia had a moderately strong signal, while those who had documented late bacteremia had a stronger signal with more protein concentrations elevated on two separate occasions a week apart.

Conclusions—Culture-confirmed early and late bacteremia are accompanied/followed by systemic inflammatory responses not seen with presumed early and late bacteremia.

Keywords

bacteremia; infant; premature; blood proteins

Corresponding author: Alan Leviton, Children's Hospital Boston, One Autumn Street (Box # 720), Boston MA 02215-5349. tel: 617 355-6491, fax: 617 730-0880. alan.leviton@childrens.harvard.edu.

None of the authors has any financial issue or conflict of interest to disclose

For Participating institutions, see supporting information.

Introduction

Bacteremia in the preterm newborn can be classified as either early or and late onset [1]. The assumption is that early-onset bacteremia has prenatal or intrapartum origins while late-onset is acquired after birth.

Because untreated bacteremia can be fatal, neonatologists often treat newborns with antibiotics if they suspect bacteremia, even when all cultures are negative [2–4]. Documented and presumed bacteremia have a similar set of antecedents, whether diagnosed in the first week or later [5]. We do not yet know, however, if they are similarly associated with indicators of systemic inflammation. Consequently, we measured the concentrations of 25 inflammation-associated proteins in perinatal blood [6–9] and compared the patterns of elevations associated with early and late bacteremia as well as the patterns associated with presumed and documented bacteremia.

Methods

The ELGAN Study

The ELGAN study was designed to identify characteristics and exposures that increase the risk of structural and functional neurologic disorders in ELGANs (the acronym for Extremely Low Gestational Age Newborns)[10]. During the years 2002–2004, women delivering before 28 weeks gestation at one of 14 participating institutions in 11 cities in 5 states were asked to enroll in the study. The enrollment and consent processes were approved by the individual institutional review boards. A full description of the methods is provided elsewhere [10]. Here we focus on those most relevant to the topic at hand.

The sample for this report consists of the 868 newborns for whom we had information about protein measurements on at least two of the three protocol days (days 1, 7, and 14) and who did not have Bell stage 3 necrotizing enterocolitis, which was strongly associated with bacteremia in this sample, or isolated bowel perforation[11].

Newborn variables

The gestational age estimates were based on a hierarchy of the quality of available information as described elsewhere [10]. Information was collected about blood cultures and sepsis therapy for each week, but not for each day. Consequently, we define early bacteremia as evident in the first week and late bacteremia as evident in weeks 2 and 3. No infant had bacteria cultured from the blood for the first time during week 4.

The recovery of an organism from blood was reported, but details about the organism were not. An infection was identified as documented when the cultured organism was considered a potential pathogen and not a contaminant. Presumed infections were culture-negative, but the infant received antibiotics for more than 72 hours.

Blood spot collection

Drops of blood were collected on filter paper (Schleicher & Schuell 903) on the first postnatal day (range: 1–3 days), the 7^{th} postnatal day (range: 5–8 days), and the 14^{th} postnatal day (range: 12–15 days). All blood was from the remainder after specimens were obtained for clinical indications. Dried blood spots were stored at −70°C in sealed bags with dessicant until processed.

Protein measurement

Details about elution of proteins from blood spots and measurement of the 25 proteins with the Meso Scale Discovery (MSD) electrochemiluminescence system are provided elsewhere [9].

Data analysis

We evaluated the generalized hypothesis that children diagnosed with bacteremia are no more likely than their peers to have a blood concentration of each inflammation-related protein in the highest quartile for gestational age and day the specimen was collected. We evaluated this hypothesis separately for early bacteremia (*i.e.*, during the first postnatal week) and for late bacteremia (*i.e.*, during postnatal weeks 2 and 3), first for elevations on each of the three protocol blood spot days, and then for elevations on one day only or for two or more days.

Using multinomial logistic regression models with adjustment for gestational age category (23–24, 25–26, and 27 weeks), we calculated odds ratios and 99% confidence intervals of a protein concentration in the top quartile comparing newborns with a diagnosis of presumed bacteremia and those with a diagnosis of documented bacteremia to newborns given neither diagnosis. To balance the risks of type 1 and type 2 errors with our many evaluations (25 proteins measured at 3 times for each of four bacteremia groups), we selected the 99% confidence interval rather than the conventional 95% confidence interval. This approach is in keeping with the view that more extreme adjustment for multiple comparisons can be counterproductive [12–16].

RESULTS

Sample characteristics (Data not shown)

Fully 40% of the 53 newborns who had documented early bacteremia also had documented late bacteremia compared to 23% of newborns who had presumed early bacteremia or no early bacteremia. In contrast, only 10% of the 210 newborns who had documented late bacteremia also had documented early bacteremia, while newborns who had presumed late bacteremia and those who had no late bacteremia diagnosis had even lower rates.

Documented and presumed early bacteremia: individual days (Table 1 in supporting information)

Newborns who had documented early bacteremia were more likely than their peers to have day-1 blood concentrations of CRP and IL-8 in the top quartile for gestational age. Children given a diagnosis of presumed early bacteremia did not show a tendency to have elevated concentrations of any protein on day1.

In addition to elevated concentrations of CRP and IL-8 as they had on day 1, newborns who had documented early bacteremia were also more likely than their peers to have elevated day-7 blood concentrations of SAA, TNF-alpha, TNF-R2, ICAM-1, and VEGF-R2. Children who had presumed early bacteremia were more likely than others to have elevated concentrations of MMP-9 and VEGF.

Children with documented or presumed early bacteremia did not have elevated concentrations of any protein in day-14 blood.

Documented and presumed late bacteremia: individual days (Table 2 in supporting information)

Newborns who had documented and presumed late bacteremia were no more likely than others to have an elevated concentration of any protein in their day-1 blood. Children who had documented late bacteremia were more likely than their peers to have day-7 elevated blood concentrations of CRP, SAA, TNF-R2, and IL-8. Infants who had presumed late bacteremia were less likely than others to have elevated concentrations of VCAM-1.

Newborns who had documented late bacteremia were more likely than their peers to have elevated concentrations in day 14 blood of CRP, SAA, IL-6, TNF-alpha, TNF-R2, IL-8, MIP-1B, I-TAC, ICAM-1, E-SEL, and VEGF-R2. In contrast, infants who had presumed late bacteremia were less likely than others to have elevated concentrations of RANTES and VCAM-1.

Documented and presumed early bacteremia: elevated concentrations on only one day or two or more days (Table 3 in supporting information)

CRP and IL-8 are the only proteins that were associated with documented early bacteremia whether the duration of elevation was on only one day or two or more days. SAA is the only protein elevated in association with documented early bacteremia on just one day, while TNF-alpha and VEGF-R2 are the only two proteins elevated in association with documented early bacteremia on two or more days.

Children who had presumed early bacteremia were more likely than children who did not show any evidence of bacteremia to have a one day elevation of MIP-1beta, and a two or more day elevation of IL-8. On the other hand, children who had presumed early bacteremia were at reduced risk of having an elevated concentration of MCP-4.

Documented and presumed late bacteremia: elevated concentrations on only one day versus two or more days (Table 4 in supporting information)

TNF-R2 and IL-8 are the only proteins associated with documented late bacteremia whether the duration of elevation was on only one day or two or more days. CRP, SAA, IL-6, and I-TAC are the four proteins elevated in association with documented early bacteremia on two or more days.

Children who had late presumed bacteremia were not at increased risk of having any protein elevations. Indeed, they were at reduced risk of having elevated concentrations of MIP-1beta, RANTES, and VCAM-1 on two days.

DISCUSSION

We have four main findings. First, children who had documented early bacteremia had a subtle inflammation-related protein signal on day 1 (CRP and IL-8), a considerably stronger day-7 signal (CRP, SAA, TNF-alpha, TNF-R2, IL-8, ICAM-1, and VEGF-R2), and no evidence of systemic inflammation on day 14. Second, children who had documented late bacteremia were at increased risk of having a moderate inflammation-related protein signal on day 7 (CRP, SAA, TNF-R2, and IL-8), a stronger inflammatory signal on day 14 (CRP, SAA, IL-6, TNF-alpha, TNF-R2, IL-8, MIP-1B, I-TAC, ICAM-1, E-SEL, and VEGF-R2), and a moderate sustained/recurrent inflammatory signal (CRP, SAA, IL-6, TNF-R2, IL-8, and I-TAC). Third, children who had presumed early bacteremia showed a subtle inflammation signal on day 7 (MMP-9 and VEGF), and had a sustained/recurrent elevation of only one protein, IL-8. Fourth, children who had presumed late bacteremia were not at increased risk of having elevated concentrations of any protein on postnatal day 1, 7, or 14.

Concurrent/content validity

One of the definitions of content validity is that a test/assessment truly reflects what it is supposed to represent [17]. Applied to our assessments, children exposed to bacteria in the blood should be much more likely than others to have elevated blood concentrations of inflammation-related proteins than their peers not exposed to inflammatory stimuli. That is what we found, providing additional documentation our protein measurements have content validity.

Differences between presumed and documented bacteremia

We have been faulted for classifying a disorder based on the therapy received, mainly because physicians vary widely in their propensity to use many therapies [18]. Yet, a physicians' decision to administer antibiotics to a very preterm newborn who does not have bacteria in his blood likely conveys information about the infant's appearing to be septic, or to be at increased risk of developing sepsis.. Perhaps some of these culture-negative infants were exposed to a non-bacterial stimulus for systemic inflammation. Nevertheless, we did not find that these newborns had systemic inflammation.

Multiple proteins are associated with documented late bacteremia

Others have reported that CRP, SAA, IL-1beta, IL-6, IL-8, and TNF-alpha are good indicators of neonatal bacteremia [19, 20]. One of the especially attractive features of CRP and SAA, for epidemiologic studies at least, is their long half-life, which means they can be identified even when opportunities to sample blood are infrequent. We found that nine proteins with presumed shorter half-lives (IL-6, TNF-alpha, TNF-R2, IL-8, MIP-1B, I-TAC, ICAM-1, E-SEL, and VEGF-R2) are also associated with bacteremia. This is not at all surprising given that an inflammatory stimulus can increase the expression of more than a thousand genes [21]. Although others encourage the quest for more specific biomarkers/tests [22], our data support the concept that elevations of multiple proteins taken together may be a better indicator of a very preterm newborn's systemic inflammation [23].

Implications for postnatal inflammation leading to organ damage

Newborns who developed sepsis are at increased risk of bronchopulmonary dysplasia/chronic lung disease [24, 25] and of indicators of brain damage [26–30] [31]. In our group of children, prolonged/recurrent elevated concentrations of inflammation-related proteins are characteristic of those who developed indicators of lung [32] and brain damage [33, 34]. Here we report that those who had late bacteremia are among the children most likely to have elevated concentrations of the proteins that have been shown to be associated with indicators of lung and brain damage. The resulting inference is that bacteria cultured from the blood sampled after the first postnatal week can be a stimulus for the inflammatory response we measured. We do not have the information to separate host and pathogen contributions to what we observed.

Strengths and limitations

Our study has several strengths. First, we included a large number of infants, making it unlikely that we have missed important associations due to lack of statistical power. Second, we selected infants based on gestational age, not birth weight, in order to minimize confounding due to factors related to fetal growth restriction [35]. Third, we collected all of our data prospectively. Fourth, our protein data are of high quality [36, 37] and have high content validity [6–9].

Our major limitation is that we did not record information about the organisms recovered from the blood of our subjects. Thus, our classification of documented bacteremia includes

such highly virulent organisms as Staphylococcus aureus along with much less virulent organisms, such as Staphylococcus epidermidis.

The other major limitation of our study is that we are not certain that the bacteremia always occurred prior to the elevation of the protein concentration. We last measured proteins on day 14 and last recorded bacteremia for the week that included days 15–21, but did not know when during that week the blood that harbored the bacteria was collected.

A limitation for those who want to compare our study to others is our definition of early bacteremia as bacteremia during the first postnatal week, rather than bacteremia limited to the first three postnatal days as defined by others. To what extent we or others misclassified the last four days of the first week remains to be determined.

CONCLUSION

Documented early and late bacteremia are accompanied/followed by systemic inflammatory responses not seen with presumed early and late bacteremia.

Key Notes

Compared to infants born before the 28th week of gestation who had no bacteremia during the first postnatal month, those who had culture-documented bacteremia had elevated blood concentrations of multiple inflammation-related proteins, sometimes on two occasions a week apart.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by a cooperative agreement with the National Institute of Neurological Disorders and Stroke (5U01NS040069-05) and a center grant award from the National Institute of Child Health and Human Development (5P30HD018655-28). The authors gratefully acknowledge the contributions of their subjects, and their subjects' families, as well as those of their colleagues.

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