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Cerebrospinal fluid cytokines in the diagnosis of bacterial meningitis in infants

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BACKGROUND: Bacterial meningitis poses diagnostic challenges in infants. Antibiotic pretreatment and low bacterial density diminish cerebrospinal fluid (CSF) culture yield, while laboratory parameters do not reliably identify bacterial meningitis. Pro and anti-inflammatory cytokines are elevated in bacterial meningitis and may be useful diagnostic adjuncts when CSF cultures are negative.

METHODS: In a prospective cohort study of infants, we used cytometric bead arrays to measure tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), IL-6, IL-8, IL-10, and IL-12 in CSF. Receiver operating characteristic (ROC) analyses and Principal component analysis (PCA) were used to determine cytokine combinations that identified bacterial meningitis.

RESULTS: Six hundred and eighty four infants < 6 mo were included; 11 had culture-proven bacterial meningitis. IL-6 and IL-10 were the individual cytokines possessing greatest accuracy in diagnosis of culture proven bacterial meningitis (ROC analyses; area under the concentration-time curve (AUC) 0.91; 0.9103 respectively), and performed as well as, or better than combinations identified using ROC and PCA. CSF cytokines were highly correlated with each other and with CSF white blood cell count (WBC) counts in infants with meningitis. A subset of antibiotic pretreated culture-negative subjects demonstrated cytokine patterns similar to culture positive subjects.

CONCLUSION: CSF cytokine levels may aid diagnosis of bacterial meningitis, and facilitate decision-making regarding treatment for culture negative meningitis.

Bacterial meningitis remains an important cause of morbidity and mortality in infants (1–3), despite advances in medical care, the availability of broad-spectrum antibiotics, and strategies for prevention of perinatal and hospital-acquired infections (4,5). Preterm and very low birth weight infants are at greater risk, due to the immaturity of the immune response, and the invasive procedures and devices necessary for survival (4,5). Mortality approaches 10%, and among survivors, meningitis significantly increases the odds of neurodevelopmental impairment (2,6).

The diagnosis of meningitis in the neonatal intensive care unit (NICU) remains fraught with challenges. Clinical signs are often nonspecific in infants. The gold standard for diagnosis is the cerebrospinal fluid (CSF) culture, obtained via a lumbar puncture (LP). However, the LP is often deferred in infants with cardiorespiratory instability, and antibiotics are initiated presumptively (4,7). Studies suggest that two-thirds of infants diagnosed with bacterial meningitis receive antibiotics prior to the LP (4,7). Infants evaluated for early onset sepsis are also frequently exposed to maternal intrapartum antibiotics. Antibiotic exposure reduces the diagnostic yield of the CSF culture (8). In the face of antibiotic pretreatment and negative CSF cultures, clinicians often rely on interpretation of CSF white blood cell count (WBC), protein, glucose, and CSF Gram stain to make the presumptive diagnosis of bacterial meningitis (9). However, no combination of CSF parameters has been shown to have ideal sensitivity and specificity (10,11). Similarly, parameters such as CSF WBC have been shown to possess suboptimal sensitivity and specificity in diagnosis of bacterial meningitis in infants with neurosurgical devices (12). Therefore, there is need for adjunctive markers that would aid in the prompt diagnosis of bacterial meningitis (including infants with neurosurgical devices), with the potential to reduce unnecessary antibiotic exposure and complications related to treatment.

Bacterial meningitis is associated with activation of the inflammatory cascade and the production of both pro and anti-inflammatory cytokines (13–16). Following the initial microbial stimulus, proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1)β, IL-6, and IL-8 amplify the inflammatory response and facilitate chemotaxis and neutrophil recruitment (15–17). This proinflammatory response is counter-balanced by the generation of anti-inflammatory (IL-10) and immune-modulating cytokines (IL-12) (15–18). Elevations of proinflammatory cytokines are sensitive markers of bacterial meningitis and may be useful in differentiating bacterial from viral disease (13,14,18–22). However, proinflammatory cytokines such as TNF-α and IL-1 remain elevated only transiently, thus limiting their diagnostic

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Received 1 April 2016; accepted 5 April 2016; advance online publication 3 August 2016. doi:10.1038/pr.2016.117

utility if the LP is performed later in the course of illness, as is often the case in neonates. While anti-inflammatory cytokines (IL-10) have been shown to be elevated in sepsis, they have been evaluated to a limited extent for their utility in the diagnosis of meningitis (17,23). Few studies have evaluated these markers in combination in the setting of meningitis in infants (19,22).

We hypothesized that measurement of a combination of pro and anti-inflammatory cytokines would aid in accurate diagnosis of culture-proven bacterial meningitis. With this study, we aimed to identify a panel of markers that would provide greatest diagnostic accuracy in identifying culture proven bacterial meningitis, and that would also improve the ability to diagnose culture-negative meningitis. Secondarily, we aimed to characterize factors (CSF blood contamination, effects of antibiotic pretreatment, and bacteremia) that may influence and modify cytokine levels in CSF in infants.

RESULTS

Six hundred and eighty four subjects were included in the analysis; 60% were male and 54% were preterm (**Table 1**). Among the infants, 77% had received antibiotics prior to the LP and 17% were diagnosed with a blood stream infection. Eleven subjects had culture proven bacterial meningitis. Most bacterial isolates from CSF were gram-positive (91%) (*Staphylococcus aureus*: $n = 4$, *Staphylococcus agalactiae*: $n = 3$, coagulase negative staphylococci: $n = 2$ and *(Escherichia faecalis*: $n = 1$); 6/11 infants with bacterial meningitis had prior neurosurgical procedures (**Table 2**). The infants who had prior neurosurgery had negative blood cultures, while the remaining five infants with meningitis also had bacteremia. Six additional subjects had positive CSF cultures, which were considered contaminants (alpha-hemolytic species, gram positive cocci not speciated, *Bacillus* species, *Staphylococcus epidermidis*-2 and *Staphylococcus hominis*), and were reclassified into the control group.

Non-parametric Analyses

CSF WBC, protein and glucose values significantly differed between subjects with culture proven bacterial meningitis vs. the remainder of subjects, as did values of the cytokines TNF-α, IL-1, IL-6, IL-8, IL-10, and IL-12 (**Table 1**, **Figure 1**). IL-6 and IL-8 showed the greatest absolute elevations in values (**Figure 1**), although all the cytokines were significantly elevated in subjects with bacterial meningitis compared with other subjects ($P < 0.05$). Of note, two subjects with bacterial meningitis (one with *S. warneri* and the other with *(Escherichia faecalis*) had much lower values of all CSF cytokines compared to the remaining nine infants in that group.

Correlations Among Cytokines and with CSF WBC

In infants with bacterial meningitis cytokine values were highly correlated with each other and with CSF WBC counts (**see Supplementary Table S1** online). In contrast, cytokines and CSF WBC counts were only weakly correlated in negative control subjects (data not shown) with the exception of IL-6 and IL-8 which were directly correlated ($R = 0.81$, $P < 0.001$).

Table 1. Demographic details and CSF parameters of cohort ($n = 684$)

Variable	Culture proven bacterial meningitis ($n = 11$)	Definite negative ($n = 151$)	Indeterminate subjects ($n = 513$)
Gestational age, wk, median (IQR)	32 (24–38)	38 (32–40)	36 (29–39)
Preterm[a], *n* (%)	7 (64%)	63 (42%)	292 (57%)
Neonates[b], *n* (%)	4 (36%)	111 (74%)	400 (78%)
Postnatal age, d, median (IQR)	40 (21–55)	8 (1–32)	4 (1–24)
Birthweight, g, median (IQR)	1,665 (660–2,665)	2,850 (1,742–3,358)	2,450 (1,300–3,260)
Male sex, *n* (%)	7 (64%)	89 (59%)	307 (60%)
Race[c], *n* (%)	AA 2 (18%), Cauc 6 (55%), Asian 0 (0%), NH/PI 0 (0%), Other 3 (27%), Unknown 0 (0%)	AA 48 (32%), Cauc 66 (44%), Asian 8 (5%), NH/PI 0 (0%), Other 23 (15%), Unknown 6 (4%)	AA 205 (40%), Cauc 190 (37%), Asian 22 (4%), NH/PI 10 (2%), Other 67 (13%), Unknown 19 (4%)
Hispanic ethnicity, *n* (%)	0 (0%)	8 (5%)	41 (8%)
Antibiotics prior to LP, *n* (%)	9 (82%)	0 (0%)	513 (100%)
Culture proven BSI, *n* (%)	5 (45%)	10 (7%)	98 (19%)
CSF WBC (cells/mm^3), median (IQR)*	215 (30–483)	4 (2–9)	4 (2–10)
CSF protein (gm/dl), median (IQR)**	322 (141–841)	88 (61–121)	101 (73–137)
CSF glucose (mg/dl), median (IQR)†	32 (20–46)	50 (42–58)	49 (42–59)

CSF, cerebrospinal fluid; IQR, interquartile ranges; BSI, blood stream infection; LP, lumbar puncture; WBC, white blood cell.
[a]Preterm: Infants born at <37 wk gestation. [b]Neonates: Infants in the first 28 d of life. [c]AA, African American; Cauc, Caucasian; NH/PI: Native Hawaiian/Pacific Islander.
P values based on Kruskal–Wallis testing: $^*P < 0.001$, $^{**}P < 0.029$, $^\dagger P < 0.004$.

Table 2. Details of infants with bacterial meningitis

Pathogen in CSF culture	GA[a] (wk)	PNA[b] (d)	Birth weight	Neurosurgical intervention	Bacteremia	CSF WBC (/cu.mm)	CSF protein (gm/dl)	CSF glucose (mg/dl)
Staphylococcus aureus[c]	23.4	123	700	VP shunt in place	None	334	75	32
S. aureus[d,e]	24.5	33	600	S/P fetal myelomeningocele repair	None	1,370	884	20
S. aureus[c]	28	55	1,070	VP shunt in place	None	57	709	20
S. aureus[d]	38	40	2,540	Ventriculostomy drain in place	None	29	45	46
Staphylococcus agalactiae	27	118	645	–	S. agalactiae	483	192	33
S. agalactiae[e]	38	27	2,665	–	S. agalactiae	215	841	19
S. agalactiae	39	46	3,160	–	GPC – NS[f]	426	322	52
Staphylococcus warneri[g]	24.2	21	660	–	S. warneri	13	141	62
Staphylococcus epidermidis	32	44	1,665	VP shunt in place	None	1500	317	19
(Escherichia faecalis	35.6	21	2,035	–	E.coli	30	453	46
(Escherichia cloacae	37.5	15	4,270	VP shunt in place	None	150	856	20

CSF, cerebrospinal fluid; VP, ventriculoperitoneal.
[a]GA, gestational age. [b]PNA, postnatal age. [c]MSSA, methicillin sensitive *S. aureus*. [d]MRSA, methicillin resistant *S. aureus*. [e]Died during hospitalization. [f]GPC-NS, Gram positive cocci, not specified. [g]CSF culture positive for S. warneri only in enrichment broth (however based on clinical symptoms and concurrent blood stream infection with same bacteria, decision made to treat for meningitis.

Effect of bacteremia, Traumatic LPs, Antibiotic Pretreatment and Neurosurgical Intervention on Cytokine Values

Bivariate analysis comparing infants with bacteremia (without bacterial or viral meningitis) with infants with negative cultures showed no significant differences in cytokine values between groups, suggesting that cytokine values are not significantly elevated in CSF during bacterial sepsis. We did not compare CSF cytokine values between infants with both bacteremia and bacterial meningitis, and infants with nonbacteremic meningitis, given the small number of culture proven cases, with neurosurgical intervention acting as a confounder. There were no linear relationships identified between CSF RBC counts and cytokine levels, except for a weak linear association with IL-8 (P-value = 0.025, R^2 = 0.0075, for all subjects). On comparing subjects with negative cultures who were pretreated with antibiotics vs. not (definite negative controls), we found slight elevations of CSF protein and IL-8, and slight decreases in TNF-α and IL-12 in the former. In culture positive subjects, there was no clear correlation between number of days on antibiotics and cytokine values. Among the infants with bacterial meningitis, the presence of neurosurgical intervention did not significantly alter cytokine values.

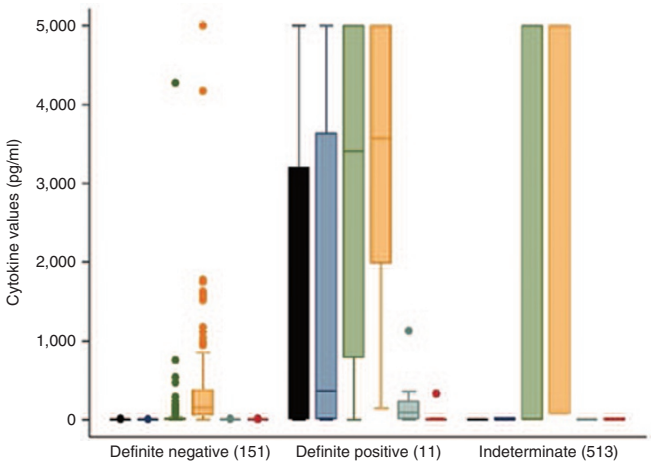

Figure 1. Box plots of cytokine values in definite positive, definite negative, and indeterminate infants. Excludes three infants with viral meningitis and six infants with contaminants in cerebrospinal fluid (CSF). To better delineate differences at lower cytokine levels, we have limited the upper limit of the graph to 5,000 pg/ml. Three values of IL-6 and 11 values of IL-8 that exceeded this limit are plotted at the upper limit of the graph. Black bars = TNF-α; blue bars = IL-1; green bars = IL-6; orange bars = IL-8; gray bars = IL-10; red bars = IL-12. TNF-α, tumor necrosis factor alpha; IL-1, interleukin 1.

ROC Analyses

Receiver operating characteristic (ROC) analyses comparing definite positive and negative samples demonstrated that five of the six cytokines had very good accuracy for the diagnosis of bacterial meningitis; among them, IL-10 and IL-6 had the greatest areas under the curve (AUC) of 0.9103 and 0.91, respectively (Figure 2). Several cytokine combinations also demonstrated excellent accuracy for discrimination of bacterial meningitis, however none provided improved accuracy beyond that derived from individual cytokine analysis (see **Supplementary Table S2** online). On applying cut-off levels derived from the ROC analysis of individual cytokines, an IL-6 level of 790 pg/ml provided a sensitivity of 81.8% and a specificity of 99.3%, while classifying 11 indeterminate subjects as positive (see **Supplementary Table S3** online). In contrast, an IL-10 level of 7.4 pg/ml, which provided an identical sensitivity of 81.8%, had a lower specificity of 91.4%, and classified 33 indeterminate subjects as positive (see **Supplementary Table S3** online).

Principal Component Analysis

In order to develop a classification tool for indeterminate subjects, we initially limited the sample set to definite positives

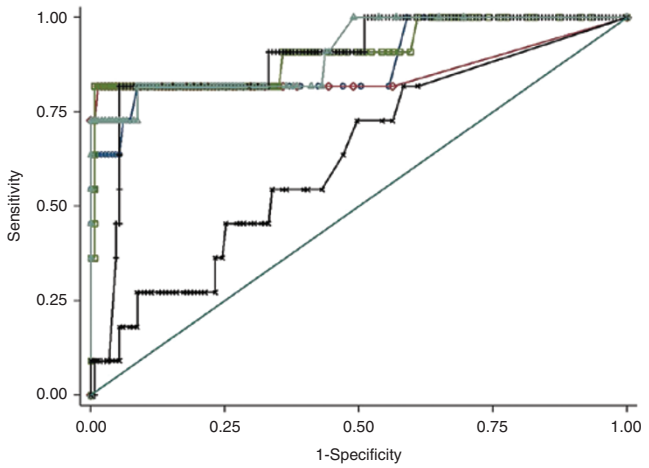

Figure 2. Receiver operating characteristic (ROC) analyses of individual cytokines in discrimination of culture proven meningitis. The ROC analysis compares definite positive and definite negative infants; excludes three infants with viral meningitis. ROC curves are shown for tumor necrosis factor alpha (TNF-α) (circles, AUC 0.88), IL-1 (diamonds, AUC 0.86), IL-6 (squares, AUC 0.91), IL-8 (plus signs, AUC 0.89), IL-10 (triangles, AUC 0.91), and IL-12 (X signs, AUC 0.63). AUC, area under the curve; IL-1, interleukin 1.

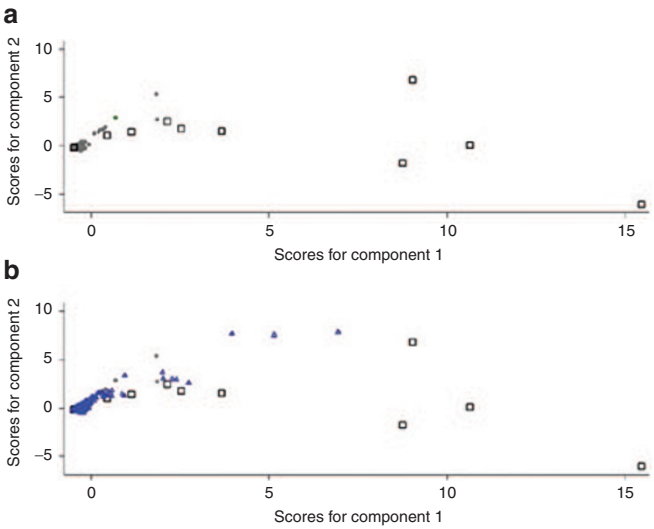

Figure 3. Score plots based on principal components 1 and 2. (**a**) Score plot of definite negatives and positives. (**b**) Scores of indeterminate subjects overlaid on score plot of definite negatives and positives. Composite scores were assigned to each sample for Principal component (PC)1 and 2. These are shown plotted in the two-way graphs. Panel **a** contains plots of the scores of the 11 definite positives (squares), and 151 definite negatives (circles). Panel **b** adds the overlay of scores of indeterminate subjects (triangles); the majority of these clustered close to the negative subjects. Fourteen indeterminate subjects had PC1 scores > 0.45, compared with the definite positive subjects, thus suggestive of meningitis.

(11 subjects) and definite negative controls (151 subjects). The first principal component (PC1) explained nearly 60% of the variance. Most definite positive subjects had distinctly different scores when compared with definite negative subjects (**Figure 3a**). **Figure 3b** demonstrates the scores of the indeterminate subjects overlaid on those of definite positive and definite negative subjects. All subjects classified as positive by PC1 cutoff (except one) were previously identified using IL-6 or IL-10, supporting the inference that PCA did not substantially alter or augment results of ROC analysis (see **Supplementary Table S3** online).

DISCUSSION

Our prospective multisite study demonstrates that TNF-α, IL-1, IL-6, IL-8, IL-10, and IL-12 levels are significantly elevated in bacterial meningitis in infants. Our ROC and PCA analyses identified individual cytokines and combinations that could diagnose culture proven meningitis, and are likely to aid in discrimination of meningitis in infants with negative CSF cultures. Furthermore, CSF cytokine values were not elevated in infants with bacteremia but without meningitis, highlighting the potential for this approach to improve our management of infants evaluated by LP.

Previous studies have demonstrated good sensitivity and specificity for TNF-α, IL-6, IL-8, and IL-12 individually in the diagnosis of bacterial meningitis (8,10,11,13,16,22). However, these authors mostly used univariate analyses and ROC analysis (8,10,11,13,16,22). A traditional ROC-based approach to assess diagnostic accuracy requires comparison against a valid reference standard. CSF culture, the generally accepted gold standard, has diminished sensitivity in antibiotic-exposed populations such as NICU infants. Thus, this approach may underestimate sensitivity and specificity of markers due to misclassification bias; infants with partially treated culture negative meningitis are classified along with culture negative infants, which could lead to blunting of differences in cytokine values between the "positive" and "negative" groups.

An alternative approach is to employ a classification system to sort confirmed positive and negative subjects, and subsequently use this system to identify "latent" or hidden positive subjects (i.e., infants with partially-treated meningitis whose cultures are negative, but whose cytokine values classify them closest to true positive subjects). We approached this question using two complementary analytic methods. We initially performed a modified ROC analysis using CSF culture as gold standard, but compared only definite positive and definite negative subjects, to limit misclassification bias. IL-10 and IL-6 were the individual cytokines with the best areas under the curve, while combinations of cytokines performed marginally better at diagnosing culture proven meningitis. The ROC approach confirmed that these cytokines possessed good diagnostic accuracy in meningitis and justified the inclusion of these markers in additional analyses.

We observed a high correlation among cytokines in infants with culture proven meningitis, suggesting overlap in measurement. A modality such as PCA has the potential to minimize redundancy by simplifying data from multiple contributing variables into a few major composite variables. Recent studies of sepsis, systemic inflammatory response syndrome and meningitis have employed a PCA approach to evaluate the diagnostic ability of multiple cytokines measured simultaneously (24,25). In our study TNF-α, IL-1 and IL-6 were the major elements of

the first PC1 score, which is consistent with previous literature in children and adults with meningitis (see **Supplementary Figure S1** online) (19,22). While these results differ somewhat from our ROC analyses, we speculate that this may be due to the correlation of IL-1 and IL-6 with IL-10, thus leading to the elimination of the latter cytokine from the model. As the PCA model did not substantially improve upon the diagnostic accuracy provided by the individual cytokines IL-6 and IL-10, we suggest that measurements of these two cytokines could best aid in decision-making regarding which antibiotic-pretreated subjects might benefit from a longer course of antibiotics befitting treatment of bacterial meningitis.

Additionally, we have elucidated the impact of various factors on CSF cytokine elevations in infants. We have shown that the presence of blood in CSF minimally impacts CSF cytokine values. Although IL-8 showed an association with CSF red blood cell (RBC) counts, the small coefficient of determination (R^2) suggests that CSF RBC explains very little of the variation in IL-8 values. Correlations between CSF WBC and CSF cytokine levels were weak in nonmeningitic subjects but strong in infants with meningitis, suggesting that WBCs in CSF play an important role in mediating elevations of CSF cytokines in the setting of bacterial meningitis. The antibiotic pretreated culture-negative group likely includes some infants with partially treated meningitis whose CSF samples are obtained beyond the onset of their disease process, which may serve to explain why CSF protein levels are higher in this indeterminate group of subjects, while TNF-α levels (which rise and fall rapidly) are not elevated. Our data also demonstrate that bloodstream infections without culture proven meningitis do not alter CSF cytokine levels. These findings suggest that there may exist a "compartmentalization" of cytokine responses whereas cytokines produced in response to noncentral nervous system infection which does not cross the blood-brain barrier. Therefore, we speculate that the adverse neurodevelopmental outcomes in premature infants with noncentral nervous system infections may be mediated by hemodynamic or metabolic pathways rather than by direct transmission of cytokine alterations from plasma to CSF.

We recognize that six infants with bacterial meningitis in our study required neurosurgical intervention, and believe this to be representative of the NICU population at greatest risk of acquiring meningitis. There were no significant differences in cytokine values in bacterial meningitis between the infants with and without neurosurgical intervention, justifying their categorization as one group. Interestingly, all infants without neurosurgical intervention who developed bacterial meningitis demonstrated bacteremia in our study. This finding, though limited by the small number of subjects, contrasts with prior studies of neonatal meningitis that have suggested that up to 25% of infants develop meningitis in the absence of bacteremia (4,26). Furthermore, two subjects deemed to have bacterial meningitis based on their CSF cultures and clinical presentation demonstrated much lower cytokine values than the rest (and plotted far away from the remaining positive subjects in PCA), leading us to consider the possibility that these two subjects could have been misclassified as having meningitis by the medical teams caring for them. The addition of cytokine based prediction rules may thus improve our ability to distinguish true infections from contamination.

Our study possesses several strengths. To our knowledge, this is the largest sample of infants under 6 mo in whom CSF cytokine levels have been measured. In contrast to many case-control studies, we employed a prospective cohort design, which allowed a more robust estimate of the diagnostic ability of cytokines without risk of selection bias. Use of a multianalyte profiling method enabled us to measure several cytokines simultaneously. Our large sample size provided us with the unique ability to examine the impact of multiple factors on cytokine levels in the CSF, including the effect of antibiotic pretreatment, traumatic taps, and bacteremia. The large antibiotic pretreated population afforded us the ability to examine the effect of antibiotics on cytokine values. We also employed two different analytic techniques to assess diagnostic ability of cytokines: ROC analyses as well as PCA.

The limitations of this study include the small number of infants with culture proven meningitis. Although we collected data on results when these were available, viral studies were not performed on all samples. In addition, a large proportion of infants in the study were pretreated with antibiotics. However, these study limitations are reflective of the real world dilemmas that surround diagnosis of bacterial meningitis. We have therefore attempted to utilize the information available about these factors to improve our understanding of how they influence CSF inflammation and cytokine values. Given these issues, we acknowledge the possibility of misclassification bias despite using rigorous definitions of bacterial meningitis, contaminants, and definite negative controls.

We believe that this study adds valuable information regarding the potential role of cytokines in the diagnosis of bacterial meningitis in the NICU. The cytokines IL-6 and IL-10 appear especially discriminatory, and may be useful in identifying infants with culture negative meningitis. With further validation, we believe that this combination of markers may be an useful tool for decision-making regarding course of treatment in culture negative but antibiotic pretreated subjects.

METHODS

Study Design and Setting

We conducted a multicenter prospective observational study. Participating sites included the NICUs at the Children's Hospital of Philadelphia (CHOP), the Hospital of the University of Pennsylvania and Pennsylvania Hospital. The Institutional Review Boards at the Children's Hospital of Philadelphia and the University of Pennsylvania approved this study. Informed consent was obtained from families prior to analysis of CSF samples and data.

Subjects

Infants born at any gestational age, who received LP between 0to 180 postnatal days of life, were eligible for enrollment. In infants undergoing multiple LPs, only data from the first LP was included in the analysis. Six subjects with unknown CSF culture results were excluded.

Study Definitions

Definite positive or culture-proven bacterial meningitis was defined by the identification of bacterial pathogens in CSF culture. CSF

cultures positive for coagulase-negative staphylococcal species needed to meet the following criteria in order to be considered true bacterial meningitis: presence of blood stream infection with the same bacteria or a documented ventriculoperitoneal shunt infection, plus a decision made by the clinical team to treat the infant for meningitis. Definite negative controls were defined as infants with negative CSF cultures and viral studies in the absence of antibiotic pretreatment. Indeterminate subjects were defined as infants with negative CSF cultures obtained after antibiotic pretreatment. Contaminants were defined based on pathogen identity and absence of clinical and laboratory signs of meningitis. Bacteremia was defined as a positive blood culture during the sepsis event for which the LP was performed.

Study Procedures

An additional sample of CSF was obtained at the time of the LP performed for clinical indications. This sample was coded and transferred to the research lab for storage at −70 °C. Data was collected from the infant's medical record and stored in a coded electronic database.

Measurements: CSF Culture

The three centers used similar methods for CSF analysis. Of which the laboratory (CHOP) performed an additional enrichment step for detection of bacteria in CSF cultures (see **Table 2** footnote for cases where only enrichment broth cultures were positive).

Cytokine Analysis

Cytokine levels (TNF-α, IL-1β, IL-6, IL-8, IL-10, and IL-12) were measured at the Children's Hospital of Philadelphia using a cytometric bead array kit (Becton Dickinson, Franklin Lakes, NJ). This method has an analytical sensitivity compared with conventional enzyme-linked immunosorbent assay, while measuring multiple analytes simultaneously using very small volumes of CSF (27). Study personnel performing the cytokine analysis were blinded to the results of CSF culture.

Sample Size Calculation

In order to achieve a precision of 0.1 with 95% confidence levels, we calculated that 10 infants with proven meningitis would be required. We enrolled subjects until we were able to achieve our target of positive cultures.

Data Analysis

Demographic, clinical, and laboratory data were summarized as follows: continuous data were presented as means and SDs (if parametric), and as medians and interquartile ranges (IQR) (if nonparametric). Categorical data were presented as proportions and percentages. Analyses of nonparametric data were performed using Kruskal–Wallis and Wilcoxon rank sum testing. Cytokine values were compared between infants with and without bacterial meningitis; as well as between subgroups of culture negative infants with and without bacteremia; and with and without antibiotic pretreatment. We examined the correlation of CSF cytokine values with each other, with CSF WBC counts, CSF RBC counts, and the number of days on antibiotics. We also compared cytokine values in infants with neurosurgical intervention who developed meningitis, to the remaining infants with meningitis, and to other infants with neurosurgical intervention but without meningitis.

We initially limited our population to definite culture positive and definite culture negative subjects, in order to identify the best markers that distinguish subjects with culture proven meningitis from noninfected controls. We first performed Receiver Operating Characteristic (ROC) analyses for each cytokine and for all possible combinations of cytokines, to verify their accuracy in diagnosis of culture-proven bacterial meningitis. We then employed principal component analysis (PCA), which provides a reductive strategy to collate the various cytokines into their most discriminatory composite components, to determine if combinations identified by PCA showed improved sensitivity and specificity over combinations identified on ROC analysis. Next, we planned to identify the subset of antibiotic pretreated subjects with negative culture results who demonstrated patterns of cytokine elevations closest to culture positive subjects. We determined cut-off values based on ROC and PCA analyses that provided good discrimination, and applied these to the entire study population, in order to identify antibiotic pretreated culture negative subjects whose values clustered close to subjects with bacterial meningitis. All analyses were performed using STATA 11.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr.

ACKNOWLEDGMENTS

We extend our sincere thanks to Caitlin LaRussa, Kaitilin Mahoney, Lavanya Madhusudan, Sharon Zirin, and Linda Corcoran for their help with study coordination, sample and data collection. We also thank Eric Reidel for his help with performance of the cytokine analysis, and Xianqun Luan for his statistical advice. Contributors' statement: All authors contributed substantially to the design, analysis, and interpretation of data. All authors provided important intellectual input in drafting or revising the manuscript.

STATEMENT OF FINANCIAL SUPPORT

This study was supported by funding by the Foerderer-Murray Award (The Children's Hospital of Philadelphia) and the University Research Foundation grant (the University of Pennsylvania).

Disclosure: None of the authors have any financial ties to products or conflicts of interest relevant to the research presented in this publication.

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