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High Pneumococcal DNA Loads Are Associated With Mortality in Malawian Children With Invasive Pneumococcal Disease

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Abstract

Background—In bacteremia owing to *Streptococcus pneumoniae*, high bacterial counts at presentation have been shown to be predictive of the development of serious invasive disease. Using real-time PCR, we aimed to determine pneumococcal DNA loads in blood and CSF, and their relationship to cytokine concentrations, clinical presentation and outcome.

Methods—Children with confirmed meningitis (n = 82) or pneumonia (n = 13) were prospectively recruited, and blood and CSF samples taken for pneumococcal bacterial DNA loads and cytokine determination.

Results—At the time of admission, the median bacterial load in blood was 1.6×10^3 DNA copies/mL (range 0.00–1.54×10^6) and in CSF it was 5.77×10^7 DNA copies/mL (range 4.42×10^2 to 6.15×10^8). Median blood and CSF bacterial loads (log DNA copies/mL) were significantly higher in nonsurvivors than in survivors; blood (3.80 vs. 2.97, $P = 0.003$), CSF (8.17 vs. 7.50, $P = 0.03$). In HIV-infected children (n = 59), blood and CSF loads and plasma tumor necrosis factor-α, interleukin-1β (IL-1β), IL-6 and IL-10 were all significantly higher in nonsurvivors than in survivors, but in HIV-uninfected children (n = 36) this difference was not significant. Blood bacterial loads and plasma cytokine concentrations were significantly associated, and were all significantly higher in children with meningitis than in those with pneumonia. In children with meningitis, median CSF cytokine concentrations were significantly higher than median plasma cytokine concentrations ($P < 0.001$) and CSF bacterial loads were significantly associated with CSF IL-1β ($P = 0.002$) and IL-10 ($P = 0.001$) concentrations.

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Conclusions—Pneumococcal DNA loads are associated with plasma cytokine concentrations, and are higher in meningitis than in pneumonia. High blood and CSF pneumococcal DNA loads are associated with a fatal outcome.

Keywords

bacterial load; pneumococcal; cytokine; meningitis; pneumonia

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The pneumococcus is a major cause of meningitis and severe pneumonia in the developing world. The burden of invasive pneumococcal disease (IPD) has increased in areas with a high prevalence of HIV infection.1 In The Gambia (with a low HIV prevalence rate), the incidence of IPD has been estimated to be 240/100,000/yr in children less than 5 years and 554/100,000/yr in children less than 1 year of age.2 By contrast, in South Africa (with a high HIV prevalence rate) the incidence of IPD in children under 2 years was found to be 73/100,000/yr in HIV-uninfected children and 3036/100,000/yr in HIV-infected children.3 The increased susceptibility of HIV-infected children to IPD is predominantly because of their increased susceptibility to disease caused by pediatric serotypes (serotypes 6, 9, 14, 19, 23).

Tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, macrophage inflammatory protein-1α, monocyte chemoattractant protein-1 and nitric oxide have been shown to be elevated in murine models of pneumococcal pneumonia.[4–6] IL-10 appears to down-regulate pathophysiologic changes in pneumococcal meningitis and this may be mediated by interfering with the production of reactive nitrogen intermediates.7 It is now recognized that the net biologic response of pro- and antiinflammatory cytokines may affect the outcome of certain inflammatory diseases.8 An improved understanding of the critical mediators in the host response to IPD is an important step in developing new therapeutic strategies.

In children with bacteremia owing to *Haemophilus influenzae* or *Streptococcus pneumoniae*, high bacterial counts (greater than 100 organisms per milliliter of blood), at presentation, based on quantitative blood culture methods, were shown to be highly predictive of the development of serious invasive disease, such as epiglottitis or meningitis.9 This was confirmed in another study which demonstrated that the magnitude of bacteremia correlated with the severity of infection.10 Patients with *H. influenzae* meningitis with greater than or equal to 10^7 colony forming units of *H. influenzae* type b per milliliter of CSF had a significantly greater frequency of neurologic sequelae.11 In children the magnitude of bacteremia is usually higher than that in adults, the level of bacteremia being inversely correlated with the patients age.12

We used a modification of a multiplex real-time PCR assay13 to detect and quantify pneumococcal DNA in blood, CSF and lung aspirate samples, and to determine the relationship between bacterial loads, cytokine concentrations, disease presentation and outcome.

METHODS

Study Setting

Study participants were recruited from Queen Elizabeth Central Hospital, Blantyre, Southern Malawi, which serves a population of ~1 million. This is a government-funded teaching and referral hospital with 150 pediatric beds, although pediatric inpatients can total in excess of 300. The children's accident and emergency unit manages about 90,000 patients a year, of whom about 20% are admitted to the wards. The prevalence of HIV infection in

pediatric inpatients in Blantyre has been estimated to be 19% overall,14 and 34% in children with bacterial meningitis.15 None of the children recruited into the study were receiving trimethoprim-sulfamethoxazole prophylaxis or antiretroviral therapy at the time of admission, and pneumococcal conjugate vaccine is not available.

The Malawi-Liverpool-Wellcome Trust Clinical Research Programme has a research pediatric ward adjacent to the government pediatric unit with a capacity for 14 beds. All children in this study were recruited either from the accident and emergency unit (during normal working hours) or the admissions unit (out of hours), and managed on the Malawi-Liverpool-Wellcome Trust research ward. The admission and management protocols are identical in both the accident and emergency and admission units.

Study Population

Children aged 2 months to 16 years identified as a possible case of pneumonia (respiratory rate ≥50 min^{-1} for children <12 months and ≥40 min^{-1} for children ≥12 months) or meningitis (stiff neck, bulging fontanelle, fever and convulsions) were recruited into the study. All children who met the study criteria between April 2004 and April 2005 were included in this cohort, and are representative of children with IPD in Malawi. The primary purpose of this study was to gain insight into the pathogenesis of IPD in children. Some of the children were also recruited into a clinical trial assessing duration of ceftriaxone treatment (see under Management Protocols). Only children with confirmed disease were included in the analysis for this study. We defined IPD when *S. pneumoniae* was identified (by culture, microscopy or PCR) from 1 or more of the following normally sterile body sites; blood, cerebrospinal fluid, lung aspirate.

Confirmed pneumococcal pneumonia was defined as radiologic evidence of pneumonia (focal or lobar consolidation) plus 1 or more of the following: blood or lung culture aspirate positive for pneumococci, lung aspirate positive for pneumococcal polysaccharide antigen or pneumococcal DNA.

Confirmed pneumococcal meningitis was defined as abnormal CSF cell count, >10 μL^{-1} plus 1 or more of the following: CSF culture positive for pneumococci, CSF Gram stain consistent with pneumococci, CSF positive for pneumococcal polysaccharide antigen, CSF positive for pneumococcal DNA.

Controls were healthy afebrile children from the same villages as the cases, and were aparasitemic. Controls were selected from neighbors of the index case (as part of a larger genetic susceptibility study) and were as closely age matched as possible to the index case. Children admitted to hospital for elective shunt surgery, who had CSF microbiologic analysis before surgery, and had no CSF pleocytosis were used as controls for CSF samples.

The primary outcome measure was death/survival in hospital. Ethical approval for this study was granted from The College of Medicine Research Committee, Malawi and The Liverpool School of Tropical Medicine Local Research Ethics Committee.

Procedures

On admission, the study was explained to the parent or guardian of each child and written informed consent was obtained. A complete history was taken which included demographic details, medical history, vaccination status and details of recent antibiotic use. All children were fully examined, and weight and height were measured. Blood sample was taken from all children for blood culture, full blood count, malaria parasites, blood glucose, blood lactate, HIV serology/PCR, pneumococcal PCR and cytokine analyses. Urine or serum samples were not tested at the time of admission for the presence of antimicrobials. A

lumbar puncture was performed for children with suspected meningitis and CSF was taken for microscopy, culture, latex agglutination and pneumococcal PCR and cytokine determination. All CSF samples were taken before antibiotics were administered. A lung aspirate was performed for children with focal or lobar pneumonia in an area identified by a combination of chest radiograph findings and physical signs. CD4 counts and HIV viral loads were not available for children recruited into the study.

Management Protocols

Children with meningitis were treated with intravenous ceftriaxone 100 mg/kg once daily for 5 days. Thereafter, if repeat lumbar puncture on day 2 did not show continued presence of bacteria, they were recruited into a randomized controlled trial on day 6 to receive either placebo or ceftriaxone 100 mg/kg once daily for the next 5 days (10 days in total). Of the 82 children in this study with meningitis, only 35 children were randomized into the trial (trial still blinded). Of those who died, only 1 child was randomized into the trial, the rest were either not randomized or died before randomization; therefore it is unlikely that the coexistence of a randomized controlled trial might have affected the main outcome of this study.

Children with pneumonia had regular monitoring of oxygen saturations, and oxygen was administered if saturations were less than 94%. The antibiotic regimen for pneumonia was intravenous benzyl penicillin (50,000 IU/kg every 6 hours) and gentamicin (6 mg/kg/d once daily) for at least 48 hours, with a switch to second line therapy, intravenous cefuroxime (50 mg/kg every 8 hours) and gentamicin (6 mg/kg/d once daily) if no improvement after 48 hours. On discharge, all children with pneumonia received oral amoxicillin to complete 10 days of treatment (125 mg 8 hourly <5 years and 250 mg 8 hourly >5 years).

Laboratory Methods

HIV status was assessed in children ≥18 months using at least 2 of the following tests; Unigold (Trinity Biotech, Ireland), Serocard (Trinity Biotech, Wick-low, Ireland) or Determine-HIV (Abbott Laboratories, Abbott Park, IL). At least 2 tests were required to be positive for a subject to be classified as HIV-infected. In children less than 18 months, and those with discordant antibody tests, HIV status was determined using Amplicor HIV-1 DNA Test version 1.5 (Roche Diagnostics, Indianapolis, IN).

Pneumococcal bacterial DNA was amplified and quantified using a real-time PCR assay for *Neisseria meningitidis, H. influenzae* and *S. pneumoniae* using the ABI 7700 Sequence detection system (Taqman) technology which has been shown to improve nonculture diagnosis and case ascertainment.13 For blood samples, 100 μL of whole blood was used. To quantify the amount of bacterial DNA present in each sample, standards were prepared using a suspension of bacteria in PBS from an overnight culture of *S. pneumoniae* and numbers of bacteria estimated using Miles and Mistra plating. The optical density was read at 650 nm and the suspension was diluted in PBS to obtain a bacterial load of 10^8 mL^{-1}. The suspension of bacteria was extracted using the Qiagen MinElute kit (Qiagen, Germany) and 10-fold dilutions prepared in PBS to 10^3 mL^{-1} to generate standards for use in the Taqman PCR. Each of the quantification standards was then amplified using the same real-time PCR assay to obtain Ct values at different concentrations of the pneumococcal control sample. Ct values were then plotted against different serial dilutions of a pneumococcal control sample to construct a standard curve. The amount of bacterial DNA present in each sample was obtained by direct extrapolation of its Ct value to the amount of DNA (in copies/mL) as read from the concentration versus Ct standard curve.

Multiple cytokine determination was performed in plasma and CSF samples using Luminex 100 technology in the Bio-plex Protein Array System (Bio-Rad Laboratories Inc., Hercules, CA). A 17-plex Bioplex Cytokine kit (Bio-Rad Laboratories Inc.) was used to measure the following cytokines: IL-1β, IL-6, IL-10 and TNF-α. CSF concentrations of IL-6 for all but 4 cases were above the maximum level of detection for the Bioplex assay even with further dilutions and extrapolation of the standard curve. We therefore measured CSF IL-6 using the R&D IL-6 Quantikine ELISA according to the manufacturer's instructions (R&D Systems, Minneapolis, MN).

Statistical Analysis

On statistical analysis, the cytokine and bacterial DNA load data had very skewed distributions; therefore, nonparametric statistical tests were used. Median levels of cytokine parameters were compared between 2 groups using the Mann–Whitney U tests. Fisher exact test was used to compare proportions of categorical variables. As the concentrations of pneumococcal DNA are exponential in nature, DNA loads were log (base 10) transformed, and associations between cytokines and bacterial DNA loads were examined using Spearman correlation coefficient. Reported P values are 2-tailed. Analysis of covariance was used to adjust for the influence of age in the analysis of bacterial loads. Multivariate logistic regression was used to adjust for potentially confounding variables with death as the outcome parameter. Statistical Package for Social Sciences software, version 13.0 (Chicago, IL) was used for all analyses, except the 95% confidence intervals for Spearman correlation coefficients, which were derived using a program in Intercooled STATA version 9.

RESULTS

In total 95 children with confirmed pneumonia or meningitis were recruited, of whom 52 (54.7%) were male. A total of 82 children (86.3%) had meningitis, and 13 had pneumonia. There were 59 (62.1%) HIV-infected children, of whom 17 (28.8%) died, and 36 (37.9%) HIV-uninfected children, of whom 8 died (22.2%), ($P = 0.63$). Table 1 compares clinical features in children with meningitis and pneumonia. Blood culture was positive in 52 of 95 (55%) cases, 50 of 82 (61%) with meningitis and 2 of 13 (15%) with pneumonia. Figure 1 (on-line version only: flow diagram showing microbiology investigations in blood, CSF and lung aspirate (LA) samples, with positivity rates) shows the proportions of positive microbiology results on blood, CSF and lung aspirate samples, and Table 1 shows the proportions of children with meningitis and pneumonia diagnosed by DNA detection alone.

There were no significant differences in blood or CSF bacterial loads and cytokine concentrations between children that had received antibiotics before admission and those that had not. Duration of symptoms before admission did not significantly influence either cytokine profile or bacterial loads. There was no significant difference in duration of symptoms between survivors and nonsurvivors, nor between HIV-infected and HIV-uninfected children.

Median admission blood bacterial load (all cases) was 1.6×10^3 DNA copies/mL (blood: range 0.00–1.54×10^6 DNA copies/mL) and CSF bacterial load (meningitis cases) was 5.77×10^7 DNA copies/mL (CSF: range 4.42×10^2 to 6.15×10^8 DNA copies/mL). For the meningitis cases, CSF bacterial loads were significantly higher than those in blood using the Wilcoxon signed rank test ($P < 0.0005$). The median (IQR) ratio of CSF to blood bacterial loads was 8384 (2764–47,512). Median blood and CSF bacterial loads (log DNA copies/mL) were significantly higher in nonsurvivors than in survivors (Table 2) and were higher in HIV-infected children than in HIV-uninfected children (blood: 3.20 vs. 3.16, $P = 0.6$ and CSF: 8.02 vs. 7.46, $P = 0.04$). In children with meningitis, there was a weak but significant correlation between blood and CSF bacterial loads ($r = 0.38$, 95% CI: [0.08, 0.62]). In HIV-

infected children, blood and CSF bacterial loads and plasma TNF-α, IL-1β, IL-6 and IL-10 were all significantly higher in nonsurvivors than that in survivors, but in HIV-uninfected children these differences were not significant (Table 2). Median plasma IL-6 and IL-10 concentrations were significantly higher in cases than that in controls (14,892.30 vs. 60.40 pg/mL $P < 0.0005$ and 91.61 vs. 8.11 pg/mL, $P = 0.003$ respectively), but for TNF-α and IL-1β (3.0 vs. 0.87 pg/mL and 3.49 vs. 1.34 pg/mL respectively), these differences were not significant.

There were significant inverse correlations between age and blood ($r = -0.33$, 95% CI: [−0.50 to −0.13]) and CSF ($r = -0.36$, 95% CI: [−0.61 to −0.06]) bacterial loads. Blood bacterial loads were significantly associated with plasma TNF-α, IL-1β, IL-6 and IL-10 [Fig. 2; on-line version only: Scatter plots of blood bacterial load (log DNA copies/ml) versus plasma (a) TNF-α, (b) IL-1β, (c) IL-6 and (d) IL-10 with Spearman's correlation coefficients (r) and 95% confidence intervals (CI). Each plot shows distribution of non-survivors (□) and survivors (▲)]. Blood bacterial loads and plasma TNF-α, IL-1β, IL-6 and IL-10 were significantly higher in children with meningitis than that in those with pneumonia (Table 2). In HIV-infected children, these parameters were significantly higher in meningitis than that in pneumonia, but there were no significant differences in HIV-uninfected children. Plasma concentrations of TNF-α ($P = 0.01$), IL-1β ($P = 0.08$), IL-6 ($P = 0.03$) and IL-10 ($P = 0.08$) were higher in HIV-infected than HIV-uninfected children. The differences in blood bacterial loads between survivors and nonsurvivors, and between meningitis and pneumonia remain significant after adjusting for age. On multivariate logistic regression, only female sex and high plasma TNF-α were significantly associated with death after controlling for age, HIV status, duration of symptoms, previous antibiotics, blood bacterial load and other plasma cytokine concentrations.

A total of 6 of 13 with pneumonia were not bacteremic by culture or PCR, and so it could be argued that these cases should be excluded, to see whether the results above hold true for the meningitis group alone. Table 2 also shows the analysis for the meningitis group alone (there were no pneumonia nonsurvivors), and demonstrates that overall blood and CSF bacterial loads and plasma TNF-α, IL-1β, and IL-10 were all significantly higher in nonsurvivors than survivors. Blood and CSF bacterial loads and TNF-α, IL-1β, IL-6 and IL-10 were also higher in HIV-infected children with meningitis than HIV-uninfected, but this was only significant for CSF bacterial load ($P = 0.04$), TNF-α ($P = 0.02$) and IL-6 ($P = 0.01$).

In children with meningitis, CSF TNF-α, IL-1β, IL-6 and IL-10 concentrations were significantly higher than the plasma concentrations ($P < 0.0005$) and were significantly higher in cases than in controls ($P < 0.0005$). CSF concentrations of TNF-α ($P = 0.008$) and IL-1β ($P = 0.02$) were significantly higher in HIV-infected than in HIV-uninfected children, but for IL-6 and IL-10 this difference was not significant. CSF TNF-α, IL-1β and IL-10 and were lower in nonsurvivors than in survivors, and CSF IL-6 higher; but these differences were not significant. There was a significant positive correlation between CSF bacterial loads (log DNA copies/mL) and CSF IL-1β ($r = 0.52$, 95% CI: [0.21–0.73]), and IL-10 ($r = 0.55$, 95% CI: [0.25–0.75]), but not CSF TNF-α and IL-6 [Fig. 3; on-line version only: Scatter plots of CSF bacterial load (log DNA copies/ml) versus CSF (a) TNF-α, (b) IL-1β and (c) IL-6 and D) IL-10 with Spearman's correlation coefficients (r) and 95% confidence intervals (CI). Each plot shows distribution of non-survivors (▲) and survivors (□)]. In both HIV-infected and HIV-uninfected children, there were no significant differences in median CSF cytokine concentrations between survivors and non-survivors. There were no significant correlations between CSF bacterial loads and either CSF total white cell count or CSF neutrophil count.

DISCUSSION

This study has demonstrated for the first time correlation of pneumococcal bacterial loads with disease severity and cytokines. We have also shown higher bacterial loads in IPD than previously determined with quantitative culture methods,9,10,16 which could be explained by the fact that PCR detects both viable and dead bacteria. We have also demonstrated a significant inverse correlation between age and both blood and CSF bacterial loads. This could reflect the development of naturally acquired immunity with increasing age. Both blood and CSF bacterial loads showed associations with mortality, and with HIV infection. It appears that in HIV-infected children, there are higher bacterial loads and a more intense inflammatory response than in HIV-uninfected children, but despite this, the inflammatory response is unable to contain the infection, resulting in death.

The finding that survivors and nonsurvivors differ in their bacterial loads cannot simply be explained by a delayed presentation in nonsurvivors, resulting in higher bacterial loads, because there was no significant difference in duration of symptoms before admission in the 2 groups. The data on the cytokine response demonstrate that although blood and CSF can be sterilized quickly with effective antibiotics, by the time the patient reaches hospital the cytokine cascade is already in a state of advanced activation.

The data presented in this study might suggest that the inflammatory response to invading pneumococci is "compartmentalized"17; pneumococci in the circulation stimulate a cytokine response within the circulation, and pneumococci within the CSF stimulate a separate cytokine response within the CSF compartment. Three results from this study would support the argument for a compartmentalized response in pneumococcal meningitis; (1) higher CSF bacterial loads than blood loads, (2) correlations between CSF bacterial load and CSF cytokines, and (3) higher CSF cytokine concentrations than plasma cytokine concentrations. The higher blood loads in meningitis compared with pneumonia might suggest spill over from the subarachnoid space into the circulation, or simply reflect the fact that sustained high-grade bacteremia is thought necessary for microbial entry into the subarachnoid space.18 Once pathogens cross the blood–brain barrier into the subarachnoid space, host defenses within the CSF are ineffective at containing encapsulated organisms such as *S. pneumoniae* because of suboptimal concentrations of complement and capsule-specific antibody.19 It is important to understand whether the cytokine response is indeed compartmentalized, as this may influence whether new adjunctive therapies are effective or not. In meningitis, for example, any experimental adjunctive therapies must cross the blood–brain barrier and accumulate within the CSF in high enough concentrations to achieve a therapeutic effect; high concentrations in the blood alone, will not suffice.

The cytokine data are consistent with data from other studies showing elevated CSF concentrations of TNF-α, IL-1β, IL-6 and IL-10 in the CSF of patients with bacterial meningitis.[20–22] These cytokines are produced by cells within the central nervous system such as astrocytes, cerebromicrovascular endothelial cells and microglial cells.23 In keeping with data from La Scolea et al24 (using quantitative blood culture techniques), we found a correlation between blood and CSF bacterial loads, but did not find a correlation between CSF bacterial load and number of polymorphonuclear leukocytes on microscopy.

We analyzed the meningitis group separately, because 6 of 13 of the pneumonia group were not bacteremic (by culture or PCR), and some authors believe that blood PCR positivity may be because of pneumococcal carriage rather than invasive disease.25 On analysis of the meningitis group alone, the conclusions remain the same; that pneumococcal bacterial loads and proinflammatory cytokines are higher in nonsurvivors and HIV-infected children with IPD.

A major limitation of the study was the extremely complex interactions, mainly because of numerous confounding variables, and so we have attempted to explore the influence of confounders such as duration of symptoms, previous antibiotic administration and antiretroviral therapy. Female sex and high plasma TNF-α concentrations are significantly associated with death. The phenomenon of higher female mortality in the postneonatal period has been described in some rural areas of the developing world, and has been attributed to male preference in parental care, food allocation and utilization of health services.[26–28] This is the converse of the situation in the Western world where male gender is associated with postneonatal mortality from infection.29 Age is also an important confounder (the disease incidence is highest in children less than 5 years of age), but the significant differences in blood bacterial loads observed between survivors and nonsurvivors, meningitis and pneumonia and HIV-infected and un-infected, remain significant when adjusted for age. The effects of other comorbidities or coinfection with other viruses in HIV-infected children are difficult to ascertain.

One of the challenges of assessing the impact of the pneumococcal conjugate vaccine on pneumonia has been the lack of standardized definitions of confirmed pneumonia. Although the numbers involved are too small to draw any conclusions, our study demonstrated that PCR of lung aspirate substantially increased the diagnostic confirmation of pneumococcal pneumonia. As this technique is expensive, and the technology required is not available in most of the developing world, funding will be required from sources such as the Gates Foundation (through PNEUMO-ADIP www.preventpneumonia.com) or the World Health Organization, to use this technique in epidemiological studies on disease burden and in the evaluation of new vaccines. Quantitative pneumococcal DNA estimation using real-time PCR and correlation with both pro- and antiinflammatory cytokines is an important step forward in improving the understanding of the pathophysiology of IPD. In addition, our data support the concept of a compartmentalized response in pneumococcal meningitis, with intrinsic production of cytokines.

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Pediatr Infect Dis J. Author manuscript; available in PMC 2010 January 25.

TABLE 1

Clinical Features of Children With Meningitis and Pneumonia

Characteristic	Meningitis	Pneumonia	*P*
Male, N (%)	43 (52.4)	9 (69.2)	NS
Age (yr), median (IQR)	2.4 (0.5–6.6)	3.92 (1.6–6.6)	NS
Duration of symptoms (d), median (IQR)	3.0 (2.0–4.0)	4.0 (2.5–7.0)	NS
Previous antibiotics, N (%)	30 (36.6)	5 (38.5)	NS
Deaths, N (%)	25 (30.5)	0	N/A
Time to death (h), median (IQR)	10.0 (4.0–30.0)	N/A	N/A
Death within 24 h, N (%)	20/25 (80.0)	0	N/A
HIV infected, N (%)	51 (62.2)	8 (61.5)	NS
Detection by PCR alone, N (%)	2 (2.4)*	9† (69.2)	<0.0005
Total	*82*	*13*	

IQR indicates interquartile range; NS, not significant; N/A, not applicable as no pneumonia deaths.

*Meningitis cases diagnosed by PCR alone: 1 by CSF PCR alone, 1 by both blood and CSF PCR.

†Pneumonia cases diagnosed by PCR alone: 1 by blood PCR alone (blood pneumococcal DNA load = 1.54×10^6 copies/mL), 5 by lung aspirate PCR alone, and 3 by both blood and lung aspirate PCR.

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Pediatr Infect Dis J. Author manuscript; available in PMC 2010 January 25.

TABLE 2

Summary of Bacterial Loads and Plasma Cytokine Concentrations by Diagnosis, Survival and HIV Status

Parameters	Meningitis Survivors, Median (IQR) (n = 57)	Meningitis Nonsurvivors, Median (IQR) (n = 25)	Pneumonia Survivors, Median (IQR) (n = 13)	*P*
Overall (n = 95)				
Blood load (log DNA copies/mL)	3.22 (2.55–4.09)	3.80 (2.91–4.49)	0.42 (0.00–2.19)	0.003 *†, <0.0005 *‡, 0.02 §
CSF load (log DNA copies/mL)	7.50 (6.55–8.14)	8.17 (7.53–8.54)	N/A	0.03 †, NA ‡, 0.03 §
Plasma TNF-α (pg/mL)	3.22 (1.77–5.36)	5.78 (2.18–16.83)	1.82 (1.09–2.77)	0.01 †, 0.009 ‡, 0.04 §
Plasma IL-1β (pg/mL)	3.03 (1.50–5.81)	6.96 (3.81–50.91)	1.71 (1.63–2.13)	0.001 *†, 0.02 ‡, 0.004 *§
Plasma IL-6 (pg/mL)	16,510.27 (4,182.40–65,945.45)	27,116.40 (7,678.50–101,341.18)	2114.65 (1,705.59–11,118.01)	0.03 †, 0.001 *‡, NS §
Plasma IL-10 (pg/mL)	97.26 (21.82–211.98)	291.80 (61.35–931.33)	14.93 (9.51–18.90)	0.002 *†, <0.0005 *‡, 0.02 §
HIV-infected (n = 59)				
Blood load (log DNA copies/mL)	3.05 (2.16–4.02)	4.10 (3.08–4.64)	0.76 (0.00–2.20)	0.001 *†, 0.006 *‡, 0.006 *§
CSF load (log DNA copies/mL)	7.63 (6.49–8.17)	8.33 (7.58–8.58)	N/A	0.03 †, NA ‡, 0.03 §
Plasma TNF-α (pg/mL)	4.19 (2.49–5.71)	5.90 (2.68–16.72)	2.07 (1.77–2.97)	0.04 †, 0.004 *‡, NS §
Plasma IL-1β (pg/mL)	4.41 (1.36–6.84)	12.46 (4.04–70.84)	1.82 (1.63–2.17)	0.001 *†, 0.02 ‡, 0.004 *§
Plasma IL-6 (pg/mL)	29,379.14 (3,865.53–92,049.62)	72371.50 (16,741.95–15,2312.23)	1,826.94 (1,686.30–11,794.33)	0.01 †, 0.003 *‡, 0.05 §
Plasma IL-10 (pg/mL)	134.62 (17.26–407.52)	546.88 (166.89–997.34)	18.25 (10.75–19.10)	0.002 *†, 0.002 *‡, 0.01 §
HIV-uninfected (n = 36)				
Blood load (log DNA copies/mL)	3.31 (2.63–4.14)	3.30 (2.57–3.93)	0.42 (0.00–2.07)	NS †, <0.0005 *‡, NS §
CSF load (log DNA copies/mL)	7.46 (6.53–8.00)	7.02 (5.97–8.24)	N/A	NS †, NA ‡, NS §
Plasma TNF-α (pg/mL)	2.18 (0.78–4.29)	5.78 (1.26–29.01)	1.23 (0.93–3.25)	NS †, NS ‡, NS §
Plasma IL-1β (pg/mL)	2.17 (1.53–4.36)	3.82 (1.37–23.27)	1.70 (1.43–3.43)	NS †, NS ‡, NS §
Plasma IL-6 (pg/mL)	14,317.44 (6,231.52–26,649.69)	8,241.57 (3,293.29–24,251.84)	2,151.50 (1,596.78–19,166.30)	NS †, NS ‡, NS §
Plasma IL-10 (pg/mL)	56.59 (28.04–150.72)	114.43 (21.25–377.16)	10.67 (6.08–26.76)	NS †, 0.004 *‡, NS §

Note: There were no pneumonia nonsurvivors.

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IQR indicates interquartile range; NA, not applicable; NS, not significant.

*Significant with Bonferroni correction.

†Comparison between survivors and nonsurvivors.

‡Comparison between meningitis and pneumonia.

§Comparison between meningitis survivors and meningitis nonsurvivors.