Pretreatment Cerebrospinal Fluid Bacterial Load Correlates With Inflammatory Response and Predicts Neurological Events During Tuberculous Meningitis Treatment

Nguyen T. T. Thuong,1 Dao N. Vinh,1 Hoang T. Hai,1 Do D. A. Thu,1 Le T. H. Nhat,1 Dorothee Heemskerk,1,4 Nguyen D. Bang,2 Maxine Caws,1,5
1Oxford University Clinical Research Unit, 2Pham Ngoc Thach Hospital, and 3Hospital for Tropical Diseases, Ho Chi Minh City, Viet Nam; and 4Nuffield Department of Medicine, University of Oxford, and 5Liverpool School of Tropical Medicine, United Kingdom

**Background.** The Mycobacterium tuberculosis load in the brain of individuals with tuberculous meningitis (TBM) may reflect the host’s ability to control the pathogen, determine disease severity, and determine treatment outcomes.

**Methods.** We used the GeneXpert assay to measure the pretreatment M. tuberculosis load in cerebrospinal fluid (CSF) specimens from 692 adults with TBM. We sought to understand the relationship between CSF bacterial load and inflammation, and their respective impact on disease severity and treatment outcomes.

**Results.** A 10-fold higher M. tuberculosis load was associated with increased disease severity (odds ratio, 1.59; P = .001 for the comparison between grade 1 and grade 3 severity), CSF neutrophil count (r = 0.364 and P < .0001), and cytokine concentrations (r = 0.438 and P < .0001). A high M. tuberculosis load predicted new neurological events after starting treatment (P = .005, by multinomial logistic regression) but not death. Patients who died had an attenuated inflammatory response at the start of treatment, with reduced cytokine concentrations as compared to survivors. In contrast, patients with high pretreatment CSF bacterial loads, cytokine concentrations, and neutrophil counts were more likely to subsequently experience neurological events.

**Conclusions.** The pretreatment GeneXpert-determined M. tuberculosis load may be a useful predictor of neurological complications occurring during TBM treatment. Given the evidence for the divergent pathogenesis of TBM-associated neurological complications and deaths, therapeutic strategies to reduce them may need reassessment.

**Keywords.** Bacterial load; tuberculous meningitis; inflammatory response; cytokines; neurological events.

Tuberculous meningitis (TBM) is the most severe form of tuberculosis. It is caused by dissemination of Mycobacterium tuberculosis to the brain, resulting in meningoencephalitis with necrotizing, granulomatous inflammation predominantly affecting the basal meninges. Inflammation can lead to life-threatening complications of hydrocephalus, infarcts, and tuberculomas [1, 2]. Death or neurological disability still occur in half of all cases.

The pathogenesis of TBM is not well understood. Much of the pathology is thought to arise from the immune response to replicating bacteria, but the nature of that response and its relationship to bacterial numbers is presently poorly defined [3].

Recent studies in large, well-characterized cohorts of human immunodeficiency virus (HIV)–uninfected Vietnamese adults with TBM demonstrated that elevated cerebrospinal fluid (CSF) concentrations of 8 of 10 tested cytokines were associated with more-severe disease [4]. Death, however, was associated with an attenuated inflammatory response, lower CSF cytokine concentrations and leukocyte counts [4], and higher CSF neutrophil counts [5]. HIV infection and polymorphism rs17525495 in the gene encoding leukotriene A4 hydrolase (LTA4H) influenced both pretreatment CSF inflammatory phenotype and survival from TBM in Vietnamese individuals [4], but the polymorphism was not associated with survival in Indonesians [5].

Neutrophils appear to play an important role in TBM pathogenesis [5]. Higher CSF neutrophil numbers have been associated with an increased likelihood of culturing M. tuberculosis from the CSF [6, 7] and of cerebral immune reconstitution inflammatory syndrome (IRIS) in those coincfected with HIV [8, 9].

One of the fundamental questions concerning TBM pathophysiology is how the M. tuberculosis load relates to intracerebral inflammation and outcome. Very low bacterial numbers in CSF and inadequate laboratory methods have to date made this
question largely refractory to investigation. CSF culture positivity may indicate higher bacterial loads and was associated with mortality in HIV-uninfected patients with TBM [5]. More recent molecular assays, such as GeneXpert, quantify bacterial nucleic acid in CSF and are now widely used for TBM diagnosis. Although GeneXpert's sensitivity for TBM diagnosis is around 60% as compared to clinical diagnosis [10], the bacterial load in CSF specimens that test positive can be categorized as very low, low, medium, or high, thus offering a new way to assess the CSF \( M. \) \( \text{tuberculosis} \) load in clinical practice. The aim of our study was to use GeneXpert to define the CSF bacterial load in a large cohort of well-characterized Vietnamese adults with TBM and to investigate the relationship between bacterial load and CSF cytokine concentrations, leukocyte numbers and types, and the occurrence of new neurological events and death after the start of antituberculosis treatment.

**METHODS**

**Participants**

Adults (age, >17 years) with TBM were enrolled into a randomized controlled trial of intensified antituberculosis chemotherapy between April 2011 and June 2014 [11]. Of the 817 trial participants, 692 had CSF GeneXpert data and were included in the current study. The other 125 participants had missing data because of an insufficient volume of CSF samples or because of GeneXpert test errors.

Written informed consent was obtained from each participant or from an accompanying relative if the participant could not provide consent. Protocols were approved by the Oxford Tropical Research Ethics Committee in the United Kingdom, the institutional review boards of the Hospital for Tropical Diseases and Pham Ngoc Thach Hospital for Tuberculosis and Lung Disease, and the Ethical Committee of the Ministry of Health in Vietnam.

**Treatment**

Participants were randomly allocated to treatment with (1) a standard antituberculosis regimen for 3 months, followed by rifampicin and isoniazid at the same doses for a further 6 months, or (2) an intensified regimen that consisted of the standard regimen with an additional higher dose of rifampicin (15 mg/kg/day) and levofloxacin (20 mg/kg/day) for the first 8 weeks of treatment. All participants also received adjunctive dexamethasone for the first 6–8 weeks of treatment [11].

**Clinical and CSF Characteristics**

We extracted data on participant age, weight, duration of illness (days of symptoms), Glasgow coma scale (GCS), British Medical Research Council (BMRC) grading for TBM, antituberculosis treatment, and HIV status on the date of TBM diagnosis. All participants were followed for 9 months, with careful characterization of their clinical response to treatment. New neurological events were defined as the new occurrence of any of the following: cerebellar symptoms; mono-, hemi-, para, or tetraplegia; seizures; cranial nerve palsy; or a ≥2-point decrease in the GCS for ≥2 days after the highest previously recorded score [11]. These events encompass paradoxical treatment reactions, which are thought to be caused by an excessive inflammatory response to dead or dying bacteria [12] and by death of brain tissue.

Laboratory data were collected from samples collected on the date of enrollment and included blood and CSF cell counts and cytokine concentrations. A panel of 10 cytokines important in inflammation in tuberculosis, comprising 6 proinflammatory cytokines (interleukin 1β [IL-1β], interleukin 2 [IL-2], interleukin 6 [IL-6], interleukin 12p70 [IL-12p70], interferon γ [IFN-γ], and tumor necrosis factor α [TNF-α]) and 4 antiinflammatory cytokines (interleukin 4 [IL-4], interleukin 5 [IL-5], interleukin 6 [IL-6], and interleukin 13 [IL-13]), was measured in stored CSF specimens, using the Luminex multiplex bead-based assay [4]. The \( \text{LTA4H} \) rs17525495 polymorphism was genotyped by the TaqMan genotyping assay [3].

GeneXpert MTB/RIF, which detects \( M. \) \( \text{tuberculosis} \) complex and rifampicin resistance, was performed on CSF specimens before the start of antituberculosis treatment [10]. Briefly, 3–5 mL of CSF was centrifuged for 20 minutes at 3000×g; 200 μL (about one third of the deposit) was resuspended in phosphate-buffered saline and mixed with 1.5 mL of sample reagent, vortexed for 30 seconds, incubated for 15 minutes at room temperature, and then transferred to a GeneXpert cartridge for measurement. The GeneXpert Dx software (version 4.0; Cepheid) reported semiquantitative mycobacterial load results as cycle threshold (Ct) values, representing the number of PCR cycles required for the signal to reach a detection threshold. \( M. \) \( \text{tuberculosis} \) loads were classified as high (Ct value, <16), medium (16–22), low (22–28), and very low (>28). \( \text{Mycobacterium bovis} \) (bacillus Calmette-Guérin; NCTC 5692) was used to generate a standard curve of GeneXpert Ct values versus bacterial numbers by the Miles and Misra method [13]. For each sample, the average Ct value of 5 probes (excluding any delayed values due to rifampicin resistance) was used to estimate the bacterial load and analyze further.

**Statistical Analysis**

Data were analyzed using R, version 3.0.1 [14], MATLAB and Statistics Toolbox, release 2013a (MathWorks, Natick, MA), and GraphPad Prism, version 6 (GraphPad Software, San Diego, CA). In statistical analyses, to preserve statistical power we used continuous comparisons of the whole data set, with negative results of GeneXpert assigned a Ct value of 40; most of the graphical representations present bacterial loads in categories, for clarity.

Baseline concentrations of the 10 CSF cytokines were analyzed using principal component analysis (PCA), a method of...
transforming complex correlated data sets into a new coordina-
tor system (ie, the component space) in which 2 or 3 first prin-
cipal components (PCs) can cover up to 70%–80% variance of the
whole data set. By this means we visualized our multivariate data
set and avoided multiple comparisons [15, 16]. Associations
between \textit{M. tuberculosis} load and cytokine PCs, blood counts,
or CSF cell counts were examined using the Spearman rank cor-
relation coefficient, in which log-transformed cell counts were
used to analyze correlations. Comparisons of bacterial loads
by GCS or disease severity, CSF neutrophil counts, and cyto-
kines were performed by a linear trend test implemented using
robust linear regression. We classified outcomes into 3 groups:
(1) “survival,” defined as survival without new neurological
events; (2) “new neurological events,” defined as survival with
new neurological events occurring during treatment; and (3)
“death,” defined as death during treatment. Comparisons of the
bacterial load between the survival group and the new neuro-
logical events or death groups used Mann-Whitney tests. The
outcome was modeled using multinomial logistic regression
dependent on \textit{M. tuberculosis} bacterial load and the detection
limit indicator. The model was later adjusted for predefined risk
factors, including age, weight, GCS, CSF leukocyte numbers,
antituberculosis regimen, antiretroviral therapy, HIV infection,
and \textit{LTA4H} genotype. Separate analysis was also performed for
each HIV status. We used the Hochberg method to correct \(P\)
values for multiple testing.

\section*{RESULTS}

\subsection*{Characteristics of Participants}
The baseline clinical characteristics of the 692 adults with
TBM for whom the CSF \textit{M. tuberculosis} load was assessed by
GeneXpert are described in Table 1. A total of 293 (42.3%) were
GeneXpert positive, and these individuals were significantly
more likely than the GeneXpert-negative participants to be
HIV infected and to have more-severe disease. The majority
of participants (568 [82.1%]) had BMRC grade 1 or 2 disease.

| Characteristic                  | Overall | GeneXpert Negative | GeneXpert Positive |
|--------------------------------|---------|---------------------|--------------------|
| Age, y                         | 692     | 35 (29–46)          | 399 36 (29–50)     | 293 35 (29–42) |
| Male sex                       | 692     | 475 (68.6)          | 399 261 (65.4)     | 293 214 (73.0) |
| Weight, kg                     | 692     | 48 (44–54.5)        | 399 49 (45–55)     | 293 48 (43–54) |
| Duration of illness, d         | 692     | 15 (10–30)          | 399 15 (10–30)     | 293 15 (10–30) |
| Glasgow coma scale             | 692     | 15 (12–15)          | 399 15 (13–15)     | 293 14 (11–15) |
| HIV infected, no. (%)          | 692     | 288 (41.6)          | 399 123 (30.8)     | 293 165 (56.3) |
| Standard treatment arm         | 692     | 345 (49.9)          | 399 199 (49.9)     | 293 146 (49.8) |
| BMRC grade\textsuperscript{a}  | 692     | ...                 | ...                | 293 ... |
| I                              | ...     | 266 (38.4)          | ... 162 (40.6)     | 293 ... |
| II                             | ...     | 302 (43.6)          | ... 184 (46.1)     | 293 ... |
| III                            | ...     | 124 (179)           | ... 53 (13.3)      | 293 ... |
| CSF smear positive             | 684     | 257 (37.6)          | 394 69 (175)       | 290 188 (64.8) |
| CSF MGIT culture positive      | 682     | 281 (41.2)          | 389 53 (13.6)      | 293 228 (77.8) |
| Diagnostic category\textsuperscript{b} | 692 | 399 | 293 | <.0001 |
| Definite TBM                   | ...     | 397 (57.4)          | ... 104 (26.1)     | 293 (100) |
| Probable TBM                   | ...     | 170 (24.6)          | ... 170 (42.6)     | 0 (0) |
| Possible TBM                   | ...     | 125 (18.0)          | ... 125 (31.3)     | 0 (0) |
| Total leukocyte count, \(x 10^3\) cells/mL | 686 | 115 (35–284) | 395 76 (22–198) | 291 200 (64–388) |
| Neutrophils, %                 | 655     | 10 (0–32)           | 371 3 (0–15)       | 284 20 (5–56) |
| Lymphocytes, %                 | 656     | 90.0 (68–100)       | 372 975 (85–100)   | 284 79.5 (44–95) |
| Protein level, g/L             | 666     | 1.2 (0.6–1.9)       | 379 1.0 (0.5–1.7)  | 287 1.4 (0.9–2.4) |
| Glucose level, mmol/L          | 666     | 1.9 (1.3–2.7)       | 379 2.2 (1.4–3.0)  | 287 1.6 (1.0–2.2) |
| Lactate level, mmol/L          | 638     | 4.8 (3.5–6.5)       | 357 4.1 (2.9–5.6)  | 281 5.9 (4.5–74) |

\textsuperscript{a}P-values are descriptive only and based on the \(\chi^2\) test (for categorical data) and the Mann-Whitney test (for continuous data).

\textsuperscript{b}Data denote the number of patients with nonmissing data for the corresponding variable.

\textsuperscript{c}Modified British Medical Research Council (BMRC) grade I indicates a GCS of 15 with no neurologic signs (baseline), grade II indicates a score of 11–14 (or 15 with focal neurologic signs), and grade III indicates a score of c10.

\textsuperscript{d}Diagnostic categories were assigned according to the consensus case definition [17]. Patients with an unlikely diagnosis of TBM had a score of <6. Confirmed other diagnosis was only made on the basis of microbiological evidence.
Association Between Pretreatment CSF M. tuberculosis Load and Inflammation

To investigate whether the M. tuberculosis load before treatment was related to inflammatory responses, we first explored the relationship between M. tuberculosis load and blood and CSF cell counts. Bacterial loads stratified by Ct values showed negative correlations with neutrophil numbers in blood and CSF specimens from both HIV-infected participants and uninfected participants (Table 2 and Figure 2B). These correlations were stronger in CSF (r = −0.395 and P < .0001; Supplementary Figure 3) than in blood (Table 2), suggesting that neutrophil recruitment correlated more strongly with bacterial replication at the infected site.

PCA was used to evaluate 10 baseline CSF cytokine concentrations, to identify correlated measurements that accounted for the variance of the data set. The first component, PC1, covered 71.4% variance of the cytokine profile, PC2 covered 6.4%, PC3 covered 4.9%, PC4 covered 4.4%, and other PCs covered less (Supplementary Figure 2). In other words, the majority of the variance was reflected by just the first 2 PCs (ie, PC1 and PC2). Because all of the coefficients were located in quadrant I and quadrant IV, a positive value of PC1 implies a high concentration across all cytokines. In other words, levels of these cytokines were in strong cocorrelation with each other. Meanwhile, PC2 explains the difference in concentrations of 2 groups of cytokines: IL-13, IFN-γ, IL-10, and IL-5 versus IL-2, IL-6, and other PCs covered less (Supplementary Figure 2). In other words, the majority of the variance was reflected by just the first 2 PCs (ie, PC1 and PC2). Because all of the coefficients were located in quadrant I and quadrant IV, a positive value of PC1 implies a high concentration across all cytokines. In other words, levels of these cytokines were in strong cocorrelation with each other. Meanwhile, PC2 explains the difference in concentrations of 2 groups of cytokines: IL-13, IFN-γ, IL-10, and IL-5 versus IL-2, IL-6, and IL-1β. We used the first 2 PCs to represent the cytokine profile, to assess whether bacterial load was related to cytokine concentrations. M. tuberculosis loads stratified by Ct values showed a negative relationship with PC1 overall (r = −0.493 and P < .0001) and with respect to HIV infection status (r = −0.448

![Figure 1](https://academic.oup.com/jid/advance-article-abstract/doi/10.1093/infdis/jiy588/5123684)
and $-0.487 \ [P < .0001]$ for HIV-uninfected and HIV-infected groups, respectively; Table 2). M. tuberculosis loads were not correlated with PC2 in participants stratified by HIV status. These results suggest that participants with a high M. tuberculosis load tended to have high concentrations of measured cytokines. This can be seen particularly in the strong relationship between M. tuberculosis load and the key proinflammatory cytokines IL-1β and TNF-α (Table 2, Figure 2C and 2D, and Supplementary Figure 3). Interestingly, the CSF neutrophil count was positively correlated with PC1 regardless of HIV status ($r = 0.381$ and $P < .0001$ for the overall group, $r = 0.385$ and $P < .0001$ for the HIV-uninfected group, and $r = 0.317$ and $P = .0003$ for the HIV-infected group).

**Association Between LTA4H Genotype and M. tuberculosis Load**

Previously, we showed that, in HIV-uninfected adults with TBM, LTA4H genotype was associated with CSF IL-1β, IL-2, and IL-6 concentrations, with low concentrations for genotype CC, intermediate concentrations for genotype CT, and high concentrations for genotype TT [4]. Laarhoven et al reported that the TT genotype was associated with decreased CSF culture positivity [5]. We analyzed the relationship of LTA4H genotype and bacterial load and found that the M. tuberculosis load in participants with genotype TT was slightly lower than that in those with genotype CC ($P = .051$) or CT ($P = .015$), but the linear trend test showed that the LTA4H genotype was not
associated with the *M. tuberculosis* load overall (*P* = .302; Supplementary Figure 4).

Relationship Between CSF *M. tuberculosis* Load, Inflammation, and New Neurological Events

Study participants were treated with intensive (n = 347) or standard (n = 345) antituberculosis treatment. As previously reported, the intensive regimen was not associated with any improvement in any measure of treatment response or outcomes, including survival [11]. During 9 months of antituberculosis treatment, new neurological events developed in 103 participants (14.9%), and the median time to new events after the start of treatment was 9 days (interquartile range, 3–43 days; Supplementary Figure 5A).

New neurological events were significantly associated with higher pretreatment *M. tuberculosis* loads in the GeneXpert-positive data set (*P* = .004 in HIV-uninfected individuals, and *P* = .022 in HIV-infected individuals; Figure 3A). In the whole data set, an elevated *M. tuberculosis* load increased the risk of new neurological events (OR, 1.68 [95% CI, 1.26–2.25; *P* = .004] per 10-fold increase in bacterial load; Table 3). In both HIV-uninfected participants and HIV-infected participants, these events were also associated with high baseline CSF neutrophil counts, high baseline PC1 scores, and high baseline TNF-α concentrations (Figure 3B–D).

The pathogenesis of neurological events may differ according to their timing during treatment. Therefore, we categorized participants by the median time to new neurological events (ie, <9 days or ≥29 days) and compared the bacterial load between these 2 groups. Bacterial loads were not significantly different between early and late neurological events (*P* = .196 for the HIV-uninfected group, and *P* = .061 for the HIV-infected group; Supplementary Figure 6A).

Predictors of New Neurological Events

The results (Table 3) indicated that increased *M. tuberculosis* load was independently associated with new neurological events in all participants (OR, 1.56 [95% CI 1.14–2.11; *P* = .005 and adjusted *P* = .045] per 10-fold load increase). The *M. tuberculosis* load was significantly associated with new neurological events in HIV-uninfected participants (OR, 1.97; 95% CI, 1.18–3.28; *P* = .009) and with a trend in HIV-infected participants (OR, 1.43; 95% CI, .95–2.15; *P* = .082). Results stratified by HIV infection lost statistical significance after adjustment for multiple testing. GCS was also strongly associated with new neurological events (OR, 0.77 [95% CI, .71–.84; *P* < .0001] per 1-point increase). These findings highlight that pretreatment CSF *M. tuberculosis* load can be used as a predictor, together with GCS, of new neurological events occurring during TBM treatment.

Relationship Between CSF *M. tuberculosis* Load, Inflammation, and Death

Death occurred in 192 participants (27.7%), with a median time to death of 39 days (interquartile range, 9–103 days) after the start of treatment (Supplementary Figure 5B). The bacterial load was not significantly associated with death, with or without neurological events, in either HIV-uninfected participants or HIV-infected participants in the GeneXpert-positive data set (Figure 3A). Although CSF neutrophil counts were similar between those who died and those who survived (Figure 3B), measured cytokine concentrations were significantly lower in those who died, with or without neurological events, among HIV-uninfected participants (Figure 3C and 3D) in the whole group.

Table 2. Relationship of *Mycobacterial tuberculosis* Load and White Blood Cell Numbers and Cytokine Levels in Blood and Cerebrospinal Fluid Specimens From Patients With Tuberculous Meningitis, by Spearman Correlation

| Group                              | Blood     | Cerebrospinal Fluid |
|------------------------------------|-----------|---------------------|
|                                    | Leukocytes | Lymphocytes | Neutrophils | Leukocytes | Lymphocytes | Neutrophils | IL-1β | TNF-α | PC1 | PC2 |
| Overall, no.                        | 650       | 649         | 650         | 682        | 653         | 651         | 521   | 522   | 518 | 518 |
| *r*                                | -0.027    | 0.242b      | -0.085      | -0.273b    | -0.11b      | -0.395b     | -0.523b| -0.48b| -0.493b| 0.185b |
| *P*                                | .486      | <.0001      | .031        | <.0001     | <.0001      | <.0001      | <.0001| <.0001| <.0001| <.0001 |
| Adjusted *P*                       | .486      | <.0001      | .062        | <.0001     | <.0001      | <.0001      | <.0001| <.0001| <.0001| <.0001 |
| HIV uninfected, no.                | 382       | 382         | 382         | 401        | 390         | 389         | 304   | 304   | 300 | 300 |
| *r*                                | -0.116b   | 0.24b       | -0.182b     | -0.199b    | -0.048      | -0.342b     | -0.456b| -0.413b| -0.448b| 0.127 |
| *P*                                | .024      | <.0001      | <.0001      | <.0001     | <.0001      | <.0001      | <.0001| <.0001| <.0001| <.028 |
| Adjusted *P*                       | .056      | <.0001      | .001        | <.0001     | <.0001      | <.0001      | <.0001| <.0001| <.056 |         |
| HIV infected, no.                  | 268       | 267         | 268         | 281        | 263         | 262         | 217   | 218   | 218 | 218 |
| *r*                                | -0.164b   | 0.129       | -0.204b     | -0.35b     | -0.206b     | -0.388b     | -0.499b| -0.515b| -0.487b| 0.114 |
| *P*                                | .007      | .035        | .001        | <.0001     | <.0001      | <.0001      | <.0001| <.0001| <.092 |         |
| Adjusted *P*                       | .021      | .070        | .003        | <.0001     | <.0001      | <.0001      | <.0001| <.0001| <.092 |         |

Analyses were performed by using GeneXpert cycle threshold values and log10-transformed cell counts, IL-1β concentrations, and TNF-α concentrations.

Abbreviations: IL-1β, interleukin 1β; PC, principal component; TNF-α, tumor necrosis factor α.

*a* Spearman’s *ρ* correlation coefficient.

*b* The adjusted *P* value is <.05.

*c* Adjusted with the Hochberg method.
data set. To examine whether early death was associated with bacterial load, we categorized participants by the median time to death (ie, <39 days or ≥39 days). Bacterial loads were not significantly different between those dying early and those dying late (P = .731 for the HIV-uninfected group, and P = .099 for the HIV-infected group; Supplementary Figure 6B).

Taken together, our data showed that a high Mycobacterium tuberculosis load was associated with more-severe disease at baseline and strongly predicted new neurological events after the start of treatment, but it did not influence 9-month mortality (Table 3).

**DISCUSSION**

TBM is one of the most difficult forms of tuberculosis to treat, with outcomes dependent on killing the bacteria with antituberculosis drugs and controlling intracerebral inflammation. However, the relationships between bacterial numbers, inflammation, and treatment response are poorly defined, primarily because of the difficulties assessing the M. tuberculosis load at the site of infection. We overcame some of these difficulties by quantifying bacterial numbers in cerebrospinal fluid (CSF) by GeneXpert in a large cohort of well-characterized patients recruited into a randomized controlled trial of intensive antituberculosis therapy [11]. We found that the pretreatment CSF M. tuberculosis load was associated with increased CSF neutrophil numbers, enhanced inflammation, and disease severity and predicted the likelihood of new neurological events after the start of antituberculosis treatment.

The CSF bacterial load showed significant correlations with neutrophil numbers in blood and CSF specimens from both...
HIV-infected participants and HIV-uninfected participants. Previously, CSF neutrophil numbers have been associated with *M. tuberculosis*–positive CSF cultures [6, 18], and studies in HIV-coinfected individuals have linked the presence of *M. tuberculosis* in the CSF with a neutrophil-mediated inflammatory response and an increased risk of intracerebral IRIS [9]. Neutrophils may be protective in early *M. tuberculosis* infection [19], although animal models suggest that they may exacerbate pathology in the later stages of disease [20, 21]. Clinical studies in active tuberculosis have suggested that neutrophils may influence disease progression and outcome: higher blood neutrophil counts before treatment have been associated with increased risk of mortality in patients with tuberculosis (of whom 49.4% had pulmonary tuberculosis) [22] and slower conversion of sputum to *M. tuberculosis* negativity during therapy in pulmonary tuberculosis [23]. In transcripational profiling of blood specimens, the signature of patients with active tuberculosis was characterized by neutrophil-driven interferon-inducible gene expression [24]. Taken together, these findings all suggest that neutrophils play an important role in tuberculosis pathogenesis.

Almost half the participants had serious clinical complications during treatment (14.9% had new neurological events, and 27% died). Surprisingly, we found that the CSF *M. tuberculosis* load influenced the likelihood of new neurological events but not mortality, suggesting different underlying mechanisms for these outcomes. In support of this assertion, current and previous data suggest that new neurological events are associated

### Table 3. Association Between Pretreatment Variables and New Neurological Events and Death, Overall and by Human Immunodeficiency Virus (HIV) Status, by Multinomial Logistic Regression Analysis

| Variable | Overall (n = 692) | HIV Uninfected (n = 404) | HIV Infected (n = 288) |
|----------|------------------|-------------------------|-----------------------|
|          | HR (95% CI)      | P                       | HR (95% CI)           | P                       | HR (95% CI)            | P                       |
| New neurological events (*M. tuberculosis* load effect) | | | | | | |
| *M. tuberculosis* load (per 10-fold increase) | 1.68 (1.26–2.25) | .0004 | 2.02 (1.26–3.23) | .003 | 0.67 (1.16–9.98) | .308 |
| *M. tuberculosis* below LOD<sup>a</sup> | 3.12 (0.96–10.1) | .057 | 6.83 (1.10–42.4) | .039 | 1.32 (2.5–703) | .454 |
| New neurological events (adjusted effect)<sup>b</sup> | | | | | | |
| *M. tuberculosis* load (per 10-fold increase)<sup>c</sup> | 1.56 (1.14–2.11) | .005 | 1.97 (1.18–3.28) | .009 | 1.43 (0.95–2.15) | .082 |
| *M. tuberculosis* below LOD<sup>c</sup> | 2.84 (0.83–9.68) | .096 | 7.60 (1.05–54.9) | .044 | 1.55 (2.7–87.5) | .617 |
| Age (per 10-y increase) | 1.07 (0.91–1.27) | .416 | 1.09 (0.90–1.31) | .367 | 1.16 (0.67–2.03) | .593 |
| Weight (per 10-kg increase) | 0.14 (0.87–14.9) | .325 | 1.04 (0.74–1.46) | .831 | 1.33 (0.83–2.12) | .228 |
| Glasgow coma scale (per 1-point increase) | 0.77 (.71–84) | < .0001 | 0.77 (.69–85) | < .0001 | 0.75 (.63–89) | .001 |
| CSF leukocyte count (per 10-fold increase) | 1.00 (0.99–100) | .552 | 1.00 (0.99–100) | .733 | 1.00 (0.99–101) | .393 |
| Intensified regimen | 0.84 (53–133) | .453 | 0.53 (29–96) | .037 | 1.76 (80–390) | .159 |
| HIV infected | 1.07 (64–180) | .791 | ... | ... | 1.83 (80–418) | .149 |
| Antiretroviral therapy at enrollment | ... | ... | 1.36 (49–377) | .547 | 0.65 (20–210) | .473 |
| LTA4H genotype: CC vs TT | 1.08 (51–2.28) | .830 | 0.84 (30–234) | .741 | 0.9 (28–866) | .856 |
| LTA4H genotype: CT vs TT | 0.96 (46–202) | .915 | ... | ... | ... | ... |
| Mortality (*M. tuberculosis* load effect) | | | | | | |
| *M. tuberculosis* load (per 10-fold increase) | 1.49 (1.15–1.92) | .002 | 1.54 (96–248) | .072 | 0.73 (54–99) | .045 |
| *M. tuberculosis* below LOD<sup>a</sup> | 3.06 (114–822) | .026 | 3.82 (66–221) | .134 | 3.22 (94–1105) | .063 |
| Mortality (adjusted effect) | | | | | | |
| *M. tuberculosis* load (per 10-fold increase)<sup>d</sup> | 1.25 (94–168) | .127 | 1.43 (82–520) | .207 | 1.14 (79–164) | .480 |
| *M. tuberculosis* below LOD<sup>d</sup> | 2.76 (89–853) | .077 | 2.72 (35–213) | .341 | 2.45 (58–102) | .220 |
| Age (per 10-y increase) | 1.52 (130–178) | < .0001 | 1.88 (140–201) | < .0001 | 0.91 (59–141) | .675 |
| Weight (per 10-kg increase) | 0.67 (51–88) | .003 | 0.87 (59–127) | .475 | 0.58 (39–88) | .010 |
| Glasgow coma score (per point increase) | 0.69 (64–75) | < .0001 | 0.71 (64–80) | < .0001 | 0.65 (56–75) | < .0001 |
| CSF leukocyte count (per 10-fold increase) | 1.00 (99–100) | .270 | 0.98 (97–100) | .094 | 1.00 (99–100) | .933 |
| Intensified regimen | 0.91 (60–138) | .667 | 0.77 (42–141) | .405 | 1.15 (64–208) | .637 |
| HIV infected | 5.50 (338–896) | < .0001 | ... | ... | 0.80 (43–150) | .487 |
| Antiretroviral therapy at enrollment | ... | ... | 3.82 (102–143) | .046 | 1.66 (64–345) | .297 |
| LTA4H genotype: CC vs TT | 2.24 (106–476) | .033 | 3.82 (102–143) | .046 | 1.66 (64–345) | .297 |
| LTA4H genotype: CT vs TT | 1.86 (87–396) | .108 | 2.04 (54–765) | .289 | 1.83 (69–487) | .225 |

Abbreviations: CI, confidence interval; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; HR, hazard ratio; LOD, limit of detection; *M. tuberculosis*, Mycobacterium tuberculosis.

<sup>a</sup>The *M. tuberculosis* load was considered below the limit of detection (LOD) if GeneXpert results were negative. The *M. tuberculosis* load covariate and indicator of LOD were used in the model.

<sup>b</sup>Adjusted for risk factors in the multivariate model.

<sup>c</sup>Values were adjusted by the Hochberg method for the association of *M. tuberculosis* load and new neurological events: *P* = .045 (all patients), *P* = .075 (HIV-uninfected patients), and *P* = .823 (HIV-infected patients).

<sup>d</sup>Values were adjusted by the Hochberg method for the association of *M. tuberculosis* load and death: *P* = .382 (all patients), *P* = .475 (HIV-uninfected patients), and *P* = .933 (HIV-infected patients).
with a high concentration of CSF cytokines before the start of treatment, whereas death is associated with an attenuated inflammatory responses [4].

One of the limitations of our study was that we were unable to define the likely cause of the new neurological events, using brain imaging. However, given the nature and timing of the events and their association with increased inflammation, it is reasonable to assume that many could be defined as paradoxical treatment reactions [12]. Therefore, taken together with the recent finding by Marais et al [9], our data provide further evidence for the importance of bacterial load and neutrophils in treatment-associated inflammatory complications in adults with TBM who are or are not infected with HIV. Our findings support the hypothesis that the pathogenesis of IRIS and paradoxical reactions are similar, resulting from excessive neutrophil-mediated inflammation and driven by M. tuberculosis load. Drugs with the potential to target neutrophils (eg, roflumilast, ibuprofen, and doxycycline [12]) may be more effective than corticosteroids in the treatment of these common complications.

An additional limitation of our study was that we could not follow the decline of bacterial loads after the start of treatment. Even before the start of antituberculosis treatment, CSF bacillary loads are very low. In our participants, pretreatment bacillary loads in CSF specimens were at least 100-fold lower than loads reported in sputum specimens from patients with pulmonary tuberculosis [25]. Furthermore, repeated CSF sampling early in treatment is rarely clinically justifiable, which further restricts the information available. We routinely sample CSF after 30 and 60 days of therapy, by which time bacterial loads are almost always below the detection threshold.

In summary, our data shed new light on the pathogenesis of TBM and suggest divergent mechanisms that lead to death and neurological events occurring after the start of treatment. Death from TBM is associated with an attenuated inflammatory response at the start of treatment, with reduced CSF leukocytes and cytokine concentrations, compared with those in survivors. In contrast, patients with high pretreatment CSF bacterial loads, cytokines, and neutrophils are more likely to experience new neurological events or paradoxical inflammatory complications. These findings may have important implications for the selection of future host-directed therapies.

**Notes**

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