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BRIEF REPORT

Differences in Immunologic Factors Among Patients Presenting with Altered Mental Status During Cryptococcal Meningitis

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Altered mental status in cryptococcal meningitis results in poorer survival, but underlying causes of altered mentation are poorly understood. Within two clinical trials, we assessed risk factors for altered mental status (GCS score<15) considering baseline clinical characteristics, CSF cytokines/chemokines, and antiretroviral therapy. Among 326 enrolled participants, 97 (30%) had GCS<15 and these patients had lower median CSF cryptococcal antigen titers (P = .042) and CCL2 (P = .005) but higher opening pressures (320 vs. 269 mm H2O; P = .016), IL-10 (P = .044), and CCL3 (P = .008) compared with persons with GCS=15. Altered mental status may be associated with host immune response rather than Cryptococcus burden.

Keywords. Cryptococcal meningitis; HIV; immunology; altered mental status; cytokines.

Cryptococcal meningitis causes approximately 15% of AIDS-related deaths and is the most common cause of adult meningitis in Africa [1, 2]. Altered mental status is consistently the strongest risk factor for death in persons with cryptococcal meningitis, increasing the odds of death by 5-fold [3, 4]. Although the association with higher mortality is known, factors associated with altered mental status at presentation are not well described.

We evaluated survival, clinical, and immunologic differences between human immunodeficiency virus (HIV)–infected persons with cryptococcal meningitis presenting with and those presenting without altered mental status. We hypothesized that fungal burden and the immunologic response in persons with altered mental status differed from findings for persons with normal mental status.

METHODS

For this study, we included adult participants from 2 prospective clinical trials in Uganda and South Africa between 2010 and 2014 presenting with their first episode of HIV-associated cryptococcal meningitis [3, 5]. In each trial, we defined altered mental status as a Glasgow Coma Scale (GCS) score of <15 at the time of diagnosis of cryptococcal meningitis. Participants or a proxy provided written informed consent, and applicable institutional review board approvals were obtained.

Participants were enrolled in the Cryptococcal Optimal Antiretroviral Therapy (ART) Timing trial between November 2010 and April 2012 in Kampala and Mbarara, Uganda, and Cape Town, South Africa (clinical trials registration NCT01075152) [6]. In total, 177 ART-naive persons with cryptococcal meningitis were randomly assigned to initiate ART early or to have ART initiation deferred (7–13 days or 5 ± 1 weeks after meningitis diagnosis, respectively).

The Adjunctive Sertraline Treatment of Cryptococcal Meningitis (ASTRO-CM) pilot study enrolled 149 HIV-infected participants with cryptococcal meningitis in Kampala from August 2013 through August 2014 [5]. The ASTRO-CM pilot was a dose-finding phase 2 clinical study of adjunctive sertraline 100–400 mg/day added to amphotericin 0.7–1.0 mg/kg/day with fluconazole 800 mg/day. The participants included both ART-naive and ART-experienced individuals; however, only participants with a first episode of cryptococcal meningitis were included.

Laboratory Procedures

Lumbar punctures were performed on patients at the time of presentation, and cryptococcal meningitis was diagnosed on the basis of CSF cryptococcal antigen test results, which were confirmed with a quantitative CSF culture that measured the number of *Cryptococcus* colony-forming units (CFU) per milliliter of CSF [7]. CSF cell counts were determined and chemistry analyses performed at on-site laboratories participating in external quality assurance testing. CSF specimens were centrifuged, and supernatants were frozen at −80°C. Frozen CSF specimens were shipped on dry ice (temperature, −20°C) to the University of Minnesota, thawed, and analyzed via Luminex magnetic bead technology (Bio-Rad Laboratories, Hercules, CA). This analysis included 19 CSF cytokines and chemokines (granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, interferon γ, tumor necrosis factor α, interleukin [IL] 1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, monocyte chemoattractant protein 1 [MCP-1; CCL2],

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macrophage inflammatory protein 1α [MIP-1α; CCL3], MIP-1β [CCL4], and vascular endothelial growth factor) and was performed according to manufacturers' protocols. Three soluble markers of macrophage activation (sCD163, sCD14, and CCL22) were also measured, using commercial enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN) according to the protocol recommended by the manufacturer [6].

Statistical Analysis

Normal mental status was defined as a GCS score of 15 and altered mental status as a GCS score of <15, as assessed at the time the patient was admitted to the hospital. Baseline demographic and other characteristics were summarized by altered mental status groups, using proportions or median values with interquartile ranges (IQRs), and were compared using χ^2 or Mann–Whitney tests, as appropriate. Survival was compared by means of Kaplan-Meier curves and Cox proportional hazards regression. Levels of CSF biomarkers analyzed as continuous variables were $\log_2$ transformed for normalization and then compared between the altered mental status groups, using linear regression. Low (ie, out-of-range) values were set to half of the manufacturer's listed assay limit of detection. IL-2 and IL-5 had many undetectable values, so these were evaluated as present or not and compared using a χ^2 test. *P* values were not adjusted for multiple comparisons as analyses were hypothesis generating.

We evaluated risk factors for altered mental status, using univariate linear regression, including patient demographic characteristics, clinical characteristics, CSF cytokine/chemokine levels, CSF fungal burden (based on cryptococcal antigen titers and results of quantitative culture), and receipt of ART as possible risk factors. Analysis was performed using SPSS Statistics 22,24 (IBM, Armonk, NY).

RESULTS

Baseline Characteristics

We enrolled 326 HIV-infected participants with cryptococcal meningitis, of whom 30% (97) had altered mental status (GCS score, <15) and 70% (n = 229) had normal mental status (GCS score, 15). The median GCS score for patients with altered mental status was 14 (IQR, 12–14; minimum, 4). The median age of participants was 35 years (IQR, 30–41 years), and 58% were men. Baseline characteristics by mental status are shown in Table 1.

Survival was compared with a Kaplan-Meier curve in Supplemental Figure 2. A Cox regression analysis found that those with altered mental status at presentation had a significantly worse survival ($P < .001$).

We observed no association between mental status and either baseline CD4$^+$ T-cell count ($P = .18$), baseline viral load ($P = .41$), or initiation of ART at presentation ($P = .91$). There was also not a significant difference in altered mental status between study cohorts ($P = .19$). Those with altered mental status had lower median CSF cryptococcal antigen titers than those with normal mental status (1:3000 vs 1:4000; $P = .04$). Similarly, the

Table 1. Baseline Characteristics of Patients, Stratified by Mental Status at Diagnosis

Characteristic	Altered Mental Status (GCS Score, <15)	Normal Mental Status (GCS Score, 15)	*P*[a]
Patients, no. (%)	97 (30)	229 (70)	
GCS score	14 (12–14)	15	
Age, y	36 (30–42)	35 (29–40)	.10
Headache duration, d	14 (7–30)	14 (7–21)	.29
Men	60 (31)	129 (56)	.36
Ugandan	94 (97)	205 (90)	.03
Receiving ART	17 (18)	39 (17)	.91
CD4$^+$ T-cell count, cells/μL	27 (10–78)	20 (7–61)	.18
Plasma HIV load, $\log_{10}$ copies/mL	4.8 (5.1–5.9)	5.2 (5.2–5.8)	.41
CSF opening pressure			
Overall, cm H_2O	32 (20–51)	27 (17–37)	.02
>25 cm H_2O	56 (64)	116 (58)	.19
CSF WBC count			
Overall, cells/μL	30 (4–63)	10 (4–75)	.23
<5 cells/μL	46 (48)	118 (56)	.61
CSF lymphocyte percentage	28.3 (18.7–100)	28.2 (19.2–43.5)	.29
CSF protein level, mg/dL	148 (43–181)	79 (37–146)	.68
CSF CrAg titer	1:3000 (1:300–1:8192)	1:4000 (1:1000–1:12 800)	.04
CSF quantitative culture, $\log_{10}$ CFU/mL	4.3 (3.5–5.2)	5.0 (4.1–5.8)	.11
Survival at 10 weeks, %	45	65	<.001

Data are no. (%) of patients or median (interquartile range).

Abbreviations: ART, antiretroviral therapy; CFU, colony-forming units; CrAg, cryptococcal antigen; CSF, cerebrospinal fluid; GCS, Glasgow Coma Scale; HIV, human immunodeficiency virus.

[a] *P* values of ≤.05 were considered statistically significant.

quantitative CSF culture counts were slightly lower in persons with altered mental status (median 4.33 $\log_{10}$ CFU/mL), compared with normal mental status (median 4.96 $\log_{10}$ CFU/mL; P = .11). Participants with altered mental status had higher median CSF opening pressures (32 cm H_2O [IQR, 20–51 cm H_2O] vs 27 cm H_2O [IQR, 17–37 cm H_2O]; P = .016).

Immunologic Characteristics

We also compared mental status groups by their CSF immune responses by measuring soluble cytokines and chemokines in each mental status group among 250 participants (Table 2). Participants with altered mental status had higher concentrations of CCL3 (MIP-1α) (P = .01) and IL-10 (P = .04) when compared to persons with normal mental status (Table 2). Median levels of CCL2 (MCP-1) were lower among those with altered mental status (P = .01). Persons lacking CSF pleocytosis (<5 white cells/μL) had higher levels of CCL2 (P = .038) and CCL3 (P < .001) than persons with CSF pleocytosis. Yet CSF white cells total counts did not differ between groups (P = .23).

DISCUSSION

Among HIV-infected persons with cryptococcal meningitis, we found that altered mental status at presentation was associated with the immune response in CSF and not a higher fungal burden. Altered mental status has been shown to be a risk factor for increased mortality in patients with HIV-related cryptococcal meningitis [4], which we found, as well; however, little is known about the pathophysiology of altered mental status in cryptococcal meningitis.

We found that the fungal burden was not increased in CSF among persons presenting with altered mental status, and in fact the cryptococcal antigen titers were slightly lower compared to those with normal mental status. Yet, the CSF opening pressure was higher in persons with altered mental status.

We found significantly higher MIP-1α (CCL3) and IL-10 levels and significantly lower MCP-1 (CCL2) levels among persons with altered mental status compared with persons with normal mental status. CCL2 and CCL3 levels were higher among persons who lacked CSF pleocytosis, yet the presence or absence of CSF pleocytosis alone was not associated with altered mental status.

MIP-1α (CCL3) plays important roles in the immune response during infection, recruiting mononuclear cells and neutrophils and modulating cytokine production [8]. MIP-1α knockout mice have decreased recruitment of leukocytes and slower clearance of *Cryptococcus* from the brain [9]; however, data in humans have been limited and suggest a different picture. Investigation of T-cell responses in 44 patients with cryptococcal meningitis from a prospective South African cohort

Table 2. Cerebrospinal Fluid (CSF) Immunologic Factors Associated with Altered Mental Status

CSF Biomarker	Available for Testing, No.	Altered Mental Status (GCS Score, <15) (n = 76)	Normal Mental Status (GCS Score, 15) (n = 174)	P[a]
CD14 level, pg/mL	250	87 (20–590)	129 (15–615)	.66
CD163 level, pg/mL	123	420 (181–845)	510 (293–917)	.22
G-CSF level, pg/mL	241	47 (24–123)	54 (28–117)	.97
GM-CSF level, pg/mL	241	347 (282–494)	337 (253–524)	.41
INF-γ level, pg/mL	241	45 (11–126)	37 (14–82)	.51
TNF-α level, pg/mL	241	8 (4–26)	8 (4–18)	.72
IL-2 level, pg/mL	241	1.7 (0.6–5.8)	1.6 (0.6–4.6)	.38
IL-4 level, pg/mL	241	0.8 (0.4–1.8)	0.8 (0.3–1.8)	.22
IL-5 level, pg/mL	241	0.5 (0.4–1.9)	0.5 (0.4–1.4)	.46
IL-6 level, pg/mL	241	215 (67–899)	133 (37–574)	.10
IL-7 level, pg/mL	241	5 (3–16)	4 (2–15)	.81
IL-8 level, pg/mL	241	525 (279–1487)	554 (268–1205)	.94
IL-10 level, pg/mL	241	16 (8–30)	10 (5–21)	.04
IL-12 level, pg/mL	241	11(6–17)	10 (4–17)	.17
IL-13 level, pg/mL	241	30 (6–98)	25 (4–93)	.66
IL-17 level, pg/mL	241	13 (5–24)	14 (4–29)	.45
MCP-1 level, pg/mL	241	383 (114–961)	495 (218–1631)	.008
MIP-1α level, pg/mL	155	128 (76–225)	72 (10–148)	.005
MIP-1β level, pg/mL	241	41 (2–100)	55 (3–120)	.23
VEGF level, pg/mL	118	46 (6–96)	47 (8–100)	.90
MIP-1α/MCP-1 ratio	155	3.2 (0.9–14.7)	14.8 (2.6–63.4)	.001

Data are median values (interquartile ranges).

Abbreviations: GCS, Glasgow Coma Scale; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; VEGF, vascular endothelial growth factor.

[a] P values were calculated using linear regression on $\log_2$-transformed data, and values ≤.05 were considered statistically significant. Supplementary Figure 1 displays CSF IL-10, MCP-1, and MIP-1α levels visually.

showed that a higher, predominant MIP-1α response was associated with higher mortality [10]. Our data showing increased MIP-1α levels in patients with altered mental status are also consistent with this. MIP-1α is likely produced as a compensatory response by the innate immune system in an to attempt to clear *Cryptococcus* [11]. We hypothesize that production of MIP-1α as a single signaling chemokine is an appropriate response yet reflects an insufficient T-helper type 1 response to disseminated *Cryptococcus.* MIP-1α is most likely a marker of a dysfunctional immune response.

We report that those with altered mental status also had lower MCP-1 (CCL2) levels. MCP-1 is a proinflammatory chemokine involved in signaling for monocytes, memory T cells, and natural killer cells, which all play a role in the control of cryptococcal infection [12]. MCP-1 can be secreted by monocytes and macrophages, as well as by astrocytes and microglial cells [12]. Lower levels of MCP-1 may be associated with lower levels of monocytes and other cells being recruited into the central nervous system to control *Cryptococcus*. In a prior South African cohort, CCL2 and CCL3 levels both had an inverse relationship with immune reconstitution inflammatory syndrome (IRIS) and mortality [13]. This is consistent with our data for CCL3 MIP-1α (CCL3) but inconsistent with our findings for MCP-1 (CCL2). Our investigation was in relation to altered mental status, not IRIS or mortality. Overall, chemokine signaling in cryptococcal meningitis requires further study.

Last, we also found possibly higher IL-10 levels in persons with altered mental status. IL-10 is primarily an antiinflammatory cytokine that inhibits the protective T-helper type 1 immunologic response in cryptococcosis [14]. In murine models, blocking IL-10 significantly increased the rate of fungal clearance in cryptococcal lung disease [15]. Persons with altered mental status and a disseminated infection, who have a higher mortality risk, may have higher levels of IL-10, representing a dysfunctional immune response.

Our study is subject to limitations. First, the study was large in the context of other studies of cryptococcal meningitis but relatively small given the number of demographic, clinical, and immunologic markers we assessed. This is exploratory work, and we hope these data can inform and be confirmed in a future prospective cohort. Second, the large number of comparisons could have yielded results that appear to be statistically significant but are actually attributable to chance, owing to multiple testing. For example, persons with altered mental status had slightly lower CSF cryptococcal antigen titers ($P = .04$). Whether this is a true or spurious result, the larger implication was that persons with altered mental status did not simply have a higher fungal burden than persons presenting with normal mental status. Future work should include validating these findings and assessing how changes in immunologic risk factors impact the evolution of mental status.

In summary, 30% of patients with cryptococcal meningitis presented with altered mental status. Survival was significantly worse in those with altered mental status, compared with that among patients with normal mental status. Those with altered mental status had a higher intracranial pressure at baseline but a slightly lower fungal burden than those with normal mental status. Altered mental status was also associated with higher MIP-1α and IL-10 levels and a lower MCP-1 level at baseline. These results suggest that patients with altered mental status have a dysfunctional immune response at a time when they need increased immune function to control disseminated *Cryptococcus*. Dysfunctional immunologic responses, rather than fungal burden, may inform why such patients with altered mental status have worse outcomes.

STUDY GROUP MEMBERS

COAT and ASTRO trial team members are as follows: Jane Francis Ndyetukira, Cynthia Ahimbisibwe, Florence Kugonza, Liberica Ndyatunga, Busingye Noeme, Brian Memela, Yolisa Sigila, James E. Scriven, Renee D. Carlson, Kate Birkenkamp, Elissa K Butler, Jonathan Dyal, and A. Wendy Fujita (provision of patient care); Alisat Sadiq, Monica Magwayi, and Busingye Noeme (HIV and ART counseling); Richard Kwizera, Emily Ninsiima, Ali Elbireer, and Robert Lukande (laboratory support); Kirsten Nielsen, Andrew Akampurira, James Mwesigy, Robert Wagubi, Henry Kajumbula, Tami McDonald, Anna Strain, Darin Wiesner, Grace Najjuka, and Tihana Bicanic (microbiology support); Leya Hassanally, Catherine Nanteza, Rhina Mushagara, Maximilian von Hohenberg, Ann Vogt Lima, Kosuke Yasukawa, Bilal Jawed, and Katelyn Pastick (logistical support); and Mariam Namawejje, Mark Ssennono, and Agnes Kiragga (data management).

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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