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Latent *Toxoplasma* Infection and Higher *Toxoplasma gondii* Immunoglobulin G Levels Are Associated With Worse Neurocognitive Functioning in HIV-Infected Adults

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Background. Human immunodeficiency virus (HIV)–associated neurocognitive disorders persist despite suppressive antiretroviral therapy (ART). Because latent *Toxoplasma* infection (LTI) may adversely impact brain function, we investigated its impact on neurocognitive impairment (NCI) in people living with HIV disease.

Methods. Two hundred sixty-three HIV-infected adults underwent comprehensive neurocognitive assessments and had anti-*Toxoplasma gondii* immunoglobulin G (anti-Toxo IgG) measured by qualitative and quantitative enzyme-linked immunosorbent assays.

Results. Participants were mostly middle-aged white men who were taking ART (70%). LTI was detected in 30 (11.4%) participants and was associated with a significantly greater prevalence of global NCI (LTI positive [LTI^+] = 57% and LTI negative [LTI^-] = 34%) (odds ratio, 1.67; 95% confidence interval, 1.17–2.40; $P = .017$). Deficits were more prevalent in the LTI^+ vs the LTI^- group in 6 of 7 cognitive domains with statistical significance reached for delayed recall ($P < .01$). The probability of NCI increased with higher $CD4^+$ T-cell counts among LTI^+ individuals but with lower $CD4^+$ T-cell counts in LTI^- persons. A strong correlation ($r = .93$) between anti-Toxo IgG levels and global deficit score was found in a subgroup of 9 patients. Biomarkers indicative of central nervous system inflammation did not differ between LTI^+ and LTI^- participants.

Conclusions. In this cross-sectional analysis, LTI was associated with NCI, especially in those with higher $CD4^+$ T-cell counts. Longitudinal studies to investigate the role of neuroinflammation and neuronal injury in LTI patients with NCI and trials of anti-*Toxoplasma* therapy should be pursued.

Keywords. latent *Toxoplasma* infection; HIV-1 infection; Anti–*Toxoplasma gondii* IgG; latent toxoplasmosis; neurocognitive impairment.

Human immunodeficiency virus (HIV)–associated neurocognitive disorder (HAND) is a well-recognized complication of HIV-1 infection that can impede employment, activities of daily living, and ultimately survival [1]. Antiretroviral therapy (ART) alone is often not sufficient to restore full cognitive functioning, suggesting that the cause of persisting neurocognitive impairment (NCI) may not be fully understood. Chronic coinfections, such as cytomegalovirus [2] or hepatitis C virus [3], are associated with NCI and may contribute to persistent NCI during ART.

Another chronic coinfection, *Toxoplasma gondii*, infects up to one-third of the world's population [4]. *Toxoplasma gondii* is an obligate intracellular protozoal parasite and humans acquire infection after ingesting the cysts in undercooked meat or contaminated fruits or vegetables. The rapidly replicating tachyzoite form disseminates throughout the body before transforming into the slowly replicating bradyzoites within cysts found mainly in the brain and skeletal muscle [5]. Latent *Toxoplasma* infection (LTI) produces no symptoms. Waning cell-mediated immunity, as in AIDS, can result in reactivation of LTI and cause encephalitis [6].

Recent evidence from animal and human studies suggests that LTI can result in behavioral changes, including increased impulsivity, aggression, and suicide attempts [7, 8]; difficulties with learning in mice [9]; and with memory [10], reaction time [11], and higher risk of traffic accidents in humans [12]. Whether LTI contributes to NCI in HIV-infected adults in the absence of clinical encephalitis is unknown. To address this, we analyzed the associations between LTI and (*i*) NCI; (*ii*) anti-*Toxoplasma* immunoglobulin G (anti-Toxo IgG) levels; and (*iii*) cerebrospinal fluid (CSF) inflammatory biomarkers.

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METHODS

Participants

We identified 263 HIV-infected participants, enrolled in several National Institutes of Health–funded cohort studies at the University of California, San Diego (UCSD) HIV Neurobehavioral Research Program, all of whom had undergone comprehensive neurocognitive assessments and had anti-Toxo IgG measured by qualitative assay for routine screening in HIV clinical care at the UCSD Owen Clinic. *Toxoplasma* encephalitis presents as an acute illness with new-onset seizures, hemiparesis, or other acute focal neurological signs [13]. Participants in the analysis described here were ambulatory patients without acute illness or focal neurologic disturbances on medical examination. All protocols were approved by the UCSD Human Research Protections Program, and all subjects provided written informed consent.

Neurocognitive Functioning Assessments

All participants were tested using a comprehensive neurocognitive test battery to assess 7 cognitive domains as previously described [14]: learning, recall, attention/working memory, speed of information processing, verbal fluency, executive functions, and motor skills. Individual test scores were standardized using published, normative data that adjust for age, education, sex, and ethnicity and were combined to create global- and domain-specific deficit scores (GDS and DDS, respectively) that range from 0 (normal) to 5 (severely impaired) [15]. The GDS is an automated method to detect impairment, requires lower-than-expected performance in several domains, and ignores higher-than-expected performance. Consistent with published, well-validated procedures, global NCI was defined as a GDS $\geq$ 0.5 and domain-specific NCI was defined as DDS > 0.5.

Laboratory Assays

Blood and, in subjects who consented to lumbar puncture, CSF specimens were collected at the time of neurocognitive function testing and stored at −80°C. LTI diagnosis was defined by qualitative detection of anti-Toxo IgG (TX022G assay, Calbiotech, Spring Valley, California) with a manufacturer-specified qualitative cutoff of 1.1. Levels of anti-Toxo IgG in IgG-positive participants were estimated based on the colorimetric signal intensity per the manufacturer's instructions. Soluble biomarkers in CSF were measured by bead suspension arrays (Millipore, Billerica, Massachusetts) on a BioPlex 100 platform (Bio-Rad, Hercules, California) for monocyte chemoattractant protein 1 (MCP-1) and interferon γ–induced protein 10 (IP-10), and enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota) for soluble CD14 (sCD14) and neopterin. HIV RNA levels were quantified in plasma and CSF by reverse transcription–polymerase chain reaction (Roche Amplicor, version 1.5, lower limit of quantitation 50 copies/mL). $CD4^+$ T-cell counts were measured in blood by flow cytometry.

Statistical Analysis

Differences between LTI-positive (LTI^+) and LTI-negative (LTI^-) participants and between trimethoprim-sulfamethoxazole (TMP-SMX) users and nonusers were compared using *t* tests or Wilcoxon rank-sum tests for means and medians, or χ^2 or Fisher exact tests for proportions. Demographic, disease, and treatment variables were screened by univariable logistic regression to estimate NCI at a 15% significance level. Variables below this screening level were combined with LTI status and their interaction in a multivariable logistic model and tested. The correlation of anti-Toxo IgG with each of $CD4^+$ T-cell count, GDS, and DDS was estimated using Pearson product moment or Spearman correlation coefficients. For all testing, required parametric assumptions were verified, and data transformations or nonparametric methods were applied when needed. Two-tailed tests and a 5% significance level were used for hypothesis testing unless indicated otherwise. Because adjustments were not made for multiple comparisons, the statistical significance of our findings must be interpreted accordingly.

RESULTS

Demographic and Disease Characteristics

Demographic, disease, and treatment characteristics are shown in Table 1. Participants had a mean age of 42 years (SD,

Table 1. Demographic, Disease, and Treatment Characteristics of Subjects With and Without Latent *Toxoplasma gondii* Infection

Characteristic	LTI^- (n = 233)	LTI^+ (n = 30)	*P* Value
Age, y, mean (SD)	41.8 (9.1)	44.6 (10.4)	.12
Education, y, mean (SD)	12.6 (2.4)	12.4 (2.9)	.82
Male sex, No. (%)	211 (91)	26 (87)	.51
Ethnicity, No. (%)			.81
Black	36 (15)	5 (17)	
Hispanic	49 (21)	7 (23)	
White	138 (59)	16 (53)	
Other	10 (4)	2 (7)	
HIV RNA in plasma, log_{10} copies/mL[a], mean (SD)	3.0 (1.4)	2.7 (1.3)	.34
Undetectable[a], No. (%)	94 (44)	17 (57)	.18
ART use[b], No. (%)	161 (69)	22 (76)	.47
On ART and RNA undetectable[c], No. (%)	92 (43)	17 (59)	.11
$CD4^+$ count, cells/μL[d], median (IQR)	395 (214–583)	330 (238–535)	.98
$CD4^+$ nadir, cells/μL[e], median (IQR)	211 (63–280)	195 (176–281)	.91
GDS $\geq$ 0.5, No. (%)	79 (34)	17 (57)	.017

Abbreviations: –, negative; +, positive; ART, antiretroviral therapy; GDS, global deficit score; HIV, human immunodeficiency virus; IQR, interquartile range; LTI, latent *Toxoplasma* infection; SD, standard deviation.

[a] n = 216 for LTI^-.

[b] n = 232 for LTI^- and n = 29 for LTI^+.

[c] n = 215 for LTI^- and n = 29 for LTI^+.

[d] n = 221 for LTI^- and n = 29 for LTI^+.

[e] n = 105 for LTI^- and n = 12 for LTI^+.

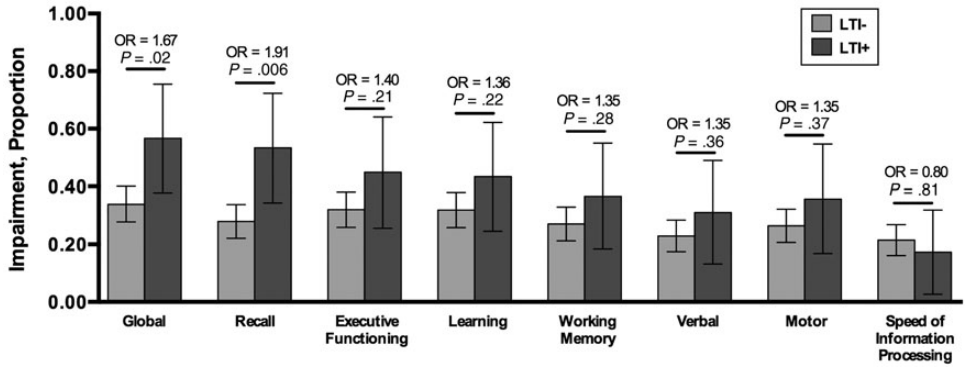

Figure 1. Global and domain impairment by latent *Toxoplasma* infection (LTI) status. Deficits were more prevalent in the LTI-positive (LTI^+) vs the LTI-negative (LTI^-) group in 6 of 7 cognitive domains, and these differences reach statistical significance for global impairment and delayed recall. Abbreviation: OR, odds ratio.

9.3 years), a mean education of 12.6 years (SD, 2.4 years), and were predominantly white (59%), male (90%), and taking ART (70%). Less than half of patients had undetectable levels of HIV RNA in blood, but the percentage was higher in LTI^+ than LTI^- participants (57% vs 44%; $P = .18$). LTI was detected in 30 (11.4%) participants. No differences in race/ethnicity were seen between LTI^- and LTI^+ individuals ($P > .8$). The median current $CD4^+$ T-cell count was 387 cells/μL (interquartile range, 231–582 cells/μL). Neither $CD4^+$ T-cell counts nor $CD4^+$ T-cell nadir was significantly associated with LTI status ($CD4^+$ T-cell count: $P = .98$; $CD4^+$ nadir: $P = .91$). Overall, LTI^+ and LTI^- participants had similar demographics and HIV disease characteristics.

Neurocognitive Impairment by LTI Status

Figure 1 displays global and domain impairment by LTI status. LTI was associated with a significantly greater prevalence of global NCI ($LTI^+ = 57\%$ and $LTI^- = 34\%$; odds ratio [OR], 1.67; 95% confidence interval [CI], 1.17–2.40; $P = .017$). Prevalence of impairment in the domain of delayed recall also differed strikingly ($LTI^+ = 53\%$ and $LTI^- = 28\%$; OR, 0.006; $P = .006$). For 6 of 7 cognitive domains, deficits were more prevalent in the LTI^+ than the LTI^- group.

Probability of NCI by LTI Status and $CD4^+$ T-Cell Counts

LTI^+ and LTI^- patients had similar mean current or nadir $CD4^+$ T-cell counts. $CD4^+$ T-cell count was significantly related to NCI ($P < .001$) univariably and, in a multivariable logistic regression model of risk of NCI (overall $P < .001$; Figure 2), the probability of NCI increased with increasing $CD4^+$ T-cell counts in LTI^+ patients, whereas NCI was inversely related to $CD4^+$ T-cell counts among those without LTI (LTI^- $P = .021$, $CD4^+$ $P = .001$; and their interaction $P = .019$). $CD4^+$ T-cell count was the only demographic, disease, or treatment variable significantly correlated to NCI in univariate analysis.

Neurocognitive Functioning and $CD4^+$ T-Cell Count Correlation With Anti-Toxo IgG Levels

In a small subgroup of anti-Toxo IgG–positive participants with stored serum specimens who underwent quantitative anti-Toxo IgG testing (n = 9), higher anti-Toxo IgG levels correlated with higher $CD4^+$ T-cell counts ($r = 0.67$, $P < .05$; Figure 3*A*). Anti-Toxo IgG levels and GDS were highly correlated ($r = 0.93$, $P < .001$, Figure 3*B*). In this subgroup, higher anti-Toxo IgG levels were also correlated with worse scores in 3 of 7 cognitive domains: (*i*) delayed recall ($r = 0.82$, $P = .007$), (*ii*) learning ($r = 0.70$, $P = .035$), and (*iii*) motor ability ($r = 0.81$, $P = .009$).

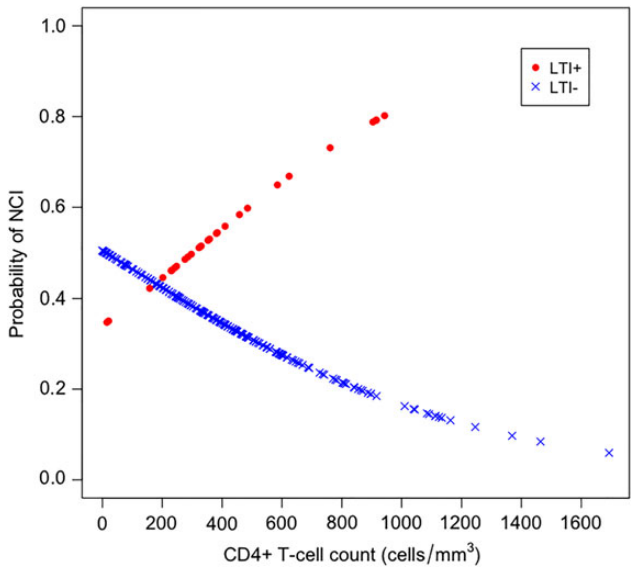

Figure 2. Logistic regression model of the probability of neurocognitive impairment (NCI) by latent *Toxoplasma* infection (LTI) status and $CD4^+$ T-cell counts. While risk of NCI decreases with higher $CD4^+$ T-cell counts in LTI-negative (LTI^-) persons (blue crosses), the opposite effect is found in LTI-positive (LTI^+) persons (red dots). At $CD4^+$ T-cell counts near 200 cells/μL, the levels of risk converge.

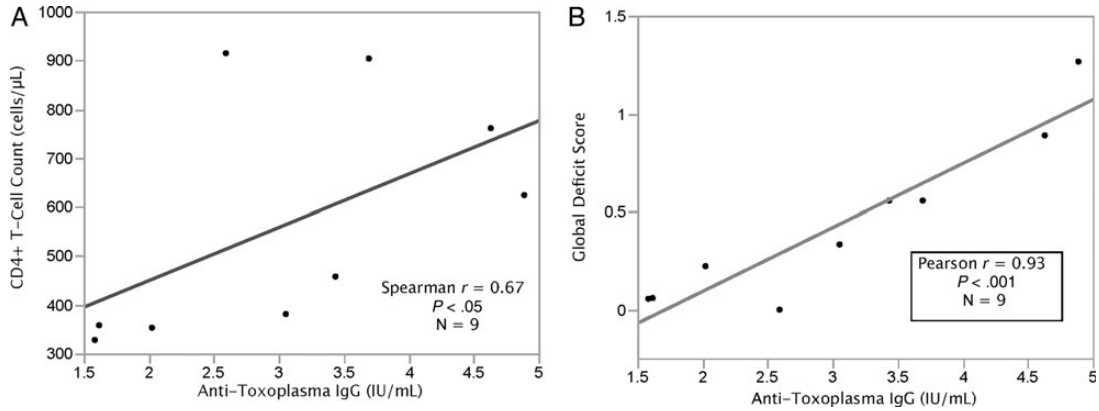

Figure 3. CD4 counts and global deficit scores correlate with anti-*Toxoplasma gondii* immunoglobulin G (IgG) levels. *A,* Higher anti-*Toxoplasma* IgG levels correlated with higher CD4$^+$ T-cell counts. *B,* Anti-*Toxoplasma* IgG levels were highly correlated with global deficit score.

LTI and CSF Biomarkers

To examine a possible role for central nervous system (CNS) inflammation in the pathogenesis of these relationships, we studied 58 participants (5 LTI$^+$ and 53 LTI$^-$) who previously had 4 biomarkers of inflammation (MCP-1, IP-10, sCD14, and neopterin) measured in CSF. These biomarkers have previously been associated with CNS inflammation or cognitive impairment in HIV infection [16]. None of the biomarkers differed between LTI$^+$ and LTI$^-$ participants (all *t* test *P* values >.2).

TMP-SMX and Neurocognitive Performance Among LTI$^+$ Participants

Because of its activity against *Toxoplasma* and use in prevention of HIV-related clinical *Toxoplasma* infections, we examined the impact of current TMP-SMX use on neurocognitive performance among the 21 of 30 LTI$^+$ participants for whom data were available. Because this cohort was predominately immune-reconstituted on ART and not at high risk of reactivation of *Toxoplasma* or other opportunistic infections, only 4 participants reported TMP-SMX use. One of these participants was excluded for hepatitis C virus seropositivity. Although not statistically significant, the remaining 3 TMP-SMX users appeared to perform substantially better (mean GDS, 0.29) than the 17 who did not report TMP-SMX use (mean GDS, 0.77) ($P = .21$; Figure 4). While our sample size is small and the difference not significant, the Cohen d value of 0.77 suggests that TMP-SMX might have a medium-to-large effect.

DISCUSSION

We investigated the effects of LTI on neurocognitive functioning, among people living with HIV disease and without a history or current signs or symptoms of *Toxoplasma* encephalitis, using a robust cognitive battery that assesses multiple domains. We found that LTI was associated with NCI, especially in those with higher CD4$^+$ T-cell counts, but not with CSF biomarkers of inflammation. In a small subgroup of HIV$^+$ patients, levels of anti-Toxo IgG correlated with NCI based on GDS.

The impact of LTI on brain functioning has been studied extensively in rodent models, a frequent mammalian intermediate host of *T. gondii*. Studies in humans have shown that persons with LTI have impaired reaction time [11] and are at a high risk of traffic accidents [12]. A recent population-based study reported impairment of memory in older adults (≥65 years) with LTI [10]. Their multivariate models evaluating only main effects demonstrated no association between LTI and cognitive function, but adding interactions revealed that LTI may decrease cognitive function in some demographic, educational, and ethnic/racial subgroups [17]. LTI is also associated with behavioral changes such as impulsivity and aggression [7] and increased suicide attempts [8].

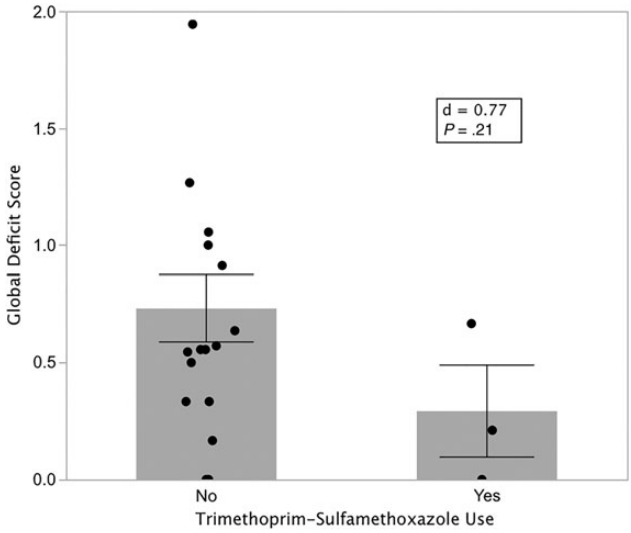

Figure 4. Effect of trimethoprim-sulfamethoxazole (TMP-SMX) treatment on neurocognitive performance. Latent *Toxoplasma* infection–positive participants who reported TMP-SMX use (n = 3) performed substantially better than nonusers (n = 17), but the power of this comparison was low due to small numbers of participants. Heights of bars are means; lines are standard errors.

The prevalence of LTI in the US population ranges from 9.5% to 13.2% [18, 19], and our *T. gondii* IgG positivity rate of 11.4% is consistent with these findings. Advanced immunosuppression due to HIV can lead to *Toxoplasma* encephalitis, a clinically obvious, progressive, and sometimes fatal opportunistic disease. Elevated HIV-related morbidity and mortality in LTI patients could falsely lower cross-sectional estimates of LTI prevalence. However, the facts that markers of HIV disease did not differ in those with and without LTI and that symptomatic *Toxoplasma* infections are relatively rare in the United States in the era of ART is evidence against such an effect. Although increased LTI in HIV-infected vs HIV-uninfected individuals has been reported [20, 21], other studies conducted in the United States, a low-prevalence country, and in Ethiopia, a very high-prevalence country, have found no differences [18, 22]. Thus, the prevalence of LTI in the general population is similar to the prevalence of LTI in the HIV population, consistent with the concept that HIV does not influence the rate of acquisition of LTI.

The LTI^+ group was more likely to have undetectable levels of HIV RNA in blood compared with the LTI^- group. Thus, they are less likely to have HIV-induced cognitive impairment based on inadequate treatment of their HIV infections. Our LTI^+ group tended to be older, although not statistically significantly so, than the LTI^- group, consistent with an expected age-related increase in prevalence of *T. gondii* infection [4, 19]. Age is unlikely to have confounded our analysis because scores on cognitive tests were adjusted for age. Because 90% of our study population were men, we were unable to determine an association between LTI and sex, but in the literature, sex as well as race/ethnicity and country of birth have not been found to increase LTI risk among HIV^+ persons [19]. In contrast, in the general population, the risk of LTI is higher among non-Hispanic blacks and Mexican Americans than non-Hispanic whites, foreign-born than US-born persons, and those below the poverty index [23].

In the brains of latently infected mice and in tissue culture systems, toxoplasmic bradyzoites (*T. gondii* cysts) commonly rupture releasing tachyzoites that subsequently infect new neurons [24, 25]. Although normal immune responses virtually always contain the parasites, these reactions and consequent inflammation may damage the brain and degrade cognition. A cycle of reactivation and reinfection of new neurons may result in host responses that cumulatively damage and deplete neurons in both HIV-infected and -uninfected persons. This cycle could be enhanced in HIV patients during immune recovery on ART.

LTI appeared to be unrelated to nadir or current $CD4^+$ T-cell count. However, in modeling NCI as a function of $CD4^+$ T-cell count (Figure 2), we found that current levels of immune competence ($CD4^+$ T-cell counts) had highly divergent effects on the probability of NCI in LTI^+ and LTI^- HIV-infected persons. In LTI^- participants, the probability of NCI increases with lower $CD4^+$ T-cell counts, consistent with prior findings [26]. The probability of NCI in LTI^+ persons was higher overall and, in contrast to LTI^- individuals, increased with higher $CD4^+$ T-cell counts. We hypothesize that LTI^+ individuals may incur subclinical brain damage because their immune reactions to toxoplasmic tachyzoites released from cysts is increased during immune reconstitution induced by ART. Our observation that anti-Toxo IgG levels correlate with NCI and with higher $CD4^+$ T-cell counts is consistent with this hypothesis. During treatment-enabled immune recovery, improving IgG responses to toxoplasmic antigens could raise their blood levels. Thus, higher anti-Toxo IgG levels may reflect greater exposure to *T. gondii* antigen—that is, more frequent or substantial reactivations of LTI during periods of low $CD4^+$ T-cell counts, or more robust antibody responses after ART-induced immune reconstitution, or both.

Because multiple biomarkers are associated with HAND [27], we measured biomarker levels in CSF where they might reflect inflammatory reactions to *T. gondii* in the brain better than blood levels. However, no differences between LTI^+ and LTI^- groups were seen in our panel of 4 biomarkers that are indicative of HIV-related neuroinflammation. Possible explanations include that (*i*) *T. gondii* leads to brain injury via mechanisms not reflected by these biomarkers; (*ii*) our cross-sectional study did not capture the brief periods after *T. gondii* cysts had ruptured and provoked inflammation; or (*iii*) power was limited by the small number of LTI^+ persons analyzed (n = 5). A larger prospective study with frequent CSF collection could better address this issue.

LTI may be a treatable cause of persistent NCI in antiretroviral-treated, HIV-infected patients, although no evidence for the efficacy of drugs in suppressing subclinical reactivation of or clearing LTI is currently available. Older drug combinations such as TMP-SMX and pyrimethamine-sulfadiazine that are used to treat or prevent *Toxoplasma* encephalitis and newer drugs such as endochin-like quinolones may be effective in treating LTI [28]. LTI treatment could be initiated as soon as it is diagnosed in HIV patients and continued until complete ART-enabled immune reconstitution. Current guidelines support this practice for prevention of *Pneumocystis* pneumonia, *Toxoplasma* infections, and malaria in HIV adults with $CD4^+$ T-cell counts <200 cells/μL. Prevention of LTI-induced NCI may be an additional benefit, especially in low-income countries, which generally have higher prevalence of LTI.

Limitations of this study include (*i*) lack of longitudinal data, (*ii*) lack of HIV-uninfected controls, and (*iii*) a small number of participants for subgroup analyses (eg, quantification of anti-Toxo IgG levels in serum, CSF biomarkers, and TMP-SMX users).

CONCLUSIONS

In this cross-sectional analysis of HIV-infected, predominately antiretroviral-treated patients, LTI was associated with risk of NCI, especially in those with $CD4^+$ T-cell counts >200 cells/μL. Higher levels of $CD4^+$ T cells and of anti-Toxo IgG also

correlated with NCI, possibly reflecting damage from improved immune responses to subclinical reactivation of *Toxoplasma* in brain during ART.

Longitudinal studies of patients before and after ART initiation could test our hypothesis that immune reconstitution plays a role in the pathogenesis of cognitive impairment in patients with LTI. Interventional trials with existing or investigational drugs active against *Toxoplasma* cysts would further strengthen our findings and could lead to new treatments for HAND in people living with LTI.

Notes

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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