The influence of *Strongyloides stercoralis* co-infection on the presentation, pathogenesis and outcome of tuberculous meningitis

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40-word summary: In Vietnamese adults with TBM, active *S. stercoralis* co-infection was significantly associated with reduced intracerebral inflammation, and reduced neurological complications by 3 months, vs. *S. stercoralis* uninfected participants. *S. stercoralis* co-infection may modulate neuroinflammatory response, and improve outcome, in TBM.

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Abstract

Background: Helminth infections may modulate the inflammatory response to *Mycobacterium tuberculosis* and influence disease presentation and outcome. *Strongyloides stercoralis* is common amongst populations with high tuberculosis prevalence. Our aim was to determine if *S. stercoralis* co-infection influenced clinical presentation, cerebrospinal fluid (CSF) inflammation, and outcome from tuberculous meningitis (TBM).

Methods: From June 2017 to December 2019, 668 Vietnamese adults with TBM, enrolled in the ACT HIV or LAST ACT trials (NCT03092817; NCT03100786), underwent pre-treatment *S. stercoralis* testing by serology, stool microscopy, and/or stool PCR. Comparisons of pre-treatment TBM severity, CSF inflammation (including cytokines), and 3-month clinical endpoints were performed in active *S. stercoralis* infected and uninfected groups.

Results: Overall, 9.4% (63/668) participants tested positive for *S. stercoralis*. Active *S. stercoralis* infection was significantly associated with reduced pre-treatment CSF neutrophils (3 cells/mm$^3$[0-25] vs. 14 cells/mm$^3$[1-83], p=0.04), and with reduced CSF IFN-γ, IL-2, and TNF-α concentrations (11.4 vs. 56.0pg/mL p=0.01; 33.1 vs. 54.5pg/mL p=0.03; 4.5 vs. 11.9pg/mL p=0.02, respectively), compared with uninfected participants. Neurological complications by 3 months were significantly reduced in active *S. stercoralis* infection vs. uninfected participants (3.8%[1/26] vs. 30.0%[33/110], respectively, p=0.01).

Conclusions: *S. stercoralis* co-infection may modulate the intracerebral inflammatory response to *M. tuberculosis* and improve TBM clinical outcomes.

Key words: *Strongyloides stercoralis*, tuberculous meningitis, immunomodulation, cytokines, inflammation, outcome
Background

The soil transmitted helminth *Strongyloides stercoralis* causes strongyloidiasis, a neglected chronic parasitic disease of humans. Found throughout tropical and subtropical regions of the world, *S. stercoralis* infects an estimated 30-100 million individuals globally.[1] The geographical distribution of *S. stercoralis* overlaps with that of tuberculosis (TB). Tuberculous meningitis (TBM) is the most severe form of TB, and results in death in almost half of all cases despite effective anti-TB chemotherapy.[2–5] TBM is characterized by intracerebral inflammation which can lead to fatal complications.

Helminth co-infection appears to modulate the host immune response to *Mycobacterium tuberculosis* infection and may increase susceptibility to developing disease (TB) and worsen its severity.[6] Helminth infections typically induce a Th2 immune response, with an IgE antibody class switch, production and activation of eosinophils, mast cell degranulation,[7] and marked elevation of IL-4, IL-5 and IL-13.[6] Th2 responses appear to be cross-inhibitory with the pro-inflammatory Th1 immune responses associated with TB.[8,9] In a case-control study of 40 individuals with pulmonary TB, significantly lower blood IFN-γ levels and a non-significant trend towards more severe disease were found in helminth co-infected individuals compared with helminth uninfected controls.[10] A study of pro-inflammatory cytokines in patients with pulmonary TB (n=88, 42/88 co-infected with *S. stercoralis*) and latent TB (n=88, 44/88 co-infected with *S. stercoralis*) found significantly lower plasma TNF-α, IFN-γ and IL-2 in *S. stercoralis* co-infected individuals, compared with a TB-only control group.[11] In addition plasma concentrations of anti-inflammatory cytokines IL-4, IL-5, IL-10 and IL-13 were significantly elevated in individuals with latent TB and *S. stercoralis* vs. in individuals with latent TB alone.

The intracerebral inflammation of TBM is poorly understood. A Th1 immune response is typical, with phagocytosis, intracellular killing of microbes,[7] and elevated cerebrospinal fluid (CSF) concentrations of pro-inflammatory cytokines[12–14] such as TNF-α and IFN-γ. However, previous
studies have shown substantial heterogeneity in the response, with poor outcomes associated with both excessive and attenuated inflammatory responses.[15–17] The determinants of this heterogeneity are uncertain; host genetic variation in Leukotriene A4 hydrolase (LTA4H) may play a role in some populations,[18,19] but other determinants are likely. Here, we examine the hypothesis that helminth co-infection modulates the intracerebral inflammatory response to M. tuberculosis and thus influences the clinical presentation and outcomes of TBM.

Methods

Participants

We performed a prospective study in Vietnamese adults with TBM to evaluate the frequency and effect of S. stercoralis co-infection on presenting clinical phenotype, CSF inflammatory parameters, CSF cytokine concentrations, and clinical endpoints. Participants were enrolled from two on-going randomised placebo-controlled phase III trials of adjunctive corticosteroid therapy for HIV co-infected and uninfected adults with TBM (ACT HIV; NCT03092817[20], LAST ACT; NCT03100786[21]).

Participants were ≥18 years old, with a diagnosis of TBM based on consistent clinical and CSF findings, with or without HIV co-infection, and admitted to the Hospital for Tropical Diseases (HTD), or Pham Ngoc Thach Hospital for Tuberculosis and Lung Disease, both in Ho Chi Minh City, Vietnam. Patients were excluded if an additional brain infection to TBM was suspected, more than 6 consecutive days of anti-TB chemotherapy or systemic corticosteroid were received, or corticosteroid was mandatory or contraindicated.

Written informed consent was obtained from all participants or from a relative if they were incapacitated. Ethical approvals for ACT HIV and LAST ACT were obtained from the Oxford Tropical Research Ethics Committee (36-16 and 52-16, respectively), the ethical committees of HTD (14/ HDDD and 37/ HDDD, respectively) and Pham Ngoc Thach Hospital for Tuberculosis and Lung Disease (1033/ HDDD-PNT and 460/ HDDD-PNT, respectively), and from the Vietnam Ministry of Health (108/CN-BDGDD and 151/CN-BDGDD, respectively).
Clinical data

Demographic data (age, gender), baseline Modified Research Council (MRC) TBM severity grade[22] and HIV status were recorded. Study participants were followed up for 3 months. Death and neurological complications by 3 months were recorded. Neurological complications were defined as any of a fall in Glasgow coma score (GCS) of ≥ 2 points for ≥ 48 hours, a focal neurological sign, seizure, cerebellar signs, coma, or cerebral herniation.

Laboratory testing

All participants enrolled in this study underwent at least one test for *S. stercoralis* infection. *S. stercoralis* serology (NovaTec Immunodagnostica GmbH, Dietzenbach, Germany) was performed in participants at baseline (date of signing informed consent). Routine wet preparation stool microscopy was performed in participants within 7 days of baseline. Stool *S. stercoralis* PCR testing was performed in a sub-group of participants (those testing positive for *S. stercoralis* by serology or stool microscopy [allowing comparison of diagnostic tests], and in consecutively enrolled participants until a total of 200 PCR tests had been performed). Blood eosinophil count was measured at baseline in all participants.

At least 6mls of lumbar CSF was sampled (if available) at baseline in all participants. CSF processing and testing followed previously described procedures.[23] CSF supernatant was removed and stored at -80°C for future CSF cytokine testing. Cytokines were selected for CSF analysis based on previous CSF cytokine studies in TBM as previously described, cytokines predicted to be affected by *S. stercoralis* co-infection,[11] and availability of testing kits; CSF TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL10, IL-12p70 and IL-13 were measured. CSF cytokine testing was performed by magnetic microbead immunoassay (RnD Systems, Inc, Minnesota, USA) following manufacturer instructions.[24] Cytokine concentrations were measured using a Luminex 200 (Luminex, Texas, USA). The Luminex 200 xPONENT version 3.1.971.0 was used for analysis.
Treatment

All participants received anti-TB chemotherapy following national guidelines. Rifampicin, isoniazid, pyrazinamide and ethambutol were given for at least the first 2 months, if drug resistance was not suspected or proven. Pyrazinamide was stopped after 2 months. At least 12 months anti-TB chemotherapy was received in total. Anti-TB chemotherapy regimens are further described in supplementary material 1. Participants with a positive stool microscopy or PCR test for S. stercoralis received oral ivermectin 200µg/kg daily for 10-14 days, with repeat stool microscopy required to demonstrate absence of S. stercoralis larvae. Participants with positive S. stercoralis serology were treated on a case-by-case basis. Additionally, all participants were randomised to dexamethasone or placebo (termed ‘study drug’), a double blinded allocation following 1:1 randomisation (except LTA4H TT-genotype HIV uninfected participants from LAST ACT [~7% total participants] who all received open-label dexamethasone). Study drug was administered over 6-8 weeks, following a tapering course, with weekly reductions (supplementary table 1). The ACT HIV and LAST ACT trials are ongoing and treatment allocations remain blinded. Permission to publish these study data was obtained by the respective Trial Steering Committees.

Statistical analysis

Primary analysis populations were selected based on clinical categories of S. stercoralis infection. A S. stercoralis ‘uninfected’ group consisted of participants tested by all of S. stercoralis serology, stool microscopy, and stool PCR; with all negative. This approach gave the highest certainty of a S. stercoralis uninfected status. A ‘past infection’ group consisted of participants with positive S. stercoralis serology, with no positive stool result (but at least one of stool microscopy or stool PCR performed). An ‘active infection’ group consisted of participants with positive S. stercoralis stool microscopy or stool PCR, regardless of other testing.

Secondary analyses were performed on two additional sub-populations; participants who had serology performed, and participants who had both serology and stool microscopy performed
(divided into groups A-C) (supplementary tables 2&3). Secondary analyses compared baseline TBM severity, CSF inflammatory parameters, and clinical endpoints between participants with and without positive S. stercoralis tests, for each sub-population. CSF cytokine analysis was performed only for primary analysis populations.

Where CSF cytokine concentrations were undetected, either the lowest limit of extrapolation divided by 2, or the lowest limit of detection divided by two, was used, whichever was lowest. CSF cytokine testing was performed across two 96-well plates, and value extrapolation was plate-specific. Rarely, where cytokine concentrations were too high for quantification, the upper limit of detection multiplied by 2 was used; samples and testing kits were unavailable for sample dilution and repeat testing. Log2 calculations of CSF cytokine concentration were performed. Given the unknown magnitude of immunomodulation by S. stercoralis on CSF cytokine concentrations, a sample size calculation was not possible. The number of CSF samples undergoing CSF cytokine analysis was based upon availability of sample and testing kits and was therefore exploratory.

Comparison between clinical data proportions was assessed by the Chi squared test. CSF cytokine concentrations were compared using the Wilcoxon rank sum test. A multivariate analysis (with odds ratios and 95% confidence intervals [CI]) was performed to evaluate whether age, MRC TBM grade, HIV co-infection and active S. stercoralis infection predicted neurological complications by 3 months.

Data were analysed using R (version 3.6).

Results

The study population

From June 2017 to December 2019 inclusive, 668 participants with TBM underwent baseline testing for S. stercoralis co-infection, by one or more of serology, stool microscopy, and stool PCR. The median age of the study population was 39 (IQR 31-50) years. 67.5% (451/668) participants were male. MRC TBM severity grades[22,25] amongst the study population were, Grade 1: 45.1% (n=301), Grade 2: 43.3% (n=289), Grade 3: 11.7% (n=78). 43.4% (n=290) study participants were diagnosed

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with definite TBM, 38.3% (n=256) with probable TBM, and 16.0% (n=107) with possible TBM. 44.6% (298/668) participants were HIV co-infected.

**S. stercoralis testing**

The total numbers of *S. stercoralis* tests performed are shown in figure 1. Overall, 9.4% (63/668) participants tested positive for *S. stercoralis* by at least one of serology (n=53), stool microscopy (n=11), or stool PCR (n=17) (figure 2). All three diagnostic tests were performed in 141/668 (21.1%) participants. A positive *S. stercoralis* diagnosis was made by stool microscopy alone in 3 participants and by stool PCR alone in 6 participants. The median age of participants testing positive for *S. stercoralis* by any method was 49 (IQR 37-59) years vs. 40 (IQR 32-51) years in participants who tested negative for *S. stercoralis* by serology, stool microscopy, and stool PCR. 55/63 (87.3%) of *S. stercoralis* positive participants were male vs. 75/110 (68.2%) of *S. stercoralis* negative participants. HIV co-infection was present in 16/63 (25.4%) *S. stercoralis* positive participants vs. in 37/110 (33.6%) *S. stercoralis* negative participants.

Influence of *S. stercoralis* infection on TBM presentation and routine CSF parameters

A comparison of baseline TBM severity and routine CSF parameters between primary analysis populations is shown in table 1. Baseline blood eosinophils were significantly elevated in active *S. stercoralis* infection compared with *S. stercoralis* uninfected participants; 0.10 x10⁹/L (0-0.38) vs. 0 x10⁹/L (0-0.10) respectively, (p=0.02). Median CSF neutrophil count and neutrophil percentage were reduced in active *S. stercoralis* infection compared with uninfected participants; 3 cells/mm³ (0-25) vs. 14 cells/mm³ (1-83) respectively, p=0.04, and 5% (0-14) vs. 10% (5-27) respectively (p=0.04).

Additionally, trends towards reduced grade 3 disease (3.8% [1/26] vs. 19.1% [21/110]), reduced total CSF WCC cells/mm³ (70 [7-168] vs. 123 cells/mm³ [29-297]), reduced CSF protein (0.94g/L [0.60-1.84] vs. 1.45g/L [0.95-2.18]), and elevated CSF/blood glucose ratio (0.45 [0.31-0.59] vs. 0.38 [0.26-0.52]), were seen with active *S. stercoralis* infection vs. uninfected participants, respectively. There was a reduced proportion of definite TBM in the active *S. stercoralis* group (19.2% [5/26]) vs. the S.
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Stercoralis uninfected group (52.7% [58/110]) (p=0.01 for definite vs. probable TBM). Additionally, there was reduced Xpert positivity in the active S. stercoralis group (3.8% [1/26]) vs. the S. stercoralis uninfected group (28.2% [31/110]) (p=0.03). There was significantly higher CSF/blood glucose, and significantly lower CSF protein, in the active S. stercoralis group vs. the S. stercoralis uninfected group, in a HIV co-infected subgroup (supplementary table 4).

Baseline TBM severity and CSF inflammatory parameter analyses performed in sub-populations of participants who had serology performed, or both serology and stool microscopy performed, are shown in supplementary tables 2&3. In participants who had S. stercoralis serology performed, HIV co-infection was less common in those with positive S. stercoralis serology compared with those with negative serology (20.8% [11/53] vs. 46.2% [280/606], respectively, p=0.001).

Baseline CSF cytokine concentrations in Strongyloides stercoralis co-infection

We hypothesized that in those with active S. stercoralis infection CSF concentrations of the pro-inflammatory cytokines IFN-γ, IL-2, and TNF-α would be reduced, and CSF concentrations of the regulatory cytokines IL-4, IL-5, IL-10, and IL-13 would be increased, compared with S. stercoralis uninfected participants. These cytokines, in addition to IL-1 β, IL-6 and IL-12p70 (as exploratory analyses) were measured in CSF, and compared between primary analysis populations.

CSF cytokine testing populations are shown in figure 1. CSF concentrations of pro-inflammatory cytokines were significantly reduced in participants with active S. stercoralis infection (n=25), vs. in uninfected participants (n=105); IFN-γ: 11.4 vs. 56.0pg/mL, p=0.01; IL-2: 33.1 vs. 54.5pg/mL, p=0.03; TNF-α: 4.5 vs. 11.9pg/mL, p=0.02; IL-6: 12.2 vs. 655.6pg/mL, p=0.01 (figure 3). Additionally CSF concentrations of IFN-γ, TNF-α, IL-2, but not IL-6, were significantly reduced in participants with past S. stercoralis infection (n=26) vs. in uninfected participants; IFN-γ: 13.8 vs. 56.0pg/mL, p=0.02; IL-2: 28.3 vs. 54.5pg/mL, p=0.03; TNF-α: 4.6 vs. 11.9pg/mL, p=0.02; IL-6: 55.4 vs. 655.6pg/mL, p=0.10.

Contrary to our hypothesis, CSF concentrations of IL-10 and IL-4 were significantly reduced in active S. stercoralis infection vs. in S. stercoralis uninfected participants; IL-10: 6.5 vs. 12.0pg/mL, p=0.004;
IL-4: 4.1 vs. 8.9 pg/mL, p=0.01 (figure 4). Seventy percent of participants had undetectable IL-5 in their CSF samples. In participants with past S. stercoralis infection CSF concentrations of IL-13 were reduced (7.5 vs. 23.9 pg/mL, p=0.03), and CSF concentrations of IL-5 were increased (0.37 vs. 0.37 pg/mL, p=0.02), vs. in uninfected participants. Median cytokine concentrations for S. stercoralis uninfected, past infection and active infection groups, together with ratio of change and statistical comparison between groups, are shown in supplementary table 5. IL-6 concentrations experienced the greatest ratio of reduction, ~54x reduction in active S. stercoralis infection compared with uninfected participants. CSF concentrations of TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-6, and IL-10 were significantly reduced in participants with active S. stercoralis infection, vs. in uninfected participants, in an HIV co-infected subgroup; yet these significant differences were not seen in an HIV uninfected subgroup (supplementary table 6).

S. stercoralis co-infection and outcome from TBM

A comparison of neurological complications by 3 months, and death by 3 months, between primary analysis populations, is shown in table 2. Neurological complications by 3 months were significantly reduced in participants with active S. stercoralis infection (3.8% [1/26]), vs. uninfected participants (30.0% [33/110]) (p=0.01). Neurological complications are listed in supplementary table 7. A fall in GCS ≥2 points for ≥48 hours was the most common neurological complication recorded, accounting for 80% (4/5) neurological complications in past S. stercoralis infection, and 78.8% (26/33) neurological complications in S. stercoralis uninfected participants. In a multivariate logistic regression active S. stercoralis infection was significantly and independently associated with reduced neurological events by 3 months (p=0.01) (supplementary table 8). Death by 3 months was not significantly reduced between active S. stercoralis infection and uninfected groups; 15.4% (4/26) vs. 28.2% (31/110), respectively, p=0.27. Neurological complications by 3 months remained significantly reduced in participants with active S. stercoralis infection vs. uninfected participants in HIV co-infected individuals, but not in those HIV uninfected (supplementary table 9).
Additional secondary sub-population comparisons of neurological complications by 3 months, and of death by 3 months, in participants with and without positive *S. stercoralis* tests, are shown supplementary tables 10&11. In participants who had serology performed (n=659), a reduction in death at 3 months was suggested in those with positive serology; (15.1% [8/53] vs. 25.7% [156/606] respectively, p=0.12). For participants who had both *S. stercoralis* serology and stool microscopy performed, neither neurological events by 3 months, nor death by 3 months, were significantly different between participants with positive *S. stercoralis* serology and positive stool microscopy, positive *S. stercoralis* serology but negative stool microscopy, and participants negative for *S. stercoralis* (supplementary table 13).

**Discussion**

*S. stercoralis* is a neglected tropical infection with a huge global disease burden. The ability of helminths to modulate host immunity is well recognised; however immunomodulation of the intracerebral inflammatory responses associated with TBM has not previously been described. In our study of 686 Vietnamese adults with TBM, active *S. stercoralis* infection was associated with reduced intracerebral inflammation and reduced neurological events by 3 months, compared with *S. stercoralis* uninfected participants. This association was strongest in HIV co-infected participants.

In TBM, intracerebral inflammation manifests as abnormal routine CSF parameters (elevated total WCC, neutrophils, and protein, and reduced glucose) and elevated pro-inflammatory CSF cytokine concentrations.[12,13,26] In our study active *S. stercoralis* infection was associated with significant
reductions in absolute CSF neutrophil count and neutrophil proportion, and non-significant reductions in CSF total WCC, CSF protein, and an increase in CSF/blood glucose ratio. The reduced inflammatory CSF profile in active *S. stercoralis* infection was consistent with the trend towards reduced grade 3 TBM disease in this group, compared with *S. stercoralis* uninfected participants.

Significantly reduced ‘definite’ TBM cases (these require a microbiological confirmation of *M. tuberculosis*) and positive Xpert results in active *S. stercoralis* infection suggest reduced mycobacterial burden in these participants. We speculate that these findings may reflect better host immunological control of TBM disease in the context of *S. stercoralis* infection.

The CSF cytokine analysis further supports a model of reduced intracerebral inflammation in TBM in active *S. stercoralis* co-infection. Pre-treatment CSF cytokine concentration analysis showed significantly reduced concentrations of the pro-inflammatory cytokines IFN-γ, IL-2, and TNF-α, in active *S. stercoralis* co-infection. *S. stercoralis* co-infection in TBM was also associated with significantly reduced CSF IL-4 and IL-10, cytokines associated with a Th2 immune response. The suppression of these cytokines does not fit our prior hypothesis, indicating more work to understand the mechanisms of *S. stercoralis* immunomodulation is needed. Previous data in fact show IL-10 to be elevated in TBM, decreasing after anti-TB chemotherapy.[12,13] Neutrophils highly express IL-4 and IL-10 in *M. tuberculosis* infection;[27] therefore a reduction in CSF IL-4 and IL-10 concentrations in *S. stercoralis* co-infected TBM be may mediated through reduced CSF neutrophils. Interestingly CSF cytokine suppression was greater in HIV co-infected participants than in HIV uninfected participants. HIV co-infection is associated with globally increased CSF cytokines in TBM,[19] yet why helminth co-infection would control CSF cytokines more in the context of HIV co-infection is unknown and a topic for future research.

Our data showed a significant reduction in neurological complications by 3 months in active *S. stercoralis* infection, compared with in *S. stercoralis* uninfected participants. This finding is consistent with the associations observed between *S. stercoralis* co-infection, reduced bacterial burden and
reduced intracerebral inflammation. In our multivariate analysis reduced neurological complications could not be explained by differences in age or HIV co-infection between groups. Elevated CSF neutrophils have been linked to neurological IRIS in TBM HIV co-infection, and death in HIV negative TBM disease.[16,28] Given the known detrimental consequences of excessive intracerebral inflammation to TBM outcomes,[19] it is plausible that reduction of neuroinflammation secondary to helminth downregulation of proinflammatory TBM immune responses reduces neurological complications. Indeed therapies in severe TBM often attempt to suppress excessive host immune responses. Corticosteroids, infliximab and thalidomide have all been either trialled or postulated as immunomodulating therapies in TBM.

This study has limitations. True *S. stercoralis* co-infection in our study population may be higher than described, given not all participants received all of serology, stool microscopy and stool PCR. This resulted in the creation of sub-populations for analysis. Additionally, the performances of diagnostic tests for *S. stercoralis* are sub-optimal. Stool microscopy sensitivity is low (<30%) [29] due to intermittent larval shedding. Stool PCR is more sensitive (~65%) [30], yet *S. stercoralis* co-infection will still be missed. *S. stercoralis* serological tests are affected by reduced sensitivity in advanced immunosuppression[31,32] or persistence of serological positivity despite parasite clearance.[30] In our sub-population where all participants underwent *S. stercoralis* serology testing, *S. stercoralis* serology was less likely to be positive in HIV co-infection, possibly reflecting false negative *S. stercoralis* serology in this group. Follow up in our study was limited to 3 months; longer term impact on neurological complications or death therefore cannot be assessed. Additionally repeat CSF cytokine analysis, to assess immune responses after *S. stercoralis* eradication, were not performed.

Finally, the study drug allocation (dexamethasone or placebo) of the trial participants remains unknown. This will not influence baseline phenotype, or pre-treatment CSF analyses; all of which represent data or sampling prior to study drug administration. Given the randomised study drug allocation (1:1), dexamethasone and placebo are expected to be evenly distributed within each individual analysis population.
The strengths of the study are that it is large and prospective, with careful clinical characterization of TBM and *S. stercoralis* co-infection. This study is part of two clinical trials with precise treatment protocols and standardised testing and data collection procedures. In this study of TBM, CSF is used for routine parameter and cytokine measurement, allowing a study of inflammation at the site of the disease instead of using blood inflammatory changes to assess intracerebral inflammation.

In conclusion, in our study active *S. stercoralis* co-infection in TBM was associated with reduced intracerebral inflammation and reduced neurological events. Further understanding of these immunomodulatory processes may aid the development of novel host directed therapies to manage excessive and damaging inflammation of TBM.
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Footnote

The authors declare no conflicts of interest.

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This work has not been presented at any national or international meetings.

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Table 1: A comparison of baseline TBM severity and CSF inflammatory parameters for cytokine testing groups

|                | S. stercoralis testing |                |                |
|----------------|------------------------|----------------|----------------|
|                | Uninfected | Past infection | Active infection |
| **Patients (No.)** | 110 | 30 | 26 |
| **HIV status (No. [%])** | | | |
| - Positive | 37 (33.6%) | 4 (13.3%) | 0.05 | 9 (34.6%) | 1.0 |
| - Negative | 73 (66.4%) | 26 (86.7%) | | 17 (65.4%) | |
| **Final diagnosis** | | | |
| - Definite | 58 (52.7%) | 9 (30.0%) | Ref | 5 (19.2%) | Ref |
| - Probable | 38 (34.5%) | 14 (46.7%) | 0.11 | 16 (61.5%) | 0.01 |
| - Possible | 13 (11.8%) | 6 (20.0%) | 0.13 | 5 (19.2%) | 0.06 |
| **MRC TBM Grade (No. [%])** | | | |
| - 1 | 44 (40.0%) | 16 (53.3%) | Ref | 13 (50.0%) | Ref |
| - 2 | 45 (40.9%) | 12 (40.0%) | 0.62 | 12 (46.2%) | 1.0 |
### Table: Comparison of Clinical and Laboratory Findings

| Parameter                          | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|-----------------------------------|---------|---------|---------|---------|---------|
| **Baseline eosinophil count (10^9/L)** | 0       | 0.2     | <0.001  | 0.1     | 0.02    |
| (Median[IQR])                     | (0-0.10)| (0.08-0.20)| (0-0.38)|         |         |
| **CSF WBC (cells/mm³)**           | 123     | 74      | 70      |         |         |
| (Median[IQR])                     | (29-297)| (9-254) | (7-168) |         |         |
| **CSF neutrophil count (cells/mm³)** | 14      | 6       | 3       |         |         |
| (Median[IQR])                     | (1-83)  | (0-36)  | (0-25)  |         |         |
| **CSF neutrophil percentage**     | 10      | 11      | 5       |         |         |
| (Median[IQR])                     | (5-27)  | (0-15)  | (0-14)  |         |         |
| **CSF/blood glucose ratio**       | 0.38    | 0.43    | 0.34    | 0.45    | 0.16    |
| (Median[IQR])                     | (0.26-0.52)| (0.33-0.52)| (0.31-0.59) |         |         |
| **CSF protein (g/L)**             | 1.45    | 1.39    | 0.69    | 0.94    | 0.08    |
| (Median[IQR])                     | (0.95-2.18)| (1.13-1.94)| (0.60-1.84) |         |         |
| **Xpert (No. [%])**               |         |         |         |         |         |
| - Positive                        | 31 (28.2%) | 6 (20.0%) | 0.50 | 1 (3.8%) | 0.03 |
| - Negative                        | 68 (61.8%) | 21 (70.0%) | 24 (92.3%) |         |         |

P values are shown for group comparison with *S. stercoralis* uninfected group in each case. The chi-squared test was used to compare categorical data. Ref: with final diagnosis, P values represent...
comparison of each of probable and possible TBM, with definite TBM. Definite, probable and possible TBM labels are allocated based on the published uniform case definition for TBM.[33] With MRC TBM Grade P values represent comparison of each of Grade 2 and Grade 3 TBM, with Grade 1 TBM. With final diagnosis, n=1 (uninfected group) did not have CSF parameters available and could not be classified as definite, probable or possible TBM. With final diagnosis, n=1 (past infection group) participant scored < 6 points for the TBM diagnostic score.[33] Both these cases were considered to be TBM by the treating clinician and were treated as such. The Wilcoxon rank sum test was used to compare continuous data. Uninfected = all 3 testing methods used, and all negative. Past infection = positive S. stercoralis serology with no positive stool testing (but at least one of stool microscopy of stool PCR performed). Active infection = Positive stool microscopy or stool PCR for S. stercoralis, regardless of other testing performed. ‘Ref’ refers to the final diagnosis or grade against which comparison was made.

CSF=Cerebrospinal fluid. HIV=Human immunodeficiency virus. IQR=Interquartile range. MRC=Modified Research Council. TBM=Tuberculous meningitis. WBC=White blood cell. Xpert=GeneXpert MTB/RIF
Table 2: A comparison of neurological complications and death by 3 months for cytokine testing groups

|                          | S. stercoralis testing |               |               |               |               |
|--------------------------|------------------------|---------------|---------------|---------------|---------------|
|                          | Uninfected             | Past infection| Active infection|               |               |
| Patients (No.)           | 110                    | 30            | 26            |               |               |
| Neurological complications by 3 months |               |               |               |               |               |
| - Yes (%)                | 33 (30.0 %)            | 5 (16.7 %)    | 1 (3.8 %)     | 0.22          | 0.01          |
| - No (%)                 | 77 (70.0 %)            | 25 (83.3 %)   | 25 (96.2 %)   |               |               |
| Death by 3 months        |                        |               |               |               |               |
| - Yes (%)                | 31 (28.2 %)            | 5 (16.7 %)    | 4 (15.4 %)    | 0.30          | 0.27          |
| - No (%)                 | 79 (71.8 %)            | 25 (83.3 %)   | 22 (84.6 %)   |               |               |

P values are shown for group comparison with S. stercoralis uninfected group in each case. The chi-squared test was used to compare categorical data. Uninfected = all 3 testing methods used, and all negative. Past infection = positive S. stercoralis serology with no positive stool testing (but at least one of stool microscopy of stool PCR performed). Active infection = Positive stool microscopy or stool PCR for S. stercoralis, regardless of other testing performed.
Figure 1: *S. stercoralis* testing populations

**S. stercoralis** tests performed

- Tested by serology, stool microscopy or stool PCR (N=668) → subgroup
- Serology performed (N=659) → subgroup
- Serology and stool microscopy performed (N=523) → subgroup
- Serology and stool microscopy and stool PCR performed (N=141)

**Primary analysis populations**

- N=166
  - Past *S. stercoralis* infection (N=30)
  - Active *S. stercoralis* infection (N=26)
  - *S. stercoralis* uninfected (N=110)
    - Variable *S. stercoralis* tests performed
    - All three *S. stercoralis* tests performed

**Cytokine testing populations**

- N=163 *
  - Past *S. stercoralis* infection (N=26)
  - Active *S. stercoralis* infection (N=25)
  - Active *S. stercoralis* infection (N=105)
  - Other status # (N=7)
A total of 668 participants underwent at least one *S. stercoralis* test. 659 participants had serology performed. 523 participants had serology and stool microscopy performed. 141 participants had serology and stool microscopy and stool PCR performed. Following the blue arrows, each group is a subgroup of the previous group. Red arrows show how primary analysis populations were developed. All *S. stercoralis* uninfected participants had all of serology, stool microscopy, and stool PCR performed. Past *S. stercoralis* infection and active *S. stercoralis* infection groups were selected independently of the number of *S. stercoralis* tests performed; therefore these are taken from the population where at least one *S. stercoralis* test was performed (N=668). Green arrows show how cytokine testing populations were formed. *173 patients were initially eligible for cytokine testing. Of these, 4 samples were excluded from testing when no stored CSF sample was available. 5 samples were not tested as *S. stercoralis* tests returned a positive result after cytokine testing had been arranged and set up. A computer error during the cytokine analysis led to loss of one sample result. 163 CSF samples therefore underwent cytokine testing, of which samples from 156 participants fitted into primary analysis population (uninfected, past infection, active infection) definitions. *Other status* refers to participants who underwent cytokine testing but did not fit into any of the three primary analysis population categories. Uninfected = all 3 testing methods used, and all negative. Past infection = positive *S. stercoralis* serology with no positive stool testing (but at least one of stool microscopy or stool PCR performed). Active infection = positive stool microscopy or stool PCR for *S. stercoralis*, regardless of other testing performed. Other=not meeting criteria for the uninfected, past infection, or active infection, groups. CSF=cerebrospinal fluid. N=number of participants undergoing testing.
Figure 2: Venn diagram of positive S. stercoralis tests

Positive tests: 53 serology, 11 stool microscopy, 17 stool PCR. 81 positive S. stercoralis tests represented in 63 participants testing positive for S. stercoralis by serology, stool microscopy, stool PCR or combinations of these tests. These 63 participants include the ‘past infection’ (N=30) and ‘active infection’ (N=26) primary analysis populations, plus participants with a positive S. stercoralis test not meeting either of these definitions.

PCR = Polymerase chain reaction
Figure 3: Log2 CSF IFN-γ, IL-2 and TNF-α concentrations in participants uninfected with *S. stercoralis*, with past *S. stercoralis* infection, or with active *S. stercoralis* infection.

Log2 cytokine concentrations (15, 10, 5, 0, -5) correspond with measured cytokine concentrations in pg/mL as follows; 32768, 1024, 32, 1, 0.03, respectively. For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is...
within 1.5x the vertical height of the box. Dots represent individual data points. Uninfected = all 3 testing methods used, and all negative. Past infection = positive S. stercoralis serology with no positive stool testing (but at least one of stool microscopy of stool PCR performed). Active infection = Positive stool microscopy or stool PCR for S. stercoralis, regardless of other testing performed. Statistical comparison of cytokine concentrations was performed by the Wilcoxon rank sum test.
Figure 4: Log2 CSF IL-4, IL-10, and IL-13 concentrations in participants uninfected with *S. stercoralis*, with past *S. stercoralis* infection, or with active *S. stercoralis* infection

For each individual boxplot, the central horizontal bar represents the median value. The box contains data between 3rd quartile (upper end of box) and 1st quartile (lower end of box). Vertical lines above and below each box extend to the most extreme data point that is within 1.5x the
vertical height of the box. Dots represent individual data points. Uninfected = all 3 testing methods used, and all negative. Past infection = positive *S. stercoralis* serology with no positive stool testing (but at least one of stool microscopy or stool PCR performed). Active infection = Positive stool microscopy or stool PCR for *S. stercoralis*, regardless of other testing performed. Cytokine concentrations are shown in pg/mL. Statistical comparison of cytokine concentrations was performed by the Wilcoxon rank sum test.
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