under-indication of the predictive values, and these values might actually be very different in high-risk groups with different criteria.

It is regrettable that the opportunity to do sequential tests of immune responses during follow-up was missed. Dynamic changes in immune markers might reveal better indicators for disease emerging from latent infection. It has been shown that early CD4 T-cell activation was correlated with tuberculosis disease risk after infection,[7] and we found that the expression of KLRG1, a marker of terminally differentiated T cells, was increased in patients with tuberculosis.[8] Therefore, further studies combining assays of defined immune markers with TST, IGRAs, or ESAT-6–CFP10 skin test might reveal a better method to predict progression to disease.

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1 Abubakar I, Drobniewski F, Southern J, et al. Prognostic value of interferon-gamma release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study. *Lancet Infect Dis* 2018; **18:** 1077–87.

2 Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. *PLoS Med* 2016; **13:** e1002152.

3 Barry CR, Boshoff HI, Dartois V, et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat Rev Microbiol* 2009; **7:** 845–55.

4 Menzies D, Adjobimey M, Ruslami R, et al. Four months of rifampin or nine months of isoniazid for latent tuberculosis in adults. *N Engl J Med* 2018; **379:** 440–53.

5 Li F, Xu M, Qin C, et al. Recombinant fusion ESAT6-CFP10 immunogen as a skin test reagent for tuberculosis diagnosis: an open-label, randomized, two-centre phase 2a clinical trial. *Clin Microbiol Infect* 2016; **22:** 889.e9–16.

6 Li F, Xu M, Zhou L, et al. Safety of recombinant fusion protein ESAT6-CFP10 as a skin test reagent for tuberculosis diagnosis: an open-label, randomized, single-center phase I clinical trial. *Clin Vaccine Immunol* 2016; **23:** 767–73.

7 Fletcher HA, Snowden MA, Landry B, et al. T-cell activation is an immune correlate of risk in BCG vaccinated infants. *Nat Commun* 2016; **7:** 11290.

8 Hu Z, Zhao HM, Li CL, et al. The Role of KLRG1 in human CD4+ T-cell immunity against tuberculosis. *J Infect Dis* 2018; **217:** 1491–503.

Treatment recommendations for trichomoniasis in women

Multidose metronidazole has been found to be superior to single-dose metronidazole for the treatment of trichomoniasis in a randomised controlled trial done in women with HIV infection,[1] in a meta-analysis of published comparisons,[2] and in our randomised controlled trial done in women without HIV infection, published in *The Lancet Infectious Diseases*.[3] The abundance of data indicates that multidose metronidazole should be the first line of treatment for trichomoniasis in women.

Zhen-Zhou Luo and colleagues[4] suggest that false positives from nucleic acid amplification tests (NAAT), re-infection, and non-adherence to treatment could have influenced the results of our study,[3] and suggest that more data are needed before changing treatment recommendations to multidose metronidazole. We disagree. In addition to our intention-to-treat analysis, we did numerous sensitivity analyses that showed that the findings of multidose metronidazole superiority were robust. In one of the analyses, we used culture alone as the outcome, to remove possible misclassifications that could occur from detection of remnant RNA during NAAT, and these findings corroborated our intention-to-treat analysis. Additionally, no test of cure was done before 3 weeks,[5] therefore we do not think that the use of NAAT as an outcome biased our results.

Regarding sexual exposure and medication non-adherence, 33·5% of women reported having sex between enrolment and test of cure, and 2·6% reported not taking all of the medication. Of 540 women followed, 137 (25·4%) had sex during follow-up with a partner they had at baseline, 33 (6·1%) with a new partner, and 15 (2·8%) with a partner from baseline and a new partner. There was no difference between groups in sexual exposure during follow-up (86 [31·9%] of 270 women in the single-dose group *vs* 95 [35·2%] of 270 in the multidose group; p=0·412). Unsurprisingly, women in the single-dose group were more likely to adhere to their medication (264 [99·2%] of 266 women) than those in the multidose group (253 [95·5%] of 265; p=0·007). However, when re-examining our data to remove those who had sex with any partner during follow-up and who did not adhere to their medication, we still found that multidose remained superior (37 [20·8%] of 179 women in the multidose group *vs* 16 [9·8%] of 164 in the single-dose group; relative risk 1·70, 95% CI 1·11–2·59; p=0·005]. Earlier data suggest that most repeat infections were due to treatment failure and not reinfection.[6] Sexual activity with an untreated partner could be driving high re-infection rates post-treatment, though it is unlikely that this occurrence explains the consistent finding of multidose superiority.

We agree that the high rate of test-of-cure positives, even among those receiving multidose metronidazole (not explained by organism in-susceptibility), is concerning and a better understanding of factors that interfere with metronidazole treatment and development of more efficacious low-cost treatments for trichomoniasis are needed.

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1 Kissinger P, Mena L, Levison J, et al. A randomized treatment trial: single versus 7-day dose of metronidazole for the treatment of *Trichomonas vaginalis* among HIV-infected women. *J Acquir Immune Defic Syndr* 2010; **55:** 565–71.
2 Howe K, Kissinger PJ. Single-dose compared with multidose metronidazole for the treatment of trichomoniasis in women: a meta-analysis. *Sex Transm Dis* 2017; **44:** 29–34.
3 Kissinger P, Muzny CA, Mena LA, et al. Single-dose versus 7-day-dose metronidazole for the treatment of trichomoniasis in women: an open-label, randomised controlled trial. *Lancet Infect Dis* 2018; **18:** 1251–59.
4 Luo Z-Z, Cai Y-M, Chen X-S. New treatment recommendations for trichomoniasis in women. *Lancet Infect Dis* 2018; **18:** 1307–08.
5 Williams JA, Ofner S, Batteiger BE, Fortenberry JD, Van Der Pol B. Duration of polymerase chain reaction-detectable DNA after treatment of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* infections in women. *Sex Transm Dis* 2014; **41:** 215–19.
6 Kissinger P, Secor WE, Leichliter JS, et al. Early repeated infections with *Trichomonas vaginalis* among HIV-positive and HIV-negative women. *Clin Infect Dis* 2008; **46:** 994–99.

Onchocerciasis-associated epilepsy

In the context of the ongoing debate about the degree of responsibility of *Onchocerca volvulus* in human cases of epilepsy, Cédric Chesnais and colleagues support a relationship between *O volvulus* microfilariae and the development of epilepsy.[1] Regarding the causes that could trigger onchocerciasis-associated epilepsy, three mechanisms have been proposed: microfilariae present in the central nervous system, the human immune response, and sleep deprivation due to intense itching.[2] Different pathogenic capabilities of *O volvulus* strains, general concomitant viral, bacterial, or parasitic infections, genetic variation in how the body produces antibodies, and measles infection followed by malnutrition have also been considered as possible causes of onchocerciasis-associated epilepsy.[1–3]

Surprisingly, the major cause of pathogenicity associated with onchocerciasis, *Wolbachia* spp, the endosymbiont bacteria of *O volvulus*, has not been considered as a potential trigger of onchocerciasis-associated epilepsy. The worm might not be the direct culprit of the neurological disorders, but only the accomplice for harbouring the bacteria that, when released, could be a potential triggering factor for epilepsy. If onchocerciasis-associated epilepsy is mainly caused by the *Wolbachia* spp, then the virulence could be related to the different wolbachia supergroups or to bacterial burdens present in different *O volvulus* populations,[4] similar to what occurs in blindness caused by *O volvulus*, which depends on the wolbachia burden.

The hallmark of filarial pathogenesis is the inflammatory response of the host, provoked by the death of the parasite and the subsequent release of wolbachia. Extracts from *Brugia malayi* and *O volvulus* infected with *Wolbachia* spp induce inflammation, whereas extracts of rodent filariasis devoid of *Wolbachia* spp did not induce inflammation in rodent models.[5] In humans and in murine models, the release of bacteria has been shown to be associated to the upregulation of proinflammatory cytokines, such as tumour necrosis factor, and neutrophil recruitment.[6] Indeed, the immune system and its associated inflammatory reactions seem to have an important role in epileptogenesis.[7]

PCR tests done in cerebrospinal fluid (CSF) samples of patients with onchocerciasis-associated epilepsy did not identify *O volvulus* DNA so far. Considering that *Wolbachia* spp have a somatic tissue tropism able to invade the CNS (intracellularly and extracellularly),[8] the presence of *Wolbachia* spp DNA should be investigated in the CSF of patients with epilepsy.

There are contradictory results regarding the effectiveness of ivermectin in the decrease of onchocerciasis-associated epilepsy in other study areas.[9] If *Wolbachia* spp were indeed an epileptogenetic factor, larger loads of bacteria released after treatments were done during an onchocerciasis control programme could explain the unknown reason why epilepsy suddenly appeared in a region where onchocerciasis is likely to have been common for centuries.[3]

To confirm the role of *Wolbachia* spp in onchocerciasis-associated epilepsy, anti-wolbachia treatment should be administered in affected areas, therefore preventing the release of bacteria and the subsequent consequences that might ensue after treatment with anti-microfilarial drugs in microfilaria populations with high wolbachia burden.

A first step to ascertain the role of *Wolbachia* spp in onchocerciasis-associated epilepsy is being taken in Uganda by a study being done in 2016–19.[4] Therefore, unless the results of this study confirm otherwise, it seems opportune to consider *Wolbachia* spp as a potential cause of onchocerciasis-associated epilepsy.

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1 Chesnais CB, Nana-Djeunga HC, Njamnshi AK, et al. The temporal relationship between onchocerciasis and epilepsy: a population-based cohort study. *Lancet Infect Dis* 2018; **18:** 1278–86.
2 Vezzani A, Fujinami RS, White HS, et al. Infections, inflammation and epilepsy. *Acta Neuropathol* 2016; **131:** 211–34.
3 Vogel G. Parasitic worm may trigger mystery nodding syndrome. *Science* 2017; **355:** 678.
4 Idro R, Opar B, Wamala J, et al. Is nodding syndrome an *Onchocerca volvulus*-induced neuroinflammatory disorder? Uganda's story of research in understanding the disease. *Int J Infect Dis* 2016; **45:** 112–17.
5 Dreyer G, Noroes J, Figuerdo-Silva J. New insights into the natural history and pathology of bancroftian filariasis: implications for clinical management and filariasis control programmes. *Trans R Soc Trop Med Hyg* 2000; **94:** 594–96.