Reply to Gupta-Wright et al

We thank Gupta-Wright et al for their insightful comments on our article and enthusiastically echo many of their points [1, 2]. Indeed, we share their concern regarding the difficulty of ruling out tuberculosis coinfection among patients with confirmed disseminated nontuberculous mycobacterial (NTM) infection who have a positive Determine TB-LAM lateral flow assay (LF-LAM) result. For that reason, in our high-burden tuberculosis setting, it is our policy to always err on the side of initially treating such patients for both infections while continuing to search for definitive evidence of tuberculosis coinfection.

However, unless the possibility that disseminated NTM infection could cause positive LF-LAM results is appreciated, patients who may only have NTM infection could be mistakenly diagnosed and treated for tuberculosis alone with therapy that is likely to prove inadequate. The high mortality rate (23%) in our study of patients at a tertiary referral hospital underscores the importance of quickly and correctly identifying this important subset of patients. Thus, while we agree that a positive LF-LAM should continue to be presumed indicative of disseminated tuberculosis until proven otherwise in high-burden tuberculosis settings, clinicians should remain vigilant in confirming the diagnosis among patients who are at risk for disseminated NTM infection.

Gupta-Wright et al argue that the clinical impact of LF-LAM’s potential inability to distinguish between mycobacterial species should be viewed in the context of the relative incidence of tuberculosis and disseminated NTM infection in human immunodeficiency virus (HIV)-infected patients. While we fully agree, the gap between the prevalence of tuberculosis and NTM infection in clinical practice may not be as wide as implied by the evidence they cite, for 3 reasons. First, the prevalence of disseminated NTM disease in HIV-infected patients in sub-Saharan Africa is largely unknown, is likely to be geographically heterogeneous, and may be higher in many areas than generally appreciated. For instance, in a study of patients from Johannesburg, South Africa, who presented with symptoms of tuberculosis, 12% of HIV-infected individuals were shown to have disseminated Mycobacterium avium complex (MAC) infection [3]. Disseminated MAC also accounted for 10% of hospitalized patients with CD4 counts <100 cells/µL in another South African study [4]. Likewise, in our own hospital, well into the era of antiretroviral therapy, NTM disease still accounts for more than 12% of positive mycobacterial blood cultures. Second, a comparison of the prevalence of tuberculosis and disseminated NTM infection is in any case a poor guide to the proportion of NTM disease seen in LF-LAM–positive cases, specifically. Patients with a positive LF-LAM represent a select subgroup of HIV-infected patients with very low CD4 counts, and the burden of NTM disease can be expected to be relatively higher in this population accordingly. Finally, the order where LF-LAM is placed within the tuberculosis diagnostic algorithm can impact the proportion of LF-LAM–positive cases attributable to NTM infection. When LF-LAM is used as an add-on test if initial samples for tuberculosis molecular testing are negative or unobtainable, the resulting referral bias will further inflate the proportion of disseminated NTM cases seen. This seems to be the case at our hospital, where 10% of the positive LF-LAM cases that had a mycobacterial blood culture subsequently cultured an NTM. The majority (88%) of these patients had no evidence of tuberculosis coinfection, and thus further demonstrate the importance of continuing to investigate for disseminated NTM disease in LF-LAM–positive patients at risk of this condition.

Note

Potential conflicts of interest. All authors: No reported conflicts of interest. 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.

References


The Critical Importance of Sampling Fraction to Inferences of Mycobacterium tuberculosis Transmission

To the Editor—In the study by Manson et al [1], whole-genome sequencing was performed on 223 Mycobacterium tuberculosis isolates from the Tiruvallur and Madurai districts of India. They subsequently examined local strain diversity and mutations associated with phenotypic drug resistance in these regions. In this important study, the authors show that lists of published resistance mutations (including [2–4]) have lower positive predictive values for phenotypic resistance in this context; lineages 1 and 3 predominate in India [1], but these published mutations were largely identified using strains from different M. tuberculosis lineages. This highlights a key obstacle to the implementation of
genomics for resistance prediction in India and potentially other endemic regions with diverse lineages of *M. tuberculosis*. By extension, this study emphasizes the critical need to collect strains and categorize the mutations circulating in these regions to better inform such predictions.

Although we reiterate the importance of this work, we have some considerations that warrant further attention. First, we note that the authors apply a threshold of 10 single nucleotide polymorphisms (SNPs) distance for “recent transmission,” stating this threshold was derived in previous publications. Given this, they then conclude that transmission “was occurring among patients from the same and not different regions.” We would argue that this inference cannot be made from the data available in this study. The sampling fraction, which corresponds to the proportion of total cases included in the study, is an essential (yet often overlooked) consideration in genomic epidemiology. Previous studies have shown that, as sampling fraction decreases, clustering is underestimated [5, 6]. With a low sampling fraction, numerous potential transmission events may be missed due to failure to observe source or secondary cases. When making inferences about transmission in genomic epidemiology (or deciding which inferences should be made), this is therefore a critical consideration [7]. In the Manson et al. study, the authors included samples from 196 unique patients from 2 districts of India that were collected over a 6-year period. Because India accounts for >2 million cases of tuberculosis per year [8], the Manson et al. study clearly includes only a small proportion of the total cases that would have been diagnosed in this time. We therefore argue that transmission between districts cannot and should not be excluded. To do so not only sends a potentially erroneous message to regional public health units but also risks promoting a “silence effect,” wherein public health officials within regions overlook risk factors for transmission beyond their administrative borders, which may ultimately prove detrimental to tuberculosis control in India and elsewhere.

We would also caution about the general application of SNP thresholds derived from external studies. Although such thresholds are useful from a public health perspective, it is important to note that their sensitivity and specificity for transmission often depends on local strain diversity (eg, [9]) and may not be readily transferrable across settings. We agree that ≤10 SNPs distance does suggest a close genetic relationship; however, it is important to keep in mind that direct person-to-person transmission cannot be ruled in absent more detailed epidemiologic and contextual data.

**Notes**

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**References**


**Reply to Lee and Howden**

To the Editor—We thank Dr Lee and Prof Howden for their letter and for giving us an opportunity to better articulate our interpretation of findings, especially with respect to transmission of *Mycobacterium tuberculosis* among patients within 2 southern Indian districts. Based on analysis of single nucleotide polymorphism (SNP) differences between 223 *M. tuberculosis* strains from 196 patients within the Thiruvallur and Madurai districts of Tamil Nadu, we report recent intradistrict, but no recent interdistrict, transmission of strains among patients. In drawing these conclusions, we limited our interpretation to the data available to us, which showed that the closest SNP distance between *M. tuberculosis* isolated from patients in different districts was 85 SNPs, which is substantially higher than the very small numbers of SNPs (as few as 0) observed when comparing isolates from patients treated in the same district and treatment center.

However, the absence of highly related *M. tuberculosis* between the 2 districts (Madurai and Thiruvallur) does not exclude the possibility of interdistrict transmission. We fully agree that our sample size was extremely small relative to the number of isolates circulating in either studied region and that a larger sample size could...