Reviewer’s report

Title: Infection with pulmonary nontuberculous mycobacteria affects performance of tuberculosis lipoarabinomannan point-of-care test: Experiences from the Danish Cystic Fibrosis Cohort Study

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Reviewer: Stephen Lawn

Reviewer’s report:

There are increasing data emerging on the utility of the Determine TB-LAM assay for the diagnosis of HIV-associated TB in sub-Saharan Africa. The potential for NTM disease in HIV+ patients to affect assay specificity has been questioned. However, there have previously been no published reports on urine LAM detection in patients with confirmed NTM disease. The study by Qvist, although in a very different study population, is therefore important and interesting.

The study is well conducted and the results clearly reported. The cohort is well characterized. Multiple sampling and thorough investigation was used to define those with and without NTM disease. These data are of value to the field. However, I think that the authors need to significantly modify the conclusions and inferences that they draw from these data as discussed below.

Major modifications:

1. Reading the test strips. The authors appropriately assessed the LAM status using more than one cut-off on the reference card. However, they must state more precisely how the test strips were read. The product insert instructions for reading the test strips were previously very vague until they were modified in 2014. Consensus definitions for LAM-positivity were published last year: (Lawn SD, Dheda K, Kerkhoff AD, Peter J, Dorman S, Boehme CC, Nicol M. Determine TB-LAM lateral flow urine antigen assay for HIV-associated tuberculosis: recommendations on the design and reporting of clinical studies. BMC Infect Dis 2013; 13: 407). When discussing among investigators of previous studies, it was clear that test strips had been read in different ways. Some had interpreted any band as positive regardless of intensity. Others had required that the test band be at least as intense as the reference band to score positive at that level. Others scored the band of most similar intensity. This, in part, explains some of the considerable heterogeneity in the specificity reported in the literature. Thus it is essential for the authors to describe exactly how the bands were interpreted. To optimize specificity and ease of reading the test strips, we recommended in this publication that bands should only be scored as positive if the test band was at least as intense as grade 2.

2. Relevance of grade 1 vs grade 2 bands. Following the above publication, the manufacturers from early 2014 have now modified the assay such that the grade 1 band has been removed. Thus the authors’ data on grade 2 has little relevance.
with respect to the use of this assay in the future. The manuscript should be reinterpreted in this light.

3. Main findings. In light of the points I have made above, the authors key finding is that only 2 of 23 (95%CI, 8.7%; 1.3-28.1%) patients with NTM disease were urine LAM positive (grade 2). This is actually a very low rate of cross-reactivity. The overwhelming proportion of mycobacterial disease in HIV+ patients in Africa is TB and not NTM (see comments below). Thus, these data are reassuring rather than concerning with regard to the PPV of a positive LAM result in Africa. I think that this is a really important finding and I would encourage the authors to reinterpret their findings accordingly.

4. Figure 1. I would amend this to show the numbers LAM+ using grade 2 (and show grade 1 results in parentheses).

5. Title and conclusions. The authors have studied the use of Determine TB-LAM for diagnosis of NTM in HIV-neg patients with pulmonary NTM disease in Europe. They should take care not to over-interpret these data and conclude that these data have major implications for the use of the assay for diagnosis of TB in HIV-infected patients in high TB burden settings. This is a discussion point. However, it does not seem appropriate that it is stated that NTM ‘affects the performance’ of a TB diagnostic assay. The title should not be interpretive in this way, but rather state what the authors have actually done.

6. Burden of NTM disease in Africa. I think the authors should carefully review and cite the post-mortem studies done among HIV+ individuals in Africa. The burden of TB, much of which is undiagnosed at the time of death, is extraordinary (present in 32%-67% of deaths). Evidence of NTM disease is comparatively very rare. Thus, the problem in Africa is overwhelmingly TB and not NTM. I cannot understand the paper from Abidjan by Bonard et al AIDS 2004 which the authors cite. Sebastian Lucas’ post mortem studies from the same setting did not find NTM disease. In my own studies reported at CROI 2014, 427 HIV+ medical admissions in South Africa had 2,391 samples cultured (including blood cultures from all). Not a single patient had NTM disease. 32.6% had M. tuberculosis. So I think the authors should clarify the relative importance of NTM disease and TB in high HIV+ burden settings where the urine LAM assay will likely be used. At present they overemphasize the importance of NTMs although this is not supported by the breadth of literature available.

7. “Grave consequences for patients who receive inappropriate treatment”. This statement appears in both the abstract and final conclusions. I disagree. As stated above, the major problem in resource-limited settings (especially sub-Saharan Africa) is under-diagnosis of TB in HIV+ patients. Undiagnosed TB and lack of treatment is a primary driver of mortality. This is so much the case that there are several ongoing trials of empiric TB treatment among HIV+ patients in Africa. NTM disease is rare and if it were diagnosed, the appropriate drugs in many settings would not be available. I think the discussion and conclusions should be tempered accordingly.
Minor Essential Revisions
8. Line 129. References should be [12,23] and not [12-23].
9. Line 137. These patients had NTM cultured from sputum but were not investigated to confirm true NTM disease.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
'I declare that I have no competing interests'