Title: *New molecular RNA-based test simplifies diagnosis and assessment of the efficacy of anti-tuberculosis medicine*

Key findings: In a study evaluating the accuracy of a new molecular RNA-based test, tuberculosis Molecular Bacterial Load Assay (TB-MBLA) in Kampala, Uganda, we found that:

- TB-MBLA accurately detected and quantified tuberculosis (TB) bacteria in patients before and during treatment, thereby providing advantage over current standard-of-care tests.
- Not all TB patients require 6-month treatment course because TB-MBLA showed that irrespective of pre-treatment bacterial load, a proportion of patients who were positive before start of treatment tested negative (no TB) at week two of treatment and remained so for the rest of the 6-month treatment period.
- Current standard-of-care Smear microscopy and GeneXpert MTB/RIF Ultra are unsuitable for monitoring response to TB treatment (measuring efficacy of anti-TB medicine) because they cannot distinguish between TB bacteria that are killed- or not yet killed by the anti-TB medicine.
- Two-month smear microscopy result is inaccurate and may be insufficient to inform extension of the intensive TB treatment phase.

Recommendation: Support operational research to accelerate adoption of TB-MBLA by the National Tuberculosis and Leprosy Programme for the benefit of TB patients in Uganda.

Background: The global and national TB case detection, treatment success and case notification rates are still lower than the expected targets, in part, because of the less effective diagnostic tools currently in use[1]. Drug resistant TB is increasing partly because of patients overstaying on inappropriate medication or being discharged prematurely before TB bacteria are cleared. Current standard-of-care tests are either inaccurate, failing to distinguish live from dead bacteria or take long for results to be realised. Therefore, the need for tests that can accurately assess efficacy of anti-TB treatment and give timely results to guide doctors and nurses on how to manage the patients cannot be more emphasised. Following several multi-site evaluations, the WHO recognised TB-molecular bacterial load assay (TB-MBLA) as a potential laboratory test[2] which may replace smear microscopy and culture for monitoring response to TB treatment [3]. TB-MBLA is a new RNA-based diagnostic tool that detects and rapidly quantifies live TB bacteria in patient sample, and measures whether the anti-TB medicine is killing these bacteria or not as the patient progresses on treatment.

References:
1. Global Tuberculosis Report 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
2. World Health Organization. Global tuberculosis report 2018. World Health Organization; 2018.
3. Honeyborne I, Mtafya B, Phillips PP, Hoelscher M, Ntinginya EN, Kohlenberg A, et al. (2014) The molecular bacterial load assay replaces solid culture for measuring early bactericidal response to anti-tuberculosis treatment. J Clin Microbiol. 52: 3064-7.

With funds from the Government of Uganda through Makerere University Research and Innovations Fund (MakRIF), and the University of St Andrews (UK), researchers at the Department of Biochemistry & Sports Science, College of Natural Sciences and colleagues from the College of Health Sciences, Makerere University in collaboration with researchers at University of St Andrews, evaluated the accuracy and usefulness of the TB-MBLA in comparison with the current standard-of-care tests.

Methodology: The study was conducted at the Uganda-China friendship Hospital, Naguru Kampala and had two arms: A cross sectional arm to evaluate diagnostic accuracy of TB-MBLA among presumptive cases of TB and longitudinal arm to assess accuracy of TB-MBLA in measuring treatment response. In each arm, each consented participant provided three sputum samples which were pooled together and tested for TB using TB-MBLA in comparison to smear microscopy and GeneXpert MTB/RIF Ultra. MGIT liquid culture was used as the reference test to determine the sensitivity, specificity, positive and negative predictive values of TB-MBLA vis-à-vis smear microscopy and GeneXpert MTB/RIF Ultra. Participants who tested positive for TB using GeneXpert MTB/RIF Ultra were enrolled into a longitudinal treatment arm and assessed for treatment response at 14 days-, month two-, four- and six- of TB treatment. Treatment response was measured as either fall in number of TB bacteria measured by TB-MBLA or change from positive to negative by all tests including smear microscopy, GeneXpert MTB/RIF Ultra and culture.

Findings:

Diagnostic accuracy among 210 presumptive cases of TB:

- The sensitivity of TB-MBLA was 99%, like GeneXpert MTB/RIF Ultra but higher than 75% for smear microscopy.
- TB-MBLA specificity was 91%, compared to 78% and 98% of GeneXpert MTB/RIF Ultra and smear microscopy, respectively.
- In a sub-population of smear microscopy-negative and HIV-positive participants, TB-MBLA sensitivity was 92%.

Treatment response measuring accuracy among 129 TB positive cases:

- Positivity for TB dropped with treatment in all tests, but the rate was slower with GeneXpert MTB/RIF. Consequently, 33% of participants were still Xpert-Ultra positive at the end of treatment but were clinically well at 3-months post-treatment (*Figure 1*).
- TB-MBLA remained specific at 93% as patient TB bacillary load reduced and was consistent with the gold standard test, MGIT culture at 94% agreement.
- TB-MBLA-measured bacillary load, among month-2 SM-positive cases who received extra month of intensive treatment was not different from SM negative cases.

References:

1. Global Tuberculosis Report 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
2. World Health Organization. Global tuberculosis report 2018. World Health Organization; 2018.
3. Honeyborne I, Mtafya B, Phillips PP, Hoelscher M, Ntinginya EN, Kohlenberg A, et al. (2014) The molecular bacterial load assay replaces solid culture for measuring early bactericidal response to anti-tuberculosis treatment. J Clin Microbiol. 52: 3064-7.

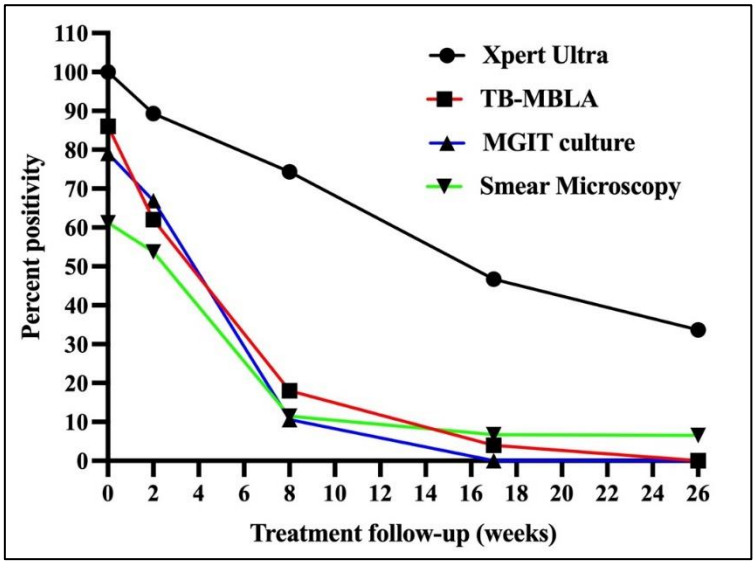

Figure 1: Change in proportion of patients who tested TB positive by TB-MBLA (red curve) compared to smear microscopy (green curve), GeneXpert MTB/RIF Ultra (black curve) and MGIT culture (blue curve) before- and during- treatment.

Implications of the study findings:

- GeneXpert MTB/RIF Ultra is a sensitive tool, suitable for pre-treatment diagnosis of TB, but not for treatment response monitoring.
- Smear microscopy is cheaper, but it is less sensitive, and quite subjective in result interpretation, making it less reliable in measuring treatment response.
- TB-MBLA closely mirrored MGIT culture (gold standard TB test) in measuring treatment response. Since it takes shorter time (four hours) to get results from the time of sample collection, TB-MBLA provides a rapid method for monitoring response to TB treatment.
- Clinical signs disappear faster or stay longer than bacteriological positivity, yet culture underestimates patient TB bacterial burden and the results take too long to come out which delays clinical decisions.
- DNA based molecular tests such as GeneXpert MTB/RIF Ultra overestimate patient TB bacterial burden during treatment which may lead to unnecessary prolonged treatment. RNA based molecular tests like TB-MBLA may be the answer.

References:
1. Global Tuberculosis Report 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
2. World Health Organization. Global tuberculosis report 2018. World Health Organization; 2018.
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Way forward: To date, TB-MBLA technology has evolved through a series of developmental levels (*Figure 2*). The next step will focus on evaluating TB-MBLA in the operational environment. The feedback from such experiments will guide, modify, or simplify technology followed by undergoing thorough standard manufacturing to ensure GMP compliance. This will be followed by Technology Readiness level (TRL) 8 and 9 – regulatory approval & licensing and subsequently adoption by the National TB Programmes for application in routine management of tuberculosis.

Figure 2: TB-MBLA is at technology readiness level 7.
Figure adapted from TWI Global. (https://www.twi-global.com/technical-knowledge/faqs/technology-readiness-levels)

What were the immediate benefits of the study to Uganda?

During this study, human resource capacity to adopt the technology at Makerere University was developed. Researchers at MSc and PhD levels were trained and equipped with knowledge to perform this assay (*Figure 3*). The trained personnel will not only perform the assay, but also lead in the manufacturing and commercialising the new technology. Ugandan researchers can take advantage of the presidential initiative to make Uganda a hub for scientific research and development (R&D). Researchers will be involved in replicating the current study in a bigger sample size and also investigate the applicability of TB-MBLA in samples other than sputum.

References:
1. Global Tuberculosis Report 2021. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO.
2. World Health Organization. Global tuberculosis report 2018. World Health Organization; 2018.
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Figure 3: Training of volunteer Biochemistry graduates at the Molecular laboratory under the Department of Immunology & Molecular Biology, College of Health Sciences, Makerere University, Uganda.

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Contacts: Wamutu Samuel <samuel.wamutu@mak.ac.ug>
Emmanuel Musisi <em303@st-andrews.ac.uk>
Wilber Sabiiti <ws31@st-andrews.ac.uk>

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