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Editorials

The NanoDisk-MS Assay: A New Frontier in Biomarker-Based Tuberculosis Diagnostics?

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Tuberculosis (TB)[2] is a chronic, progressive infection caused by microorganisms within the *Mycobacterium tuberculosis* (MTB) complex. Despite global initiatives, TB remains a significant public health threat and a major cause of morbidity and mortality worldwide. According to the 2017 World Health Organization (WHO) Global Tuberculosis Report, TB was the ninth leading cause of death worldwide, causing an estimated 1.6 million deaths, including 374 000 deaths among those with HIV infection. Additionally, an estimated 10.4 million people developed TB in 2016. The WHO's END TB Strategy aims to reduce the number of TB deaths by 90% and reduce the number of new cases by 80% by 2030. To achieve these goals, the WHO has outlined 3 key areas of need, including the need for major advancements in TB diagnostics *(1)*.

Rapid and accurate diagnosis of active TB and initiation of treatment are critical to control the spread of disease. TB diagnostics, however, continue to rely on traditional techniques, including microscopy and mycobacterial culture. The diagnosis of TB begins with an assessment of symptoms and exposure history. Patients determined to be at risk for TB should be evaluated with a tuberculin skin test or interferon-γ release assay, and, if TB is suspected, specimens should be collected for examination by microscopy and culture *(2–4)*.

Detection of acid fast bacilli (AFB) on microscopic examination of stained specimens is a rapid and inexpensive diagnostic tool, but it is limited in its analytical sensitivity, which has been reported to range from 20% to 80% for sputum *(5)*. In addition, clinical sensitivity is reduced in individuals with a small organism burden, such as in children, individuals with HIV infection, and in individuals with extrapulmonary TB (EPTB) infection. In addition, microscopy has limited analytical specificity because AFB detected by microscopy may represent MTB or non-TB mycobacteria (NTM). Despite these limitations, smear microscopy remains the mainstay of TB diagnosis in many resource-limited settings and is recommended for patients with suspected pulmonary TB in all settings *(2–5)*.

A diagnosis of TB made by isolation of MTB from a specimen and culture is regarded as the "gold standard" for the diagnosis of pulmonary TB and EPTB. Culture is more analytically sensitive than smear microscopy and is necessary for species identification and drug susceptibility testing. However, owing to the slow growth rate of MTB, the culture results may take up to 8 weeks and the culture may not be routinely available in resource-poor settings *(2–5)*.

The most recent advancement in the diagnosis of active TB is the use of PCR-based diagnostic assays. One such assay, the GeneXpert MTB/RIF (Cepheid), was endorsed by the WHO in 2011 and received U.S. Food and Drug Administration approval in 2013. The Xpert MTB/RIF can detect MTB and rifampin resistance within hours and has been shown to be highly accurate in high- and low-incidence settings. The assay is analytically more sensitive than AFB smear microscopy and shows high specificity *(6–8)*. However, the Xpert MTB/RIF assay is approved for the diagnosis of only pulmonary TB and has higher analytical sensitivity in smear-positive than in smear-negative patients *(7)*. In addition, PCR-based diagnostics cannot distinguish viable from nonviable bacteria and require specialized equipment. Recently, the WHO recommended the Xpert MTB/RIF Ultra as a replacement for the Xpert MTB/RIF assay, where available, as the initial diagnostic test for TB in adults and children owing to its higher sensitivity and for use in the diagnosis EPTB *(1)*.

To facilitate rapid identification of both pulmonary TB and EPTB, new rapid diagnostics with improved analytical sensitivity are needed. In a 2014 consensus report, the WHO identified the development of a nonsputum-based biomarker test as 1 of 4 diagnostic priorities. Ideally, this biomarker-based test could diagnose both pulmonary TB and EPTB and can be used as a point-of-care test so that TB treatment could be initiated

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[2] Nonstandard abbreviations: TB, tuberculosis; MTB, *Mycobacterium tuberculosis*; WHO, World Health Organization; AFB, acid fast bacilli; EPTB, extrapulmonary TB; NTM, non-TB mycobacteria; LAM, lipoarabinomannan; CFP 10-kDa, culture filtrate antigen; ESAT-6, 6-KDa early secretory antigenic target; MS, mass spectrometry.

on the same day as diagnosis *(9)*. Although there are numerous studies on new host- and pathogen-derived biomarkers, few have moved into the clinic *(10)*. One such assay, the Alere Determine TB LAM Ag (Alere) lateral flow assay, detects mycobacterial liporabinomannan (LAM) antigen in urine. LAM is a cell wall glycolipid antigen of mycobacteria that is released from metabolically active or degenerating mycobacteria and excreted in the urine. Because of its overall poor diagnostic accuracy, however, a 2015 WHO policy recommended LF (lateral flow)-LAM be restricted for use in only HIV-positive patients with a low CD4 count or who are seriously ill *(11)*.

Recently, Liu and colleagues described a blood-based biomarker assay that holds promise for the detection of pulmonary TB and EPTB and for use in measuring response to treatment *(12)*. This assay, the NanoDisk-MS, relies on the detection of the following 2 MTB antigens: CFP 10-kDa (culture filtrate antigen) and ESAT-6 (6-KDa early secretory antigenic target). ESAT-6 and CFP-10 are promising candidates for use in biomarker-based assays because they are essential for MTB virulence and are recognized by T cells in human infection *(12, 13)*. These proteins are not unique to MTB, however, because homologs of both antigens are expressed by NTM. To get around this limitation, the investigators identified tryptic ESAT-6 and CFP-10 peptides with little homology to many NTM strains. Antibodies specific to these tryptic peptides were conjugated to NanoDisk particles and incubated with digested serum samples. Antibody binding enriched the peptides, facilitating detection by MALDI-TOF mass spectrometry (MS). Because of the inclusion of internal standards, both antigens could be quantified, suggesting that this assay could be used to monitor treatment response and clearance of infection.

The investigators tested the diagnostic performance of the NanoDisk-MS assay in a cohort of 50 HIV-negative patients. Blood was collected from 25 patients with active TB and from 25 non-TB controls. The assay was found to have high analytical sensitivity in both smear-positive and smear-negative cases (100% and 91%, respectively), and the assay outperformed the culture for the detection of pulmonary TB and EPTB infection in HIV-positive patients. The assay, however, encountered limitations in its analytical specificity, giving a positive result for a subset of patients with latent TB infection or infection with NTM *(12)*.

In a study appearing in this issue of *Clinical Chemistry*, Liu et al. expand on their previous work and assess the clinical performance of the NanoDisk-MS assay in 376 HIV-negative adult patients in China *(14)*. Patients were evaluated for the risk of active TB infection on the basis of compatible symptoms, chest radiography, and medical history. Patients suspected of having active pulmonary TB or EPTB infection were further evaluated using AFB smear and culture. A patient was considered to have active TB infection by the NanoDisk-MS assay if a signal for either ESAT-6 or CFP-10 was detected.

In this study, the NanoDisk-MS assay was found to readily distinguish active TB cases from non-TB cases, with an overall analytical sensitivity of 88.3% and specificity of 95.8%. In addition, the Nano-Disk MS assay vastly outperformed both AFB smear microscopy and culture, detecting ESAT-6 or CFP-10 peptide in 85.3% of culture-negative and 87.2% of smear-negative cases. The diagnosis of EPTB is especially challenging, requiring collection of invasive specimens that often yield negative results by microscopy and culture owing to low organism burden in extrapulmonary specimens. A particularly exciting feature of the NanoDisk-MS assay is its performance in patients with EPTB infection, showing similar analytical sensitivity for the detection of EPTB as for pulmonary TB. In addition, pulmonary TB and EPTB can be diagnosed with a blood specimen, eliminating the need for collection of invasive specimens for EPTB diagnosis.

Although the NanoDisk-MS assay holds promise as an accurate, rapid, quantitative blood-based biomarker assay for the diagnosis of pulmonary TB and EPTB, further studies, including the assessment of performance in children and in those with latent TB infection, are warranted. Additional studies are also needed to further test the assay's analytical specificity. In both studies conducted to date, positive NanoDisk-MS results were given for patients without clinical or laboratory evidence of active TB infection *(12)*. In some cases, it is unclear if these results were false-positive results owing to NTM infection or if the assay detected MTB antigens in patients with subclinical infection. Larger studies to assess the analytical specificity of the assay in individuals with known latent or NTM infections should be undertaken to robustly evaluate the assay's specificity.

A major limitation of the NanoDisk-MS assay as a point-of-care test is its dependency on a MALDI-TOF MS instrument. Although MALDI-TOF MS is available in an increasing number of clinical laboratories, the instrument may not be available in many resource-limited settings and, therefore, widespread use of this assay in such settings will be limited until portable MS systems are developed. Nevertheless, the NanoDisk-MS is a promising step toward achieving the WHO's goal of a nonsputum, sensitive biomarker-based TB diagnostic assay. Although a more extensive study of the assay's specificity is required, current studies have shown Nano-Disk MS assay to be both a sensitive and specific assay for the diagnosis of pulmonary TB and EPTB. Quantification of MTB antigen concentration in successive blood samples could permit rapid monitoring of response to treatment, with the potential to help prevent TB-associated morbid-

ity and mortality. Thus, NanoDisk-MS is a significant step toward a TB biomarker assay with features including high sensitivity, quick time to result, and ability to monitor disease that may help accomplish the reductions in TB transmission and mortality set forth in the WHO's END TB Strategy.

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