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COMMENTARY

# Serological Diagnostic Assays for HIV-Associated Tuberculosis in Sub-Saharan Africa?

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**In this issue of *Clinical and Vaccine Immunology*, Siev and colleagues present an evaluation of antibody responses to four immunodominant proteins of *Mycobacterium tuberculosis* in patients with HIV-associated pulmonary tuberculosis (TB) in South Africa (M. Siev, D. Wilson, S. Kainth, V. O. Kasprowicz, C. M. Feintuch, E. Jenny-Avital, and J. J. Achkar, 21:791–798, 2014, doi:http://dx.doi.org/10.1128/CVI.00805-13). This commentary discusses the enormous need for simple point-of-care assays for tuberculosis (TB) diagnosis in patients with and without HIV coinfection in high-burden settings and considers the potential role of serological assays and the huge challenges inherent in developing and validating such assays.**

Sub-Saharan Africa bears the brunt of the HIV-associated tuberculosis (TB) epidemic, accounting for 830,000 (75%) of the 1.1 million new cases occurring worldwide each year (1). Incidence rates are highest toward the south and east of the continent, where HIV prevalence among new TB cases exceeds 50% in a total of 9 countries. In the worst-affected countries, South Africa and Swaziland, an estimated 1% or more of the national population develops TB each year, much of this disease fuelled by HIV (1). The TB notification rate is even higher in impoverished South African townships, where up to 2% to 3% of young adults may develop TB annually (2). Risk is greater still among those who are HIV infected, as their CD4 counts decrease, with rates reaching as high as 25 cases per 100 person years in those with CD4 counts of less than 50 cells/μl either before or during the early stages of antiretroviral therapy (3, 4). Thus, the HIV-associated TB epidemic presents an unprecedented challenge to public health in the region.

Development of new effective diagnostic assays has long been identified as a critical need to improve the response to the TB epidemic in high-burden settings. Major progress has been made within the developmental pipeline for TB diagnostics over the past 10 years (5, 6). This includes the development of the Xpert MTB/RIF rapid molecular assay (Cepheid Inc., Sunnyvale, CA, USA), which was endorsed by the World Health Organization (WHO) in 2010 (7). This diagnostic platform detects with high specificity the vast majority of smear-positive and two-thirds of smear-negative pulmonary-TB cases and a variable proportion of extrapulmonary cases depending on the sample type (6). The Xpert MTB/RIF assay is now being widely implemented around the world, and in 2011, South Africa took the bold decision to implement this nationwide as a replacement for sputum smear microscopy.

The Xpert MTB/RIF assay, however, is an imperfect solution. High cost, sophisticated hardware, linkage to a computer, the need for an uninterrupted power supply, annual calibration, and operator training requirements are all disadvantages in resource-limited settings (8). Although the assay can be completed within 2 h, same-day diagnosis and treatment initiation are logistically challenging to achieve within overcrowded African clinics. Economic and logistical issues mean that implementation of this assay will largely be constrained to the laboratory environment and away from the clinical interface. If the results of a diagnostic assay are not immediately available, clinical impact is readily undermined and clinical outcomes compromised (9). Thus, to directly overcome this problem, rapid assays that can be readily used at the point of care are urgently needed.

One such development is the Determine TB-LAM assay (Alere Inc., Waltham, MA, USA). This simple, low-cost, lateral-flow assay detects the mycobacterial cell wall glycolipid, lipoarabinomannan (LAM), in urine samples, permitting a diagnosis of TB to be made with high specificity within 30 min (10, 11). However, useful sensitivity is limited to adult patients with very low CD4 cell counts and poor prognosis, (12–14). Thus, the utility of this assay is likely to be restricted to accelerating the diagnosis of TB among sick HIV-infected patients prior to starting antiretroviral therapy or among those requiring medical admission to a hospital (12). Since the assay has no useful diagnostic accuracy among patients with CD4 counts of >200 cells/μl or among those without HIV coinfection, this assay will not be appropriate to use for routine investigation or screening of unselected patients. Other point-of-care assays are urgently needed to fill this void to accelerate TB diagnosis in the community, reduce TB transmission, and effect TB control.

While development of rapid diagnostic tests has transformed the diagnosis of HIV-1 infection, malaria, and other endemic tropical diseases, systematic reviews of a large range of commercially available serodiagnostic assays for TB revealed that they had very limited accuracy and were of no clinical value (15, 16). In 2011, the WHO took the unprecedented step of issuing a negative recommendation against their use (17). However, future use of suitable serodiagnostic assays has not been discounted, and active research and development are encouraged. The huge potential advantages of a simple, rapid, low-cost, and noninstrumented as-

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say that uses a finger prick blood sample and can be used in the very lowest tier of the health care system and within the community are self-evident.

The challenges to the development and validation of serological assays for use in countries with the highest HIV prevalence and TB incidence rates in the world are immense, however. A multiplicity of infection and disease state may exist (18, 19), and each may be associated with different host humoral immune responses. Individuals may have never been exposed to *Mycobacterium tuberculosis*, they may have been exposed but remain uninfected, they may be infected but with bacilli remaining under immunological control, infection may have occurred recently or remotely, patients may have been infected once or more than once and have a high or low mycobacterial burden, infection may be active (with failure of immune control of mycobacterial replication) and yet remain undetectable either by assays for the organism or by clinico-radiological means, patients may have pulmonary TB disease or disease at a wide range of extrapulmonary locations, diseases may vary in severity from asymptomatic subclinical disease to fulminant life-threatening disease, patients may have previously received treatment for TB or received chemoprophylaxis for latent TB, they may be adults or children, they may be HIV infected or HIV negative, those living with HIV may have a spectrum of CD4 cell counts, and they may or may not be receiving antiretroviral therapy with various degrees of immune recovery. Many of these clinical states are likely to form a continuous spectrum, ranging from quiescent infection to overt disease, with a reciprocal relationship between bacterial load and immunological control gradually changing over prolonged periods of time (18, 19). Additional complexity arises from other comorbidities, the multiplicity of strains of *M. tuberculosis* causing disease, exposure to nontuberculous *Mycobacterium* spp., and receipt of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) vaccine during childhood.

In countries with low TB burdens and negligible TB transmission risks, many of the clinical states described above are infrequent, whereas in townships with high TB burdens in southern Africa, a high prevalence of these diverse infection and disease states is the norm. Here, gamma interferon (IFN-$\gamma$) responses to RD-1-encoded antigens of *M. tuberculosis* in 7-day whole-blood assays are positive in over 90% of HIV-negative adults, suggesting almost universal cumulative exposure to *M. tuberculosis* (20). The prevalence of positive tuberculin skin test (TST) responses rises steadily throughout childhood, with an annual risk of infection of approximately 4% and a force of infection of almost 8% among adolescents (21, 22). By the age of 15 years, one in two adolescents in such communities has evidence of *M. tuberculosis* infection, and this proportion rises to over 70% in adulthood (21). Of routinely notified active TB cases, approximately one-quarter are due to recurrent disease, largely resulting from reinfection (23). Nearly two-thirds of adults with notified TB are HIV coinfected, and more than one-half of these have CD4 cell counts of $<$200 cells/$\mu$l at diagnosis (24). Active-case finding among HIV-infected people detects a significant prevalence of subclinical sputum culture-positive disease, which progresses to symptomatic disease over time (25–27). Postmortem studies reveal that more than one-half of AIDS-related medical deaths in South Africa are associated with active TB, and much of this disease remains undiagnosed at the time of death (28, 29). In the context of this extraordinary diversity in the host-pathogen relationship and associated immune responses, can serological assays provide simple answers, accurately differentiating between those who need full TB treatment, those who need chemoprophylaxis, and those who are uninfected? This is a tall order but one that needs to be pursued.

In this issue of *Clinical and Vaccine Immunology*, Siev and colleagues present an evaluation of antibody responses to several immunodominant proteins of *Mycobacterium tuberculosis* in patients with HIV-associated TB in South Africa (30). Among the range of purified immunodominant antigens that have previously been evaluated for serological detection of *M. tuberculosis* (31), selected proteins which demonstrate greater potential utility as biomarkers of TB among HIV-infected patients have been studied by Siev and colleagues (32, 33). Antibody responses to two of these antigens, malate synthase (MS) and MPT51, have been detected among patients months before the development of overt clinical TB, suggesting that they may serve as biomarkers for detection of both active and subclinical TB (34, 35). Many previous studies of serological responses to *M. tuberculosis* antigens have studied patients with sputum smear-positive pulmonary TB (31). In contrast, Siev and colleagues have examined serological responses among a diagnostically more challenging group, HIV-infected individuals who have sputum smear-negative disease (30). Furthermore, the study population was recruited in a setting in KwaZulu-Natal, South Africa, where there is an extremely high burden of HIV-associated TB. This is precisely the environment where effective new diagnostic assays are most needed and where such studies should be conducted.

Siev and colleagues evaluated serological responses to MPT51 and MS and two other immunodominant proteins (echA1 and the 38-kDa protein) in patients with HIV-associated sputum smear-negative TB and CD4 counts exceeding 400 cells/$\mu$l (30). In this case-control study, positive antibody responses to MPT51 and echA1 were observed in more than one-half of the TB cases and in a minority of the control group subjects studied at a single time point. In the context of high specificities, the moderate sensitivities observed would nevertheless be useful in this diagnostically challenging group. However, the Achilles heel of serological assays to date has been poor specificity (15). The true specificity of responses to these proteins cannot be adequately defined from this study. Responses were almost universally absent from the small group of HIV-negative patients attending the same health facility in South Africa, despite the likelihood of latent infection with *M. tuberculosis* in many of them. However, reactivity in sera was much more frequently observed in HIV-infected controls, with higher proportions among South African controls than among TST-positive controls living in the United States. It cannot be concluded whether these responses simply denote poor assay specificity or are indicative of active mycobacterial replication and likely progression to active TB disease as reported in previous studies (34, 35).

To assess the diagnostic accuracy of an assay such as Xpert MTB/RIF, which directly detects *M. tuberculosis* in samples, the reference standard typically used is culture of the same clinical sample. This is straightforward. However, if serological assays detect emerging active disease some time before culture-based diagnosis is possible, then this reference standard is inadequate. Instead, prospective studies with clinical follow-up and serial sampling and culture will be essential. Another real weakness of a majority of studies of serological diagnostics to date is the use of a case-control design with typically small groups of selected partic-

ipants being studied. Case selection inevitably fails to capture the huge heterogeneity in the disease process and in the patient characteristics. Moreover, the true infection status of the selected control groups often remains uncertain, especially among those with HIV infection.

The extraordinary burden of HIV-associated TB in South Africa presents an opportunity to prospectively evaluate new diagnostics and biomarkers. Here it would be possible to identify large prospective cohorts of HIV-infected patients in whom there is a very high prevalence of active TB at baseline, a high incidence of TB in those initially thought to be disease-free at enrollment, and a subset who remain TB-free during follow-up. Use of IFN-$\gamma$ release assays, Xpert MTB/RIF, and liquid culture now make it increasingly possible to carefully document infection and disease status at baseline and during prospective follow-up. Such large cohort studies are logistically difficult and expensive to conduct and might best be achieved through large collaborative efforts evaluating a wide range of types of potential biomarkers. Although the challenges are formidable, the very slow progress in global TB control mandates that we redouble our efforts to discover appropriate biomarkers and develop a simple point-of-care assay (1).

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