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Evaluation of *Mycobacterium tuberculosis*-specific antibody responses for the discrimination of active and latent tuberculosis infection

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ABSTRACT

Objectives: The serological antibody detection tests offer several advantages for the rapid diagnosis of tuberculosis (TB). The *Mycobacterium tuberculosis*-specific antibody responses associated with different stages of TB infection remain to be investigated.
Methods: The Pathozyme-Myco IgG (Myco G), Pathozyme TB Complex Plus (TB Complex), IBL *M. tuberculosis* IgG ELISA (IBL), Anda Biologicals TB IgG (Anda-TB), and T-SPOT.*TB* (T-SPOT) tests were performed for 133 active TB patients (ATB group), 131 controls (CON group), and 95 subjects with latent TB infection (LTBI group).
Results: The four serological tests all showed relatively low sensitivity in the ATB group but high specificity in the LTBI and CON groups. The antibody levels of the four serological tests were significantly higher in the ATB group than in the LTBI group. The same trend was observed between the LTBI and CON groups. The four serological tests demonstrated potential diagnostic value in discriminating ATB from LTBI. A combination of the Anda-TB and TB Complex tests exhibited the best diagnostic potential in discriminating ATB from LTBI, with a sensitivity of 89.4% and a specificity of 94.7%. Further, the diagnostic value of Anda-TB and TB Complex were validated in a prospective cohort including 106 patients with suspected ATB. Combined with the T-SPOT test, the tests showed a sensitivity of 87.2% and a specificity of 92.5% for discriminating ATB patients from all ATB suspected cases in the validation group.
Conclusions: The antibody responses of the serological tests all showed significant differences between the ATB and LTBI groups. A combination of Anda-TB and the TB Complex test demonstrated high diagnostic potential in discriminating ATB from LTBI and may be an additional diagnostic tool in the diagnosis of *M. tuberculosis* infection.

Introduction

The global tuberculosis (TB) epidemic has resulted in nearly two million deaths and nine million new cases of the disease per year. It is estimated that two billion people live with latent *Mycobacterium tuberculosis* infection (LTBI) and represent a potential source of future active TB cases (Corbett et al., 2003). Thus, the development of rapid and accurate new diagnostic methods is vital for the global control of TB. However, the diagnostic accuracy of existing tests is inadequate (Wallis et al., 2013). The microscopy method has high specificity in TB-endemic countries, but modest sensitivity and variable results in many settings (Steingart et al., 2006; Urbanczik, 1985). The current immunodiagnostic tests for TB also have considerable limitations. The tuberculin skin test (TST) has limited specificity, and false-positive results can occur as a result of prior bacillus Calmette–Guérin (BCG) vaccination or infection with non-tuberculous mycobacteria (NTM) (Latorre et al., 2010). The T-cell-based interferon-gamma (IFN-γ) release assays (IGRAs) are just as sensitive as and more specific than the TST (Pai et al., 2008). However, the current IGRAs cannot discriminate active TB (ATB) from LTBI, which makes them unsuitable for diagnosing active

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disease, particularly in TB-endemic areas (Mazurek et al., 2007; Mazurek et al., 2010).

In addition to these methods, the development of immune-based tests for the detection of the humoral (serological) antibody immune response to *M. tuberculosis*-specific antigens has been ongoing for decades. The serological antibody detection tests (hereafter referred to as serological tests) offer several advantages (Gennaro, 2000). Detection of the presence of specific antibodies is faster and simpler to perform than most sputum-based and T-cell-based methods (Silva et al., 2003; Rasolofo and Chanteau, 1999; Andersen et al., 2000). In patients suspected of extrapulmonary TB, serological tests may be able to detect antibodies from different sample sources such as urine, cerebrospinal fluid, and pleural fluid, which may reduce or eliminate the need for more invasive tests.

Over the past decade, serological tests for TB have been investigated extensively and several promising candidate antigens have been identified and evaluated, such as 12 kDa, 38 kDa, LAM, 16 kDa, CFP-10, Rv3425, and antigen 60 (Gennaro, 2000; Chan et al., 2000). A number of commercial antibody detection tests have also been developed and evaluated in regions with different TB endemicity rates. However, according to systematic reviews of these studies, the diagnostic accuracy of the commercial serological tests varies widely in performance, with highly inconsistent estimates of sensitivity and specificity, and none of the commercial tests has performed well enough to replace sputum smear microscopy (Steingart et al., 2007a; Steingart et al., 2007c; Steingart et al., 2011). In 2011, the World Health Organization (WHO) policy recommended against the use of these tests for the diagnosis of pulmonary and extrapulmonary TB (World Health Organization, 2011). In spite of this, future use of the serological tests has not been discounted (Ivanyi, 2012; Jacqueline and Anke, 2012). The WHO still strongly encourages improved research on the serological diagnosis of TB, because it may provide many advantages in high-burden, resource-limited regions in the form of point-of-care rapid tests, which are currently missing in the TB diagnostic pipeline (World Health Organization, 2011; Morris, 2011).

The serological tests are also attractive because they may have the diagnostic potential to distinguish between active disease and latent infection, which offers a significant improvement over current routine test methods. The specific antibody responses are rarely detected in non-symptomatic individuals (Daniel et al., 1991). For the most frequently evaluated A60 antigen, several studies have shown that the percentage of positivity and the A60 IgG titers are much lower in inactive post-primary TB patients than in ATB patients (Luh et al., 1996; Gevaudan et al., 1992; Zou et al., 1994). In one study, the antibody levels of 38 kDa and 16 kDa antigens were found to be significantly lower in patients with inactive TB compared to those with ATB, but no significant difference was found between patients with inactive TB and control cases (Senol et al., 2007). A study by Anderson et al. also indicated that the positive rate of the serological test was lower in subjects with latent infection identified by IGRA, and that the serological test may have the ability to aid in the differentiation between ATB and LTBI (Anderson et al., 2008).

These differences in antibody response between active and latent TB are striking, but the diagnostic potential of serological tests to discriminate between active and latent TB infection still requires thorough investigation. Moreover, whether a combination of serological tests and T-cell-based IGRAs could achieve the highest diagnostic efficacy has also not been evaluated to date.

This study recruited subjects with different stages of TB infection, including ATB patients, subjects with LTBI, and controls, to evaluate the diagnostic accuracy of four serological tests. Focus was placed on the evaluation of the serological tests as potential biomarkers in discriminating active TB from latent infection. The serological tests were also used in combination with the T-SPOT.*TB* test (Oxford Immunotech, Oxford, UK) to determine whether the diagnostic performance could be improved. In the next stage of the study, an independent clinical-based validation cohort was recruited to confirm the high performance of the serological tests in discriminating ATB from LTBI.

Materials and methods

Study design and participant selection

In order to investigate the diagnostic performance of the serological tests, two independent study groups were recruited. The subjects in group I were recruited from a cross-sectional study that consisted of three subgroups: active TB patients (ATB group), subjects with LTBI (LTBI group), and non-ATB controls (CON group). Study group II subjects were recruited prospectively from a clinical setting-oriented study to further evaluate the diagnostic performance of the serological tests. The demographic characteristics of the study populations are described in Table 1 and in

Table 1
Characteristics of the study participants.

Characteristic	Study group I			Study group II	
	ATB	LTBI	CON	ATB	NTB
Total number	133	95	131	39	67
Male, *n* (%)	81 (60.9)	49 (51.6)	72 (55.0)	22 (56.4)	32 (47.8)
Median age (range), years	45 (18–75)	47 (22–67)	41 (19–62)	36 (21–73)	41 (19–77)
BCG status					
Unvaccinated	34 (25.6)	16 (16.8)	21 (16.0)	11 (28.2)	15 (22.4)
Vaccinated	95 (71.4)	77 (81.1)	107 (81.7)	25 (64.1)	45 (67.2)
Unknown	4 (3.0)	2 (2.1)	3 (2.3)	3 (7.7)	7 (10.4)
Preexisting conditions and illnesses, *n* (%)					
Previous tuberculosis	5 (3.8)	3 (3.2)	0 (0)	2 (5.1)	7 (10.4)
Diabetes	12 (9.0)	9 (9.5)	7 (5.3)	4 (10.3)	11 (16.4)
HIV-positive	0 (0)	0 (0)	0 (0)	1 (2.6)	2 (3.0)
History of hepatitis B/C	7 (5.3)	6 (6.3)	6 (4.6)	2 (5.1)	5 (7.5)
Pneumonia	3 (2.3)	1 (1.1)	0 (0)	2 (5.1)	1 (1.5)
Chronic liver disease	2 (1.5)	1 (1.1)	1 (0.8)	2 (5.1)	3 (4.5)
Chronic renal failure	1 (0.8)	0 (0)	0 (0)	0 (0)	1 (1.5)
Coronary heart disease	4 (3.0)	2 (2.1)	1 (0.8)	1 (2.6)	2 (3.0)

ATB, active TB patients; BCG, bacillus Calmette–Guérin; CON, controls; LTBI, latent TB infection; NTB, no active TB disease.