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The complexity of diagnosing latent tuberculosis infection in older adults in long-term care facilities

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SUMMARY

Objectives: In the USA, tuberculosis disease rates are highest in older adults. Diagnostic testing for latent tuberculosis infection (LTBI) has not been evaluated carefully in this group. The aim of this study was to define the relationship between tuberculin skin test (TST) results, T-SPOT.*TB* results, and T-cell responses to *Mycobacterium tuberculosis* antigens.

Methods: Long-term care facility residents with known prior TST results (positive or negative) were retested with TSTs and T-SPOT.*TB*. Prior exposure to *M. tuberculosis* was assessed by quantifying T-cell activation to mycobacterial antigens in vitro.

Results: The median age of the 37 participants was 77 years (range 57–98 years). Among 18 participants with a prior positive TST, three (16.7%) had a negative TST when retested (TST reversion); two had a negative T-SPOT.*TB*. Of the 15 who were historically and currently TST-positive, four (26.7%) had a negative T-SPOT.*TB* and one (6.7%) had a borderline result. Percentages of CD4+ T-cells responding to mycobacterial antigens were higher in participants with positive TST and T-SPOT.*TB* (18.2%) compared to those with a positive TST but negative T-SPOT.*TB* (6.4%, $p = 0.16$) and negative TST and T-SPOT.*TB* (5.9%, $p < 0.001$).

Conclusions: LTBI testing in older adults is complicated by TST reversion and TST-positive/T-SPOT.*TB*-negative discordance, *which may reflect clearance of infection or waning immunity.*

1. Introduction

An estimated 32% of the world's population, or 1.86 billion people, are infected with *Mycobacterium tuberculosis*,[1] and more than eight million new cases of tuberculosis disease (TB) occur each year.[2] Between 1993 and 2008, 21.9% of TB cases in the USA involved older adults (≥65 years of age),[3] so continued progress towards the elimination of TB in the USA will require that the substantial burden of TB in older adults is addressed. Average yearly TB rates between 1993 and 2008 were 1.5 times higher in older adults than in those aged 21–64 years and were 2.3 times higher in older adults residing in long-term care facilities (LTCF) than in those in the community.[3] Older adults comprise the fastest growing sector of the global population and are expected to account for 20% of the US population by the year 2050.[4] The population of older adults in need of long-term care is predicted to rise from eight million in 2000 to 19 million in 2050.[5] Because most TB in older adults likely results from reactivation of latent TB infection (LTBI) rather than new infection,[6,7] accurate diagnosis of LTBI in this population is critical, particularly if linked to the treatment of LTBI.

Diagnosing LTBI in older adults is complicated because of the limited specificity of the tuberculin skin test (TST) and reversion over time. TSTs are known to have reduced specificity among persons with non-tuberculous mycobacterial exposure, those with a history of residence in the southern USA, and bacillus Calmette–Guérin (BCG)-vaccinated individuals.[8–11] The use of boosted (two-step) TSTs, recommended for LTCF residents,[6,9] further reduces test specificity; a study from Hong Kong showed that boosted TST results are not predictive of TB disease.[12] The sensitivity of the TST

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in older adults may also decrease due to waning immunity,[13–17] with a 5% decline in test positivity per decade after age 65 years, up to 9% annually in those >60 years of age.[14,16] Comorbidities associated with older age, including being malnourished or underweight[12] and requiring assisted feeding,[14] can also reduce TST sensitivity. What is unknown is the degree to which the waning TST response reflects cleared infection and to what degree the waning TST response reflects a false-negative test.

Data on the use of interferon-gamma release assays (IGRAs), including the T-SPOT.*TB* and QuantiFERON-TB Gold In-Tube (QFT-GIT) assays, in older adults are limited, but the specificity for all ages is greater than the TST when BCG-vaccinated individuals are tested.[8,11,18] For low-risk populations, pooled specificity estimates range from 93% for T-SPOT.*TB* to 98% for QFT-GIT,[11] and it appears that IGRA sensitivity is less affected by age than the TST. In one study, older adults (defined as age greater than 50 years) were 3.8 times as likely to be QFT-GIT-positive/TST-negative as younger adults.[13] Older age was associated with positive IGRA (but not TST) results in persons screened prior to renal transplant,[19] Japanese healthcare workers,[20] and persons with radiographic evidence of healed TB.[21] Similarly, IGRA results were not affected by age in patients with silicosis[22] and in the older population in the UK (odds ratio 5.3, 95% confidence interval 2.9–9.8 for IGRA positivity with increasing age).[23] This suggests that with age, the TST response may wane, but the IGRA response persists.

Persistent immune responses to *M. tuberculosis* antigens can be assessed by evaluating antigen-specific memory cell response and activation. CD69 is an early activation marker on the surface of lymphocytes[24,25] and a costimulatory molecule for T-cell activation and proliferation.[25,26] The expression of CD69 on CD4+ T-cells correlates with TST induration, lymphocyte blastogenesis, and IGRA results.[25,27] CD69 has been found in greater amounts on CD4+ T-cells (after *M. tuberculosis* antigen stimulation) in persons who are TST-positive or have clinically inactive/treated TB compared with those who are TST-negative.[25,27,28] It was therefore predicted that CD69 upregulation could potentially be used to quantify T-cell activation in vitro to identify infection in older adults, thereby differentiating 'true-positive' from 'false-positive' TST and T-SPOT.*TB* results.

Given the potential for LTBI reactivation within LTCFs and spread to other residents, the diagnosis and treatment of LTBI is a public health priority. The goal of this study was to improve our understanding of LTBI diagnostics in this population and determine the relationship between TST responsiveness in older adults, T-SPOT.*TB* test results, and immunologic evidence of memory T-cell response.

2. Methods

2.1. Study location and data collection

This cross-sectional study was performed between September 2011 and August 2012 in three Boston-area LTCFs. Records were reviewed to identify all HIV-uninfected residents with a history of a positive TST performed on admission to the facility, or documented as positive prior to admission as part of the patient's routine healthcare. The specific setting and indication for prior testing was not always known and was not recorded. Controls with known prior negative TSTs (and no known positive TST) were matched to the TST-positive individuals by sex and age (within 5 years). Study personnel performed a medical records review and administered a standardized questionnaire to participants, or to the legal authorized representatives (i.e., healthcare proxies) of those persons without capacity to provide consent. Questionnaires addressed known history of TB disease, LTBI history, BCG vaccination, and comorbidities known to be associated with LTBI.

The TST was performed using the Mantoux technique with 2 TU of purified protein derivative (Sanofi Pasteur, Cambridge, MA, USA) and read by trained personnel at 48–72 h; results ≥10 mm were considered positive,[29] and any questionable result was confirmed by a second reader. TSTs were repeated ('boosted') once after 7–14 days for those persons with an initial negative TST. T-SPOT.*TB* assays were performed at the first study visit and were processed by Oxford Immunotech (Marlborough, MA, USA) in accordance with the manufacturer's guidelines. Results, interpreted by subtracting the spot count in the negative control from the spot count in panels A and B, were reported as positive (>8 spots), negative (<4 spots), borderline (5–7 spots), or invalid. Blood samples were collected at the first study visit for immunological assessment.

2.2. Immunological assays

Peripheral blood mononuclear cells (PBMCs), isolated from peripheral blood by Ficoll density gradient (Sigma-Aldrich, St. Louis, MO, USA), were cultured in 10% fetal bovine serum in RPMI 1640 supplemented with 2 mM L-glutamine and 1 mM penicillin/streptomycin (Invitrogen, Woburn, MA, USA). Cells were stimulated with *M. tuberculosis* whole cell lysate (WCL) (BEI Resources, Manassas, VA, USA) for 3 days. Cells were harvested and prepared for flow cytometry to quantify T-cell activation using anti-CD69, CD3, CD4, and CD8 antibodies (BD Pharmingen, San Diego, CA, USA). The positive control for T-cell activation was stimulation with anti-CD3/CD28 (eBioscience, San Diego, CA, USA); CD3+CD4+ T-cells and CD3+CD8+ T-cells were assessed for expression of CD69 by flow cytometry. Supernatants were analyzed for expression of interferon-gamma (IFN-γ) by ELISA (R&D Systems, Minneapolis, MN, USA).

2.3. Statistical analyses

All analyses were performed using SAS 9.1.3 (SAS Institute, Cary, NC, USA). *p*-Values were calculated using the exact Wilcoxon two-sample test for continuous variables and Fisher's exact test for categorical variables. CD69 expression on CD4+ T-cells and CD8+ T-cells was analyzed in two ways: (1) percentage expression, and (2) the ratio of percentage expression following WCL stimulation to percentage expression following stimulation with media (with a cut-off of 3 denoted as 'positive' CD69 expression). The Institutional Review Board of Boston University approved this study.

3. Results

3.1. Participant characteristics

One hundred and twenty-one persons were screened for participation and 38 persons were enrolled in the study (31.4% participation rate). Among the 83 who did not participate, the reasons for non-participation included legally authorized representative unwilling to consent (n = 32, 38.5%), inability to contact legally authorized representative despite numerous attempts (n = 19, 22.9%), subject unwilling to consent (n = 18, 21.8%), and other (n = 14, 16.9%). Laboratory data were not available for one participant. Among the 37 with data, 29 (78.4%) were male, and the median age was 77 years (range 57–98 years) (Table 1). Among those with data on race/ethnicity, 25/33 (75.8%) were black, 8/33 (24.2%) were white, and 5/34 (14.7%) were Hispanic; 12/27 (44.4%) were born outside the USA. The median duration of residence in the LTCF was 5 years (range 0–19 years).