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A TB Antigen-Stimulated CXCR3 Ligand Assay for the Diagnosis of Active Pulmonary TB

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BACKGROUND: The ligands for CXC chemokine receptor 3 (CXCR3) recruit T-helper type 1 cells, which play a major role in cell-mediated immunity in TB.

METHODS: A total of 409 subjects were enrolled. The study population comprised 186 patients with active TB, 58 patients with non-TB pulmonary diseases, 50 control subjects with a positive interferon (IFN)-γ release assay (IGRA) result, and 115 control subjects with a negative IGRA result. Whole-blood samples were collected using IGRA methodology. After incubation, plasma IFN-γ levels and two CXCR3 ligands, IFN-inducible T-cell α-chemoattractant (I-TAC, CXCL11) and monokine induced by IFN-γ (MIG, CXCL9), were measured by enzyme-linked immunosorbent assay. Receiver operating characteristic (ROC) analysis was performed. Sensitivity and specificity were based on cutoff values selected to maximize the Youden index.

RESULTS: The TB antigen-stimulated levels of IFN-γ, I-TAC, and MIG were significantly increased in the active pulmonary TB group compared with all other groups. From ROC analysis, for the diagnosis of active TB, I-TAC and MIG outperformed IFN-γ in all comparisons with the IGRA-positive and -negative control groups and the non-TB pulmonary disease group. The areas under the curve (95% CI) for differentiating active pulmonary TB from all other groups were 0.893 (0.864-0.924) for IFN-γ, 0.962 (0.946-0.978) for I-TAC, and 0.944 (0.922-0.965) for MIG. Sensitivity and specificity were 90.3% and 90.7%, respectively, for I-TAC; 92.5% and 85.2% for MIG; and 84.9% and 79.8% for IFN-γ.

CONCLUSIONS: TB antigen-stimulated assays of I-TAC and MIG may be useful surrogate markers in the diagnosis of active pulmonary TB. CHEST 2014; 146(2):283-291

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ABBREVIATIONS: AUC = area under the curve; CXCR3 = CXC chemokine receptor 3; IFN = interferon; IGRA = interferon-γ release assay; IP-10 = interferon-γ-inducible 10-kDa protein; I-TAC = interferon-inducible T-cell α-chemoattractant; LTBI = latent TB infection; MIG = monokine induced by interferon-γ; ROC = receiver operating characteristic; Th1 = T-helper type 1; TST = tuberculin skin test

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TB has long been one of the most important public health concerns worldwide.[1] Effective control of TB relies on early detection and adequate treatment of the infection. However, it is difficult to achieve these goals using conventional laboratory tests.[2]

Because of the need for better diagnostic tools, the interferon (IFN)-γ release assay (IGRA) was developed as an alternative to the classic, century-old methodology, the tuberculin skin test (TST).[3,4] Many studies have reported that the IGRA has superior sensitivity and specificity to TST for the diagnosis of active TB infection and latent TB infection (LTBI).[5-8] However, IGRA fails to differentiate between active TB infection and LTBI, as is the case for TST.[2,8,9] This limitation may be more problematic in high-endemic countries, where LTBI is prevalent and difficulties in treatment decisions are often encountered.[10] Furthermore, despite earlier expectations that IGRA would possess high diagnostic accuracy, a meta-analysis revealed increasingly unsatisfactory results, including in countries with various levels of TB prevalence.[8] According to this report, pooled sensitivity and specificity were 80% and 79%, respectively.

Cell-mediated immunity, particularly the T-helper type 1 (Th1) lymphocyte pathway is closely related to the pathogenesis of TB.[11-13] CXC chemokine receptor 3 (CXCR3) ligands act downstream of IFN-γ and more specifically toward the Th1 pathway.[14,15] CXCR3 ligands comprise three chemokines: IFN-γ-inducible 10-kDa protein (IP-10, CXCL10), monokine induced by IFN-γ (MIG, CXCL9), and IFN-inducible T-cell α-chemoattractant (I-TAC, CXCL11). IP-10 has been evaluated in a whole-blood release assay in patients with TB but was not superior to IFN-γ for the diagnosis of active TB.[12,16] I-TAC exhibits the most potent activity but has not been assessed in patients with TB.[17] We screened the blood of the patients with TB for Th1-related chemokines and found higher levels of I-TAC and MIG than of IP-10.

We evaluated the clinical utility of I-TAC and MIG in patients suspected of having TB as well as in a control group. The former was categorized into TB and non-TB groups and the latter into IGRA-positive and -negative groups. We evaluated the diagnostic utility of these markers in Korea, where the prevalence of both active TB and LTBI remains high.[18]

Materials and Methods

Subjects

This study was conducted at Ajou University Hospital from January 2010 to April 2012. In total, 252 patients with suspected pulmonary TB were enrolled consecutively. The control group comprised 165 volunteer subjects who received a general health examination. All subjects were without HIV. All samples were collected from patients before the initiation of treatment. The study was approved by the Ajou University Hospital Institutional Review Board (approval number MED-SMP-12-068), and all subjects provided written informed consent.

A diagnosis of active pulmonary TB was made when *Mycobacterium tuberculosis* was identified in clinical specimen cultures, or in the case of negative culture results, when suggestive clinicoradiologic features were reinforced by responses to anti-TB therapy. TB lymphadenitis was diagnosed based on the presence of *M tuberculosis* or observation of typical pathologic findings with an appropriate response to treatment. TB pleural effusion was diagnosed based on the presence of *M tuberculosis*, observation of typical pathologic findings in pleural tissues, or compatible findings in cellular and biochemical analyses of the pleural fluid with an appropriate response to treatment. The diagnosis of meningeal TB was supported by cerebrospinal fluid biochemical findings. Chest CT scan was performed in 220 patients with suspected TB. The patients were assigned to a non-TB pulmonary disease group if they did not have active TB infection. The diagnosis of active TB was initially made by the physician in charge and two other investigators. The patients were followed continuously until October 2013, and the cases were independently reviewed by one radiologist and two other respiratory medicine specialists; a final decision was made by consensus.

The presence of a fibrotic lesion denotes preexisting, inactive TB. Subjects in this group presented with chest roentgenograms showing fibrotic sequelae, which were verified as stable for at least 6 months and if the sputum culture for *M tuberculosis* was negative. Of the control subjects, one had a history of treatment for TB, and four had fibrotic lesions.

The control subjects were subdivided into IGRA-positive and -negative groups according to their IGRA results from QuantiFERON-TB Gold In-Tube tests (Cellestis, a QIAGEN Company). To support the diagnosis of LTBI in the control subjects, the contact history of TB was assessed and stratified according to the epidemiologic risk of infection.[19]

Whole Blood Stimulation

Blood (1 mL) was drawn and placed into three vacutainer tubes from the QuantiFERON-TB Gold In-Tube test. The tubes were precoated with three components: saline (nonstimulated; nil tube); *M tuberculosis* ESAT-6, CFP10, and TB 7.7 antigens (stimulated; TB-antigen tube); or phytohemagglutinin (nonspecific positive control; mitogen tube). The tubes were incubated for 20 to 24 h at 37°C, and plasma was harvested and frozen until further analysis.

Measurement of Biomarkers

I-TAC and MIG were measured in the samples collected using the Quantikine ELISA Kit (R&D Systems, Inc). IFN-γ levels were measured using the QuantiFERON-TB Gold In-Tube ELISA (enzyme-linked immunosorbent assay).[20]

Statistical Analysis

Data were analyzed with SPSS, version 18 (IBM) and MedCalc, version 12.3.0.0 (MedCalc Software bvba) software. Nonnormal data are presented as median (interquartile range), unless otherwise indicated. The Kruskal-Wallis test was used for comparisons among groups, followed by Dunn post hoc test for pairwise multiple comparisons. The predictive performance was evaluated using nonparametric receiver operating characteristic (ROC) analysis. The optimal cutoff values were determined to maximize the Youden index. The Youden index is based on selecting a point that maximizes the number of subjects who are classified correctly and gives equal weight to sensitivity and specificity.[21] $P < .05$ was considered statistically significant.