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ORIGINAL ARTICLE

Improvement in the Diagnosis of Tuberculosis Combining *Mycobacterium Tuberculosis* Immunodominant Peptides and Serum Host Biomarkers

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Background. Pulmonary tuberculosis (PTB) is a public health problem with 10.4 million new cases reported in 2017 (1). According to the World Health Organization (WHO), accurate diagnostic tests based in serum biomarkers to detect new cases of tuberculosis are necessary.

Aim of the study. To evaluate antibodies against *Mycobacterium. tuberculosis* (Mtb) peptides (Ab-Mtb) and three soluble host biomarkers by ELISA serial multiple test in sera from non-infected controls (NIC, $n = 31$), latent tuberculosis (LTB, $n = 37$) and PTB ($n = 28$) patients in a diagnosis tuberculosis assay.

Materials and methods. Levels of four Ab-Mtb peptides derived from Mtb and three host response molecules in serum from NIC, LTB and PTB were evaluated by ELISA as tuberculosis biomarkers. Multiple comparisons tests, determination of diagnostic values and ROC curves were performed. Serial and parallel multiple tests were performed with the biomarkers with the highest discriminatory capacity to improve diagnostic values of the test.

Results. We found significant differences between biomarkers levels in PTB comparing LTB and NIC to all candidate biomarkers; peptides P12033, P12037, and serum biomarkers such as sCD14 and chemokine CXCL9 showed the best sensitivity and specificity, the highest discriminatory power, and the best area under the curve (AUC) individually. In serial multiple tests, P12037 and sCD14 together have 92% of sensitivity and 91% of specificity, with positive and negative likelihood ratios greater than 10.

Conclusions. Ab-Mtb peptide P12037 and sCD14 could be applied in a diagnostic test for suspected PTB to improve accuracy and time to diagnosis and could be implemented in a POCT device which can be affordable.

Key Words: Tuberculosis, Host response biomarkers, Antibody detection, ELISA, Serodiagnosis tests.

Introduction

The World Health Organization (WHO) reported in 2017 that Tuberculosis (TB), mainly caused by *Mycobacterium tuberculosis,* remains as one of the top 10 causes of death worldwide in 2016, provoking more deaths than HIV and

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malaria; this makes Mtb the most lethal infectious pathogen (1). There were an estimated 10.4 million new (incident) TB cases worldwide, of which 65% were men, 29.1% were women and 6.9% were children (1). Also, an estimated of 490,000 people developed multiple drug resistance TB (MDR-TB) worldwide in 2016, and out of these 490,000 people, 240,000 people died (2). MDR-Mtb strains with additional resistance have now been found in every region of the world (3).

Approaches to decreasing TB morbidity, mortality, and Mtb transmission rely on correct and timely diagnosis, effective treatment, and prevention of infection. Up until recently, LTB was thought to represent a uniform state (4). However, it has become clear that LTBI and TB have to be considered as a broad spectrum of states that differ by the degree of pathogen replication, host resistance, and inflammation (4−6), making more difficult to have a TB diagnosis.

Conventional LTB diagnosis relies on the tuberculin skin test (TST) and interferon-gamma release assays (IGRA). Neither TST nor IGRA can discriminate latent infection from active disease. For the diagnosis of PTB, sputum smear microscopy, cultures and, nucleic acid amplification tests (NAAT) are the currently used tools. However, smear microscopy lacks sensitivity and cannot detect paucibacillary cases as the culture can do it, but cultures are expensive, require BSL3 labs, and obtaining results takes a long time, while NAAT are expensive and not easily deployable at the peripheral level. Recently, Pai reviewed the current best diagnostic tools available for TB diagnosis and described the most important gaps and translational challenges for developing innovative tuberculosis tests (7). He suggests a sputum-based replacement test for smear-microscopy and a non-sputum-based biomarker test for all forms of TB.

The translational challenge for several NAAT is to convert them into more affordable assays. For the non-sputum TB test, the biggest challenge is the lack of validated biomarkers. Although considerable efforts are being made to identify biomarkers that can meet some of these needs, progress has been slow (7,8). Thus, the discovery of TB biomarkers is an important goal in current TB research; unfortunately, no such markers are currently available.

In a previous work, using an experimental animal model of PTB (9), we identified a transcriptome of the host response associated with the progression of tuberculosis. In this specific transcriptome, sUPAR, sCD14, and CXCL9 were highly up-regulated in the blood of patients with active tuberculosis (Patent submitted, 2012). Moreover, on another parallel study, we demonstrated the usefulness of an enzyme-linked immunosorbent assay (ELISA) for the detection Ab-Mtb peptides in the serum of patients with PTB and extrapulmonary TB (EPTB) (10). This assay uses 20 amino acid- long, non-overlapped synthetic peptides that spanned Mtb ESAT-6 and Ag85A sequences. The validation cohort included patients with PTB, patients with EPTB, individuals without EPTB, individuals with leprosy and NIC. For the PTB group, two ESAT-6 peptides (12033 and 12034) had the best sensitivity values, while the best specificity values were for an ESAT-6 peptide (12037) and an Ag85A-peptide (29878).

Here, we evaluated the levels of Ab-Mtb peptides of these four different derived peptides from Mtb and we combined the results with the evaluation of three soluble host biomarkers in serial multiple tests in sera from NIC, LTBI and PTB patients.

Materials and Methods

Study Participants

We included 96 adult individuals, recruited between September 2006 and May 2011. Groups were stratified as NIC ($n = 31$), LTB ($n = 37$) and PTB ($n = 28$). The inclusion criteria for the NIC group included TST (<9mm) and QuantiFERON-Gold® (QFN) negative. The LTB group comprised asymptomatic subjects with TST$>$ 10 mm and/or positive to Quantiferon *in tube* IGRA test. The PTB group was confirmed by either culture (positive) and/or AFB (acid-fast bacilli) smears.

Each of the individuals was clinically evaluated by a certified pneumologist and answered a standardized clinical-epidemiological questionnaire. All participants signed an informed consent letter fulfilling all international regulations and requirements of the ethics and the National Research Committee at IMSS (protocols approval: IMSS CNIC 2005 3301-18 and IMSS CNIC 2005 3301-19).

Serum samples and ELISA determinations

Two blood samples without anticoagulant were obtained from each participant. Samples were centrifuged for 5 min at 300 × g to obtain serum aliquots were stored at −20°C until use.

Antibodies levels of Ab-Mtb peptides derived from *Mtb* (P12033, P12034, P12037 and P29878) and three serum host response proteins (sCD14, sCXCL9, sUPAR) were evaluated by ELISA (BioAssays Systems, USA and R&D systems) following manufacturer's instructions. One hundred microliters of each standard and serum samples (diluted and not diluted) were applied separately to each well in duplicates. Plates were read on a microplate reader (Multiskan Ascent 96/384 Plate Reader, MTX Lab Systems, Inc. USA).

The Mtb derived synthetic peptides sequences and ELISA methodology to quantify serum Ab-Mtb peptides were previously described (10). Serum samples were used diluted and the OD values for each well was measured in an ELISA microplate reader as described previously.