MESA: Novedades en métodos diagnósticos y en tratamiento

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IGRAs' evolution

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The tuberculin skin test (TST), that recalls the delayed-type hypersensitivity response to the intradermal inoculation of purified protein derivate (PPD), has been used to diagnose TB infection for the last hundred years. The PPD contains a mixture of more than 200 antigens that are widely shared by mycobacteria other than *Mycobacterium tuberculosis*, including the vaccinal strain of *Mycobacterium bovis* bacilli Calmette-Guérin (BCG) and many non-tuberculous mycobacteria (NTM). As a result, individuals sensitized by previous exposure to NTM or BCG vaccine may respond immunologically to PPD. The other main limitation of the TST is its low sensitivity in certain groups of individuals, such as immunosuppressed patients and young children.

Immunodiagnostic methods have been developed based on the *in vitro* quantification of the cellular immune response, by detecting interferon-gamma (IFN-γ) released by sensitized T-cells stimulated with specific *M. tuberculosis* antigens. The two main antigens used are the 6-kD *M. tuberculosis* early-secreted antigenic target protein (ESAT-6) and the 10-kD culture filtrate protein (CFP-10), encoded in the region of difference 1 (RD1), which is present in *M. tuberculosis* but not in BCG or in most NTM. This *in vitro* technology was adapted from initial in-house methods to two commercial techniques: QuantiFERON-TB Gold assays (QFT-Gold) (Qiagen, Germany) and T-SPOT.TB assay (Oxford Immunotec, UK). Both tests, collectively known as IGRAs (Interferon-Gamma Release Assays), were approved for sale in Europe and received final approval from the U.S. Food and Drug Administration (FDA) as an aid for diagnosing *M. tuberculosis* infection. T-SPOT.TB detects the number of IFN-γ producing T-cells after stimulating a definite number of isolated peripheral blood mononuclear cells (PBMCs) with ESAT-6 and CFP-10 separately by means of enzyme-linked immunospot assay (ELISPOT). QFT-Gold tests are whole blood assays that use an enzyme-linked immunosorbent assay (ELISA) to detect IFN-γ produced in supernatants by stimulated T-cells. The QFT-G In Tube version (QFT-IT) includes a third antigen, TB7.7. This antigen is encoded in RD11 and is missing from the BCG strains as well as most common environmental mycobacteria. Both *in vitro* tests include a positive control that detects the capacity of T cells to produce IFN-γ upon stimulation with a mitogen, in order to distinguish false-negatives from indeterminate results.

IFN-γ-based assays have become a reliable alternative to the old TST for the diagnosis of TB infection[1]. Both tests, QFT-IT and T-SPOT.TB, have a higher specificity than TST, and a better correlation with risk factors for TB and the degree of contact with a TB case. Although their sensitivity may be affected to some extent by immunosuppression and extreme ages of life, they perform better than TST in these situations.

In addition, in the last years, some of the important questions concerning the prognostic value of a positive/negative result for the development of active TB, the significance of discordant results, and the conversion/reversion phenomenon, has been resolved. The prognostic value of a positive IFN-γ result predicts progression to active disease similar than TST, and therefore most people with a positive result will not develop TB[2]. Because of the discordance between IFN-γ tests and TST results, practitioners are reluctant to use them in everyday clinical practice. Studies focusing specifically on understanding the discordant results between IFN-γ tests and the TST, and between IFN-γ tests themselves, have been done, showing that, specifically in childhood,

the impact of NTM sensitization should play an important role[3]. It has been also described which parameters are involved in the variability of the IGRAs results. The potential conversion/reversion have been described more frequently in borderline results[4].

The new advances in this field resides in the use of new specific antigens more focused in latency[5] that allows distinguish between latent and active TB, the detection of new cytokines[6] or new molecular strategies as the blood RNA signature analysis[7]. On the other hand, the new generation of QFN, the QFN-TB Gold Plus assay, includes two tubes containing specific antigen cocktails (TB1, containing peptides derived from ESAT-6 and CFP-10; similar to the previously used TB tube). As this mixture of peptide has been shown to primarily induce cytokine release from CD4 T cells, the QFT-Plus assay includes a second *M. tuberculosis* specific tube (TB2) that not only contains the ESAT-6 and CFP-10 derived peptides that elicit CD4 T cells from tube TB1, but also additional peptides optimized to stimulate CD8 T cells. As many immunodeficiencies are associated with either general lymphopenia or specific depletion of CD4 T cells, it is hypothesised that this additional tube will increase sensitivity of the diagnosis for LTBI and active tuberculosis across various groups of immunocompromised patients, and may also decrease the percentage of indeterminate results which were found to be high in patients with T-cell associated immunodeficiencies. However, recent published studies did not observe clear differences with the previous version[8].

It will also be considered a novel *M. tuberculosis*-specific skin test that contains ESAT-6 and CFP-10 antigens (C-Tb. Statens Serum Institut, Denmark) as a real alternative for the LTBI detection. The first publications reported similar sensitivity than QFN in active TB patients[9].

While waiting to the new generations of tests, the use of the IFN-γ-based tests in clinical practice should be guided by clinical judgement and evidence-based guidelines for different groups of patients. In this regards, SEIMC and SEPAR has recently published the clinical practice guidelines of the IGRAs in the different clinical situations[10].

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References

1. Dominguez J, Ruiz-Manzano J, De Souza-Galvao M, Latorre I, Mila C, Blanco S, *et al*. Comparison of two commercially available gamma interferon blood tests for immunodiagnosis of tuberculosis. *Clin Vaccine Immunol*. 2008;15:168-71.
2. Altet N, Dominguez J, Souza-Galvao ML, Jimenez-Fuentes MA, Mila C, Solsona J, *et al*. Predicting the Development of Tuberculosis with the Tuberculin Skin Test and QuantiFERON Testing. *Annals of the American Thoracic Society*. 2015;12:680-8.
3. Latorre I, De Souza-Galvao M, Ruiz-Manzano J, Lacoma A, Prat C, Altet N, *et al*. Evaluating the non-tuberculous mycobacteria effect in the tuberculosis infection diagnosis. *Eur Respir J*. 2010;35:338-42.
4. Pai M, Denkinger CM, Kik SV, Rangaka MX, Zwerling A, Oxlade O, *et al*. Gamma interferon release assays for detection of Mycobacterium tuberculosis infection. *Clinical microbiology reviews*. 2014;27:3-20.
5. Serra-Vidal MM, Latorre I, Franken KL, Diaz J, de Souza-Galvao ML, Casas I, *et al*. Immunogenicity of 60 novel latency-related antigens of Mycobacterium tuberculosis. *Frontiers in microbiology*. 2014;5:517.
6. Latorre I, Diaz J, Mialdea I, Serra-Vidal M, Altet N, Prat C, *et al*. IP-10 is an accurate biomarker for the diagnosis of tuberculosis in children. *J Infect*. 2014;69:590-9.
7. Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, Amon LM, *et al*. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. *Lancet*. 2016;387:2312-22.
8. Petruccioli E, Chiacchio T, Pepponi I, Vanini V, Urso R, Cuzzi G, *et al*. First characterization of the CD4 and CD8 T-cell responses to QuantiFERON-TB Plus. *J Infect*. 2016.
9. Hoff ST, Peter JG, Theron G, Pascoe M, Tingskov PN, Aggerbeck H, *et al*. Sensitivity of C-Tb: a novel RD-1-specific skin test for the diagnosis of tuberculosis infection. *Eur Respir J*. 2016;47:919-28.
10. Santin M, Garcia-Garcia JM, Rigau D, Altet N, Anibarro L, Casas I, *et al*. Panel of experts from the Mycobacteria Study Group of the Spanish Society of Infectious D, Clinical M, the Spanish Society of Respiratory D, Thoracic S. Executive summary of the guidelines for the use of interferon-gamma release assays in the diagnosis of tuberculosis infection. *Enfermedades infecciosas y microbiología clínica*. 2016;34:304-8.

C-Tb skin test: next steps

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Rationale

The WHO post-2015 global tuberculosis strategy emphasize detection and preventive treatment of *M.tuberculosis* infected individuals at risk. Current diagnostic options including the interferon gamma release assays (IGRA) and PPD Tuberculin Skin Test (TST) are either too complex or unspecific for implementation in the populations at highest need. Statens Serum Institut has developed a novel specific skin test, C-Tb, based on the antigens ESAT-6 and CFP10. C-Tb combines the field friendliness of TST, with the high specificity of the IGRA. This presentation gives and overview of results from two recently completed Phase III trials (TESEC-05 and -06) and outline current status of development at SSI.

Methods

The TESEC-06 trial included 979 participants from 13 clinical trial sites in Catalonia, Galicia and Basque Country with various risk of *M.tuberculosis* infection. The TESEC-05 trial included 1090 participants with symptoms of TB and 100 endemic controls both from Cape Town (South Africa). In both trials, C-Tb and TST were administered in a double-blinded fashion to one or the other forearm. Skin indurations were read 2-3 days later, a reading ≥5mm was considered positive for TST and C-Tb (cut off determined in Phase II trials). Blood for IGRA testing (Quantiferon, QFT-GIT) was drawn prior to skin testing.

Results

Test specificity was assessed in 212 presumed unexposed Spanish controls. Here, C-Tb had comparable specificity to QFT-GIT (both 97%, p=1.0, and there was no impact of BCG vaccination. In contrast, previous BCG vaccination had a strong negative impact on TST specificity, 62% (67/108) compared to 95% (99/104) in BCG unvaccinated (p<0.001). Sensitivity of C-Tb and QFT-GIT was comparable in patients with confirmed TB 77% (235/307) vs 81% (250/307) (p=0.08). In contacts, there was a strong trend in increasing C-Tb test positivity with *M.tuberculosis* exposure, at-par with QFT-GIT. The impact of age and HIV infection on C-Tb reactivity was assessed in 1090 individuals with symptoms suspect of TB disease. Young age (<5years) was associated with reduced size of induration for both C-Tb and TST. In HIV infected, C-Tb appeared more robust than IGRA with significantly higher number of positive responders and less impact of a low CD4 T cell count.

Discussion

These phase III trial results demonstrates that C-Tb has comparable diagnostic performance to QFT-GIT, and addresses the problem of false positive TST results in BCG vaccinated. The field-friendliness and high specificity offered by the C-Tb test, could allow for improved target treatment of *M.tuberculosis* infected in resource restraint settings, where IGRAs are too complicated to implement due to laboratory issues.

SSI are currently commercializing C-Tb and preparing for regulatory approval.

The future of latent TB screening

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There are scientific and practical (implementation) roadblocks to improved latent TB screening. The main scientific roadblock is that current diagnostic tests (TST and IGRAs) do not distinguish latently-infected persons at increased risk of progression to active disease. Immunological risk-stratification of latent TB will be discussed with reference to the strongest single risk factor for progression to diseases in immunocompetent adults. The main practical roadblock is to latent TB screening is poor participation by hard-to-reach and immigrant communities. The UK recently launched a free nationwide latent TB screening programme for all new immigrants from high-burden countries but participation rates have turned out to be low. Innovative, community-based approaches to enhance screening uptake will be discussed.

Rifapentine (Priftin®): A significant step forward in Tuberculosis control

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In recent years, the WHO End TB strategy started highlighting the importance of including the management of Latent Tuberculosis Infection (LTBI) to achieve elimination of Tuberculosis (TB) by 2035. Sanofi has been involved in the TB field for many years, starting with the discovery of rifamycins including rifapentine, and is still actively involved in the search for new compounds through its R&D. In 2011, a decade of research conducted by the United States Centers for Disease Control and Prevention in partnership with Sanofi resulted in the establishment of the shorter rifapentine plus isoniazid once a week for 3 months, so called "3HP" , regimen's efficacy and safety[1]. The 3HP regimen promises to make the treatment of LTBI easier to tolerate and complete, including for some of the populations most at risk of TB disease: young children and people living with HIV, thereby significantly contributing to the 2035 WHO achievement.

In 2014, the United States Food and Drug Administration (FDA) approved rifapentine (Priftin®) in combination with isoniazid for LTBI[2]. The 3HP regimen is included in the WHO's first-ever Guidelines on the Management of Latent Tuberculosis Infection, released in 2015[3]. In the same year, the WHO added rifapentine to its model Lists of Essential Medicines for adults and children[4]. In 2016, rifapentine was listed on the product catalogue of the Stop TB Partnership's Global Drug Facility, giving countries that receive support from the Global Fund to fight AIDS, TB, and Malaria a direct route to purchase the drug[5].

Based on the recent WHO and national recommendations, Sanofi intends to submit registration dossiers for rifapentine in several parts of the world, in line with current local treatment recommendations, regulatory needs and regulations which will give the best likelihood of meeting

the expectations of the regulatory agencies and national TB Control Programs.

Regarding EU submission and in line with current pediatric regulation, the European Medicines Agency requires that a Pediatric Investigation Plan (PIP) be submitted and approved by The Paediatric Committee (PDCO) before the dossier can be submitted. Sanofi is working on developing this PIP, which will be submitted in the best possible timeframe.

Sanofi acknowledges the invaluable support granted by US academics and public institutions, supported by partners in many countries, in the development of rifapentine in LTBI. We remain willing to establish similar public-private partnerships in other parts of the world in order to address the public health challenges posed by LTBI worldwide, extend disease awareness in national strategic plans and advocate for the importance of treating LTBI to reach WHO goals to eliminate TB.

References

1. Sterling TR, Villarino ME, Borisov AS, et al. Three months of rifapentine and isoniazid for latent tuberculosis infection. N Engl J Med. 2011 Dec 8; 365(23):2155–66. doi: 10.1056/NEJMoa1104875
2. Sanofi, "Sanofi receives FDA approval of Priftin® (rifapentine) tablets for the treatment of latent tuberculosis infection," 2 December 2014. Available from: http://www.news.sanofi.us/press-releases?item=136875
3. World Health Organization. Guidelines on the management of latent tuberculosis infection. Geneva: World Health Organization, 2015. Available from: http://www.who.int/tb/publications/ltbi_document_page/en/.
4. World Health Organization, "WHO moves to improve access to lifesaving medicines for hepatitis C, drug-resistant TB and cancers," 8 May 2015. Available from: http://www.who.int/mediacentre/news/releases/2015/new-essential-medicines-list/en/
5. See Global Drug Facility catalogue at http://www.stoptb.org/gdf/.