IVD_11 - Optimizing usage of remaining blood from Interferon Gamma Releasing Assay (IGRA) to search for immune signatures and biomarkers for Latent Tuberculosis Infection (LTBI)

Bárbara Suéllen Guimarães Marin Ferreira[1]; Marisa Aparecida Cairiac Nunes[2]; Denise do Socorro da Silva Rodrigues[2]; Paula Ordonhez Rigato[3].
[1]IAL;
[2]Clemente Ferreira Institute;
[3]Adolfo Lutz Institute/University of São Paulo.

Introduction: Tuberculosis (TB) is a serious public health problem, killing more than 1.5 million people a year. The WHO estimates that ¼ of the global population is infected with *M. tuberculosis* (Mtb). To end the TB epidemic, in addition to treating symptomatic cases, it is important to diagnose and treat latent TB (LTBI). LTBI is characterized by a persistent immune response to Mtb antigens without clinical evidence of TB. Interferon Gamma Release Assays (IGRA) are used to detect LTBI, one of them is the QuantiFERON-TB-Gold-Plus (QTF). In addition to diagnosing and treating LTBI, it is important to define biomarkers that characterize the risk of developing TB. Reusing blood remaining from clinical diagnostic tests to increase knowledge in TB is useful and avoids wasting the sample and recalling patients.

Objective: Optimize the use of IGRA remnant blood and define new biomarkers for Latent TB, correlating the expression of T cell activation molecules with IFN-g production in response to QTF Mtb antigens.

Methodology: Pheripheral blood samples from LTBI suspected subjects (n=38) was collected in tubes QTF-TB-Gold-Plus, incubated at 37°C, overnight. Plasma was collect and stored between 2-8ºC until perform the IFN-g. The remaining blood in the tubes was stained with monoclonal antibodies to CD45, CD3, CD4, CD8, CD69, CD71 and HLA-DR, lysates and evaluated in flow cytometer.

Results: We immunophenotyped the remaining blood cells used in the QTF test. Our preliminary results show that, as expected, most of the cells present in the lymphocyte gate are CD45+/CD3+ with a median of 77.8% (Tube N, Nill); 82.5% (Mitogen, M); 74.03% (TB1) and 76.75% (TB2). Considering CD4+ T cells, we detected 56% (N), 44% (M), 56% (TB1) and 55% (TB2). On the other hand, we showed that there were 28% (N), 25% (M), 31% (TB1) and 29% (TB2) of CD8+ T cells in the tubes. When we looked for activation markers (CD69, CD71 and HLA-DR) we showed that when T cells are stimulated with mitogen there are higher percentages of T cells, TCD4+ TCD8+ expressing these markers. For the antigen-specific tubes (TB1 and TB2) there were not enough QTF positive samples to conclude the correlation of IFN-g production with the expression of these activation markers.

Conclusion: We conclude that it is possible to better characterize the immune phenotype of remaining blood from IGRA.

Keywords: Latent tuberculosis; Interferon-Gamma Release Assays; Biomarkers