Accuracy of combined immune experimental tools to distinguish active tuberculosis from latent infection

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INTRODDUCTION: Tuberculosis (TB) represents a major public health problem, responsible for 9.6 million cases and 1.5 million deaths annually. Moreover, latent TB infection (LTBI), which is estimated to affect one-third of the world's population, may progress to active disease in about 3-10% of the LTBI individuals. Microscopic and cultural detection of *M. tuberculosis* (Mtb) in biological fluids are the commonly recommended tests for diagnosing and monitoring TB. However, these tests do not detect active TB patients who do not expectorate mycobacteria from the airways and their use is problematic in subjects with extra-pulmonary localizations. Therefore, blood-based host biomarkers for diagnosing active TB are attractive alternatives to tests depending on mycobacteria detection. Recently, using several concomitant cytometry approaches, we showed that evaluating CD27 surface expression on Mtb-specific-$CD4^+$T-cells (1) and the proportion of Mtb-specific $IFN\gamma^+TNF\alpha^+CD4^+$T-cells (2) are useful biomarkers for discriminating active disease from LTBI in a low TB-endemic country.

AIM

To evaluate if the combined assessment of different cytometric tests for CD27 assessment and cytokine profile characterization may improve the accuracy of active TB diagnosis.

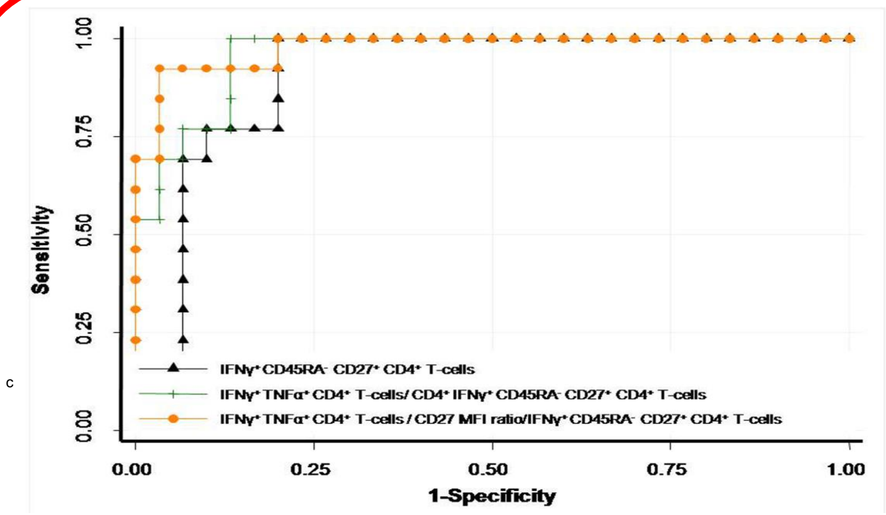
MEHOTDS: We enrolled 13 active TB and 30 LTBI subjects. The active TB enrolled within 7 days of starting therapy (Mtb isolation from sputum culture and drug-sensitive to first line drugs). LTBI were QFT-IT positivite with noo clinical, microbiological and radiological signs of active TB. Whole blood cells were stimulated with recombinant proteins ESAT-6 and CFP-10 to induce the $CD4^+$T-cell specific response. Cytokine profile ($IFN\gamma^+TNF\alpha^+$) and phenotype ($CD45RA^{-/+}CD27^{-/+}$) of Mtb-specific $CD4^+$T-cells were characterized by cytometry. The immunological markers evaluated were: (a) $IFN\gamma^+TNF\alpha^+CD4^+$T-cells; (b) $CD45RA^-CD27^{-/+}IFN\gamma^+CD4^+$T-cells; (c) $CD27^{-/+}IFN\gamma^+CD4^+$T-cells; (d) CD27 MFI RATIO, which is the median fluorescence intensity of CD27 in the $CD4^+$T-cell gate over the MFI of CD27 in the $CD4^+IFN\gamma^+$T-cells (3). By Logistic Regression (LR) we modelled the probability of active TB given several immunological markers (14). To begin, each pairwise combinations of tests were explored and evaluated for collinearity. When collinearity arose, only markers without collinearity were included for further analysis. After each LR's fitting, the predicted probability of active TB was estimated. The linear score obtained was used to build the ROC Curve. The Area Under the ROC Curve (AUC) was the parameter chosen to evaluate and compare the different biomarker combinations. Moreover, tests were performed to evaluate the equality of the ROC areas.

Table.1. Accuracy of biomarkers evaluated as a "single test" or "combined tests" for discriminating active TB from LTBI

	Tests	AUC	95% CI	P
Single test	$IFN\gamma^+TNF\alpha^+CD4^+$T-cells	0.828	0.688-0.969	0.483
	CD27 MFI ratio	0.841	0.681-1.000	
	$IFN\gamma^+CD45RA^-CD27^-$ T-cells	0.849	0.731-0.967	
	$IFN\gamma^+CD27^-$ T-cells	0.903	0.813-0.993	
	$IFN\gamma^+CD45RA^-CD27^+$ T-cells	0.905	0.812-0.998	
Pairwise test combinations	$IFN\gamma^+TNF\alpha^+$ T-cells CD27 MFI ratio	0.918	0.836-0.999	0.619
	$IFN\gamma^+CD45RA^-CD27^+$ T-cells CD27 MFI ratio	0.954	0.895-1.000	
	$IFN\gamma^+TNF\alpha^+$ T-cells $IFN\gamma^+CD45RA^-CD27^+$ T-cells	0.959	0.909-1.000	
Combined tests	$IFN\gamma^+TNF\alpha^+$ T-cells CD27 MFI ratio $IFN\gamma^+CD45RA^-CD27^+$ T-cells	0.977	0.940-1.000	-

Footnotes: IFNγ: interferon γ; TNFα: tumor necrosis factor α, AUC: area under the curve; CI: confidence interval

RESULTS: Moving from the single test analysis to the pairwise combination and finally to the identification of a new variable (score) to discriminate patients with active TB and LTBI, we show that: (i) different cytometric tests based on the modulation of CD27 discriminate active TB from LTBI (CD45RA- CD27-/+ IFNγ+ CD4+ T-cells, CD27-/+ IFNγ+ CD4+ T-cells, CD27 MFI RATIO AUC from 0.841 to 0.905); (ii) the test based on IFNγ+ TNFα+ CD4+ T-cells discriminates active TB from LTBI (AUC 0.828); (iii) the pairwise combination of the different tests shows a better AUC compared to the single test (combination of: CD45RA- CD27+ IFNγ+ CD4+ T-cells, CD27 MFI RATIO, IFNγ+TNFα+ CD4+ T-cells, AUC from 0.918 to 0.959); (iv) the best AUC is reached generating a new variable measure by combining the three different tests (CD45RA-CD27+IFNγ+CD4+T-cells/CD27 MFI RATIO/ IFNγ+TNFα+ CD4+ T-cells; AUC 0.977).



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Comparison of the AUC of the three different immunological tests. The graph shows the AUC of the three different tests based on $IFN\gamma^+CD45RA^-CD27^+CD4^+$T-cells; $IFN\gamma^+TNF\alpha^+CD4^+$T-cells/$IFN\gamma^+CD45RA^-$; $IFN\gamma^+TNF\alpha^+CD4^+$T-cells/CD27 MFI ratio/$IFN\gamma^+CD45RA^+$ $CD27^+CD4^+$T-cells. No significant difference was obtained (p=0.185)

CONCLUSIONS: The combination of the 3 immunological tests based on the CD27 evaluation and cytokine profile characterization may improve the accuracy of active TB diagnosis.

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