Potential LTBI biomarkers in ESAT-6 and CFP10 stimulated plasma

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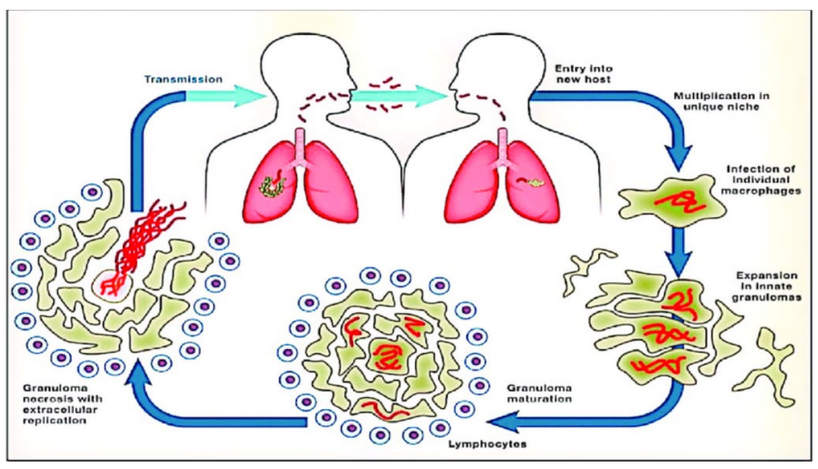
1. Introduction

- One third of world population is latently infected with *Mycobacterium tuberculosis* (MTB) (1,2)
- It is estimated that 5-10% of the latently infected individuals will advance to active TB
- WHO has recommended diagnosis and treatment of latent TB infection (LTBI) to prevent progression to active tuberculosis(2)
- Thus, there is urgent need for development of diagnostic biomarkers to distinguish LTBI from acute TB and healthy individuals

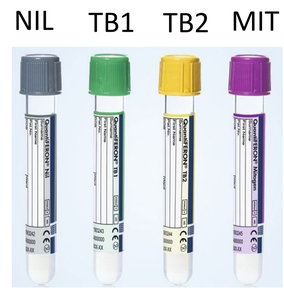
Pathogenesis of LTBI



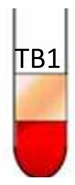
Aim: To identify blood-based LTBI biomarkers in CFP-10/ESAT6 stimulated plasma

2. Methods

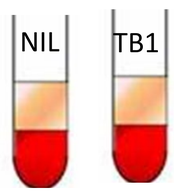
- Blood from 20 healthy and 20 LTBI individuals were collected, both groups were all HIV negative and TB negative on GeneXpert MTB/RIF Ultra
- IGRA assay was performed on all samples
- 1 mL of whole blood was collected directly into each IGRA tube (NIL, TB1, TB2 and Mitogen)
- Tubes were incubated at 37°C for 20 hours and plasma was isolated



- 27 Multiplex Elisa was performed on plasma from TB1 tube (stimulated by ESAT-6 and CFP-10)

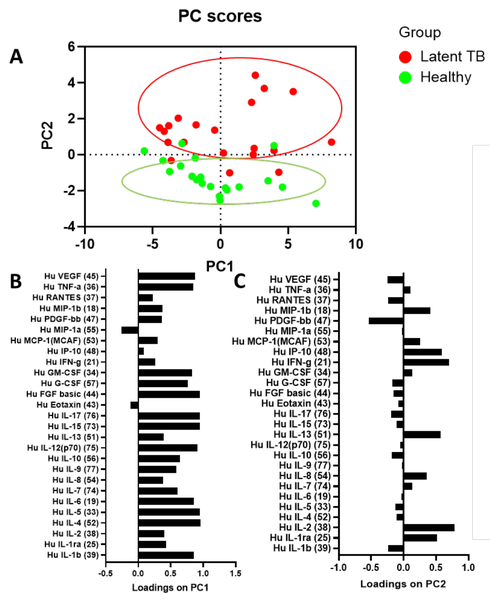


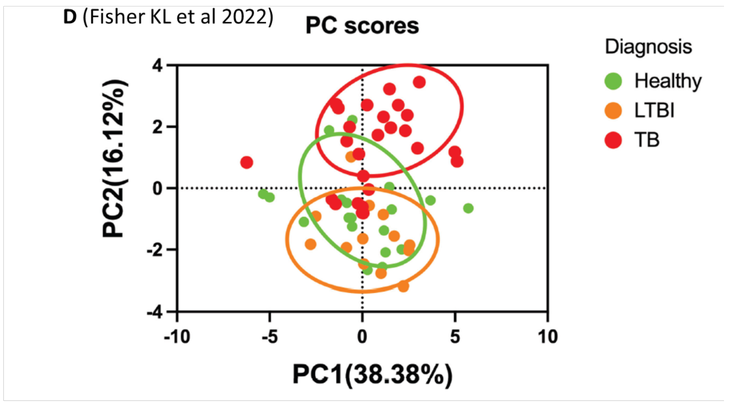
- IL-2, IP-10, and INF-γ Elisa assay was done on plasma from the NIL tube (unstimulated) and plasma from the TB1 tube (stimulated by ESAT-6 and CFP-10)



3. Results

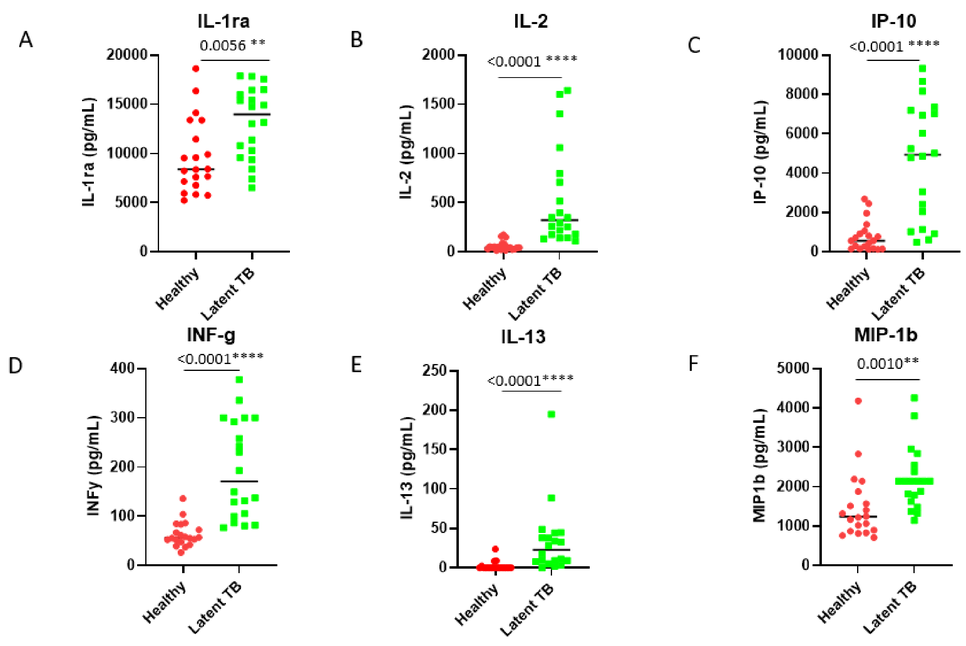
Circulatory cytokines may distinguish between LTBI and healthy individuals





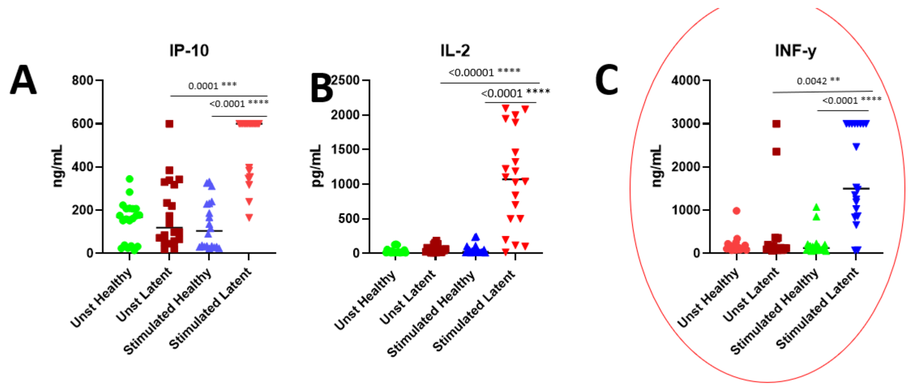
- A, B and C: Biomarkers IL-1ra, IL-2, IL-13, INF-γ, IP-10, and MIP1b, segregated according to LTBI along PC2 after stimulation
- D: Data by Fisher KL et al.(3) show no difference between unstimulated plasma cytokines from healthy and LTBI individuals

IL-1ra, IL-2, IP-10, INF-γ, IL-13, and MIP-1b are abundant in LTBI compared to healthy individuals



- Markers associated with LTBI were identified by Multiplex ELISA.
- There were significant differences between LTBI and healthy for IL-1ra (p=0.0056), IL-2 (p<0.0001), IP-10 (p<0.0001), INF-γ (p<0.0001), IL-13 (p<0.0001), and MIP-1b (p=0.0010)

Whole blood stimulation with MTB-specific antigens induces unique cytokines in LTBI



- To determine the effect of antigen stimulation on IP-10, IL-2 and INF-γ production, we compared unstimulated and stimulated plasma among healthy and LTBI groups.
- IP-10, IL-2 and INF-γ were significantly increased in stimulated LTBI compared to the stimulated healthy group and both unstimulated healthy and LTBI groups

4. Summary

- The measurements of IL-1ra, IL-2, IL-13, IP-10, INF-γ and MIP-1b in ESAT-6 and CFP-10 stimulated plasma can distinguish LTBI from healthy individuals
- These cytokines could be considered for further trials as potential diagnostic biomarkers of LTBI at point-of-care.

Next Experiments:

- Fluorescence activated cell sorting (FACS) intracellular staining for IL-2, INF-γ and IP-10 on stimulated and unstimulated peripheral blood mononuclear cells (PBMC)

References:
1. WHO. Global Tuberculosis Report 2020. Geneva: World Health Organization; 2020.
2. WHO. Latent tuberculosis infection. 2018.
3. Fisher KL et al. Elevated IP-10 at the Protein and Gene Level Associates With Pulmonary TB. Front Cell Infect Microbiol. 2022;12:908144.