

10 ABSTRACT

Latent tuberculosis infection (LTBI) remains one of the major challenges in the global fight against tuberculosis (TB). Despite significant advancements in the diagnosis and treatment of active TB, LTBI continues to affect approximately one-quarter of the world's population. Although a gradual decline in prevalence has been observed in recent years, the rate of decline remains insufficient to meet the World Health Organization's (WHO) End TB Strategy targets. An estimated 5–10% of individuals with LTBI is at risk of progression to active disease, especially in the presence of risk factors such as immunosuppression, chronic illnesses, or malnutrition. Therefore, effective identification of individuals with LTBI is one of the foundations of contemporary programmes of fight against TB.

Current diagnostic tools for LTBI including the tuberculin skin test (TST), interferon- gamma release assays (IGRAs), and emerging skin tests are indirect and rely on an intact immune response. None of these methods can differentiate latent from active TB infection or predict disease reactivation. Nonetheless, individuals with LTBI represent a substantial reservoir of future TB cases, justifying the urgent need for novel biomarkers and diagnostic tools allowing for more accurate evaluation of a patient's clinical condition.

This study aimed to evaluate the diagnostic performance of the IGRA, QuantiFERON- TB Gold Plus (QFT-Plus) and its automated version, Liaison QFT-Plus, and to identify biomarkers capable of distinguishing LTBI from active TB.

A total of 5,443 IGRA results collected between 2017 and 2024 at the Department of Microbiology, National Institute of Tuberculosis and Lung Diseases in Warsaw, was analyzed. The study population was divided into three cohorts: individuals with single QFT-Plus test result ($n = 712$), individuals tested with both QFT-Plus and Liaison QFT-Plus ($n = 269$) and individuals whose plasma samples were analyzed for 20 cytokines after QFT-Plus testing ($n = 145$).

Positive QFT-Plus results were significantly more frequent in females and children, which suggests age- and sex-related differences in immune response. Moreover, individuals with a positive QFT-Plus result had seven-fold increased risk of developing active TB ($RR = 7.2$), reinforcing QFT-Plus value in identifying LTBI cases with

high- risk of progression. IFN- γ levels in TB1 and TB2 tubes of QFT-Plus were comparable, which indicates similar contributions of CD4⁺ and CD8⁺ T cells in *Mycobacterium tuberculosis*-specific immune responses. The diagnostic accuracy of QFT-Plus was higher in children, and optimal IFN- γ cut-off values closely approximated the recommended threshold of 0.35 IU/ml.

Comparison between QFT-Plus and Liaison QFT-Plus showed over 89.5% agreement, with the best concordance achieved at a 0.63 IU/ml threshold Liaison QFT-Plus. These findings support the use of Liaison QFT-Plus as a reliable alternative, particularly in highthroughput diagnostic laboratories.

The most innovative aspect of this study was the in-depth analysis of cytokine profiles. It was demonstrated that the combination of IL-7 and IFN- γ plasma concentrations makes it possible to effectively differentiate LTBI from active TB. The developed diagnostic model reached AUROC 98.1%, sensitivity 94.6%, specificity 96.5%. Furthermore, levels of MIG/CXCL9 were significantly higher in active TB compared to LTBI and healthy controls, supporting its role as a promising biomarker of active stage of infection.

From a public health perspective, the obtained results may contribute to effective identification of patients requiring preventive therapy, optimisation of therapeutic decisions, as well as limiting TB transmission. The incorporation of novel biomarkers into routine diagnostics may significantly support TB elimination and make it possible to meet the targets of global WHO strategy for 2035.