Improving interferon-γ release assay interpretation: are IP-10 and MIG the solution?

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As a gold standard to diagnose TB infection remains elusive, the need to identify new biomarkers to support the tools we currently rely on endures. IP-10 and MIG detection could improve IGRA interpretation. https://bit.ly/3wUGD0Z

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It is a bumpy road, to develop a tuberculosis infection (TBI) test with the capability of predicting progression to active tuberculosis (TB). As a gold standard for TBI diagnosis with these characteristics is still missing, tuberculin skin tests (TSTs) and the interferon-γ (IFN-γ) release assays (IGRAs), including QuantiFERON-TB Gold Plus (QFT-plus) and T-SPOT.TB, remain the main tests used to identify people at higher risk of developing TB disease [1].

Although IGRAs have overcome some of the TST limitations, high costs and the need for adequate infrastructure and skilled personnel still represent major shortcomings to these tests' widespread implementation [2, 3]. Furthermore, several studies evaluating IGRA performance reported a high conversion or reversion rate for the results close to the test cut-off, primarily due to high variability in pre-analytical aspects [4, 5]. A borderline zone, for results close to the clinical cut-off, has been proposed by several international and national organisations, to reduce the risks related to transmission and following clinical action of false-negative and false-positive results [4, 6].

Even with several new TBI diagnostic tests being introduced to the market in the past few years, and with some of them presenting operational advantages [2, 7], none fulfils, so far, the requirements to be considered a gold standard for TBI diagnosis [1, 2]. Therefore, the need for the identification of new biomarkers or a multiple marker biosignature to improve the tools we currently have for TBI diagnosis endures [8].

In this context, several studies have explored the diagnostic potential of different *Mycobacterium tuberculosis* (MTB)-antigen stimulated cytokines or, better, different combinations of cytokines and chemokines as possible biomarkers to identify TB-infected individuals [9–13]. However, even if numerous cytokine biomarkers have been suggested to differentiate TBI and active TB, to evaluate therapy efficacy and to predict the progression from TBI to active TB, wide heterogeneity in performance and role of these markers has been reported [14].

The study by Uzorka *et al.* [15] enters this crowded landscape with the specific aim of finding a possible biosignature or a single biomarker to better predict true TBI and distinguish it from random test variation in the context of borderline QFT-plus results. A panel of 48 cytokines, chemokines and growth factors has been tested on the supernatants of QFT-plus samples collected from a cohort of 195 patients. This analysis revealed three biomarkers: IFN-γ inducible protein 10 (IP-10), monokine induced by IFN-γ (MIG) and interleukin 1 receptor antagonist (IL-1ra), for which values were higher in patients with a clearly positive

QFT-plus in comparison to individuals with a low-negative QFT-plus result. The statistical analysis performed demonstrated that IP-10 and MIG had a positive correlation and were extremely accurate in predicting TBI as defined by a high positive QFT-plus result. In contrast, IL-1ra had only moderate accuracy and moderate to low correlation with the other two cytokines. Moreover, IL-1ra levels in borderline samples were not significantly different from the ones detected in the samples tested as low negative with QFT-plus. The developed prediction model, including only MIG and IP-10, predicted that almost two-thirds of the patients with a borderline result have TBI, in line with precedent estimates performed by the same group in different patient cohorts [16].

IP-10 and MIG are both recognised actors of the host's defence against an infection sustained by MTB and target of several studies that over the years have tried to use these biomarkers for TB diagnosis [17]. In particular, an involvement of IP-10 and MIG has been reported in the immunity cascade linked to granuloma formation [18, 19], as well as an increase in the serum levels of IP-10 and MIG during TB infection [20]. MIG and IP-10 level decrease has also been associated with response to TB therapy [21] and lateral flow assays employing IP-10 as readout marker are currently under development, even if little information is available on their performance [2]. Furthermore, both MIG and IP-10 have been evaluated as possible biomarkers to discriminate between active TB and TB infection [22–24], and agonist and antagonist forms of IP-10 have been identified both in blood and urine of people with active TB [25, 26].

Interestingly, Uzorka *et al.* [15] reported that the IP-10/MIG signature allocated five TB active cases identified during the study in the QFT-plus high positive group. Nonetheless, the authors decided not to include a TB active cohort into the study. Expanding the performed analysis to include a cohort of TB active patients, as well as for the TBI cohort subgroups by BCG vaccination status and by likelihood of exposure to nontuberculous mycobacteria [1], could be an important next step to assess the sensitivity and specificity of these markers. This would help to further validate the markers and the predictive model. Moreover, host-based biomarkers can be influenced by the immune status of the host. In future studies, a sufficient number of immunocompromised and/or immunosuppressed patients could be included in order to evaluate and draw conclusions on the utility of these host biomarkers in this population.

Although the reported study shows some limitations, the proposed model presents an attractive operational advantage: the possibility of measuring IP-10 and MIG levels as reflex tests directly on the previously collected QFT-plus tubes, without the need to recall patients to obtain a second blood sample. In low endemicity settings, where IGRA tests are primarily performed, TBI screening usually targets immunocompromised individuals, patients initiating therapy with tumour necrosis factor-α inhibitors or those preparing for organ or haematological transplant [27]. In these risk groups, the need to start immunosuppressive therapy as soon as possible is well recognised, as well as the difficulties in draining several blood samples from patients who have undergone numerous infusion treatments. Therefore, the detection of IP-10 and MIG as a reflex test to be performed in case of borderline results, or the integration of IP-10 and MIG into IGRAs alongside IFN-γ, could simplify diagnosis in this population and allow faster access to life-saving therapies.

Conflict of interest: The authors have nothing to disclose.

References

1 Hamada Y, den Boon S, Cirillo DM, *et al.* Framework for the evaluation of new tests for tuberculosis infection. *Eur Respir J* 2021; 58: 2004078.
2 Migliori GB, Ong CWM, Petrone L, *et al.* The definition of tuberculosis infection based on the spectrum of tuberculosis disease. *Breathe* 2021; 17: 210079.
3 Goletti D, Delogu G, Matteelli A, *et al.* The role of IGRA in the diagnosis of tuberculosis infection, differentiating from active tuberculosis, and decision making for initiating treatment or preventive therapy of tuberculosis infection. *Int J Infect Dis* 2022; 7: 39.
4 Folkvardsen DB, Holicka Y, Ködmön C, *et al.* Reporting of interferon-gamma release assay results close to cut-off values. *Int J Tuberculosis Lung Dis* 2021; 25: 66–68.
5 Tagmouti S, Slater M, Benedetti A, *et al.* Reproducibility of interferon gamma (IFN-γ) release assays. A systematic review. *Ann Am Thorac Soc* 2014; 11: 1267–1276.
6 Wikell A, Åberg H, Shedrawy J, *et al.* Diagnostic pathways and delay among tuberculosis patients in Stockholm, Sweden: a retrospective observational study. *BMC Public Health* 2019; 19: 151.
7 Saluzzo F, Mantegani P, Poletti de Chaurand V, *et al.* QIAreach™ QuantiFERON®-TB for the diagnosis of M. tuberculosis infection. *Eur Respir J* 2021; 59: 2102563.

8 MacLean E, Broger T. A systematic review of biomarkers to detect active tuberculosis. *Nat Microbiol* 2019; 4: 748–758.
9 Kim SY, Park MS, Kim YS, *et al.* The responses of multiple cytokines following incubation of whole blood from TB patients, latently infected individuals and controls with the TB antigens ESAT-6, CFP-10 and TB7.7. *Scand J Immunol* 2012; 76: 580–586.
10 Borgström E, Andersen P, Atterfelt F, *et al.* Immune responses to ESAT-6 and CFP-10 by FASCIA and multiplex technology for diagnosis of *M. tuberculosis* infection; IP-10 is a promising marker. *PLoS ONE* 2012; 7: e43438.
11 Walzl G, Ronacher K, Hanekom W, *et al.* Immunological biomarkers of tuberculosis. *Nat Rev Immunol* 2011; 11: 343–354.
12 Clifford V, Tebruegge M, Zufferey C, *et al.* Cytokine biomarkers for the diagnosis of tuberculosis infection and disease in adults in a low prevalence setting. *Tuberculosis* 2019; 114: 91–102.
13 Chegou NN, Heyckendorf J, Walzl G, *et al.* Beyond the IFN-γ horizon: biomarkers for immunodiagnosis of infection with *Mycobacterium tuberculosis*. *Eur Respir J* 2014; 43: 1472–1486.
14 Wei Z, Li Y, Wei C, *et al.* The meta-analysis for ideal cytokines to distinguish the latent and active TB infection. *BMC Pulm Med* 2020; 20: 248.
15 Uzorka JW, Bakker JA, van Meijgaarden KE, *et al.* Biomarkers to identify *Mycobacterium tuberculosis* infection among borderline QuantiFERON results. *Eur Respir J* 2022; 60: 2102665.
16 Uzorka JW, Bossink AWJ, Franken WPJ, *et al.* Borderline QuantiFERON results and the distinction between specific responses and test variability. *Tuberculosis (Edinb)* 2018; 111: 102–108.
17 Santos VS, Goletti D, Kontogianni K, *et al.* Acute phase proteins and IP-10 as triage tests for the diagnosis of tuberculosis: systematic review and meta-analysis. *Clin Microbiol Infect* 2019; 25: 169–177.
18 Whittaker E, Gordon A, Kampmann B. Is IP-10 a better biomarker for active and latent tuberculosis in children than IFNγ? *PLoS ONE* 2008; 3: e3901.
19 Chung W-Y, Lee K-S, Park J-H, *et al.* TB antigen-stimulated CXCR3 ligand assay for diagnosis of tuberculous lymphadenitis. *Int J Environ Res Public Health* 2021; 18: 8020.
20 Bai X, Chmura K, Ovrutsky AR, *et al. Mycobacterium tuberculosis* increases IP-10 and MIG protein despite inhibition of IP-10 and MIG transcription. *Tuberculosis* 2011; 91: 26–35.
21 Zimmer AJ, Lainati F, Aguilera Vasquez N, *et al.* Biomarkers that correlate with active pulmonary tuberculosis treatment response: a systematic review and meta-analysis. Date last updated: 31 Aug 2021. Date last accessed: 28 Mar 2022. https://github.com/stmcg/tmsr
22 Li Y, He D, Che Y, *et al.* Monokine induced by gamma interferon for detecting pulmonary tuberculosis: a diagnostic meta-analysis. *Medicine* 2020; 99: e23302.
23 Chung WY, Lee KS, Jung YJ, *et al.* A TB antigen-stimulated CXCR3 ligand assay for the diagnosis of active pulmonary TB. *Chest* 2014; 146: 283–291.
24 Petrone L, Vanini V, Chiacchio T, *et al.* Evaluation of IP-10 in Quantiferon-Plus as biomarker for the diagnosis of latent tuberculosis infection. *Tuberculosis* 2018; 111: 147–153.
25 Blauenfeldt T, Petrone L, del Nonno F, *et al.* Interplay of DDP4 and IP-10 as a potential mechanism for cell recruitment to tuberculosis lesions. *Front Microbiol* 2018; 9: 1456.
26 Petrone L, Bondet V, Vanini V, *et al.* First description of agonist and antagonist IP-10 in urine of patients with active TB. *Int J Infect Dis* 2019; 78: 15–21.
27 Migliori GB, Wu SJ, Matteelli A, *et al.* Clinical standards for the diagnosis, treatment and prevention of TB infection. *Int J Tuberculosis Lung Dis* 2022; 26: 190.