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**Title:** The performance of flow cytometry in the diagnosis of latent tuberculosis infection in patients under treatment with anti-TNF $\alpha$  and other biological agents

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**Body:** Introduction: Detection of IFN- $\gamma$  synthesis in the cytoplasm of activated CD4+ T cells by flow cytometry [intracellular cytokine flow cytometry (ICCFC)] is a new method to diagnose tuberculosis (TB) infection. The aim of this study is to investigate the performance of Mantoux, Quantiferon Gold In-tube (QFN-G-IT) and ICCFC in patients with collagen vascular diseases who are on treatment with biological agents, including anti-TNF $\alpha$ . Methods: Mantoux, QFN-G-IT and ICCFC were performed in 54 medically immunosuppressed patients and in a cohort of immunocompetent individuals consisting of a group of 39 close contacts with an index case of TB. Results: In patients under anti-TNF $\alpha$  treatment, 28/54 (52%) were ICCFC ESAT-6 (+), compared with QFN-G-IT (+) who were 3.7% ( $p < 0.0001$ ), and Mantoux positive who were 15% ( $p < 0.0001$ ). Additionally, 35/54 (65%) were ICCFC PPD (+) ( $p < 0.0001$  in comparison with QFN-G-IT (+) and  $p < 0.0001$  in comparison with Mantoux positive). In close contacts, 27 were ICCFC ESAT (+) (69%) in comparison with QFN-G-IT (+) who were 41% ( $p < 0.02$ ). 29 were ICCFC PPD (+) (74%) ( $p = 0.0047$  vs QFN-G-IT (+)). However, there was no statistical difference between ICCFC and Mantoux. ICCFC indeterminate results were observed in the immunosuppressed (5/54) and in 5 out of 39 immunocompetent patients ( $p = ns$ ). Conclusion: The performance of ICCFC for the diagnosis of TB infection is better than QFN-G-IT and Mantoux in patients under immunosuppressive treatment and in immunocompetent close contacts. The robustness of ICCFC test is not substantially affected by the administration of immunosuppressive treatment.

