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**Title:** Application of intracellular cytokine flow cytometry in the diagnosis of active tuberculosis

Jaehee 23249 Lee rainforfrog@daum.net MD <sup>1</sup>, Eun Jin 23250 Kim kejean@medimail.co.kr MD <sup>2</sup>, Seung Soo 23251 Yoo 007md@hanmail.net MD <sup>1</sup>, Shin Yup 23252 Lee shinyup@knu.ac.kr MD <sup>1</sup>, Seung Ick 23253 Cha sicha@knu.ac.kr MD <sup>1</sup>, Jae Yong 23259 Park jaeyong@knu.ac.kr MD <sup>1</sup> and Chang Ho 23260 Kim kimch@knu.ac.kr MD <sup>1</sup>. <sup>1</sup> Internal Medicine, Kyungpook National University, School of Medicine, Daegu, Korea and <sup>2</sup> Internal Medicine, Cha University Hospital, Gumi, Korea .

**Body:** Background: Intracellular cytokine flow cytometry (ICCFC) has been introduced to detect the T cell response to M. tuberculosis antigen (MTB Ag) to overcome the limits shown by whole-blood interferon-gamma (IFN- $\gamma$ ) release assays (IGRA). Given the important role of CD4+T cells as well as IFN- $\gamma$  and TNF- $\alpha$  in pathogenesis of TB, we compared the diagnostic accuracy between ICCFC measuring frequencies of MTB-specific Ag stimulated IFN- $\gamma$ +TNF- $\alpha$ +CD4+T cells and IGRA to confirm the usefulness of application of ICCFC for TB diagnosis in clinical practice. Methods: Both QuantiFERON<sup>®</sup> TB Gold In-Tube (QFT-IT) test and ICCFC analysis were performed in 80 patients who were suspected of having pulmonary TB or TB pleurisy and 10 controls with no known exposure to TB. Results: (1) Sixty one and 19 out of total 80 patients were diagnosed with active TB and non-TB, respectively. (2) Double IFN- $\gamma$ +TNF- $\alpha$ +CD4+T cells among all T cell subsets analyzed by ICCFC showed the highest sensitivity (90%) for diagnosis of TB. (3) Sensitivity of QFT-IT test and ICCFC assay were 77% and 90%, respectively (p = 0.021). (4) Specificity of QFT-IT and ICCFC assay were 73.7% and 89.5%, respectively. (5) There was a good correlation between the quantity of IFN- $\gamma$ , as detected by the QFT-IT test, and the frequencies of IFN- $\gamma$ +TNF- $\alpha$ +CD4+T cell measured by the ICCFC assay in TB patients (p=0.012). (6) The frequencies of IFN- $\gamma$ +TNF- $\alpha$ +CD4+T cells were significantly decreased after 6 months of treatment compared with pretreatment (p=0.026). Conclusions: The ICCFC assay with T cells stimulation by MTB-specific Ag may be a useful additional tool for the diagnosis of active TB, although further study is needed for more convincing data.