M. Tebruegge*,#,¶, T. Connell*,#,¶, N. Ritz*,#,¶, P.A. Bryant*,#,¶ and N. Curtis*,#,¶

*Dept of Paediatrics, The University of Melbourne, #Infectious Diseases Unit, Dept of General Medicine, and ¶Murdoch Children's Research Institute, Royal Children's Hospital Melbourne, Parkville, Victoria, Australia.

Correspondence: M. Tebruegge, Dept of Paediatrics, The University of Melbourne, Royal Children's Hospital Melbourne, Parkville, VIC 3052, Australia. E-mail: marc.tebruegge@rch.org.au

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From the authors:

We do appreciate the comments by M. Tebruegge and co-workers about our recently published manuscript in the *European Respiratory Journal* [1]. The aim of this reply is to clarify some points in order to interpret better the results of the study, given that we think there were some misunderstandings.

In vitro assays for measuring interferon (IFN)-γ released by the T-cells after specific *Mycobacterium tuberculosis* stimulation have demonstrated promising results in adults and also in children for diagnosing tuberculosis (TB) infection [2, 3]. However, there are discordant results between IFN-γ based assays and the tuberculin skin test (TST) that require clarification in order to assess the real utility of the *in vitro* tests in the management of patients [2, 4].

In our study we determined the potential role of non-tuberculous mycobacteria (NTM) sensitisation in children as a factor of discordant results between TST and an *in vitro* T-cell based assay (T.SPOT.*TB*; Oxford Immunotec, Oxford, UK). We enrolled 21 non-bacille Calmette–Guérin vaccinated paediatric patients for suspicion of latent TB infection (LTBI). These patients yielded a positive TST and a negative T.SPOT.*TB*. Cells were stimulated with *Mycobacterium avium* sensitin (MAS) and the presence of reactive T-cells was determined by an *ex vivo* enzyme-linked immunospot assay. From the 16 patients with a valid result, in 10 cases we obtained a positive ELISPOT result after stimulation with MAS.

Our main disagreement with the argumentation by M. Tebruegge and co-workers resides in the fact that we are not using MAS for distinguishing *M. tuberculosis* from NTM infection. For this objective, we used the specific *M. tuberculosis* RD1 antigens included in the T.SPOT.*TB* test, and, as no response against RD1 antigens was obtained, we assessed T-cell sensitisation against MAS antigens to investigate if NTM sensitisation could be responsible for TST positivity. Indeed, LEIN *et al.* [5], also referred to in the letter by M. Tebruegge and co-workers, obtained significant immune responses to ESAT-6 from 59% of pulmonary *M. tuberculosis* disease patients diagnosed, but no response was obtained from patients with *M. avium* complex pulmonary disease.

However, we agree with M. Tebruegge and co-workers that in some cases alternative explanation can also be possible. Given that MAS are not totally specific, and cross-reactions with other mycobacteria species have been described, we cannot totally exclude the possibility that we are detecting, in some cases, a response of specific T-cells against some *M. tuberculosis* antigens different from ESAT-6 and CFP-10; or a false-negative result of the T.SPOT.*TB*.

On the one hand, M. Tebruegge and co-workers have shown some concerns about our group of children with positive TST and positive T.SPOT.*TB* where 50% of children responded to the MAS. The results are in concordance with the known cross-reaction between MAS and other mycobacteria. Nevertheless, we cannot totally reject simultaneous infection of *M. tuberculosis* and NTM. Furthermore, these results are in total agreement with those obtained by LEIN *et al.* [5], where they found response against MAS in 24 out of 27 *M. tuberculosis* disease patients.

On the other hand, we want to point out that the main MAS positive results were obtained in children enrolled during LTBI screening at school with TST induration >5 mm and <10 mm. In all these children a complete medical exploration, including clinical and radiographic studies, was performed, and active TB was excluded. In the subsequent contact tracing studies no index case was found. Based on the classical studies performed by NYBOE [6], the main guidelines in this kind of child population consider as a cut-off for *M. tuberculosis* infection a TST induration $\geqslant 10$ mm, in order to avoid false-positive TST results induced by NTM immunisation [7]. Nevertheless, indurations >15 mm [8] and 20 mm [9] have been reported in children with NTM infections. Therefore, our results reinforce, in part, the guidelines in that unnecessary chemoprophylaxis treatment in this unexposed population could be avoided, and that IFN-γ based assays could help to confirm a positive TST result.

Children from contact-tracing studies truly exposed to an active TB case merit special consideration as they can develop the disease very quickly after primary infection, with the most severe forms prevailing in younger children [10]. For this child population we did not recommend withholding the chemoprophylaxis;

but we stated that, according to our results, IFN-γ based assays could reduce unnecessary chemoprophylaxis in non-*M. tuberculosis* infected children. In fact, BAKIR *et al.* [11] in a recent study concluded that a positive IFN-γ based assay result predicted the development of active TB as well as the TST, allowing more focused preventive therapy to fewer contacts.

In conclusion, we believe our results provide enough evidence that previous NTM sensitisation induces false-positive results in the TST for diagnosing LTBI; but, we also strongly agree with TEBRUEGGE *et al.* that additional studies are needed in order to clarify different issues related to the discordant IFN-γ based assay results, and to assess the real utility in the management and benefit of a child population.

I. Latorre*,¶,+, M. De Souza-Galvão¶,§, J. Ruiz-Manzano#,¶,+, A. Lacoma*,¶,+, C. Prat*,¶,+, N. Altet§, V. Ausina*,¶,+ and J. Domínguez*,¶,+

*Servei de Microbiologia, and #Servei de Pneumologia, Hospital Universitari ''Germans Trias i Pujol'', Fundació Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, +Ciber Enfermedades Respiratorias, Instituto de Salud Carlos III, Badalona, ¶Universitat Autònoma de Barcelona, Bellaterra, and §Unidad de Prevención y Control de la Tuberculosis de Barcelona, Barcelona, Spain.

Correspondence: J. Domínguez, Servei de Microbiologia, Fundació Institut d'Investigació en Ciències de la Salut ''Germans Trias i Pujol'', Carretera del Canyet s/n, 08916 Badalona, Barcelona. Spain. E-mail: jadomb@gmail.com

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Complete smoking cessation is beneficial in older and more advanced COPD patients

To the Editors:

We read with great interest the article by TASHKIN *et al.* [1] in a recent issue of the *European Respiratory Journal* evaluating effects of smoking status on long-term responses to maintenance bronchodilator therapy in the Understanding Potential Long-term Impacts on Function with Tiotropium (UPLIFT®) trial. The UPLIFT® trial [2] is a recent investigation in a long series of clinical trials assessing, among other things, the effects of different drugs on long-term forced expiratory volume in 1 s (FEV_1) decline in patients with COPD, a ''holy grail'' of the pulmonological community.

As tobacco smoking is the most frequent risk factor for COPD, researchers in the UPLIFT® trial paid the utmost attention to smoking status of the investigated cohort. They registered smoking status at inclusion, offered smoking cessation to every smoking patient before entry and checked smoking status at each follow-up visit during the 4 yrs of study. Study participants were classified into three subgroups: continuing current smokers (CS), continuing ex-smokers (CE) and intermittent smokers (IS).

The authors concentrate on analysis of effects of tiotropium in relation to smoking status on bronchodilation, exacerbation