From the authors:

We agree with D. Gallagher and colleagues on the need to corroborate our recently published preliminary findings with larger and well-designed studies targeting different risk groups and performed in settings with low and high burdens of tuberculosis (TB). Indeed, we do hope that our first assessment of the QuantiFERON-TB Plus (QFT-Plus) (QIAGEN GmbH, Hilden, Germany) performance characteristics, together with the data presented by D. Gallagher and colleagues, may constitute the first body of evidence necessary to guide clinical and programmatic use of QFT-Plus within the context of TB infection management and TB elimination strategy [1].

Including $CD8^+$ T-cell stimulation in a commercial diagnostic assay is a major breakthrough, which opens the field to new research possibilities. In fact, several bodies of evidence support the involvement of $CD8^+$ T-cells as a front-line defence in the response to *Mycobacterium tuberculosis* [2]. This includes the proposed correlation with the mycobacterial load in sputum samples [3], and the functional and phenotypic changes they undergo during different phases of the infection in HIV-uninfected and [4, 5] -infected subjects [6]. In this context, QFT-Plus, despite all limitations, becomes an interesting blueprint for exploring the use of interferon-γ release assay (IGRA)-related $CD8^+$ signals in programmatic TB management, as well in the investigation of new-generation parameters for the interpretation of the test in other contexts.

The results on intersubject variability reported by D. Gallagher and colleagues are likely to be important, as IGRA reproducibility issues may limit their use in settings where repeated testing is necessary [7]. Going in the same direction, we reported, in the active TB patient group, an overall increased TB2 antigen interferon (IFN)-γ response when compared to TB1 (median (interquartile range): TB1 2.09 (0.83–6.52), TB2 2.88 (1–7.89) $IU{\cdot}mL^{-1}$; Wilcoxon test p=0.0002) [8]. The significant increase in the IFN-γ release in the samples from active TB after addition of the novel antigens to the QFT-Plus assay responds to the need for high IFN-γ responses for a reliable analytical accuracy in the measurements and might help in interpreting mild fluctuations in IFN-γ responses observed during serial testing with the QuantiFERON Gold In-Tube (QFT-GIT) version.

Furthermore, the addition of the second antigen tube may be valuable in the interpretation of the borderline results in patients with unclear TB risk factors, especially in the low-risk population. However, functional T-cell assays are highly susceptible to the variability induced by numerous factors at multiple levels (manufacturing, pre-analytic, analytic and immunological) [9] and the dichotomous nature of IGRAs make them intrinsically prone to conversion/reversion phenomena.

Interpretation of data on IGRA reproducibility is challenged by the different methods used to assess variability. Traditionally, manufacturers report variability as the coefficient of variation, determined by dividing the pooled standard deviation by the overall mean TB response. As a consequence, persons with highly positive test results have less variability (percentage of the mean) when compared to persons with negative or borderline tests results and this may account for the discrepancies in QFT-GIT variability reported in literature by various investigators [10].

Therefore, despite the encouraging preliminary findings, the variability of QFT-Plus response still needs to be determined with large studies to establish appropriate cut-offs for conversion, in order to optimise the interpretation of test results in low-incidence settings.

@ERSpublications
Variability of QFT-Plus response still needs to be determined with large studies to establish appropriate cut-offs http://ow.ly/60bP301kLRT

Daniela Maria Cirillo[1], Lucia Barcellini[1] and Delia Goletti[2]
[1]IRCCS San Raffaele Scientific Institute, Emerging Bacterial Pathogens Unit, Milan, Italy. [2]UOC Epidemiologia Clinica, Istituto Nazionale Malattie Infettive "L. Spallanzani", Rome, Italy.

Correspondence: Daniela Maria Cirillo, Emerging Bacterial Pathogens Unit, IRCCS San Raffaele Scientific Institute Emerging Bacterial Pathogens Unit, Via Olgettina 60, 20132 Milano, Italy. E-mail: cirillo.daniela@hsr.it

Received: May 30 2016 | Accepted after revision: June 04 2016

Conflict of interest: None declared.

References

1 Lönnroth K, Migliori GB, Abubakar I, *et al.*Towards tuberculosis elimination: an action framework for low-incidence countries. *Eur Respir J* 2015; 45: 928–952.
2 Lin PL, Flynn JL. CD8T cells and *Mycobacterium tuberculosis* infection. *Semin Immunopathol* 2015; 37: 239–249.
3 Lewinsohn DA, Heinzel AS, Gardner JM, *et al.Mycobacterium tuberculosis*-specific $CD8^+$ T cells preferentially recognize heavily infected cells. *Am J Respir Crit Care Med* 2003; 168: 1346–1352.

4 Rozot V, Vigano S, Mazza-Stalder J, *et al.Mycobacterium tuberculosis*-specific $CD8^+$ T cells are functionally and phenotypically different between latent infection and active disease. *Eur J Immunol* 2013; 43: 1568–1577.
5 Prezzemolo T, Guggino G, La Manna MP, *et al.*Functional signatures of human CD4 and CD8T cell responses to *Mycobacterium tuberculosis*. *Front Immunol* 2014; 5: 180.
6 Chiacchio T, Petruccioli E, Vanini V, *et al.*Polyfunctional T-cells and effector memory phenotype are associated with active TB in HIV-infected patients. *J Infect* 2014; 69: 533–545.
7 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.
8 Barcellini L, Borroni E, Brown J, *et al.*First independent evaluation of QuantiFERON-TB Plus performance. *Eur Respir J* 2016; 47: 1587–1590.
9 Pai M, Denkinger CM, Kik SV, *et al.*Gamma interferon release assays for detection of *Mycobacterium tuberculosis* infection. *Clin Microbiol Rev* 2014; 27: 3–20.
10 Metcalfe JZ, Cattamanchi A, McCulloch CE, *et al.*Test variability of the QuantiFERON-TB Gold In-Tube assay in clinical practice. *Am J Respir Crit Care Med* 2013; 187: 206–211.

Eur Respir J 2016; 48: 955–956 | DOI: 10.1183/13993003.01084-2016 | Copyright ©ERS 2016

Paediatric study of bedaquiline remains an "open issue"

To the Editor:

In their editorial, "Bedaquiline and multidrug-resistant tuberculosis: a systematic and critical analysis of the evidence", Pontali *et al.* [1] detail and analyse the existing evidence for bedaquiline for multidrug-resistant tuberculosis (MDR-TB). The authors also discuss "open issues" or areas where additional research is required to inform use, including in children. Pontali *et al.* [1] mention the planned phase II paediatric study of bedaquiline (www.clinicaltrials.gov identifier number NCT 02354014). This study to evaluate the pharmacokinetics and safety of bedaquiline in children is crucial to guiding treatment in people under 18 years of age, who have previously been excluded from trials of bedaquiline.

An estimated 32 000 children develop MDR-TB each year [2] but evidence-based dosing guidelines, formulations and data on interactions with antiretrovirals to appropriately treat them are lacking [3]. The planned phase II paediatric study of bedaquiline would help address this gap. This study has been in development for over 5 years. In that time, an estimated 160 000 new cases of paediatric MDR-TB have appeared.

In a consensus statement published in *The Lancet*, a group of experts convened by the US National Institutes of Health recommended that paediatric investigation of new tuberculosis drugs and regimens begin as soon as efficacy and safety have been established in adults (phase IIb studies) [4]. Planning for and starting paediatric investigations earlier can help close gaps in access that currently exist between adults, adolescents and children. Favourable phase IIb data were available for bedaquiline in 2011 and the US Food and Drug Administration conditionally approved bedaquiline for use in adults in December 2012 [5]. Given the time it will take to enrol and complete the study, and file for regulatory approval for the paediatric formulation, there will be, at best, a 5-year gap between adult and paediatric formulation availability.

Several factors appear to have contributed to this delay. Janssen Pharmaceuticals (Titusville, NJ, USA), the sponsor of bedaquiline, worked for 3 years with the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) network of the US National Institute of Allergy and Infectious Diseases to complete a protocol to study bedaquiline in HIV-positive and -negative children. In June 2014, Janssen ended this collaboration in favour of creating their own independent study in HIV-negative children only [6]. This decision necessitated the creation of a new protocol and introduced new challenges, as most trial site infrastructure and expertise for paediatric MDR-TB studies are concentrated in the IMPAACT network [7]. Janssen received an infusion of $1.5 million in public funds from UNITAID's STEP-TB project to support the development of a paediatric formulation of bedaquiline and, as recently as December 2015, Janssen reported that its paediatric study would open in January 2016; however, at the time of writing it had yet to start [7, 8].

In contrast, delamanid, the other newly approved drug for MDR-TB, has gone through pharmacokinetic and safety investigations in HIV-negative children as young as 6 years old, and is currently under study in children 3–5 years old. Though delamanid has not been widely registered for adults or children, children have received delamanid under compassionate use and had favourable outcomes [9].