OPEN

# Added Value of IP-10 as a Read-Out of *Mycobacterium tuberculosis*

## *Specific Immunity in Young Children*

*Synne Jenum, MD, PhD,*† Sivakumaran Dhanasekaran, PhD,‡ Christian Ritz, PhD,§ Ragini Macaden, MB BS, MD,¶ T. Mark Doherty, PhD,‖ and Harleen M. S. Grewal, MD, PhD,‡ for the TB Trials Study Group*

**Abstract:** We have explored the added value of interferon-γ (IFNγ)–inducible protein 10 as a read-out of *Mycobacterium tuberculosis*–specific immunity in young Indian children, where the sensitivity of the IFNγ release assays for tuberculosis is poor. Reduced frequency of indeterminate results and an increased sensitivity for tuberculosis suggest a potential for fewer missed cases with a combined IFNγ/inducible protein 10 read-out in a 4th generation IFNγ release assays.

**Key Words:** tuberculosis, child, tuberculin skin test, interferon-γ release assay, cytokines, chemokines

(*Pediatr Infect Dis J* 2016;35:1336–1338)

The interferon-γ (IFNγ) release assays (IGRAs) are current state-of-the-art tests for *M. tuberculosis* (MTB) infection but have low sensitivity in young children.[1] The IFNγ-inducible protein 10 (IP-10) has emerged as an alternative read-out to increase the sensitivity of the IGRAs without compromising specificity. IP-10 is expressed by antigen presenting cells and can be considered a downstream marker of IFNγ—the standard read-out of cell-mediated immunity. The advantage of IP-10 is that it is expressed at higher levels than IFNγ while retaining the signal-to-noise ratio,[2] and the stability of the protein in dried blood spots implies a potential for a point-of-care diagnostic test.[3]

IGRAs are acknowledged supplements in diagnosis when tuberculosis (TB) is suspected in children but remains unconfirmed by smear/culture,[4] as a positive test provides evidence of recent MTB infection. In a cohort of Indian children aged <3 years referred on suspicion of TB, the QuantiFERON® Gold In-Tube (QFT) assay, showed low sensitivity for clinical TB (23%).[5] Obviously, a negative QFT is unsuited to rule out MTB infection in this population. The increase in indeterminate QFT tests with decreasing age is also a concern.[2,5] We, therefore, wanted to explore the possibility of increased sensitivity for the IP-10 read-out compared with IFNγ in this population. As indeterminate test results are related to mitogen response, we compared IP-10 and IFNγ concentrations in response to TB-specific antigens and mitogen. The problem in evaluating these read-outs is the lack of a gold standard for MTB infection. The stringent categorization of MTB-infection status based on the results from the Tuberculin skin test (TST) and QFT recently proposed by Tebruegge et al[6] was, therefore, applied. We evaluated single versus duplex analyses of the analytes before choosing a multiplex analysis platform because some have suggested to include both IFNγ and IP-10 as read-outs of cell-mediated immunity in order to increase the sensitivity.[2]

## MATERIALS AND METHODS

As part of a prospective cohort study of 4382 neonates in South India, 746 children were evaluated for TB after referral based on predefined criteria.[5]

For this substudy, MTB-infection status was categorized according to Tebruegge et al.[6] Of the 746 children, all children having an assignment of active clinical TB (ATB; n=13) and MTB infection (TST ≥10 mm and QFT positive; n=18), common discordance (TST ≥10 mm and QFT negative; n=53), and reverse discordance (TST <10 mm and QFT positive; n=19) were included, whereas children classified as possible discordance (TST 5–9 mm and QFT negative; n=22) as well as probably MTB uninfected (TST 1–4 mm and QFT negative; n=24) and uninfected (TST 0 and QFT negative; n=7) were randomly selected from the larger population of study children with available samples.

IFNγ and IP-10 were analyzed in QFT supernatants from the TB antigen and mitogen tube. We applied a customized 2-plex (IFNγ and IP-10) cytokine/chemokine kit (Bio-Rad Laboratories Inc., Berkeley, CA), according to the manufacturer's instructions. For the duplex assay, the optimal dilution of the QFT supernatants was 1:4. The difference in measured concentrations of the analytes between single and duplex assays was assessed by a pilot study (n=16), where a head-to-head comparison of paired samples (Wilcoxon Signed Rank test), revealed equal or better detection of the analytes on the more cost-effective duplex platform (Fig. 1A and B).

Clinical characteristics were summarized for children within the defined categories of MTB infection. IFNγ and IP-10 concentration levels and their fold change were compared between the following MTB-infection categories (Mann–Whitney *U* test): ATB, MTB infected, common discordance, and unlikely MTB infected (probably MTB uninfected and uninfected clubbed). Area under curve (AUC) was calculated to evaluate sensitivity and specificity. A $P$ value <0.05 was considered significant. SPSS software version 23 and GraphPad Prism 5 were used for statistical analyses and figures.

Accepted for publication April 13, 2016.

From the *Department of Clinical Science, University of Bergen, Bergen, Norway; †Department of Infectious Diseases, Oslo University Hospital, Oslo, Norway; ‡Department of Clinical Science, Faculty of Medicine and Dentistry, University of Bergen, Bergen, Norway; §Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark; ¶Division of Infectious Diseases, St. John's Research Institute, Koramangala, Bangalore, India; and ‖GlaxoSmithKline, Vaccines, Wavre, Belgium.

This work was supported by the Research Council of Norway (grants 179342 192534), the University of Bergen (Norway), Aeras Global Vaccine Foundation (USA) and St. John's Research Institute (India). QFT kits were supported, in part, by the Foundation for Innovative New Diagnostics (FIND).

The authors have no conflicts of interest.

Address for correspondence: Synne Jenum, MD, PhD, Department of Infectious Diseases, Oslo University Hospital, PO Box 4956 Nydalen, 0424 Oslo, Norway. E-mail: synnejenum@gmail.com

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (www.pidj.com).



ISSN: 0891-3668/16/3512-1336
DOI: 10.1097/INF.0000000000001328

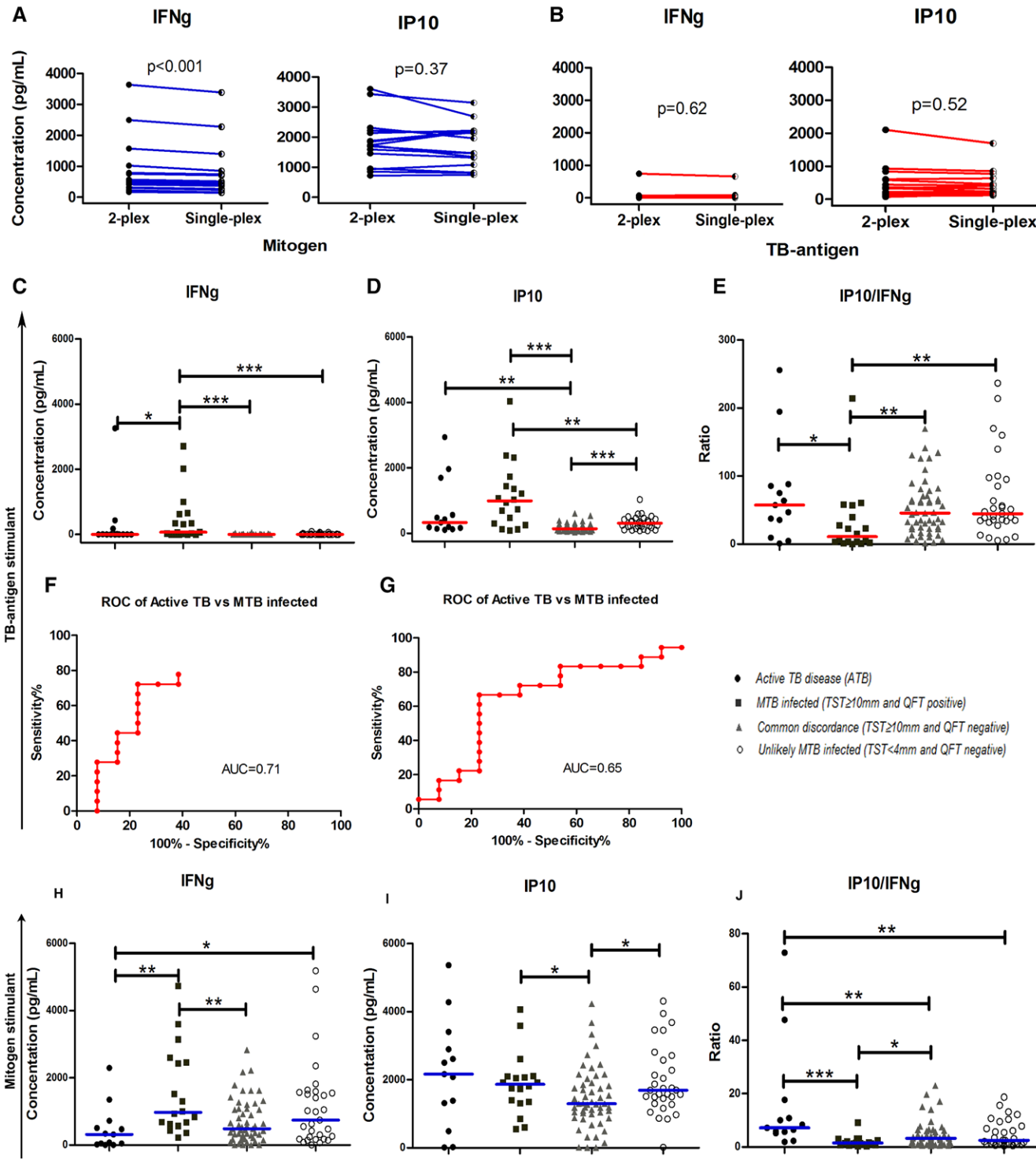


**FIGURE 1.** Comparison of the concentration of IFNγ and IP10 in 2-plex and single-plex set-ups in response to A: Mitogen and B: TB-antigen. The concentration of the analytes within the MTB infection categories in response to tuberculosis (TB) antigen [C: interferon-γ (IFNγ) and D: IFNγ-inducible protein 10 (IP-10)] and mitogen (H: IFNγ and I: IP-10). IP-10:IFNγ ratio for the MTB infection categories are depicted in response to TB antigen (E) and mitogen (J). Discriminatory capacity between children with active TB versus MTB infection is shown for IFNγ (F) and IP-10 (G) concentrations, respectively. The *P* values <0.05 (*), <0.01 (**), <0.001 (***) were considered to be significant. AUC indicates area under curve; ROC, receiver operating characteristic. full color online

## Ethics Approval

The study was conducted according to the Helsinki (4th revision) declaration and approved by the institutional ethical review board of the St. John's Medical College, an independent ethics committee contracted by the Aeras Global TB Vaccine Foundation, and the Ministry of Health Screening Committee of the Government of India (No. 5/8/9/60/2006-ECD-I).

## RESULTS

The 156 included children had a median age of 23 months, and 92 (60%) were male. As many as 102 (65.4%) had failure to thrive, and 57 (36.5%) had evidence of chronic malnutrition (height-for-age Z score <2). Overall, 11 (7%) children had known TB exposure, whereas the proportion in ATB- and MTB-infected children was 39 and 7.7%, respectively. Longstanding (≥2 weeks) cough was present in 18 (11.5%), most frequently in ATB children (31%), and fever was present in 10 (6.4%), most frequently in ATB children (31%) and reverse discordance (16%; Table, Supplemental Digital Content 1, http://links.lww.com/INF/C533).

The concentration of IFNγ in response to TB antigen was significantly higher in MTB-infected children compared with all other groups. As expected from the poor sensitivity of QFT in ATB children, no difference was observed compared with common discordance or unlikely MTB-infected children (Fig. 1C). The ability of IFNγ to discriminate between ATB- and MTB-infected children gave an AUC of 0.71 [sensitivity: 76.9% (46.2–94.9%) and specificity: 72.2% (46.5–90.3%); Fig. 1F]. No difference in the concentration of IP-10 in response to TB antigen was observed between ATB- and MTB-infected children, but both the groups had significantly higher IP-10 levels than the children with common discordance (Fig. 1D). The ability of IP-10 to discriminate between ATB- and MTB-infected children was somewhat lower than that of IFNγ; AUC 0.65 [sensitivity: 66.7% (41.0–86.7%) and specificity 76.9% (46.2–95.0%); Fig. 1G].

The concentration of IFNγ in response to mitogen was significantly lower in ATB children compared with MTB-infected and unlikely MTB-infected children, but this was not observed for IP-10. MTB-infected children had higher levels of both analytes than did children with common discordance (Fig. 1G and H).[2]

The IP-10:IFNγ ratio was significantly higher in ATB compared with MTB-infected children both in response to TB antigen and mitogen. Interestingly, the ratio was significantly lower in MTB infected children compared with all other groups.

## DISCUSSION

Whereas Tebruegge et al,[6] analyzed IFNγ in undiluted supernatants and IP-10 in 1:20 diluted samples, we found that IFNγ and IP-10 could be analyzed on a duplex platform using a 1:4 dilution of the QFT supernatants despite IP-10 being secreted at higher magnitudes. The possibility of analyzing both analytes in 1 tube by the same assay provides simplification and cost-reduction. In line with other studies, our study provides no evidence for increased discrimination between ATB and MTB infection by IP-10 alone.[2] Our study supports a higher sensitivity for the detection of ATB by TB antigen–induced IP-10 compared with IFNγ as the concentrations were significantly higher compared with children with common discordance. A recent study in a similar African population contradicts this finding, but IFNγ and IP-10 were analyzed in separate enzyme-linked immunosorbent assays (ELISAs).[7] But maybe more noteworthy when considering a combined IFNγ/IP-10 read-out in a 4th IGRAs[2] is the finding of a more compromised IFNγ than IP-10 response to mitogen in ATB children who had an increased IP-10:IFNγ ratio compared with all other groups. The pattern of blunted immune responses with increasing disease severity[8,9] may be part of the picture. Combined, these findings suggest that a reduced frequency of indeterminate results and an increased discrimination between ATB and common discordance in children could be achieved by a combined IFNγ/IP-10 read-out.

MTB-infected children seem to have the most robust overall immune responses in our population. It should be noted that the robustness of responses in MTB-infected children may be the result of a selection bias as the groups where, in part, defined by the QFT result.

Currently, IGRAs and TST are used as indicative tests in the diagnosis of TB in infants but cannot be used as "rule-out tests". Our data suggest a possibility of improving the sensitivity of the IGRA in children particularly vulnerable to TB, which implies a potential to identify TB cases that might otherwise have been missed.

## ACKNOWLEDGMENTS

*TB Trials Study Group: Doherty TM, Grewal HMS, Hesseling AC, Jacob A, Jahnsen F, D' Souza G, Kurpad AV, Lau E, Lindtjorn B, Macaden R, Vaz M.*

## REFERENCES

1. 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.
2. Ruhwald M, Aabye MG, Ravn P. IP-10 release assays in the diagnosis of tuberculosis infection: current status and future directions. *Expert Rev Mol Diagn*. 2012;12:175–187.
3. Aabye MG, Latorre I, Diaz J, et al. Dried plasma spots in the diagnosis of tuberculosis: IP-10 release assay on filter paper. *Eur Respir J*. 2013;42:495–503.
4. Basu Roy R, Whittaker E, Kampmann B. Current understanding of the immune response to tuberculosis in children. *Curr Opin Infect Dis*. 2012;25:250–257.
5. Jenum S, Selvam S, Mahelai D, et al. Influence of age and nutritional status on the performance of the tuberculin skin test and QuantiFERON-TB gold in-tube in young children evaluated for tuberculosis in Southern India. *Pediatr Infect Dis J*. 2014;33:e260–e269.
6. Tebruegge M, Dutta B, Donath S, et al. Mycobacteria-specific cytokine responses detect tuberculosis infection and distinguish latent from active tuberculosis. *Am J Respir Crit Care Med*. 2015;192:485–499.
7. Holm LL, Rose MV, Kimaro G, et al. A comparison of interferon-γ and IP-10 for the diagnosis of tuberculosis. *Pediatrics*. 2014;134:e1568–e1575.
8. Ellner JJ. Review: the immune response in human tuberculosis–implications for tuberculosis control. *J Infect Dis*. 1997;176:1351–1359.
9. Hirsch CS, Toossi Z, Othieno C, et al. Depressed T-cell interferon-gamma responses in pulmonary tuberculosis: analysis of underlying mechanisms and modulation with therapy. *J Infect Dis*. 1999;180:2069–2073.