Evaluation of QuantiFERON-TB Gold Plus for Predicting Incident Tuberculosis among Recent Contacts: A Prospective Cohort Study

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Screening for latent tuberculosis infection (LTBI) among recent tuberculosis (TB) contacts is an important component of TB control, particularly in settings with low TB incidence aiming towards pre-elimination(1). However, currently available LTBI diagnostics lack sensitivity, and have poor predictive value for incident TB(2–8) Consequently, prevention of one incident TB case requires treatment of many for LTBI. This is true for both interferon gamma release assays (IGRAs) and the tuberculin skin test (TST); a recent evaluation found that QuantiFERON Gold-In-Tube (QFT-GIT; Qiagen, Hilden, Germany) and T-SPOT.TB (Oxford Immunotec, UK) perform similarly to the TST when a BCG-stratified TST cut-off is used(8).

A newer generation QuantiFERON (QuantiFERON-TB Gold Plus; QFT-Plus) was recently launched, adding a second TB antigen tube (TB2) that incorporates short peptides designed to stimulate a $CD8^+$ T-cell response, in addition to the $CD4^+$-response tube (TB1) included in previous versions. The proposed rationale for this is that $CD8^+$-responses have been associated with mycobacterial load and recent TB exposure(9, 10). Initial independent evaluations have suggested QFT-Plus may have improved test sensitivity in active TB compared to QFT-GIT(11), and that the $CD8^+$-targeted antigen tube response may be associated with proxy measures of degree of TB exposure among contacts(12). However, no studies have examined the prognostic value of QFT-Plus for predicting incident TB. We aimed to address this key knowledge gap in a prospective cohort of UK TB contacts.

Methods

We recruited adult (≥16 years) contacts of pulmonary and extrapulmonary TB index cases from ten London TB clinics, when attending for routine contact screening (07/07/2015-22/11/2016).

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Participants completed a questionnaire and had blood sampling for QFT-Plus (at least six weeks from last known TB exposure). Contacts with evidence of prevalent TB disease (defined as TB diagnosed within 21 days of enrolment as per previous work(8)), and those accepting preventative therapy (offered in accordance with contemporary national guidance(13, 14)) were excluded from analysis.

Participants were linked to national TB surveillance records held by Public Health England, including all statutory TB notifications, to identify those notified with TB (until 31/12/2017). Notified TB cases were validated by local record review and included culture-confirmed TB or clinically diagnosed with radiological or histological evidence of TB, where a clinician had prescribed treatment with a full course of anti-TB treatment.

QFT-Plus results were interpreted as per manufacturer guidance, with TB antigen responses calculated as TB antigen interferon-γ minus unstimulated control interferon-γ. We calculated incidence rates and rate ratios relative to the negative test category, along with sensitivity, specificity and predictive values, including the full duration of follow-up.

To assess the incremental value of adding the $CD8^+$-stimulating tube in predicting incident TB cases, we compared receiver operating characteristic (ROC) curves and areas under the curves (AUCs) when using TB1 only, TB2 only, and the maximal TB antigen tube (higher of TB1 and TB2). We also plotted a ROC curve for the calculated difference between the TB1 and TB2 tubes (TB2-TB1) as a surrogate for the $CD8^+$-specific response, since it has been hypothesised that this may identify contacts with recently acquired *M. tuberculosis* infection, who are at highest risk of TB disease(12).

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Results

A total of 623 contacts were recruited, of whom 532 (85.4%) had QFT-Plus results (89 missing; 2 indeterminate) and were followed-up for a median 1.93 years (interquartile range (IQR) 1.65-2.21). QFT-Plus results were positive in 180/532 (33.8%) (Table 1), of whom 39 (21.7%) commenced preventative therapy. One patient was notified with prevalent TB (3 days after recruitment). A total of 492 participants were therefore included in the analysis. Baseline characteristics were similar between included and excluded participants, except for those who commenced preventative therapy being younger than those who did not (Table 1).

Ten incident TB cases were notified during follow-up (median 222 days after recruitment; range 90-688). Of these, median age was 27 (IQR 21-33), 3/10 (30%) were female, the majority (7/10; 70%) were of black African or South Asian ethnicity and all were non-UK born. All ten cases completed at least six months of TB therapy. A total of 2/10 cases (20.0%) were pulmonary in site (both were culture-confirmed), and eight exclusively extra-pulmonary (of which 2/8 were culture-confirmed). One participant with TB was diabetic; the remaining TB cases were not immunocompromised, and none were HIV-infected. TB incidence rates (per 1,000 person-years) were 30.6 (95% CI 15.3-61.1) and 3.0 (0.8-12.1) in the QFT-Plus positive and negative groups, respectively (incidence rate ratio (IRR) 10.1 (2.2-47.7)). QFT-Plus sensitivity for incident TB was 80.0% (44.4-97.5). The positive and negative predictive values (PPV/NPV) were 5.7% (2.5-10.9) and 99.4% (98.0-99.9), respectively. Characteristics of QFT-Plus for predicting microbiologically-confirmed TB cases are reported in Table 2.

ROC curves for prediction of incident TB during all follow-up were similar for the TB1, TB2 and maximal TB antigen responses (AUCs 0.80-0.82; Figure 1a). TB2 minus TB1, however,

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did not discriminate TB progressors from non-progressors (AUC 0.44 (95% CI 0.20-0.68)). There was very strong correlation between the TB1 and TB2 interferon-γ responses ($r = 0.993$; $p < 0.001$; Figure 1b).

Discussion

We found that QFT-Plus performance appeared comparable to published evaluations of QFT-GIT and T-SPOT.TB, with an IRR of 10.1, 80% sensitivity for detection of incident TB, and an overall PPV for incident TB of 5.7%(8). Interferon-γ responses in the TB1 and TB2 tubes were strongly correlated, and ROC curves showed minimal difference between them for predicting incident TB. As a result, the calculated difference between TB1 and TB2 responses, as a proxy for the CD8-specific response, did not predict incident TB. However, despite our sample size of 492 recent TB contacts, the number of TB progressors was small, reflecting low progression risk even among contacts. Thus, a larger-scale study is indicated to investigate subtle differences in the relative prognostic contributions of the TB1 and TB2 antigen tubes.

This is the first evaluation of the prognostic value of QFT-Plus test. The prospective design allowed the collection of detailed clinical, demographic and laboratory data. Participants were recruited while attending routine contact-tracing services, ensuring the study population was representative of TB contacts. Our findings are therefore likely generalizable to other low incidence settings globally. Moreover, follow-up was robust through linkage to national surveillance records using a validated matching algorithm(15), minimising risk of missing incident TB cases.

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A limitation was that the provision of preventative therapy to a subset of the QFT-Plus positive patients could have led to selection bias. However, while the subgroup who received preventative therapy were younger than those who did not (reflecting national policy at the time of the study(13, 14)), other characteristics were similar, suggesting the impact of this bias was likely small. Secondly, the TB contacts included both pulmonary and extra-pulmonary index cases, reflecting national contact screening policy during the study period(13). PPV of QFT-Plus may be higher among populations including only pulmonary TB contacts, due to higher pre-test probability of incident TB. However, previous evaluations of the QFT-GIT and T-SPOT.TB have also included extra-pulmonary contacts, which allows the current study findings to be put into this context(8). Thirdly, serial testing (before and after exposure) was not performed, meaning that we are unable to assess QFT-Plus conversions over time, which may provide a more reliable measure of recent *M. tuberculosis* infection. This reflects the reality of contact screening practices; the ability of assays to accurately stratify TB risk from a single baseline test is therefore a key attribute. The absence of serial testing also means that participants who developed incident TB may have been re-exposed to *M. tuberculosis* during the interval between testing and disease, though the overall risk of exposure in the UK (a low TB incidence setting) is likely small. Fourthly, QFT-Plus results were missing or indeterminate for 91/623 patients (14.6%), in keeping with the proportion of missing results for other IGRAs in our recent evaluation(8). However, these patients' characteristics were similar to the overall study population, suggesting that risk of subsequent selection bias was likely small. Finally, we included both microbiologically-confirmed and clinically-diagnosed TB cases in our outcome definition, in keeping with previous IGRA evaluations(3–8). The rationale for this is that extra-

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pulmonary TB, which occurs frequently among TB cases occurring in foreign-born people living in the UK(16), is often challenging to prove microbiologically. However, all TB cases diagnosed during the study received a full course of TB therapy, and none were de-notified. It is therefore likely that these represented true TB cases, with low risk of outcome misclassification.

In summary, in this first evaluation of the predictive value of QFT-Plus for incident TB, performance was comparable to other commercial IGRAs. Better biomarkers are required to transform management of TB contacts.

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ANNALSATS Articles in Press. Published February 21, 2020 as 10.1513/AnnalsATS.201905-407RL

References

1. World Health Organization. WHO | Latent TB Infection : Updated and consolidated guidelines for programmatic management. *WHO* 2018;at <http://www.who.int/tb/publications/2018/latent-tuberculosis-infection/en/>.
2. Pai M, Denkinger CM, Kik S V., Rangaka MX, Zwerling A, Oxlade O, Metcalfe JZ, Cattamanchi A, Dowdy DW, Dheda K, Banaei N. Gamma Interferon Release Assays for Detection of Mycobacterium tuberculosis Infection. *Clin Microbiol Rev* 2014;27:3–20.
3. Zellweger J-PP, Sotgiu G, Block M, Dore S, Altet N, Blunschi R, Bogyi M, Bothamley G, Bothe C, Codecasa L, Costa P, Dominguez J, Duarte R, Fløe A, Fresard I, García-García J-M, Goletti D, Halm P, Hellwig D, Henninger E, Heykes-Uden H, Horn L, Kruczak K, Latorre I, Pache GG, Rath H, Ringshausen FC, Ruiz ASAS, Solovic I, *et al.* Risk Assessment of Tuberculosis in Contacts by IFN-gamma Release Assays. A Tuberculosis Network European Trials Group Study. In: Aeby M Girardi E, Vanini V, Vecchi E, Lauria FN, Vecchi M, Ferrarese M, Repossi A, Gerdes S, Haller M, Glaewe A, Krabbe L, Hilberg O, Janssens JP, Rubsamen C, Schlosser S, Villanueva-Montes MA CG, editor. *Am J Respir Crit Care Med* 2015;191:1176–1184.
4. Sloot R, Van Der Loeff MFS, Kouw PM, Borgdorff MW. Risk of tuberculosis after recent exposure: A 10-year follow-up study of contacts in Amsterdam. *Am J Respir Crit Care Med* 2014;190:1044–1052.
5. Haldar P, Thuraisingam H, Patel H, Pereira N, Free RC, Entwisle J, Wiselka M, Hoskyns EW, Monk P, Barer MR, Woltmann G. Single-step QuantiFERON screening of adult contacts: a prospective cohort study of tuberculosis risk. *Thorax* 2013;68:240–246.
6. Erkens CGM, Slump E, Verhagen M, Schimmel H, Cobelens F, van den Hof S. Risk of developing tuberculosis disease among persons diagnosed with latent tuberculosis infection in the Netherlands. *Eur Respir J* 2016;48:1420–1428.
7. Diel R, Loddenkemper R, Niemann S, Meywald-Walter K, Nienhaus A. Negative and positive predictive value of a whole-blood interferon-gamma release assay for developing active tuberculosis: an update. *Am J Respir Crit Care Med* 2011;183:88–95.
8. Abubakar I, Drobniewski F, Southern J, Sitch AJ, Jackson C, Lipman M, Deeks JJ, Griffiths C, Bothamley G, Lynn W, Burgess H, Mann B, Imran A, Sridhar S, Tsou C-YY, Nikolayevskyy V, Rees-Roberts M, Whitworth H, Kon OM, Haldar P, Kunst H, Anderson S, Hayward A, Watson JM, Milburn H, Lalvani A, Team PS, Adeboyeku D, Bari N, *et al.* Prognostic value of interferon-gamma release assays and tuberculin skin test in predicting the development of active tuberculosis (UK PREDICT TB): a prospective cohort study. In: Adeboyeku D Barker J, Booth H, Chua F, Creer D, Darmalingam M, Davidson RN, Dedicoat M, Dunleavy A, Figueroa J, Haseldean M, Johnson N, Losewicz S, Lord J, Moore-Gillon J, Packe G, Pareek M, Tiberi S, Pozniak A, Sanderson F BN, editor. *Lancet Infect Dis* 2018;18:1077–1087.
9. Day CL, Abrahams DA, Lerumo L, Janse van Rensburg E, Stone L, O'rie T, Pienaar B, de Kock M, Kaplan G, Mahomed H, Dheda K, Hanekom WA. Functional capacity of Mycobacterium tuberculosis-specific T cell responses in humans is associated with mycobacterial load. *J*

ANNALSATS Articles in Press. Published February 21, 2020 as 10.1513/AnnalsATS.201905-407RL

Immunol 2011;187:2222–32.

10. Nikolova M, Markova R, Drenska R, Muhtarova M, Todorova Y, Dimitrov V, Taskov H, Saltini C, Amicosante M. Antigen-specific CD4- and CD8-positive signatures in different phases of Mycobacterium tuberculosis infection. *Diagn Microbiol Infect Dis* 2013;75:277–281.
11. Barcellini L, Borroni E, Brown J, Brunetti E, Codecasa L, Cugnata F, Dal Monte P, Di Serio C, Goletti D, Lombardi G, Lipman M, Rancoita PMV, Tadolini M, Cirillo DM, Monte PD, Serio C Di, Goletti D, Lombardi G, Lipman M, Rancoita PMV, Tadolini M, Cirillo DM. First independent evaluation of QuantiFERON-TB Plus performance. *Eur Respir J* 2016;47:1587–1590.
12. Barcellini L, Borroni E, Brown J, Brunetti E, Campisi D, Castellotti PF, Codecasa LR, Cugnata F, Di Serio C, Ferrarese M, Goletti D, Lipman M, Rancoita PM V, Russo G, Tadolini M, Vanino E, Cirillo DM. First evaluation of QuantiFERON-TB Gold Plus performance in contact screening. *Eur Respir J* 2016;48:1411–1419.
13. National Institute for Clinical Excellence. Tuberculosis Clinical diagnosis and management of tuberculosis, and measures for its prevention and control. 2011;at <http://www.nice.org.uk/guidance/cg117/resources/guidance-tuberculosis-pdf>.
14. NICE Guidance and Guidelines. Tuberculosis. 2016;
15. Aldridge RW, Shaji K, Hayward AC, Abubakar I. Accuracy of Probabilistic Linkage Using the Enhanced Matching System for Public Health and Epidemiological Studies. In: Pacheco AG, editor. *PLoS One* 2015;10:e0136179.
16. Kruijshaar ME, Abubakar I. Increase in extrapulmonary tuberculosis in England and Wales 1999-2006. *Thorax* 2009;64:1090–5.

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Table 1: Baseline characteristics of study cohort, stratified by Quantiferon-TB Gold Plus (QFT-Plus) results and provision of preventative therapy (PT). Data presented as n(%), unless stated otherwise. IQR = interquartile range.

	QFT Plus -[#]	QFT Plus +		QFT Plus missing*	All
		No PT[#]	*PT*		
Age					
Median (IQR)	31 (25-43)	43 (32-54)	30 (26-35)	31.5 (23.7-49)	33 (25-46)
Missing	3 (0.9)	2 (1.4)	0 (0)	1 (1.1)	6 (1)
Gender					
Male	165 (46.9)	76 (53.9)	24 (61.5)	37 (40.7)	302 (48.5)
Female	180 (51.1)	62 (44)	15 (38.5)	51 (56)	308 (49.4)
Missing	7 (2)	3 (2.1)	0 (0)	3 (3.3)	13 (2.1)
Ethnicity					
White	95 (27)	27 (19.1)	9 (23.1)	31 (34.1)	162 (26)
South Asian	117 (33.2)	55 (39)	13 (33.3)	33 (36.3)	218 (35)
Black African or Caribbean	67 (19)	30 (21.3)	7 (17.9)	15 (16.5)	119 (19.1)
Other	63 (17.9)	24 (17)	10 (25.6)	9 (9.9)	106 (17)
Missing	10 (2.8)	5 (3.5)	0 (0)	3 (3.3)	18 (2.9)
UK born					
No	235 (66.8)	126 (89.4)	33 (84.6)	66 (72.5)	460 (73.8)
Yes	111 (31.5)	11 (7.8)	6 (15.4)	24 (26.4)	152 (24.4)
Missing	6 (1.7)	4 (2.8)	0 (0)	1 (1.1)	11 (1.8)
Contact type					
Household	210 (59.7)	96 (68.1)	30 (76.9)	49 (53.8)	385 (61.8)
Family non-household	19 (5.4)	7 (5)	2 (5.1)	3 (3.3)	31 (5)
Work or Social	62 (17.6)	19 (13.5)	4 (10.3)	14 (15.4)	99 (15.9)
Other	13 (3.7)	3 (2.1)	2 (5.1)	2 (2.2)	20 (3.2)
Missing	48 (13.6)	16 (11.3)	1 (2.6)	23 (25.3)	88 (14.1)
Diabetes					
No	318 (90.3)	120 (85.1)	38 (97.4)	83 (91.2)	559 (89.7)
Yes	20 (5.7)	18 (12.8)	0 (0)	6 (6.6)	44 (7.1)
Missing	14 (4)	3 (2.1)	1 (2.6)	2 (2.2)	20 (3.2)
HIV					
No	331 (94)	137 (97.2)	37 (94.9)	84 (92.3)	589 (94.5)
Yes	4 (1.1)	0 (0)	1 (2.6)	2 (2.2)	7 (1.1)

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Missing	17 (4.8)	4 (2.8)	1 (2.6)	5 (5.5)	27 (4.3)
Follow-up (years)					
Median (IQR)	1.94 (1.64-2.21)	1.92 (1.66-2.21)	1.85 (1.67-2.25)	1.56 (1.25-2.06)	1.88 (1.58-2.20)
Total	**352**	**141**	**39**	**91**	**623**

*Includes 2 patients with indeterminate QFT-Plus results.
#Included in primary analysis

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Table 2: Incidence rates, rate ratios, and predictive values for incident tuberculosis (TB) during follow-up, stratified by Quantiferon-TB Gold Plus (QFT-Plus) result. Data presented as point estimates (95% confidence interval).

	QFT Plus +	QFT Plus -
TB cases (microbiologically confirmed and/or clinically diagnosed)	8	2
Participants	140	352
Person-years	261.6	663.0
Incidence rate per 1,000 person-years	30.6 (15.3-61.1)	3.0 (0.8-12.1)
Incidence rate ratio	10.1 (2.2-47.7)	
Positive predictive value	5.7 (2.5-10.9)	
Negative predictive value	99.4 (98-99.9)	
Sensitivity	80.0 (44.4-97.5)	
Specificity	72.6 (68.4-76.5)	
TB cases (microbiologically confirmed only)	3	1
Incidence rate per 1,000 person-years	11.5 (3.7-35.6)	1.5 (0.2-10.7)
Incidence rate ratio	7.6 (0.8-73.1)	
Positive predictive value	2.1 (0.4-6.1)	
Negative predictive value	99.7 (98.4-100)	
Sensitivity	75.0 (19.4-99.4)	
Specificity	71.9 (67.7-75.9)	

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Figure Legend:

Figure 1: (a) Receiver operating characteristic curves showing performance of QuantiFERON-TB Gold Plus for predicting incident tuberculosis during the duration of follow-up, stratified by antigen tube interferon-γ responses. TB max = higher of TB1 and TB2; AUC = area under the curve (95% confidence interval). (b) Scatterplot showing association of interferon-γ responses in the TB1 and TB2 tubes.

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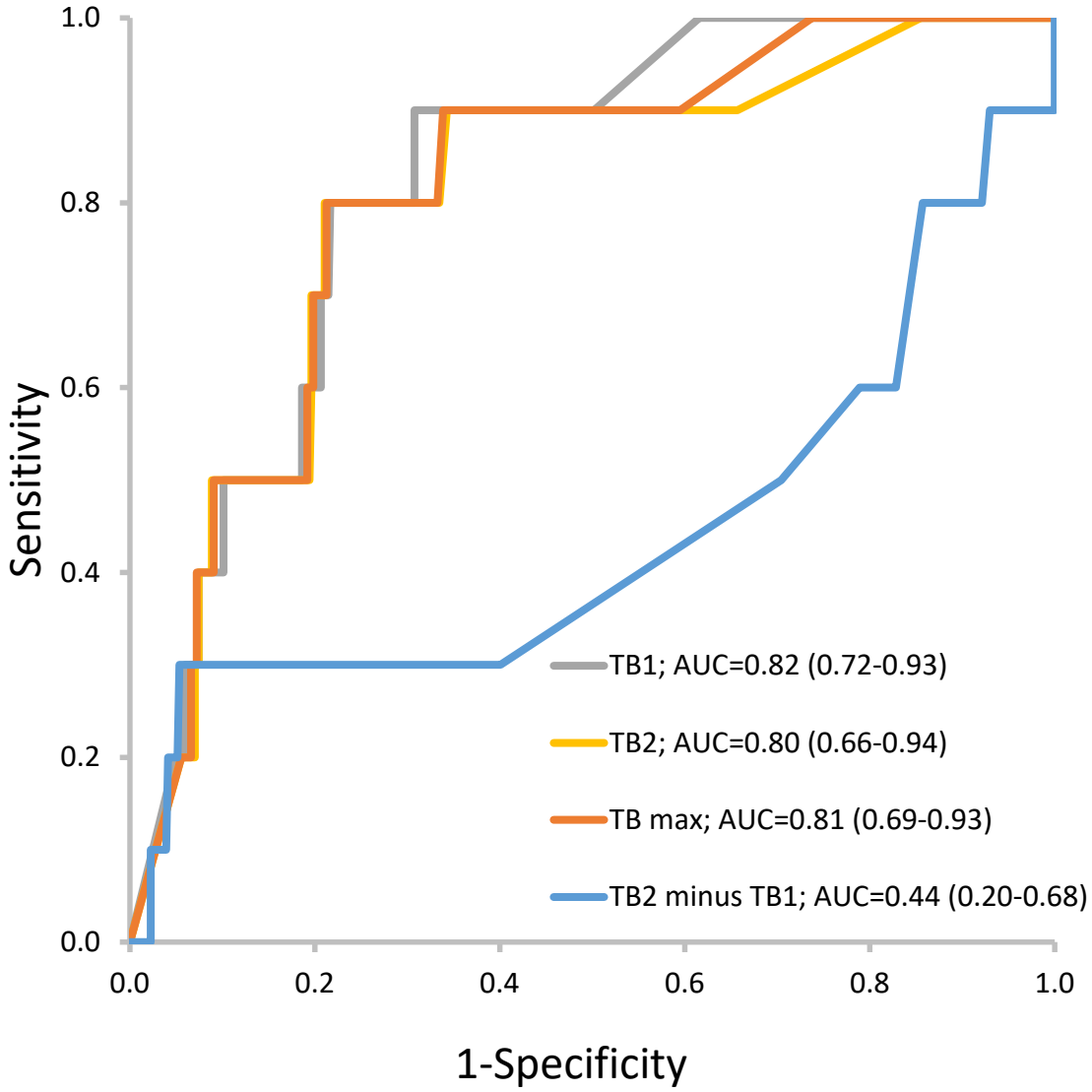
a)



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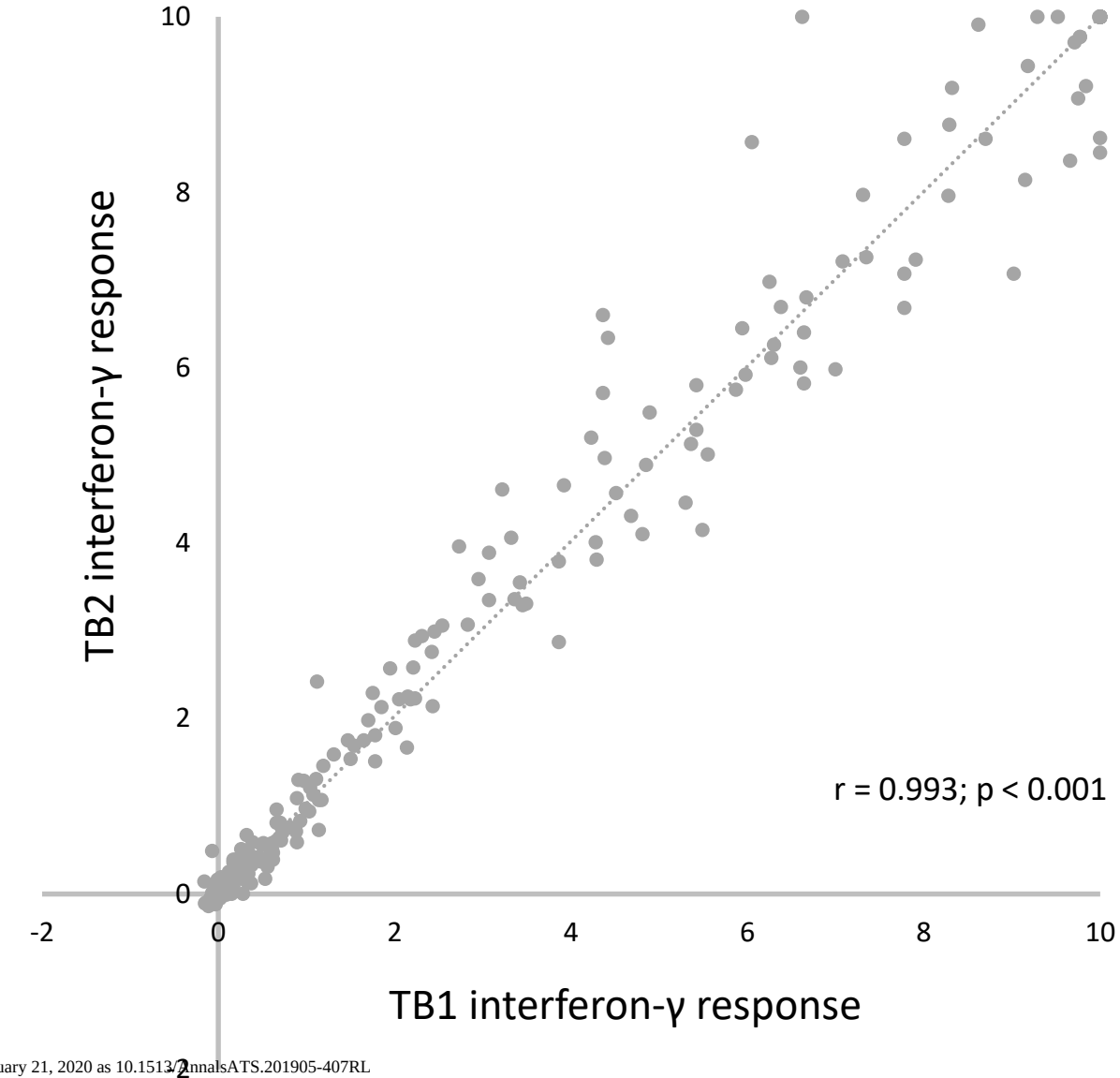

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