Baseline IL-6 is a biomarker for unfavourable tuberculosis treatment outcomes: a multisite discovery and validation study

Akshay N. Gupte[1,2], Pavan Kumar[3], Mariana Araújo-Pereira[4,5,6], Vandana Kulkarni[2,7,8], Mandar Paradkar[2,7,8], Neeta Pradhan[2,7,8], Pradeep Menon[3], Chandrasekaran Padmapriyadarsini[3], Luke-Elizabeth Hanna[3], Shri Vijay Bala Yogendra Shivakumar[8], Neesha Rockwood[9,10,11], Elsa Du Bruyn[9,12], Rajesh Karyakarte[13], Sanjay Gaikwad[14], Robert Bollinger[1,2], Jonathan Golub[1,15], Nikhil Gupte[1,2], Vijay Viswanathan[16], Robert J. Wilkinson[9,11,12,17], Vidya Mave[1,2], Subash Babu[18], Hardy Kornfeld[19], Bruno B. Andrade[4,5,6] and Amita Gupta[1,2]

[1]Division of Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, MD, USA. [2]Center for Clinical Global Health Education, Johns Hopkins University School of Medicine, Baltimore, MD, USA. [3]National Institute for Research in Tuberculosis, Chennai, India. [4]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ), Salvador, Brazil. [5]Multinational Organization Network Sponsoring Translational and Epidemiological Research, Salvador, Brazil. [6]Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Brazil. [7]Byramjee-Jeejeebhoy Government Medical College – Johns Hopkins University Clinical Research Site, Pune, India. [8]Johns Hopkins India Private Limited, Pune, India. [9]Wellcome Centre for Infectious Disease Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa. [10]Dept of Microbiology, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka. [11]Dept of Infectious Diseases, Imperial College London, London, UK. [12]Dept of Infectious Diseases, University of Cape Town, Cape Town, South Africa. [13]Dept of Microbiology, Byramjee-Jeejeebhoy Government Medical College, Pune, India. [14]Dept of Pulmonary Medicine, Byramjee-Jeejeebhoy Government Medical College, Pune, India. [15]Center for Tuberculosis Research, Johns Hopkins University, Baltimore, MD, USA. [16]Professor M. Viswanathan Diabetes Research Centre, Chennai, India. [17]The Francis Crick Institute, London, UK. [18]National Institutes of Health – National Institute for Research in Tuberculosis – International Center for Excellence in Research, Chennai, India. [19]Division of Pulmonary Medicine, University of Massachusetts Medical School, Worcester, MA, USA.

Corresponding author: Akshay N. Gupte (agupte1@jhmi.edu)

Shareable abstract (@ERSpublications)
Pre-treatment IL-6 is a biomarker for unfavourable tuberculosis treatment outcomes independent of disease severity and improves the performance of risk prediction models comprised of established clinical predictors https://bit.ly/38394xE

Cite this article as: Gupte AN, Kumar P, Araújo-Pereira M, *et al*. Baseline IL-6 is a biomarker for unfavourable tuberculosis treatment outcomes: a multisite discovery and validation study. *Eur Respir J* 2022; 59: 2100905 [DOI: 10.1183/13993003.00905-2021].

Received: 7 April 2021
Accepted: 18 Aug 2021

Abstract

Background Biomarkers of unfavourable tuberculosis (TB) treatment outcomes are needed to accelerate new drug and regimen development. Whether plasma cytokine levels can predict unfavourable TB treatment outcomes is unclear.

Methods We identified and internally validated the association between 20 *a priori* selected plasma inflammatory markers and unfavourable treatment outcomes of failure, recurrence and all-cause mortality among adults with drug-sensitive pulmonary TB in India. We externally validated these findings in two independent cohorts of predominantly diabetic and HIV co-infected TB patients in India and South Africa, respectively.

Results Pre-treatment interferon-γ, interleukin (IL)-13 and IL-6 were associated with treatment failure in the discovery analysis. Internal validation confirmed higher pre-treatment IL-6 concentrations among failure cases compared with controls. External validation among predominantly diabetic TB patients found an association between pre-treatment IL-6 concentrations and subsequent recurrence and death. Similarly, external validation among predominantly HIV co-infected TB patients found an association between pre-treatment IL-6 concentrations and subsequent treatment failure and death. In a pooled analysis of 363 TB cases from the Indian and South African validation cohorts, high pre-treatment IL-6 concentrations were associated with higher risk of failure (adjusted OR (aOR) 2.16, 95% CI 1.08–4.33; p=0.02), recurrence (aOR 5.36, 95% CI 2.48–11.57; p<0.001) and death (aOR 4.62, 95% CI 1.95–10.95; p<0.001). Adding

baseline IL-6 to a risk prediction model comprised of low body mass index, high smear grade and cavitation improved model performance by 15% (C-statistic 0.66 *versus* 0.76; p=0.02).
Conclusions Pre-treatment IL-6 is a biomarker for unfavourable TB treatment outcomes. Future studies should identify optimal IL-6 concentrations for point-of-care risk prediction.

Introduction

Tuberculosis (TB) is the leading infectious cause of death worldwide with over 10 million new cases and 1.5 million deaths annually [1]. Drug-sensitive cases require 6 months of multidrug therapy for durable cure. Although effective in the majority of cases, implementing this relatively long and complex treatment regimen can increase the risk of default and overutilisation of health system resources. Developing shorter and simpler TB treatment regimens is therefore a research priority. Biomarkers have the potential to accelerate new drug and regimen discovery by identifying TB patients at high risk of unfavourable treatment outcomes who can preferentially be enrolled into early-phase clinical trials or, conversely, characterise a subgroup of low-risk patients who may benefit from shorter duration of therapy [2].

Sputum culture conversion at 2 months of treatment is the most widely used biomarker of treatment response; however, its validity in predicting subsequent clinical outcomes has been questioned in recent treatment-shortening trials [3, 4]. Blood-based biomarkers have an advantage as they overcome the difficulty of reliably obtaining high-quality sputum and can be translated into point-of-care tests. A recent study by Kumar *et al.* [5] identified an association between a combination of six chemokines and unfavourable TB treatment outcomes, suggesting a role of plasma inflammatory markers as predictors of unfavourable treatment outcomes. However, translating such a "signature" comprised of multiple and often correlated immune markers, each with their different concentration threshold for optimal risk prediction, to a point-of-care test is challenging. Whether a parsimonious selection of inflammatory markers can predict unfavourable treatment outcomes in TB patients is unclear.

We have previously identified plasma cytokines associated with lung injury in pulmonary TB cases in India [6]. We now report on a multisite discovery–validation analysis in India and South Africa to identify plasma cytokines associated with treatment failure, recurrence and death.

Methods

Study design and population

We conducted a prospective cohort study to discover plasma cytokines associated with unfavourable treatment outcomes among adult drug-sensitive pulmonary TB patients enrolled in the Cohort for Tuberculosis Research by the Indo-US Medical Partnership (CTRIUMPH) study in Pune, India [7]. We internally validated these results among CTRIUMPH participants not previously included in the discovery cohort by nesting a 1:1 age- and sex-matched case–control analysis among adult drug-sensitive pulmonary TB patients who failed treatment (cases) and those who were cured (controls). We subsequently conducted external validation analyses in two independent cohorts from India and South Africa. The Indian external validation cohort was comprised of predominantly diabetic drug-sensitive pulmonary TB patients from the Effect of Diabetes on Tuberculosis Severity (EDOTS) study in Chennai, India [8]. In this cohort, we nested a 1:2 age-, sex- and body mass index (BMI)-matched case–control analysis among adult drug-sensitive pulmonary TB patients who experienced a composite unfavourable treatment outcome (cases) of failure, recurrence or death and those with recurrence-free cure over 18 months of follow-up (controls). The South African external validation cohort was comprised of predominantly HIV co-infected drug-sensitive pulmonary TB patients from Khayelitsha, South Africa [9, 10]. In this cohort, we conducted an unmatched case–control analysis among adult drug-sensitive pulmonary TB patients who experienced a composite unfavourable treatment outcome (cases) of failure, recurrence or death and those with recurrence-free cure over 18 months of follow-up (controls). Finally, we pooled data from the Indian and South African cohorts for a combined validation analysis. Detailed descriptions of the discovery, internal validation, Indian external validation and South African external validation cohorts are provided in the supplementary material. In addition to different study populations, we used different laboratories for measuring cytokine concentrations for the internal and external validation studies to assess the generalisability of our findings (supplementary table S1). This study was approved by the ethics committees at the participating study sites in India and South Africa.

Cytokine measurement

For the discovery analysis, plasma samples collected at treatment initiation, 2 months and 6 months underwent cytokine testing, in duplicates, using multiplex ELISA by Luminex assay (Bio-Rad, Hercules, CA, USA) at the National Institutes of Health (NIH) – National Institute for Research in Tuberculosis (NIRT) – International Center for Excellence in Research (ICER) laboratory in Chennai, India. Cytokines

analysed were selected *a priori* for their role in the host inflammatory response to *Mycobacterium tuberculosis* (interferon (IFN)-γ, tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-4, IL-6, IL-10, CXCL-10, IL-12, IL-13, IL-17) [11], lung pathology (matrix metalloproteinase (MMP)-1, MMP-3, MMP-7, tissue inhibitor of metalloprotease (TIMP)-1, TIMP-2, TIMP-3, TIMP-4) [12] and fibrous remodelling (transforming growth factor (TGF)-β1, TGF-β2, TGF-β3) [13]. IL-6, IL-13 and IFN-γ measured at treatment initiation were statistically significantly associated with treatment failure in the discovery analysis and were therefore selected for internal validation. For the internal validation analysis, plasma samples collected at treatment initiation underwent cytokine testing, in duplicates, using multiplex ELISA by Luminex assay (Bio-Rad) at the Byramjee-Jeejeebhoy Government Medical College laboratory in Pune, India. IL-6 measured at treatment initiation was statistically significantly associated with treatment failure during internal validation and was therefore selected for independent external validation. For the Indian external validation cohort, plasma samples collected at treatment initiation underwent IL-6 testing by Luminex assay (R&D Systems, Minneapolis, MN, USA) at the NIH-NIRT-ICER laboratory in Chennai, India. For the South African external validation cohort, plasma samples collected at TB treatment initiation underwent IL-6 testing by Luminex assay (MilliporeSigma, Burlington, MA, USA) at the Wellcome Centre for Infectious Disease Research, Cape Town, South Africa. The lower limit of IL-6 detection was 0.31 $pg \cdot mL^{-1}$ and a nonlinear curve fit model was used to estimate IL-6 values less than the lower standard values.

Outcomes

Unfavourable TB treatment outcomes included failure, recurrence and death. Treatment failure was defined as culture confirmation of *M. tuberculosis* during the last 2 months of treatment. Recurrence was defined in a TB patient who did not fail treatment but was subsequently found to have culture-positive TB during 18 months of post-treatment follow-up. Death included all-cause mortality.

Statistical analysis

Discovery

We compared cytokine expression at treatment initiation, 2 months and 6 months between TB cases with and without an unfavourable treatment outcome using one-way hierarchical cluster analysis by Ward's method with 100 times bootstrap. Median concentrations of cytokines, stratified by treatment outcomes and duration, were used to describe the overall change in cytokine expression in response to TB treatment. Cytokine concentrations were log_{10}-transformed or z-score-normalised for analysis. Data were compared using the Mann–Whitney U-test and p-values were adjusted for multiple comparisons using the Holm–Bonferroni method [14].

Internal and external validation

We compared log_{10}-transformed cytokine concentrations between participants with and without an unfavourable treatment outcome using the Wilcoxon sign-rank test. Receiver operator characteristic (ROC) curve analysis was used to assess the ability of individual cytokines to discriminate between participants with and without unfavourable treatment outcomes. Random effects linear regression, with matched case–control pairs as random effects, was used to compare cytokine concentrations between participants with and without unfavourable treatment outcomes. Multivariable analyses were adjusted for TB disease severity assessed by BMI, chest radiography score including cavitation and sputum smear grade. We further adjusted for pre-treatment illness duration to account for differences in the timing of patient presentation relative to disease onset and its possible impact on cytokine concentrations, glycated haemoglobin (HbA1c) among participants with diabetes, and CD4 cell counts and receipt of antiretroviral therapy (ART) among HIV co-infected participants.

Pooled validation

We pooled data from the internal and external validation cohorts to measure the association between baseline IL-6 concentrations and unfavourable TB treatment outcomes. Since each of the three validation cohorts utilised different laboratories, ELISA kits and testing protocols, there was substantial variability in $pg \cdot mL^{-1}$ IL-6 quantification across the study sites. To account for this variability, we used z-scores and percentiles to standardise IL-6 concentrations across the three validation cohorts. For each validation cohort, we calculated a z-score for $pg \cdot mL^{-1}$ IL-6 concentrations using the formula z=(observed concentration−mean concentration)/standard deviation. This standardised z-score was used for ROC analysis in the pooled validation cohort. Similarly, we classified "high" IL-6 concentrations as having $pg \cdot mL^{-1}$ concentrations in the highest quartile for a given study. "Low" IL-6 concentrations were defined as having $pg \cdot mL^{-1}$ concentrations in the lower three quartiles for a given study. To account for clustering by study location and case–control matching, we used random effects logistic regression with bootstrap confidence intervals, to measure the association between "high" baseline IL-6 concentrations and

unfavourable TB treatment outcomes of failure, recurrence or death. Multivariable analyses adjusted for age, sex, BMI, smear grade, chest radiography score including cavitation, pre-treatment illness duration, HIV and diabetes. Furthermore, cavitary disease, low BMI and higher smear grade have previously been shown to predict unfavourable TB treatment outcomes [15]. Therefore, we calculated the incremental gain

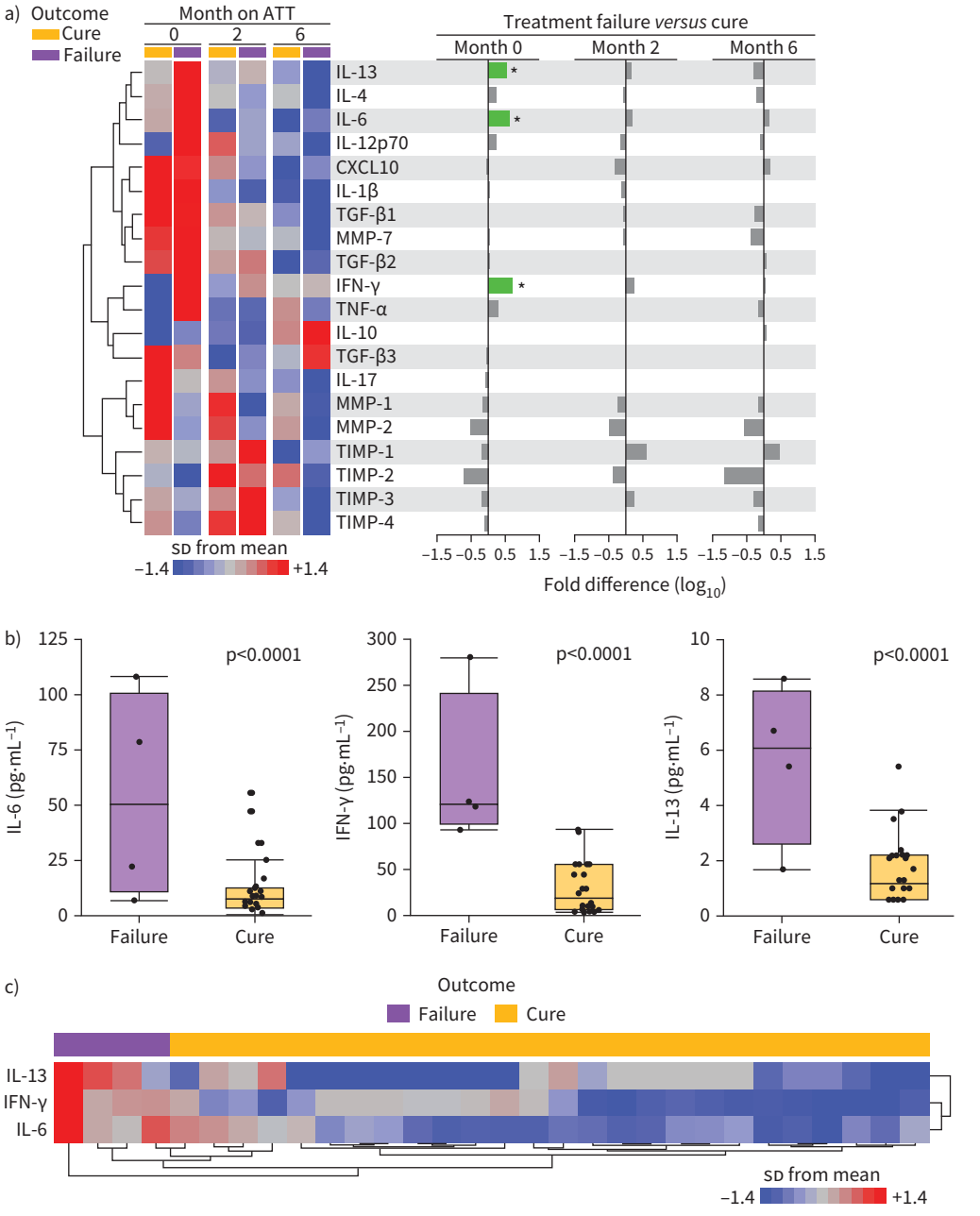

FIGURE 1 Cytokines associated with treatment failure in the discovery cohort. a) Differences in cytokine concentrations (z-score-standardised and $\log_{10}$-transformed) between participants who failed treatment and those who were cured, stratified by duration of treatment. *: p<0.05 after adjustment for multiple comparisons. ATT: antituberculosis treatment; IL: interleukin; CXCL: CXC motif ligand; TGF: transforming growth factor; MMP: matrix metalloproteinase; IFN: interferon; TIMP: tissue inhibitor of metalloprotease. b) Differences in absolute cytokine concentrations ($pg \cdot mL^{-1}$) at enrolment (month 0) between participants who failed treatment and those who were cured. c) A two-way unsupervised hierarchical cluster analysis using concentrations of IL-6, IFN-γ and IL-13 measured at enrolment (month 0) to identify a unique combined profile of biomarker protein expression that could distinguish participants based on treatment outcome.

in discriminatory ability, measured by the C-statistic, by adding baseline IL-6 to a risk prediction model comprised of cavitation, BMI and smear grade.

Results

Baseline characteristics of the discovery subcohort did not differ significantly from the full cohort and are summarised in supplementary table S2. Of the 30 participants selected in the discovery subcohort, four (13%) failed treatment and none had recurrence or died. Participants who failed treatment had significantly higher baseline concentrations of IL-6, IFN-γ and IL-13 relative to those without failure ($p<0.001$ for all); however, we did not find statistically significant differences in cytokine levels measured at 2 and 6 months of treatment (figure 1). The internal validation cohort was comprised of 20 participants with culture-confirmed treatment failure and 20 age- and sex-matched participants with culture-confirmed cure. Overall, three (8%) participants were HIV co-infected and three (8%) had diabetes. Except for lower median BMI (16.0 *versus* 17.4 $kg \cdot m^{-2}$; p=0.02) and longer pre-treatment illness duration (35 *versus* 30 days; p=0.03), cases were comparable to controls with respect to their baseline characteristics (supplementary table S3). After adjusting for markers of disease severity and pre-treatment illness duration, cases had significantly higher IL-6 concentrations at baseline relative to controls (0.26 log higher concentration, 95% CI 0.01–0.52; p=0.04) with an area under the curve (AUC) of 0.70 (95% CI 0.52–0.88). We did not find differences in IFN-γ and IL-13 concentrations by treatment outcomes (table 1 and figure 2).

The Indian external validation cohort was comprised of 72 cases with an unfavourable treatment outcome and 122 age-, sex- and BMI-matched controls with recurrence-free cure. Overall, 115 (59%) participants had diabetes and HIV was an exclusion criterion. Cases were comparable to controls with respect to their baseline characteristics, except for a higher proportion of ever-smokers (supplementary table S4). Overall, 18 (9%) participants failed treatment, 35 (18%) had recurrence and 19 (10%) died. Cases with an unfavourable treatment outcome had significantly higher concentrations of IL-6 at baseline relative to controls (0.26 log higher concentration, 95% CI 0.17–0.36; p<0.001), after adjusting for markers of disease severity, pre-treatment illness duration, smoking status and diabetes. Results were similar when restricted to TB patients with diabetes and adjusting for HbA1c levels; cases experiencing recurrence or death had higher baseline IL-6 concentrations compared with controls (table 2 and figure 3). IL-6 could effectively discriminate TB patients with and without unfavourable treatment outcomes with AUC 0.73 (95% CI 0.66–0.80) for all participants and AUC 0.73 (95% CI 0.64–0.83) when restricted to participants with diabetes (figure 3).

Of the 129 participants enrolled in the South African external validation cohort, 76 (59%) had HIV, 29 (38%) were receiving ART and the median (interquartile range) CD4 count was 192 (66–366) $cells \cdot mm^{-3}$ (supplementary table S5). Overall, 18 (14%) participants experienced an unfavourable treatment outcome: nine (7%) failed treatment, four (3%) had recurrence and five (4%) died. After adjusting for age, sex, HIV status, ART and markers of disease severity, participants who experienced an unfavourable treatment outcome had significantly higher concentrations of IL-6 at baseline compared with those with recurrence-free cure (0.38 log higher concentration, 95% CI 0.13–0.63; p=0.003). However, we found marginally significant associations between higher baseline IL-6 concentrations and unfavourable TB treatment outcomes when restricted to HIV co-infected participants (table 3). IL-6 could discriminate TB patients with and without unfavourable treatment outcomes with AUC 0.69 (95% CI 0.55–0.85) for all participants and AUC 0.66 (95% CI 0.42–0.85) when restricted to HIV co-infected participants (figure 4).

TABLE 1 Difference in cytokine concentrations between tuberculosis patients who failed treatment (cases) and those who were cured (controls) in the internal validation cohort

Cytokines	Log_{10} difference in baseline cytokine levels comparing cases with controls			
	Unadjusted difference (95% CI)	p-value	Adjusted difference (95% CI)	p-value
Interleukin-6	0.29 (0.06 to 0.52)	0.01	0.26 (0.01 to 0.52)	0.04
Interferon-γ	−0.12 (−0.68 to 0.42)	0.65	−0.38 (−0.98 to 0.21)	0.21
Interleukin-13	0.09 (−0.20 to 0.39)	0.52	0.13 (−0.22 to 0.50)	0.45

Adjusted analyses accounting for body mass index, chest radiography score including cavitation, smear grade and pre-treatment illness duration. Age and sex are adjusted by study design.

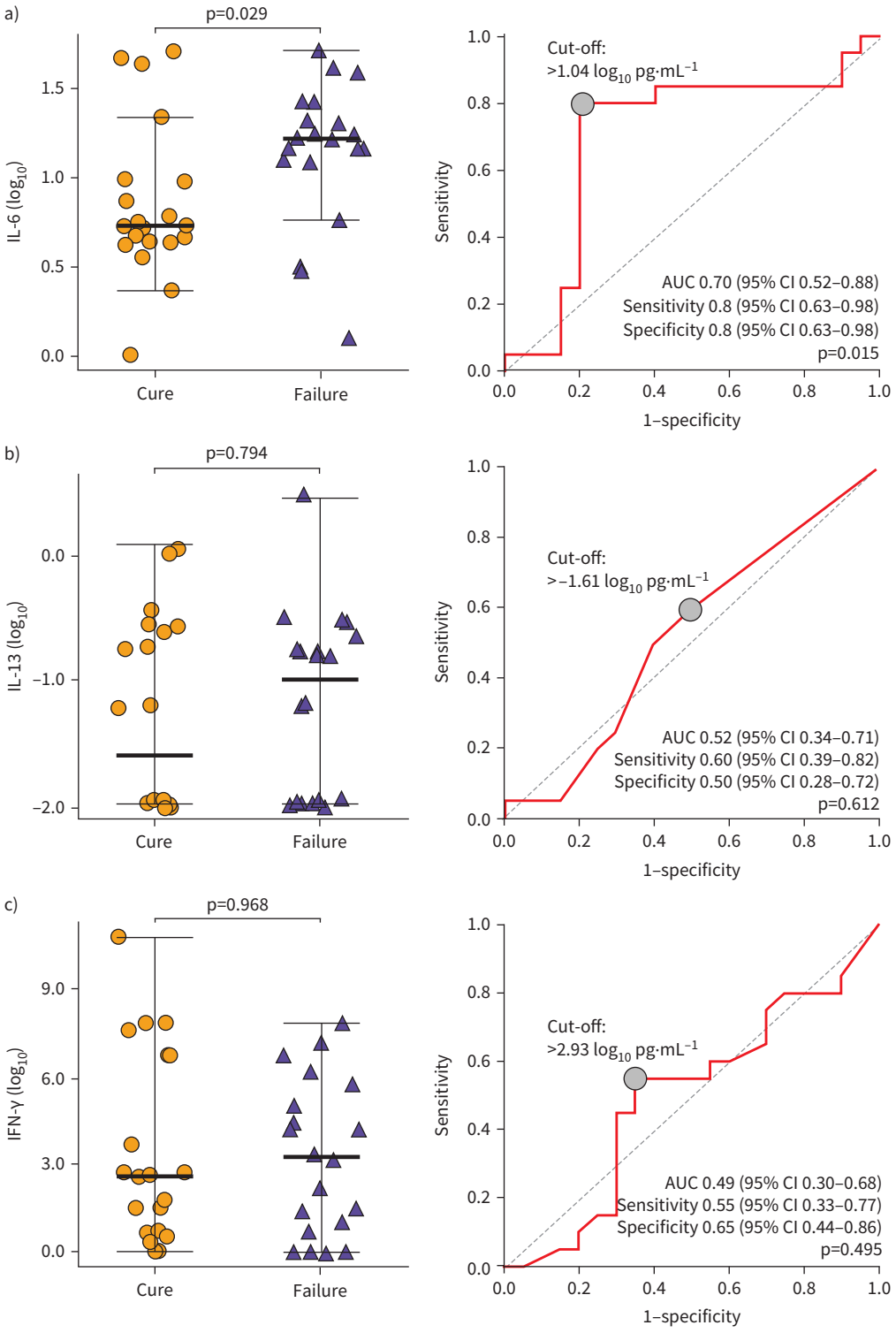

FIGURE 2 Difference in a) interleukin (IL)-6, b) IL-13 and c) interferon (IFN)-γ concentrations between tuberculosis treatment failures and cures and associated area under the curve (AUC) for failure–cure classification in the internal validation cohort.

The pooled validation from Indian and South African cohorts was comprised of 363 participants experiencing 110 unfavourable outcomes: 47 (13%) had failure, 39 (11%) had recurrence and 24 (6%) died. High IL-6 concentration at baseline was present in 91 (25%) participants and was associated with

TABLE 2 Difference in interleukin-6 concentrations between tuberculosis patients with and without unfavourable treatment outcomes in the Indian external validation cohort

Treatment outcomes	Log_{10} difference in baseline cytokine levels comparing cases with controls			
	Unadjusted difference (95% CI)	p-value	Adjusted difference (95% CI)	p-value
All participants				
Composite	0.24 (0.16 to 0.32)	<0.001	0.26 (0.17 to 0.36)	<0.001
Failure	0.10 (−0.02 to 0.24)	0.10	0.16 (−0.02 to 0.36)	0.07
Recurrence	0.26 (0.17 to 0.35)	<0.001	0.27 (0.16 to 0.38)	<0.001
Death	0.32 (0.18 to 0.46)	<0.001	0.32 (0.14 to 0.50)	<0.001
Diabetes only				
Composite	0.23 (0.12 to 0.35)	<0.001	0.22 (0.07 to 0.37)	0.01
Failure	0.15 (−0.03 to 0.34)	0.11	0.15 (−0.12 to 0.43)	0.27
Recurrence	0.22 (0.10 to 0.36)	0.001	0.25 (0.05 to 0.44)	0.01
Death	0.30 (0.10 to 0.50)	0.004	0.26 (0.01 to 0.51)	0.04

Adjusted analyses accounting for chest radiography score including cavitation, smear grade, ever-smoking, diabetes and pre-treatment illness duration. Age, sex and body mass index are adjusted by study design. Diabetes-restricted analysis further adjusted for glycated haemoglobin levels.

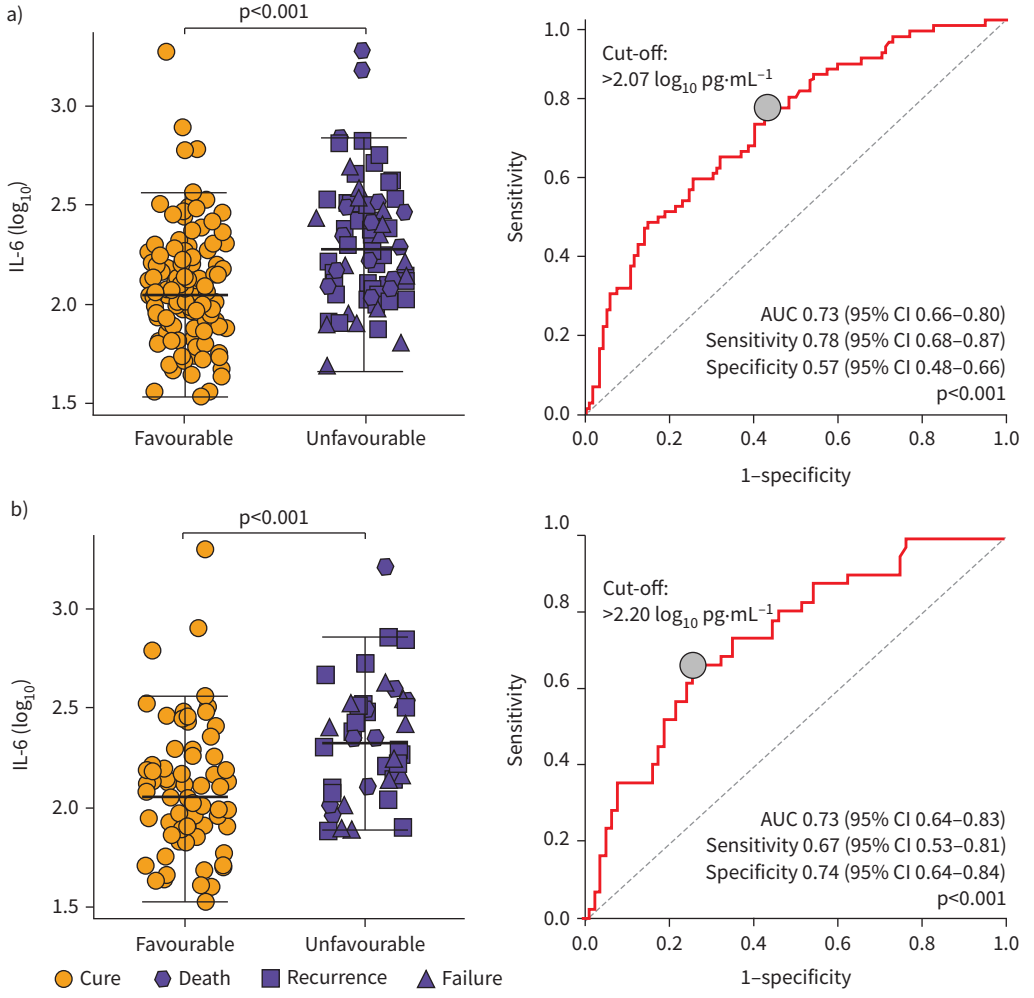

FIGURE 3 Difference in interleukin (IL)-6 concentrations between tuberculosis patients with and without unfavourable treatment outcomes and associated area under the curve (AUC) among a) all participants and b) restricted to patients with diabetes only in the Indian external validation cohort.

TABLE 3 Difference in interleukin-6 concentrations between tuberculosis patients with and without unfavourable treatment outcomes after adjusting for markers of disease severity in the South African external validation cohort

Treatment outcomes	Log_{10} difference in baseline cytokine levels comparing participants with unfavourable treatment outcomes with cures			
	Unadjusted difference (95% CI)	p-value	Adjusted difference (95% CI)	p-value
All participants				
Composite	0.33 (0.06 to 0.60)	0.01	0.38 (0.13 to 0.63)	0.003
Failure	0.41 (0.05 to 0.76)	0.02	0.49 (0.15 to 0.82)	0.004
Recurrence	0.10 (−0.43 to 0.64)	0.70	−0.08 (−0.60 to 0.42)	0.73
Death	0.38 (−0.01 to 0.86)	0.10	0.50 (0.06 to 0.94)	0.02
HIV co-infected only				
Composite	0.22 (−0.10 to 0.55)	0.15	0.29 (−0.01 to 0.60)	0.06
Failure	0.39 (−0.04 to 0.83)	0.06	0.46 (0.01 to 0.90)	0.04
Recurrence	−0.64 (−1.39 to 0.10)	0.10	−0.56 (−1.23 to 0.12)	0.10
Death	0.36 (−0.12 to 0.85)	0.13	0.48 (0.05 to 0.92)	0.03

Adjusted analyses accounting for age, sex, body mass index, chest radiography score including cavitation, smear grade and HIV. HIV-restricted analyses further adjusted for CD4 cell count and antiretroviral therapy receipt.

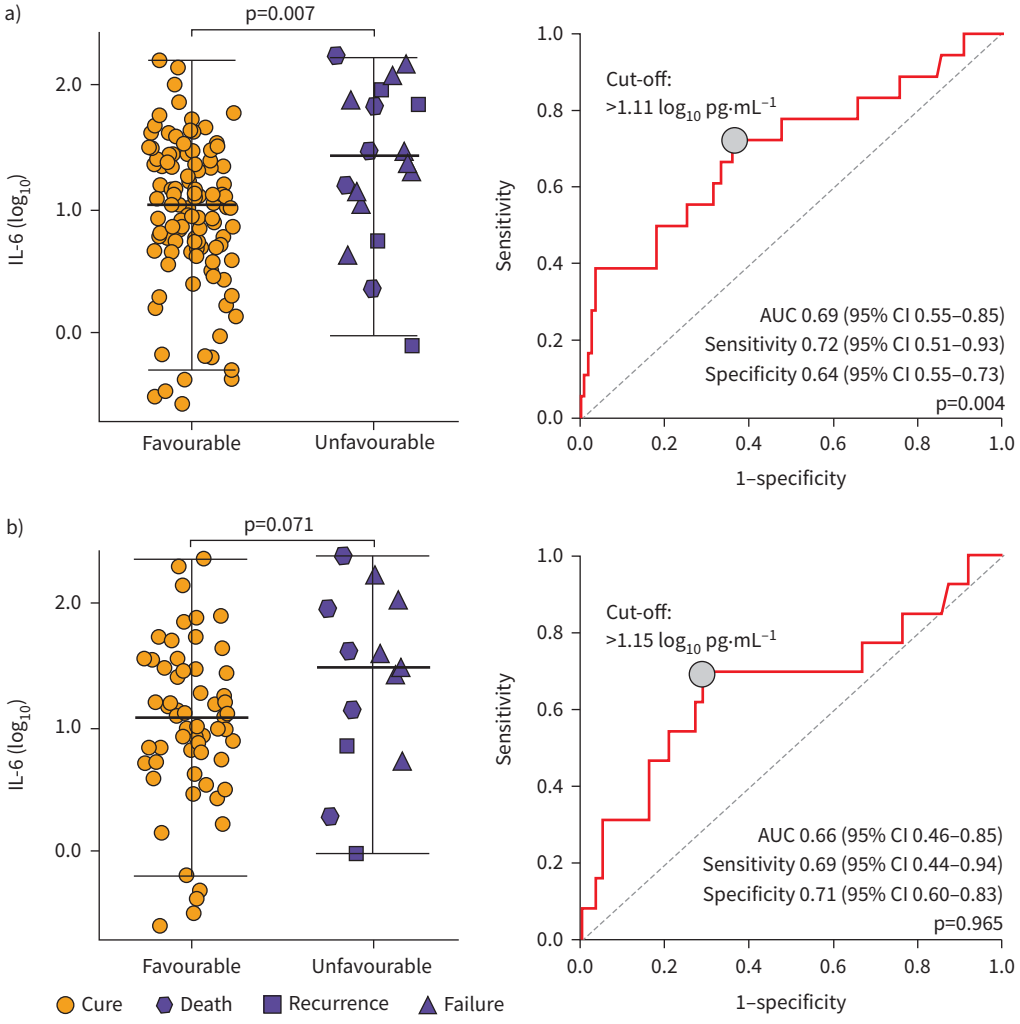

FIGURE 4 Difference in interleukin (IL)-6 concentrations between tuberculosis patients with and without unfavourable treatment outcomes and associated area under the curve (AUC) among a) all participants and b) restricted to HIV co-infected patients only in the South African external validation cohort.

3-fold higher odds of any subsequent unfavourable treatment outcome (95% CI 2.03–5.89; p<0.001) after adjusting for age, sex, BMI, chest radiography score including cavitation, smear grade, pre-treatment illness duration, HIV, diabetes and correlations within each contributing study (table 4). Similarly, high baseline IL-6 concentrations were significantly associated with subsequent failure (adjusted OR (aOR) 2.22, 95% CI 1.04–4.75; p=0.03), recurrence (aOR 5.92, 95% CI 2.52–13.90; p<0.001) and death (aOR 5.53, 95% CI 2.08–14.68; p<0.001) in adjusted analyses.

Low BMI, high smear grade (⩾2+) and cavitation on chest radiography were poor baseline predictors of unfavourable TB treatment outcomes in our cohort. A risk prediction model comprised of these variables had a C-statistic (AUC) of 0.66 (95% CI 0.56–0.77) for discriminating between TB patients with and without unfavourable treatment outcomes. However, baseline IL-6 could discriminate TB patients with and without unfavourable treatment outcomes with AUC 0.71 (95% CI 0.65–0.77); a z-score cut-off of −0.32 identified by the Youden's method had a sensitivity of 76%, specificity of 60% and a negative predictive value of 85% for ruling out subsequent unfavourable TB treatment outcomes. Furthermore, the addition of baseline IL-6 improved performance of the prediction model comprised of low BMI, high smear grade and cavitation by 15% (C-statistic 0.76, 95% CI 0.67–0.85; p=0.02) (supplementary table S6).

Discussion

Our discovery–validation analyses among geographically and epidemiologically diverse populations of pulmonary TB cases found an association between higher baseline IL-6 concentrations in plasma and subsequent treatment failure, recurrence and death. This association was generalisable despite different testing laboratories, comorbidities of diabetes and HIV, and after adjusting for disease severity. Furthermore, the inclusion of baseline IL-6 significantly improved the performance of established risk prediction models for poor clinical outcomes. Our data support the role of IL-6 as a biomarker for unfavourable TB treatment outcomes.

IL-6 exhibits a pleotropic immunoregulatory role by promoting neutrophil and macrophage recruitment and survival, inducing the acute-phase response in the liver, and facilitating tissue damage by stimulating protease secretion and matrix deposition [16, 17]. IL-6 has long been considered a nonspecific marker of TB disease activity, and prior studies have identified a correlation between IL-6 concentrations, bacterial burden and lung pathology [6, 18, 19]. However, few studies have reported an association between IL-6 and TB treatment outcomes. A secondary analysis of an early-phase treatment trial reported correlations between TB disease severity and baseline IL-6 concentrations, and between greater declines in IL-6 concentrations and sputum culture conversion during the first 2 months of treatment [20]. However, this study did not report on final treatment outcomes. Across the three independent cohorts analysed in our study, we found a consistent association between higher baseline IL-6 concentrations and subsequent unfavourable TB treatment outcomes. This association remained significant after adjusting for markers of disease severity and suggests an independent predictive role of baseline IL-6 for risk stratification in TB patients.

While our study found an association of baseline IL-6 concentrations with recurrent TB in participants with and without diabetes, we did not find a similar association among HIV co-infected participants. This finding is inconsistent with a recent study by Sivro *et al*. [21], which reported an association between IL-6 concentrations, measured after completion of TB treatment, and recurrent disease among ART-naïve HIV co-infected patients. This inconsistency may be due to differences in the underlying study populations and timing of IL-6 measurement, *i.e.* treatment initiation in our study compared with after treatment completion in the Sivro *et al*. [21] study. However, our sample size for HIV-restricted analysis was limited and further

TABLE 4 Association between high concentration of interleukin-6 at baseline and unfavourable tuberculosis treatment outcomes in the pooled validation analysis

Treatment outcomes	OR for unfavourable treatment outcomes			
	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Composite	3.32 (2.02–5.46)	<0.001	3.45 (2.03–5.89)	<0.001
Failure	2.16 (1.08–4.33)	0.02	2.22 (1.04–4.75)	0.03
Recurrence	5.36 (2.48–11.57)	<0.001	5.92 (2.52–13.90)	<0.001
Death	4.62 (1.95–10.95)	<0.001	5.53 (2.08–14.68)	<0.001

Regression analyses are adjusted for age, sex, body mass index, chest radiography score including cavitation, smear grade, HIV and diabetes.

validation in larger cohorts of HIV co-infected TB patients is needed. Conversely, we found higher baseline IL-6 concentrations among TB patients with diabetes who had recurrence or died; we did not find similar differences by treatment failure. The precise reasons for this are unclear. The internal validation cohort was specifically designed to examine an outcome of treatment failure, while the external validation cohorts did not perform *a priori* sampling on treatment outcomes. A relatively smaller number of treatment failure cases in the external validation cohorts may explain a weaker association of baseline IL-6 with treatment failure. Furthermore, diabetes is characterised by hyperinflammation and higher overall IL-6 concentrations among participants with diabetes at the start of TB treatment could explain this finding [22–24]. Nevertheless, further study is needed explore longitudinal changes in the immunological profile of TB patients with diabetes who fail treatment.

A consistent finding in our study, regardless of HIV or diabetes comorbidity, was the association between baseline IL-6 and all-cause mortality. The ability of IL-6 to predict mortality has previously been reported in several noncommunicable and infectious diseases, and more recently in COVID-19 [25–29]. While studies among TB patients with advanced HIV do suggest a role of IL-6 in explaining excess mortality [30, 31], similar associations among HIV-uninfected TB patients have yet to be investigated. Our study addresses this knowledge gap by reporting a consistent and significant association between high baseline IL-6 concentrations and subsequent all-cause mortality in HIV-uninfected TB patients with and without diabetes. Importantly, the majority of deaths in our study occurred during TB treatment, and the association between IL-6 and mortality was independent of baseline disease severity and duration of symptomatic illness prior to testing. These data suggest an independent role of IL-6-mediated immune pathways in mortality during TB treatment.

An important limitation of our study was the relatively small discovery cohort and a limited selection of cytokines. We selected cytokines *a priori* based on their biological plausibility; however, a nontargeted approach may identify additional cytokines predictive of unfavourable outcomes. Furthermore, our randomly selected discovery cohort was exploratory in nature and did not include participants with recurrence or death. Therefore, we are likely underpowered to detect a statistically significant association of a smaller magnitude for selected cytokines. We did not objectively assess treatment adherence. However, we did measure self-reported treatment adherence and all participants reported taking >95% of their prescribed doses. Finally, each of the validation cohorts used a different laboratory, ELISA kits and testing protocols, which led to a high variability in IL-6 quantification. Therefore, we were unable to identify a $pg{\cdot}mL^{-1}$ IL-6 concentration cut-off for point-of-care risk prediction.

Despite these limitations, our study found a significant and generalisable association between high baseline IL-6 concentrations and subsequent unfavourable TB treatment outcomes in a relatively large and epidemiologically diverse population. Although the overall discriminatory ability of IL-6 in our study was modest, it was nevertheless consistent across the Indian and South African validation cohorts. Identifying concentration thresholds to optimise either sensitivity or specificity could facilitate the use of baseline IL-6 as a rule-out or rule-in test, respectively, for risk stratification at treatment initiation. Such an approach may be important given the poor performance of low BMI, high smear grade and cavitary disease, clinical characteristics that have recently been shown to identify hard-to-treat phenotypes of TB patients in clinical trial settings [15], in predicting unfavourable treatment outcomes in programmatic settings as seen in this study. For instance, the inclusion of baseline IL-6 in a risk prediction model comprised of these clinical variables significantly improved performance of the prediction model by nearly 15% in our study, supporting a strategy of combining baseline IL-6 with established clinical predictors of unfavourable outcomes for risk stratification in TB. Furthermore, in contrast to prior studies which identified predictive signatures comprised of multiple immune markers, our data identified a single cytokine predictive of unfavourable TB treatment outcomes, potentially simplifying its translation to a point-of-care assay. Future studies should focus on standardising IL-6 measurements across laboratories for point-of-care translation and identify optimal predictive thresholds of IL-6 concentrations for risk stratification.

Conflict of interest: A.N. Gupte has nothing to disclose. P. Kumar has nothing to disclose. M. Araújo-Pereira has nothing to disclose. V. Kulkarni has nothing to disclose. M. Paradkar has nothing to disclose. N. Pradhan has nothing to disclose. P. Menon has nothing to disclose. C. Padmapriyadarsini reports funding from the Dept of Biotechnology, Government of India, within the scope of the present manuscript. L-E. Hanna has nothing to disclose. S.V.B. Yogendra Shivakumar has nothing to disclose. N. Rockwood has nothing to disclose. E. Du Bruyn has nothing to disclose. R. Karyakarte has nothing to disclose. S. Gaikwad has nothing to disclose. R.C. Bollinger reports research support from the NIH and Ujala Foundation, within the scope of the present manuscript. J. Golub reports grants to their institution from the NIH, within the scope of the present manuscript. N. Gupte reports

grants to their institution from the NIH, within the scope of the present manuscript. V. Viswanathan has nothing to disclose. R.J. Wilkinson has nothing to disclose. V. Mave has nothing to disclose. S. Babu has nothing to disclose. H. Kornfeld has nothing to disclose. B.B. Andrade has nothing to disclose. A. Gupta reports grants to their institution from the National Institutes of Health within the scope of the present manuscript; grants to their institution from CRDF outside the scope of the present manuscript; and membership of an NIH/NIAID Advisory Council and the Indo-US Science Technology Forum Board.

Support statement: Data were collected as part of the Regional Prospective Observational Research for Tuberculosis (RePORT) India Consortium. This project has been funded in whole or in part with Federal funds from the Government of India's Dept of Biotechnology (DBT), Indian Council of Medical Research (ICMR), NIH, NIAID, Office of AIDS Research (OAR), and distributed in part by CRDF Global. Research reported in this publication was also supported by the NIAID (R01AI097494 and UM1AI069465), the Wyncote Foundation, the Gilead Foundation and the Ujala Foundation. This research was also funded in part by a 2019 developmental grant from the Johns Hopkins University Center for AIDS Research, an NIH-funded program (1P30AI094189), which is supported by the following NIH Co-Funding and Participating Institutes and Centers: NIAID, NCI, NICHD, NHLBI, NIDA, NIA, NIGMS, NIDDK and NIMHD. A.N. Gupte was supported by the NIAID (K99AI151094). M. Araújo-Pereira received a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (finance code 001). The work of B.B. Andrade and R.J. Wilkinson was supported by a grant from the NIAID (U01AI115940), by the Intramural Research Program of the Oswaldo Cruz Foundation (FIOCRUZ) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil. R.J. Wilkinson is supported by The Francis Crick Institute which receives support from the Medical Research Council (FC001218), Cancer Research UK (FC001218) and Wellcome (FC001218). Additional support was provided by the Wellcome Trust (203135 and 104803), the European and Developing Countries Clinical Trials Partnership (SRIA 2015-1065), and the Foundation for the NIH (WILKI16PTB). The funders had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. The contents of this publication are solely the responsibility of the authors and do not represent the official views of the DBT, ICMR, MRC, NIH or CRDF Global. Any mention of trade names, commercial projects or organisations does not imply endorsement by any of the sponsoring organisations. The authors also acknowledge support from Persistent Systems in kind. Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 World Health Organization. Global Tuberculosis Report. Geneva, WHO, 2020.
2 Wallis RS, Kim P, Cole S, *et al.* Tuberculosis biomarkers discovery: developments, needs, and challenges. *Lancet Infect Dis* 2013; 13: 362–372.
3 Phillips PP, Fielding K, Nunn AJ. An evaluation of culture results during treatment for tuberculosis as surrogate endpoints for treatment failure and relapse. *PLoS One* 2013; 8: e63840.
4 Wallis RS, Peppard T, Hermann D. Month 2 culture status and treatment duration as predictors of recurrence in pulmonary tuberculosis: model validation and update. *PLoS One* 2015; 10: e0125403.
5 Kumar NP, Moideen K, Nancy A, *et al.* Plasma chemokines are baseline predictors of unfavorable treatment outcomes in pulmonary tuberculosis. *Clin Infect Dis* 2021; 73: e3419–e3427.
6 Gupte AN, Selvaraju S, Gaikwad S, *et al.* Higher IL-6 levels and changes in TGF-β are associated with lung impairment in pulmonary tuberculosis. *ERJ Open Res* 2021; 7: 00390-2020.
7 Gupte A, Padmapriyadarsini C, Mave V, *et al.* Cohort for Tuberculosis Research by the Indo-US Medical Partnership (CTRIUMPH): protocol for a multicentric prospective observational study. *BMJ Open* 2016; 6: e010542.
8 Kornfeld H, West K, Kane K, *et al.* High prevalence and heterogeneity of diabetes in patients with TB in South India: a report from the Effects of Diabetes on Tuberculosis Severity (EDOTS) study. *Chest* 2016; 149: 1501–1508.
9 Rockwood N, Costa DL, Amaral EP, *et al. Mycobacterium tuberculosis* induction of heme oxygenase-1 expression is dependent on oxidative stress and reflects treatment outcomes. *Front Immunol* 2017; 8: 542.
10 Rockwood N, Sirgel F, Streicher E, *et al.* Low frequency of acquired isoniazid and rifampicin resistance in rifampicin-susceptible pulmonary tuberculosis in a setting of high HIV-1 infection and tuberculosis coprevalence. *J Infect Dis* 2017; 216: 632–640.
11 O'Garra A, Redford PS, McNab FW, *et al.* The immune response in tuberculosis. *Annu Rev Immunol* 2013; 31: 475–527.
12 Ong CW, Elkington PT, Friedland JS. Tuberculosis, pulmonary cavitation, and matrix metalloproteinases. *Am J Respir Crit Care Med* 2014; 190: 9–18.
13 Tatler AL, Jenkins G. TGF-beta activation and lung fibrosis. *Proc Am Thorac Soc* 2012; 9: 130–136.
14 Holm S. A simple sequentially rejective multiple test procedure. *Scand J Stat* 1979; 6: 65–70.
15 Imperial MZ, Nahid P, Phillips PPJ, *et al.* A patient-level pooled analysis of treatment-shortening regimens for drug-susceptible pulmonary tuberculosis. *Nat Med* 2018; 24: 1708–1715.

16 Rincon M. Interleukin-6: from an inflammatory marker to a target for inflammatory diseases. *Trends Immunol* 2012; 33: 571–577.
17 Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nat Immunol* 2015; 16: 448–457.
18 Casarini M, Ameglio F, Alemanno L, *et al.* Cytokine levels correlate with a radiologic score in active pulmonary tuberculosis. *Am J Respir Crit Care Med* 1999; 159: 143–148.
19 Mesquita ED, Gil-Santana L, Ramalho D, *et al.* Associations between systemic inflammation, mycobacterial loads in sputum and radiological improvement after treatment initiation in pulmonary TB patients from Brazil: a prospective cohort study. *BMC Infect Dis* 2016; 16: 368.
20 Sigal GB, Segal MR, Mathew A, *et al.* Biomarkers of tuberculosis severity and treatment effect: a directed screen of 70 host markers in a randomized clinical trial. *EBioMedicine* 2017; 25: 112–121.
21 Sivro A, McKinnon LR, Yende-Zuma N, *et al.* Plasma cytokine predictors of tuberculosis recurrence in antiretroviral-treated human immunodeficiency virus-infected individuals from Durban, South Africa. *Clin Infect Dis* 2017; 65: 819–826.
22 Restrepo BI, Fisher-Hoch SP, Pino PA, *et al.* Tuberculosis in poorly controlled type 2 diabetes: altered cytokine expression in peripheral white blood cells. *Clin Infect Dis* 2008; 47: 634–641.
23 Kumar NP, Sridhar R, Banurekha VV, *et al.* Type 2 diabetes mellitus coincident with pulmonary tuberculosis is associated with heightened systemic type 1, type 17, and other proinflammatory cytokines. *Ann Am Thorac Soc* 2013; 10: 441–449.
24 Prada-Medina CA, Fukutani KF, Pavan Kumar N, *et al.* Systems immunology of diabetes-tuberculosis comorbidity reveals signatures of disease complications. *Sci Rep* 2017; 7: 1999.
25 Baune BT, Rothermundt M, Ladwig KH, *et al.* Systemic inflammation (interleukin 6) predicts all-cause mortality in men: results from a 9-year follow-up of the MEMO Study. *Age* 2011; 33: 209–217.
26 Lindmark E, Diderholm E, Wallentin L, *et al.* Relationship between interleukin 6 and mortality in patients with unstable coronary artery disease: effects of an early invasive or noninvasive strategy. *JAMA* 2001; 286: 2107–2113.
27 Srisangthong P, Wongsa A, Kittiworawitkul P, *et al.* Early IL-6 response in sepsis is correlated with mortality and severity score. *Crit Care* 2013; 17: Suppl. 2, P34.
28 Leisman DE, Ronner L, Pinotti R, *et al.* Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. *Lancet Respir Med* 2020; 8: 1233–1244.
29 McElvaney OJ, McEvoy NL, McElvaney OF, *et al.* Characterization of the inflammatory response to severe COVID-19 illness. *Am J Respir Crit Care Med* 2020; 202: 812–821.
30 Manabe YC, Andrade BB, Gupte N, *et al.* A parsimonious host inflammatory biomarker signature predicts incident TB and mortality in advanced HIV. *Clin Infect Dis* 2019; 69: 352–356.
31 Ravimohan S, Tamuhla N, Steenhoff AP, *et al.* Immunological profiling of tuberculosis-associated immune reconstitution inflammatory syndrome and non-immune reconstitution inflammatory syndrome death in HIV-infected adults with pulmonary tuberculosis starting antiretroviral therapy: a prospective observational cohort study. *Lancet Infect Dis* 2015; 15: 429–438.