**Matrix degradation in HIV-1-associated tuberculosis and tuberculosis immune reconstitution inflammatory syndrome: a prospective, observational study**

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Key points: Matrix metalloproteinase activity in TB diverges according to HIV serostatus and compartment, releasing the matrix degradation product PIIINP into plasma. Plasma PIIINP and MMP-8 are potential predictive biomarkers of TB-IRIS, while doxycycline inhibits TB-driven matrix degradation.

Key words: HIV-1, tuberculosis, immune reconstitution inflammatory syndrome, matrix metalloproteinase, matrix degradation product, procollagen III N-terminal propeptide.

Word Count = 2993

**Abstract**

Background: Extensive immunopathology occurs in HIV-tuberculosis (TB) co-infection but the underlying molecular mechanisms are not well-defined. Excessive matrix metalloproteinase (MMP) activity is emerging as a key process but has not been systematically studied in HIV-associated TB.

Methods: We performed a cross-sectional study of matrix turnover in HIV-1-infected and -uninfected TB patients and controls, and a prospective cohort study of HIV-1-infected TB patients at risk of TB immune reconstitution inflammatory syndrome (TB-IRIS), in Cape Town, South Africa. Sputum and plasma MMP concentrations were quantified by Luminex, plasma procollagen III N-terminal propeptide (PIIINP) by ELISA and urinary lipoarabinomannan (LAM) by Alere Determine TB LAM assay. Peripheral blood mononuclear cells from healthy donors were cultured with *Mycobacterium tuberculosis* (Mtb) and extracellular matrix in a 3-D model of TB granuloma formation.

Findings: MMP activity differed between HIV-1-infected and -uninfected TB patients and corresponded with specific TB clinical phenotypes. HIV-1-infected TB patients had reduced pulmonary MMP concentrations, associated with reduced cavitation, but increased plasma PIIINP, compared to HIV-1-uninfected TB patients. Elevated extra-pulmonary extracellular matrix turnover associated with TB-IRIS, both before and during TB-IRIS onset. The predominant collagenase was MMP-8, which was likely neutrophil-derived and Mtb-antigen-driven. Mtb-induced matrix degradation was suppressed by the MMP inhibitor doxycycline *in-vitro*.

Interpretation: MMP activity in TB differs by HIV-1 status and compartment, and releases matrix degradation products. Matrix turnover in HIV-1-infected patients is increased before and during TB-IRIS, informing novel diagnostic strategies. MMP inhibition is a potential host-directed therapy strategy for prevention and treatment of TB-IRIS.

**Introduction**

Tuberculosis (TB) and HIV-1 infection are global pandemics with complex interplay. HIV-1 increases the risk of TB, while TB accelerates HIV-1 progression and is the commonest cause of HIV-related death [[1](#_ENREF_1)]. Initiation of antiretroviral therapy (ART) reduces mortality, but is frequently complicated by development of tuberculosis immune reconstitution inflammatory syndrome (TB-IRIS) [[2](#_ENREF_2)]. TB-IRIS is characterised by an acute inflammatory response to *Mycobacterium tuberculosis* (Mtb) presenting either in an uncharacteristically exaggerated form (unmasking IRIS), or as deterioration in a patient already receiving TB treatment (paradoxical IRIS) [[3](#_ENREF_3)].

In immunocompetent adults, Mtbtypically causes apical pulmonary disease with cavitation, which drives transmission and spread [[4](#_ENREF_4)]. Conversely, in advanced HIV-1 infection, disseminated disease is more common and pulmonary cavitation less frequent [[1](#_ENREF_1), [5](#_ENREF_5)]. In paradoxical TB-IRIS, focal inflammatory pathology primarily affects the lung and lymph nodes, causing tissue damage [[6](#_ENREF_6)]. Whilst specific features have been described, such as hypercytokinaemia and inflammasome activation, the final effectors of this immunopathology are poorly defined [[2](#_ENREF_2), [6-9](#_ENREF_6)]. In HIV-uninfected TB patients, pulmonary immunopathology is driven by matrix metalloproteinases (MMPs), in particular the collagenase MMP-1, releasing matrix degradation products [[10](#_ENREF_10), [11](#_ENREF_11)]. Pulmonary MMPs are suppressed in advanced HIV-1 infection, providing a mechanism for reduced lung cavitation [[12](#_ENREF_12)].

In this study, we systematically explored MMP activity and immunopathology in HIV-1-associated TB. We hypothesized that HIV-1-associated TB would be characterised by reduced MMP activity at TB diagnosis compared to HIV-uninfected TB, but that increased MMP activity would associate with inflammatory pathology during TB-IRIS. Our insights inform novel approaches to risk stratify and diagnose TB-IRIS, and also host-directed interventions to prevent pathology.

**Materials and Methods**

Full methods are provided in the online supplement. The study was approved by the University of Cape Town Human Research Ethics Committee (REF 516/2011). Cross-sectional study participants were healthy volunteers, patients with symptoms requiring assessment, or recently diagnosed TB patients (Supplementary Table S1). Longitudinal study participants were ART naïve HIV-1-infected patients with a CD4 count <200 cells/mm3 and recently diagnosed TB. Longitudinal study visits occurred at TB diagnosis (TB0), ART initiation (ARV0), two (ARV2) and four (ARV4) weeks of ART. Induced sputum and venous blood were collected. TB-IRIS diagnosis was assigned retrospectively on case review, using INSHI criteria [[3](#_ENREF_3)]. Chest x-ray inflammation (0-10) and sputum acid-fast bacilli (0-6) were scored as previously described [[12](#_ENREF_12)].

**Laboratory analyses**

Sputum and plasma samples were analysed by Bio-Rad Bio-Plex 200 using MMP beads (R&D Systems, Abingdon, UK). Procollagen III N-terminal propeptide (PIIINP) ELISAs (Cloud Clone Corp, USA) and urine lipoarabinomannan (LAM) assays (Alere Determine TB LAM assay) were performed as per manufacturer’s instructions.

**PBMC stimulation with H37Rv**

Cryopreserved peripheral blood mononuclear cells (PBMC) from a separate cohort of 22 TB-IRIS patients and 22 non-IRIS controls were stimulated with heat-killed H37Rv Mtb, as previously described [[13](#_ENREF_13)]. Culture supernatants were harvested at 24 hours and MMP-8 quantified by Luminex.

**Extracellular matrix 3-D modelling**

Alginate microspheres incorporating healthy donor PBMC and collagen were generated by bioelectrospray methodology using a Nisco encapsulator [[14](#_ENREF_14)]. Stimulated cells were pre-infected with ultraviolet-killed Mtb. DQ-labelled fluorescent gelatin or collagen (Invitrogen) was incorporated into the microspheres to quantitate matrix destruction [[15](#_ENREF_15)]. Doxycycline (10 μg/ml or 20 μg/ml) was added to media after microsphere generation.

**Statistical analysis**

Statistical analysis was performed using Prism 6 (GraphPad, UK) and STATA 14. Two-tailed Fisher’s Exact or Mann–Whitney U analysis were performed for key comparisons. Correlations were assessed by Spearman rank-order correlation coefficients. Unadjusted and adjusted linear regression models were fitted to quantify effects and adjust for age, sex and smoking status. Repeated measures two-way ANOVA with Tukey’s post-test comparison compared time-points and conditions in the TB granuloma model.

**Results**

**Cross-sectional study participants**

In the cross-sectional study, 227 participants were enrolled. Of these, 17 were excluded (unable to obtain samples n=8, diagnostic uncertainty n=9), leaving 210 for analysis (Supplementary Figure S1). Participant demographic and clinical characteristics are described in Table 1. HIV-infected TB patients had a median CD4 count of 172 (IQR 91-351) cells/mm3. Age, sex and body mass index (BMI) were similar in TB (HIV-) and TB (HIV+). However, smoking was more prevalent in TB (HIV-). TB (HIV-) and TB (HIV+) associated with diverse pulmonary pathologies on chest radiograph. Frequency of cavities and median chest x-ray inflammation score were both reduced in TB (HIV+) compared to TB (HIV-). CD4 count and the number of cavities positively correlated (r=0·357, p=0·016), suggesting that destructive pulmonary pathology is reduced in advanced TB (HIV+). Microbiological confirmation of TB was similar for TB (HIV-) and TB (HIV+) (Supplementary Table S2). However, sputum smear positivity was more common in TB (HIV-).

**Pulmonary MMP profile differs between TB (HIV-) and TB (HIV+)**

In sputum, we found multiple MMPs to be elevated in TB patients compared to controls (Figure 1A-G). In TB (HIV-), median MMP-1 was increased 35-fold and 33-fold compared to HIV-1-uninfected respiratory symptomatics and healthy controls respectively. However, in TB (HIV+), lower median sputum MMP-1, -2, -3, and -9 concentrations were observed compared to TB (HIV-) (Figure 1A-C and F). Unadjusted and adjusted linear regression modelling of the effect of HIV and TB infection on log-transformed sputum MMP concentrations provided further evidence of elevated sputum MMPs with TB, and reduced sputum MMPs in TB (HIV+) compared to TB (HIV-), after adjusting for age, sex and smoking status (Supplementary Table S3). The greatest effect was for MMP-1 and MMP-3. We did not adjust for BMI as no association was observed with MMP concentrations. Sputum MMP concentrations by sex are reported in Supplementary Figure S2 and Supplementary Table S4.

We related radiographic features with MMP concentrations. Sputum MMP-1 positively correlated with cavity frequency in TB (HIV-) and TB (HIV+) (r=0·592 and r=0·533 respectively, both p<0·0001). In TB (HIV-), the chest x-ray inflammation score positively correlated with sputum MMP-1 and MMP-3 (r=0·452 and r=0·453, both p=0·011). However, in TB (HIV+), no such correlation was evident (Supplementary Table S5). In TB (HIV-) patients with bilateral chest x-ray lesions, median sputum MMP-1 was increased 85-fold compared to those with unilateral lesions. However, in TB (HIV+) patients with bilateral chest x-ray lesions, sputum MMP-1 was not increased compared to patients with unilateral lesions (Figure 1F).

In TB patients, sputum smear and culture positivity were associated with increased sputum MMP-1 and MMP-3, which were positively correlated with AFB score (Supplementary Figure S3 and S4). Together, these data support a role for sputum MMPs in pulmonary TB-driven matrix degradation. However, divergent MMP upregulation occurs in TB (HIV-) and TB (HIV+), with reduced sputum MMP-1 in TB (HIV+) associated with lesser pulmonary matrix destruction.

**Plasma PIIINP is elevated in TB, and further increased in HIV-1-associated TB**

MMP activity releases matrix degradation products such as PIIINP from type III collagen. Analysis of plasma in a subgroup of 73 patients of mixed HIV serostatus in the cross-sectional study (39 TB, 34 control) showed PIIINP concentrations were elevated in TB patients compared to control patients (Figure 2A). Median PIIINP values were 25278 pg/ml (IQR 11787-45071) and 3888 pg/ml (IQR 1278-10367) respectively (p<0·0001). When analysed according to HIV status, both TB (HIV-) and TB (HIV+) patients had higher plasma PIIINP concentrations than corresponding controls. However, we unexpectedly found that plasma PIIINP was further elevated in TB (HIV+) compared to TB (HIV-) (Figure 2B), despite the reduced sputum MMP concentrations. To investigate further, we related plasma PIIINP to clinical features. Plasma PIIINP negatively correlated with peripheral blood CD4 count (r=-0·435, p=0·006, Figure 2C), and haemoglobin concentration (r=-0·557, p<0·0001), and positively correlated with HIV-1 viral load (r=0·544 p=0·002, Figure 2D). Plasma PIIINP was significantly elevated in TB patients with extra-pulmonary TB compared to those without (Figure 2E). Taken together with the sputum MMP analysis, this suggested that elevated plasma PIIINP in TB (HIV+) was due to increased extra-pulmonary MMP activity.

**HIV co-infection does not suppress systemic MMP activity in TB**

We therefore measured plasma MMP concentrations in the cross-sectional cohort (Figure 2F, 2G and Supplementary Figure S5 and S6). In TB (HIV-), MMP-1, MMP-7 and MMP-8 were elevated compared to HIV-uninfected controls, while plasma MMP-3, -9 and -10 were similar and MMP-2 was reduced. In TB (HIV+), the collagenases MMP-1 and MMP-8 were elevated in TB (HIV+) compared to HIV-infected controls (Figure 2F and 2G) and, in contrast to the findings in sputum, were not reduced compared to TB (HIV-).

**Paradoxical TB-IRIS is associated with systemic inflammation at TB diagnosis**

In the longitudinal cohort, 57 ART-naïve TB patients with advanced HIV-1 (CD4 count <200 cells/mm3) were enrolled (Supplementary Figure S1). Paradoxical TB-IRIS was diagnosed in 29 (59·2%) of 49 patients who completed follow up. Of these, 25 met the INSHI criteria for TB-IRIS and four were probable TB-IRIS. 20 patients did not develop TB-IRIS and were designated non-IRIS controls. Two non-IRIS controls were excluded (one likely an elite controller having an undetectable HIV-1 viral load and therefore considered immunologically distinct, one developed hepatotoxicity on TB treatment delaying ART initiation), leaving 47 (29 TB-IRIS, 18 non-IRIS) patients in the final analysis.

Demographic and clinical characteristics of longitudinal study participants are reported in Supplementary Table S6. The median time to TB-IRIS symptom onset was six days (IQR 3·5-9·5, range 1-23) post-ART initiation and patients presented with symptoms at a median of 14 days (IQR 9-15, range 4-29). Clinical features of TB-IRIS presentations are reported in Supplementary Table S7. Predominant symptoms and signs were constitutional (n=29, 100%) and pulmonary (n=27, 93·1%). Patients with TB-IRIS were unwell: hospital admission was required in 13 (45%) TB-IRIS patients during study follow-up, compared to only one (6%) non-IRIS control (p=0·007).

Clinical signs that characterised TB-IRIS patients were elevated heart rate (Figure 3A) and respiratory rate (Figure 3B) at TB diagnosis, as well as at TB-IRIS presentation, compared to non-IRIS controls. In both TB-IRIS and non-IRIS patients, CD4 count increased in the first two weeks of ART (Figure 3C) and HIV-1 viral load reduced (Figure 3D), although median HIV-1 viral load was higher in TB-IRIS patients than in non-IRIS patients at TB diagnosis and at ARV2. Markedly increased C-reactive protein occurred at TB-IRIS presentation (Figure 3E). Median lymphocyte counts were lower in TB-IRIS patients at all timepoints (Figure 3F), whereas neutrophil counts (Figure 3G) and monocyte counts (Figure 3H) were increased at ARV2. Therefore, TB-IRIS was frequent, associated with significant morbidity and characterised by marked features of systemic inflammation at TB diagnosis, which partially resolved with TB treatment but recurred at the time of TB-IRIS.

**Immunopathology in paradoxical TB-IRIS is associated with increased MMP activity**

To investigate the mechanism of immunopathology in TB-IRIS, we measured sputum MMPs and plasma PIIINP longitudinally. We observed no consistent association of increased sputum MMPs with TB-IRIS diagnosis (Supplementary Figure S7 and Supplementary Tables S8 and S9). In contrast, plasma PIIINP was elevated in TB-IRIS compared to non-IRIS patients both at the time of TB diagnosis, and during TB-IRIS (ARV2 and ARV4), but not at ART initiation (Figure 4A). At TB0, patients who later developed TB-IRIS had a median plasma PIIINP more than double that in non-IRIS controls, 43600 pg/ml (IQR 30021-63913) compared to 21651 pg/ml (IQR 17757-33196) respectively (p=0·036).

To investigate the hypothesis that the elevated PIIINP resulted from systemic MMP activity in TB-IRIS, we examined plasma MMP concentrations. Plasma MMP-1, -3, and -8 were elevated in TB-IRIS compared to non-IRIS patients (Figure 4B-D, Supplementary Tables S8 and S10). MMP-8 (neutrophil collagenase) was the most significantly increased, in a similar pattern to PIIINP, suggesting that systemic collagenase activity caused matrix degradation and PIIINP production during TB-IRIS. Supporting this, MMP-8 correlated with plasma PIIINP concentration (r=0·435, p<0·0001) (Figure 4E). As neutrophils may be a source of MMP-8 and neutrophil counts were elevated in TB-IRIS patients, we assessed the correlation between plasma MMP-8 and neutrophil count and percentage. MMP-8 concentration correlated with neutrophil count (r=0·617, p<0·0001, Figure 4F) and percentage (r=0·664, p<0·0001).

**Mtb antigen associates with elevated MMP concentrations in TB-IRIS**

We further hypothesised that increased MMP activity in TB-IRIS patients was secondary to increased mycobacterial antigen load. The frequency of sputum smear positivity, culture positivity, smear score, and time to culture positivity was similar between TB-IRIS and non-IRIS patients. However, these indices represent Mtb antigen in the pulmonary compartment. We therefore measured urinary LAM, indicative of disseminated TB, in patients for whom a urine sample was available. In an adjusted regression analysis, IRIS was associated with increased odds of a positive urine LAM finding (odds ratio 10·9, 95% CI 1·02-115·88, p=0·048), Supplementary Table S11. In an analysis of TB-IRIS patients only, those LAM positive had higher plasma MMP-3, MMP-7 and MMP-8 than TB-IRIS patients who were LAM negative (Figure 5A).

We next examined the effect of antigen stimulation on MMP activity in TB-IRIS patients. We studied MMP-8 concentrations in PBMC culture supernatants from a previously published cohort of TB-IRIS and non-IRIS controls sampled at the time of TB-IRIS onset [[13](#_ENREF_13)]. In TB-IRIS patients, MMP-8 secretion was increased following stimulation with heat-killed H37Rv Mtb compared to non-IRIS controls (Figure 5B).

**Doxycycline suppresses Mtb-driven matrix degradation**

Doxycycline is a licenced MMP inhibitor and reduces collagenase activity. We studied the inhibitory effect of doxycycline in a 3-dimensional cell culture model of TB which recapitulates key components of human granuloma formation using a functional readout of matrix destruction [[15](#_ENREF_15)]. Stimulation of PBMC with ultraviolet-killed Mtb increased degradation of gelatin within microspheres over time compared to uninfected cells (Figure 5C). Doxycycline in cell culture media around microspheres inhibited this breakdown (Figure 5D). Similarly, doxycycline suppressed Mtb-driven collagen degradation in a dose-dependent manner (Figure 5E).

**Discussion**

Despite some advances, treatment of TB remains a great challenge, due to lengthy regimens, poor side-effect profiles, drug interactions and drug resistance [[16](#_ENREF_16)]. These problems are further compounded by HIV-1 co-infection [[17](#_ENREF_17)]. Host-directed therapies have been proposed as a novel strategy to improve treatment outcome, but their development requires greater understanding of pathological and protective immune responses [[16](#_ENREF_16), [18](#_ENREF_18)]. In this study, we identified key differences between MMPs that cause immunopathology in TB dependent on HIV-1 status and characterised MMPs in TB-IRIS. In HIV-uninfected TB patients, MMP activity was prominent in the pulmonary compartment and MMP-1 was dominant, whereas in HIV-1-infected patients, higher plasma PIIINP may represent MMP-driven tissue destruction at extra-pulmonary sites, with MMP-8 as the principal protease. We identified PIIINP as a pathological marker of excessive MMP activity at TB diagnosis and during TB-IRIS, with potential to risk stratify individuals prior to ART and also to diagnose TB-IRIS. In addition, the MMP inhibitor doxycycline has potential as a host-directed therapy to prevent TB-IRIS.

Our findings of increased sputum MMPs in TB, and reduced sputum MMPs in HIV-1-associated TB, concur with findings in previous smaller studies [[10](#_ENREF_10), [12](#_ENREF_12)]. We previously reported that multiple MMP genes were upregulated in TB-IRIS PBMC re-stimulated with heat-killed H37Rv Mtb [[13](#_ENREF_13)]. MMP-8 was not amongst the upregulated genes. However, while most MMPs are regulated at the transcriptional level, MMP-8 is predominantly pre-synthesised in neutrophils and therefore may not be identified by gene expression analysis. We have previously reported compartmentalised inflammatory responses in HIV-infected patients with TB meningitis (TBM), in CSF and plasma, with elevated CSF MMP-1, -7 and -10 in TBM-IRIS compared to non-IRIS controls, although MMP-8 was not studied [[19](#_ENREF_19)]. Ravimohan *et al.* found that an increase in plasma MMP-8 at week 4 of ART relative to baseline pre-ART levels associated with increased TB-IRIS risk and abnormal pulmonary function tests after TB treatment completion, consistent with our finding that MMP-8 is a key collagenase in TB-IRIS [[20](#_ENREF_20)]. In addition, we have previously demonstrated that neutrophils were an important source of MMP-8 in TB, and that neutrophilia was associated with poor outcomes [[21](#_ENREF_21), [22](#_ENREF_22)].

Dysregulated innate immune responses have been implicated in TB-IRIS pathophysiology [[6](#_ENREF_6), [7](#_ENREF_7), [9](#_ENREF_9), [23](#_ENREF_23)]. Elevated innate pro-inflammatory cytokines, monocyte activation, cytotoxicity and inflammasome activation have been associated with TB-IRIS, implying a global activation of the innate immune response [[6](#_ENREF_6), [8](#_ENREF_8), [9](#_ENREF_9), [24](#_ENREF_24), [25](#_ENREF_25)], but these studies do not identify the ultimate effectors of tissue destruction. An association between TB-IRIS and mycobacterial antigen load has been demonstrated [[9](#_ENREF_9), [26](#_ENREF_26)]. Our results suggest that either Mtb antigen leads to increased MMP activity, or that increased MMP activity in patients who develop TB-IRIS causes increased extracellular matrix destruction, thereby increasing detection of urinary antigen. In HIV-1-infected TB patients on ART, elevated hyaluronic acid, a glycosaminoglycan component of the extracellular matrix, was associated with poor outcomes including death [[25](#_ENREF_25)]. Therefore, matrix degradation products may have a prognostic role in HIV-1-associated TB and indicate high TB-IRIS risk.

Currently, the only established immunomodulatory strategy for TB or TB-IRIS is corticosteroid therapy, used adjunctively in central nervous system and pericardial TB, and in treatment of paradoxical TB-IRIS [[27](#_ENREF_27)]. Corticosteroids suppress Mtb-driven MMPs and this may contribute to their beneficial effects [[28-30](#_ENREF_28)]. Our 3-D model of TB granuloma formation demonstrates the potential of doxycycline to inhibit Mtb-driven matrix degradation. Extracellular matrix integrity favours host cell survival in Mtb infection, and therefore matrix-protective strategies may improve outcome in TB without exacerbating HIV-1-related immune compromise [[31](#_ENREF_31)]. Doxycycline, a licenced MMP inhibitor, is cheap, safe and widely available and could be studied as an immunomodulatory adjuvant in TB treatment, with the added benefit of bacteriostatic anti-mycobacterial activity [[12](#_ENREF_12), [32](#_ENREF_32)].

We report a large study investigating mechanisms of immunopathology in TB. However, as an observational study, we cannot attribute causality, nor exclude the possibility that unmeasured confounding factors contributed to measured associations. We adjusted for sex and smoking status, factors that have been associated with divergent MMP responses, but cannot exclude alternative confounders [[33](#_ENREF_33), [34](#_ENREF_34)]. The incidence of TB-IRIS (59%) was high, causing significant morbidity. Sputum samples were not available from some severely unwell TB-IRIS patients who were unable to expectorate, which may have resulted in an under-estimation of effect in the longitudinal sputum analysis.

In summary, our work supports a central role for MMPs in causing tissue damage in TB and TB-IRIS, generating matrix degradation products. Differential MMP expression and compartmentalisation occurs in HIV-1-infected patients. Systemic MMP-8, is the dominant protease in TB-IRIS, in contrast to pulmonary-localised MMP-1 in HIV-uninfected TB patients. Matrix degradation products are promising biomarkers of TB-IRIS risk prior to and during clinical onset. Doxycycline, an MMP inhibitor, may prevent immunopathology in TB-IRIS.

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**Author contributions**

NFW, KAW, GM, RJW, JSF and PTE conceived and designed the clinical study. NFW, GM, RG, JMP and RJW recruited the clinical cohort. NFW, KAW and AC performed laboratory analyses of cross-sectional and longitudinal study samples. KAW, GM, RT and RJW conceived and performed TB-IRIS PBMC Mtb stimulation experiments and LBT and PTE conceived and performed the 3-D TB granuloma experiments. NW and CO performed analysis of data. NFW and PTE hold all primary data and are responsible for the integrity of the data. All authors contributed to the writing of the manuscript and approved the final submitted version.

**Declaration of Interests**

All authors declare no conflicts of interest.

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**Table 1** Demographics and clinical characteristics of cross-sectional study participants

Body mass index (BMI); interquartile range (IQR); not applicable (N/A); p values are for Fisher’s exact or Mann-Whitney U analysis.

**Figure Legends**

**Figure 1 Pulmonary MMP concentrations are increased in TB and differ by HIV serostatus**

Pulmonary TB is associated with increased MMP-1, -2, -3, -7, -8, -9 and -10 concentrations in sputum in comparison to respiratory symptomatic and healthy controls (A-G). Comparison of TB (HIV-) with TB (HIV+) demonstrated lower median sputum MMP-1 (A), -2 (B), -3 (C) and -9 (F) concentrations in TB (HIV+). Median MMP-8 was also reduced in TB (HIV+) compared to TB (HIV-) although to a lesser extent (E). TB (HIV-) patients with bilateral radiographic abnormalities had elevated sputum MMP-1, compared to TB (HIV-) patients with unilateral abnormalities (H). However, in TB (HIV+), MMP-1 was similar in patients with bilateral and unilateral chest x-ray involvement (H). In A-G, boxes represent the 1st and 3rd quartiles and horizontal bars within indicate median values, whiskers indicate minimum and maximum values. Horizontal bars between the datasets indicate Mann-Whitney U comparisons; in A-G comparisons between TB (HIV-) and HC (HIV-), TB (HIV+) and HC (HIV+), and TB (HIV+) and TB (HIV-) are shown. P values are shown by asterisks \*<0·05, \*\*<0·01, \*\*\*<0·001, \*\*\*\*<0·0001. TB = tuberculosis patient; RS = respiratory symptomatic; HC = healthy control, bilateral = bilateral inflammatory abnormalities on chest radiograph.

**Figure 2 TB increases systemic extracellular matrix turnover, which is further augmented by HIV co-infection**

Plasma PIIINP concentration was elevated in TB patients compared to control (healthy (HC) and respiratory symptomatic (RS)) participants (A). TB (HIV+) and TB (HIV-) patients had higher plasma PIIINP concentrations than corresponding controls (B). In contrast to sputum MMPs, plasma PIIINP was further elevated in TB (HIV+) compared to TB (HIV-). In HIV-infected patients, plasma PIIINP concentration and peripheral blood CD4 count negatively correlated (C), while HIV-1 viral load positively correlated with PIIINP concentration (D). Plasma PIIINP was elevated in patients with extra-pulmonary TB (EPTB) compared to those without EPTB (E). Plasma MMP-1 (F) and plasma MMP-8 (G) were elevated in TB (HIV-) and TB (HIV+) compared to respective RS and HC, and did not differ by HIV serostatus. Boxes represent the 1st and 3rd quartiles and horizontal bars within indicate median values, whiskers indicate minimum and maximum values. Horizontal bars between the datasets indicate Mann-Whitney U comparisons. P values are indicated by asterisks \*<0·05, \*\*<0·01, \*\*\*<0·001, \*\*\*\*<0·0001. Correlations were performed using Spearman rank-order correlation co-efficient.

**Figure 3 Paradoxical TB-IRIS is characterized by systemic inflammation at TB diagnosis and during TB-IRIS**

We studied 47 ART naïve TB patients with advanced HIV (CD4 count <200 cells/mm3) at enrolment, who underwent clinical observation at TB diagnosis (TB0) and bi-weekly for the first 4 weeks of ART (ARV0, ARV2, ARV4). TB-IRIS patients were characterized by elevated heart rate (A) and elevated respiratory rate (B), compared to non-IRIS controls. CD4 count increased in both TB-IRIS patients and non-IRIS controls following ART initiation (C) and concurrently HIV-1 viral load reduced (D), although median HIV-1 viral load was higher in TB-IRIS patients than in non-IRIS patients at TB diagnosis and at ARV2. Elevated C-reactive protein was a feature of TB-IRIS onset (E). Median lymphocyte counts were lower in TB-IRIS patients at all timepoints (F), whereas neutrophil counts (G) and monocyte counts (H) were increased at ARV2 and to a lesser extent at ARV4. In (A), (B), (F), (G) and (H), boxes represent the 1st and 3rd quartiles, horizontal bars within the median values, whiskers minimum and maximum values. In (C) and (D) data are median values (TB-IRIS filled circles; non-IRIS, open squares) linked by horizontal lines and interquartile ranges are shown by vertical bars. In (E) individual data points are shown, including results for unscheduled visits (ARV1, 3, 5 and 6 representing visits at one, three, five and six weeks of ART respectively) and horizontal lines represent the median. In all panels, asterisks indicate Mann-Whitney U comparisons; summary p values \*<0·05, \*\*<0·01, \*\*\*<0·001.

**Figure 4 Immunopathology in paradoxical TB-IRIS is associated with increased MMP activity**

Plasma PIIINP was elevated in TB-IRIS patients compared to non-IRIS control patients at the time of TB diagnosis and also at IRIS onset (A). Plasma MMPs were elevated in TB-IRIS compared to non-IRIS controls, including MMP-1 (B), MMP-3 (C), and most consistently MMP-8 (D). Plasma MMP-8 positively correlated with plasma PIIINP (E) and also with neutrophil count (F). In (A-D), boxes represent the 1st and 3rd quartiles, horizontal bars within median values and whiskers minimum and maximum values. Comparisons are by Mann-Whitney U analysis, with asterisks representing p values, \*<0·05, \*\*<0·01, \*\*\*<0·001. In (E) and (F) individual data points are plotted by filled circles. Spearman rank-order correlation coefficient r and p values are reported for correlations.

**Figure 5 Elevated MMPs associate with increased TB antigen load and Mtb-driven MMP activity is inhibited by doxycycline**

TB-IRIS patients with positive urinary lipoarabinomannan (LAM) had increased plasma MMP-3, MMP-7 and MMP-8 compared to TB-IRIS patients who were LAM negative (A). Plasma MMP-3 was higher at TB0 but not at ARV2, whereas MMP-7 and MMP-8 were most significantly increased at ARV2. MMP-8 concentrations were measured in culture supernatants of PBMC stimulated with heat-killed H37Rv *Mycobacterium tuberculosis* (Mtb) in a cohort of 22 TB-IRIS patients and 22 non-IRIS controls (B). After stimulation, MMP-8 secretion was greater from TB-IRIS PBMC than non-IRIS controls. In a 3-dimensional cell culture model of TB, microspheres were impregnated with ultraviolet-killed Mtb-stimulated PBMC and either DQ-gelatin or DQ-collagen, which increase in fluorescence when cleaved. Mtb stimulation increased total gelatin degradation within microspheres compared to control PBMC (C). Addition of doxycycline to the surrounding cell culture media inhibited extracellular matrix breakdown (D). Similarly, doxycycline suppressed Mtb-driven collagen degradation in a dose-dependent manner (E). (A, B), horizontal lines indicate medians and comparisons between groups are by Mann-Whitney U analysis; (C-E) means and standard error of mean are shown, analyses are by two-way repeated measures ANOVA, with Tukey’s post-test comparison. Summary p values \*<0·05, \*\*<0·01, \*\*\*\*<0·0001.





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