Elevated Plasma Matrix Metalloproteinase-8 associates with Sputum Culture Positivity in Pulmonary Tuberculosis

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Short title: Elevated MMP-8 and TB culture positivity

Summary: In this cohort study, plasma MMP-8 was increased in sputum culture positive versus culture negative participants at TB diagnosis and after 6 months of TB treatment, demonstrating potential as a biomarker of sputum culture positivity, to enhance TB treatment monitoring.

Abstract

Current methods for tuberculosis (TB) treatment monitoring are suboptimal. We evaluated plasma matrix metalloproteinase (MMP) and procollagen III N-terminal propeptide concentrations before and during TB treatment as biomarkers. Plasma MMP-1, -8 and -10 significantly decreased during treatment. Plasma MMP-8 was increased in sputum *Mycobacterium tuberculosis* culture positive relative to culture negative participants, prior to (median 4993 pg/ml, IQR 2542-9188 vs 698 pg/ml, IQR 218-4060, p=0.004) and after 6 months (median 3650, IQR 1214-3888 vs 720, IQR 551-1321, p=0.008) of TB treatment. Consequently, plasma MMP-8 is a potential biomarker to enhance TB treatment monitoring and screen for possible culture positivity.

Abstract Word Count: 99

Key words: Tuberculosis; HIV; matrix metalloproteinase; procollagen III N-terminal propeptide; diagnosis; immunopathology

Background

Tuberculosis (TB) is a major cause of morbidity and mortality worldwide, causing an estimated 9.9 million cases and approximately 1.5 million deaths in 2020, disproportionately affecting low resource settings (1). The treatment success rate for first line TB treatment was only 86% in 2019. For people living with HIV and those with multidrug-resistant (MDR) /rifampicin-resistant TB, treatment success rates are considerably lower (77% and 59% respectively) (1).

Monitoring response to TB treatment is a challenge for national TB programmes, especially in resource-limited settings. World Health Organization (WHO) recommendations are for repeat sputum smear microscopy for acid-fast bacilli after two months of TB treatment for people with a new diagnosis of pulmonary TB on first line treatment. Sputum culture for *Mycobacterium tuberculosis* (*Mtb*) is reserved for cases where sputum smear positivity persists or develops/recurs later in treatment (2). Patients who are sputum smear positive at diagnosis but smear negative at month two are recommended to have repeat smears at the end of month five and six. Both sputum smear and culture require appropriate laboratory facilities and trained personnel. Culture is limited by considerable time to result availability. Poor specificity for viable organisms precludes use of molecular tests such as Xpert MTB/RIF for treatment monitoring (3). Monitoring reliant on sputum production has diminished utility in sputum non-productive patients, including those too unwell, those with extrapulmonary TB and those whose cough has resolved on treatment. The WHO End TB strategy highlighted the need for new tools, including non-sputum-based diagnostics, to support effective patient-centred care (4).

Matrix metalloproteinases (MMPs) are host enzymes collectively capable of degrading the lung extracellular matrix at neutral pH. They are tightly regulated *in vivo,* but have the potential to cause immunopathology (5). We have previously demonstrated that MMP dysregulation is a key feature of

TB immunopathology (6-10). We have shown that sputum MMP-1 and MMP-8 are significantly elevated in TB patients at diagnosis compared to controls, irrespective of HIV serostatus (7-9). In plasma, MMP-1, MMP-8 and procollagen III N-terminal propeptide (PIIINP), a matrix degradation product released during collagen turnover, are elevated in TB patients compared to healthy or respiratory symptomatic controls (8, 11). In patients without HIV infection, elevated sputum MMP-1, -2, -3 and -8 decrease after just two weeks of TB treatment (12). Here, in an exploratory study, we evaluated plasma MMP and PIIINP concentrations and their association with sputum smear and culture status in South African TB patients longitudinally, to determine potential utility as novel peripheral biomarkers of treatment response.

Methods

This was a retrospective analysis of the Collection of Sputum, Urine and Blood Samples for Research (CUBS) Study (see also Supplementary Methods). CUBS prospectively recruited adult participants in health facilities in eTheKwini municipality, KwaZulu-Natal, South Africa. Inclusion in this analysis required a diagnosis of TB (clinical and/or microbiological) leading to TB treatment initiation. All CUBS participants enrolled at the Prince Cyril Zulu Communicable Disease Centre (December 2013 - May 2014) were included. Plasma was collected at baseline (TB diagnosis) and visits at the end of month 2 (week eight) and 6 (week 24) of TB treatment. HIV testing was offered if status was unknown. Sputum was collected for mycobacterial analysis including smear, *Mtb* culture and drug susceptibility testing (DST) at each visit. Culture was performed on solid (7H11) and liquid (MGIT) media. Plasma MMP-1, -3, -8, -9, and -10 were quantified by Luminex array (Bio-Rad Bio-Plex 200, assay from R&D Systems, UK) and PIIINP by ELISA (Cloud clone corp, China). The study was approved by University of KwaZulu-Natal and London School of Hygiene & Tropical Medicine research ethics committees (REFs BE022/13 and 11710 - 1 respectively).

Analysis was performed in Prism 8 (GraphPad, USA). Comparisons between two groups were by Mann-Whitney U test. Comparisons between multiple groups were by Kruskal-Wallis test with Dunn's multiple test comparison. Diagnostic accuracy was assessed by receiver operating characteristic (ROC) curve analysis and associations between analytes by Spearman correlation.

Results

Participant (n=85) characteristics at TB diagnosis are reported in Supplementary Table S1 and S2. HIV serostatus was known to be positive in 43.5% (n=37) and unknown in 11.8% (n=10). The majority of participants were male (72.9%, n=62). Median age was 35 years (IQR 28.5-42.0, range 18.0-60.0). TB diagnosis was confirmed on sputum culture in 89.4% (n=76). Baseline DST results were available for 80% (n=68) participants and in 86.8% (n=59) were fully sensitive.

Plasma collagenases & PIIINP decrease during TB treatment

The collagenases, MMP-1 (interstitial collagenase) and -8 (neutrophil collagenase) decreased significantly between baseline and month 2, as did the stromelysin, MMP-10 (Figure 1A-C). Concordantly, the matrix degradation product PIIINP, which is released during collagen turnover, decreased over the first two months of TB treatment (Figure 1D). No further significant reductions between month 2 and month 6 were observed. Conversely, plasma MMP-9 significantly increased between baseline and month 2, whilst plasma MMP-3 and MMP-7 did not significantly change (Supplementary Figure S1). Assessing correlations between analytes including data from all timepoints revealed a positive correlation between plasma PIIINP and the collagenases MMP-1 ($r=0.759$, $p<0.001$)

and MMP-8 (r=0.224, p<0.001). MMP-1 and MMP-8 were also positively correlated (r=0.377, p<0.001). Full correlation results are reported in Supplementary Table S3.

Elevated plasma MMP-1 & PIIINP in smear positive disease

Plasma MMPs and PIIINP were compared in sputum smear positive and smear negative participants at baseline (Figure 2A). MMP-1 and PIIINP were significantly increased in sputum smear positive compared to sputum smear negative participants. MMP-3, -8, -9 and -10 did not differ by smear status. A longitudinal analysis by smear status was not performed as only one participant had a subsequent smear positive result during treatment.

Plasma MMP-8 associates with sputum culture status at TB diagnosis and month 6.

At the end of month 6, five (5.8%) participants remained sputum culture positive for *Mtb*, on liquid culture. Four out of five of these participants were HIV negative. *Mtb* culture confirmed that in two cases drug-sensitive isolates at diagnosis remained drug-sensitive at month 6, whilst in two cases isolates that were isoniazid-resistant at TB diagnosis were additionally also rifampicin-resistant at month 6, indicating the development of MDR TB. In one case, drug susceptibility test results were available neither at diagnosis, nor later timepoints. Only one of these patients had a positive result on smear microscopy at month 6, and all cases were smear negative at month 2, indicating that the majority of these cases would not have been identified by current microscopy-based methods of screening for treatment failure.

Plasma MMPs were compared in sputum culture positive and culture negative participants at each timepoint (Figure 2B and Supplementary Figure S2). Plasma MMP-8 was significantly increased in *Mtb*

culture positive compared to culture negative participants at baseline (median 4993 pg/ml, IQR 2542-9188 vs median 698 pg/ml, IQR 281-4060, p=0.004) and also month 6 (median 3650 pg/ml, IQR 1214-3888 vs median 720 pg/ml, IQR 551-1321, p=0.008). However, there was no significant difference found at month 2 (median 1295 pg/ml, IQR 754-4294 for culture positive vs 870 pg/ml, IQR 499-1986 for culture negative, p=0.298). Analysis by HIV status was limited in power, however, a similar pattern of elevated MMP-8 associated with culture positivity at baseline and month 6 was seen in both HIV negative and positive subgroups (Supplementary Table S4). There was a trend towards an association at month 2 in a subgroup analysis of male participants (Supplementary Figure S3). No other MMP, nor PIIINP concentration, differed by sputum culture status at any timepoint. Plasma MMP-8 at month 6 predicted month 6 sputum culture status with an area under the curve of 0.844, corresponding to a sensitivity of 100% and a specificity of 65% at the optimal cut-off (>920 pg/ml) (Figure 2C).

Discussion

In this longitudinal analysis of TB patients on treatment, we found that plasma MMP-1, -8, -10 and PIIINP decreased with effective TB treatment over two months. Whilst all but one participant in this study converted to smear negative by the end of six months of TB treatment, five participants were culture positive at six months. Elevated plasma MMP-8 at TB diagnosis and at the end of six months TB treatment was associated with sputum culture positivity, indicating that plasma MMP-8 is a candidate biomarker for monitoring treatment response.

Neutrophils are a potential source of MMP-8, which may be stored in granules before release. *In vitro*, neutrophils secrete MMP-8 directly in response to *Mtb* infection in a dose-dependent manner, and in response to cellular networks (13). We have previously demonstrated that elevated plasma MMP-8 is associated with lipoarabinomannan positivity and neutrophil count in HIV-associated TB (8). In patients starting TB treatment and then antiretroviral therapy for HIV who go on to develop

paradoxical TB-IRIS, plasma MMP-8 is also increased at TB diagnosis and at TB-IRIS presentation (8). Together, these findings suggest that plasma MMP-8 may be a surrogate plasma marker of mycobacterial load and neutrophil-driven immune responses in TB.

This study highlights the problem of identifying treatment failure in TB. Despite being started on TB treatment, five patients in the study remained culture positive for *Mtb* at six months. The majority of these would not have been identified by standard smear-based methods of assessing for treatment failure. A plasma biomarker, such as MMP-8, could provide a useful additional objective risk indicator to alert treating clinicians to the possibility of treatment failure, especially where resources are limited and in the case of sputum non-productive patients. If further developed for measurement using a low-cost point-of-care tool, for example a lateral flow device, this could be implemented at the community level as a rule-out triage test, whereby a low reading supports treatment success, and a high reading prompts repeat culture.

This study is not the first to identify an association of MMP-8 with culture positivity in TB patients on treatment. Sigal *et al*. reported an association of elevated ratios of serum MMP-8 at week 8 to baseline with culture positivity at week 8 and week 12 but did not examine later timepoints (14). Lee *et al.* evaluated a number of potential biomarkers in plasma at baseline and two months (15). At month 2, MMP-8 concentrations were increased in patients who were culture positive compared to culture negative, with an AUC of 0.632 on ROC curve analysis. This is consistent with our findings, but at a different time point. Lee *et al.* included only participants with drug-sensitive TB, without HIV infection. Here, we report a cohort of patients of mixed HIV serostatus. The sample size limited our ability to perform subgroup analyses to explore the impact of HIV infection and ART status on plasma MMP concentrations during TB treatment and we did not evaluate the occurrence of TB-IRIS, but we hypothesise that these factors may influence plasma MMP concentrations, supported by findings in our previous study (8).

A strength of this cohort study was the detailed microbiological follow up and inclusion of participants of mixed HIV serostatus, as well as drug-susceptible and drug-resistant TB cases. However, this was an exploratory study as opposed to a diagnostic accuracy assessment, and further evaluation is required to discern the clinical utility of these findings. The specificity of high plasma MMP-8, especially in the context of other respiratory infections, requires further study. It is important to recognise that additional clinical factors, including symptoms and BMI monitoring may indicate patients who are failing TB treatment. We did not evaluate these indicators in this cohort. The sample size limited our ability to perform subgroup analyses, including in women and patients with drug-resistant TB, and we cannot exclude a role for unmeasured potential confounders (for example smoking).

In conclusion, we describe an association of plasma MMP-8 with sputum *Mtb* culture positivity at the beginning and after 6 months of TB treatment, in a cohort of patients of mixed HIV serostatus. We advocate for the further evaluation of plasma MMP-8 as a biomarker of culture positivity to support TB treatment monitoring as a triage test, with the aim of early identification of treatment failure and appropriate allocation of diagnostic resources, to better support care of patients and improve TB treatment outcomes.

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Conflicts of interest

All authors: no conflicts of interest.

Previous reporting of this work

This work in part has been reported as a poster presentation at the 11th European Congress on Tropical Medicine and International Health, in Liverpool, UK and a version of this manuscript has been uploaded to the preprint server, Medrxiv, available at https://doi.org/10.1101/2021.11.15.21265734.

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Legend to Figures

Figure 1 Plasma MMP-1, -8, -10 & PIIINP concentrations during TB treatment

Plasma MMP-1 (A), -8 (B), -10 (C) and PIIINP (D) concentrations decreased between baseline (TB diagnosis) and the end of month 2 of TB treatment but did not decrease further between month 2 and month 6. Analysis was by Kruskal-Wallis test with Dunn's Multiple Test comparison. P values are summarised: ** $p<0.001$, **** $p<0.0001$. Where no p value is reported, $p>0.05$. Abbreviations: Matrix metalloproteinase (MMP); procollagen III N-terminal propeptide (PIIINP).

Figure 2 Plasma MMP-8 is increased in culture positive TB at baseline and month 6

Plasma MMP-1 and PIIINP, but not plasma MMP-8, were increased in participants who were smear positive compared to smear negative at TB diagnosis (A). Plasma MMP-8 was increased in *Mycobacterium tuberculosis* sputum culture positive compared to culture negative participants at TB diagnosis and at the end of month 6 of TB treatment (B). Receiver operating characteristic curve analysis of MMP-8 concentration at month 6 post-TB treatment initiation for identification of culture positivity at month 6 (C). In A and B, analysis was by Mann-Whitney U test. P values are summarised: * $p<0.05$, ** $p<0.001$. Where no p value is reported, $p>0.05$. Abbreviations: Area under the curve (AUC); Confidence interval (CI; Matrix metalloproteinase (MMP); procollagen III N-terminal propeptide (PIIINP).

Supplementary Figure S1 Plasma MMP-9, MMP-3 & MMP-7 concentrations during TB treatment

Plasma matrix metalloproteinase (MMP)-9 (A) increased between baseline (TB diagnosis) and month 2 following TB treatment initiation. Plasma MMP-3 (B) and MMP-7 (C) concentrations did not change

over time with tuberculosis (TB) treatment. Analysis was by Kruskal-Wallis test with Dunn's Multiple Test comparison. P values: ** p<0.001; where no p value is reported, p>0.05.

Supplementary Figure S2 Plasma MMPs and PIIINP by sputum *Mycobacterium tuberculosis* culture status

Plasma MMP concentrations were compared by *Mycobacterium tuberculosis* culture status (positive + and negative -) at each timepoint. Analysis was by Mann-Whitney U test. P values are summarised: *p<0.05; ** p<0.001; where no p value is reported, p>0.05. Abbreviations: Matrix metalloproteinase (MMP); procollagen III N-terminal propeptide (PIIINP).

Supplementary Figure S3 Plasma MMP-8 by culture status in male participants

Plasma MMP concentrations were compared by *Mycobacterium tuberculosis* culture status (positive + and negative -) at each timepoint in male participants only. Analysis was by Mann-Whitney U test. P values are summarised: *p<0.05; ** p<0.001 or are stated where >0.05. Abbreviations: Matrix metalloproteinase (MMP); procollagen III N-terminal propeptide (PIIINP).

Tables

Supplementary Table S1 Participant demographic and clinical characteristics

Supplementary Table S2 Clinical status of participants with HIV infection

Supplementary Table S3 Spearman r correlation between analytes

Supplementary Table S4 Median plasma MMP-8 concentrations by culture result and HIV serostatus

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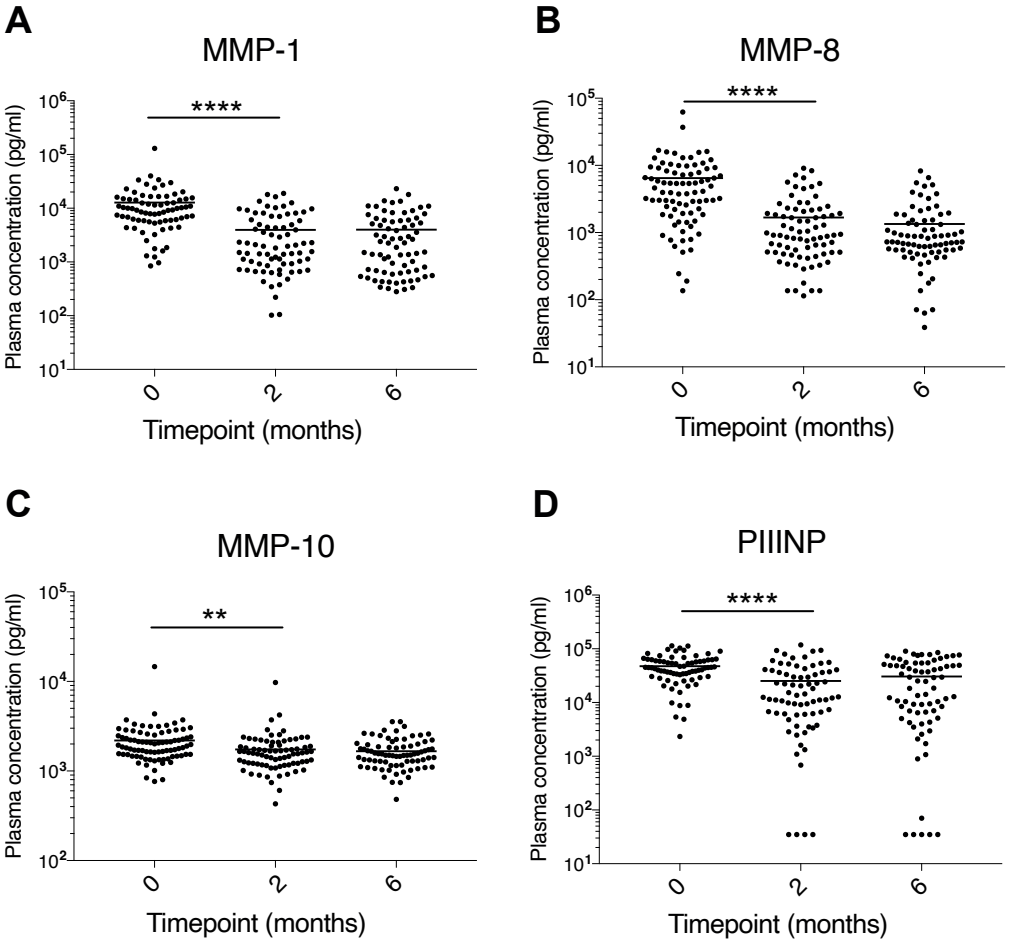
A
MMP-1
****
Plasma concentration (pg/ml)
Timepoint (months)
B
MMP-8
****
Plasma concentration (pg/ml)
Timepoint (months)
C
MMP-10
**
Plasma concentration (pg/ml)
Timepoint (months)
D
PIIINP
****
Plasma concentration (pg/ml)
Timepoint (months)

Figure 1

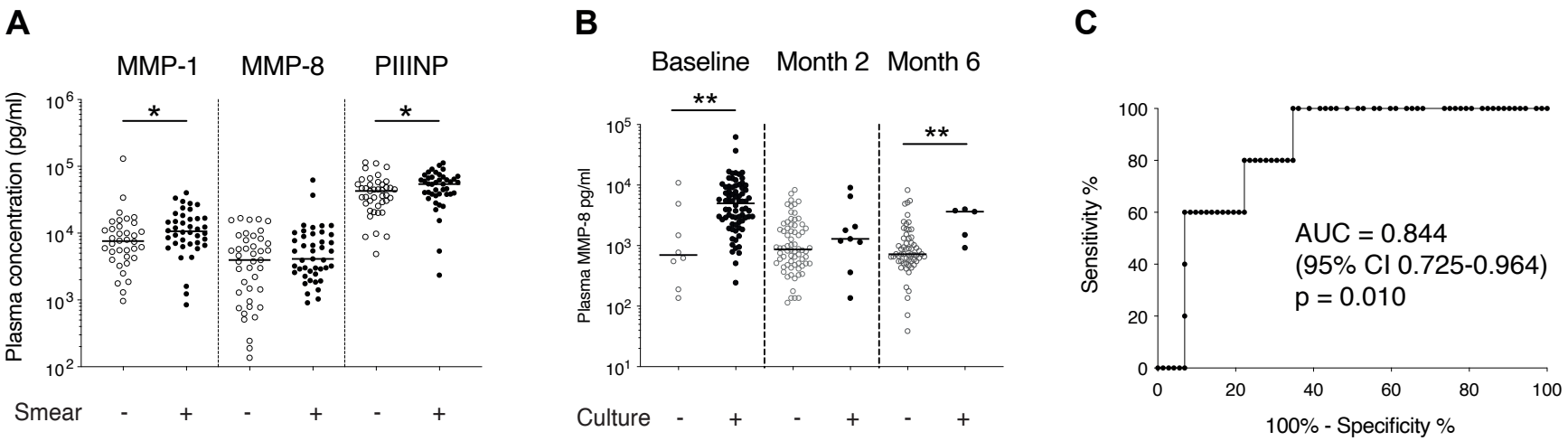
A
MMP-1
MMP-8
PIIINP
Plasma concentration (pg/ml)
10^6
10^5
10^4
10^3
10^2
*
*
Smear - + - + - +
B
Baseline
Month 2
Month 6
Plasma MMP-8 pg/ml
10^5
10^4
10^3
10^2
10^1
**
**
Culture - + - + - +
C
Sensitivity %
100
80
60
40
20
0
0
20
40
60
80
100
100% - Specificity %
AUC = 0.844
(95% CI 0.725-0.964)
p = 0.010

Figure 2