Elevated plasma matrix metalloproteinases associate with *Mycobacterium tuberculosis* blood stream infection and mortality in HIV-associated tuberculosis

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40-word summary: In HIV-associated TB, plasma MMP, including MMP-8, and procollagen III N-terminal propeptide associate with *Mycobacterium tuberculosis* blood stream infection and 12-week mortality. This implicates MMP dysregulation in pathophysiology of advanced HIV-TB and supports MMP inhibition as a host-directed therapeutic strategy.

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Word count = 2000

Abstract

Mortality from HIV-associated tuberculosis (HIV-TB) is high, particularly among hospitalised patients. In 433 people living with HIV hospitalised with symptoms of TB, we investigated plasma matrix metalloproteinases (MMP) and matrix-derived biomarkers in relation to TB diagnosis, mortality and *Mycobacterium tuberculosis* (*Mtb)* blood stream infection (BSI). Compared to other diagnoses, MMP-8 was elevated in confirmed TB and in *Mtb*-BSI, positively correlating with extracellular matrix breakdown products. Baseline MMP-3, -7, -8, -10 and PIIINP associated with *Mtb*-BSI and 12-week mortality. These findings implicate MMP dysregulation in pathophysiology of advanced HIV-TB and support MMP inhibition as a host-directed therapeutic strategy for HIV-TB.

Abstract word count = 99

Background

Tuberculosis (TB) is a leading infectious cause of death worldwide, resulting in an estimated 1.3 million deaths annually (1). The End TB Strategy aims to reduce TB deaths by 95% between 2015 and 2035 (2). In people living with HIV (PLWH), TB is the leading cause of death (3). To meet End TB targets, identification of and interventions for those at highest risk of poor outcomes are needed, particularly for use in low-resource settings where the burden of TB falls most heavily. Further understanding of the causes of mortality and pathophysiology of TB disease is required to prevent TB deaths in PLWH.

We have previously reported that plasma matrix metalloproteinase (MMP)-1, MMP-8 (neutrophil collagenase) and procollagen N-terminal propeptide (PIIINP), a matrix degradation product released during collagen turnover, are elevated in patients with active TB compared to patients without TB, highlighting collagen turnover as a feature of TB disease, in both HIV positive and negative cohorts (4, 5). Plasma PIIINP was significantly higher in HIV positive patients with newly diagnosed active TB compared to HIV negative patients, positively correlated with HIV viral load and was elevated during TB immune reconstitution inflammatory syndrome (IRIS) (4).

Here, we evaluated plasma MMP, PIIINP and extracellular matrix components, collagen type IV alpha 1 chain (Col4α1) and hyaluronic acid (HA), in a cohort of patients hospitalised with advanced HIV and TB symptoms, thereby focusing on the population most requiring interventions to reduce mortality. We aimed to evaluate potential as diagnostic biomarkers

and hypothesised that disseminated *Mycobacterium tuberculosis* (*Mtb*) in HIV-TB may drive systemic MMP upregulation and consequently tissue damage. We report a novel association of elevated plasma MMP with *Mtb*-blood stream infection (BSI) and mortality in advanced HIV-TB, providing pathophysiological insights.

Methods

The study was approved by the University of Cape Town Human Research Ethics Committee (REC, reference 057/2013) and London School of Hygiene and Tropical Medicine REC (reference 11710). Full methods have been reported elsewhere (6). See also Supplementary Methods. Eligible patients were adults with HIV infection and a CD4 count ≤ 350 cells/µl, admitted to Khayelitsha Hospital, Cape Town, with a probable new diagnosis of TB. Exclusion criteria were pregnancy, TB treatment within one month prior to admission or more than 3 doses of TB treatment prior to enrolment, or unknown HIV status (declined testing). Participants provided written informed consent. Participants were investigated by TB culture (sputum, blood), Xpert (sputum, urine) and urine lipoarabinomannan (LAM, Alere Determine TB LAM assay) prior to initiation of TB treatment. Vital status was determined at 12 weeks.

Participants were classified retrospectively as microbiologically **confirmed TB** if *Mtb* was identified in clinical samples, **clinical TB** if TB was likely and treatment for TB was given following WHO guidelines but no microbiological confirmation was obtained, **no TB**, if TB was

105 excluded on clinical and microbiological grounds, or **LAM TB** if criteria for no TB was met but
106 urine LAM was positive >=2 by two independent readers (6).

107

108 Inclusion required the availability of EDTA plasma at enrolment. Plasma MMP were quantified
109 by Luminex array (Bio-Rad Bio-Plex 200 system; R&D Systems, United Kingdom). PIIINP,
110 Col4α1 and HA were quantified by enzyme-linked immunosorbent assays (Cloud Clone Corp,
111 China). HA and Col4α1 measurement were limited to a subset of 73 randomly selected
112 participants. Statistical analysis was performed in Prism 9 and R Studio (2023.03.1).
113 Comparisons of analytes by TB diagnosis was between no TB and either confirmed TB or
114 clinical TB and a Bonferroni correction was performed as two groups were compared for one
115 analyte. Statistical significance was inferred by a p value <0.05. Comparisons between two
116 groups were by Mann-Whitney U test unless otherwise stated.

117

118 The relationship between MMP/PIIINP concentrations and *Mtb*-BSI or mortality was depicted
119 using a Loess non-parametric smoother and assessed using a mixed effects logistic regression
120 model, including a random effect on intercept for plate, to account for technical variation
121 between plates, as analytes were measured across multiple plates. Hierarchical clustering
122 analysis was performed by Ward's method based on Euclidean distance, to assess the
123 association between analytes, including neutrophil count, neutrophil percentage and
124 procalcitonin, but excluding Col4α1 and HA, as they had only been measured in a subset of
125 patients. This was on scaled data, excluding extreme outliers (observations with a value
126 greater than four median absolute deviations from the variable median applied after

transformation). Pearson correlation to assess the association between analytes was performed on log-transformed values, excluding extreme outliers.

Results

Plasma samples were available for 437 participants (Supplementary Table S1). TB diagnosis was confirmed in 313 (71.6%) and clinical TB in 48 (11.0%). Only 4 participants met the criteria for LAM TB, so these were excluded from subsequent analyses. In no TB (n=72, 16.5%), community acquired pneumonia (n=34, 47.2%) was the most frequent diagnosis, followed by other blood pathogen (n=8, 11.1%), *Pneumocystis jirovecii* pneumonia (n=6, 8.33%) and cryptococcal disease (n=6, 8.33%). The median CD4 count was 56 cells/mm^3 (IQR 18.0-112) in confirmed TB, 93 (IQR 53.8-182) in clinical TB and 89.5 (IQR 29.0-224) in no TB.

Vital status at 12 weeks was known for 427/433 (98.6%) participants. Death occurred in 82/433 (18.9%) participants at a median of 16.0 (IQR 2.75-44.3) days. Mortality at 12 weeks in confirmed TB was 19.8% (62/313). *Mtb*-BSI was present in 133/313 (42.5%) participants with confirmed TB and was associated with increased mortality: 37/133 (27.8%) participants with *Mtb*-BSI died compared to 23/173 (13.3%) without (p = 0.002 by Fisher's Exact Test). Participant characteristics are reported by vital status and *Mtb*-BSI in Supplementary Table S2. More *Mtb*-BSI occurred in men than women, but there was no difference in mortality. Older age associated with mortality, but not *Mtb*-BSI. Being ART naïve or having defaulted was associated with increased *Mtb*-BSI, compared to being ART treated. *Mtb*-BSI and mortality were associated with lower CD4 counts.

Plasma MMP-8 is elevated in patients hospitalised with HIV-TB

We first examined plasma MMP and matrix-derived biomarker concentrations in no TB in comparison to confirmed TB or clinical TB. Plasma MMP-8 was significantly elevated in confirmed TB compared to no TB (median 23712 pg/ml, IQR 7688-47571 vs median 10602, IQR 2019-32205; p=0.003; Figure 1A and Supplementary Table S3), as was plasma Col4α1 (Figure 1B). Plasma MMP-3 and -10 were lower in participants with confirmed TB compared to no TB and clinical TB, whilst MMP-1, -7 and -9, PIIINP and HA did not differ between groups. These findings were similar in male and female participants (Supplementary Figure S1). MMP-8 did not differ significantly by sex (Supplementary Table S4 and Supplementary Figure S2). Analyte associations with age and CD4 count for confirmed TB are shown in Supplementary Figure S2.

Plasma MMP-8 associates with Mtb-BSI and mortality in HIV-TB

MMP are primarily transcriptionally regulated however, MMP-8 may be stored in neutrophil granules. Exploring the hypothesis that disseminated *Mtb* drives MMP-8 upregulation and release from neutrophils and that this associates with poor outcomes in HIV-TB, we next examined MMP-8 in the presence or absence of *Mtb*-BSI and by vital status at 12 weeks. We found that in confirmed TB, those with *Mtb*-BSI had elevated plasma MMP-8 (Figure 1C) compared to those without (median 40003 pg/ml, IQR 20006-70583 vs median 11451, IQR 4697-30789; p<0.001). Plasma MMP-8 was elevated in participants with confirmed TB who died compared to those who survived (median 32811 pg/ml IQR 12060-66934 vs median

20201, IQR 6050-40561, p=0.002, Figure 1D). Col4α1 and HA did not differ by vital status (data not shown), although Col4α1 was elevated in confirmed TB with *Mtb*-BSI compared to without (median 11.2 ng/ml, IQR 7.76-9.92 vs median 8.75 IQR 9.76-13.5, p<0.001). Plasma MMP-8 positively correlated with Col4α1 (r = 0.535, p<0.001) and PIIINP (r = 0.404, p<0.001).

Multiple MMP and PIIINP associate with HIV-TB severity

We next examined the association of other plasma MMP and PIIINP with *Mtb*-BSI and mortality in confirmed TB. We found a positive association between plasma MMP-3, -7, -8, -10 and PIIINP for *Mtb*-BSI and mortality (Figure 2A-B). We performed hierarchical clustering analysis including neutrophil count, neutrophil percentage, and procalcitonin as biomarkers of acute inflammation (Supplementary Figure S2). MMP-8 most closely clustered with PIIINP and procalcitonin. Procalcitonin, but not neutrophil count, positively associated with *Mtb*-BSI and mortality (Figure 2C-D).

Discussion

Patients with advanced HIV-1 hospitalised with symptoms of TB are at high risk of mortality. We found that baseline plasma MMP-8 and Col4α1 were increased in hospitalised patients with HIV infection who were confirmed to have TB compared to those who eventually received an alternative diagnosis. Contrary to our previous finding in outpatients, in this

hospitalised cohort, plasma PIIINP was not elevated in patients with TB compared to symptomatic patients with alternative diagnoses.

Elevated plasma MMP-8, together with MMP-3, -7, -10 and PIIINP associated with *Mtb*-BSI and mortality at 12 weeks, suggesting that MMP upregulation and matrix turnover are features of TB disease severity. This is in keeping with our recently reported finding that elevated plasma MMP-8 concentrations at the end of TB treatment are associated with persistent *Mtb* culture positivity (7). MMP-8 was most closely associated with procalcitonin (an acute phase reactant) and the matrix degradation product, PIIINP (released during Type III collagen turnover), suggesting that MMP-8 activity, collagen turnover and acute inflammation are closely related processes in HIV-TB (5, 8). HA, a polysaccharide component of the extracellular matrix which is released during extracellular matrix turnover has previously been found to predict AIDS events or death in PLWH commencing antiretroviral therapy (9). Plasma HA was not associated with mortality in this study.

Whilst we have demonstrated an association between plasma MMP and mortality in HIV-TB, our study does not prove a causal relationship. Elevated MMP may be a function of *Mtb* bacterial load or *Mtb* dissemination, which is itself associated with mortality. *In vitro*, virulent *Mtb* is able to induce neutrophil-derived MMP-8 secretion, via an NF-kB-dependent mechanism, so it is possible that blood stream *Mtb* directly stimulates neutrophil MMP-8 release (10). However, elevated MMP-8 may also occur via upregulation of pro-inflammatory cellular networks and the finding that MMP-8 associated more closely with procalcitonin than neutrophil count or percentage is consistent with networks upregulating MMP-8 (11).

Increased MMP-8 in TB was positively associated with PIIINP, a matrix degradation product released during type III collagen turnover and Col4α1, a component of type IV collagen. MMP inhibition with doxycycline has been shown to be safe and effective for TB in HIV negative patients (12). Our results support the case for clinical trials of MMP inhibition with doxycycline as a host-directed therapy for HIV-TB.

Prior studies have examined MMP-8 in patients with TB, predominantly in those who are HIV negative (11). Our finding of elevated plasma MMP-8 in participants with confirmed TB compared to those symptomatic with other illnesses is consistent with previous reports in outpatients, although this is the first report in PLWH who are hospitalised (4, 5, 13, 14). MMP-8 is emerging as a biomarker that may help identify patients with active TB, in some settings if used in combination with other screening tools. Here, we also report elevated plasma Col4α1 in HIV-TB. Elevated Col4α1 was also associated with *Mtb*-BSI. Col4α1 is a subunit of Type IV collagen, a key component of basement membranes that has not previously been studied in human TB, to our knowledge.

A strength of this study is the combination of rigorous clinical and mycobacterial analyses that was employed to determine TB status. A limitation pertains to the use of a single blood culture to determine the presence or absence of *Mtb*-BSI. It is likely that sequential blood cultures would have a higher diagnostic accuracy for *Mtb*-BSI (15). We have not adjusted for known predictors of mortality in our analysis, as causal pathways are not well understood.

In summary, in PLWH hospitalised with a clinical syndrome compatible with TB, plasma MMP-8 was elevated in confirmed TB, likely due to *Mtb*-driven tissue damage, compared to other diagnoses. MMP-3, -7, -8, -10 and PIIINP associated with *Mtb*-BSI and mortality at 12 weeks, implicating MMP dysregulation in HIV-TB morbidity. MMP inhibition is a potential therapeutic target for people with HIV-TB, who urgently need improved treatment outcomes.

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collection, data analysis, data interpretation, or writing of this report. The opinions, findings and conclusions expressed in this manuscript reflect those of the authors alone.

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Results were presented in part at Keystone Symposia *X7: Tuberculosis: Translating Scientific Findings for Clinical and Public Health Impact; Whistler, Canada, April 2018.*

Conflicts of Interest

All authors: No conflicts of interest to declare.

Author contributions

GM, CS, AW, DB conceived the clinical study and recruited the clinical cohort. NFW, GM, PE, DB conceived the laboratory study. NFW, KAW, MS, DB conducted laboratory analyses. NFW, CS, CO, DB performed data analysis. NFW wrote the first draft of the manuscript. All authors contributed to the manuscript and approved the final submitted report.

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Figure Legends

Figure 1 Elevated matrix metalloproteinase-8 in HIV-TB, *Mtb*-blood stream infection and TB mortality

Plasma matrix metalloproteinase (MMP)-8 (A) and Col4α1 (B) were elevated in hospitalised participants with HIV infection and microbiologically-confirmed TB, compared to hospitalised HIV positive participants with symptoms due to other diagnoses (no TB). Differences between confirmed TB and TB diagnosed clinically were not statistically significant. Plasma MMP-8 was increased in participants with confirmed TB and *Mycobacterium tuberculosis* blood stream infection (MTBBSI) compared to those without (C) and in those who had died compared to those who had survived at 12 weeks (D). Col4α1 was measured in a subset of 73 participants. *p<0.05, **p<0.01, ***p<0.001.

Figure 2 Plasma matrix metalloproteinase (MMP) and procollagen III N-terminal propeptide (PIIINP) associate with *Mycobacterium tuberculosis* blood stream infection and mortality in HIV-associated TB.

Probability (dependent variable, y axis) of *Mycobacterium tuberculosis* blood stream infection (MTBBSI, A/C) and mortality (B/D) by analyte log concentration (predictor variable, x-axis). Loess fit to data (coloured lines with shaded 95% confidence intervals) demonstrates the functional relationship, with measured concentrations shown as a jittered scatterplot at 0 (survived/no MTBBSI) or 1 (died/MTBBSI) on the y axis. Odds ratios (OR) and 95% confidence

346 interval (CI) are from logistic regression models, with adjustment for random effects by plate.

347 In A and B, log MMP or PIIINP concentrations are in pg/ml. In C/D, log neutrophil count is

348 $x10^9$/L and log procalcitonin concentration in μg/L.

Figures

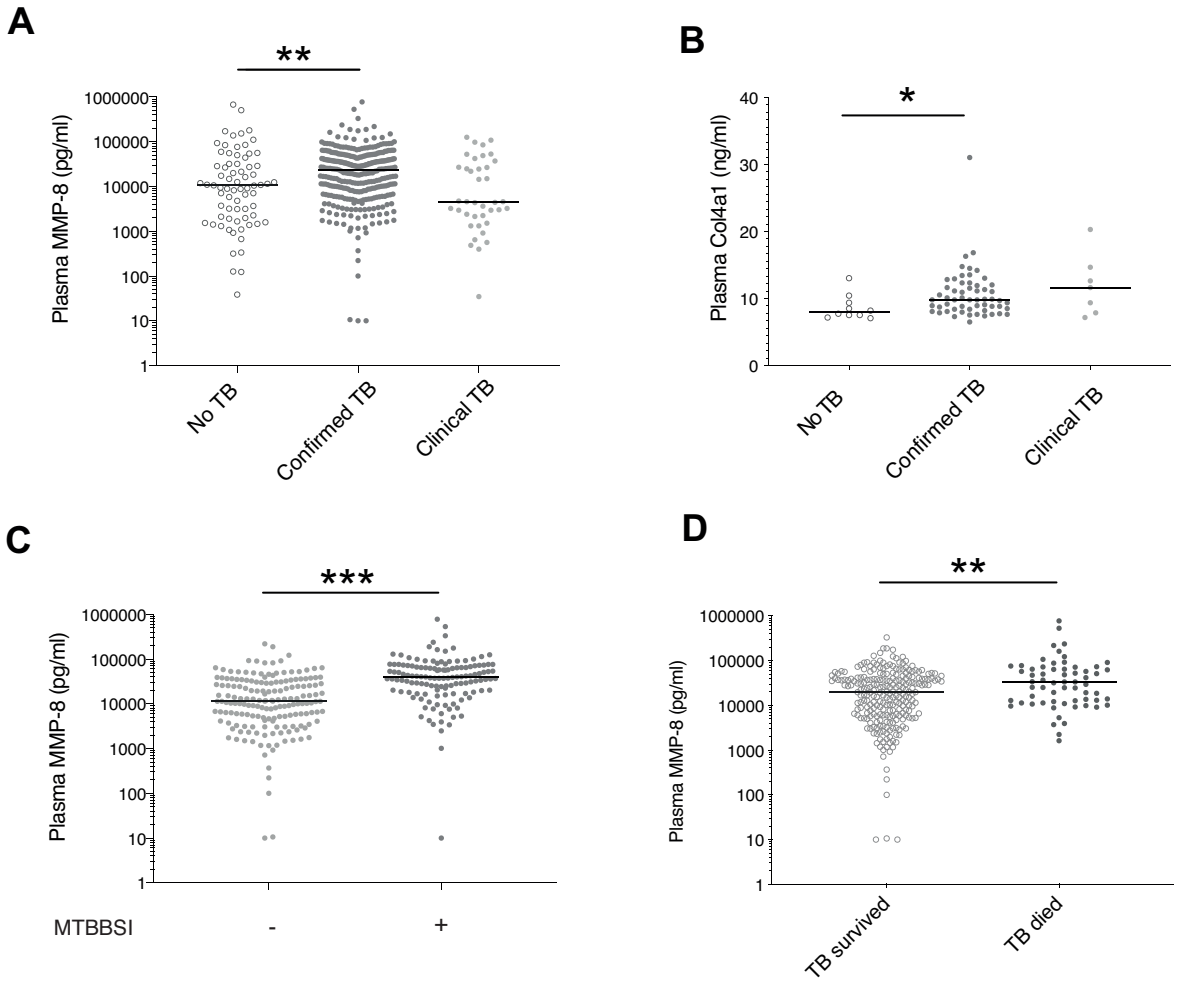


Figure 1

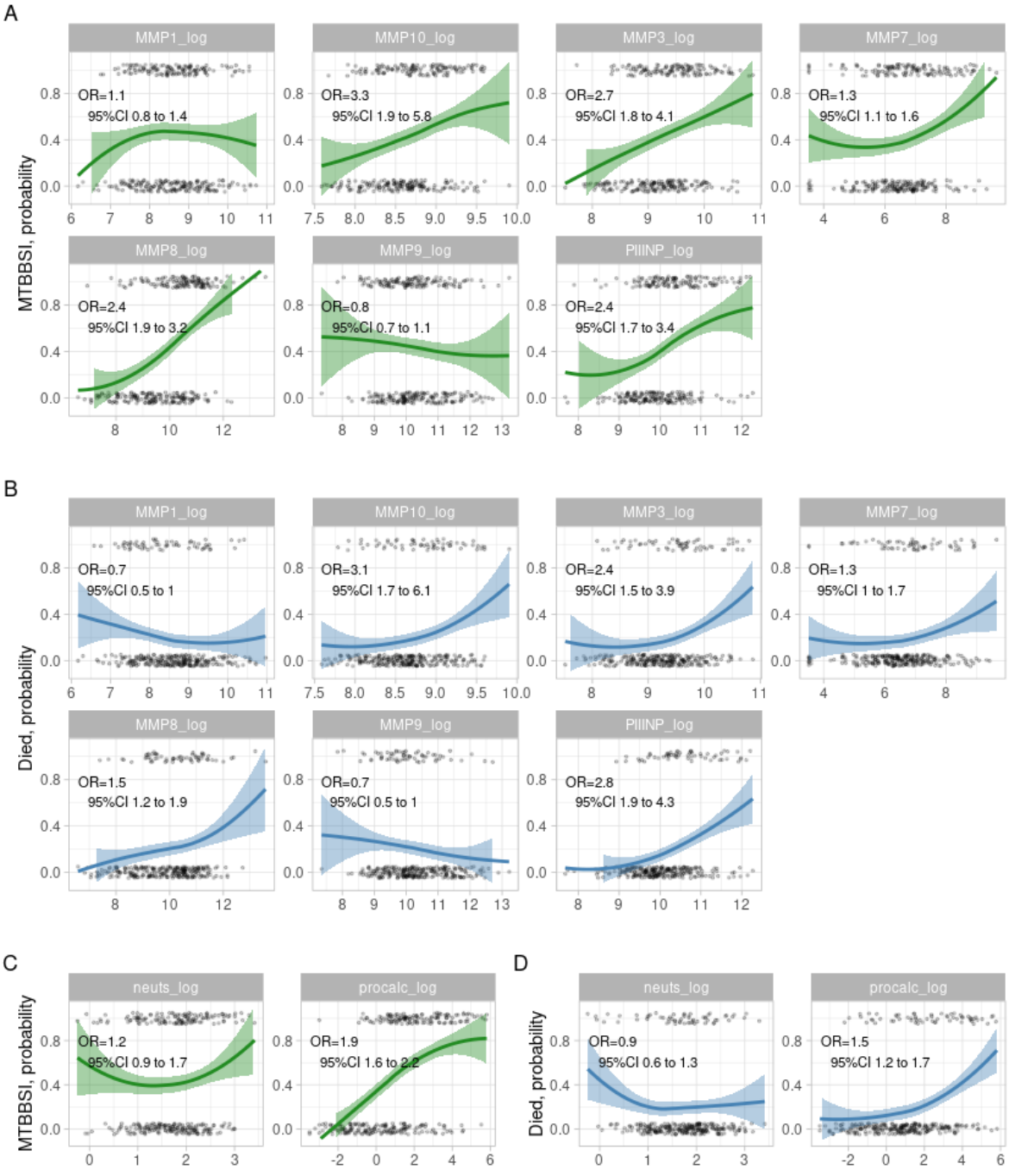

A
MMP1_log
OR=1.1
95%CI 0.8 to 1.4
MMP10_log
OR=3.3
95%CI 1.9 to 5.8
MMP3_log
OR=2.7
95%CI 1.8 to 4.1
MMP7_log
OR=1.3
95%CI 1.1 to 1.6
MMP8_log
OR=2.4
95%CI 1.9 to 3.2
MMP9_log
OR=0.8
95%CI 0.7 to 1.1
PIIINP_log
OR=2.4
95%CI 1.7 to 3.4
MTBBSI, probability
B
MMP1_log
OR=0.7
95%CI 0.5 to 1
MMP10_log
OR=3.1
95%CI 1.7 to 6.1
MMP3_log
OR=2.4
95%CI 1.5 to 3.9
MMP7_log
OR=1.3
95%CI 1 to 1.7
MMP8_log
OR=1.5
95%CI 1.2 to 1.9
MMP9_log
OR=0.7
95%CI 0.5 to 1
PIIINP_log
OR=2.8
95%CI 1.9 to 4.3
Died, probability
C
neuts_log
OR=1.2
95%CI 0.9 to 1.7
procalc_log
OR=1.9
95%CI 1.6 to 2.2
MTBBSI, probability
D
neuts_log
OR=0.9
95%CI 0.6 to 1.3
procalc_log
OR=1.5
95%CI 1.2 to 1.7
Died, probability

Figure 2

Elevated plasma matrix metalloproteinases associate with *Mycobacterium tuberculosis* blood stream infection and mortality in HIV-associated tuberculosis

Supplementary Information

Supplementary Methods

Participants provided written informed consent. Eligible patients with decreased capacity to consent were enrolled and followed up daily to obtain consent according to the approved protocol. All results were made available to the clinical team and participants remained in routine clinical care. Inclusion in this analysis required the availability of EDTA plasma samples at enrolment. MMP -1, -3, -7, -8, -9 and -10 were quantified by Luminex array (Bio-Rad Bio-Plex 200 system; R&D Systems, United Kingdom) on EDTA plasma. PIIINP, Col4α1 and HA were quantified by enzyme-linked immunosorbent assays (Cloud Clone Corp, China) on EDTA plasma, as per manufacturers' instructions.

After log transformation, data were normally distributed, with the exception of a few datapoints (less than ten per analyte), likely reflecting technical rather than biological factors. For hierarchical clustering analysis and Pearson correlation extreme outliers (observations with a value greater than four median absolute deviations from the variable median applied after transformation) were excluded. For scaling of data for hierarchical clustering analysis, data was mean subtracted and divided by standard deviation.

List of Supplementary Tables and Figures

Supplementary Table S1 Demographic and Clinical Features of Study Participants

	No TB	Confirmed TB	Clinical TB	LAM TB*	All participants
Frequency, n (%)	72 (16.5)	313 (71.6)	48 (11.0)	4 (0.9)	437 (100)
Median age (years), IQR	39.9 (30.9-49.3)	35.7 (30.5-43.0)	38.0 (31.6-42.5)	36.8 (34.3-37.9)	36.1 (30.8-43.9)
Female, n (%)	40 (55.6)	159 (50.8)	29 (60.4)	2 (50.0)	230 (52.6)
Median CD4 count (cells/mm^3), IQR	89.5 (29.0-224)	56 (18.0-112)	93.0 (53.8-182)	88.0 (26.8-137)	62.0 (22.5-133)
On ART, n (%)	28 (38.9)	95 (30.4)	19 (39.6)	0 (0)	142 (32.5)
Mortality at 12 weeks, n (%)	11 (15.3)	62 (19.8)	9 (18.8)	1 (25.0)	83 (19.0)

Abbreviations: ART = antiretroviral therapy; IQR = interquartile range; LAM = lipoarabinomannan.
*This group were excluded from laboratory analyses

Supplementary Table S2 Participant characteristics by *Mycobacterium tuberculosis* blood stream infection and vital status

Participant characteristics	No *Mtb*-BSI	*Mtb*-BSI	p	Survived	Died	p
Age, median years (Q1, Q3)	35.4 (30.4, 43.9)	35.8 (30.7, 41.5)	0.835	35.4 (30.3, 42.1)	38.4 (31.9, 47.2)	**0.021**
Female, n (%)	100 (57.8)	56 (42.1)		125 (50.6)	32 (51.6)	
Male, n (%)	73 (42.2)	77 (57.9)	0.008*	122 (49.4)	30 (48.4)	1.000*
CD4 count, median cells/mm^3 (Q1, Q3)	83 (33.5, 162)	28 (9.0, 59)	**<0.001**	57 (23, 125)	31 (8.75, 84.3)	**0.002**
ART status, on ART, n (%)	62 (43.7)	30 (27.3)		74 (36.3)	19 (38)	
ART status, ART naïve, n (%)	55 (38.7)	42 (39.1)		84 (41.2)	14 (28)	
ART status, defaulted, n (%)	25 (17.6)	38 (34.5)	**0.005*$**	46 (22.5)	17 (34)	0.870*$

*p values by Fisher's Exact Test (otherwise by Mann-Whitney U test); $given for *on ART* versus *ART naïve or defaulted.*
ART = Antiretroviral therapy (ART); *Mtb*-BSI = *Mycobacterium tuber*culosis blood stream infection; Q1, Q3 = First quartile, third quartile

Supplementary Table S3 Matrix metalloproteinase and extracellular matrix breakdown product concentrations

	No TB		Confirmed TB		Clinical TB		p values[$]	
Plasma	Median	Q1, Q3	Median	Q1, Q3	Median	Q1, Q3	No TB vs Confirmed TB	No TB vs Clinical TB
MMP-1 (pg/ml)	4477	1988, 7640	5316	2686, 9756	5163	2671, 9799	0.112	0.162
MMP-3 (pg/ml)	12840	8180, 22951	10716	6934, 15935	8535	5528, 14080	**0.021**	**0.006**
MMP-7 (pg/ml)	1071	455, 1967	712	330, 1406	525	174, 1589	0.046	0.088
MMP-8 (pg/ml)	10602	2019, 32205	23712	7688, 47571	4430	2156, 27099	**0.003**	0.324
MMP-9 (pg/ml)	26512	11079, 45181	26574	14400, 58120	23853	12909, 50802	0.220	0.667
MMP-10 (pg/ml)	7809	4990, 11089	5807	4147, 8009	5310	3798, 7897	**<0.001**	**0.001**
PIIINP (pg/ml)	21380	14323, 41866	22712	13978, 40724	19308	12082, 28705	0.941	0.193
HA (ng/ml)	8.26	5.24, 12.9	8.287	5.815, 10.88	8.815	5.29, 15.5	0.640	0.872
Col4α1 (ng/ml)	7.98	7.45, 9.65	9.81	8.42, 12.0	11.7	7.87, 14.7	0.017	0.103

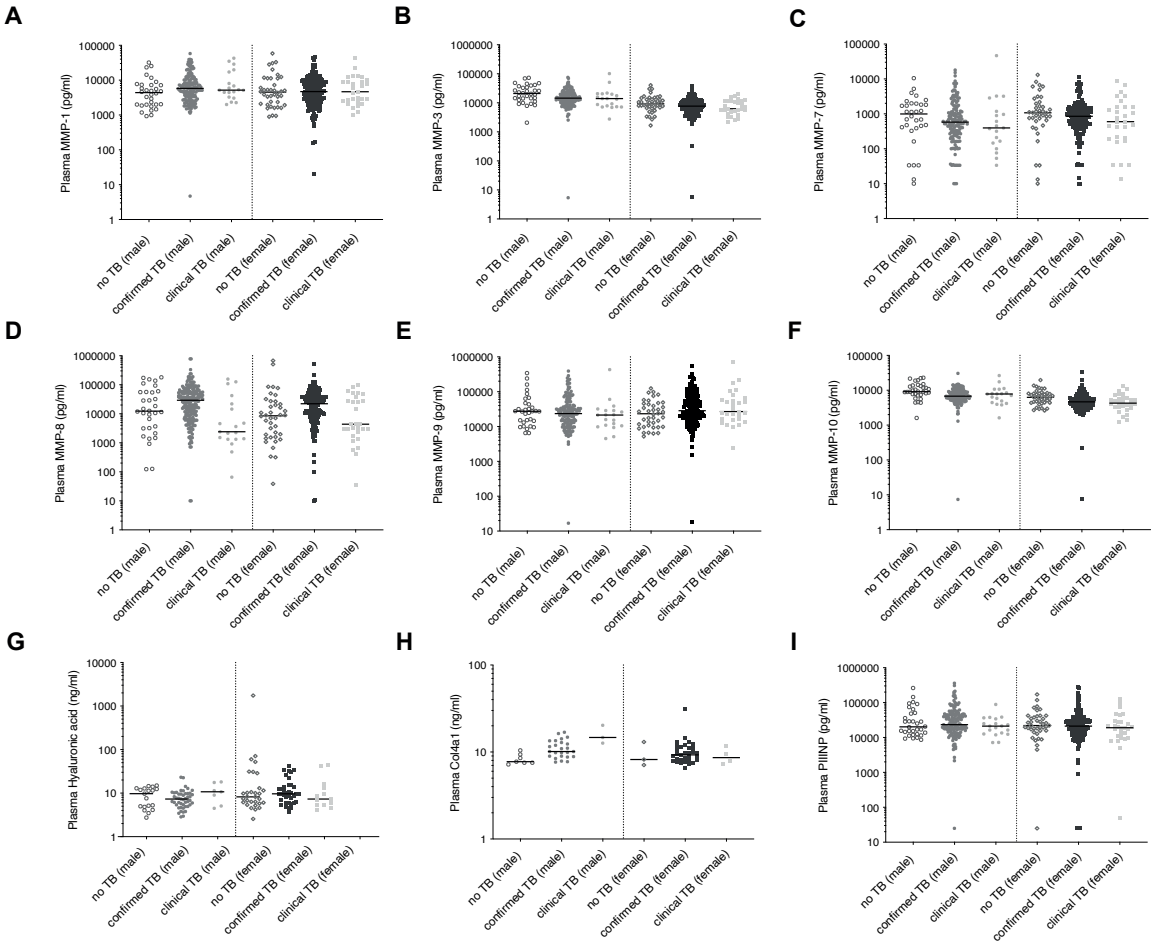
Confirmed TB*: Mycobacterium tuberculosis* identified in clinical samples; Clinical TB*:* TB was likely and the patient was treated for TB but no microbiological confirmation was obtained; No TB: TB was excluded on clinical and microbiological grounds. Abbreviations: Col4α1 = collagen IV alpha 1; HA = Hyaluronic Acid (HA); Q1,Q3 = first quartile, third quartile; MMP = matrix metalloproteinase; PIIINP = procollagen N-terminal propeptide. HA and Col4α1 were measured on a random subgroup of 73 participants.

[$]A Bonferroni correction for multiple comparisons indicated that a p value of <0.025 was equivalent to a significance threshold of <0.05. Significant p values are shown in bold.

Supplementary Table S4 Matrix metalloproteinase and procollagen III N-terminal propeptide concentration by sex (all participants)

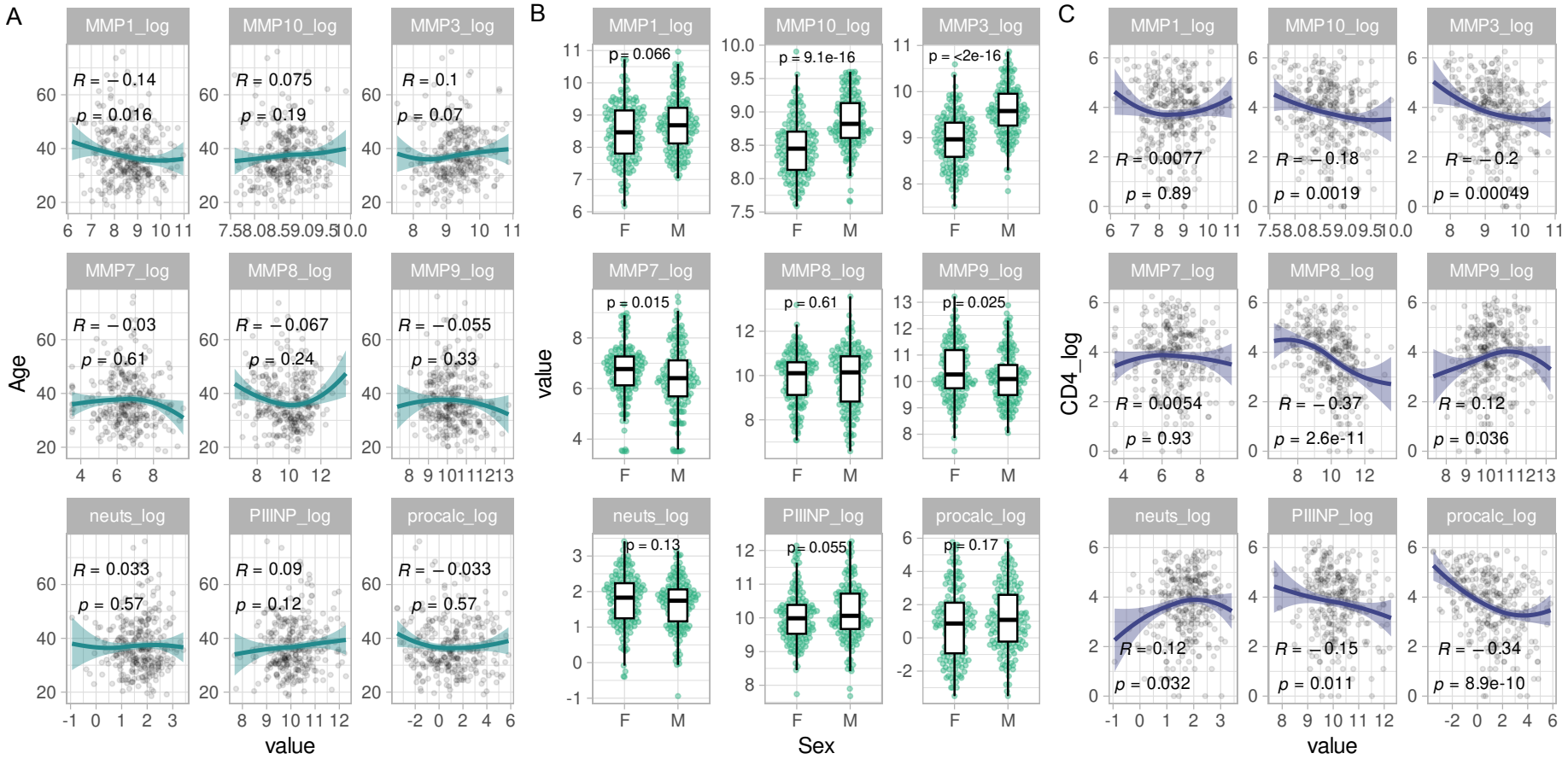
Plasma analyte concentration median pg/ml (Q1, Q3)							
	MMP-1	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	PIIINP
Male, n=207	5354 9(2853, 9923)	14753 (10334, 21972)	608 (273, 1468)	18690 (5027, 52615)	24126 (12836, 39473)	7388 (5602, 10188)	22744 (14617, 45025)
Female, n=230	4683 (2367, 33147)	7842 (2367, 11757)	871 (406, 1498)	15830 (4754, 37854)	26888 (15085, 61994)	4803 (3397, 6593)	20373 (12625, 33147)
p value	0.078	**<0.001**	**0.023**	0.115	**0.043**	**<0.001**	**0.034**

MMP = matrix metalloproteinase; PIIINP = procollagen N-terminal propeptide; Q1,Q3 = first quartile, third quartile.



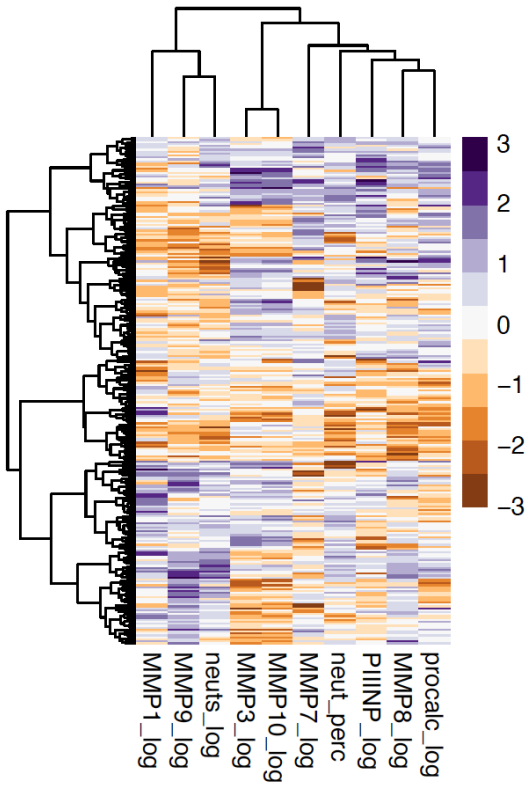
Supplementary Figure S1 Plasma matrix metalloproteinase (MMP) and matrix-derived biomarker concentrations by diagnostic category in male and female participants

Plasma MMP and extracellular matrix breakdown products were measured in hospitalised participants with HIV infection and symptoms suggestive of TB (either subsequently microbiologically confirmed or otherwise clinically diagnosed) or in hospitalised HIV positive participants with symptoms due to other diagnoses (no TB), demonstrating similar findings in male and female participants. No statistical tests are reported. Col4α1 = collagen IV alpha 1; PIIINP = procollagen III N-terminal propeptide.



Supplementary Figure S2 Biomarkers by age, sex and CD4 count in confirmed TB

Plasma matrix metalloproteinase concentrations (MMP), procollagen III N-terminal propeptide concentrations (PIIINP), neutrophil (neuts) count and procalcitonin (procalc) are shown by age (A), sex (B) and CD4 count (C). A and C, Loess fit to data (coloured lines with shaded 95% confidence intervals) with measured log concentrations plotted on the x axis and analysis by Pearson correlation with extreme outliers (observations with a value greater than four median absolute deviations from the variable median applied after transformation) excluded. In B, concentration/count is on the y axis and sex is on the x axis (M=male, F=female) with comparisons by Mann-Whitney U test. MMP/PIIINP concentrations are in pg/ml, procalcitonin concentrations are in µg/L and neutrophil count is $x10^9$/L.



Supplementary Figure S3 Plasma matrix metalloproteinase-8 associates with collagen turnover and acute inflammation

Hierarchical clustering analysis demonstrates that plasma matrix metalloproteinase-8 (MMP-8) most closely associated with procalcitonin (procalc) concentrations. Plasma MMP-3 and MMP-10 clustered together, whilst MMP-9 mostly closely clustered with neutrophil count (neuts). The analysis was on scaled data, excluding extreme outliers, which were less than ten data points for any one analyte. Neuts_perc = neutrophil percentage; PIIINP = procollagen III N-terminal propeptide.