


# Elevated circulating levels of monocyte activation markers among tuberculosis patients with diabetes co-morbidity

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## Summary

Alteration in the frequency of monocyte subsets is a hallmark of tuberculosis–diabetes co-morbidity (TB-DM). To study this association, we examined the plasma levels of sCD14, sCD163, C-reactive protein (CRP) and soluble tissue factor (sTF) in individuals with TB-DM, TB or diabetes mellitus (DM), and in healthy controls (HC). Circulating levels of sCD14, sCD163 and sTF were significantly increased in TB-DM and DM compared with TB and HC; however, CRP was significantly increased in TB-DM and TB compared with DM and HC. During longitudinal follow up, sCD14, CRP and sTF levels remained significantly increased in TB-DM compared with TB from baseline (pre-treatment), during treatment (2nd month) and at the completion (6th month) of anti-TB treatment (ATT), whereas sCD163 was significantly higher in TB-DM compared with TB only at baseline. Moreover, the levels of sCD14 and sCD163 were significantly higher in TB-DM individuals with bilateral and cavitary disease and exhibited a significant positive relationship with bacterial burden. Levels of sCD14, sCD163 and CRP exhibited a positive relationship with HbA1c levels. Within the TB-DM group, those with known diabetes before incident TB (KDM) exhibited significantly higher levels of sCD14 and sCD163 compared with individuals with newly diagnosed DM with TB (NDM). Finally, KDM individuals on metformin treatment exhibited significantly lower levels of sCD14, sCD163 and CRP compared with those on non-metformin-containing regimens. Our data demonstrate that systemic monocyte activation marker levels reflect baseline disease severity and extent in TB-DM, differentiate KDM from NDM and are modulated by ATT and metformin therapy.

**Keywords:** diabetes mellitus; monocytes; tuberculosis.

## Introduction

Tuberculosis (TB) and diabetes mellitus (DM) are two common diseases that independently have immense public health significance globally. Their association and consequences are well-established,<sup>1–3</sup> but some aspects need further research. It is not completely understood why patients with DM, predominantly those with poor glycemic control, are at highest risk of TB, although alterations have been found in both innate and adaptive immune responses.<sup>4,5</sup>

Moreover, individuals with diabetes who have smear-positive pulmonary TB have a higher possibility of failure during smear conversion after 2 months of therapy than patients without DM.<sup>6</sup> On the one hand, DM constitutes a risk factor for active TB and might impact the disease presentation and response to treatment;<sup>1,7</sup> while on the other hand, TB may stimulate glucose intolerance and exacerbate dysglycemia in patients with DM.<sup>8,9</sup> The exact mechanisms underlying this interaction are still relatively vague and are in need of comprehensive evaluation.

Abbreviations: ATT, anti-TB treatment; CRP, C-reactive protein; DM, diabetes mellitus; HbA1c, glycated hemoglobin; HC, healthy controls; KDM, diabetic before incident TB; NDM, newly diagnosed with DM; sTF, soluble tissue factor; TB, tuberculosis

Monocytes play a vital role in TB given their rapid migration to the lung upon initial *Mycobacterium tuberculosis* infection, where they differentiate into macrophages and dendritic cells for antigen presentation and secretion of cytokines.<sup>10</sup> Monocytes are important to the innate immune system and play a crucial role in several inflammatory conditions associated with chronic infection, including TB.<sup>11</sup> Plasma levels of soluble (s) CD14 have been established as a strong indicator of monocyte activation.<sup>12</sup> A soluble form of CD163 was present in normal plasma<sup>13</sup> and a heightened plasma concentration of sCD163 was seen in diseases linked to macrophage activity, including acute and chronic inflammation.<sup>14</sup> Expression of soluble tissue factor (sTF) by cells of the monocyte/macrophage lineage was also shown to be involved in the development and progression of local and systemic inflammatory reactions.<sup>15,16</sup> C-reactive protein (CRP) is an established marker of acute inflammation and its serum concentration correlates with the severity of systemic inflammation.<sup>17</sup> Soluble markers derived from monocyte activation such as sCD14, sCD163, CRP and sTF in individuals have been broadly studied in the context of HIV infection.<sup>17–21</sup> However, a detailed examination of the association of monocyte activation markers with tuberculosis–diabetes co-morbidity (TB-DM) or TB, and their relationship to disease pathology or bacterial burden, is presently unknown. We postulated that one potential mechanism for the increased severity of TB in individuals with diabetes could be excessive monocyte activation that promotes tissue injury and adverse TB disease outcomes. As macrophages and monocytes play a pivotal role in the progression of TB, the aim of this study was to elucidate the association of the systemic levels of monocyte activation markers, sCD14, sCD163, CRP and sTF in individuals with TB-DM and those with TB and compare them with individuals with no TB but DM alone (DM) or healthy controls (HC).

## Materials and methods

### Ethics statement

This study was approved by the Ethics Committees of the Prof. M. Viswanathan Diabetes Research Center and the National Institute for Research in Tuberculosis. Informed written consent was obtained from all participants.

### Study population

Plasma samples were collected from 44 individuals with active pulmonary TB with DM (TB-DM), 44 individuals with active pulmonary TB (TB), 44 individuals with diabetes mellitus (DM), and 30 healthy control individuals with no TB or diabetes (HC) recruited in Chennai, India. All the enrolled DM and HC participants were negative for

Quantiferon TB gold assay with no clinical symptoms of TB and normal chest X-rays. The diagnosis of pulmonary TB was based on smear and culture positivity for *M. tuberculosis*. Chest X-rays were used to define cavitory disease (TB-DM,  $n = 13$  and TB,  $n = 10$ ) and non-cavitory disease (TB-DM,  $n = 31$  and TB,  $n = 34$ ) as well as unilateral (TB-DM,  $n = 24$  and TB,  $n = 25$ ) versus bilateral (TB-DM,  $n = 20$  and TB,  $n = 19$ ) lung involvement. Smear grades were used to determine bacterial burdens and were classified as 1+ (TB-DM,  $n = 14$  and TB,  $n = 19$ ), 2+ (TB-DM,  $n = 19$  and TB,  $n = 14$ ) and 3+ (TB-DM,  $n = 11$  and TB,  $n = 11$ ). At the time of enrolment, all individuals with active TB had no record of prior TB disease or anti-TB treatment (ATT). Glycemic status (DM or normoglycemia) was diagnosed on the basis of oral glucose tolerance test and/or glycated hemoglobin (HbA1c) levels (for those known to have diabetes), according to the WHO criteria. Among the 44 TB-DM individuals, 22 were known diabetics (KDM) and 22 were newly diagnosed diabetics (NDM). Among the KDM individuals, 11 were on metformin-containing anti-diabetic medication and 11 were not. This was at the discretion of the treating physician and not related to extent or severity of diabetic disease. All the enrolled DM and HC individuals were negative for the Quantiferon TB gold assay with no clinical symptoms of TB and with normal chest X-rays. The study groups were similar with regard to age and gender and the baseline characteristics of the study participants are shown in Table 1. Standard ATT was administered to TB-DM and TB individuals using the directly observed treatment, short course strategy. At 2 and 6 months following ATT initiation, fresh plasma samples were obtained from TB-DM and TB individuals. All TB-DM and TB individuals were culture negative at the end of ATT.

### ELISA

Circulating levels of sCD14, sCD163 and CRP were measured using the Duoset ELISA Development System (R&D Systems, Minneapolis, MN). Quantikine ELISA kit (R&D Systems) was used for measuring sTF. The lowest detection limits were as follows: sCD14, 62.5 pg/ml; sCD163, 8.75 pg/ml; CRP, 0.781 pg/ml; sTF, 7.81 pg/ml.

### Statistical analysis

Geometric means were used for measurements of central tendency. Statistically significant differences between the four groups were analyzed using the Kruskal–Wallis test with Dunn's correction for multiple comparisons. The Mann–Whitney *U*-test was used to compare monocyte activation marker concentrations between pulmonary TB patients with and without DM, unilateral or bilateral lung lesions and cavitory or non-cavitory disease. Linear trend post-test was used to compare monocyte marker concentrations with smear grades (reflecting bacterial burdens)

**Table 1.** Demographics of the study groups and biochemical parameters in TB-DM, TB non-DM, DM and HC

Study demographics	TB-DM		TB	DM	HC
	KDM	NDM			
No. of participants recruited	22	22	44	44	30
Gender (male/female)	16/6	18/4	27/17	30/14	15/15
Median age, years (range)	52 (25–70)	42 (29–70)	39 (24–67)	44 (33–68)	34 (23–55)
Smear grade: 0/1+/2+/3+	0/4/10/8	0/10/9/3	0/19/14/11	NA	NA
Cavitary disease (Y/N)	9/13	4/18	10/34	NA	NA
Lung lesions (unilateral/bilateral)	13/9	11/11	25/19	NA	NA
Fasting blood glucose, mg/dl	166 (120–417)	139 (111–375)	93 (73–103)	144 (95–405)	75 (70–109)
Post-prandial glucose, mg/dl	350 (217–550)	311 (202–505)	110 (68–137)	341 (210–543)	98 (72–139)
Glycated hemoglobin level, %	12.3 (8–15.6)	10 (7.3–13.9)	5.6 (5.0–5.8)	10 (6.8–12.6)	5.5 (5.0–5.9)

DM, diabetes mellitus; HC, healthy controls; KDM, known DM with incident TB; NDM, newly diagnosed DM; TB, tuberculosis; TB-DM, tuberculosis and diabetes mellitus co-morbidity.

Values represent the geometric mean (and the 95% confidence intervals) except for age where the median (and the range) are depicted.

and Spearman rank correlation was used to compare monocyte marker concentrations with HbA1c levels. Analyses were performed using GRAPHPAD PRISM Version 7 (GraphPad, San Diego, CA, USA).

## Results

### Study population characteristics

The baseline characteristics including demographic and biochemical features of the study population are shown in Tables 1 and 2. No significant differences were observed in age, sex, smear or culture grades at baseline between the TB-DM and TB groups (Table 1).

### Heightened levels of circulating monocyte activation markers in TB-DM

To determine the systemic levels of circulating monocyte activation markers in TB-DM and TB, we measured the circulating levels of sCD14, sCD163, CRP and sTF in TB-DM, TB, DM and HC individuals (Fig. 1). As shown, the levels of sCD14 (median 3253 pg/ml in TB-DM versus

2520 pg/ml in TB, 2778 pg/ml in DM and 2441 pg/ml in HC) were significantly higher in TB-DM compared with TB and HC individuals and levels in DM were significantly increased compared with HC. Also, the levels of sCD163 (median 294.2 pg/ml in TB-DM versus 241.4 pg/ml in TB, 334.1 pg/ml in DM and 132.7 pg/ml in HC) and sTF (median 10.8 pg/ml in TB-DM versus 8.8 pg/ml in TB, 16.2 pg/ml in DM and 8.5 pg/ml in HC) were significantly higher in TB-DM and DM compared with TB and HC individuals. However, CRP (median 91.8 pg/ml in TB-DM versus 84.3 pg/ml in TB, 51 pg/ml in DM and 51.2 pg/ml in HC) was significantly increased in TB-DM and TB compared with DM and HC. Hence, TB-DM is associated with elevated systemic levels of circulating monocyte activation markers.

### Heightened circulating levels of monocyte activation markers in TB patients with diabetes compared with TB patients without diabetes during TB treatment

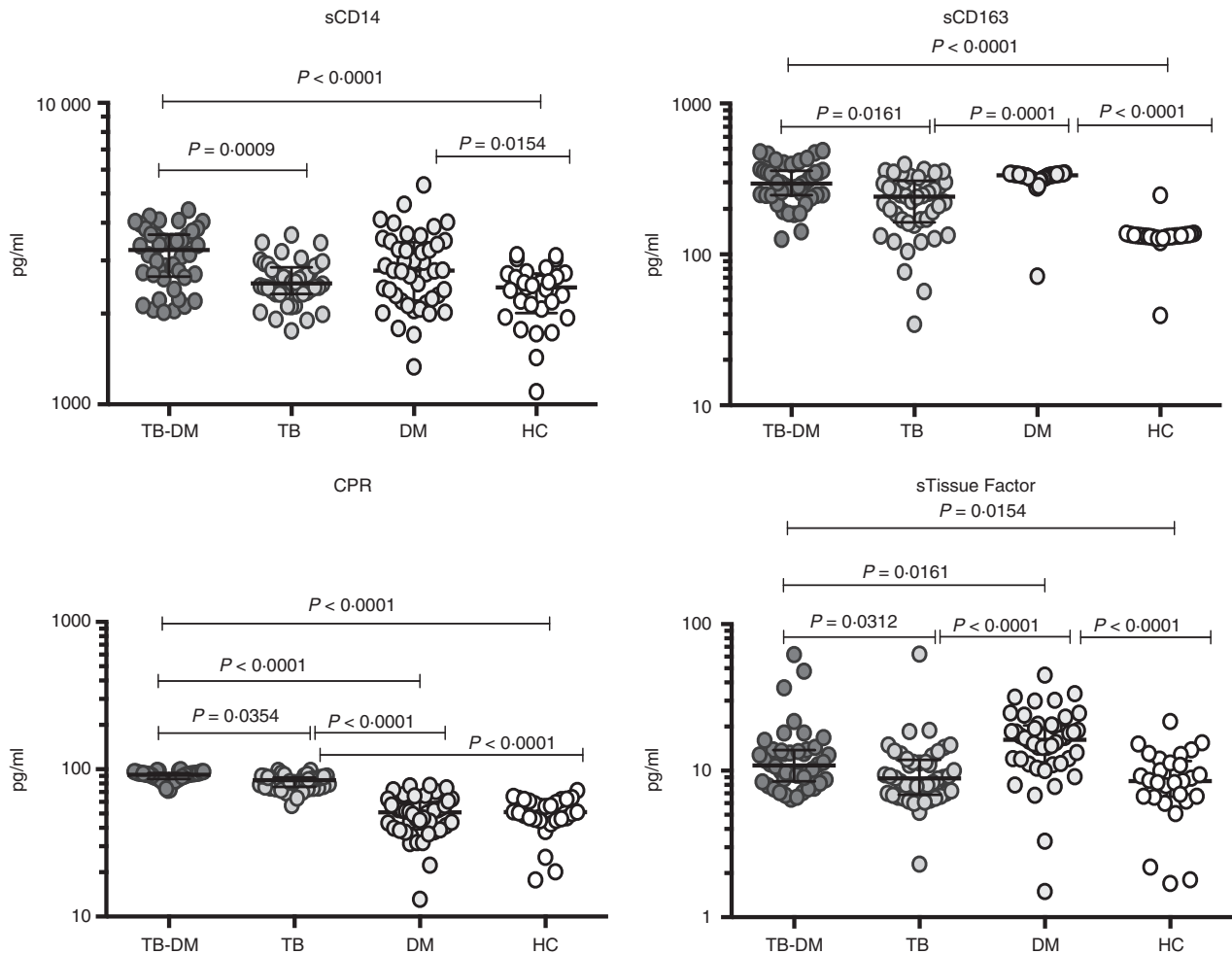
To determine whether the monocyte activation markers were altered by TB treatment, we measured the circulating levels of sCD14, sCD163, CRP and sTF in TB-DM

**Table 2.** Demographics of the clinical data in TB-DM

Study demographics	TB-DM		
	KDM		NDM
	Metformin	Non-metformin	
No. of participants recruited	11	11	22
Smear grade: 0/1+/2+/3+	0/3/4/4	0/1/6/4	0/10/9/3
Cavitary disease (Y/N)	4/7	5/6	4/18
Lung lesions (Unilateral/Bilateral)	7/4	6/5	11/11
Glycated hemoglobin level, %	11 (7.7–15.6)	10.2 (7.9–14.3)	10 (7.3–13.9)

KDM, known DM with incident TB; NDM, newly diagnosed DM; TB-DM, tuberculosis and diabetes mellitus co-morbidity.

Values represent the geometric mean (and the 95% confidence intervals).



**Figure 1.** Elevated circulating levels of monocyte activation markers in tuberculosis–diabetes mellitus co-morbidity (TB–DM) and DM individuals. Plasma levels of sCD14, sCD163, C-reactive protein (CRP) and soluble tissue factor (sTF) were measured in TB–DM ( $n = 44$ ), TB ( $n = 44$ ) and DM ( $n = 44$ ) individuals, and in healthy controls (HC) ( $n = 30$ ) at baseline. Data are represented as scatter plots with each circle representing a single individual.  $P$  values were calculated using the Kruskal–Wallis test with Dunn’s *post hoc* for multiple comparisons.

and TB at baseline (pre-treatment), during treatment (2nd month) and at the completion of ATT (6th month). As shown Fig. 2, sCD14 (median 3253 pg/ml in TB–DM versus 2520 pg/ml in TB); sCD163 (median 294.2 pg/ml in TB–DM versus 241.4 pg/ml in TB); CRP (median 91.8 pg/ml in TB–DM versus 84.3 pg/ml in TB) and sTF (median 10.8 pg/ml in TB–DM versus 8.8 pg/ml in TB) levels were significantly higher in TB–DM compared with TB pre-treatment. Similarly, sCD14 (median 4029 pg/ml in TB–DM versus 2197 pg/ml in TB); CRP (median 83 pg/ml in TB–DM versus 73.6 pg/ml in TB) and sTF (median 11.2 pg/ml in TB–DM versus 7.2 pg/ml in TB) levels were also significantly higher at 2 months of treatment. Finally, sCD14 (median 3732 pg/ml in TB–DM versus 2746 pg/ml in TB); CRP (median 63.6 pg/ml in TB–DM versus 49.9 pg/ml in TB) and sTF (median 12.7 pg/ml in TB–DM versus 9.3 pg/ml in TB) levels were also significantly higher after 6 months of treatment.

### Circulating monocyte activation markers as markers of disease severity and bacterial burdens in TB–DM

To determine the association between the systemic levels of circulating monocyte markers and disease severity in TB–DM, we measured the circulating levels of sCD14, sCD163, CRP and sTF in TB–DM and TB individuals with unilateral versus bilateral disease and cavitory versus non-cavitory disease based on chest X-ray. As shown in Fig. 3(a), the circulating levels of sCD14 (median 3474 pg/ml in bilateral versus 2888 pg/ml in unilateral disease) and sCD163 (median 342.1 pg/ml in bilateral versus 276.3 pg/ml in unilateral disease) were significantly higher in TB–DM individuals with bilateral disease compared with those with unilateral disease. Similarly, as shown in Fig. 3(b), the circulating levels of sCD14 (median 3838 pg/ml in cavitory versus 2901 pg/ml in non-cavitory disease) and sCD163 (median 359.6 pg/ml in bilateral versus 276.7 pg/ml in

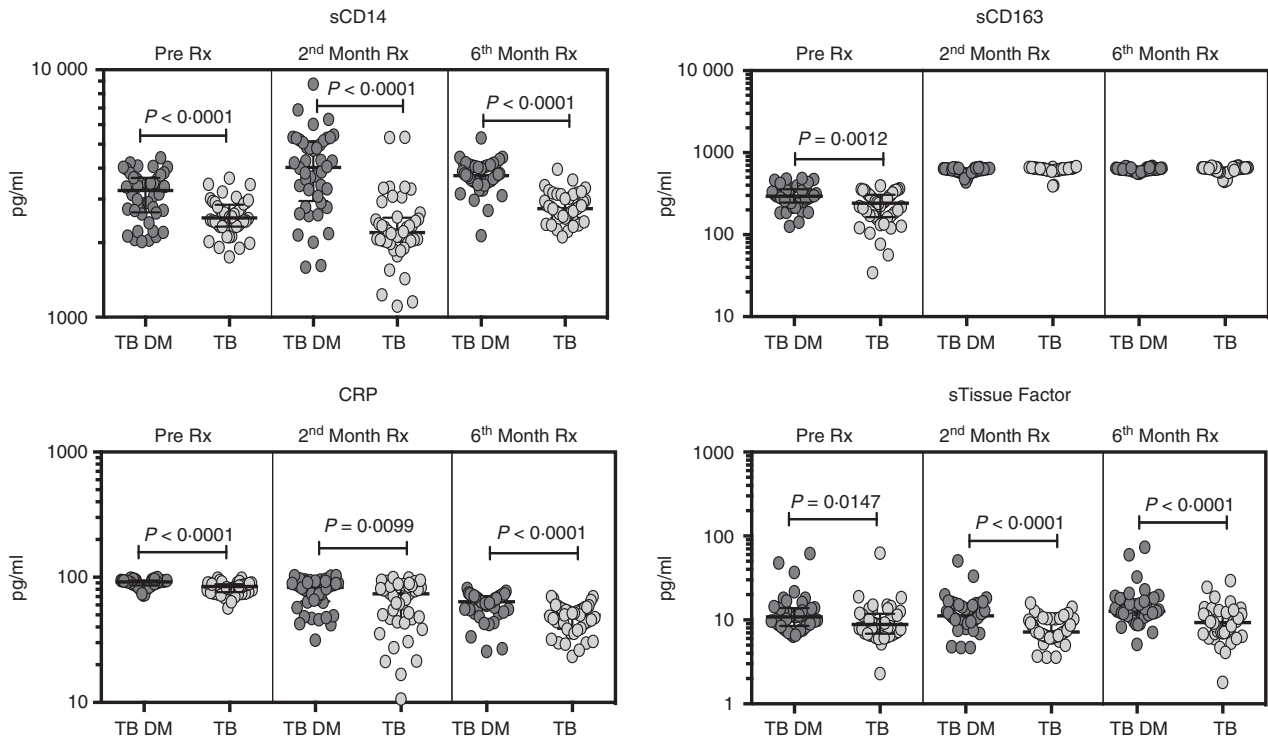


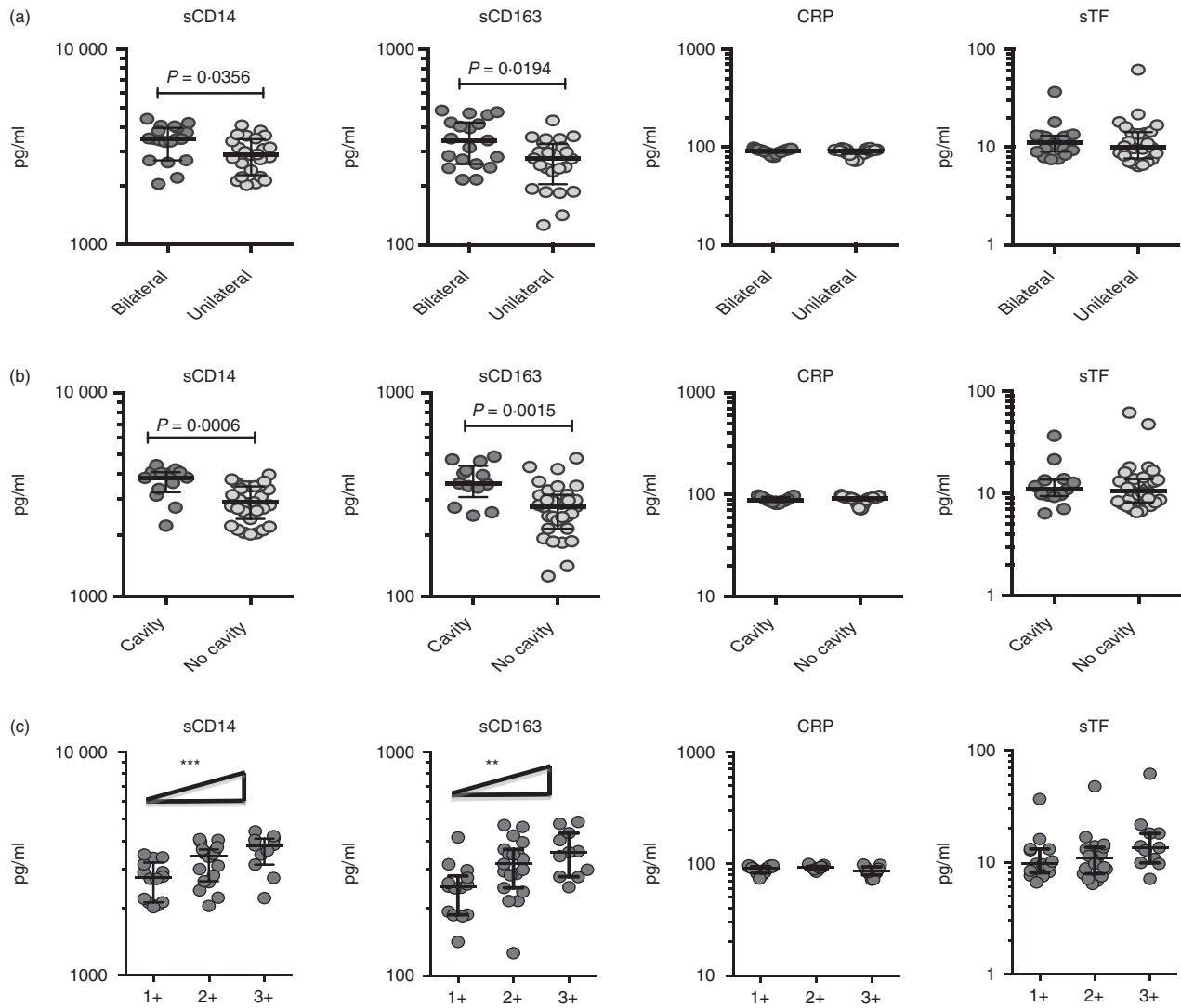
Figure 2. Tuberculosis–diabetes mellitus co-morbidity (TB-DM) is associated with increased frequencies of monocyte activation markers pre-treatment and following treatment. Circulating levels of monocyte activation markers in TB-DM ( $n = 44$ ) and TB ( $n = 44$ ) individuals at pre-treatment and at 2 and 6 months following treatment are shown. Data are represented as scatter plots with each circle representing a single individual.  $P$  values were calculated using the Mann–Whitney test.

unilateral disease) were significantly higher in TB-DM individuals with cavitory disease compared with those without. In contrast, as shown in the Supplementary material (Fig. S1A,B), no significant differences were observed in TB individuals between unilateral and bilateral disease and between cavitory and non-cavitory disease. To determine the association of circulating monocyte markers and bacterial burdens, we performed a correlation of the circulating levels of sCD14, sCD163, CRP and sTF in TB-DM and TB individuals with smear grades. As shown in Fig. 3(c), both sCD14 and sCD163 exhibited a significant positive correlation with smear grades in TB-DM individuals, indicating a positive association of these factors with bacterial burdens. In contrast, as shown in Fig. S1(C), no significant correlation with smear grades was observed in individuals with TB. Hence, both disease severity and bacterial burden in TB-DM are associated with elevated systemic levels of circulating monocyte-associated activation markers.

**Circulating monocyte activation markers exhibit a positive relationship with HbA1c in TB-DM, are increased in KDM individuals, and decreased by metformin treatment**

To determine the association between systemic levels of circulating monocyte markers and glycemic control in

TB-DM, we examined the relationship between the circulating levels of sCD14, sCD163, CRP and sTF in all TB individuals with and without DM with baseline HbA1c levels (Fig. 4a). As shown, the systemic levels of sCD14, sCD163 and CRP exhibited a significant positive relationship with HbA1c levels in TB individuals with and without DM, indicating a significant association of these factors with poor glycemic control. To determine whether monocyte marker levels differ between KDM ( $n = 22$ ) and NDM ( $n = 22$ ) in TB-DM individuals, we first measured HbA1c levels in KDM and NDM and observed that HbA1c % was significantly higher in KDM compared with NDM (geometric mean 11.4% in KDM versus 8.9% in NDM,  $P = 0.0028$ ). Second, we measured the baseline levels of sCD14, sCD163, CRP and sTF in KDM and NDM individuals. As shown in Fig. 4(b), systemic levels of sCD14 (median 3487 pg/ml in KDM versus 2836 pg/ml in NDM) and sCD163 (median 340.3 pg/ml in KDM versus 266.4 pg/ml in NDM) were significantly higher in KDM compared with NDM individuals. Hence, KDM is associated with elevated systemic levels of circulating monocyte markers at baseline. As the anti-diabetic drug metformin is associated with protection against mortality in TB-DM, we wanted to examine the circulating levels of sCD14, sCD163, CRP and sTF in KDM individuals on metformin treatment



**Figure 3.** Elevated circulating levels of sCD14 and sCD163 in bilateral and cavitary disease and also marker of bacterial burden in individuals with tuberculosis–diabetes mellitus co-morbidity (TB-DM). (a) Plasma levels of sCD14, sCD163, C-reactive protein (CRP) and soluble tissue factor (sTF) were measured in TB-DM individuals with bilateral versus unilateral disease. (b) Plasma levels of sCD14, sCD163, CRP and sTF were measured in TB-DM individuals with cavitary versus non-cavitary disease. (c) Relationship between the plasma levels of sCD14, sCD163, CRP and sTF and smear grades as estimated by sputum smears was examined in TB-DM individuals. Data are represented as scatter plots with each circle representing a single individual. *P* values were calculated using the Mann–Whitney test with Holm’s correction for multiple comparisons. For bacterial burden correlation, *P* values were calculated using the linear trend post-test.

( $n = 11$ ) compared with those on non-metformin regimens ( $n = 11$ ). No significant differences were observed in HbA1c levels between KDM individuals on metformin compared with KDM individuals not on metformin. As shown in Fig. 4(c), systemic levels of sCD14 (median 2874 pg/ml in metformin versus 4035 pg/ml in non-metformin), sCD163 (median 292 pg/ml in metformin versus 349.6 pg/ml in non-metformin) and CRP (median 86.3 pg/ml in metformin versus 93.2 pg/ml in non-metformin) were significantly diminished in KDM individuals on metformin compared with KDM individuals not on metformin. Hence, metformin therapy in

KDM individuals is associated with diminished systemic levels of monocyte-associated activation markers.

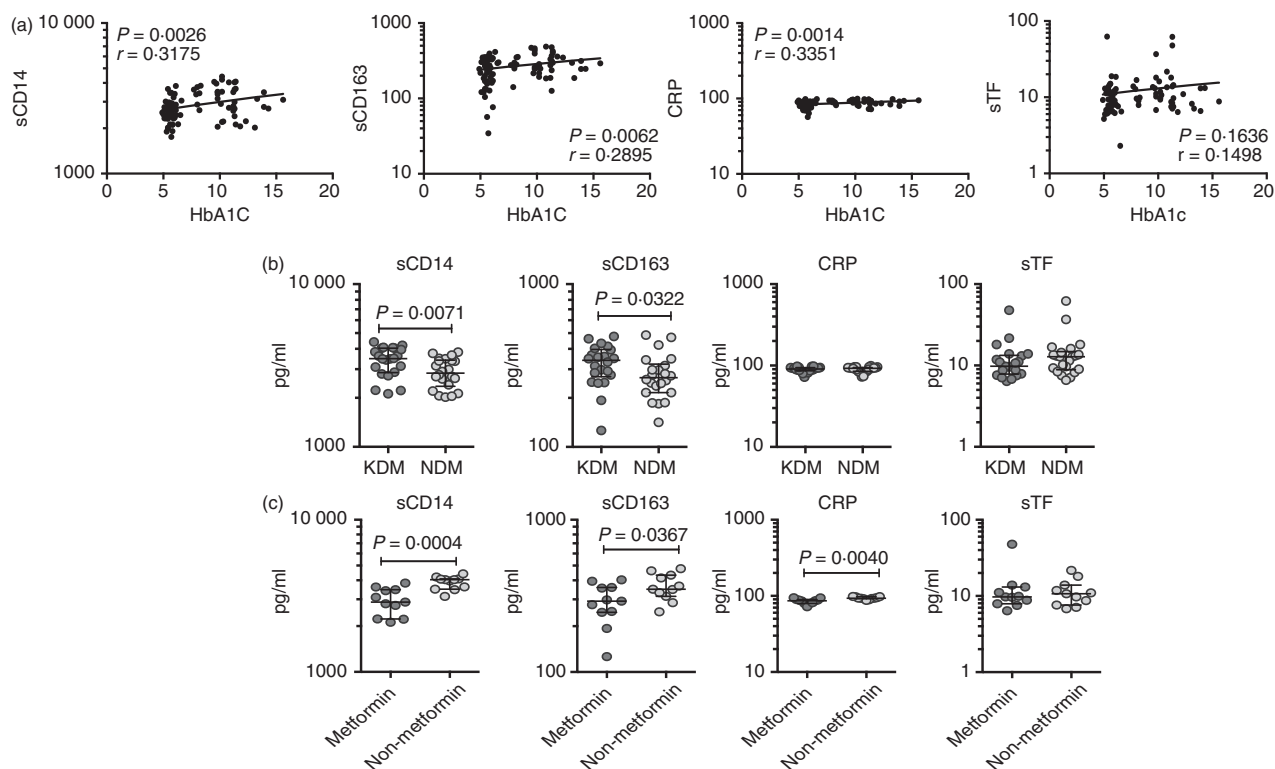
## Discussion

Diabetes mellitus is one of the most important risk factors in the worsening of TB and triples the risk of developing active TB disease.<sup>1</sup> Now, approximately 15% of global TB cases can be attributed to DM co-morbidity.<sup>22</sup> Clinically, DM increases TB severity and worsens TB treatment outcomes,<sup>7</sup> while equally TB hampers glycemic control.<sup>1</sup> Monocytes and macrophages play an important

role in TB immunopathogenesis.<sup>23</sup> Pathogens, including *M. tuberculosis*, can influence macrophage differentiation and activation. Monocytes/macrophages have key roles in TB pathogenesis, as hosts for productive intracellular replication of *M. tuberculosis*,<sup>24</sup> as mediators of tissue injury at sites of infection,<sup>25</sup> and as effectors that contribute to an effective immune response. In the current study, we found that plasma biomarkers of monocyte activation are present in higher levels in individuals with TB-DM compared with TB alone. Whether the increased monocyte activation reflects a causal mechanism of TB susceptibility in DM, or is a marker of increased TB disease severity and risk for tissue injury, remains to be determined. In this regard, we found that levels of sCD14 were higher in DM than HC individuals without active TB disease (Fig. 1). This suggests the potential that dysregulated monocyte activation in DM promotes TB susceptibility.

CD14 is a component of the innate immune system that functions as a co-receptor for bacterial lipopolysaccharide and exists in membrane-bound and soluble

forms.<sup>26</sup> Monocytes are an important source of sCD14 and upon cell activation release sCD14 into the circulation.<sup>27</sup> It has been previously reported that sCD14 serum levels were significantly increased in individuals with pulmonary TB alone and pulmonary TB and HIV co-infection compared with healthy controls.<sup>20</sup> Earlier studies have also demonstrated that sCD14 levels were higher in HIV/TB with high ( $\geq 350$  cells/ $\mu$ l) CD4 T-cell counts compared with low CD4 T-cell counts. In addition, sCD14 levels remained elevated in HIV/TB individuals with lower CD4 T-cell counts despite treatment of TB.<sup>28</sup> Finally, studies have also reported that sCD14 is elevated in the serum of patients with diabetes compared with those without.<sup>29,30</sup> In agreement with these reports, our study revealed that TB-DM and DM individuals exhibited significantly higher systemic levels of sCD14 compared with TB and HC. In addition, before, during and after ATT, sCD14 levels remained elevated in TB-DM compared with TB. Our data also revealed a novel association of sCD14 levels with the severity of TB disease (as estimated by bilateral and cavitary disease) and with



**Figure 4.** Significant correlation between circulating levels of monocyte activation markers and glycemic parameters and elevated circulating levels of monocyte activation markers in individuals with known diabetes mellitus (DM) before incident tuberculosis (TB) (i.e. KDM individuals). (a) Relationship between the plasma levels of sCD14, sCD163, C-reactive protein (CRP) and soluble tissue factor (sTF) and HbA1c levels was examined in all TB individuals with and without DM at baseline. (b) Plasma levels of sCD14, sCD163, CRP and sTF were measured in individuals with tuberculosis–diabetes mellitus co-morbidity (TB-DM) with known diabetes (KDM) versus newly diagnosed diabetes (NDM) (c) Plasma levels of sCD14, sCD163, CRP and sTF were measured in KDM individuals on metformin treatment versus no metformin treatment. Data are represented as scatter plots with each circle representing a single individual. For HbA1c correlations,  $P$  values were calculated using the Spearman rank correlation. For KDM,  $P$  values were calculated using the Mann–Whitney test with Holm’s correction for multiple comparisons.

estimated bacterial burden. Of additional interest was the finding that sCD14 levels correlated positively with HbA1c, indicating an association with poor glycemic control that to our knowledge has not previously been reported. It is of interest that sCD14 and sCD163 levels were not significantly different between TBDM and DM. Our interpretation of these data is that both TB and DM independently affect the monocyte markers. However, the effect of DM is powerful enough to override any significant differences that TB per se might cause. Apart from TB infection, it has also been previously reported that sCD14 expression was also significantly higher in other bacterial infections, such as pneumonia, and in cystic fibrosis and asthma compared with controls.<sup>31</sup>

CD163 is a scavenger receptor for the hemoglobin-haptoglobin complex. It is expressed exclusively on activated cells of the macrophage/monocyte lineage, especially on M2 phenotype cells.<sup>32,33</sup> Published studies have demonstrated that both proliferation and inflammatory activation of macrophages result in increased sCD163 levels.<sup>34,35</sup> Previous studies have shown increased serum concentrations of sCD163 in patients with active pulmonary TB compared with control participants.<sup>36</sup> Other studies have also reported that during chronic HIV infection CD14<sup>+</sup> CD16<sup>+</sup> monocytes were correlated with high levels of sCD163<sup>37,38</sup> and published studies have also reported that TB patients with immune reconstitution inflammatory syndrome (TB-IRIS) exhibited increased levels of sCD163 compared with non-IRIS patients.<sup>11</sup> Although sCD163 expression is induced by monocyte/macrophages in mycobacterial infection and HIV infections, little is known about the role of sCD163 in TB-DM. Our data reveal that sCD163 levels are present at significantly enhanced levels in TB-DM and DM compared with the other groups. TB-DM is also characterized by elevated sCD163 levels at pre-treatment, 2 and 6 months of ATT in comparison with TB individuals. Our study also reveals that sCD163 correlates with severity of disease and/or bacterial burdens in TB-DM and that sCD163 exhibits a positive relationship with HbA1c levels. Hence, sCD163 appears to be associated with pathology and bacterial burdens in TB-DM and could serve as a biomarker for severity of disease and therapeutic responses following treatment.

C-Reactive protein is an acute-phase reactant synthesized by hepatocytes under the influence of interleukin-1 arising at sites of infection and inflammation<sup>39</sup> and it is also used as a biomarker for the detection of inflammation in various active infections.<sup>40,41</sup> CRP has been shown to be useful in the clinical evaluation of respiratory tract infections in adults.<sup>42,43</sup> We previously reported that pulmonary TB displayed heightened serum CRP levels compared with latent TB and extrapulmonary TB.<sup>44</sup> Previously published data also indicate that circulating CRP levels were significantly increased in active TB

disease compared with controls.<sup>45,46</sup> During *M. tuberculosis* infection, CRP levels are increased in TB patients starting ATT and decreased during the first 2 months of treatment, and these elevated CRP levels reflected higher AFB smear grades and correlated with disease severity.<sup>44,46</sup> Similar to earlier findings, our data recapitulate those results and show that CRP levels are present at significantly enhanced levels in TB-DM and TB compared with the other groups. In addition, as reported earlier in active TB, our findings also reveal that at baseline (pre-treatment), CRP levels were increased and diminished following the 2nd month of ATT in the TB-DM group. Finally, we found that CRP levels exhibited a positive correlation with HbA1c levels. This observation leads us to speculate that *M. tuberculosis*-driven inflammatory tissue damage may initiate strong induction of CRP production.

Activated monocytes in the circulation and tissue macrophages are a major source of tissue factor (TF).<sup>47</sup> Increased expression of TF by cells of the monocyte/macrophage lineage to the development and progression of local and systemic inflammatory reactions in many diseases.<sup>15,22</sup> Previously, it has been reported that *M. tuberculosis* induces TF expression in macrophages and *M. tuberculosis* signaling pathways that stimulate TF require the cooperation of multiple receptors and co-factors including Toll-like receptors.<sup>21</sup> Our results demonstrate that sTF in plasma is significantly elevated in TB-DM and DM compared with the other groups. In addition, before, during and after ATT, sTF levels remained elevated in TB-DM compared with TB. However, sTF did not exhibit any correlation with severity of disease and/or bacterial burdens or with HbA1c levels. Hence, sTF levels appear to be associated with active TB with or without DM, although not directly associated with either severity of disease or bacterial burden.

A previously published study from our group reported that there was a bimodal distribution of baseline HbA1c between KDM and NDM individuals in our study cohort, with significantly higher baseline HbA1c in the KDM group.<sup>48</sup> Our present study adds to this apparent heterogeneity in the presentation of TB-DM co-morbidity. We found significantly higher levels of sCD14 and sCD163 in KDM compared with NDM groups, suggesting increased severity of TB disease in KDM individuals. Metformin is the recommended first-line anti-hyperglycemic drug for the treatment of type 2 diabetes.<sup>49</sup> Recently published studies revealed a potential role of metformin in improving the effective treatment of TB and indicated that metformin is a promising candidate adjunctive therapy for individuals with TB.<sup>49</sup> A previously published study reports that metformin improves the chronic low-grade inflammatory state and also alters the macrophage polarization both *in vivo* and *in vitro*.<sup>50</sup> Our current findings provide additional evidence of a host-directed therapeutic effect for metformin based on decreased systemic levels of



sCD14, sCD163 and CRP. Also, as the non-metformin groups of TB-DM individuals were only on insulin or sulfonyl-urea, it would not result in any confounding effect. Also, the duration of diabetes was not significantly different between the two groups.

In summary, our study provides additional evidence for an adverse effect of poorly controlled type 2 DM on TB disease severity as reflected by increased monocyte activation. The data also add to the growing body of evidence indicating heightened immune activation in this immune-metabolic nexus. Longitudinal examination of monocyte markers demonstrated the evolution of these markers over the course of TB treatment and also provided evidence of unresolved inflammation at treatment completion in the TB-DM group. We identified a positive correlation of monocyte activation with HbA1c, suggesting that chronic hyperglycemia drives this complication of DM as is the case for microvascular and macrovascular complications. Finally, we describe an anti-inflammatory effect of metformin in TB that might relate to its reported disease-modifying effects in TB.

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## Author contributions

SB, HK and PKN designed the study; PKN, KM, YB and AN conducted experiments; PKN and KM acquired data; PKN, KM and SS analyzed data; VV, HK and SB contributed reagents and also revised subsequent drafts of the manuscript; VV, BSS and MN are responsible for the enrolment of participants and also contributed to acquisition and interpretation of clinical data; SB and PKN wrote the manuscript. All authors read and approved the final manuscript.

## Disclosures

The authors declare that they have no competing interests.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** No significant alterations in levels of monocyte activation markers in lung lesions, cavitory disease and bacterial burden in individuals with tuberculosis. (A) The plasma levels of sCD14, sCD163, C-reactive protein (CRP) and soluble tissue factor (sTF) were measured in TB individuals with bilateral versus unilateral disease. (B) The plasma levels of sCD14, sCD163, CRP and sTF were measured in individuals with TB with cavitory versus non-cavitory disease. (C) The relationship between the plasma levels of sCD14, sCD163, CRP and sTF and smear grades as estimated by sputum smears was examined in individuals with TB. The data are represented as scatter plots with each circle representing a single individual. *P* values were calculated using the Mann–Whitney test with Holm’s correction for multiple comparisons. For bacterial burden correlation, *P* values were calculated using the linear trend post-test.