Background

- Tuberculosis (TB) is the leading infectious killer worldwide with over 10.4 million incident cases and 1.7 million deaths in 2016.
- While culture conversion by 2 months of anti-tuberculosis treatment (ATT) is widely used as a surrogate marker for microbiological response, recent clinical trials have shown suboptimal performance of 2-month culture in predicting unfavorable treatment outcomes, particularly treatment failure.
- Novel biomarkers predictive of unfavorable treatment outcomes are needed for the early identification and risk-stratification of TB cases.
- The objective of this study was to identify systematic inflammatory markers associated with treatment failure in newly diagnosed adult pulmonary TB (PTB) cases in India.

Methods

Study population:
- We randomly selected 30 new adult (>18 years) drug-sensitive PTB cases within 1 week of ATT initiation from the ongoing CTRI-689 study in Pune and Chennai, India.
- Participants were prospectively evaluated at 0 weeks (≤7 days since ATT initiation), 8 weeks and 24 weeks for plasma concentrations of 20 cytokines linked to the host immune response in TB.
- Treatment failure was defined as Mycobacterium tuberculosis growth on liquid or solid culture between 17 and 24 weeks of ATT.

Cytokine analysis:
- Group A (Host immune response in TB): INF-γ, TNF-α, IL-1β, IL-6, IL-10, CXCL-10, IL-13, IL-12P70 and IL-17.
- Group B (Tissue destruction and fibrosis): MMP-1, MMP-3, MMP-7, TIMP-1, TIMP-2, TIMP-3, TGF-β1, TGF-β2 and TGF-β3.
- Cytokine concentrations were evaluated, in duplicates, using multiplex ELISA according to manufacturer’s protocols (BioRad Inc).

Statistical analysis:
- Cytokine concentrations were log-transformed and two-score normalized for analysis.
- Differentially expressed cytokines by duration of ATT and treatment failure were identified using non-parametric tests.
- P-values were adjusted for multiple comparisons using the Benjamini-Hochberg procedure and a 10% false-discovery rate.

Table 1. Baseline characteristics of study participants

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Cohort (n=30)</th>
<th>Sub-cohort (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (Q1-Q3)</td>
<td>40 (27-50)</td>
<td>36 (28-50)</td>
</tr>
<tr>
<td>Male, sex, n (%)</td>
<td>203 (64)</td>
<td>20 (74)</td>
</tr>
<tr>
<td>BMI (kg/m²), median (Q1-Q3)</td>
<td>18 (16-20)</td>
<td>18 (16-20)</td>
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<tr>
<td>Ever-smoking, n (%)</td>
<td>124 (9)</td>
<td>31 (9)</td>
</tr>
<tr>
<td>HIV co-infection, n (%)</td>
<td>19 (6)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>88 (28)</td>
<td>7 (26)</td>
</tr>
<tr>
<td>Cautivation, n (%)</td>
<td>122 (45)</td>
<td>10 (43)</td>
</tr>
<tr>
<td>Treatment outcomes, n (%)</td>
<td>35 (12)</td>
<td>4 (14)</td>
</tr>
<tr>
<td>Failure</td>
<td>14 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Recurrence</td>
<td>6 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
- No significant differences between those selected for inflammatory analysis compared to those not selected but part of the full cohort.
- Notable exception being the absence of recurrence and deaths.

Discussion

Key findings:
- TNF-α and TNF-β were overexpressed at week 8 and week 24 following ATT initiation, respectively.
- Relative to week 0 concentrations: IL-1β, IL-6 and MMP-7 concentrations declined at week 8; CXCL-10, TGF-β1, TGF-β2, TIMP-1 and TIMP-3 concentrations declined by week 24 and TGF-β3 concentrations declined at both week 8 and week 24.
- None of the participants who failed treatment had HIV coinfection or diabetes.
- Participants who failed treatment had significantly higher plasma concentrations of IL-6, IL-13 and IFN-γ at ATT initiation compared to those who were cured, however this difference was not statistically significant at 8 and 24 weeks of ATT.

Limitations:
- Limited sample size of n=4 failures.
- We could not identify cytokines associated with recurrence or death.

Future direction:
- We plan to conduct well powered validation studies measuring the performance of IL-6, IL-13 and IFN-γ as predictive markers for unfavorable treatment outcomes and lung injury.

Conclusion

- Overexpression of circulating IL-6, IL-13 and IFN-γ at treatment initiation may be associated with treatment failure among drug-susceptible pulmonary tuberculosis cases.
- Well-powered validation studies should be undertaken to evaluate the performance of these biomarkers, individually or in combination, for predicting unfavorable tuberculosis treatment outcomes.

References