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Immunological Aspects

Application of multiplexed ion mobility spectrometry towards the identification of host protein signatures of treatment effect in pulmonary tuberculosis

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ABSTRACT

Rationale: The monitoring of TB treatments in clinical practice and clinical trials relies on traditional sputum-based culture status indicators at specific time points. Accurate, predictive, blood-based protein markers would provide a simpler and more informative view of patient health and response to treatment.

Objective: We utilized sensitive, high throughput multiplexed ion mobility-mass spectrometry (IM-MS) to characterize the serum proteome of TB patients at the start of and at 8 weeks of rifamycin-based treatment. We sought to identify treatment specific signatures within patients as well as correlate the proteome signatures to various clinical markers of treatment efficacy.

Methods: Serum samples were collected from 289 subjects enrolled in CDC TB Trials Consortium Study 29 at time of enrollment and at the end of the intensive phase (after 40 doses of TB treatment). Serum proteins were immunoaffinity-depleted of high abundant components, digested to peptides and analyzed for data acquisition utilizing a unique liquid chromatography IM-MS platform (LC-IM-MS). Linear mixed models were utilized to identify serum protein changes in the host response to antibiotic treatment as well as correlations with culture status end points.

Results: A total of 10,137 peptides corresponding to 872 proteins were identified, quantified, and used for statistical analysis across the longitudinal patient cohort. In response to TB treatment, 244 proteins were significantly altered. Pathway/network comparisons helped visualize the interconnected proteins, identifying up regulated (lipid transport, coagulation cascade, endopeptidase activity) and down regulated (acute phase) processes and pathways in addition to other cross regulated networks (inflammation, cell adhesion, extracellular matrix). Detection of possible lung injury serum proteins such as HPSE, significantly downregulated upon treatment. Analyses of microbiologic data over time identified a core set of serum proteins (TTHY, AFAM, CRP, RET4, SAA1, PGRP2) which change in response to treatment and also strongly correlate with culture status. A similar set of proteins at baseline were found to be predictive of week 6 and 8 culture status.

Conclusion: A comprehensive host serum protein dataset reflective of TB treatment effect is defined. A repeating set of serum proteins (TTHY, AFAM, CRP, RET4, SAA1, PGRP2, among others) were found to change significantly in response to treatment, to strongly correlate with culture status, and at baseline to be predictive of future culture conversion. If validated in cohorts with long term follow-up to capture failure and relapse of TB, these protein markers could be developed for monitoring of treatment in clinical trials and in patient care.

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1. Introduction

Tuberculosis (TB) is the leading cause of death from a single infectious agent [1]. According to the WHO, an estimated 10.4 million people fell ill with TB in 2016 with most of the incidents concentrated in developing countries [1]. Sputum-based culture remains the gold standard for monitoring response to treatment in clinical practice as well as in clinical trials [2–4]. Despite its long history in TB drug development, 8-week culture status and culture conversion as a measure of bacterial burden has limitations. First, the reliance on sputum is limiting given sputum production decreases rapidly on treatment. Additionally, analyses of performance of treatment culture conversion for predicting long term outcomes have shown variable results [5–7]. As a consequence, there has been a call for the development of new quantitative, non-sputum, nonculture-based TB biomarkers. If proven accurate and predictive, a blood-based biomarker signature would facilitate middle and phase 2 development for new TB drugs and regimens, as well as provide information on individual response to treatment.

Recent technologic advances in mass spectrometry (MS) based proteomics now provide multiple, robust tools that allow for high-accuracy, high-throughput analysis of clinical samples [8,9]. As a result, a better depth of coverage with accurate quantification can be obtained for a global proteome in complex clinical specimens. To date, discovery-based proteomics has primarily concentrated on investigating protein biomarkers of infection for early diagnosis of TB [10]. Early surface-enhanced laser desorption/ionization (SELDI) platforms compared serum samples from active TB patients with uninfected controls to identify possible discriminatory protein profiles, with recent studies involving a more in-depth separation [11,12]. Additionally, several liquid chromatography-mass spectrometry (LC-MS/MS) based platforms have been used as well to perform this comparison, providing a complimentary list of differentiated host proteins [13,14]. Achkar et al., presented a more comprehensive diagnostic study using LC-MS/MS and multiple-reaction monitoring (MRM) based assay to identify panels of host proteins that can distinguish active TB from latent infections as well as other respiratory disorders [15]. Longitudinal studies of TB treatment and response have also been conducted using aptamer array-based methods applied to small cohorts of patients [16–18].

In the present study we evaluate treatment response through the use of a sensitive MS platform, with the addition of ion mobility separations (IM) to an LC-MS platform, providing a more robust and higher throughput discovery analysis pipeline capable of larger scale clinical study investigations [19]. We assayed a large collection of serum samples collected at baseline and after 8 weeks of treatment from participants enrolled in a phase 2 clinical trial comparing rifapentine to rifampin, as part of combination therapy [20]. In this study, we sought to provide an unbiased characterization of the serum proteome in TB patients undergoing treatment, evaluating associations with clinical and microbiologic characteristics, comparing changes in protein biomarkers relative to culture status at time points 2, 4, 6 and 8 weeks during treatment, with the objective of identifying a serum protein signature indicative of treatment effect.

2. Materials and methods

2.1. Study population

CDC TB Trials Consortium (TBTC) Study 29 (ClinicalTrials.gov Identifier NCT00694629) is a prospective, multicenter, open-label Phase 2 clinical trial comparing the antimicrobial activity and safety of standard daily regimen containing rifampin, to that of the experimental regimen with daily rifapentine (10 mg/kg/dose), both given with isoniazid, pyrazinamide and ethambutol to adults with smear positive, culture-confirmed pulmonary TB. The primary efficacy endpoint of the trial was the proportion of patients, by regimen, having negative sputum cultures at completion of 8 weeks (40 doses) of treatment. All TB treatment was administered 5 days/week for 8 weeks and directly observed. All participants underwent HIV testing. Cultures were performed using both Lowenstein-Jensen (LJ) solid media (inoculum volume, 0.2 mL) and BACTEC mycobacterial growth indicator tube (MGIT, Becton Dickinson and Co, Franklin Lakes, New Jersey) liquid media (inoculum volume, 0.5 mL) with the MGIT 960 system, and assessed for presence of *M. tuberculosis*. Additional information regarding the design, conduct, and results of TBTC Study 29 has been published [20].

2.2. Selection of participants

Out of a total of 531 participants in the parent trial, we included 289 consecutively enrolled protocol-correct participants from CDC-TB Trials Consortium clinical trials sites in North America, South Africa, Uganda, and Spain, for this sub-study. The 289 participants included in the proteomic analyses had sputum smears positive for acid fast bacilli at baseline and were culture positive for drug-susceptible pulmonary TB. Detailed demographic, clinical, radiographic and microbiologic data were collected using CDC-TBTC developed case report forms as part of the parent clinical trial.

2.3. Sample processing

Serum was collected, processed and stored at baseline (time of enrollment), and after 8 weeks (40 doses) as previously described [20], in a standardized manner according to a manual of operating procedures. The investigators conducting LC-IM-MS assays were blinded to all patient characteristics until after assay results were submitted to the TBTC Data and Coordinating Center. Individual human serum samples were partitioned and depleted of 14 specific highly abundant proteins using a ProteomeLab™ 12.7 × 79.0-mm human IgY14 LC10 affinity LC column (Beckman Coulter, Fullerton, CA), which was performed using the manufacturer's instructions to disrupt protein-protein binding and capture of additional proteins. Further processed of the flow-through portion of the depletion included automated protein isolation, denaturation, tryptic digestion, and peptide isolation [19].

2.4. LC-IM-MS analysis

Analysis of processed serum samples was performed on an in-house built instrument that couples a 1-m ion mobility separation with an Agilent 6224 TOF MS upgraded to a 1.5 m flight tube [19] coupled with LC and including a fully automated in-house built 4-column HPLC system equipped with in-house packed capillary columns [21]. A 60-min gradient with shorter columns (30 cm long with same dimensions and packing) was used with the IM-MS platform and data were collected from 100 to 3200 m/*z*. This unique platform has been previously described in detail [19,22], and has been optimized for higher throughput larger scale clinical sample analysis as it provides the benefit of the inclusion of an orthogonal ion mobility separations for depth of coverage coupled with a reduced LC separations component for faster overall analysis times (60 min per sample).

2.5. Data processing

Identification and quantification of the detected peptide peaks was performed utilizing the Accurate Mass and Time (AMT) tag approach [23]. Briefly, three pooled human serum samples were created (baseline, 8 weeks negative culture status, 8 weeks positive culture status) and divided into 24 fractions each using HPLC high pH fractionation. LC-MS/MS was performed on each fraction using the LTQ Orbitrap Velos MS and a reverse phase low pH LC separation [19]. A subsequent AMT tag database was created as previously described [24,25]. All MS/MS data can be obtained at ProteomeXchange under identifier PXD009029. Multiple in-house developed informatics tools (publicly