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Research Paper

Biomarkers of Tuberculosis Severity and Treatment Effect: A Directed Screen of 70 Host Markers in a Randomized Clinical Trial

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ABSTRACT

More efficacious treatment regimens are needed for tuberculosis, however, drug development is impeded by a lack of reliable biomarkers of disease severity and of treatment effect. We conducted a directed screen of host biomarkers in participants enrolled in a tuberculosis clinical trial to address this need. Serum samples from 319 protocol-correct, culture-confirmed pulmonary tuberculosis patients treated under direct observation as part of an international, phase 2 trial were screened for 70 markers of infection, inflammation, and metabolism. Biomarker assays were specifically developed for this study and quantified using a novel, multiplexed electrochemiluminescence assay. We evaluated the association of biomarkers with baseline characteristics, as well as with detailed microbiologic data, using Bonferroni-adjusted, linear regression models. Across numerous analyses, seven proteins, SAA1, PCT, IL-1β, IL-6, CRP, PTX-3 and MMP-8, showed recurring strong associations with markers of baseline disease severity, smear grade and cavitation; were strongly modulated by tuberculosis treatment; and had responses that were greater for patients who culture-converted at 8 weeks. With treatment, all proteins decreased, except for osteocalcin, MCP-1 and MCP-4, which significantly increased. Several previously reported putative tuberculosis-associated biomarkers (HOMX1, neopterin, and cathelicidin) were not significantly associated with treatment response. In conclusion, across a geographically diverse and large population of tuberculosis patients enrolled in a clinical trial, several previously reported putative biomarkers were not significantly associated with treatment response, however, seven proteins had recurring strong associations with baseline radiographic and microbiologic measures of disease severity, as well as with early treatment response, deserving additional study.

1. Introduction

The lack of reliable surrogate markers of efficacy has hampered tuberculosis (TB) drug development. The current standard for use as a surrogate endpoint in phase 2 studies remains focused on culture conversion and the most studied is the two month culture status, which has low sensitivity and modest specificity for predicting outcomes after treatment completion.(Horne et al., 2010; Phillips et al., 2016; Phillips et al., 2013; Nahid et al., 2011) Additionally, being dependent on sputum, culture-based monitoring can be challenging to use in extrapulmonary TB, and in patients with paucibacillary disease such as is seen in HIV-coinfected patients and in children.(Wallis et al., 2013; Zumla et al., 2013) Both sputum volume and quality decreases in response to treatment and many patients cannot provide sputum samples for culture after a few weeks of treatment. The development of non-sputum-based biomarkers of treatment response would represent an advance for individual monitoring of TB patients as well as serving as an intermediate marker for use in TB drug development.

As an alternative to sputum-based monitoring, blood-based biomarkers are appealing for several reasons. Blood is relatively easy to collect and, unlike sputum, remains an available source for biomarker measures throughout treatment. Blood-based markers of inflammation and infections are also quantitative, and provide an opportunity to improve predictive power by combining multiple biomarkers into predictive biosignatures. Finally, blood-based markers of treatment response

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could potentially be translated into point of care assays usable in the field without requiring sophisticated laboratory infrastructure, and biosignatures that at baseline could determine disease severity would also be valuable to clinical trialists and TB care providers for risk stratification purposes, as alternatives to chest radiography, for example. Efforts to identify host biomarkers predictive of treatment outcomes have resulted in the identification of a number of biomarkers that change during TB treatment, albeit most are described in observational cohorts or smaller case-control studies.(Andrade et al., 2013; Azzurri et al., 2005; Coussens et al., 2012; Djoba Siawaya et al., 2009; Djoba Siawaya et al., 2008; Huang et al., 2014; Jayakumar et al., 2015; Lee and Chang, 2003; Mihret et al., 2013; Ostrowski et al., 2006) Such studies acknowledge a variety of limitations including being single center studies, focusing on single markers, using convenience samples, having modest sample sizes, or relying on case-control designs.

In the work described herein, serum collected in a standardized manner in a rigorously conducted clinical trial sponsored by the CDC-funded TB Trials Consortium, was used to evaluate the efficacy and safety of a rifapentine-based regimen for drug-susceptible TB (Dorman et al., 2012) Study participants received treatment under direct observation, thereby enhancing and carefully measuring adherence; an important benchmark when assessing biomarkers of treatment effect. We measured the concentration, and change in concentration, of 70 potential biomarkers associated with inflammatory, antimicrobial, T-cell and acute phase responses to bacterial infections, and with tissue remodeling at infection sites. These biomarkers were selected because they have been published as indicators of TB disease and the clinical trial samples provided an opportunity to reassess these associations across diverse international sites (Andrade et al., 2013; Azzurri et al., 2005; Coussens et al., 2012; Djoba Siawaya et al., 2009; Djoba Siawaya et al., 2008; Huang et al., 2014; Jayakumar et al., 2015; Lee and Chang, 2003). Access to clinical trial-quality data allowed us to account for changes in biomarker levels across disease phenotypes, regimens, and geographic regions, in order to identify biomarkers associated with treatment response.

2. Methods

2.1. Study Design and Population

The parent study was a CDC-sponsored clinical trial, Tuberculosis Trials Consortium (TBTC) Study 29 (ClinicalTrials.gov Identifier NCT00694629). This was a randomized phase 2 trial, comparing the antimicrobial activity and safety of a standard daily regimen containing rifampin, to that of the experimental regimen with daily rifapentine (10 mg/kg), both given with isoniazid, pyrazinamide and ethambutol to adults with smear-positive, culture-confirmed pulmonary TB. All TB treatment was administered 5 days/week and directly observed. All participants underwent HIV testing. Information regarding the design, conduct, and results of TBTC Study 29 has been published (Dorman et al., 2012). Out of a total of 531 participants in the parent study, 389 consecutively enrolled protocol-correct participants (rather than the modified intention-to-treat population, since adherence may not be optimal in this population) were included in this biomarker study. Of 389 participants, 319 had paired baseline and week 8 serum samples available for biomarker testing. The parent trial excluded patients if they had received >5 days of TB treatment in the preceding 6 months, however, treatment of <5 days was permissible, and was noted in 60% of study participants; the median number of days of treatment prior to enrollment was 2 days, (IQR of 0 to 4 days). Detailed clinical, radiographic and microbiologic data including sputum culture status at 8 weeks and 12 weeks (determined on both liquid and solid media) were collected in a standardized manner as part of the parent clinical trial and used in biomarker analyses. Written informed consent was obtained from all study participants for collection of serum for TB-related research. In addition, the institutional review board at University of California, San Francisco (UCSF) approved this ancillary study to assess putative biomarkers of treatment response (approval #12-10360).

2.2. Specimen Collection

Blood was collected at enrollment (baseline), and after 8 weeks of combination drug therapy, using Becton Dickinson Serum Separator Tubes (BD Vacutainer® SST™ Tube, BD Diagnostics, Franklin Lakes, NJ, USA). BD Vacutainer® SST™ Tubes were centrifuged within 2 h of collection and processed according to manufacturer recommendations. Collection, processing and storage of sera was conducted according to a standardized manual of operating procedures that has been confirmed to provide quality samples free of processing errors (Nahid et al., 2014). Serum was aliquoted on site, frozen at −70 °C, and batch-shipped on dry ice.

2.3. Multiplexed Immunoassays

A total of 70 biomarkers were measured in 14 multiplexed assay panels using a sandwich immunoassay format (proteins) or a competitive immunoassay format (neopterin), using electrochemiluminescence (ECL) detection (Debad et al., 2004). The ECL assays employed consumables and instrumentation from Meso Scale Diagnostics, LLC (MSD). The assay components for each panel included a MSD MULTI-ARRAY® 96-well plate having an array of capture antibodies in each well, a set of labeled detection antibodies for each analyte in the panel (labeled with the MSD SULFO-TAG™ ECL label), a combined calibration standard containing a mixture of the target analytes, an assay diluent and a detection antibody diluent. For the neopterin competitive assay, a labeled neopterin analog was used in the place of the labeled detection antibody. In total, 14 biomarker panel assays were tested, six MSD commercial kits and eight custom assay panels that were newly developed for this study (see Supplemental materials on Assay Methods).

2.4. Clinical Sample Tests

MSD received 500 μL of each sample at their core facility (Gaithersburg, MD) where assays were conducted with investigators and technicians blinded to participant data. Each sample was tested in duplicate with each of the 70 assays. Concentrations were reported as the average value of the duplicate measurements; values below the LOD were assigned a concentration equal to the LOD. CVs were determined for the biomarker levels measured in the control samples run on each assay plate; the median control CV (and IQR) across the different assays was 10% (9%–13%).

2.5. Statistical Methods

Statistical analysis was conducted using the R statistical programming language (version 3.2.3). Analysis of biomarkers used $\log_{10}$-transformed concentrations. The Student's *t*-test was used for comparing means across groups. Linear regression (using the "lm" function in R) was used to determine the association of biomarker concentrations with clinical variables and to adjust for potential covariates. The two-sided t-statistic was used to determine the significance of regression coefficients with a threshold of $p < 0.05$ taken as statistically significant. To account for multiple testing, we performed a Bonferroni correction for the number of tests applied in each analysis.

Linear regression was used to identify associations of $\log_{10}$-transformed baseline biomarker levels with baseline clinical indicators of disease severity at baseline: smear grade (grade = 1 vs. grade ≥ 2), chest radiograph status (no cavities vs cavities; cavities ≤ 4 cm vs cavities >4 cm; and extent of lung involvement < 50% vs lung involvement > 50%) and MGIT time-to-detection (≤5 days vs >5 days). Two linear models were employed: (i) an unadjusted model and (ii) a model that adjusted for potential demographic and clinical covariates (gender,

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