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DIAGNOSTICS

Serum biomarkers of treatment response within a randomized clinical trial for pulmonary tuberculosis

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SUMMARY

Rationale: Biomarkers for monitoring response to anti-tuberculosis treatment are needed. We explored immune markers previously published as having predictive capability for 8 week culture status in 39 adults enrolled in a clinical trial in Kampala, Uganda.
Methods: We consecutively selected 20 HIV-negative pulmonary TB subjects with positive cultures, and 19 subjects with negative cultures at the end of intensive phase therapy. At baseline and after 8 weeks, serum was assayed for nine cytokines and soluble cytokine receptors using multiplexed platforms or ELISA. We evaluated their association with week 8 culture status first using single-variable logistic models, then using cross-validated estimates of the C-statistic, a measure of discrimination, of candidate models including 2 or 3 analytes in addition to age.
Results: All but one analyte decreased from baseline to week 8 (all $p < 0.01$). Individual biomarkers were not associated with 8 week culture status. Logistic models including increasing age, higher baseline soluble tumor necrosis factor receptor alpha 1 (sTNF-R1), and higher week 8 C-reactive protein (CRP) concentration classified subjects by culture status with up to 85% accuracy and acceptable discrimination (cross-validated C-statistic 0.76) and calibration (Hosmer–Lemeshow $P > 0.2$).
Conclusion: Exploratory post-hoc models including sTNF-R1, CRP, and age, classified 8 week culture status with promising accuracy.

1. Introduction

There is renewed interest in shortening the duration of TB treatment, currently six months for drug susceptible TB and up to 24 months for multidrug resistant TB (ref ATS statement and WHO MDR guideline), and several new drugs are in preclinical and clinical evaluation [1]. However, efficiently evaluating the efficacy of these new agents remains challenging. Testing the efficacy of new drugs and drug combinations currently relies on phase 2 trial designs that use *Mycobacterium tuberculosis* (MTB) sputum culture status (positive or negative) on solid or liquid media at week 8 of treatment. Using dichotomous endpoints in phase 2 clinical trials is inefficient, and it necessitates large sample sizes [2]. Moreover, culture-based techniques depend upon the ability of patients to produce representative sputum throughout treatment, often a challenge after several weeks of therapy [3].

Several blood-based candidate markers for monitoring TB treatment response have shown promise in prior studies on the

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bases of high sensitivity, correlation with disease severity, and decline on treatment [4,5]. We re-evaluated nine putative serum markers previously published in small cohort studies to show association with treatment effect, in participants randomized in a Phase 2 clinical trial, who underwent rigorous, standardized collection of clinical, radiographic, and microbiologic data and additionally received directly observed therapy throughout the intensive phase of treatment.

The nine candidate markers are all nonspecific indicators of inflammation: neopterin, granzyme B, C-reactive protein (CRP), soluble Intercellular Adhesion Molecule 1 (sICAM-1), soluble urokinase plasminogen activator receptor (suPAR), interferon-gamma inducible protein (IP-10), soluble interleukin-2 receptor alpha (sIL-2Rα), soluble tumor necrosis factor alpha receptor 1 (sTNF-R1), and soluble tumor necrosis factor alpha receptor 2 (sTNF-R2). Neopterin is synthesized by macrophages in response to stimulation by IFN-γ, and serves as a marker of TH1-mediated immune activation. It is elevated in body fluids of patients with TB, and correlates with disease extent and activity [6–11]. Granzyme B is a serine protease that mediates apoptosis of infected cells, and has been utilized in multivariable models to predict response to anti-TB treatment [12]. CRP is a nonspecific acute phase reactant produced by the liver that opsonizes bacterial pathogens, binds to macrophages, and promotes phagocytosis. Multiple studies have reported that CRP is elevated at TB diagnosis, is correlated with severity of disease and sputum bacillary load, and decreases during treatment [13–19]. sICAM-1 is a cellular adhesion molecule which may play a role in maintaining a pro-inflammatory environment, and is elevated in active TB. It has also been included in models for prediction of TB treatment response [19–22]. suPAR is a receptor produced by macrophages and monocytes. It is elevated in active TB and correlates with sputum bacillary burden [23]. IP-10 is a monocyte and T-cell-derived chemokine that holds promise as a biomarker for both active and latent TB [24–27]. sIL-2rα is expressed by activated T lymphocytes, and it correlated with response to anti-TB therapy in one study [28]. TNF receptors sTNF-R1 and sTNF-R2 have been shown in other models to serve as markers of innate and adaptive immunity. Levels of sTNF-R1 are increased in active TB, and sTNF-R2 polymorphisms have been implicated in susceptibility to TB [29–31].

We describe the kinetics of change of these markers during treatment, and we constructed a biomarker-based classifier using multiple markers in combination with clinical characteristics that were associated with response to treatment.

2. Materials and methods

2.1. Ethics statement

The parent study was a CDC-sponsored clinical trial, TBTC Study 29 (ClinicalTrials.gov Identifier NCT00694629). It was approved by both CDC and local institutional review boards. 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.

2.2. Study population and setting

TBTC Study 29, a randomized, phase 2 clinical trial, compared 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. Information regarding the design, conduct, and results of TBTC Study 29 has been published [32].

2.3. Selection of participants and clinical, radiographic and microbiologic features

The cohort for this biomarker study was composed of adult (age $\geq$ 18) patients with culture-confirmed pulmonary TB enrolled in Kampala, Uganda, as part of TBTC Study 29. Out of a total of 531 participants in the parent study, the first 40 consecutively enrolled participants who met the following criteria were selected for this study based on feasibility and previously published sample sizes in the literature: HIV-uninfected, 40 uninterrupted doses of intensive phase TB therapy received by week 8, and adequate serum and requisite culture data available through week 8. In addition, the participants were systematically selected such that after 8 weeks of treatment, 20 were culture negative on both solid and liquid (Mycobacterial Growth Indicator Tube, MGIT 960 system, BD, Sparks, MD) media, and 20 were culture positive on either solid or liquid media (15 were positive on liquid media only, 1 on solid media only, and 4 on both). We report on 39 patients, as subsequent quality checks on data uncovered an error in the retrieval of serum samples from the repository for one patient. Of the 39 patients included in the analysis, 37 had drug-susceptible and two had drug-resistant TB (one resistant to isoniazid and rifampin, one resistant to rifampin and streptomycin). Detailed radiographic and microbiologic data were collected in a standardized manner as part of the parent clinical trial. Laboratory technicians who conducted the assays were blinded to all patient characteristics until after assay results were submitted to the TBTC Data and Coordinating Center.

2.4. Serum collection, processing and storage

Blood was collected before anti-TB treatment (baseline), and after 8 weeks (40 doses) 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. All sites conducted collection, processing and storage of sera according to a standardized manual of operating procedures that has been confirmed to provide quality samples free of processing errors [33]. Serum was aliquoted (minimum of 500ul volume) into 1.2 ml cryovials on site in Kampala, Uganda, then frozen at −70 °C.

2.5. Immunoassays

All analytes were measured at UCSF, at the MTB Research Laboratory and the Core Immunology Laboratory at San Francisco General Hospital. Soluble receptor analytes (sIL-2Rα, sTNF-R1, and sTNF-R2) were measured on the Luminex 100 multiplex platform (Millipore Corporation, Billerica, MA, USA), with kits obtained from Millipore Corporation. Levels of neopterin (Immuno-Biological Laboratories, Inc., Minneapolis, MN, USA), Granzyme B (Bender MedSystems, San Diego, CA, USA), sICAM-1 (R&D Systems, Minneapolis, MN, USA), IP-10 (R&D Systems, Minneapolis, MN, USA), and suPAR (ViroGates, Birkerød, Denmark) were measured using enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions. CRP levels were measured using a

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