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DRUG DISCOVERY AND RESISTANCE

Pre-treatment mycobacterial sputum load influences individual on-treatment measurements

Andreas H. Diacon [a, b, *], Lize van der Merwe [c, e], Anne-Marie Demers [a], Florian Von Groote-Bidlingmaier [b], Amour Venter [c], Peter R. Donald [d]

[a] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa
[b] Task Applied Science, Bellville, Cape Town, South Africa
[c] MRC Centre for Molecular and Cellular Biology, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa
[d] Department of Paediatrics and Child Health, Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa
[e] Department of Statistics, Faculty of Natural Sciences, University of the Western Cape, Cape Town, South Africa

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SUMMARY

Time to culture positivity (TTP) in liquid medium is now widely available as a measure of viable mycobacterial sputum load. TTP correlates well with and could replace colony-forming unit (CFU) counting in studies of antituberculosis drug effects. We investigated the influence of the pre-treatment mycobacterial sputum load on 4428 CFU measurements obtained within the first 14 days of treatment. Using a prediction model we show that pre-treatment CFU counts contribute 29% to the variation of on-treatment CFU counts and increase the precision of the prediction of on-treatment CFU from TTP by 12%. On the other hand, pre-treatment TTP contributed only 12% to the variation of on-treatment TTP and only added 2% to the prediction of TTP from CFU. We conclude pre-treatment measurements are covariates that can enhance the accuracy of statistical estimates of treatment effects, particularly when measured by CFU counts.

1. Introduction

Colony forming unit (CFU) counts of *Mycobacterium tuberculosis* and time to positive (TTP) culture in liquid medium are regularly employed measurements of sputum mycobacterial load. Antituberculosis treatment effects can be estimated by comparing measurements obtained under treatment to measurements collected before treatment [1–3]. At present smears graded at least 1+ on the WHO/IUATLD scale are required for participation in studies of early antituberculosis treatment effects. This maximizes the chance for positive sputum cultures over the time needed to detect and quantify treatment activities.

It is generally accepted that sputum contains variable proportions of mycobacterial subpopulations with a spectrum of metabolic activity, and that different drugs target specific subpopulations [4,5]. A predominant population of rapidly metabolizing, extracellular mycobacteria is believed to determine the magnitude of CFU and TTP before and during the early stages of treatment [6,7]. This creates a potentially significant but underappreciated source of error in the measurement of treatment effects. The standard requirement of "sputum smear grade ≥1+" reflects a more than 100-fold range from the lowest to the highest possible pre-treatment sputum mycobacterial burden in study subjects. An abundance of metabolically highly active mycobacteria in a subject, for instance, might favour any agent targeting this bacterial phenotype but underestimate the activity of agents that target other bacterial phenotypes. The pre-treatment mycobacterial load could thus be an independent predictor for drug activity measured by a fall in CFU or increase of TTP over time [8,9].

This problem has been largely ignored in antimycobacterial activity studies because culture-based measurements of load become available too late to be prospectively controlled for. At present it is uncertain whether the pre-treatment load influences quantitative estimates of drug activity. To investigate this question we constructed prediction models for CFU and explored whether pre-treatment CFU and pre-treatment TTP values alter the model's prediction of CFU counts obtained within the first 14 days of treatment, ignoring individual patient level factors and the treatment received in order to focus on what percentage of the variation

* Corresponding author. Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 19063, 7505 Tygerberg, South Africa. Tel.: +27 21 917 1044; fax: +27 21 938 9476.
E-mail address: ahd@sun.ac.za (A.H. Diacon).

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of on-treatment measurements can be explained by the pre-treatment values.

2. Methods

2.1. Microbiology

The methodology for determining CFU counts and TTP has been described in detail previously [2]. For all measurements sputum is collected over 16 h overnight from subjects with ≥1+ smear-positive (IUATLD scale) pulmonary tuberculosis hospitalised in a research ward. For CFU counting sputum is homogenised by addition of N-acetylcystein and magnetic stirring to create a mixture from which a range of 10-fold dilutions is inoculated onto selective agar plates in quadruplicate. After 3–4 weeks incubation visible colonies are counted and expressed as $\log_{10}$ CFU/mL sputum. For TTP in liquid culture, sputum is decontaminated with sodium hydroxide and inoculated into duplicate liquid culture tubes. Cultures are automatically flagged when positive and the result recorded as TTP in hours. For each sample mean $\log_{10}$ CFU and mean TTP are calculated from up to four CFU counts and two TTPs, respectively. We studied consecutive EBA studies conducted from 2005 to 2012 with 27 experimental or control treatments of up to 14 days duration. A total of 4428 sputum specimens were available from 473 patients that were susceptible to the treatment given and had paired CFU and TTP result from at least one sputum collected at baseline (pre-treatment) and after start of drug therapy (on-treatment).

2.2. Statistics

We investigated linear models of on-treatment $\log_{10}$ CFU as a function of the predictors on-treatment TTP, day of sputum collection, baseline TTP (TTP.BL) and baseline $\log_{10}$ CFU ($\log_{10}$ CFU.BL), and vice versa for on-treatment TTP, ignoring all other individual patient level factors. We experimented with several transformations of these predictors, such as $\log_{10}$ and square root. These were considered by visual inspection and the transformation giving the

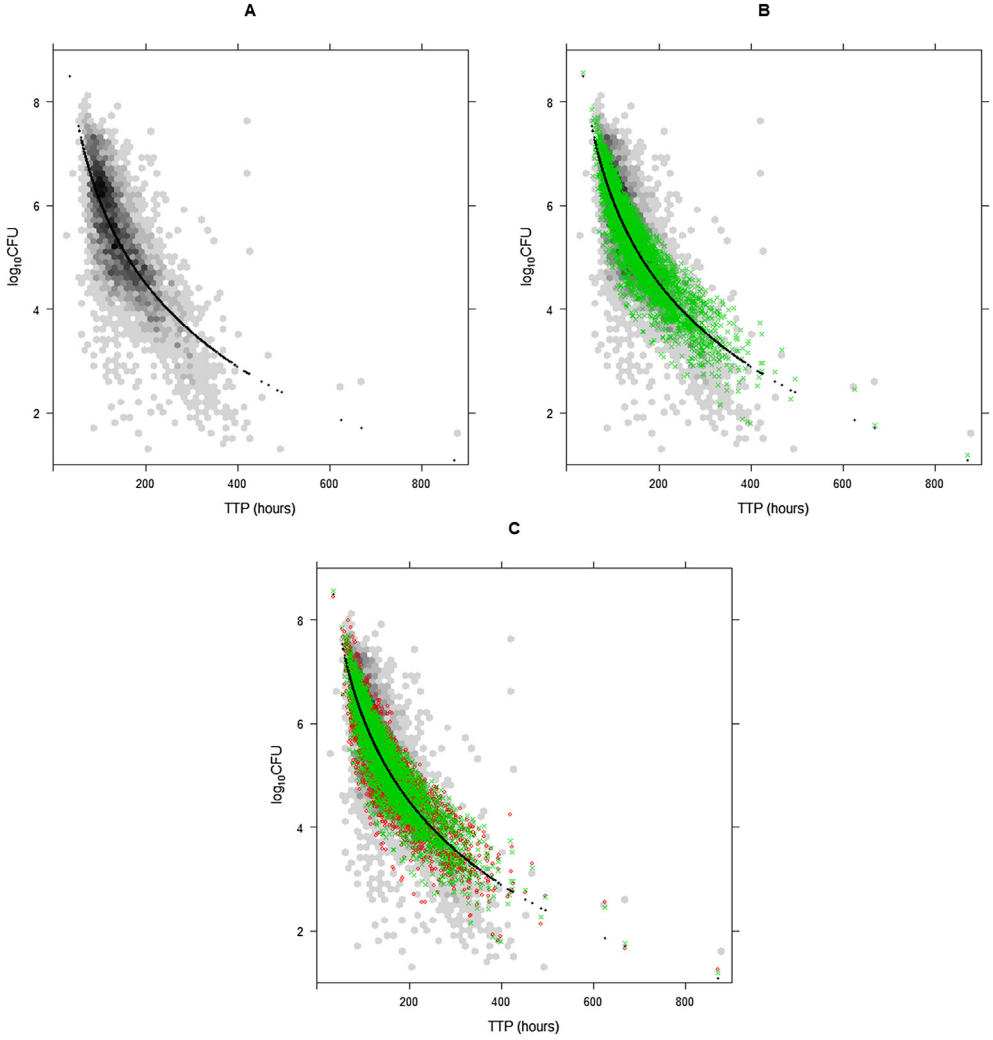

Figure 1. Observed and estimated $\log_{10}$ CFU versus TTP. The panels show improved predictions of observed $\log_{10}$ CFU with an increasing number of predictors. Panel A predictor: TTP. The observed on-treatment $\log_{10}$ CFU (perfect prediction) are shown as hexagons (hexbins) versus TTP. Darker colour of hexagons indicates a higher number of measurements. The prediction line with TTP is overlaid in black. Panel B predictors: TTP and pre-treatment $\log_{10}$ CFU. The predicted area is overlaid in green symbols (×) that move away from the simplest prediction line from panel A. The coverage of the dark area demonstrates a large improvement in prediction with pre-treatment $\log_{10}$ CFU. Panel C predictors: TTP, pre-treatment $\log_{10}$ CFU, pre-treatment TTP and treatment day. The additional prediction is overlaid in red symbols (○). The covered area still spreads away from the simplest prediction line but the increase in coverage is much smaller than from panel A to panel B. CFU = colony forming units, TTP = time to positivity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)