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Quantitative assessment of the activity of antituberculosis drugs and regimens

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Abstract

Introduction: Identification of optimal drug doses and drug combinations is crucial for optimized treatment of tuberculosis.

Areas covered: An unprecedented level of research activity involving multiple approaches is seeking to improve tuberculosis treatment. This report is a review of the quantitative methods currently used on clinical data sets to identify drug exposure targets and optimal drug combinations for tuberculosis treatment. A high-level summary of the methods, including the strengths and weaknesses of each method and potential methodological improvements is presented. Methods incorporating data generated from multiple sources such as *in vitro* and clinical studies, and their potential to provide better estimates of pharmacokinetic/pharmacodynamic (PK/PD) targets, are discussed. PK/PD relationships identified are compared between different studies and data analysis methods.

Expert opinion: The relationships between drug exposures and tuberculosis treatment outcomes are complex and require analytical methods capable of handling the multidimensional nature of the relationships. The choice of a method is guided by its complexity, interpretability of results, and type of data available.

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Declaration of interest

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Keywords

Tuberculosis; Pharmacokinetic; Pharmacodynamic; Machine learning; Classification and regression trees; Random forests; Multivariate adaptive regression splines

1. Introduction

Research exploring optimal drug doses and combinations for tuberculosis treatment has expanded enormously in recent years. Approaches used to identify pharmacokinetic/pharmacodynamic (PK/PD) targets include *in vitro* experiments, studies in animals and clinical studies, as well as integrated approaches combining different types of data together with certain assumptions in computational models. Promising systems pharmacology methods have been used to link the pharmacokinetics of antituberculosis drugs in plasma, the spatial and temporal distribution of drug concentrations in granulomas, and *in vitro* drug activity [1]. Similarly, systemic concentrations have been linked to patient TB cavity pharmacokinetics which were shown to be associated with resistance emergence at the same exposures identified as resistance-amplifying in hollow fiber PK/PD models [2]. Such models have also integrated the *in vitro* pharmacodynamic interactions between multiple drugs in a regimen, their respective pharmacokinetic distributions, and multi-state models accounting for multiple populations of *Mycobacterium tuberculosis* with different growth states [3]. Robust validation methods should be developed to assess the ability of these pharmacometric models to accurately describe the PK/PD relationships within the complex and dynamic host-pathogen system, and hence their ability to accurately predict treatment outcomes within patients. Moreover, they need to be extended or stratified to account for the heterogeneity of tuberculosis patients within the clinically relevant spectrum of disease severity, as well as for various potential sites of infection.

The *in vitro* hollow fiber system of tuberculosis (HFS-TB) generates invaluable PK/PD data by accurately measuring bacterial kill rates and resistance patterns while mimicking human plasma drug exposure [4]. Although these systems do not account for the dynamic effects of the host's immune response and are not sufficiently complex on their own to represent the dynamic heterogeneity of the pathogen's phenotype within the patient and the spatial distribution of active drug concentrations [5], they generate PK/PD targets applied to specific pathogen phenotypes, which can be compared to pharmacokinetic or PK/PD thresholds derived in clinical studies [6,7]. The quantitative forecasting accuracy (95% confidence interval [CI]) of the HFS-TB to predict optimal drug exposures and doses, susceptibility breakpoints, and optimal combination regimens was 94.4% (95% CI: 84.3% to 99.9%) based on 20 clinical studies that were published at least 6 months after the HFS-TB experiments, while bias was 1.8% (95% CI: -13.7% to 6.2%) in studies prepared for the US Food and Drug Administration and the European Medicines Agency [8].

Challenges in the analysis of clinical data include a lack of sensitive markers of treatment response, inaccurate extrapolation of pharmacokinetics over time, high-dimensional data with multiple time-varying covariates sometimes displaying multicollinearity and complex interactions, and heterogeneity within and between study populations. Classical linear

statistical methods often perform very poorly when used to analyze high-dimensional data since they were developed for parametric models involving a few input variables where the ratio of the observations to the input variables is high. These methods also make strong statistical assumptions regarding the underlying distribution of the data and the nature of the relationships between the outcome and explanatory variables [9]. Severe multicollinearity presents a challenge for parameter estimation in classical statistical methods. The direction of a covariate effect may be V-shaped with a reversal of direction within the dataset, or, the covariate effect may be discontinuous in some data spaces [10–12]. Furthermore, it is not clinically rational to assume that individual drug concentrations have an additive effect on treatment response (i.e., following the superposition principle). Treatment response may instead reflect synergism or antagonism between individual drugs in a multidrug regimen. Here, we review the quantitative methods applied to clinical data to identify the key drugs driving treatment response, characterize PK/PD relationships and define drug exposure thresholds for activity, or toxicity, in clinical data sets. We highlight the consistency of drug exposure targets associated with tuberculosis treatment outcomes between the different studies and methods. We discuss the strengths and weaknesses of the methods, as well as potential methods to improve the analysis of these data.

2. Machine learning techniques

The use of machine learning techniques has aided understanding of the relationships between antituberculosis drug exposures and treatment outcomes. With PK/PD modelling techniques, it has been used to describe the relationship between tuberculosis treatment response (as assessed by indices of mycobacteria in sputum) or treatment outcomes (e.g., treatment success versus treatment failure, relapse, or death), and drug exposure indices such as 24-hour AUC (AUC_{0-24}), maximum concentration (C_{max}), ratio of AUC_{0-24} to minimum inhibitory concentration (MIC), and time or percentage of time the concentrations are above the MIC ($T_{>MIC}$ or $\%T_{MIC}$) [13–15]. The techniques emerged as a means of responding to the exponential growth of data generated in clinical research and failure of classical statistical methods to identify important relationships in the data. They are discovery-driven (as opposed to assumption driven), i.e., the hypothesis is automatically extracted from the data, and tend to be more robust for handling complex real-world data. In general, the techniques work by sifting through a dataset in search of patterns and relationships [16]. This procedure can be mimicked with classical statistical methodology: one could define an exposure cut-off *a priori* then apply a regression model to determine the effect of higher or lower exposure on treatment outcome, as was applied to clinical data from a study conducted in Botswana [17]. However, the use of a predefined cut-off may fail to detect a significant effect if the actual cut-off that determines the treatment outcome is much higher or lower than the predefined value. The technique of sifting through the data implemented in machine learning ensures that observations are optimally assigned to homogenous groups.

Outputs from machine learning techniques are generally intuitive and based on rules that easily understood. The methods are often implemented in conjunction with generalised linear models such as logistic regression to confirm, via hypothesis testing, the PK/PD relationships, and to present the results using conventional measures of association [10,12,18]. The three commonly applied methods in the context of machine learning are

classification and regression trees (CART) with and without gradient boosting, random forests, and multivariate adaptive regression splines (MARS).

2.1 Classification and regression trees

CART is a nonparametric method that implements recursive partitioning to a data space, fitting a predictive model within each partition [19]. The recursive partitioning technique provides for exploration of the structure of a data set including outcome and predictors, and identification of easy to visualize decision rules for predicting a continuous (regression tree), time-to-event (survival tree) or categorical (classification tree) outcome. In the continuous outcome case, the objective is to predict the mean response within each partition. However, the use of regression trees to identify pharmacokinetic targets is limited mainly due to the availability and widespread use of other machine learning and PK/PD modelling methods described later. Time-to-event outcomes such as time-to-culture conversion are important markers for treatment response, but this type of data is often analysed using classical statistical techniques such as the Cox proportional hazards model. However, the Cox proportional hazards model cannot be used to describe the nonlinear interactions between the predictors of a time-to-event treatment outcome without pre-specification. In spite of its potential advantages, survival tree analysis has not been applied to clinical data from tuberculosis patients, to our knowledge. More often, tuberculosis treatment outcomes are recorded as categorical variables (including time-to-event data converted to a binary outcome), and classification trees are used to predict responses of the categorical outcome.

The output from CART is a tree-like structure with nodes at each split, and several terminal nodes where no further split is possible based on the input rules. The main elements of CART implementation are: selecting the predictor variables (frequently a large set of potential predictors is available) and the splitting criterion at each node based on the value of the selected predictor; defining the stopping rules based on the size of the node or on cross-validation; and pruning the tree to reduce overfitting. In general, the splits for a regression tree are determined by the residual sum of squares while the error rate controls the splits of a classification tree. An important feature of CART is the ability to detect nonlinear interactions between predictors of tuberculosis treatment outcomes without *a priori* specification of the interactions. Other advantages of CART include easy interpretation and communication of the results, identification of key pharmacokinetic thresholds that can be used to optimize antituberculosis drug doses, and detection of pharmacodynamic interactions.

CART has been successfully applied to clinical data of patients recruited in Africa and Asia. In a clinical trial involving patients with drug-susceptible tuberculosis conducted in South Africa, long-term tuberculosis treatment outcomes were associated with AUC_{0-24} for pyrazinamide, rifampin and isoniazid. Pyrazinamide AUC_{0-24} was identified as the most important predictor of long-term treatment outcome and an AUC_{0-24} of 363 mg·h/L optimally separated patients with poor versus favourable long-term treatment outcomes. In patients with pyrazinamide $AUC_{0-24} > 363$ mg·h/L, rifampin AUC_{0-24} (split point = 13 mg·h/L) was predictive of treatment outcomes. A poor treatment outcome was averted if a patient had pyrazinamide $AUC_{0-24} > 363$ mg·h/L and rifampin $AUC_{0-24} > 13$ mg·h/L. In

patients with pyrazinamide $AUC_{0-24} > 363$ mg·h/L and rifampin $AUC_{0-24} = 13$ mg·h/L, having a high isoniazid $AUC_{0-24} (> 52$ mg·h/L) was associated with a favourable treatment outcome [18]. In patients with pyrazinamide $AUC_{0-24} = 363$ mg·h/L an additional split was not supported by the data. In the same study, two-month culture conversion was correlated with pyrazinamide, rifampin and isoniazid exposure and displayed the same order of importance for each drug. However, the peak concentration was predictive of the response to treatment at two months. Having lower pyrazinamide C_{max} (< 58.3 mg/L), rifampin C_{max} (< 6.6 mg/L) or isoniazid C_{max} (< 8.8 mg/L) was associated with a positive sputum culture at two months of treatment. It is worth noting that patients recruited into the study, on average, received higher daily doses of pyrazinamide (25 – 35 mg/kg) than those currently recommended by the WHO (20 – 25 mg/kg) [20]. A retrospective Korean study in patients with drug-susceptible (89.2%) and drug-resistant tuberculosis used CART to analyze routine therapeutic drug monitoring (TDM) data. Effects on tuberculosis treatment outcomes (sputum result after five months of treatment) were detected for all the first-line drugs (pyrazinamide, rifampin, isoniazid and ethambutol). The study utilised two-hour blood samples (which approximate but do not match the C_{max}) which may partly account for the lower exposure split points for the three drugs: pyrazinamide (20.7 mg/L), rifampin (4.5 mg/L) and isoniazid (2.1 mg/L) for one branch of the tree [21]. Additionally, patients in this cohort received lower mg/kg doses of pyrazinamide and ethambutol compared to the South African study. Rifampin and isoniazid concentrations were key predictors of treatment success in patients with high pyrazinamide concentrations, while ethambutol concentration was an important predictor in patients with a low pyrazinamide concentration. The difference in the split points selected using CART for the South African versus the Korean study show that the split points identified in one data set may not be reproduced in another data set with a different distribution of a predictor. In another study amongst patients in Botswana with multidrug-resistant tuberculosis (MDR-TB) on an amikacin-containing regimen, amikacin AUC_{0-24} and C_{max} were found to be equally predictive of treatment failure with split points of 568.3 mg·h/L and 67 mg/L, respectively [22]. The analysis was restricted to the amikacin exposure variables AUC_{0-24} , C_{max} , and C_{trough} , dose in mg/kg, and patient characteristics. Body weight was also reported to be a key driver of treatment success after amikacin exposure (split point = 41 kg). Patients with higher weight than the split point who also had increased exposure to amikacin were more likely to achieve sputum conversion.

Nonlinear relationships detected by CART analyses can point to important pharmacodynamic interactions. Antagonism is present if a daughter node has a higher proportion of poor treatment outcomes at a higher concentration than lower concentration, as was shown by Pasipanodya et al., in a subset of West African patients with low rifampin concentrations who received gatifloxacin as part an experimental regimen [11]. A low rifampin C_{max} (< 7.01 mg/L) was associated with unfavourable treatment outcome and the effect was magnified in patients who also had high gatifloxacin $C_{max} > 29.08$ mg·h/L. Unfavourable outcomes were more likely to be observed in these patients if their pyrazinamide C_{max} was > 32.09 mg/L. The thresholds identified using CART and MARS analysis were further analysed to better inform clinical decision making using probit and logistic regression analysis. Concentration-dependent antagonism was confirmed in patients

with a rifampin $C_{\max} < 7.01$ mg/L and a gatifloxacin AUC_{0-24} of 35.48–50.29 mg·h/L. Using the same method, concentration-dependent antagonism of pyrazinamide was established in patients with a $C_{\max}$ of 33.29–54.92 mg/L who had rifampin $C_{\max} < 7.01$ mg/L. In another example, consistent with studies employing MARS technique to data sets of patients on standard first-line regimens [10,13], Swaminathan et al. described an antagonistic effect of isoniazid in children with low rifampin and pyrazinamide exposures [12]. Children with isoniazid $AUC_{0-24} > 31.8$ mg·h/L were more likely to have a poor treatment outcome compared to those with $AUC_{0-24} \leq 31.8$ mg·h/L if their pyrazinamide and rifampin $C_{\max}$ were less than 38.1 mg/L and 6.2 mg/L respectively. In the same study, an antagonistic effect of isoniazid was reported in children with high rifampin $AUC_{0-24} (> 3.7$ mg·h/L) and low pyrazinamide $C_{\max} (< 37.49$ mg/L); in this subset isoniazid $C_{\max} > 4.29$ mg/L was associated with death.

The problem of missing data is common in many data sets, and if not properly handled, the results can be biased. There are two approaches to handling missing data within CART analyses. For categorical predictors an extra category for missing data is created and could uncover hidden patterns where observations with missing values have different outcomes to those that have nonmissing values. A more general approach is based on the construction of surrogate variables, predictors with a close resemblance to the primary splitting variable. To select a splitting variable and split point only observations with nonmissing values are considered. Observations with missing values are then assigned to the nodes based on a surrogate variable that best mimics the split achieved by the primary variable. The loss of information as a result of missing data is minimized if the correlation between missing predictor and other predictors is high [23]. CART has several drawbacks that investigators have to be aware of. First, CART has a tendency to overfit data. While there are methods to prune down the tree, these are highly dependent on user-defined settings for the algorithm and are often not reported. The final tree is dependent on the size of the dataset: small changes to the dataset can have a substantial effect on the structure of the final tree. Second, CART tends to select continuous covariates because there are many possible splitting points compared to categorical variables. This arises from the failure to recognise two distinct steps in selecting a splitting point: (i) selecting the variable and (ii) selecting the splitting point. The method has been modified to include both statistical significance testing using randomization and separating the two steps via the conditional inference framework [24]. However, the use of CART to identify PK/PD targets for antituberculosis drugs within the conditional inference framework has not been explored. Third, once a variable has been assigned to one branch, it becomes unavailable for decision making in the opposite branch. While this improves the intuitiveness of CART, it tends to throw away weak learners. Combining gradient boosting with CART, however, reverses this and is recommended especially with small datasets [25,26].

2.2 Random forests

Random forests are an improvement of CART, and fall under the group of ensemble methods together with bagging, which combine the predictions from multiple machine learning algorithms together to make more accurate predictions than utilizing a single decision tree [27]. Bagging is a general approach that uses bootstrapping in conjunction with

any classification or regression model to construct an ensemble. It uses a different random subset of the original dataset for each model in the ensemble. Random forests extend the bagging technique by considering a random subset of the predictors at each split of the decision tree. Each tree is generated from a training dataset obtained by randomly sampling observations from the original dataset with replacement (using bootstrap sampling) and randomly selecting a subset of the input covariates. A proportion of the original dataset is left off from the bootstrap sample (out-of-bag sample) and is used to compute an unbiased estimate of the classification error and then compute the variable importance score, expressed as a percentage. The error rate is affected by the correlation between the trees: a higher correlation between any two trees is associated with an increased error. The error rate is also increased if the number of input covariates selected randomly for each tree is low. [27] The main advantage of random forests is improved prediction accuracy. The variable importance score is the primary output of the random forests analysis and provides information on how important a variable is in predicting the outcome. However, the downside is that the output of random forests is not directly linked to PK/PD exposure targets as it is with CART. Therefore, random forests methodology has been used as a variable reduction technique to identify a few important predictors for further analysis using CART and its variants.

Swaminathan et al. implemented random forests to identify variables associated with treatment failure or death in Indian children with tuberculosis [12]. The top four predictors of the treatment outcome, by variable importance score, were pyrazinamide $C_{\max}$ (100%), rifampin AUC_{0-24} (61%), rifampin $C_{\max}$ (59%) and pyrazinamide AUC_{0-24} (54%). Due to the lack of interpretability property of random forests the authors used the random forests as a variable reduction technique to select the top 15 out of 30 potential predictors correlated with the treatment outcome (including the four mentioned above). A modified version of CART (boosted CART) was then applied to the 15 selected predictors to obtain exposure thresholds associated with treatment outcomes. Random forests were also applied to identify predictors of therapy failure in drug-sensitive tuberculosis patients from three West African countries and South Africa randomized to a 4-month regimen of gatifloxacin, rifampicin, isoniazid and pyrazinamide [11]. The pyrazinamide $C_{\max}$, rifampicin $C_{\max}$, and gatifloxacin AUC_{0-24} were among the top predictors of therapy failure, and the result was confirmed using a boosted CART approach.

2.3 Multivariate adaptive regression splines

MARS is often used to model high-dimensional data [28]. It is a nonparametric regression method; it does not specify the overall functional relationship between a continuous outcome and a predictor. Like CART and random forests, MARS employs recursive partitioning, to partition the data space into regions, each with its own regression coefficients and basis functions. The basis functions describe the relationship between a predictor and the outcome. Related to the basis function is a knot which connects two regression lines. The knots are selected to minimize the sum of squared errors, i.e., the sum of the squared differences between the predicted and observed outcome.

MARS has been applied to clinical data from two separate studies conducted in Cape Town, South Africa. Chigutsa et al. identified nonlinear relationships between drug exposure and the sterilizing activity of first-line antituberculosis drugs comprising the standard regimen for drug-susceptible tuberculosis [13]. The key predictors (thresholds) of sterilizing activity were rifampin AUC_{0-24} (35.4 mg·h/L) and C_{max} (8.2 mg/L), pyrazinamide AUC_{0-24}/MIC ratio (11.3), ethambutol C_{max}/MIC ratio (0.46) and isoniazid C_{max} (0.72 mg/L). In patients with rifampin $AUC_{0-24} < 35$ mg·h/L, higher pyrazinamide AUC_{0-24}/MIC and ethambutol C_{max}/MIC ratios increased sterilizing activity, while isoniazid C_{max} antagonised the sterilizing activity. Higher sterilizing activity was also reported in patients with a rifampin $C_{max} > 8.2$ mg/L and pyrazinamide AUC_{0-24}/MIC ratio > 11.3 . The importance of the identified thresholds was evaluated on two-month treatment outcome, which confirmed that the odds of a negative two-month culture result were six times higher in patients with rifampin $C_{max} > 8.2$ mg/L and pyrazinamide AUC_{0-24}/MIC ratio > 11.3 compared to the rest of the cohort. The odds of a negative two-month culture result were almost four times higher in patients with a rifampin $C_{max} > 8.2$ mg/L, regardless of pyrazinamide AUC_{0-24}/MIC ratio. In a separate study, MARS identified the rifampin C_{max}/MIC ratio and isoniazid C_{max} to be the main drivers of two-month culture conversion [10]. In patients with a rifampin C_{max}/MIC ratio of 28 and under, the probability of culture conversion at two months declined as the rifampin C_{max}/MIC ratio declined, and this negative effect was exacerbated by an increasing isoniazid C_{max} up to 4.6 mg/L.

MARS analyses automatically detect interactions between predictors and, as demonstrated by the studies cited above, concentration-dependent synergism and antagonism are readily described. It is also robust with respect to outlier observations, similar to other nonparametric regression methods. However, it also tends to overfit the data, and as the model becomes complex, the results become difficult to interpret. With highly correlated predictors (e.g. AUC_{0-24} and C_{max} values for the same drug), the MARS method may encounter estimation problems leading to instability of estimates, especially when higher order interactions are included in the model [28].

3. Pharmacokinetic/pharmacodynamic modelling

PK/PD modelling is an important tool in clinical pharmacology to optimize drug doses and dosing regimens. Mathematical and statistical components may be integrated into a PK/PD model to describe mechanistic aspects and variability in pharmacology, respectively. Application of PK/PD models is important to characterize population dynamics of *M. tuberculosis* and the effect of a specific antituberculosis drug on bacterial growth states and kill rates. However, the laboratory methods commonly used to quantify the bacterial population in sputum samples fail to detect non-multiplying bacteria, and it is challenging to track bacteria in different growth states in patients, hence the need to integrate data generated from *in vitro* experiments with clinical data. A multi-state tuberculosis pharmacometric (MTP) model was developed using *in vitro* data to describe fast-, slow- and non-multiplying *M. tuberculosis* bacteria [29]. The MTP model was integrated with a rifampin pharmacokinetic model and colony forming unit (CFU) data from tuberculosis patients to characterise the effect of rifampin exposure on inhibition of the growth of fast-multiplying bacteria and stimulation of the death rate of slow- and non-multiplying bacteria

during the first 14 days of tuberculosis treatment [30]. The model estimated that rifampin completely inhibits the growth of fast-multiplying bacteria. At the same time rifampin kills slow- and non-multiplying bacteria with second-order rate constants of 0.2 and 0.106 L/mg/day respectively. Recently, a more general-purpose computational modelling framework was developed, utilising set theory mapping to predict time to extinguish *M. tuberculosis* in patients on chemotherapy based on PK/PD relationships identified in HFS-TB [31]. The modelling framework could map, using different transformation functions, the bactericidal and sterilising activity kill rate of the standard first-line regimen containing rifampin, isoniazid, pyrazinamide, and ethambutol. The estimated bactericidal activity kill rates were comparable between HFS-TB and patient sputa ($> 0.9 \log_{10}$ CFU/mL/day). Differences in sterilizing activity kill rates were observed between HFS-TB ($0.486 \log_{10}$ CFU/mL/day) and patient sputa ($0.176 \log_{10}$ CFU/mL/day). The study showed that translation of bacterial kill rates and time to extinguish *M. tuberculosis* from HFS-TB to patients is largely driven by pharmacokinetic and MIC variability. The method was validated using data from a separate clinical trial comparing a four-month and a six-month regimen, and accurately showed that the four-month regimen was suboptimal as previously reported [31]. The strength of the integrated PK/PD approach lies in the ability to model data from multiple sources under specific assumptions. As the results from PK/PD modelling are difficult to interpret, simulations of various clinical scenarios is important. Simulations using parameter estimates from the integrated MTP and pharmacokinetic model showed that the rate of decline of the bacterial populations was faster using a 20 mg/kg daily dose of rifampin compared with a 10 mg/kg dose.

The effect of rifampin exposure in the integrated PK/PD model by Svensson and Simonsson utilised switch (on or off) and linear functions. The $E_{\max}$ function is another common choice to describe the activity of drugs and regimens quantitatively. The shape of the function is driven by two parameters, the $E_{\max}$ and EC_{50} describing the maximum effect and the concentration that results in 50% of the maximum effect, respectively. For tuberculosis treatment, this function can reveal the relationship between antituberculosis drug concentrations and the probability of a favourable treatment outcome. Furthermore, a target exposure value can be determined, e.g., AUC_{0-24} associated with a 90% chance of a favourable treatment outcome. The $E_{\max}$ function could be a valuable tool to determine the dose required to achieve an exposure level associated with a favourable treatment outcome. Pasipanodya et al. implemented a similar approach, using probit regression to determine the optimal dose of gatifloxacin (800 mg/day) in patients with rifampin $C_{\max} < 7.01$ mg/L [11]. Using an *in vitro* PK/PD model with an inhibitory sigmoidal $E_{\max}$ function, the ratio of free AUC_{0-24}/MIC ($fAUC_{0-24}/MIC$) 209.08 of pyrazinamide was associated with 90% of the maximal kill [32]. Such targets should be validated using clinical data. One approach would be to develop an $E_{\max}$ model of the probability of microbial kill against free AUC_{0-24}/MIC of pyrazinamide and use the model to determine the proportion of patients with a ratio of 209.08 who are successfully treated. A relative deviation target ratio 209.08 from the exposure associated with a 90% chance of treatment success will indicate the reliability of the exposure target derived from the *in vitro* experiment. Another approach would be to include treatment response information from the HFS-TB together with patient-specific disease pathology within a translational PK/PD model and apply simulations to predict

treatment outcomes for clinical trials. This approach was successfully implemented by Bartelink et al. to predict PK/PD relationships in patients recruited in clinical trials [33]. The $E_{\max}$ function can also be implemented in a two-stage procedure to derive target drug exposure. In the first stage, the exposure parameters associated with treatment outcome are determined using either the $E_{\max}$ model, logistic regression or other statistical techniques. Simulation techniques are then applied to identify a cut-off value of exposure associated with the outcome of interest (e.g., probability of favourable treatment outcome of at least 95%). The method has been applied to data collected in a cohort of Indian children and the authors reported that the average steady-state weekly rifampin AUC is predictive of favourable outcomes [34]. A target weekly rifampin AUC of 185 mg·h/L which translates to an average daily exposure of approximately 26.4 mg·h/L was proposed. However, the proposed target is low in light of results of recent clinical trials reviewed below, where doses up to 35 mg/kg were evaluated. Thus, exposure targets identified via model-based simulations are influenced by the distribution of the data: PK/PD relationships may not be extrapolated to exposures outside those observed in the dataset.

The relationship between drug exposure and suppression of resistance to antituberculosis drugs has been reported to be a system of time-dependent quadratic functions [2,14,32,35,36]. To derive PK/PD targets, drug exposure is modelled against a drug-resistant *M. tuberculosis* subpopulation. The relationship is described by an inverted U curve: showing an increase in the drug-resistant subpopulation with increasing drug exposure from zero to a plateau before decreasing to values below those observed without the drug. In the HFS-TB, suppression of resistance to pyrazinamide was associated with %T_{MIC} and a target of 66.7% was proposed [32]. A separate *in vitro* study found that the highest isoniazid exposure evaluated ($AUC_{0-24}/MIC \approx 1300$) did not optimally suppress the development of resistance [37].

The observational approaches employed to explore the PK/PD relationships within several studies of clinical cohorts [10–13,17,22,34] have utilized the pharmacokinetic variability between patients on standard doses of the antituberculosis drugs. Optimisation of drug exposure could also be tackled from a dosing perspective. An increased range of drug doses allows exploration of the PK/PD relationships over a wider range of drug exposures. Moreover, increasing the dose of a specific drug allows determination of the proportion of patients with favourable outcomes, on various doses. Rifampin doses higher than the currently recommended 10 mg/kg daily dose, up to 40 mg/kg daily, have been evaluated. In per-protocol analyses of the HIRIF trial (NCT01408914), Velásquez et al. found that each 5 mg/kg increase in rifampin dose up to 20 mg/kg, and each 1 log increase in rifampin AUC_{0-6} , were associated with an increased elimination rate of *M. tuberculosis* from sputum cultured in solid medium [38]. In the HIGHRIF1 trial (NCT01392911), rifampin doses of 10, 20, 25, 30, and 35 mg/kg showed a more than dose-proportional increase of rifampin exposure with increasing dose [39]. The highest rifampin doses of 30 and 35 mg/kg displayed the highest 14-day early bactericidal activity on both solid and liquid media. A model-based exposure-response analysis of data from the MAMS-TB-01 trial (NCT01785186) conducted in seven sites in Tanzania and South Africa showed that the expected probability of two-month culture conversion increased from 39% with the 10 mg/kg rifampin dose to 55% with the 35 mg/kg dose. The rifampin AUC_{0-24} was close to 9-

fold higher in simulated patients receiving the 35 mg/kg dose compared to the 10 mg/kg dose [40].

4. Conclusion

We described the common quantitative methods used to identify PK/PD indices and exposure targets for antituberculosis drugs. The drug exposure targets are derived from clinical and/ or nonclinical experiments. The activity of the drug can be concentration- or time-dependent with $fAUC_{0-24}/MIC$ and fC_{max}/MIC being the main drivers of treatment outcomes for concentration-dependent activity while $T_{>MIC}$ or $\%T_{MIC}$ are key predictors for time-dependent activity. Results from the analysis of PK/PD data confirm that rifampin and pyrazinamide are key to successful treatment of drug-susceptible tuberculosis, while in some scenarios isoniazid plays an antagonistic role. Further methodological work on machine learning methods is needed to discriminate between the strength of the effect of increasing pyrazinamide exposure and its relatively lower random unexplained variability, compared with other drugs in the regimen. Analytical methods capable of handling data from multiple sources have the potential to provide more robust estimates of PK/PD targets.

5. Expert opinion

Machine learning methods are key to identifying nonlinear trends in high-dimensional data. However, some trends could be detected by chance. The findings generated should therefore be verified by statistical tests or validated using additional data sets. CART methods currently applied to identify PK/PD targets should be modified to include hypothesis testing employing the conditional inference framework [24]. PK/PD targets identified by machine learning methods are useful but have important limitations. The results between studies differ as a result of the heterogeneity of the patient populations, treatment factors and dosing strategies, pharmacokinetic methods and measures of treatment response, as well as methodological differences between the analyses employed. As a result, the PK/PD targets may not be generalizable. The distribution of the data in a particular population has a considerable influence on how CART defines a threshold for improved treatment outcome. As the PK/PD associations determined are limited by the distribution of PK measures and other covariates in that population, the threshold may not be optimal. This is true in the case of rifampin: the currently recommended dose is known to be less than the optimal, but CART analyses will still identify an optimal exposure. Meta-analyses including individual-level data for treatment outcomes and potential predictors including drug exposure could provide more broadly applicable estimates of optimal drug exposures amongst patients on approved doses. The pharmacokinetic parameters AUC_{0-24} and C_{max} are often highly correlated and distinguishing the effect of one from the other is difficult when the same dose frequency is applied. For drugs with a shorter elimination half-life such as first-line antituberculosis drugs, AUC_{0-24} -associated indices provide a better measure of overall drug exposure over time, which generally contain less random error than peak concentration measurements. The machine learning methods described in this report fail to account for the changes in pharmacokinetic parameters over time and other time-dependent covariates such as patient weight and bacterial load. However, parametric time-to-event models have been implemented to describe the time-to-positivity in liquid media among tuberculosis patients,

which is driven by the change in bacterial load during treatment [41,42]. It is encouraging that a mixed effects trees method has been developed to analyse longitudinal data within a machine learning technique [43], but the method has not been applied yet within the context of identifying PK/PD exposure targets.

Dose-ranging studies and integrated approaches combining disease models and PK distributions in pharmacometric PK/PD models will better define the optimal PK/PD targets. PK/PD modelling approaches capable of translating findings from preclinical models to tuberculosis patients have attracted attention among researchers. These approaches can be applied to identify treatment shortening drugs and drug combinations as well as accelerate the development of novel TB treatment regimens. There is need to expand the PK/PD modelling approaches to include the rate of drug penetration into TB lesions for more drugs used for the treatment of TB and to optimize drug doses using exposures observed in TB lesions [44,45]. The semi-mechanistic PK/PD models described in this review may be considered less flexible to describe the complex interaction between *M. tuberculosis*, host cells and antituberculosis drug activity. Physiologically based pharmacokinetic (PBPk) modelling is an alternative which supports a detailed description of the physiology and possibility to quantify underlying processes including simultaneous evaluation of treatment efficacy at the site of infection and toxicity in off-target organs [46].

Application of quantitative methods to establish optimal antituberculosis drug exposures and regimens has expanded in recent years. Wider application of machine learning methods to detect nonlinear interactions between drug exposures and to identify optimal drug exposures associated with time-to-event treatment outcomes is likely. The use of an ensemble of machine learning methods such as boosted CART is key to identify weak but important signals that would not be detected by ordinary CART, reduce bias and improve predictions of treatment outcomes in clinical data sets. Modifications to the traditional machine learning methods to incorporate hypothesis testing might be widely acceptable to modellers and clinicians who are more familiar with association statistics and effect sizes. Machine learning methods to account for longitudinal changes in drug exposure will provide improved estimates of drug exposure thresholds associated with treatment outcomes.

PK/PD relationships are complex and methods exploring the relationships need continuous improvement to expedite the development of optimal drug doses and regimens. The use of flexible pharmacometric modelling methods, capable of describing the complex relationships between antituberculosis drug exposure and treatment outcomes is likely to increase. Clinical trials with a nested pharmacokinetic study are becoming more commonplace which makes it possible to perform pooled PK/PD analysis, with possibly a wider range of doses that will improve the generalizability of drug exposure targets. Data generated from *in vitro* experiments, animal studies, and clinical trials can be utilized within complex integrated PK/PD models to optimize antituberculosis drug exposure. The application of PK/PD models to derive drug exposure targets while taking into account the bacterial populations, changes in resistance patterns, drug penetration at the site of infection, and time-course of PK and PD is likely to expand.

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* of interest

** of considerable interest

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Article highlights

- Pharmacokinetic differences within and between study populations together with lack of sensitive markers of tuberculosis treatment response pose a challenge to the optimization of drugs and regimens.
- Classical linear statistical models rely heavily on statistical assumptions underlying the distribution of the data and often perform poorly to describe the complex relationships between antituberculosis drug exposures and treatment outcomes.
- We describe machine learning and PK/PD modelling techniques currently used to identify drug exposure targets and optimal drug combinations for tuberculosis treatment.
- Drug exposure targets derived using machine learning techniques are sensitive to the training dataset but can be improved by applying ensemble methods.
- PK/PD modelling approach is a useful computational tool for tuberculosis treatment to identify new combination therapies, and to optimize existing drugs and regimens.