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REVIEW

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Current status and opportunities for therapeutic drug monitoring in the treatment of tuberculosis

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ABSTRACT

Introduction: Tuberculosis remains a global health problem and pharmacokinetic variability has been postulated as one of the causes of treatment failure and acquired drug resistance. New developments enable implementation of therapeutic drug monitoring, a strategy to evaluate drug exposure in order to tailor the dose to the individual patient, in tuberculosis treatment.

Areas covered: Literature on pharmacokinetics and pharmacodynamics of anti-tuberculosis drugs was explored to evaluate the effect of drug exposure in relation to drug susceptibility, toxicity and efficacy. New, down-sized strategies, like dried blood spot analysis and limited sampling strategies are reviewed. In addition, molecular resistance testing of *Mycobacteria tuberculosis*, combining a short turn-around time with relevant information on drug susceptibility of the causative pathogen was explored. Newly emerging host biomarkers provide information on the response to treatment.

Expert opinion: Therapeutic drug monitoring can minimize toxicity and increase efficacy of tuberculosis treatment and prevent the development of resistance. Dried blood spot analysis and limited sampling strategies, can be combined to provide us with a more patient friendly approach. Furthermore, rapid information on drug susceptibility by molecular testing, and information from host biomarkers on the bacteriological response, can be used to further optimize tuberculosis treatment.

1. Introduction

Although tuberculosis (TB) mortality has fallen by 47% since 1990, it will remain a huge medical, social, and financial problem throughout the world for the coming decades. The World Health Organization (WHO) estimated that in 2014 about 9.6 million children, women, and men fell ill from TB. Twelve percent of these were human immunodeficiency virus (HIV) positive and of the 1.5 million who died from TB, 400,000 were HIV positive.[1] One of the problems that facilitates ongoing transmission is the lack of early diagnosis. Only 63% of the new TB cases were diagnosed, and an even smaller proportion of multidrug-resistant (MDR)-TB disease is diagnosed. Approximately, 25% of the estimated annual number of prevalent 480,000 MDR-TB cases are detected and reported. The notification of MDR-TB and extensively drug resistant (XDR)-TB has risen in recent years, while the incidence rates of susceptible TB are slowly decreasing.[1] Most MDR-TB cases are nowadays detected by molecular testing of the infecting mycobacteria. There are no exact data on the prevalence of more extended

resistance profiles in this group. By molecular testing, we do know there is at least resistance to rifampicin and isoniazid, but molecular testing provides no information on to which alternative drugs the strain is still susceptible. Therefore, the true magnitude of the problem of drug-resistant TB in the world is in fact unknown. After the start of an effective drug regime, it was shown that transmission almost immediately stops.[2] An effective treatment regime for TB, that prevents relapse after treatment completion, needs to be based on sufficient effective drugs in an effective dose for a sufficient duration. The standardized regimen for drug-susceptible TB was found to lead to treatment success in 85% of the cases.[1] Further optimization by identifying patients that are over- or undertreated would be expected to increase treatment success rate further. Therapeutic drug monitoring (TDM) would help detect patients receiving a too high or too low drug dose and allow them to be provided with individualized treatment.[3] TDM for TB includes evaluation of both pharmacokinetics (PK) and pharmacodynamics (PD) to enable individualized treatment optimization.

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1.1. PK

PK describes what happens with a drug in the body. Generally, the drug is absorbed (A) into the systemic circulation after oral intake, after which it is distributed (D) throughout the body including to the TB infection sites. After metabolism (M) mainly by the liver, eventually the drugs are eliminated (E) mainly by the kidneys and released in the urine.[4] Integration of these parameters results in a PK model that describes these processes (ADME) (Figure 1a).

PK of TB drugs are not only influenced by the physical and chemical properties of the drug, but are also dependent on patient characteristics, such as age, gender, or kidney- or liver function.[4,6] PK variability is a well-known problem affecting drug exposure and thereby TB treatment.[7,8] For example, Pranger et al. observed a nine-fold variability in the area under the time concentration curve between 0 and 24 h (area under the curve [$AUC_{0-24\,h}$]) of moxifloxacin, possibly due to variation in protein binding. This led to suboptimal as well as potential toxic concentrations of moxifloxacin given in the same dosage of 400 mg.[9] The most relevant measure of drug exposure is $AUC_{0-24\,h}$. To assess drug exposure, generally multiple samples are collected.[7]

1.2. PD

PD describes effect of the drug on the target, for the purpose of this review on, *Mycobacterium tuberculosis* (*Mtb*). Anti-TB drugs can be subdivided in bactericidal and bacteriostatic drugs. The maximum effect of a drug is described by maximum effect (E_{max}). The EC_{50} is the concentration at 50% of E_{max} and describes the potency of the drug (Figure 1b). To describe the susceptibility of a pathogen to a drug, generally the minimal inhibitory concentration (MIC) is used, which reflects the drug concentration that is associated with >99% inhibition of growth of the pathogen.[1,10] Not only the effect of the drug on the pathogen, but also the effect of the drug
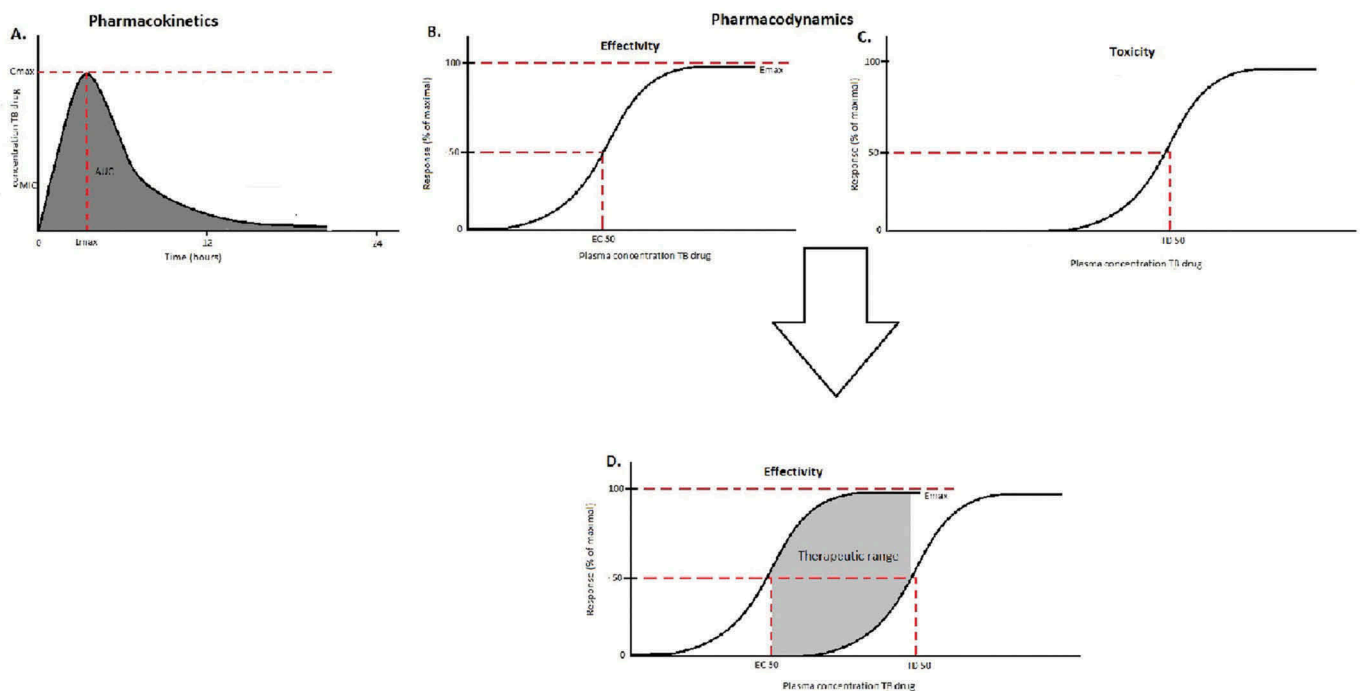
Figure 1. Pharmacokinetic/pharmacodynamic integration.
In pharmacokinetic/pharmacodynamic (PK/PD) modeling, both pharmacokinetic (PK) models and pharmacodynamic (PD) models are brought together to provide information about the time at which the concentration of an anti-tuberculosis (anti-TB) drug is within the therapeutic range A. PK model with on the x-axis the time in hours after dosage and on the y-axis the concentration of the anti-TB drug. The dark grey area is the area under the curve (AUC). The maximum of the curve is the peak concentration (C_{max}), at a certain time (t_{max}). B. PD model with on the x-axis the plasma concentration of the anti-TB drug and on the y-axis the response in percentage of the maximum effect (E_{max}). The EC_{50} is the half maximal effective concentration, which is used as a measure of the anti-TB drug's potency. C. This graph shows another PD model, but in this case the plasma concentration of the drug at which toxicity occurs is shown. The TD50 is the median toxic dose at which in 50% of the cases toxicity occurs. D. By combining Figure 1B and 1C, the plasma concentration of the anti-TB drug at which the maximum effect is reached with the lowest change of toxicity. This is known as the therapeutic range and is marked with the light grey area.[5]

on the patient, the toxicity, is described by PD (Figure 1c). When both the efficacy and the toxicity curves of the anti-TB drug are described in one figure, the therapeutic range can be derived (Figure 1d). The therapeutic range is the concentration at which the TB drug has high efficacy with low toxicity.

1.3. PK/PD

PK/PD integrates both concentration and effect of a drug in time. PK/PD focuses on the efficacy of the drug on *Mtb* as well as the toxicity on the patient. The *in vitro* Hollow Fiber infection model, in which the exposure to drugs can be varied, has been used to elucidate PK/PD indices for many anti-TB drugs (Table 1).[10–12] However, PK/PD indices can also be elucidated in preclinical and clinical studies by dose-fractionation studies and early bactericidal (EBA) studies. Dose-fractionation studies in preclinical models are often used to determine which PK/PD parameter is most important for the anti-TB drug. EBA studies measure the ability of anti-TB drugs to reduce the burden of *Mtb* in sputum to determine the efficacy of treatment. In contrast to dose-fractionation studies, EBA studies do not break the collinearity of PK/PD parameters. [10,13] For most anti-TB drugs, the $AUC_{0-24\ h}$ relative to the MIC has been suggested as the best parameter to predict *in vivo* efficacy of drugs against *Mtb*.[9–11,14] As only unbound drug is active, the free AUC is often used as a target.[9,11,15] Other PK/PD parameters are peak concentration (C_{max})/MIC ratio or the time the concentration exceeds the MIC ($T > MIC$) (Table 1).[16] If PK/PD targets are not met, this results in selection of resistant bacteria.[10,17]

2. Current status

The current programmatic TB treatment regimens use the principle of 'One size fits all.' Due to the PK variability, however, this carries the risk of hidden monotherapy that may result in treatment failure and development of drug resistance. More and more information is becoming available showing that underdosing is an important factor in the development of resistance.[34,35] Srivastava stated that pharmacological variability is also a factor that needs to be taken into consideration and could be an underlying reason for the occurrence of resistance.[36] The use of TDM is not yet standard in the treatment of TB, neither is the way it is performed. No official guidelines for TDM are available at international (e.g. WHO) or national health organizations. The WHO recommends the use of drug susceptibility testing (DST) for (resistant) TB.[1] There are only a limited number of laboratories in the world providing reliable and reproducible susceptibility testing on first-line drugs.[37] Nowadays, most MDR-TB cases are diagnosed by the detection of mutations related to rifampicin resistance, for instance by the GeneXpert.[38] This is a fast and relatively cheap diagnostic tool, but does not yield information on an alternative and effective treatment regime to be used when resistance occurs. A phenotypic resistance testing pattern, based on exact MIC's and not breakpoints, is needed to enable PK/PD-guided dosing using TDM.[39] Unfortunately, this is a time- and money-consuming procedure taking up to 10 weeks before the definite resistance pattern for second-line and

third-line drugs becomes available. This implicates that in most cases treatment is started empirically using a standardized programmatic regime or an educated guess. Once drug susceptibility data have become available, the medication regime must be revised according to the results.

Using TDM, it is possible to adapt the dose, based on the drug concentration and thereby increase efficacy or prevent toxicity. Repeated sampling of blood is often required after a change of dose. The most frequent used sampling time points are 2 ($C_{2\ h}$) and 6 h ($C_{6\ h}$) post-drug intake. The $C_{2\ h}$ is a surrogate of the peak plasma concentration (C_{max}), which is linked to a certain time at which this peak concentration occurs (t_{max}) (Table 1) [7,40] and the $C_{6\ h}$ is often collected to evaluate potential delayed absorption.[1,40] Moreover, selected sample time points can be used to estimate the AUC precisely, referred to as limited sampling.[16]

As already mentioned, to date, TDM is not recommended by the guidelines.[21] However, to assess its value, the currently available data on efficacy, toxicity, and drug susceptibility is summarized.

2.1. Efficacy

To determine the efficacy of TB treatment, several measures are available: sputum smear conversion, sputum culture conversion, time to positivity, and treatment outcome. Sputum smear and culture conversion are defined by two consecutive negative sputum cultures at least 30 days apart.[41,42] Treatment outcome is either recorded as successful, defined as cured, treatment completed or still on treatment or recorded as failure defined as defaulted, relapsed or has deteriorated during treatment.[1] Current treatment of drug susceptible TB with group 1 anti-TB drugs (Table 1) is quite successful, with a treatment success rate of 85%.[1] However, even though the treatment success target for MDR-TB was set on e75% by the WHO, this target has only been reached by a few countries.[1] A MDR-TB treatment regimen is constructed, using ethambutol (if proven susceptible) and pyrazinamide in combination with second-line anti-TB drugs: an injectable in combination with a fluoroquinolone completed with group 4 drugs (ethionamide, cycloserine, and *para*-aminosaliciylic acid) (Table 1).[21] The reference value for C_{max} at a normal dose, which needs to be reached for effective treatment with these anti-TB drugs, is described in Table 1. There are several studies that describe the relationship between low drug levels and treatment failure. For example, a retrospectively evaluated cohort showing high treatment failure and high rates of acquired resistance indicated that most of these patients had low drug levels of rifampicin and isoniazid. After TDM, dose adjustments were made in 71.8% of the patients in order to increase plasma concentrations of the drugs, which led to variable results.[43] The PK/PD index and the relationship with treatment outcome is difficult to study in 'real-life.' Technical complications with respect to drug concentration measurement and DST, long-term outcome evaluation, and budget restrains complicate these studies. Gumbo et al. did link sputum smear and the $AUC_{0-24\ h}$/MIC ratio for ciprofloxacin, which combines treatment outcome directly with PK/PD. [17] The EBA is used to evaluate the efficacy of anti-TB drugs

Table 1. Pharmacokinetic and pharmacodynamic markers of antituberculosis drugs for the use of therapeutic drug monitoring [7,10,18–20].

WHO group [21]	Drug	Adult daily dose* [7,18,20]	C_{max} [7,18,20]	T_{max} (h) [7,18,20]	PK/PD[b] [7,18,20]	PK/PD target value [19,22]	LSS[c]	DBS[c]
1	Isoniazid	300 mg	3–6 µg/ml	0.75–2	AUC/MIC	19.2 (slow) and 11.6 (fast)	No	No
1	Rifampicin	600 mg	8–24 µg/ml	2	AUC/MIC	40	Yes [11,23]	Yes [24]
1	Rifabutin	300 mg	0.45–0.9 µg/ml	3–4	N/A	N/A	No	No
1	Pyrazinamide	25–35 mg/kg	20–60 µg/ml	1–2	AUC/MIC	52	Yes [16]	No
1	Ethambutol	25 mg/kg	2–6 µg/ml	2–3	AUC/MIC or C_{max}/MIC	23 or 10	No	No
2	Streptomycin	15 mg/kg	35–45 µg/ml[d]	0.5–1.5 IM	AUC/MIC [19]	8–10[e]	No	No
2	Capreomycin	15–30 mg/kg	32 µg/ml	N/A	C_{max}/MIC	20	No	No
2	Amikacin	15–30 mg/kg	46 µg/ml	End infusion IV	C_{max}/MIC	43	No	No
2	Kanamycin	15–30 mg/kg	44 µg/ml	End infusion IV	C_{max}/MIC	20	No	No
3	Ofloxacin	800 mg	10.5 µg/ml	1	AUC/MIC	47	No	No
3	Levofloxacin	500–1000 mg	8–13 µg/ml	1–2	AUC/MIC	N/A	Yes [15]	No
3	Moxifloxacin	400 mg a day	3–5 µg/ml	1–2	AUC/MIC	110.5	Yes[25]	Yes[26]
4	p-Aminosalicylic acid	4000 mg twice daily	20–60 µg/ml (granules)	4–8 (granules)	AUC/MIC [19]	154	No	No
4	Ethionamide	250–500 mg	1.35 µg/ml	2	C_{max}/MIC or T > MIC [19]	1.6 or 1.5	No	No
4	Prothionamide	750 mg	2.5 µg/ml	3–4	N/A	N/A	No	No
4	Cycloserine	250–500 mg	8–9 µg/ml	2–3	AUC/MIC [19]	N/A	No	No
4	Terizidone	750 mg	20–30 µg/ml	N/A	N/A	N/A	No	No
5	Clofazimine	100 mg	0.5–2.0 µg/ml	2–7	N/A	N/A	No	No
5	Linezolid	300–600 mg	12–26 µg/ml	1.5	AUC/MIC [19]	80–120 and 100%	Yes [27]	Yes [28]
5	Clarithromycin	1000 mg	2–4 µg/ml	2–4	AUC/MIC	N/A	No	Yes [29]
5	Amoxicillin/ clavulanate	1750/250 mg	15–25 µg/ml	1–2.5	T > MIC	N/A	No	No
5	High-dose isoniazid	10 mg/kg (if INH MIC < 1 mg/l) or 16–20 mg/kg three times a week (if INH MIC 1–5 mg/l)	9–15 µg/ml	0.75–2	AUC/MIC	N/A	No	No
5	Thiacetazone [19]	150 mg	1–2.5 µg/ml	2–6	T > MIC	N/A	No	No
5	Imipenem	1000–1500 mg	30–40 µg/ml	2	T > MIC	N/A	No	No
6[f]	Bedaquilline [30]	400 mg[g]	5.5 µg/ml	2–6	AUC/MIC	N/A	No	No
6[f]	Rifapentin[h]	600 mg	8–30 µg/ml	5	N/A	N/A	No	Yes [31]
6[f]	Pretomanid [32]	200 mg	6 µg/ml	2	T > MIC	100%	No	No
6[f]	Delamanid [33]	200 mg	228 ng/ml	4	N/A	N/A	No	No

[a]The adult dosage is based on the dosage that is given a day, while dosing multiple times a day is not performed very often anymore because of compliance.

[b]The PK/PD parameters are based on the parameters that describe the in vivo efficacy of the drug (microbial kill) best.

[c]Limited sampling strategies and dried blood spots provide information about the anti-TB drugs that have validated methods for these strategies, marked with yes or no.

[d]Calculated C_{max} using linear regression to 1 h post-IM dose or end of IV infusion.

[e]Effective bactericidal activity. >12 is thought to result in maximum killing.

[f]Other anti-TB drugs, not included in the WHO list of drugs.

[g]First 2 weeks, thereafter 200 mg.

[h]For treatment of latent TB infection (LTBI) only.

C_{max}: Peak concentration; t_{max}: time at which the peak concentration occurs; PK/PD: pharmacodynamics/pharmacokinetics (integration); LSS: limited sampling strategy; DBS: dried blood spots; AUC: area under the time concentration curve; MIC: minimal inhibitory concentration; N/A: not applicable, no information is available.

in this study. The EBA is based on the rate of decline in the bacterial density in sputum per day for the first few days of treatment. This study shows that when a mean MIC of TB isolates is known, the EBA that is found in human EBA studies is mediated through an $AUC_{0-24 h}$/MIC ratio that is achieved in an *in vitro* TB model.[13,17]

The $AUC_{0-24 h}$ was also linked to treatment outcome in the study of Pasipanodya et al. (2013). They showed that the AUC of pyrazinamide, rifampicin, and isoniazid below a certain threshold, were predictors of a poor long-term outcome. They observed that poor outcomes were encountered in 32/78 patients with the AUC of at least one of these drugs below the threshold versus 3 out of 64 patients with AUC of all drugs above threshold.[12] Another parameter that can be used for TDM is $C_{2 h}$ which has also been found to correlate with efficacy endpoints. For example, Prahl et al. (2014) found that patients with therapy failure had significantly lower $C_{2 h}$ of isoniazid and/or pyrazinamide, despite the fact that they received a higher dosage per kg of body weight. They also observed that more patients with both low rifampicin and isoniazid concentrations experienced therapy failure.[44] These results, however, can be misinterpreted because $C_{2 h}$ might not represent the actual peak concentration. Therefore, a second sample might be necessary to make sure the drug absorption is assessed more completely.[7] Furthermore, Chideya et al. found that pyrazinamide plasma concentrations below 35 µg/ml were a predictor of poor treatment outcome, which can function as a reference value in TDM.[6] However, more studies are needed to elucidate PK/PD indices in 'real life' treatment, because the PK/PD index (Table 1) of a drug in a combination regimen may differ from the index of that particular drug given as monotherapy in an EBA study.

2.2. Toxicity

It is well known that second-line anti-TB drugs are more toxic than first-line anti-TB drugs. The most severe and frequently observed adverse drug events are hematological changes, hepatotoxicity, immunological reactions, ototoxicity, nephrotoxicity, neurotoxicity, and neuromuscular blockade.[45] Although many studies report on adverse effects of anti-TB drugs, there are relatively few that take plasma concentration into account. Ethambutol optical neuropathy, peripheral neuropathy due to isoniazid and pyrazinamide-induced hepatotoxicity, and QT-interval prolongation due to fluorquinolones are all dose-dependent,[45] taking plasma concentration into account could reduce the occurrence of these toxicities. Satyaraddi et al. have studied the plasma-drug levels of first-line anti-TB drugs in 110 TB patients, in relation to hepatotoxicity. They showed that plasma rifampicin levels were higher in patients with drug-induced hepatotoxicity (DIH) than in controls. They found that a rifampicin C_{max} at day 7 exceeding 12.50 mg/l, was associated with DIH in most patients.[46] TDM of rifampicin might possibly reduce the occurrence of DIH, or reverse the toxicity without the need to withdraw the drug for DIH. Neuropsychiatric symptoms were shown to be associated with a cycloserine concentration above 40 µg/ml in a study of Holmes et al. Neuropsychiatric toxicities frequently require changes in DOTS-Plus regimens, including temporary

suspension.[47] TDM is intended for prevention of toxicity by individual dosing, neuropsychiatric toxicities could therewith be prevented. Torün et al. showed in their retrospective review of 263 patients that in 182 cases (69.2%) at least one side effect developed, and that in 146 cases (55.5%), one or more drugs were withdrawn from treatment due to side effects. These side effects were mostly due to amikacin ototoxicity or cycloserine neuropsychiatric toxicity.[48] Other adverse effects of anti-TB drugs include ocular toxicity, which is more common with a daily dose of ethambutol above 30 mg/kg.[18] Another study found that ocular toxicity may be due to overdosing of ethambutol in obese patients. Overdosing occurred in relation to the calculation of the dosage on total body weight. Ethambutol does not accumulate in fat tissue, which causes the dosage to be too high. They suggest ethambutol dosing according to lean body weight, for which more PK studies in obese patients are necessary.[49] Linezolid is a second-line anti-TB drug that is found to have a higher degree of safety at a lower dose (≤600 mg a day), while it may still be effective at a dose of 300 mg.[50–52] Moreover, a dose of 600 mg is found to be safer than 1200 mg daily, with adverse effects occurring in respectively 14.3% and 54.4% of the cases, adverse effects mostly representing anemia and thrombocytopenia.[41] If linezolid has to be used at a higher dose to maximize its efficacy, the toxic plasma concentrations should be closely monitored to minimize its toxicity.[53] However, there is still research needed to determine reference values for toxic concentrations of most anti-TB drugs for adequate implementation of TDM.

2.3. Drug susceptibility

In order to select a treatment strategy for a TB patient, the drug susceptibility of the *Mtb* complex isolate to anti-TB drugs is determined on basis of growth inhibition in specialized laboratories. If the causative bacteria are 'susceptible,' the respective drug is included in the regimen; if the bacteria are considered 'resistant,' the drug is excluded.[54]

Nowadays, the vast majority of the DST is performed in the mycobacteria growth indicator tube (MGIT) device at the 'critical concentration.' However, as recently published by an expert group in Europe, to adjust the dose of particular drugs in treatment, especially in complicated cases involving multidrug resistance and severe side effects, there is a need to determine the true MIC.[22,55,56] This provides the possibility to increase the dose of the respective drug to overcome a reduced susceptibility.[57,58] Nowadays, MIC values of rifampicin near the breakpoint are also detected regularly.[59]

In Bangladesh, this appeared to be a risk factor for treatment failure.[60] Recent data showed that a higher dose of rifampicin is well tolerated;[61] therefore, it may be considered in complicated cases to increase the dose of rifampicin. Nevertheless, evidence on long-term outcome supporting this approach is not yet available.

Another disadvantage of the use of the MGIT method for DST is that in fact only inhibition of growth of *Mtb* is measured and not the killing of bacteria. So this method does not take into account the survival of bacteria entering a stage of reduced metabolism to survive the harsh conditions.

Furthermore, for particular drugs, like ethambutol, the reproducibility is questionable due to the close natural distribution of the MIC and the critical concentration.[22,55,62]

2.4. Biomarkers

Nonspecific circulating biomarkers, whose kinetics are associated with the initiation of effective treatment, have been identified. Some of these biomarkers have the potential for point-of-care measurement, notably IP-10.[63,64] The clinical value for treatment optimization and the predictive power of these markers needs to be established in larger trials in different patient populations. To date, no biomarkers have been included in treatment evaluation.

3. Opportunities

3.1. Dried blood spots

Dried blood spot (DBS) analysis is a method in which a small amount of blood is collected on filter paper, after which it is allowed to air dry. At the moment of analysis, a circular punch is taken from the blood spot and eluted with solvent. Hereafter, the dried blood sample is ready to be analyzed. [25] Blood concentrations of anti-TB drugs can be determined from the DBS using high performance liquid chromatography techniques coupled to mass spectrometry.[65,66] Allanson and colleagues were the first to examine the possibility of using DBS in the determination of the first-line anti-TB drug rifampicin in human plasma and blood spots.[24] Vu and co-workers thereafter examined the simultaneous determination of rifampicin, clarithromycin, and their metabolites on basis of DBS. [29] Both studies showed promising results for the use of DBS in TDM of anti-TB drugs. A recent review described which drugs used for pulmonary infections were potential candidates for the development and application of DBS monitoring from an analytical and clinical point of view.[66] DBS has several advantages in comparison to conventional plasma sampling. First, the sampling is cheap and simple, while the blood can be directly analyzed from the blood spot and does not need time-consuming sample preparation. Second, DBS can be stored for longer time periods as a dried sample. This improves the stability and hence cooled shipment is unnecessary.[28,65] For example, the study of Vu showed that the second-line TB drug clarithromycin showed no significant degradation after exposure of the DBS at 50℃ for 60 days. They also showed that rifampicin was stable for 2 months at ambient temperature.[29] Because DBS improves stability, due to the use of dried blood, it could provide an answer for patients in remote areas where the temperature and humidity are high. Stability of samples under high temperature and humidity is of the utmost importance, because most of the new TB cases in 2014 were diagnosed in Southeast Asia and western Pacific regions (58%) and the African region (29%). Furthermore, the use of dried blood reduces the biohazard risk because it reduces contact with liquid blood, making it safe to use in high HIV prevalence areas. Also, FTA DMPK-A and B DBS cards have certain characteristics which makes them able to lyse cells and denature proteins on contact, making sure the

blood is no longer virulent.[66] However, further research should be performed to determine whether *Mtb* the DBS is still viable. Finally, DBS uses smaller blood volumes than conventional plasma sampling and is less invasively collected. This is especially important in children, because even sparse sampling, taking 3 ml of blood per sample, is often too much in relation to their limited total blood volume.[67] From the 9.6 million new TB cases in 2014, an estimated 1.0 million were children.[1] Although it is widely known that the PK of children is significantly different from that of adults, PK studies are usually not performed in children. Therefore, TDM of anti-TB drugs in children is needed. Because of the use of only a limited amount of blood in DBS, collected in a minimally invasive way, DBS could provide children with optimal drug dosing. The advantages of DBS analysis above conventional sampling make it an attractive analysis method for adults as well as children.

3.2. Limited sampling

Limited sampling is a strategy that uses a limited number of optimal sampling time points. In this approach, typically two or three [11,16,23] samples are used to estimate the exposure. Optimal sampling times are calculated during limited sampling studies using PK models and Monte Carlo simulations. When the optimal sampling points have been obtained, they can be used to predict the $AUC_{0-24\,h}$, using population PK. For most anti-TB drugs, the $AUC_{0-24\,h}$/MIC ratio is the best parameter to predict efficacy.[10,17,68] To determine the $AUC_{0-24\,h}$, conventionally 10–15 sample points are needed to cover 80% of the total AUC.[11] A significant amount of blood samples are taken from the patients before- and at several time points after intake of the anti-TB drug.[11,15,16,23,26,27] However, blood sampling is invasive, and should be kept to a minimum. Limited sampling may be of help in minimizing the number of samples. Limited sampling has several other advantages over conventional blood sampling. Because it uses less samples, it is less expensive and less time consuming for the patient as well as for the laboratory analyzing the samples. Besides these advantages, there still remain some problems with limited sampling. For example, there is no qualification available on the laboratories where TDM should be performed. In contrast, according to the WHO guidelines, DST should only be performed at current good laboratory practices (cGLP) certified laboratories. It therefore seems plausible that TDM should also be performed at a cGLP laboratory. Not all laboratories in low- and middle-income countries are GLP licensed, TDM should therefore be performed at a central laboratory in the country that specializes in TDM. A central laboratory also has advantages for DBS analysis because it needs specialized equipment and standardized protocols. In the ideal situation, in each country DBS is performed at a central level along with sophisticated diagnostics. Currently, DBS samples can be sent to international labs, which is much cheaper compared to plasma samples as shipment on dry ice is not needed.[66] Second, only few studies describe limited sampling for one of multiple anti-TB drugs. Alsultan et al. (2015) showed that with limited sampling at 1 and 6 h post dose an AUC could be estimated of levofloxacin.[15] TDM could help find an optimal TB treatment by studying general optimal

dosing and this could be performed in a simpler, less expensive, and less time consuming manner by using limited sampling. Limited sampling may be used for an accurate and precise estimation of the $AUC_{0-24\ h}$ of a few anti-TB drugs, which are marked yes in Table 1. Limited sampling studies have to be performed for every anti-TB drug separately; therefore, more research is necessary to determine if limited sampling can be used for other anti-TB drugs.

3.3. Molecular testing

Because of the complications in phenotypic resistance testing, the acceptance of the molecular approach to determine the presence of resistance mutations of Mtb is increasing. Moreover, the information of molecular resistance tests is readily available. Whereas in the last two decades especially reverse line blot assays [69–71] were applied to detect or exclude the presence of particular resistance mutations in the genome of Mtb, nowadays whole genome sequencing (WGS) provides the possibility to detect all mutations previously associated with resistance.[72] However, the general problem faced at the moment is that the predictive value of resistance mutations is not always clear, especially when they are rarely encountered.[73] For the frequently observed mutations tested in reverse line blot assays, such as the ones associated with rifampicin and isoniazid, the positive- and negative-predictive value is high and this merits direct clinical use of these test results to steer the treatment.[74] For other drugs, especially of the second-line category (Table 2), the predictive value is sometimes somewhat lower and this

confuses the utility of this information. A part of the current confusion may well be caused by the low reproducibility of phenotypic resistance testing along with the fact that in reverse line blot assays only a small subset of the resistance mutations is revealed.

Nonetheless, with the introduction of WGS and hence the screening of the entire genome of Mtb for the presence of resistance mutations, the correlation of resistance indicators and the level of phenotypic resistance will improve rapidly, if this information is collected on a large scale, as is being done in several current initiatives.[72,81] It is conceivable that within a few years a large part of the phenotypic resistance testing could be avoided. Ideally also the correlation between the presence of particular resistance mutations and the level of resistance against all anti-TB drugs will be established, so the selection of the treatment regimen can be based solely on DNA analysis. This will speed up the adjustment of treatment and also avoid cumbersome, time consuming, and potentially dangerous, phenotypic resistance testing, requiring a complicated quality assurance. It has become conceivable in recent years that all information to select the proper treatment of TB in the majority of cases can be traced in the genome of the causative bacteria, also regarding the newly emerging drugs. [82,83] Just the collection of sufficient data to associate the occurrence of resistance mutations with phenotypic resistance, and eventually treatment outcome separates us from introducing this major improvement in TB control. If this becomes reality, most of the laboratory diagnosis for TB could be based on WGS analysis, that is identification of the causative agent to (sub)species- and genotype level, as well as

Table 2. Drug susceptibility test methods and critical concentrations for first- and second-line drug susceptibility tests together with molecular tests.

| | | Genotypic DST | | | Phenotypic DST | | | |
| | | | | | DST critical concentrations (µg/ml) [54] | | | |
Group	Drug	Molecular test	Gene	Genes screened with WGS [72]	Löwenstein–Jensen	Middlebook 7H10	Middlebrook 7H11	MGIT960
1	Pyrazinamide		pncA [75] panD [76]	pncA, rpsA	–	–	–	100
	Ethambutol	MTBDRsl v1[a]	embB [74]	embA, embB, embC, iniA, iniC, manB,rm1D	2	5	7.5	5
	Rifabutin	MTBDRplus[c]	rpoB [77]					0.5
	Isoniazid	MTBDRplus[c]	inhA [74] katG [74]	ahpC, fabG1, inhA, katG, ndh	0.2	0.2	0.2	0.1
	Rifampicin	MTBDRplus[c], Xpert/RIF[d] [38,54]	rpoB [74]	rpoB	40	1	1	1
2	Kanamycin	MTBDRsl v1[a], v2[b]	rrs [74] eis [78]	gidB, rrs, tlyA, eis	30	5	6	2.5
	Amikacin	MTBDRsl v1[a], v2[b]	rrs [74]	gidB, rrs, tylA	30	4	–	1
	Capreomycin	MTBDRsl v1[a], v2[b]	rrs [74]	gidB, rrs, tylA	40	4	–	2.5
	Streptomycin	MTBDRsl v1[a], v2[b]	rrs [79]	rpsL, gidB, rrs, tlyA	4	2	2	1
3	Levofloxacin				–	1	–	1.5
	Moxifloxacin	MTBDRsl v1[a], v2[b]	gyrA [74] gyrB[78]	gyrA, gyrB	–	0.5/2	–	0.5/2
	Ofloxacin	MTBDRsl v1[a], v2[b]	gyrA [78] gyrB [78]	gyrA, gyrB	4	2	2	2
4	P-aminosalicyclic acid				1	2	8	4
	Cycloserine				30	–	–	–
	Ethionamide	MTBDRplus[c]	inhA [80]		40	5	10	5
	Protionamide				40	–	–	2.5
5	Clofazimine				–	–	–	–
	Linezolid				–	–	–	1

DST: Drug susceptibility test; MGIT: mycobacterium growth indicator tube; WGS: whole genome sequencing.
[a]GenoType MTBDRsl version 1 (v1) is for the detection of M. tuberculosis complex and its resistance to fluoroquinolones, aminoglycosides/cyclic peptides, and ethambutol.
[b]GenoType MTBDRsl version 2 (v2) is for the detection of M. tuberculosis complex and its resistance to fluoroquinolones, aminoglycosides/cyclic peptides.
[c]GenoType MTBDRplus is for the detection of M. tuberculosis complex and its resistance to isoniazid and rifampicin.
Xpert/RIF this is a cartridge-based fully automated molecular diagnostic assay for the detection of M. tuberculosis complex and its resistance to rifampicin.

resistance testing and epidemiological typing. This new approach may be much easier to implement in high-prevalence settings than the conventional approach that is based on the availability of a Biosafety Level 3 laboratory and many complicated procedures, which have proven hard to standardize.

3.4. Biomarkers

Established methods to measure bacterial killing include microscopy and culture performed at diagnosis and at month 2, and thereafter monthly until repeatedly negative. This is mostly referred to as AFB and culture conversion, respectively. Thus, these methods can only detect the effect of treatment after 2 months at the earliest. The currently available automated polymerase chain reaction assays are also unsuitable for tracking early bacterial killing, although adaptations to address this weakness have been proposed. [84,85] Innovations in microscopy, able to discriminate between viable and non-viable bacilli, are also under development.[86,87] Detection of mutations associated with resistance as a form of direct susceptibility testing also provides the possibility of predicting a failure to respond to certain drugs, [88] as mentioned above. Interestingly, the possibility of objectively measuring host response by monitoring the kinetics of circulating host markers has recently received increased attention.[89,90] Measuring such markers in parallel with drug levels have the potential to facilitate and simplify personalized therapy. When biomarkers are measured in possibly infected plasma, appropriate measures for the protection of health care and laboratory workers must be implemented.

Here, we will briefly discuss the recent progression in identifying and validating host markers for treatment monitoring. These host markers used are not specific for TB but their potential for simple detection allows measurement of their kinetics in response to therapy, which may provide information to guide treatment and allow more complex specific laboratory-based assays to be targeted at the patients who are failing to respond to the therapy prescribed. Candidate biomarker selections have been based on systematic protein and mRNA profiling and on selection of cytokines/chemokines known to be associated with or involved in the immune response to TB infection.[91–93] It should be noted that for treatment monitoring circulating markers are of primary interest. The stimulated *in vitro* secretion of cytokines by white blood cells in response to challenge with TB antigens has also received considerable attention, but this is more suited to detecting previous exposure to and infection with TB.[94]

From these studies a number of promising markers have been selected, a notable example is IP-10/CXCL-10, a chemokine involved in the establishment of a proinflammatory Th1 response. TB is associated with increased serum IP-10 levels [93,95–97], and at completion of therapy, levels are significantly lower than before treatment/at diagnosis.[64,98,99] This decrease is also measurable in the first weeks of therapy,[63,93] and therefore measurement of the kinetics of this cytokine during treatment may provide useful information on whether patients are responding to the prescribed drugs. It is also notable that host markers are present and detectable in all individuals. Unlike bacterial-derived markers such as LAM, which is one of the most promising bacterial derived biomarkers, which are still undetectable in most infected patients. [100,101] In fact, IP-10 is a particularly interesting treatment response biomarker as it has been shown to be stable in dried plasma spots [64] and is present at high levels that are measurable with the new generation of simple near patient assays, [102] which should facilitate rapid repeated costs effective measurement. IP10, however, measured at a single-time point cannot specifically identify TB, nor distinguish between TB and latent-TB infection. It is possible that combinations of biomarkers may be more informative,[97,99] or for example the kinetics of osteopontin combined with IP-10,[103] which both appear to be associated with microscopic conversion.

The clinical value for treatment optimization and the predictive power of these markers for treatment monitoring need to be established in larger trials with different patient populations. To date, no biomarkers have been included in routine treatment evaluation.

3.5. Conclusion

TDM is currently not included in the management of TB treatment. To date, no randomized controlled trial has evaluated the potential benefits of this strategy for optimization of TB treatment. However, in current literature a lot of supportive evidence can be found. For example, *in vitro* studies have shown a relation between drug exposure and efficacy of treatment. In addition, acquired drug resistance could be predicted by these models. Furthermore, in actual patients a high variability in drug exposure has been shown for several drugs supporting the idea that drug exposure could play a significant role in treatment outcome. To overcome current hurdles to implement TDM in TB treatment, many new techniques have become available over the recent years. DBS analysis in combination with limited sampling strategies will enable more simple collection of patients' samples to adequately assess drug exposure. Rapid diagnosis of bacterial resistance, using molecular tests (Table 2), will be enable to start the most appropriate drug regimen up front, preventing treatment with drugs that show resistance once results from classical drug susceptibility tests have become available. Biomarker kinetics linked to clinical response, is potentially easily measurable and may also help to optimize treatment.

4. Expert opinion

4.1. DBS

DBS shows promising results for application for TDM of anti-TB drugs in developed countries and less developed countries. [29] However, some issues need to be addressed before DBS can be implemented for TDM. DBS has only been validated for a few drugs, which have been marked with yes in Table 1. The remaining anti-TB drugs must be individually validated for application of DBS.[104] The validation process of DBS is more extensive than a normal method validation, while for example hematocrit variation has to be taken into account. Another problem is the blood spot sampling, while the blood

spot has to satisfy certain requirements, such as filling the predefined 3 mm diameter circle with only one drop of blood. When these requirements are not met, analysis of the DBS sample cannot be performed. To make sure DBS is performed accurately, training of personnel is necessary. A future opportunity would be a combined LC–MS/MS method, where multiple anti-TB drug concentrations are determined from one DBS sample.[105] This would reduce analyzing time and would provide an easier method. This seems to be a possibility looking at the study of Han et al.,[106] but this need to be validated for DBS.

4.2. Limited sampling

Because PK/PD guided TDM requires an AUC for most of the anti-TB drugs, which is costly and time consuming, limited sampling could provide a long-awaited solution. Limited sampling time points need to be evaluated for each anti-TB drug individually. In order to obtain these, population PK studies are necessary, which are not readily available for all anti-TB drugs. To obtain this information, several studies remain to be conducted. In addition, it would be very helpful and an asset to TDM to have a limited sampling strategy that is suitable for more than one drug. Such a strategy is available for first-line drugs (Table 1), but has not been established for second-line drugs (Table 1).[16] Furthermore, it is likely that limited sampling strategies need to be confirmed in different populations due to variability caused by pharmacogenetic differences. However, hopefully in the future this information is readily available for limited sampling to be performed routinely, because of the many advantages of limited sampling to conventional blood sampling.

4.3. Biomarkers and molecular testing

Using culture conversion and AFB conversion as indicators of treatment, response in the era of molecular diagnostics is too slow. A simple point of care test to objectively monitor clinical response would be highly valuable, especially if data on drug levels and bacterial genetic resistance markers are also available. This is a rapidly developing field, and initial combined results of multiple groups suggest measurement of circulating host markers contain highly valuable information on response within 14 days. However, monitoring of large cohorts of patients with- and without drug resistance and follow-up on treatment outcome is required for reliable validation. Much more focus is needed on treatment outcome and strategies to optimize the treatment of individual patients on the basis of clinical, bacteriological, and PK data. Drug susceptibility and resistance testing, including conventional DST and the detection of bacteriological genetic markers of resistance, could both serve as surrogate markers for patient response. If patient response could be measured directly and linked to blood drug levels and genotypic mutations, personalized treatment could be realized on a large scale. More data are expected on the impact of drug resistance and bacteriological factors (genotype and resistance profile) on host response and the link with successful outcome. But the ultimate challenge is to find predictors of cure or measure of relapse early in

treatment. Therefore, much more effort should be put in the exploration and validation of host biomarker kinetics.

4.4. Integrated TDM approach to optimize TB treatment

To truly optimize TB treatment, an integrated approach a combination of all next generation tools is needed. The most appropriate drugs are selected, based on molecular resistance tests (Table 2) and a combination of information on drug exposure and biomarker response with clinical expertise is employed to further tailor treatment to the individual patients' needs (Figure 2).

A new strategy, in which slow phenotypic evaluation of bacterial cultures can be avoided, will allow effective treatment to be much more rapidly identified and will allow failing patients to be provided with effective drugs. In addition, biomarker and drug exposure evaluation will avoid the need to wait for classical evaluation based on sputum culture conversion. However, also with these new markers, a rapid turnaround time of results and integration of all next generation tools is key to meeting the expectations of the clinician and the patient.

Although implementing TDM will increase the direct costs of TB treatment, it can prevent hospitalization due to toxicity and it can prevent development of drug resistance leading to MDR-TB treatment, which is even more expensive. To date, no randomized controlled trials have been performed comparing TDM with standard of care. Therefore a cost-effectiveness analysis is difficult to perform. This may hinder implementation of TDM in TB treatment. [105,107] TDM using limited sampling and available AUC/ MIC indices, should be performed for isoniazid and rifampicin in selected patients to prevent the occurrence of MDR-TB. TDM has already been applied for second-line anti TB drugs to reduce toxicity and increase efficacy. However, due to lacking target values for some second-line anti-TB drugs
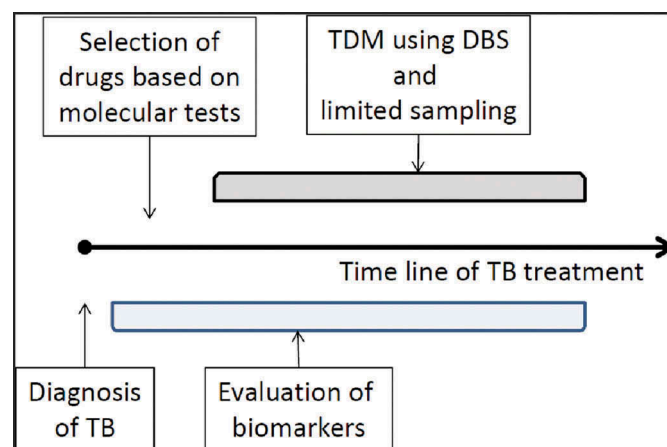
Figure 2. Next generation approach of integrated therapeutic drug monitoring approach including molecular testing and biomarker evaluation.
First, TB is diagnosed, where after drugs are selected based on molecular resistance tests. A baseline biomarker is then selected, which can be followed throughout treatment. The biomarker can help to determine whether the treatment is effective and if changes in the regimen need to be made. TDM is done during the entire course of treatment using the dried blood spot (DBS) approach. TB: tuberculosis; TDM: Therapeutic Drug Monitoring; DBS: Dried Blood Spots.

and randomized clinical trials it has not yet been included in WHO treatment guidelines. In the ideal situation, TDM will be integrated in TB treatment to optimize care in individual patients.

Declaration of interest

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•• of considerable interest

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