BMJ Open

Prospective Evaluation of impRoving Fluoroquinolone Exposure using Centralized TDM in patients with Tuberculosis (PERFECT) – a study protocol of a prospective multicentre cohort study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035350
Article Type:	Protocol
Date Submitted by the Author:	29-Oct-2019
Complete List of Authors:	van den Elsen, Simone; University of Groningen, University Medical Center Groningen, Clincal Pharmacy and Pharmacology Sturkenboom, Marieke; University of Groningen, University Medical Center Groningen, Clincal Pharmacy and Pharmacology Akkerman, Onno; University of Groningen, University Medical Center Groningen, Department of Pulmonary Diseases & Tuberculosis; University of Groningen, University Medical Center Groningen, Tuberculosis Center Beatrixoord Barkane, Linda; Riga East University Hospital TB and Lung Disease Clinic, MDR-TB department Bruchfeld, Judith; Karolinska Institutet, Division of Infectious Diseases, Department of Medicine, Solna; Karolinska University Hospital, Department of Infectious Diseases Eather, Geoffrey; Princess Alexandra Hospital, Department of Respiratory Medicine & Metro South Clinical Tuberculosis Service Heysell, Scott; University of Virginia, Division of Infectious Diseases and International Health Hurevich, Henadz; The Republican Scientific and Practical Center for Pulmonology and Tuberculosis Kuksa, Liga; Riga East University Hospital TB and Lung Disease Clinic, MDR-TB department Kunst, Heinke; Barts Health NHS Trust, Blizard Institute, Queen Mary University of London, Department of Respiratory Medicine Kuhlin, Johanna; Karolinska Institutet, Department of Medicine, Unit of Infectious Disease; Karolinska University Hospital, Department of Infectious Diseases Manika, Katerina; Aristotle University of Thessaloniki, G. Papanikolaou Hospital, Pulmonary Department, Respiratory Infections Unit Moschos, Charalampos; "Sotiria" Hospital for Chest Diseases, Drug-Resistant Tuberculosis Unit Mpagama, Stellah; Kibong'oto Infectious Diseases Hospital Muñoz Torrico, Marcela; Instituto Nacional de Enfermedades Respiratorias, Clínica de Tuberculosis Skrahina, Alena; The Republican Scientific and Practical Center for Pulmonology and Tuberculosis Sotgiu, Giovanni; University of Sassari, Clinical Epidemiology and Medical Statistics Unit, Department of Medical, Surgical and Experimental Sciences,

	Tadolini, Marina; Alma Mater Studiorum University of Bologna, Unit of Infectious Diseases, Department of Medical and Surgical Sciences Tiberi, Simon; Barts Health NHS Trust, Blizard Institute, Queen Mary University of London, Department of Infection Volpato, Francesca; Alma Mater Studiorum University of Bologna, Unit of Infectious Diseases, Department of Medical and Surgical Sciences van der Werf, Tjip S.; University Medical Center Groningen, Department of Pulmonary Diseases & Tuberculosis; University of Groningen, University Medical Center Groningen, Department of Internal Medicine Wilson, Malcolm; Princess Alexandra Hospital, Department of Respiratory Medicine & Metro South Clinical Tuberculosis Service Zúñiga , Joaquin; Instituto Nacional de Enfermedades Respiratorias, Laboratory of Immunobiology and Genetics; Escuela de Medicina y Ciencias de Salud, Tecnologico de Monterrey Touw, Daan; University of Groningen, University Medical Center Groningen, Clincal Pharmacy and Pharmacology Migliori, Giovanni; Istituti Clinici Scientifici Maugeri IRCCS, Servizio di Epidemiologia Clinica delle Malattie Respiratorie Alffenaar, Jan-Willem; University of Groningen, University Medical Center Groningen, Clincal Pharmacy and Pharmacology; The University of Sydney, Sydney Pharmacy School, Faculty of Medicine and Health
Keywords:	Tuberculosis < INFECTIOUS DISEASES, CLINICAL PHARMACOLOGY, Organisation of health services < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, INFECTIOUS DISEASES

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Prospective Evaluation of impRoving Fluoroquinolone Exposure using Centralized TDM in patients with Tuberculosis (PERFECT) – a study protocol of a prospective multicentre cohort study.

Simone HJ van den Elsen[a], Marieke GG Sturkenboom[a], Onno W Akkerman[b,c], Linda Barkane[d], Judith Bruchfeld[e,f], Geoffrey Eather[g], Scott K Heysell[h], Henadz Hurevich[i], Liga Kuksa[d], Heinke Kunst[j], Johanna Kuhlin[e,f], Katerina Manika[k], Charalampos Moschos[l], Stellah G Mpagama[m], Marcela Muñoz-Torrico[n], Alena Skrahina[o], Giovanni Sotgiu[p], Marina Tadolini[q], Simon Tiberi[r], Francesca Volpato[q], Tjip S van der Werf[c,s], Malcolm R Wilson[g], Joaquin Zuñiga[t], Daan J Touw[a] #, Giovanni B Migliori[u] #, and Jan-Willem C Alffenaar[a,v] #

[a] University of Groningen, University Medical Center Groningen, Department of Clinical Pharmacy and Pharmacology, Groningen, The Netherlands.

[b] University of Groningen, University Medical Center Groningen, Tuberculosis Center Beatrixoord, Haren, The Netherlands

[c] University of Groningen, University Medical Center Groningen, Department of Pulmonary Diseases & Tuberculosis, Groningen, The Netherlands.

[d] MDR-TB department, Riga East University Hospital TB and Lung Disease Clinic, Riga, Latvia.

[e] Division of Infectious Diseases, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden.

[f] Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden.

[g] Department of Respiratory Medicine & Metro South Clinical Tuberculosis Service, Princess Alexandra Hospital, Woolloongabba, Queensland, Australia.

[h] Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, VA, USA.

[i] The Republican Scientific and Practical Center for Pulmonology and Tuberculosis, Minsk, Belarus.

[j] Blizard Institute, Queen Mary University of London, Department of Respiratory Medicine, Barts Health NHS Trust, London, United Kingdom.

[k] Pulmonary Department, Respiratory Infections Unit, Aristotle University of Thessaloniki, G. Papanikolaou Hospital, Thessaloniki, Greece.

[l] Drug-Resistant Tuberculosis Unit, "Sotiria" Hospital for Chest Diseases, Athens, Greece

[m] Kibong'oto Infectious Diseases Hospital, Kilimanjaro, Tanzania.

[n] Clínica de Tuberculosis, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico.

[o] The Republican Scientific and Practical Center for Pulmonology and Tuberculosis, Minsk, Belarus.

[p] Clinical Epidemiology and Medical Statistics Unit, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy

[q] Unit of Infectious Diseases, Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy.

[r] Blizard Institute, Queen Mary University of London, Department of Infection, Barts Health NHS Trust, London, United Kingdom

[s] University of Groningen, University Medical Center Groningen, Department of Internal Medicine, Groningen, The Netherlands.

[t] Laboratory of Immunobiology and Genetics, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico. Tecnologico de Monterrey, Escuela de Medicina y Ciencias de Salud, Mexico City, Mexico.

[u] Servizio di Epidemiologia Clinica delle Malattie Respiratorie, Istituti Clinici Scientifici Maugeri IRCCS, Tradate, Italy.

[v] Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia

# Authors contributed equally

Corresponding author: J.W.C. Alffenaar, University of Groningen, University Medical Center Groningen, Department of Clinical Pharmacy and Pharmacology, Groningen, The Netherlands. Email: j.w.c.alffenaar@umcg.nl

Word count: 2604

Abstract

Introduction: Global multidrug-resistant tuberculosis (MDR-TB) treatment success rates remain suboptimal. Highly active World Health Organization (WHO) Group A drugs moxifloxacin and levofloxacin show intra- and inter-individual pharmacokinetic variability which can cause low drug exposure. Therefore, therapeutic drug monitoring (TDM) of fluoroquinolones is recommended to personalise the drug dosage, aiming to prevent development of drug resistance and optimize treatment. However, TDM is considered laborious and expensive, and the clinical benefit in MDR-TB has not been extensively studied. This observational multicentre study aims to determine the feasibility of centralized TDM and to investigate the impact of fluoroquinolone TDM on sputum conversion rates in patients with MDR-TB compared with historical controls.

Methods and analysis: Patients aged 18 years or older with sputum smear and culture positive pulmonary MDR-TB will be eligible for inclusion. Patients receiving TDM using a limited sampling strategy (t=0 and t=5 hours) will be matched to historical controls without TDM in a 1:2 ratio. Sample analysis and dosing advice will be performed in a centralized laboratory. Centralized TDM will be considered feasible if >80% of the dosing advices is returned within seven days after sampling and 100% within fourteen days. The number of patients who are sputum smear and culture negative after two months of treatment will be determined in the prospective TDM group and will be compared to the control group without TDM to determine the impact of TDM.

Ethics and dissemination: All participating centres obtained ethical clearance according to local procedures. Patients will be included after written informed consent. We aim to publish the study results in a peer-reviewed journal.

Trial registration: This study is registered at clinicaltrials.gov (NCT03409315)

Strengths and limitations of this study

- To our knowledge, this is the first study that investigates the impact of fluoroquinolone TDM on sputum smear and culture conversion rates in prospective patients with MDR-TB versus historical controls without TDM.
- The feasibility for centralised TDM will be evaluated due to participation of multiple health care centres located in differently resourced countries from multiple regions in the world.
- The use of limited sampling strategies will reduce the burden of TDM for patients and health care providers while still providing a reliable estimation of drug exposure.
- A limitation is that this study focuses on TDM for moxifloxacin and levofloxacin only, being core drugs in MDR-TB treatment, without assessing other (core) anti-tuberculosis drugs.

Introduction

Tuberculosis (TB) is one of the major infectious diseases worldwide with an estimated number of 10.0 million new cases in 2017.[1] In addition, multidrug-resistant TB (MDR-TB) remains a persistent problem with an estimated 458,000 new patients in 2017.[1] MDR-TB is treated from 9-20 months with a multidrug regimen.[2] The grouping of second-line anti-TB drugs was revised in 2018 by the World Health Organisation (WHO).[3] The fluoroquinolones, specifically moxifloxacin and levofloxacin, are now considered drugs of first choice (Group A drugs), together with bedaquiline and linezolid, in the treatment of MDR-TB.[2,3] The administration of Group A medicines to patients with MDR-TB has been associated with increased treatment success and reduced mortality rates in comparison with other second-line anti-TB drugs.[4] However, the estimated prevalence of fluoroquinolone-resistance among MDR-TB cases is on the rise from 14.5% in 2011 to 22% in 2017.[5,6] Mismanagement of MDR-TB treatment, especially the shorter regimen, could amplify the risk of drug resistance even further.[7] Importantly, antibiotic resistance can be acquired due to noncompliance but also insufficient drug exposures (e.g. inter-individual pharmacokinetic variability in patients treated with fluoroquinolones).[8–11] Therapeutic drug monitoring (TDM) can help to prevent acquired resistance by individualising doses based on blood drug concentrations relative to the bacterial susceptibility, ideally measured as the minimal inhibitory concentration (MIC).[7,12] Several studies described the role played by low drug concentrations on treatment outcomes.[13–15] In the light of this evidence, it can be hypothesized that TDM, which aims for adequate dosing and exposure, could improve treatment outcomes. Yet, the added value of TDM in MDR-TB treatment outcomes has not been directly studied.[16,17] One retrospective study reported the effect of TDM on the treatment results of patients with drug-susceptible TB, either with and without diabetes.[18] In the group without diabetes, TDM had a significant beneficial effect with 73% sputum culture conversion at two months amongst patients receiving TDM versus 60% in the control group. The positive effect of TDM was even larger in patients with diabetes and TB. To the best of our knowledge, such controlled studies have not yet been performed in people with MDR-TB.

The pharmacokinetic-pharmacodynamic parameter of fluoroquinolones is both time- and concentration dependent and therefore uses the ratio of area under the concentration time curve to minimal inhibitory concentration (AUC_{0-24}/MIC) with a target value of >146 for levofloxacin and free or unbound *f*AUC_{0-24}/MIC >53 for moxifloxacin.[19,20] However, multiple concentration measurements widely distributed over the dosing interval are required to compute the AUC_{0-24}. Limited sampling strategies (LSS) could be adopted to reduce the burden of frequent sampling for both patient and personnel while providing a reliable estimation of AUC_{0-24} using only two blood samples.[21,22]

Unfortunately, TDM is not always easily accessible in high TB burden areas because of practical and financial reasons. Therefore, centralized TDM could be a valuable service.[23] Large laboratories are generally well organised, have highly trained personnel with adequate performance of analytical methods leading to reliable sample analysis results.[24] In addition, centralizing the TDM procedures will engender more consistent practice from health care practitioners familiar with TDM and the provision of dosing advice for anti-tuberculosis drugs.

The aim of the present study is, firstly, to investigate the feasibility of centralized TDM of moxifloxacin and levofloxacin in the treatment of MDR-TB recruited in TB reference centres located in different continents. Secondly, the impact of TDM on treatment results will be assessed by comparing two month sputum smear and culture conversion rates among patients who received TDM compared with matched historical controls without TDM.

Methods and analysis

Study design

This observational, prospective, multicentre study aims to evaluate the feasibility of centralized TDM of moxifloxacin and levofloxacin as well as the impact of TDM on two month sputum smear and culture conversion rates of patients with MDR-TB. Study design and procedures are displayed in

Figure 1. The study was registered at clinicaltrials.gov (NCT03409315) and started on 10 February 2018.

Study location

University Medical Center Groningen (UMCG) in Groningen, The Netherlands is the coordinating centre and serves as central laboratory facility for this study. The hospitals that are involved in patient recruitment are displayed in Table 1.

Study population

Patients aged 18 years and older are eligible for inclusion if they are diagnosed with pulmonary MDR-TB, have positive sputum smear and culture samples at time of inclusion, are treated with either oral moxifloxacin or levofloxacin, and provide written informed consent. Pregnant or breast feeding women will be excluded.

A total number of 120 patients (60 with moxifloxacin, 60 with levofloxacin) will be prospectively included and compared with 240 matched historical controls (120 with moxifloxacin, 120 with levofloxacin).

Historical control patients will be matched on age, sex, *M. tuberculosis* resistance pattern of the isolate (only regimen core drugs), comorbidities (HIV, diabetes, immunosuppression), presence or absence of cavitary TB on chest radiography, and dosing of the fluoroquinolone (mg/kg body weight, ±10%) to prospectively enrolled patients in a 2:1 ratio.

Interventions

The objective of the feasibility of centralized TDM will be assessed by evaluating the process, by which a locally collected sample will be analysed in a central laboratory and subsequent dosing advice will be returned to the local physician. In brief, after at least seven days of treatment (steady state) two blood samples will be collected for TDM of moxifloxacin or levofloxacin according to a

previously developed LSS.[21,22] The first sample will be collected just before drug intake (t=0) and the other at 5 hours after drug intake (t=5). Samples will be transported to the central laboratory for drug analysis and will be accompanied by a form including key patient characteristics for personalised dosing advice (i.e. sex, age, weight, height, serum creatinine, corrected QT (QTc) interval, MIC, TB presentation, start of treatment, other anti-TB drugs, and comorbidities). AUC_{0-24} will be calculated using a population pharmacokinetic model [21,22] and Bayesian dose optimisation in MWPharm++ (version 1.7.3; Mediware, Groningen, The Netherlands).

Dosing is optimised based on AUC_{0-24}/MIC or AUC_{0-24} (in case MIC is unknown), taking into consideration comorbidities (HIV, diabetes, and immunosuppression) and clinical condition of the patient. The target AUC_{0-24}/MIC and AUC_{0-24} are shown in Table 1. If a dose change is necessary, TDM is to be repeated after at least seven days after the initiation of the new dose (steady state). Dose increases of moxifloxacin will not be advised in case of a prolonged QTc interval (>450 ms for males, >470 ms for females), because of safety reasons. As levofloxacin is less cardiotoxic than moxifloxacin, levofloxacin dose increases are permitted in case of prolonged QTc interval with frequent electrocardiogram monitoring. Patients with prolonged QTc interval will not be excluded from the study, since TDM can still be helpful to verify drug exposure. A closely monitored follow-up including MIC determination can be advised in case of AUC_{0-24} of 25 to 40 mg*h/L in combination with QTc interval prolongation. In case of very low moxifloxacin exposure (AUC_{0-24}<20 mg*h/L) in combination with a prolonged QTc interval, the physician will be advised to reconsider the anti-TB regimen as moxifloxacin may be less active than expected.

Laboratory methods

Drug analysis:

Measurement of moxifloxacin and levofloxacin plasma/serum concentrations will take place at the laboratory of the department of Clinical Pharmacy and Pharmacology in the UMCG, The Netherlands, and using validated liquid chromatography-mass spectrometry (LC-MS/MS) methods. The method for

levofloxacin has an accuracy of 0.1-12.7%, within-run precision of 1.4-2.4%, and between-run precision of 3.6-4.1%. The calibration curve is linear over a range of 0.10 to 5.00 mg/L.[25] Accuracy of the moxifloxacin method is 2.7-7.1%, within-run precision 1.4-1.6%, and between-run precision 1.0-1.6%. The calibration curve is linear over a range of 0.05 to 5.00 mg/L.[26] Only the total moxifloxacin concentration (bound and unbound) will be measured, therefore we assume a constant protein binding of 50%.[27]

Plasma and serum samples containing levofloxacin are stable for at least ten days at 50 ^{o}C and can therefore be transported to the central facility in ambient temperature, without the need of transport on dry ice.[28] The thermal stability of moxifloxacin was also tested according to the method of Ghimire *et al* and showed that moxifloxacin serum and plasma samples are stable for at least ten days at 50 ^{o}C as well (data on file).

Microbiology:

The assessment of sputum smear and culture status after two months of MDR-TB treatment will be performed according to the local procedures, but at least once a month until documented culture conversion. MIC determination is preferred but not mandatory for TDM and will be performed according to local procedures as well. To account for the differences in culture media used in drug susceptibility testing, correction factors based on the critical concentrations in the WHO-document "Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis" will be applied.[29] The target AUC_{0-24}/MIC values for each medium are shown in Table 2. Furthermore, second line molecular drug susceptibility tests will be considered in case MIC data are not available.

Data analysis plan

The primary outcome to assess the feasibility of centralized TDM will be the turn-around time, which is defined by the time between blood sampling and the peripheral centres receiving the TDM results

including the dosing advice. The procedure is considered feasible if >80% of the collected samples will be reported back to the physician within seven days and 100% within two weeks. Additionally, the feasibility will be evaluated using secondary outcomes of sample quality after shipping and completeness of required information on the sample form.

Furthermore, we will evaluate the role of TDM on MDR-TB treatment by comparing the percentages of patients with sputum smear and culture conversion at two months in the enrolled groups. In addition, we will evaluate the number of patients with low fluoroquinolone exposure requiring dose changes after TDM to estimate the potential gains.

Sample size calculation

As the primary endpoint was of descriptive nature and no data were available to perform a well-informed sample size calculation, it was decided to power the study on the clinical impact of TDM. The primary assumption was based on the detection of a proportional difference in sputum smear and culture positivity at two months of treatment in patients with MDR-TB undergoing TDM (35%) [30] and control patients (60%)[31]. Given an alpha error of 0.05 and statistical power of 80%, we calculated a sample size of 60 per single group is needed (i.e. 60 prospective and 120 historical control patients for moxifloxacin and equally for levofloxacin).

Ethics and dissemination

This study will be performed according to the Declaration of Helsinki and Good Clinical Practice.[32] In each centre ethical clearance has been granted according to local regulations and patient recruitment has begun at most sites. Written informed consent will be obtained from all patients undergoing TDM. The need of new informed consent for historical controls was waived, because of the use of retrospective anonymous data collected for programmatic purposes or previously reported data from studies for which patients had provided informed consent.

This study includes historical patients who did not receive TDM as controls instead of prospectively randomising patients to either receive or not receive TDM for ethical reasons. The evidence that TDM actually improves MDR-TB treatment outcomes has not been confirmed in randomised controlled trials, but multiple studies have described treatment failure and risk of antibiotic resistance due to sub therapeutic drug exposure of anti-TB drugs.[8,13,15,19,20] In combination with a large between-patient pharmacokinetic variability [9,10], we hypothesize that TDM is able to improve treatment outcomes by ensuring adequate exposure in individual patients. Moreover, TDM for MDR-TB is recommended in guidelines when it is available.[2,33,34] We therefore considered it unethical to withhold TDM.

Study results will be published in a peer-reviewed journal and will be presented at an international conference.

Discussion

We present an observational prospective multicentre study which aims to: a) evaluate the feasibility of centralized TDM in differently resourced settings of varying TB endemicity and geographic region and b) evaluate the role of TDM of moxifloxacin or levofloxacin on sputum smear and culture conversion rates in patients with MDR-TB after two months of treatment.

Presently, TDM is offered as an adjunctive to patients with TB in only a few hospitals worldwide and is considered to be part of the excellent clinical care.[16,23,35–37] However, general interest in TDM and MDR-TB treatment optimization has been increasing. A consensus statement on the diagnosis and treatment of MDR-TB in Europe states that TDM for second-line drugs should be used if available.[34] Moreover, the use of second-line anti-TB drugs was listed in the American Thoracic Society (ATS) guidelines as indication for TDM and TDM is also recommended in the European Union Standards for Tuberculosis Prevention and Care.[33,38] Yet, TDM is considered by some to be laborious, expensive and thus unpractical in countries with high TB incidence. Similar injurious arguments of economistic rationing of services were applied to second-line drugs for the treatment

of MDR-TB in highly endemic settings and such rationing conversely led to amplification of the MDR-TB epidemic.[39] This study will focus on the feasibility of centralized TDM, which could stimulate performing TDM more often as it requires only one qualified laboratory with validated analytical methods and devices in a central location. Other options to facilitate TDM are the implementation of LSS, urine samples, dried-blood spots and saliva-screening methods.[35,40–42] Although incorporating TDM in TB treatment has shown to give high treatment success rates in low endemic countries, like the Netherlands [30], this has not yet been evaluated in well-designed randomized controlled trials.[43] This study will provide a first-ever conclusion on the value of TDM of moxifloxacin and levofloxacin on sputum smear and culture conversion of patients with MDR-TB.

It can be considered a limitation that only TDM of fluoroquinolones is performed in this study. However, moxifloxacin and levofloxacin are currently among the core drugs in the MDR-treatment regimen together with linezolid and bedaquiline.[3] Based on TDM criteria [44], we have selected moxifloxacin and levofloxacin, because they show large inter-individual pharmacokinetic variability, which emphasizes the need for personalized dosing.[9,10] Moreover, fluoroquinolone resistance is on the rise and can develop during low drug exposure.[8] TDM of fluoroquinolones aims to find the individual patients who have low drug exposure and would benefit from dose adjustment. Therefore, it is expected that TDM of fluoroquinolones will have the largest impact on MDR-TB treatment outcomes. We did not include TDM for linezolid and bedaquiline in this study, because of unclear evidence for TDM of bedaquiline due to the novelty of the drug [45] and TDM of linezolid has focussed more on preventing toxicity.[46–48]

Another limitation is that we are only evaluating interim outcomes such as sputum conversion rates at two months and will not assess outcomes at the end of treatment. However, this study is primarily designed to determine the feasibility of centralized TDM. In addition, this is the first study to evaluate the impact of fluoroquinolone TDM. We believe that reporting the results on sputum conversion rates is relevant as bacterial load and risk of acquired resistance are highest in the first months of therapy. Fast sputum culture conversion reduces the risk of transmission of *M.*

tuberculosis strains which continues to sustain the MDR-TB epidemic.[49] With the results of this study we aim to design a future study to extensively evaluate TDM of all drugs in the regimen including the final treatment outcomes. However, such study would require substantial funding. We hope that this study will show that centralized TDM is feasible and that TDM can improve the quality of treatment in terms of faster sputum conversion rates compared to historical experience. If that might be the case, the major hesitations about TDM in TB treatment can be attenuated favouring the improvement of TB management using a personalized approach.[38]

References

1 World Health Organization. Global tuberculosis report 2018. 2018.

2 World Health Organization. WHO consolidated guidelines on drug-resistant tuberculosis treatment. 2019.

3 World Health Organization. Rapid Communication: Key changes to treatment of multidrug- and rifampicin-resistant tuberculosis (MDR/RR-TB). 2018.

4 Ahmad N, Ahuja SD, Akkerman OW, *et al.* Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis: an individual patient data meta-analysis. *Lancet* 2018;**392**:821–34. doi:10.1016/S0140-6736(18)31644-1

5 Alffenaar J-WC, Migliori GB, Gumbo T. Multidrug-resistant tuberculosis: pharmacokinetic and pharmacodynamic science. Lancet. Infect. Dis. 2017;**17**:898. doi:10.1016/S1473-3099(17)30449-8

6 World Health Organization. Global tuberculosis report 2012. 2012.

7 Davies Forsman L, Bruchfeld J, Alffenaar J-WC. Therapeutic drug monitoring to prevent acquired drug resistance of fluoroquinolones in the treatment of tuberculosis. *Eur Respir J* 2017;**49**. doi:10.1183/13993003.00173-2017

8 Srivastava S, Pasipanodya JG, Meek C, *et al.* Multidrug-resistant tuberculosis not due to noncompliance but to between-patient pharmacokinetic variability. *J Infect Dis*

2011;**204**:1951–9. doi:10.1093/infdis/jir658

9 Van't Boveneind-Vrubleuskaya N, Seuruk T, van Hateren K, *et al.* Pharmacokinetics of Levofloxacin in Multidrug- and Extensively Drug-Resistant Tuberculosis Patients. *Antimicrob Agents Chemother* 2017;**61**:e00343-17. doi:10.1128/AAC.00343-17

10 Pranger AD, van Altena R, Aarnoutse RE, *et al.* Evaluation of moxifloxacin for the treatment of tuberculosis: 3 years of experience. *Eur Respir J* 2011;**38**:888–94. doi:10.1183/09031936.00176610

11 Ghimire S, Van't Boveneind-Vrubleuskaya N, Akkerman OW, *et al.* Pharmacokinetic/pharmacodynamic-based optimization of levofloxacin administration in the treatment of MDR-TB. *J Antimicrob Chemother* 2016;**71**:2691–703. doi:10.1093/jac/dkw164

12 Alffenaar J-WC, Gumbo T, Aarnoutse RE. Acquired drug resistance: we can do more than we think! Clin. Infect. Dis. 2015;**60**:969–70. doi:10.1093/cid/ciu1146

13 Pasipanodya JG, McIlleron H, Burger A, *et al.* Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *J Infect Dis* 2013;**208**:1464–73. doi:10.1093/infdis/jit352

14 Modongo C, Pasipanodya JG, Magazi BT, *et al.* Artificial Intelligence and Amikacin Exposures Predictive of Outcomes in Multidrug-Resistant Tuberculosis Patients. *Antimicrob Agents Chemother* 2016;**60**:5928–32. doi:10.1128/AAC.00962-16

15 Sekaggya-Wiltshire C, von Braun A, Lamorde M, *et al.* Delayed Sputum Conversion in TB-HIV Co-Infected Patients with Low Isoniazid and Rifampicin Concentrations. *Clin Infect Dis* 2018;**67**:708–16. doi:10.1093/cid/ciy179

16 Alffenaar J-WC, Tiberi S, Verbeeck RK, *et al.* Therapeutic Drug Monitoring in Tuberculosis: Practical Application for Physicians. *Clin Infect Dis* 2017;**64**:104–5. doi:10.1093/cid/ciw677

17 Peloquin C. The Role of Therapeutic Drug Monitoring in Mycobacterial Infections. *Microbiol Spectr* 2017;**5**:TNMI7-0029–2016. doi:10.1128/microbiolspec.TNMI7-0029-2016

18 Alkabab Y, Keller S, Dodge D, *et al.* Early interventions for diabetes related tuberculosis associate with hastened sputum microbiological clearance in Virginia, USA. *BMC Infect Dis*

2017;**17:125**:1–8. doi:10.1186/s12879-017-2226-y

19 Deshpande D, Pasipanodya JG, Mpagama SG, *et al.* Levofloxacin Pharmacokinetics/Pharmacodynamics, Dosing, Susceptibility Breakpoints, and Artificial Intelligence in the Treatment of Multidrug-resistant Tuberculosis. *Clin Infect Dis* 2018;**67**:S293–302. doi:10.1093/cid/ciy611

20 Gumbo T, Louie A, Deziel MR, *et al.* Selection of a moxifloxacin dose that suppresses drug resistance in Mycobacterium tuberculosis, by use of an in vitro pharmacodynamic infection model and mathematical modeling. *J Infect Dis* 2004;**190**:1642–51. doi:10.1086/424849

21 van den Elsen SHJ, Sturkenboom MGG, Van't Boveneind-Vrubleuskaya N, *et al.* Population Pharmacokinetic Model and Limited Sampling Strategies for Personalized Dosing of Levofloxacin in Tuberculosis Patients. *Antimicrob Agents Chemother* 2018;**62**. doi:10.1128/AAC.01092-18

22 van den Elsen SHJ, Sturkenboom MGG, Akkerman OW, *et al.* Limited sampling strategies using linear regression and the Bayesian approach for therapeutic drug monitoring of moxifloxacin in tuberculosis patients. *Antimicrob Agents Chemother* Published Online First: April 2019. doi:10.1128/AAC.00384-19

23 Ghimire S, Bolhuis MS, Sturkenboom MGG, *et al.* Incorporating therapeutic drug monitoring into the World Health Organization hierarchy of tuberculosis diagnostics. *Eur Respir J* 2016;**47**:1867–9. doi:10.1183/13993003.00040-2016

24 Schimke I. Quality and timeliness in medical laboratory testing. *Anal Bioanal Chem* 2009;**393**:1499–504. doi:10.1007/s00216-008-2349-5

25 Ghimire S, van Hateren K, Vrubleuskaya N, *et al.* Determination of levofloxacin in human serum using liquid chromatography tandem mass spectrometry. *J Appl Bioanal* 2018;**4**:16–25. doi:10.17145/jab.18.004

26 Pranger AD, Alffenaar J-WC, Wessels AMA, *et al.* Determination of moxifloxacin in human plasma, plasma ultrafiltrate, and cerebrospinal fluid by a rapid and simple liquid

chromatography- tandem mass spectrometry method. *J Anal Toxicol* 2010;**34**:135–41.

27 Wright DH, Brown GH, Peterson ML, *et al.* Application of fluoroquinolone pharmacodynamics. *J Antimicrob Chemother* 2000;**46**:669–83.

28 Ghimire S, Jongedijk EM, van den Elsen SHJ, *et al.* Cross validation of liquid chromatography tandem mass spectrometry method for quantification of levofloxacin in saliva. *Submitted*

29 World Health Organization. Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. 2018.

30 van Altena R, de Vries G, Haar CH, *et al.* Highly successful treatment outcome of multidrug-resistant tuberculosis in the Netherlands, 2000-2009. *Int J Tuberc Lung Dis* 2015;**19**:406–12. doi:10.5588/ijtld.14.0838

31 Sotgiu G, Centis R, D'Ambrosio L, *et al.* Efficacy, safety and tolerability of linezolid containing regimens in treating MDR-TB and XDR-TB: systematic review and meta-analysis. *Eur Respir J* 2012;**40**:1430–42. doi:10.1183/09031936.00022912

32 World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013;**310**:2191–4. doi:10.1001/jama.2013.281053

33 Nahid P, Dorman SE, Alipanah N, *et al.* Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: Treatment of Drug-Susceptible Tuberculosis. *Clin Infect Dis* 2016;**63**:147–95. doi:10.1093/cid/ciw376

34 Lange C, Abubakar I, Alffenaar J-WC, *et al.* Management of patients with multidrug-resistant/extensively drug-resistant tuberculosis in Europe: a TBNET consensus statement. Eur. Respir. J. 2014;**44**:23–63. doi:10.1183/09031936.00188313

35 Alffenaar J-WC, Heysell SK, Mpagama SG. Therapeutic Drug Monitoring: The Need for Practical Guidance. *Clin Infect Dis* 2019;**68**:1065–6. doi:10.1093/cid/ciy787

36 Lange C, Dheda K, Chesov D, *et al.* Management of drug-resistant tuberculosis. *Lancet (London, England)* 2019;**394**:953–66. doi:10.1016/S0140-6736(19)31882-3

37 Alffenaar J-WC, Gumbo T, Dooley KE, *et al.* Integrating pharmacokinetics and pharmacodynamics in operational research to End TB. *Clin Infect Dis* Published Online First: September 2019. doi:10.1093/cid/ciz942

38 Migliori GB, Sotgiu G, Rosales-Klintz S, *et al.* ERS/ECDC Statement: European Union standards for tuberculosis care, 2017 update. *Eur Respir J* 2018;**51**:1702678. doi:10.1183/13993003.02678-2017

39 Nicholson T, Admay C, Shakow A, *et al.* Double Standards in Global Health: Medicine, Human Rights Law and Multidrug-Resistant TB Treatment Policy. *Health Hum Rights* 2016;**18**:85–102.

40 Ghimire S, Maharjan B, Jongedijk EM, *et al.* Evaluation of Saliva as a Potential Alternative Sampling Matrix for Therapeutic Drug Monitoring of Levofloxacin in Patients with Multidrug-Resistant Tuberculosis. *Antimicrob Agents Chemother* 2019;**63**:e02379-18. doi:10.1128/AAC.02379-18

41 Zuur MA, Bolhuis MS, Anthony R, *et al.* Current status and opportunities for therapeutic drug monitoring in the treatment of tuberculosis. *Expert Opin Drug Metab Toxicol* 2016;**12**:509–21. doi:10.1517/17425255.2016.1162785

42 Zentner I, Modongo C, Zetola NM, *et al.* Urine colorimetry for therapeutic drug monitoring of pyrazinamide during tuberculosis treatment. *Int J Infect Dis* 2018;**68**:18–23. doi:10.1016/j.ijid.2017.12.017

43 van der Burgt EPM, Sturkenboom MGG, Bolhuis MS, *et al.* End TB with precision treatment! *Eur Respir J* 2016;**47**:680 LP – 682. doi:10.1183/13993003.01285-2015

44 Figueras A. WHO report 'Review of the evidence to include TDM in the Essential in vitro Diagnostics List and prioritization of medicines to be monitored'. 2019.

45 Borisov SE, Dheda K, Enwerem M, *et al.* Effectiveness and safety of bedaquiline-containing regimens in the treatment of MDR- and XDR-TB: a multicentre study. *Eur Respir J* 2017;**49**:1700387. doi:10.1183/13993003.00387-2017

46 Bolhuis MS, Tiberi S, Sotgiu G, *et al.* Is there still room for therapeutic drug monitoring of

linezolid in patients with tuberculosis? *Eur Respir J* 2016;**47**:1288 LP – 1290. doi:10.1183/13993003.02185-2015

47 Alffenaar J-WC, van Altena R, Harmelink IM, *et al.* Comparison of the pharmacokinetics of two dosage regimens of linezolid in multidrug-resistant and extensively drug-resistant tuberculosis patients. *Clin Pharmacokinet* 2010;**49**:559–65. doi:10.2165/11532080-000000000-00000

48 Bolhuis MS, Akkerman OW, Sturkenboom MGG, *et al.* Linezolid-based Regimens for Multidrug-resistant Tuberculosis (TB): A Systematic Review to Establish or Revise the Current Recommended Dose for TB Treatment. *Clin Infect Dis* 2018;**67**:S327–35. doi:10.1093/cid/ciy625

49 Migliori GB, Nardell E, Yedilbayev A, *et al.* Reducing tuberculosis transmission: a consensus document from the World Health Organization Regional Office for Europe. *Eur Respir J* 2019;**53**:1900391. doi:10.1183/13993003.00391-2019

Acknowledgements:

The project is part of the scientific activities of the Global Tuberculosis Network (GTN; Committees on pharmacology- chair Jan-Willem C Alffenaar; Treatment- chair Marcela Muñoz-Torrico and Global TB Consilium- chairs M. Tadolini and S. Tiberi) and of the WHO Collaborating Centre for Tuberculosis and Lung Diseases, Tradate, ITA-80, 2017-2020- GBM/RC/LDA.

Author contributions:

SE, MS, DT, GB, JWA designed the major outlines of the study. OA, LB, JB, GE, SH, HH, LK, HK, JK, KM, CM, SM, MM, AS, GS, MT, ST, FV, TW, MW, JZ contributed to the study design. OA, LB, JB, GE, SH, HH, LK, HK, JK, KM, CM, SM, MM, AS, MT, ST, FV, TW, MW, JZ will include patients in the study. GS performed the sample size calculation. SE wrote the first draft of the manuscript together with MS, DT, JWA. All authors read and approved the final version of the manuscript.

Funding statement: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests: none declared

Figure 1. Workflow of study procedures in local hospitals and central laboratory facility.

Table 1. List of participating hospitals and their location

Hospital	Location
University Medical Center Groningen (central lab facility)	Groningen, The Netherlands
Tuberculosis Clinic “Beatrixoord”, UMCG	Haren, The Netherlands
Princess Alexandra Hospital	Brisbane, Australia
Karolinska University Hospital	Stockholm, Sweden
Instituto Nacional de Enfermedades Respiratorias	Mexico City, Mexico
Athens Chest Hospital "Sotiria"	Athens, Greece
Kibong’oto Infectious Diseases Hospital	Kilimanjaro, Tanzania
Republican Scientific and Practical Centre for Pulmonology and Tuberculosis	Minsk, Belarus
Barts Health NHS trust	London, United Kingdom
St. Orsola-Malpighi Hospital, University of Bologna	Bologna, Italy
Riga East University Hospital TB and Lung Disease Clinic	Riga, Latvia

Table 2. Target AUC_{0-24}/MIC and AUC_{0-24} for TDM of moxifloxacin and levofloxacin in patients with multidrug-resistant tuberculosis (MDR-TB). Standard disease is defined as non-cavitary and regular disease on radiograph.Severe disease is defined as cavitary or extensive disease on radiograph.

Fluoroquinolone	Pulmonary MDR-TB	Target AUC_{0-24}/MIC[a]			Target AUC_{0-24} (mg*h/L)
		MGIT	7H10/11	LJ	
Moxifloxacin	Standard disease	>100	>50	>25	>40
	Severe disease or comorbidities	>100	>50	>25	>60[b]
Levofloxacin	Standard disease	>150	>150[c]	>75	>150
	Severe disease or comorbidities	>150	>150[c]	>75	>200[b]

[a] Minimum inhibitory concentration (MIC) varies depending on growth media; Mycobacteria Growth Indicator Tubes (MGIT), Middlebrook 7H10/7H11, and Lowenstein Jensen (LJ) agar.

[b] Target AUC_{0-24}/MIC at site of cavity; therefore higher AUC_{0-24} is required.

[c] Levofloxacin critical concentration of 7H11 was extrapolated to 7H10.

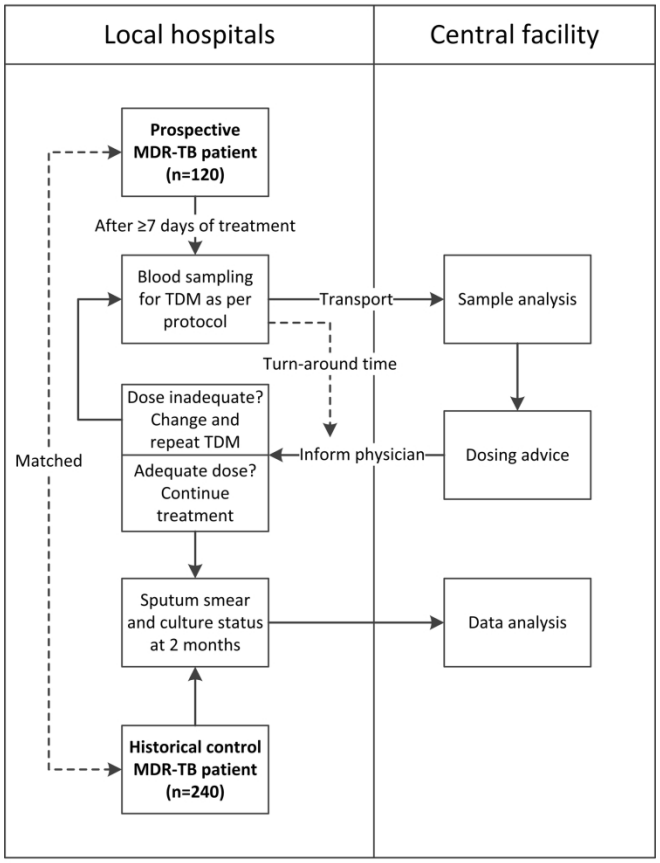


Figure 1. Workflow of study procedures in local hospitals and central laboratory facility.

70x130mm (600 x 600 DPI)

BMJ Open

Prospective Evaluation of impRoving Fluoroquinolone Exposure using Centralized Therapeutic Drug Monitoring (TDM) in patients with Tuberculosis (PERFECT) – a study protocol of a prospective multicentre cohort study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035350.R1
Article Type:	Protocol
Date Submitted by the Author:	06-Mar-2020
Complete List of Authors:	van den Elsen, Simone; University of Groningen, University Medical Center Groningen, Clincal Pharmacy and Pharmacology Sturkenboom, Marieke; University of Groningen, University Medical Center Groningen, Clincal Pharmacy and Pharmacology Akkerman, Onno; University of Groningen, University Medical Center Groningen, Department of Pulmonary Diseases & Tuberculosis; University of Groningen, University Medical Center Groningen, Tuberculosis Center Beatrixoord Barkane, Linda; Riga East University Hospital TB and Lung Disease Clinic, MDR-TB department Bruchfeld, Judith; Karolinska Institutet, Division of Infectious Diseases, Department of Medicine, Solna; Karolinska University Hospital, Department of Infectious Diseases Eather, Geoffrey; Princess Alexandra Hospital, Department of Respiratory Medicine & Metro South Clinical Tuberculosis Service Heysell, Scott; University of Virginia, Division of Infectious Diseases and International Health Hurevich, Henadz; The Republican Scientific and Practical Center for Pulmonology and Tuberculosis Kuksa, Liga; Riga East University Hospital TB and Lung Disease Clinic, MDR-TB department Kunst, Heinke; Barts Health NHS Trust, Blizard Institute, Queen Mary University of London, Department of Respiratory Medicine Kuhlin, Johanna; Karolinska Institutet, Department of Medicine, Unit of Infectious Disease; Karolinska University Hospital, Department of Infectious Diseases Manika, Katerina; Aristotle University of Thessaloniki, G. Papanikolaou Hospital, Pulmonary Department, Respiratory Infections Unit Moschos, Charalampos; "Sotiria" Hospital for Chest Diseases, Drug-Resistant Tuberculosis Unit Mpagama, Stellah; Kibong'oto Infectious Diseases Hospital Muñoz Torrico, Marcela; Instituto Nacional de Enfermedades Respiratorias, Clínica de Tuberculosis Skrahina, Alena; The Republican Scientific and Practical Center for Pulmonology and Tuberculosis Sotgiu, Giovanni; University of Sassari, Clinical Epidemiology and Medical Statistics Unit, Department of Medical, Surgical and Experimental

	Sciences, Tadolini, Marina; Alma Mater Studiorum University of Bologna, Unit of Infectious Diseases, Department of Medical and Surgical Sciences Tiberi, Simon; Barts Health NHS Trust, Blizard Institute, Queen Mary University of London, Department of Infection Volpato, Francesca; Alma Mater Studiorum University of Bologna, Unit of Infectious Diseases, Department of Medical and Surgical Sciences van der Werf, Tjip S.; University Medical Center Groningen, Department of Pulmonary Diseases & Tuberculosis; University of Groningen, University Medical Center Groningen, Department of Internal Medicine Wilson, Malcolm; Princess Alexandra Hospital, Department of Respiratory Medicine & Metro South Clinical Tuberculosis Service Zúñiga , Joaquin; Instituto Nacional de Enfermedades Respiratorias, Laboratory of Immunobiology and Genetics; Escuela de Medicina y Ciencias de Salud, Tecnologico de Monterrey Touw, Daan; University of Groningen, University Medical Center Groningen, Clincal Pharmacy and Pharmacology Migliori, Giovanni; Istituti Clinici Scientifici Maugeri IRCCS, Servizio di Epidemiologia Clinica delle Malattie Respiratorie Alffenaar, Jan-Willem; University of Groningen, University Medical Center Groningen, Clincal Pharmacy and Pharmacology; The University of Sydney, Sydney Pharmacy School, Faculty of Medicine and Health
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Pharmacology and therapeutics, Health services research
Keywords:	Tuberculosis < INFECTIOUS DISEASES, CLINICAL PHARMACOLOGY, Organisation of health services < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, INFECTIOUS DISEASES

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Prospective Evaluation of impRoving Fluoroquinolone Exposure using Centralized Therapeutic Drug Monitoring (TDM) in patients with Tuberculosis (PERFECT) – a study protocol of a prospective multicentre cohort study.

Simone HJ van den Elsen[a], Marieke GG Sturkenboom[a], Onno W Akkerman[b,c], Linda Barkane[d], Judith Bruchfeld[e,f], Geoffrey Eather[g], Scott K Heysell[h], Henadz Hurevich[i], Liga Kuksa[d], Heinke Kunst[j], Johanna Kuhlin[e,f], Katerina Manika[k], Charalampos Moschos[l], Stellah G Mpagama[m], Marcela Muñoz-Torrico[n], Alena Skrahina[o], Giovanni Sotgiu[p], Marina Tadolini[q], Simon Tiberi[r], Francesca Volpato[q], Tjip S van der Werf[c,s], Malcolm R Wilson[g], Joaquin Zuñiga[t], Daan J Touw[a] #, Giovanni B Migliori[u] #, and Jan-Willem C Alffenaar[a,v] #

[a] University of Groningen, University Medical Center Groningen, Department of Clinical Pharmacy and Pharmacology, Groningen, The Netherlands.

[b] University of Groningen, University Medical Center Groningen, Tuberculosis Center Beatrixoord, Haren, The Netherlands

[c] University of Groningen, University Medical Center Groningen, Department of Pulmonary Diseases & Tuberculosis, Groningen, The Netherlands.

[d] MDR-TB department, Riga East University Hospital TB and Lung Disease Clinic, Riga, Latvia.

[e] Division of Infectious Diseases, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden.

[f] Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden.

[g] Department of Respiratory Medicine & Metro South Clinical Tuberculosis Service, Princess Alexandra Hospital, Woolloongabba, Queensland, Australia.

[h] Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, VA, USA.

[i] The Republican Scientific and Practical Center for Pulmonology and Tuberculosis, Minsk, Belarus.

[j] Blizard Institute, Queen Mary University of London, Department of Respiratory Medicine, Barts Health NHS Trust, London, United Kingdom.

[k] Pulmonary Department, Respiratory Infections Unit, Aristotle University of Thessaloniki, G. Papanikolaou Hospital, Thessaloniki, Greece.

[l] Drug-Resistant Tuberculosis Unit, "Sotiria" Hospital for Chest Diseases, Athens, Greece

[m] Kibong'oto Infectious Diseases Hospital, Kilimanjaro, Tanzania.

[n] Clínica de Tuberculosis, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico.

[o] The Republican Scientific and Practical Center for Pulmonology and Tuberculosis, Minsk, Belarus.

[p] Clinical Epidemiology and Medical Statistics Unit, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy

[q] Unit of Infectious Diseases, Department of Medical and Surgical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy.

[r] Blizard Institute, Queen Mary University of London, Department of Infection, Barts Health NHS Trust, London, United Kingdom

[s] University of Groningen, University Medical Center Groningen, Department of Internal Medicine, Groningen, The Netherlands.

[t] Laboratory of Immunobiology and Genetics, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico. Tecnologico de Monterrey, Escuela de Medicina y Ciencias de Salud, Mexico City, Mexico.

[u] Servizio di Epidemiologia Clinica delle Malattie Respiratorie, Istituti Clinici Scientifici Maugeri IRCCS, Tradate, Italy.

[v] Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia

# Authors contributed equally

Corresponding author: J.W.C. Alffenaar, University of Sydney, Faculty of Medicine and Health, School of Pharmacy, Pharmacy Building A15, NSW 2006, Australia. Email: johannes.alffenaar@sydney.edu.au; j.w.c.alffenaar@umcg.nl

Word count: 2992

Abstract

Introduction: Global multidrug-resistant tuberculosis (MDR-TB) treatment success rates remain suboptimal. Highly active World Health Organization (WHO) Group A drugs moxifloxacin and levofloxacin show intra- and inter-individual pharmacokinetic variability which can cause low drug exposure. Therefore, therapeutic drug monitoring (TDM) of fluoroquinolones is recommended to personalise the drug dosage, aiming to prevent development of drug resistance and optimize treatment. However, TDM is considered laborious and expensive, and the clinical benefit in MDR-TB has not been extensively studied. This observational multicentre study aims to determine the feasibility of centralized TDM and to investigate the impact of fluoroquinolone TDM on sputum conversion rates in patients with MDR-TB compared with historical controls.

Methods and analysis: Patients aged 18 years or older with sputum smear and culture positive pulmonary MDR-TB will be eligible for inclusion. Patients receiving TDM using a limited sampling strategy (t=0 and t=5 hours) will be matched to historical controls without TDM in a 1:2 ratio. Sample analysis and dosing advice will be performed in a centralized laboratory. Centralized TDM will be considered feasible if >80% of the dosing recommendations are returned within seven days after sampling and 100% within fourteen days. The number of patients who are sputum smear and culture negative after two months of treatment will be determined in the prospective TDM group and will be compared to the control group without TDM to determine the impact of TDM.

Ethics and dissemination: Ethical clearance was obtained by the ethical review committees of the ten participating hospitals according to local procedures or is pending (supplementary file 1). Patients will be included after written informed consent. We aim to publish the study results in a peer-reviewed journal.

Trial registration: This study is registered at clinicaltrials.gov (NCT03409315)

Strengths and limitations of this study

- To our knowledge, this is the first study that investigates the impact of fluoroquinolone therapeutic drug monitoring (TDM) on sputum smear and culture conversion rates in prospective patients with multidrug-resistant tuberculosis (MDR-TB) versus historical controls without TDM.
- The feasibility for centralised TDM will be evaluated due to participation of multiple health care centres located in differently resourced countries from multiple regions in the world.
- The use of limited sampling strategies will reduce the burden of TDM for patients and health care providers while still providing a reliable estimation of drug exposure.
- A limitation is that this study focuses on TDM for moxifloxacin and levofloxacin only, being core drugs in MDR-TB treatment, without assessing other (core) anti-tuberculosis drugs.

Introduction

Tuberculosis (TB) is one of the major infectious diseases worldwide with an estimated number of 10.0 million new cases in 2017.[1] In addition, multidrug-resistant TB (MDR-TB) remains a persistent problem with an estimated 458,000 new patients in 2017.[1] MDR-TB is treated from 9-20 months with a multidrug regimen.[2] The grouping of second-line anti-TB drugs was revised in 2018 by the World Health Organisation (WHO).[3] The fluoroquinolones, specifically moxifloxacin and levofloxacin, are now considered drugs of first choice (Group A drugs), together with bedaquiline and linezolid, in the treatment of MDR-TB.[2,3] The administration of Group A medicines to patients with MDR-TB has been associated with increased treatment success and reduced mortality rates in comparison with other second-line anti-TB drugs.[4] However, the estimated prevalence of fluoroquinolone-resistance among MDR-TB cases is on the rise from 14.5% in 2011 to 22% in 2017.[5,6] Mismanagement of MDR-TB treatment, especially the shorter regimen, could amplify the risk of drug resistance even further.[7] Importantly, antibiotic resistance can be acquired due to noncompliance but also insufficient drug exposures (e.g. inter-individual pharmacokinetic variability in patients treated with fluoroquinolones).[8–11] Therapeutic drug monitoring (TDM) can help to prevent acquired resistance by individualising doses based on blood drug concentrations relative to the bacterial susceptibility, ideally measured as the minimal inhibitory concentration (MIC).[7,12] Several studies described the role played by low drug concentrations on treatment outcomes.[13–15] In the light of this evidence, it can be hypothesized that TDM, which aims for adequate dosing and exposure, could improve treatment outcomes. Yet, the added value of TDM in MDR-TB treatment outcomes has not been directly studied.[16,17] One retrospective study reported the effect of TDM on the treatment results of patients with drug-susceptible TB, either with and without diabetes.[18] In the group without diabetes, TDM had a significant beneficial effect with 73% sputum culture conversion at two months amongst patients receiving TDM versus 60% in the control group. The positive effect of TDM was even larger in patients with diabetes and TB. The isoniazid or rifampicin dose was adjusted in 12 out of 17 (71%) of the patients with diabetes based on peak

concentration (C_{max}) targets. However, this data is not available for the group without diabetes. To the best of our knowledge, such controlled studies have not yet been performed in people with MDR-TB.

The pharmacokinetic-pharmacodynamic parameter of fluoroquinolones is both time- and concentration dependent and therefore uses the ratio of area under the concentration-time curve to minimal inhibitory concentration (AUC_{0-24}/MIC). The target value is AUC_{0-24}/MIC >146 for levofloxacin and free or unbound $fAUC_{0-24}$/MIC >53 for moxifloxacin which corresponds to a total (bound and unbound) AUC_{0-24}/MIC >106 assuming a constant protein binding of 50%.[19,20] However, multiple concentration measurements widely distributed over the dosing interval are required to compute the area under the concentration-time curve from 0-24 h (AUC_{0-24}). Limited sampling strategies (LSS) could be adopted to reduce the burden of frequent sampling for both patient and personnel while providing a reliable estimation of AUC_{0-24} using only two blood samples.[21,22]

Unfortunately, TDM is not always easily accessible in high TB burden areas because of practical and financial reasons. Therefore, centralized TDM could be a valuable service.[23] Large laboratories are generally well organised, have highly trained personnel with adequate performance of analytical methods leading to reliable sample analysis results.[24] In addition, centralizing the TDM procedures will engender more consistent practice from health care practitioners familiar with TDM and the provision of dosing advice for anti-TB drugs.

The aim of the present study is, firstly, to investigate the feasibility of centralized TDM of moxifloxacin and levofloxacin in the treatment of MDR-TB recruited in TB reference centres located in different continents. Secondly, the impact of TDM on treatment results will be assessed by comparing two month sputum smear and culture conversion rates among patients who received TDM compared with matched historical controls without TDM.

Methods and analysis

Study design

This observational, prospective, multicentre study aims to evaluate the feasibility of centralized TDM of moxifloxacin and levofloxacin as well as the impact of TDM on two month sputum smear and culture conversion rates of patients with MDR-TB. Study design and procedures are displayed in Figure 1. The study was registered at clinicaltrials.gov (NCT03409315), recruitment started on 10 February 2018, and is expected to be completed in December 2020.

Study location

University Medical Center Groningen (UMCG) in Groningen, The Netherlands is the coordinating centre and serves as central laboratory facility for this study. The hospitals that are involved in patient recruitment are displayed in Table 1.

Study population

Patients aged 18 years and older are eligible for inclusion if they are diagnosed with pulmonary MDR-TB, have positive sputum smear and culture samples at time of inclusion, are treated with either oral moxifloxacin or levofloxacin, and provide written informed consent. Pregnant or breast feeding women will be excluded. The decision whether a patient is treated with either moxifloxacin or levofloxacin is made by the clinician at the start of TB treatment based on local guidelines. Patients will not be actively assigned to use moxifloxacin or levofloxacin since this is an observational study. A total number of 120 patients (60 with moxifloxacin, 60 with levofloxacin) will be prospectively included and compared with 240 matched historical controls (120 with moxifloxacin, 120 with levofloxacin).

The following data will be collected in both groups: sex, age, body weight, height, country of birth, country of residence, comorbidities, corrected QT interval, laboratory values (kidney and liver function, electrolytes), history of previous TB treatment, bacterial susceptibility (including MIC if available), TB presentation (cavitary or non-cavitary), current MDR-TB regimen (including drug

dosages), sputum smear and culture data, treatment outcome (if known), and details on fluoroquinolone use (duration, possible drug interactions or adverse events).

Historical control patients will be matched on age, sex, *Mycobacterium tuberculosis* resistance pattern of the isolate (only regimen core drugs), comorbidities (human immunodeficiency virus [HIV], diabetes, immunosuppression), presence or absence of cavitary TB on chest radiography, and dosing of the fluoroquinolone (mg/kg body weight, ±10%) to prospectively enrolled patients in a 2:1 ratio.

Interventions

The objective of the feasibility of centralized TDM will be assessed by evaluating the process, by which a locally collected sample will be analysed in a central laboratory and subsequent dosing advice will be returned to the local physician. In brief, after at least seven days of treatment (steady state) two blood samples will be collected for TDM of moxifloxacin or levofloxacin according to a previously developed LSS.[21,22] The first sample will be collected just before drug intake (t=0) and the other at 5 hours after drug intake (t=5). Samples will be transported to the central laboratory for drug analysis and will be accompanied by a form including key patient characteristics for personalised dosing advice (i.e. sex, age, weight, height, serum creatinine, corrected QT (QTc) interval, MIC, TB presentation, start of treatment, other anti-TB drugs, and comorbidities). AUC_{0-24} will be calculated using a population pharmacokinetic model [21,22] and Bayesian dose optimisation in MWPharm++ (version 1.7.3; Mediware, Groningen, The Netherlands).

Dosing is optimised based on AUC_{0-24}/MIC or AUC_{0-24} (in case MIC is unknown), taking into consideration comorbidities (HIV, diabetes, and immunosuppression), persistence of TB symptoms, and response to treatment so far. The Bayesian dosing software uses sex, age, height, weight, and renal function in addition to drug dose and measured drug concentrations to forecast the drug exposure after a dose change. For patients who are at risk for treatment failure due to the previously mentioned reasons, a higher drug exposure is recommended. This is especially relevant in case of an unknown individual MIC, since the actual MIC might be near the breakpoint, to prevent treatment

failure and acquired resistance. The target AUC_{0-24}/MIC and AUC_{0-24} are shown in Table 1. If a dose change is necessary, TDM is to be repeated after at least seven days after the initiation of the new dose (steady state). Dose increases of moxifloxacin will not be advised in case of a prolonged QTc interval (>450 ms for males, >470 ms for females), because of safety reasons. As levofloxacin may be less cardiotoxic than moxifloxacin, levofloxacin dose increases are permitted in case of prolonged QTc interval, but only with adequateelectrocardiogram monitoring. Patients with prolonged QTc interval will not be excluded from the study, since TDM can still be helpful to verify drug exposure. A closely monitored follow-up including MIC determination can be advised in case of AUC_{0-24} of 25 to 40 mg*h/L in combination with QTc interval prolongation. In case of very low moxifloxacin exposure (AUC_{0-24}<20 mg*h/L) in combination with a prolonged QTc interval, the physician will be advised to reconsider the anti-TB regimen as moxifloxacin may be less active than expected.

Laboratory methods

Drug analysis:

Measurement of moxifloxacin and levofloxacin plasma/serum concentrations will take place at the laboratory of the department of Clinical Pharmacy and Pharmacology in the UMCG, The Netherlands, and using validated liquid chromatography-mass spectrometry (LC-MS/MS) methods. The method for levofloxacin has an accuracy of 0.1-12.7%, within-run precision of 1.4-2.4%, and between-run precision of 3.6-4.1%. The calibration curve is linear over a range of 0.10 to 5.00 mg/L.[25] This range was successfully expanded to 0.20 to 50.0 mg/L in a recent update of the method (data on file). Accuracy of the moxifloxacin method is 2.7-7.1%, within-run precision 1.4-1.6%, and between-run precision 1.0-1.6%. The calibration curve is linear over a range of 0.05 to 5.00 mg/L.[26] For both fluoroquinolones only the total concentration (bound and unbound) will be measured. Therefore, the target AUC_{0-24}/MIC values of >150 [19] and >100 [20] will be used for levofloxacin and moxifloxacin, respectively (Table 2).

Plasma and serum samples containing levofloxacin are stable for at least ten days at 50 °C and can therefore be transported to the central facility in ambient temperature, without the need of transport on dry ice.[27] The thermal stability of moxifloxacin was also tested according to the method of Ghimire *et al* and showed that moxifloxacin serum and plasma samples are stable for at least ten days at 50 °C as well (data on file).

Microbiology:

The assessment of sputum smear and culture status after two months of MDR-TB treatment will be performed according to the local procedures, but at least once a month until documented culture conversion. MIC determination is preferred but not mandatory for TDM and will be performed according to local procedures as well. To account for the differences in culture media used in drug susceptibility testing, correction factors based on the critical concentrations in the WHO-document "Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis" will be applied.[28] The target AUC_{0-24}/MIC values for each medium are shown in Table 2. Furthermore, second line molecular drug susceptibility tests will be considered in case MIC data are not available.

Data analysis plan

The primary outcome to assess the feasibility of centralized TDM will be the turn-around time, which is defined by the time between blood sampling and the peripheral centres receiving the TDM results including the dosing advice. The procedure is considered feasible if >80% of the collected samples will be reported back to the physician within seven days and 100% within two weeks. Additionally, the feasibility will be evaluated using secondary outcomes of sample quality after shipping and completeness of required information on the sample form.

Furthermore, we will evaluate the role of TDM on MDR-TB treatment by comparing the percentages of patients with sputum smear and culture conversion at two months in the enrolled groups. In

addition, we will evaluate the number of patients with low fluoroquinolone exposure requiring dose changes after TDM to estimate the potential gains.

Sample size calculation

As the primary endpoint was of descriptive nature and no data were available to perform a well-informed sample size calculation, it was decided to power the study on the clinical impact of TDM. The primary assumption was based on the detection of a proportional difference in sputum smear and culture positivity at two months of treatment in patients with MDR-TB undergoing TDM (35%) [29] and control patients (60%)[30]. Given an alpha error of 0.05 and statistical power of 80%, we calculated a sample size of 60 per single group is needed (i.e. 60 prospective and 120 historical control patients for moxifloxacin and equally for levofloxacin).

Patient and public involvement

There has been no patient or public involvement in the design of this study.

Ethics and dissemination

This study will be performed according to the Declaration of Helsinki and Good Clinical Practice.[31] In each recruiting centre ethical clearance has been granted according to local regulations and patient recruitment has begun at most sites (supplementary file 1) . Written informed consent will be obtained from all patients undergoing TDM. The need of new informed consent for historical controls was waived, because of the use of retrospective anonymous data collected for programmatic purposes or previously reported data from studies for which patients had provided informed consent.

This study includes historical patients who did not receive TDM as controls instead of prospectively randomising patients to either receive or not receive TDM for ethical reasons. The evidence that TDM actually improves MDR-TB treatment outcomes has not been confirmed in randomised

controlled trials, but multiple studies have described treatment failure and risk of antibiotic resistance due to sub therapeutic drug exposure of anti-TB drugs.[8,13,15,19,20] In combination with a large between-patient pharmacokinetic variability [9,10], we hypothesize that TDM is able to improve treatment outcomes by ensuring adequate exposure in individual patients. Moreover, TDM for MDR-TB is recommended in guidelines when it is available.[2,32,33] We therefore considered it unethical to withhold TDM.

Study results will be published in a peer-reviewed journal and will be presented at an international conference.

Discussion

We present an observational prospective multicentre study which aims to: a) evaluate the feasibility of centralized TDM in differently resourced settings of varying TB endemicity and geographic region and b) evaluate the role of TDM of moxifloxacin or levofloxacin on sputum smear and culture conversion rates in patients with MDR-TB after two months of treatment.

Presently, TDM is offered as an adjunctive to patients with TB in only a few hospitals worldwide and is considered to be part of the excellent clinical care.[16,23,34–36] However, general interest in TDM and MDR-TB treatment optimization has been increasing. A consensus statement on the diagnosis and treatment of MDR-TB in Europe states that TDM for second-line drugs should be used if available.[33] Moreover, the use of second-line anti-TB drugs was listed in the American Thoracic Society (ATS) guidelines as indication for TDM and TDM is also recommended in the European Union Standards for Tuberculosis Prevention and Care.[32,37] Yet, TDM is considered by some to be laborious, expensive and thus unpractical in countries with high TB incidence. Similar injurious arguments of economistic rationing of services were applied to second-line drugs for the treatment of MDR-TB in highly endemic settings and such rationing conversely led to amplification of the MDR-TB epidemic.[38] This study will focus on the feasibility of centralized TDM, which could stimulate performing TDM more often as it requires only one qualified laboratory with validated analytical

methods and devices in a central location. Other options to facilitate TDM are the implementation of LSS, urine samples, dried-blood spots and saliva-screening methods.[34,39–41] This study will additionally use LSS to increase feasibility as well as to reduce the burden of TDM. The LSS for moxifloxacin and levofloxacin used in this study (0 and 5 h post-dose samples) were designed to optimise $AUC_{0\text{-}24}$ [21,22], whereas the frequently used sampling schedule at 2 and 6 h post-dose is more suitable to estimate C_{max} and identify delayed absorption.[42]

Although incorporating TDM in TB treatment has shown to give high treatment success rates in low endemic countries, like the Netherlands [29], this has not yet been evaluated in well-designed randomized controlled trials.[43] This study will provide a first-ever conclusion on the value of TDM of moxifloxacin and levofloxacin on sputum smear and culture conversion of patients with MDR-TB. It can be considered a limitation that only TDM of fluoroquinolones is performed in this study. However, moxifloxacin and levofloxacin are currently among the core drugs in the MDR-treatment regimen together with linezolid and bedaquiline.[3] Based on TDM criteria [44], we have selected moxifloxacin and levofloxacin, because they show large inter-individual pharmacokinetic variability, which emphasizes the need for personalized dosing.[9,10] Moreover, fluoroquinolone resistance is on the rise and can develop during low drug exposure.[8] TDM of fluoroquinolones aims to find the individual patients who have low drug exposure and would benefit from dose adjustment. Therefore, it is expected that TDM of fluoroquinolones will have the largest impact on MDR-TB treatment outcomes. We did not include TDM for linezolid and bedaquiline in this study, because of unclear evidence for TDM of bedaquiline due to the novelty of the drug [45] and TDM of linezolid has focussed more on preventing toxicity.[46–48]

Another limitation is that we are only evaluating interim outcomes such as sputum conversion rates at two months and will not assess outcomes at the end of treatment. However, this study is primarily designed to determine the feasibility of centralized TDM. In addition, this is the first study to evaluate the impact of fluoroquinolone TDM. We believe that reporting the results on sputum conversion rates is relevant as bacterial load and risk of acquired resistance are highest in the first

months of therapy. Fast sputum culture conversion reduces the risk of transmission of *M. tuberculosis* strains which continues to sustain the MDR-TB epidemic.[49] With the results of this study we aim to design a future study to extensively evaluate TDM of all drugs in the regimen including the final treatment outcomes. However, such study would require substantial funding. We hope that this study will show that centralized TDM is feasible and that TDM can improve the quality of treatment in terms of faster sputum conversion rates compared to historical experience. If that might be the case, the major hesitations about TDM in TB treatment can be attenuated favouring the improvement of TB management using a personalized approach.[37]

References

1 World Health Organization. Global tuberculosis report 2018. 2018.

2 World Health Organization. WHO consolidated guidelines on drug-resistant tuberculosis treatment. 2019.

3 World Health Organization. Rapid Communication: Key changes to treatment of multidrug- and rifampicin-resistant tuberculosis (MDR/RR-TB). 2018.

4 Ahmad N, Ahuja SD, Akkerman OW, *et al.* Treatment correlates of successful outcomes in pulmonary multidrug-resistant tuberculosis: an individual patient data meta-analysis. *Lancet* 2018;**392**:821–34. doi:10.1016/S0140-6736(18)31644-1

5 Alffenaar J-WC, Migliori GB, Gumbo T. Multidrug-resistant tuberculosis: pharmacokinetic and pharmacodynamic science. *Lancet Infect Dis* 2017;**17**:898. doi:10.1016/S1473-3099(17)30449-8

6 World Health Organization. Global tuberculosis report 2012. 2012.

7 Davies Forsman L, Bruchfeld J, Alffenaar J-WC. Therapeutic drug monitoring to prevent acquired drug resistance of fluoroquinolones in the treatment of tuberculosis. *Eur Respir J* 2017;**49**:1700173. doi:10.1183/13993003.00173-2017

8 Srivastava S, Pasipanodya JG, Meek C, *et al.* Multidrug-resistant tuberculosis not due to

noncompliance but to between-patient pharmacokinetic variability. *J Infect Dis* 2011;**204**:1951–9. doi:10.1093/infdis/jir658

9 Van't Boveneind-Vrubleuskaya N, Seuruk T, van Hateren K, *et al.* Pharmacokinetics of Levofloxacin in Multidrug- and Extensively Drug-Resistant Tuberculosis Patients. *Antimicrob Agents Chemother* 2017;**61**:e00343-17. doi:10.1128/AAC.00343-17

10 Pranger AD, van Altena R, Aarnoutse RE, *et al.* Evaluation of moxifloxacin for the treatment of tuberculosis: 3 years of experience. *Eur Respir J* 2011;**38**:888–94. doi:10.1183/09031936.00176610

11 Ghimire S, Van't Boveneind-Vrubleuskaya N, Akkerman OW, *et al.* Pharmacokinetic/pharmacodynamic-based optimization of levofloxacin administration in the treatment of MDR-TB. *J Antimicrob Chemother* 2016;**71**:2691–703. doi:10.1093/jac/dkw164

12 Alffenaar J-WC, Gumbo T, Aarnoutse RE. Acquired drug resistance: we can do more than we think! *Clin Infect Dis* 2015;**60**:969–70. doi:10.1093/cid/ciu1146

13 Pasipanodya JG, McIlleron H, Burger A, *et al.* Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *J Infect Dis* 2013;**208**:1464–73. doi:10.1093/infdis/jit352

14 Modongo C, Pasipanodya JG, Magazi BT, *et al.* Artificial Intelligence and Amikacin Exposures Predictive of Outcomes in Multidrug-Resistant Tuberculosis Patients. *Antimicrob Agents Chemother* 2016;**60**:5928–32. doi:10.1128/AAC.00962-16

15 Sekaggya-Wiltshire C, von Braun A, Lamorde M, *et al.* Delayed Sputum Conversion in TB-HIV Co-Infected Patients with Low Isoniazid and Rifampicin Concentrations. *Clin Infect Dis* 2018;**67**:708–16. doi:10.1093/cid/ciy179

16 Alffenaar J-WC, Tiberi S, Verbeeck RK, *et al.* Therapeutic Drug Monitoring in Tuberculosis: Practical Application for Physicians. *Clin Infect Dis* 2017;**64**:104–5. doi:10.1093/cid/ciw677

17 Peloquin C. The Role of Therapeutic Drug Monitoring in Mycobacterial Infections. *Microbiol Spectr* 2017;**5**:TNMI7-0029–2016. doi:10.1128/microbiolspec.TNMI7-0029-2016

18 Alkabab Y, Keller S, Dodge D, *et al.* Early interventions for diabetes related tuberculosis

associate with hastened sputum microbiological clearance in Virginia, USA. *BMC Infect Dis* 2017;**17**:125. doi:10.1186/s12879-017-2226-y

19 Deshpande D, Pasipanodya JG, Mpagama SG, *et al.* Levofloxacin Pharmacokinetics/Pharmacodynamics, Dosing, Susceptibility Breakpoints, and Artificial Intelligence in the Treatment of Multidrug-resistant Tuberculosis. *Clin Infect Dis* 2018;**67**:S293–302. doi:10.1093/cid/ciy611

20 Gumbo T, Louie A, Deziel MR, *et al.* Selection of a moxifloxacin dose that suppresses drug resistance in Mycobacterium tuberculosis, by use of an in vitro pharmacodynamic infection model and mathematical modeling. *J Infect Dis* 2004;**190**:1642–51. doi:10.1086/424849

21 van den Elsen SHJ, Sturkenboom MGG, Van't Boveneind-Vrubleuskaya N, *et al.* Population Pharmacokinetic Model and Limited Sampling Strategies for Personalized Dosing of Levofloxacin in Tuberculosis Patients. *Antimicrob Agents Chemother* 2018;**62**:e01092-18. doi:10.1128/AAC.01092-18

22 van den Elsen SHJ, Sturkenboom MGG, Akkerman OW, *et al.* Limited sampling strategies using linear regression and the Bayesian approach for therapeutic drug monitoring of moxifloxacin in tuberculosis patients. *Antimicrob Agents Chemother* 2019;**63**:e00384-19. doi:10.1128/AAC.00384-19

23 Ghimire S, Bolhuis MS, Sturkenboom MGG, *et al.* Incorporating therapeutic drug monitoring into the World Health Organization hierarchy of tuberculosis diagnostics. *Eur Respir J* 2016;**47**:1867–9. doi:10.1183/13993003.00040-2016

24 Schimke I. Quality and timeliness in medical laboratory testing. *Anal Bioanal Chem* 2009;**393**:1499–504. doi:10.1007/s00216-008-2349-5

25 Ghimire S, van Hateren K, Vrubleuskaya N, *et al.* Determination of levofloxacin in human serum using liquid chromatography tandem mass spectrometry. *J Appl Bioanal* 2018;**4**:16–25. doi:10.17145/jab.18.004

26 Pranger AD, Alffenaar J-WC, Wessels AMA, *et al.* Determination of moxifloxacin in human

plasma, plasma ultrafiltrate, and cerebrospinal fluid by a rapid and simple liquid chromatography- tandem mass spectrometry method. *J Anal Toxicol* 2010;**34**:135–41.

27 Ghimire S, Jongedijk EM, van den Elsen SHJ, *et al.* Cross validation of liquid chromatography tandem mass spectrometry method for quantification of levofloxacin in saliva. *Submitted*

28 World Health Organization. Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. 2018.

29 van Altena R, de Vries G, Haar CH, *et al.* Highly successful treatment outcome of multidrug-resistant tuberculosis in the Netherlands, 2000-2009. *Int J Tuberc Lung Dis* 2015;**19**:406–12. doi:10.5588/ijtld.14.0838

30 Sotgiu G, Centis R, D'Ambrosio L, *et al.* Efficacy, safety and tolerability of linezolid containing regimens in treating MDR-TB and XDR-TB: systematic review and meta-analysis. *Eur Respir J* 2012;**40**:1430–42. doi:10.1183/09031936.00022912

31 World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA* 2013;**310**:2191–4. doi:10.1001/jama.2013.281053

32 Nahid P, Dorman SE, Alipanah N, *et al.* Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: Treatment of Drug-Susceptible Tuberculosis. *Clin Infect Dis* 2016;**63**:147–95. doi:10.1093/cid/ciw376

33 Lange C, Abubakar I, Alffenaar J-WC, *et al.* Management of patients with multidrug-resistant/extensively drug-resistant tuberculosis in Europe: a TBNET consensus statement. *Eur Respir J* 2014;**44**:23–63. doi:10.1183/09031936.00188313

34 Alffenaar J-WC, Heysell SK, Mpagama SG. Therapeutic Drug Monitoring: The Need for Practical Guidance. *Clin Infect Dis* 2019;**68**:1065–6. doi:10.1093/cid/ciy787

35 Lange C, Dheda K, Chesov D, *et al.* Management of drug-resistant tuberculosis. *Lancet* 2019;**394**:953–66. doi:10.1016/S0140-6736(19)31882-3

36 Alffenaar J-WC, Gumbo T, Dooley KE, *et al.* Integrating pharmacokinetics and

pharmacodynamics in operational research to End TB. *Clin Infect Dis* 2019;:ciz942. doi:10.1093/cid/ciz942

37 Migliori GB, Sotgiu G, Rosales-Klintz S, *et al.* ERS/ECDC Statement: European Union standards for tuberculosis care, 2017 update. *Eur Respir J* 2018;**51**:1702678. doi:10.1183/13993003.02678-2017

38 Nicholson T, Admay C, Shakow A, *et al.* Double Standards in Global Health: Medicine, Human Rights Law and Multidrug-Resistant TB Treatment Policy. *Health Hum Rights* 2016;**18**:85–102.

39 Ghimire S, Maharjan B, Jongedijk EM, *et al.* Evaluation of Saliva as a Potential Alternative Sampling Matrix for Therapeutic Drug Monitoring of Levofloxacin in Patients with Multidrug-Resistant Tuberculosis. *Antimicrob Agents Chemother* 2019;**63**:e02379-18. doi:10.1128/AAC.02379-18

40 Zuur MA, Bolhuis MS, Anthony R, *et al.* Current status and opportunities for therapeutic drug monitoring in the treatment of tuberculosis. *Expert Opin Drug Metab Toxicol* 2016;**12**:509–21. doi:10.1517/17425255.2016.1162785

41 Zentner I, Modongo C, Zetola NM, *et al.* Urine colorimetry for therapeutic drug monitoring of pyrazinamide during tuberculosis treatment. *Int J Infect Dis* 2018;**68**:18–23. doi:10.1016/j.ijid.2017.12.017

42 Lange C, Aarnoutse RE, Alffenaar JWC, *et al.* Management of patients with multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis* 2019;**23**:645–62. doi:10.5588/ijtld.18.0622

43 van der Burgt EPM, Sturkenboom MGG, Bolhuis MS, *et al.* End TB with precision treatment! *Eur Respir J* 2016;**47**:680 LP – 682. doi:10.1183/13993003.01285-2015

44 Figueras A. WHO report 'Review of the evidence to include TDM in the Essential in vitro Diagnostics List and prioritization of medicines to be monitored'. 2019.

45 Borisov SE, Dheda K, Enwerem M, *et al.* Effectiveness and safety of bedaquiline-containing regimens in the treatment of MDR- and XDR-TB: a multicentre study. *Eur Respir J* 2017;**49**:1700387. doi:10.1183/13993003.00387-2017

46 Bolhuis MS, Tiberi S, Sotgiu G, *et al.* Is there still room for therapeutic drug monitoring of linezolid in patients with tuberculosis? *Eur Respir J* 2016;**47**:1288 LP – 1290. doi:10.1183/13993003.02185-2015

47 Alffenaar J-WC, van Altena R, Harmelink IM, *et al.* Comparison of the pharmacokinetics of two dosage regimens of linezolid in multidrug-resistant and extensively drug-resistant tuberculosis patients. *Clin Pharmacokinet* 2010;**49**:559–65. doi:10.2165/11532080-000000000-00000

48 Bolhuis MS, Akkerman OW, Sturkenboom MGG, *et al.* Linezolid-based Regimens for Multidrug-resistant Tuberculosis (TB): A Systematic Review to Establish or Revise the Current Recommended Dose for TB Treatment. *Clin Infect Dis* 2018;**67**:S327–35. doi:10.1093/cid/ciy625

49 Migliori GB, Nardell E, Yedilbayev A, *et al.* Reducing tuberculosis transmission: a consensus document from the World Health Organization Regional Office for Europe. *Eur Respir J* 2019;**53**:1900391. doi:10.1183/13993003.00391-2019

Acknowledgements:

The project is part of the scientific activities of the Global Tuberculosis Network (GTN; Committees on pharmacology- chair Jan-Willem C Alffenaar; Treatment- chair Marcela Muñoz-Torrico and Global TB Consilium- chairs M. Tadolini and S. Tiberi) and of the WHO Collaborating Centre for Tuberculosis and Lung Diseases, Tradate, ITA-80, 2017-2020- GBM/RC/LDA.

Author contributions:

SE, MS, DT, GB, JWA designed the major outlines of the study. OA, LB, JB, GE, SH, HH, LK, HK, JK, KM, CM, SM, MM, AS, GS, MT, ST, FV, TW, MW, JZ contributed to the study design. OA, LB, JB, GE, SH, HH, LK, HK, JK, KM, CM, SM, MM, AS, MT, ST, FV, TW, MW, JZ will include patients in the study. GS performed the sample size calculation. SE wrote the first draft of the manuscript together with MS, DT, JWA. All authors read and approved the final version of the manuscript.

Funding statement: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests: none declared

Figure 1. Workflow of study procedures in local hospitals and central laboratory facility.

Table 1. List of participating hospitals and their location

Hospital	Location
University Medical Center Groningen (central lab facility)	Groningen, The Netherlands
Tuberculosis Clinic “Beatrixoord”, UMCG	Haren, The Netherlands
Princess Alexandra Hospital	Brisbane, Australia
Karolinska University Hospital	Stockholm, Sweden
Instituto Nacional de Enfermedades Respiratorias	Mexico City, Mexico
Athens Chest Hospital "Sotiria"	Athens, Greece
Kibong’oto Infectious Diseases Hospital	Kilimanjaro, Tanzania
Republican Scientific and Practical Centre for Pulmonology and Tuberculosis	Minsk, Belarus
Barts Health NHS trust	London, United Kingdom
St. Orsola-Malpighi Hospital, University of Bologna	Bologna, Italy
Riga East University Hospital TB and Lung Disease Clinic	Riga, Latvia

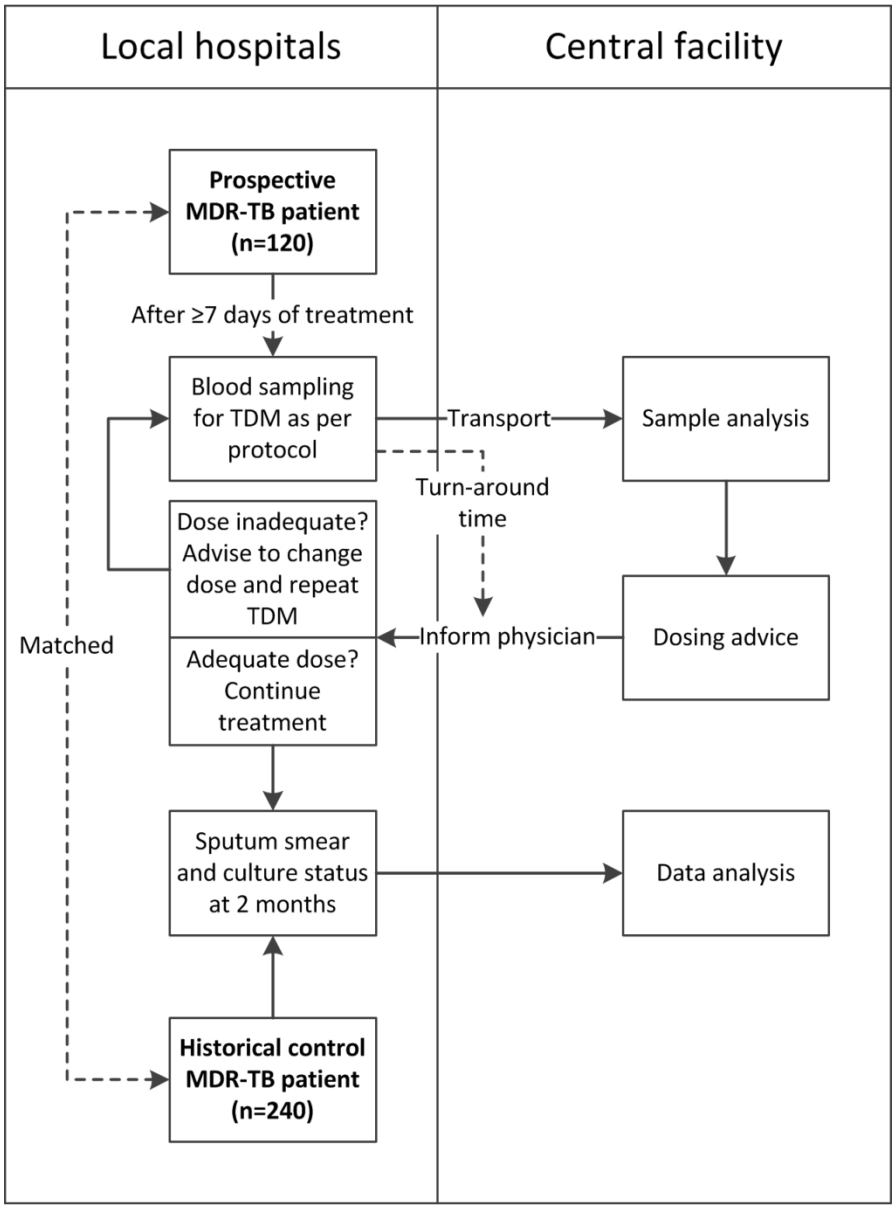
Table 2. Target AUC_{0-24}/MIC and AUC_{0-24} for TDM of moxifloxacin and levofloxacin in patients with multidrug-resistant tuberculosis (MDR-TB). Standard disease is defined as non-cavitary and regular disease on radiograph.Severe disease is defined as cavitary or extensive disease on radiograph.

Fluoroquinolone	Pulmonary MDR-TB	Target AUC_{0-24}/MIC[a]			Target AUC_{0-24} (mg*h/L)
		MGIT	7H10/11	LJ	
Moxifloxacin	Standard disease	>100	>50	>25	>40
	Severe disease or comorbidities	>100	>50	>25	>60[b]
Levofloxacin	Standard disease	>150	>150[c]	>75	>150
	Severe disease or comorbidities	>150	>150[c]	>75	>200[b]

[a] Minimum inhibitory concentration (MIC) varies depending on growth media; Mycobacteria Growth Indicator Tubes (MGIT), Middlebrook 7H10/7H11, and Lowenstein Jensen (LJ) agar.

[b] Target AUC_{0-24}/MIC at site of cavity; therefore higher AUC_{0-24} is required.

[c] Levofloxacin critical concentration of 7H11 was extrapolated to 7H10.



Workflow of study procedures in local hospitals and central laboratory facility.

107x145mm (300 x 300 DPI)

Supplementary file 1

Hospital	Ethical review committee	Reference number
University Medical Center Groningen (central lab facility)	Medical Ethics Review Board of University Medical Center Groningen	2018/029
Tuberculosis Clinic “Beatrixoord”, University Medical Center Groningen	Medical Ethics Review Board of University Medical Center Groningen	2018/029
Princess Alexandra Hospital	Metro South Human Research Ethics Committee	HREC/18/QPAH/218
Karolinska University Hospital	Regional ERB Stockholm	2018/1115-31/2
Instituto Nacional de Enfermedades Respiratorias	Medical Ethics Review Board of Instituto Nacional de Enfermedades Respiratorias	C24-18
Athens Chest Hospital "Sotiria"	Medical Ethics Review Board of Athens Chest Hospital	6000421/14-03-2018
Kibong’oto Infectious Diseases Hospital	National Institute for Medical Research	NIMR/HQ/R.8c/Vol.11/706
Republican Scientific and Practical Centre for Pulmonology and Tuberculosis	Ethics pending	Ethics pending
Barts Health NHS trust	Ethics pending	Ethics pending
St. Orsola-Malpighi Hospital, University of Bologna	Ethics pending	Ethics pending
Riga East University Hospital TB and Lung Disease Clinic	The Research Ethics Committee of Rīga Stradiņš University	68/22.02.2018