PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	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.
AUTHORS	van den Elsen, Simone; Sturkenboom, Marieke; Akkerman, Onno; Barkane, Linda; Bruchfeld, Judith; Eather, Geoffrey; Heysell, Scott; Hurevich, Henadz; Kuksa, Liga; Kunst, Heinke; Kuhlin, Johanna; Manika, Katerina; Moschos, Charalampos; Mpagama, Stellah; Muñoz Torrico, Marcela; Skrahina, Alena; Sotgiu, Giovanni; Tadolini, Marina; Tiberi, Simon; Volpato, Francesca; van der Werf, Tjip S.; Wilson, Malcolm; Zúñiga, Joaquin; Touw, Daan; Migliori, Giovanni; Alffenaar, Jan-Willem

VERSION 1 – REVIEW

REVIEWER	Shadi Baniasadi National Research Institute of Tuberculosis and Lung Diseases, Tehran, Iran
REVIEW RETURNED	11-Dec-2019

GENERAL COMMENTS	of centralized therapeutic drug monitoring of fluoroquinolones (moxifloxacin and levofloxacin) in multi-drug resistant tuberculosis patients. According to the methods, 60 patients with moxifloxacin and 60 with levofloxacin will be included in the study. I would like to ask what is the criteria to choose moxifloxacin or levofloxacin for the patients. In addition, please clarify how comorbidities and clinical condition of the patients affect dosing of fluroquinolones (as mentioned in methods, line 180). Is there any software to calculate personalized dosing (based on patient characteristics mentioned in methods, line 176)?

REVIEWER	Charles Peloquin, Mohammad Alshaer, Catherine Vu University of Florida, USA
REVIEW RETURNED	25-Jan-2020

GENERAL COMMENTS	BMJ review The paper is well writtent. The authors taken on the difficult task of performing a TDM trial. The following points of clarification should help the reader understand the procedures. Abstract line 72: Recommend "…>80 of the dosing recommendations are returned…" Introduction – Included an appropriate amount of background on the topic, and provided sufficient rationale for TDM. It explained

	clear benefits from studying the feasibility and efficacy outcome data. L115-119: The authors should highlight whether there were dose modifications and if certain PKPD targets were achieved in the referenced study. Page 9, lines 121-125: It is not clear why LEVO is assessed as total drug and MOXI as free drug. Please explain. Obviously, a literature value for free drug percentage could be applied to both. L173-174: 0 and 5 hr sampling was proposed based on a previously developed model. Caution is advised regarding letting the software estimating the peak concentration. The sampling scheme provides very limited data for ka and for V, and focuses primarily on Cl. For all of its limitations, a 2 and 6 hour sampling for oral drugs allows one to distinguish among normal, delayed and malabsorption. A trough (0 or 24 h) could be added to further strengthen the data in terms of Cl. L181: How will you take comorbidities and clinical condition into consideration? Are these covariates in the developed PK model? What do the authors mean by clinical condition? All of these should be explained in the methods. Page 11, lines 181-185. It is widely held that LEVO has less effect on QTc than MOXI, including review articles and meta-analyses. However, some cardiologists have challenged that, and assert that this is a class effect. Further, they assert that, depending on the measurement used, LEVO is equal or greater than MOXI in that regard. While the protocol already is in effect, the authors may consider discussing this topic towards the end of the paper. The reference below unfortunately is not available on line. There was a good discussion of the topic during the call. Source: US National Webinar TB Expert Network: Unplugged! conference December 19, 2019 “Quinolone-Associated Complications in a Patient on TB Treatment” Case Presenter: Sean O’Neil, MD, Texas Center for Infectious Diseases Hosted by: Heartland National TB Center Moderators: Neela D. Goswami, MD, MPH, Division of TB Elimination, CDC and Lisa Armitige, MD & Barbara Seaworth, MD, Heartland National TB Center L200: LEVO assay range is up to 5 mg/L. This might be low for this drug. What is the dilution protocol? L203: Are you going to measure free concentrations of LEVO? L229: Please clarify, are you going to compare patients enrolled prospectively among themselves, or to the historic group? The latter is limited in that there are no exposure data in the historic group. Comparing TDM vs non-TDM groups may be inconclusive, since the primary driver is (presumed to be) how much exposure is achieved (at the site of infection). Table 2: AUC should be changed to f AUC

	Figure 1: The middle box “Dose inadequate? Change dose and repeat TDM” The way it is written, it looks like an interventional study. Based on the methods, the decision will be based on the clinician and not per study protocol. Consider changing to clarify. Additional comments: 1. Methods a. Please clarify the timeframe of historical arm patients. New companion drugs may be very important in affecting the response. b. Data collection – clarify the baseline demographics: i. Factors that affect PK variability, such as kidney or liver dysfunction ii. There is not much information about exclusion criteria - will past treatment regimens or prior treatment failures be collected or incorporated into data analysis? 2. Data analysis plan a. The methods listed patient factors that would be included in historical matching and needed for the Bayesian model. However, there are no specific patient demographics listed in the discussion of data analysis – are you planning to control for covariates that impact sputum conversion? What statistical tests/software will be used? b. Historical control is predicted to be 60%, and a paper studying linezolid in MDR TB was cited – do you have any historical data on the sputum conversion rate or can you justify how your population is similar to this control c. You mention that patients will be evaluated for impact of dose increases in subtherapeutic patients. What is the plan/timeline for evaluating these patients? Are you evaluating the same outcomes? d. Is there any plan for cost analysis? Feasibility is the primary outcome of this study and cost is one of the major challenges for TDM implementation that the paper has cited

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Shadi Baniasadi

Institution and Country: National Research Institute of Tuberculosis and Lung Diseases, Tehran, Iran

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

This is a well-designed study to evaluate feasibility of centralized therapeutic drug monitoring of fluoroquinolones (moxifloxacin and levofloxacin) in multi-drug resistant tuberculosis patients.

According to the methods, 60 patients with moxifloxacin and 60 with levofloxacin will be included in the study.

- I would like to ask what is the criteria to choose moxifloxacin or levofloxacin for the patients.
  Response: We will not actively assign either moxifloxacin or levofloxacin to the patients. This decision will be made at the start of treatment by the clinicians and will be based on the availability of the two fluoroquinolones and local preference or guidelines. In general, levofloxacin and moxifloxacin are considered equally effective, but levofloxacin can be preferred in case of QTC >450 ms, while moxifloxacin can be preferred in case of renal failure.

  We added a statement on the drug choice in the methods section (lines 169-171): "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."

- In addition, please clarify how comorbidities and clinical condition of the patients affect dosing of fluoroquinolones (as mentioned in methods, line 180).
  Response: By clinical condition we mean the persistence of TB symptoms and the response to treatment so far. The comorbidities, TB symptoms and response to treatment (so far) will be taken into consideration for dosing advice. A higher exposure can be targeted in patients with comorbidities, persisting TB symptoms or decreased response to treatment because of the increased risk for treatment failure. This especially is important if the individual MIC is unknown, because it is possible that in these cases the MIC is higher, therefore higher AUC is required and thus also a higher dose. We have adjusted this part of the methods to clarify.

  Previous: "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."
  Revised (lines 198-205): "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."

- Is there any software to calculate personalized dosing (based on patient characteristics mentioned in methods, line 176)?
  Response: The central facility (University Medical Center Groningen, the Netherlands) uses Bayesian dosing software (MWPharm ++) to calculate individual AUC using patient characteristics, drug dosage information, and measured drug concentrations in blood. Previously developed and validated population pharmacokinetic models for levofloxacin and moxifloxacin are included in the software (Van den Elsen et al. AAC 2018, Van den Elsen et al. AAC 2019.

Reviewer: 2

Reviewer Name: Charles Peloquin, Mohammad Alshaer, Catherine Vu

Institution and Country: University of Florida, USA

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below BMJ review

The paper is well written. The authors taken on the difficult task of performing a TDM trial.

The following points of clarification should help the reader understand the procedures.

- Abstract line 72: Recommend “…>80 of the dosing recommendations are returned…”
  Response: We made the recommended adjustment.

  Previous: “Centralized TDM will be considered feasible if >80% of the dosing advices is returned within seven days after sampling and 100% within fourteen days.”
  Revised (lines 74-76): “Centralized TDM will be considered feasible if >80% of the dosing recommendations are returned within seven days after sampling and 100% within fourteen days.”

Introduction – Included an appropriate amount of background on the topic, and provided sufficient rationale for TDM. It explained clear benefits from studying the feasibility and efficacy outcome data.

- L115-119: The authors should highlight whether there were dose modifications and if certain PKPD targets were achieved in the referenced study.
  Response: We included this data as requested.

  Previous: 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.
  Revised (lines 128-132): 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 (Cmax) 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.

- Page 9, lines 121-125: It is not clear why LEVO is assessed as total drug and MOXI as free drug. Please explain. Obviously, a literature value for free drug percentage could be applied to both.
  Response: We understand that this might be confusing. Because we will only measure total drug concentration, we added a target of total (bound and unbound) AUC/MIC for moxifloxacin based on a 50% protein binding in the introduction. We clarified both the introduction and methods.

  Previous: “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 $fAUC_{0-24}$/MIC >53 for moxifloxacin.”
  Revised (lines 129-133): “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%."

Previous: "Only the total moxifloxacin concentration (bound and unbound) will be measured, therefore we assume a constant protein binding of 50% for moxifloxacin."
Revised (lines 227-230): "For both fluoroquinolones only the total drug concentration (bound and unbound) will be measured. Therefore, the target AUC_{0-24}/MIC values of >150 and >100 will be used for levofloxacin and moxifloxacin, respectively (Table 2)."

- L173-174: 0 and 5 hr sampling was proposed based on a previously developed model. Caution is advised regarding letting the software estimating the peak concentration. The sampling scheme provides very limited data for ka and for V, and focuses primarily on Cl. For all of its limitations, a 2 and 6 hour sampling for oral drugs allows one to distinguish among normal, delayed and malabsorption. A trough (0 or 24 h) could be added to further strengthen the data in terms of Cl.
  Response: We agree with the reviewer that a LSS using 0 and 5 h samples is not suitable to estimate Cmax. The LSS using 0 and 5 h samples was developed to optimize AUC0-24 estimation, but not Cmax estimation (Van den Elsen et al AAC 2019, Van den Elsen et al AAC 2018). We added a sentence on the debate when to collect TDM samples to the discussion.(Lange et al IJTLD 2019.)
  Lines 309-313: "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_{0-24}, whereas the frequently used sampling schedule at 2 and 6 h post-dose is more suitable to estimate C_{max} and identify delayed absorption."

- L181: How will you take comorbidities and clinical condition into consideration? Are these covariates in the developed PK model? What do the authors mean by clinical condition? All of these should be explained in the methods.
  Response: We understand that the term "clinical condition" is unclear. By clinical condition we mean the persistence of TB symptoms and the response to treatment so far. The developed popPK models (Van den Elsen et al AAC 2018, Van den Elsen et al AAC 2019) included a range of patients with different comorbidities and was able to fit the data adequately. The comorbidities, TB symptoms and response to treatment (so far) will be taken into consideration for dosing advice. A higher exposure can be targeted in patients with comorbidities, persisting TB symptoms or decreased response to treatment because of the increased risk for treatment failure. This especially is important if the individual MIC is unknown, because it is possible that in these cases the MIC is higher, therefore higher AUC is required and thus also a higher dose. We have adjusted this part of the methods to clarify.

  Previous: "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."
  Revised (lines 198-205): "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."

- Page 11, lines 181-185. It is widely held that LEVO has less effect on QTc than MOXI, including review articles and meta-analyses. However, some cardiologists have challenged

that, and assert that this is a class effect. Further, they assert that, depending on the measurement used, LEVO is equal or greater than MOXI in that regard. While the protocol already is in effect, the authors may consider discussing this topic towards the end of the paper. The reference below unfortunately is not available on line. There was a good discussion of the topic during the call. Source: US National Webinar TB Expert Network: Unplugged! conference December 19, 2019 “Quinolone-Associated Complications in a Patient on TB Treatment” Case Presenter: Sean O’Neil, MD, Texas Center for Infectious Diseases Hosted by: Heartland National TB Center Moderators: Neela D. Goswami, MD, MPH, Division of TB Elimination, CDC and Lisa Armitige, MD & Barbara Seaworth, MD, Heartland National TB Center
Response: We provided some nuance in our statement on levofloxacin and QTc prolongation.

Previous: As levofloxacin is less cardiotoxic than moxifloxacin, levofloxacin dose increases are permitted in case of prolonged QTc interval with frequent electrocardiogram monitoring.
Revised (lines 209-211): “As levofloxacin may be less cardiotoxic than moxifloxacin, levofloxacin dose increases are permitted in case of prolonged QTc interval, but only with adequate electrocardiogram monitoring.”

- L200: LEVO assay range is up to 5 mg/L. This might be low for this drug. What is the dilution protocol?
  Response: Recently our assay has been updated and the range has been expanded to 50.0 mg/L. We do not expect any levofloxacin concentrations above 50 mg/L and therefore we think sample dilution will not necessary. The general practice in our lab is as follows: if a drug concentration is higher than the validated assay range, the sample will be 10-fold diluted with blank matrix (either serum or plasma). We included a statement in lines 224-225: “This range was successfully expanded to 0.20 to 50.0 mg/L in a recent update of the method (data on file).”

- L203: Are you going to measure free concentrations of LEVO?
  Response: No, we will not measure free concentrations of either moxifloxacin or levofloxacin. Instead, we will used total (bound and unbound) AUC/MIC targets of >150 for levo and >100 for moxi (assuming 50% protein binding). We clarified the introduction (lines 129-133) and methods (lines 227-230). Analysis of the free concentrations is foreseen in a later separate project.

- L229: Please clarify, are you going to compare patients enrolled prospectively among themselves, or to the historic group? The latter is limited in that there are no exposure data in the historic group. Comparing TDM vs non-TDM groups may be inconclusive, since the primary driver is (presumed to be) how much exposure is achieved (at the site of infection).
  Response: We will compare the prospective patients (who receive TDM) to the historic group (who did not receive TDM). We do realise that exposure data is not available for the historic group, but the historical control patients will be matched to the prospective TDM patients on multiple characteristics to increase the comparability between these two groups. Additionally, we feel that using this design we will include a very diverse control group, presumably with varying drug exposures and similar to real life data in the programmatic settings. Ideally, a randomized controlled trial is used to provide evidence for the effect of TDM on treatment outcomes, but we consider this unfeasible because of practical, ethical, and financial reasons (see ethics and discussion).

- Table 2: AUC should be changed to f AUC
  Response: These targets are total (bound and unbound) AUC/MIC based on the references of Deshpande et al. 2018 and Gumbo et al. 2004. We clarified the targets in the methods section (lines 227-230).

- Figure 1: The middle box “Dose inadequate? Change dose and repeat TDM” The way it is written, it looks like an interventional study. Based on the methods, the decision will be based

on the clinician and not per study protocol. Consider changing to clarify.
Response: We agree with the reviewer and changed the figure.
Previous: "Dose inadequate? Change dose and repeat TDM"
Revised (Figure 1): "Dose inadequate? Advise to change dose and repeat TDM"

Additional comments:

- 1. Methods a. Please clarify the timeframe of historical arm patients. New companion drugs may be very important in affecting the response.
  Response: We do agree that applying a time frame to the historical control patients would be a good idea to avoid possible bias caused by new drugs. This will be most relevant for bedaquiline and linezolid. We plan to take this into account when matching prospective cases to historical controls.

- b. Data collection – clarify the baseline demographics: i. Factors that affect PK variability, such as kidney or liver dysfunction ii. There is not much information about exclusion criteria - will past treatment regimens or prior treatment failures be collected or incorporated into data analysis?
  Response: We included the data collected in both groups (lines 175-180): "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)."
  The only exclusion criteria are breastfeeding and pregnancy (see lines 168-169).
  We will try to match the historical controls to the prospective patients on drug regimen and prior TB treatment history in addition to other listed criteria (lines 181-184).

- 2. Data analysis plan a. The methods listed patient factors that would be included in historical matching and needed for the Bayesian model. However, there are no specific patient demographics listed in the discussion of data analysis – are you planning to control for covariates that impact sputum conversion? What statistical tests/software will be used?
  Response: We will test for impact of the covariates in the final analysis. However, the main study aim is to evaluate the feasibility of centralized TDM and the estimation of the effect of TDM is only a secondary aim. Additionally, this will be the first study to investigate the effect of TDM of fluoroquinolones in patients with MDR-TB on treatment results and is considered a proof of concept study to guide the design of a future randomized controlled study.

- b. Historical control is predicted to be 60%, and a paper studying linezolid in MDR TB was cited – do you have any historical data on the sputum conversion rate or can you justify how your population is similar to this control
  Response: Our study includes both low and high burdened settings. By selecting historical controls from each setting differences caused by the setting can be taken into account. Our estimation of historical controls is based on data from a meta-analysis including 12 studies mostly located in low burdened settings and is therefore considered to be conservative (Sotgiu et al. ERJ 2012). Although we have not collected historical data from each site yet we can expect some variability. However, this is the main reason for collecting controls from each site matched to the prospective cases.

- c. You mention that patients will be evaluated for impact of dose increases in subtherapeutic patients. What is the plan/timeline for evaluating these patients? Are you evaluating the same outcomes?
  Response: Treatment evaluation is similar for all patients. By increasing the dose in patients with low drug exposure we expect to reduce time to sputum culture conversion compared to historical controls.
  In lines 256-258 we mean that the possible gain of TDM will also be evaluated by determining the number (%) of the prospective patients who showed low drug exposure in the TDM results

and required a dose increase to meet the drug exposure targets. If these patients wouldn't have received TDM, this information would not have been available and they would have been treated with insufficient drug dosages, leading to increased risk of treatment failure and acquired drug resistance. The more patients appear to have insufficient drug exposures in TDM, the larger the potential gain of TDM in this patient population.

- d. Is there any plan for cost analysis? Feasibility is the primary outcome of this study and cost is one of the major challenges for TDM implementation that the paper has cited.
  Response: We plan to calculate direct costs related to the offered centralised service. Our study is unfortunately not designed for cost-effectiveness analysis. However, our study will provide important data that can be used in a scenario analysis comparable to an earlier study in which we studied cost-effectiveness of higher dosages of intermediate susceptible isolates (Zuur et al IJTLD 2018).

VERSION 2 – REVIEW

REVIEWER	Charles Peloquin University of Florida, USA
REVIEW RETURNED	15-Mar-2020

GENERAL COMMENTS	The authors have addressed all of the comments.