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

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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)

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3 83 **Strengths and limitations of this study**
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- 5 84 • To our knowledge, this is the first study that investigates the impact of fluoroquinolone TDM
6
7 on sputum smear and culture conversion rates in prospective patients with MDR-TB versus
8 85
9 historical controls without TDM.
10 86
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12 87 • The feasibility for centralised TDM will be evaluated due to participation of multiple health
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14 care centres located in differently resourced countries from multiple regions in the world.
15 88
16
17 89 • The use of limited sampling strategies will reduce the burden of TDM for patients and health
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19 care providers while still providing a reliable estimation of drug exposure.
20 90
21
22 91 • A limitation is that this study focuses on TDM for moxifloxacin and levofloxacin only, being
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24 core drugs in MDR-TB treatment, without assessing other (core) anti-tuberculosis drugs.
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95 Introduction

96 Tuberculosis (TB) is one of the major infectious diseases worldwide with an estimated number of
97 10.0 million new cases in 2017.[1] In addition, multidrug-resistant TB (MDR-TB) remains a persistent
98 problem with an estimated 458,000 new patients in 2017.[1] MDR-TB is treated from 9-20 months
99 with a multidrug regimen.[2] The grouping of second-line anti-TB drugs was revised in 2018 by the
100 World Health Organisation (WHO).[3] The fluoroquinolones, specifically moxifloxacin and
101 levofloxacin, are now considered drugs of first choice (Group A drugs), together with bedaquiline and
102 linezolid, in the treatment of MDR-TB.[2,3] The administration of Group A medicines to patients with
103 MDR-TB has been associated with increased treatment success and reduced mortality rates in
104 comparison with other second-line anti-TB drugs.[4] However, the estimated prevalence of
105 fluoroquinolone-resistance among MDR-TB cases is on the rise from 14.5% in 2011 to 22% in
106 2017.[5,6] Mismanagement of MDR-TB treatment, especially the shorter regimen, could amplify the
107 risk of drug resistance even further.[7] Importantly, antibiotic resistance can be acquired due to
108 noncompliance but also insufficient drug exposures (e.g. inter-individual pharmacokinetic variability
109 in patients treated with fluoroquinolones).[8–11] Therapeutic drug monitoring (TDM) can help to
110 prevent acquired resistance by individualising doses based on blood drug concentrations relative to
111 the bacterial susceptibility, ideally measured as the minimal inhibitory concentration (MIC).[7,12]
112 Several studies described the role played by low drug concentrations on treatment outcomes.[13–
113 15] In the light of this evidence, it can be hypothesized that TDM, which aims for adequate dosing
114 and exposure, could improve treatment outcomes. Yet, the added value of TDM in MDR-TB
115 treatment outcomes has not been directly studied.[16,17] One retrospective study reported the
116 effect of TDM on the treatment results of patients with drug-susceptible TB, either with and without
117 diabetes.[18] In the group without diabetes, TDM had a significant beneficial effect with 73%
118 sputum culture conversion at two months amongst patients receiving TDM versus 60% in the control
119 group. The positive effect of TDM was even larger in patients with diabetes and TB. To the best of
120 our knowledge, such controlled studies have not yet been performed in people with MDR-TB.

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3 121 The pharmacokinetic-pharmacodynamic parameter of fluoroquinolones is both time- and
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5 122 concentration dependent and therefore uses the ratio of area under the concentration time curve to
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7 123 minimal inhibitory concentration (AUC_{0-24}/MIC) with a target value of >146 for levofloxacin and free
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10 124 or unbound $fAUC_{0-24}/MIC >53$ for moxifloxacin.[19,20] However, multiple concentration
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12 125 measurements widely distributed over the dosing interval are required to compute the AUC_{0-24} .
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14 126 Limited sampling strategies (LSS) could be adopted to reduce the burden of frequent sampling for
15
16 127 both patient and personnel while providing a reliable estimation of AUC_{0-24} using only two blood
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18 128 samples.[21,22]
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20 129 Unfortunately, TDM is not always easily accessible in high TB burden areas because of practical and
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22 130 financial reasons. Therefore, centralized TDM could be a valuable service.[23] Large laboratories are
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24 131 generally well organised, have highly trained personnel with adequate performance of analytical
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26 132 methods leading to reliable sample analysis results.[24] In addition, centralizing the TDM procedures
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28 133 will engender more consistent practice from health care practitioners familiar with TDM and the
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30 134 provision of dosing advice for anti-tuberculosis drugs.
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32 135 The aim of the present study is, firstly, to investigate the feasibility of centralized TDM of
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34 136 moxifloxacin and levofloxacin in the treatment of MDR-TB recruited in TB reference centres located
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36 137 in different continents. Secondly, the impact of TDM on treatment results will be assessed by
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38 138 comparing two month sputum smear and culture conversion rates among patients who received
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40 139 TDM compared with matched historical controls without TDM.
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141 **Methods and analysis**

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143 Study design

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

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2
3 147 Figure 1. The study was registered at clinicaltrials.gov (NCT03409315) and started on 10 February
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5 148 2018.

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10 150 Study location

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12 151 University Medical Center Groningen (UMCG) in Groningen, The Netherlands is the coordinating
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14 152 centre and serves as central laboratory facility for this study. The hospitals that are involved in
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16 153 patient recruitment are displayed in Table 1.

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21 155 Study population

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23 156 Patients aged 18 years and older are eligible for inclusion if they are diagnosed with pulmonary MDR-
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25 157 TB, have positive sputum smear and culture samples at time of inclusion, are treated with either oral
26
27 158 moxifloxacin or levofloxacin, and provide written informed consent. Pregnant or breast feeding
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29 159 women will be excluded.

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32 160 A total number of 120 patients (60 with moxifloxacin, 60 with levofloxacin) will be prospectively
33
34 161 included and compared with 240 matched historical controls (120 with moxifloxacin, 120 with
35
36 162 levofloxacin).

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39 163 Historical control patients will be matched on age, sex, *M. tuberculosis* resistance pattern of the
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41 164 isolate (only regimen core drugs), comorbidities (HIV, diabetes, immunosuppression), presence or
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43 165 absence of cavitary TB on chest radiography, and dosing of the fluoroquinolone (mg/kg body weight,
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45 166 $\pm 10\%$) to prospectively enrolled patients in a 2:1 ratio.

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50 168 Interventions

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52 169 The objective of the feasibility of centralized TDM will be assessed by evaluating the process, by
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54 170 which a locally collected sample will be analysed in a central laboratory and subsequent dosing
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56 171 advice will be returned to the local physician. In brief, after at least seven days of treatment (steady
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58 172 state) two blood samples will be collected for TDM of moxifloxacin or levofloxacin according to a
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3 173 previously developed LSS.[21,22] The first sample will be collected just before drug intake (t=0) and
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5 174 the other at 5 hours after drug intake (t=5). Samples will be transported to the central laboratory for
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7 175 drug analysis and will be accompanied by a form including key patient characteristics for
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10 176 personalised dosing advice (i.e. sex, age, weight, height, serum creatinine, corrected QT (QTc)
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12 177 interval, MIC, TB presentation, start of treatment, other anti-TB drugs, and comorbidities). AUC_{0-24}
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14 178 will be calculated using a population pharmacokinetic model [21,22] and Bayesian dose optimisation
15
16 179 in MWPharm++ (version 1.7.3; Mediware, Groningen, The Netherlands).
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19 180 Dosing is optimised based on AUC_{0-24}/MIC or AUC_{0-24} (in case MIC is unknown), taking into
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21 181 consideration comorbidities (HIV, diabetes, and immunosuppression) and clinical condition of the
22
23 182 patient. The target AUC_{0-24}/MIC and AUC_{0-24} are shown in Table 1. If a dose change is necessary, TDM
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25 183 is to be repeated after at least seven days after the initiation of the new dose (steady state). Dose
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27 184 increases of moxifloxacin will not be advised in case of a prolonged QTc interval (>450 ms for males,
28
29 >470 ms for females), because of safety reasons. As levofloxacin is less cardiotoxic than moxifloxacin,
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31 185 levofloxacin dose increases are permitted in case of prolonged QTc interval with frequent
32
33 186 electrocardiogram monitoring. Patients with prolonged QTc interval will not be excluded from the
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35 187 study, since TDM can still be helpful to verify drug exposure. A closely monitored follow-up including
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37 188 MIC determination can be advised in case of AUC_{0-24} of 25 to 40 mg*h/L in combination with QTc
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39 189 interval prolongation. In case of very low moxifloxacin exposure ($AUC_{0-24}<20$ mg*h/L) in combination
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41 190 with a prolonged QTc interval, the physician will be advised to reconsider the anti-TB regimen as
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43 191 moxifloxacin may be less active than expected.
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194 Laboratory methods

195 Drug analysis:

196 Measurement of moxifloxacin and levofloxacin plasma/serum concentrations will take place at the
197 laboratory of the department of Clinical Pharmacy and Pharmacology in the UMCG, The Netherlands,
198 and using validated liquid chromatography-mass spectrometry (LC-MS/MS) methods. The method for
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3 199 levofloxacin has an accuracy of 0.1-12.7%, within-run precision of 1.4-2.4%, and between-run
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5 200 precision of 3.6-4.1%. The calibration curve is linear over a range of 0.10 to 5.00 mg/L.[25] Accuracy
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7 201 of the moxifloxacin method is 2.7-7.1%, within-run precision 1.4-1.6%, and between-run precision
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9 202 1.0-1.6%. The calibration curve is linear over a range of 0.05 to 5.00 mg/L.[26] Only the total
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11 203 moxifloxacin concentration (bound and unbound) will be measured, therefore we assume a constant
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13 204 protein binding of 50%.[27]
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16 205 Plasma and serum samples containing levofloxacin are stable for at least ten days at 50 °C and can
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18 206 therefore be transported to the central facility in ambient temperature, without the need of
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20 207 transport on dry ice.[28] The thermal stability of moxifloxacin was also tested according to the
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22 208 method of Ghimire *et al* and showed that moxifloxacin serum and plasma samples are stable for at
23
24 209 least ten days at 50 °C as well (data on file).
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30 211 Microbiology:

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32 212 The assessment of sputum smear and culture status after two months of MDR-TB treatment will be
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34 213 performed according to the local procedures, but at least once a month until documented culture
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36 214 conversion. MIC determination is preferred but not mandatory for TDM and will be performed
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38 215 according to local procedures as well. To account for the differences in culture media used in drug
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40 216 susceptibility testing, correction factors based on the critical concentrations in the WHO-document
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42 217 “Technical Report on critical concentrations for drug susceptibility testing of medicines used in the
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44 218 treatment of drug-resistant tuberculosis” will be applied.[29] The target AUC_{0-24}/MIC values for each
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46 219 medium are shown in Table 2. Furthermore, second line molecular drug susceptibility tests will be
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48 220 considered in case MIC data are not available.
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53 222 Data analysis plan

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56 223 The primary outcome to assess the feasibility of centralized TDM will be the turn-around time, which
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58 224 is defined by the time between blood sampling and the peripheral centres receiving the TDM results
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3 225 including the dosing advice. The procedure is considered feasible if >80% of the collected samples
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5 226 will be reported back to the physician within seven days and 100% within two weeks. Additionally,
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7 227 the feasibility will be evaluated using secondary outcomes of sample quality after shipping and
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9 228 completeness of required information on the sample form.
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12 229 Furthermore, we will evaluate the role of TDM on MDR-TB treatment by comparing the percentages
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14 230 of patients with sputum smear and culture conversion at two months in the enrolled groups. In
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16 231 addition, we will evaluate the number of patients with low fluoroquinolone exposure requiring dose
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18 232 changes after TDM to estimate the potential gains.
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23 234 Sample size calculation

25 235 As the primary endpoint was of descriptive nature and no data were available to perform a well-
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27 236 informed sample size calculation, it was decided to power the study on the clinical impact of TDM.
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29 237 The primary assumption was based on the detection of a proportional difference in sputum smear
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31 238 and culture positivity at two months of treatment in patients with MDR-TB undergoing TDM (35%)
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33 239 [30] and control patients (60%)[31]. Given an alpha error of 0.05 and statistical power of 80%, we
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35 240 calculated a sample size of 60 per single group is needed (i.e. 60 prospective and 120 historical
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37 241 control patients for moxifloxacin and equally for levofloxacin).
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43 243 **Ethics and dissemination**

45 244 This study will be performed according to the Declaration of Helsinki and Good Clinical Practice.[32]
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48 245 In each centre ethical clearance has been granted according to local regulations and patient
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50 246 recruitment has begun at most sites. Written informed consent will be obtained from all patients
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52 247 undergoing TDM. The need of new informed consent for historical controls was waived, because of
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54 248 the use of retrospective anonymous data collected for programmatic purposes or previously
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56 249 reported data from studies for which patients had provided informed consent.
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3 250 This study includes historical patients who did not receive TDM as controls instead of prospectively
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5 251 randomising patients to either receive or not receive TDM for ethical reasons. The evidence that
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7 252 TDM actually improves MDR-TB treatment outcomes has not been confirmed in randomised
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9 253 controlled trials, but multiple studies have described treatment failure and risk of antibiotic
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11 254 resistance due to sub therapeutic drug exposure of anti-TB drugs.[8,13,15,19,20] In combination with
12
13 255 a large between-patient pharmacokinetic variability [9,10], we hypothesize that TDM is able to
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15 256 improve treatment outcomes by ensuring adequate exposure in individual patients. Moreover, TDM
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17 257 for MDR-TB is recommended in guidelines when it is available.[2,33,34] We therefore considered it
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19 258 unethical to withhold TDM.
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21 259 Study results will be published in a peer-reviewed journal and will be presented at an international
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23 260 conference.
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30 262 **Discussion**

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32 263 We present an observational prospective multicentre study which aims to: a) evaluate the feasibility
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34 264 of centralized TDM in differently resourced settings of varying TB endemicity and geographic region
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36 265 and b) evaluate the role of TDM of moxifloxacin or levofloxacin on sputum smear and culture
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38 266 conversion rates in patients with MDR-TB after two months of treatment.
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40 267 Presently, TDM is offered as an adjunctive to patients with TB in only a few hospitals worldwide and
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42 268 is considered to be part of the excellent clinical care.[16,23,35–37] However, general interest in TDM
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44 269 and MDR-TB treatment optimization has been increasing. A consensus statement on the diagnosis
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46 270 and treatment of MDR-TB in Europe states that TDM for second-line drugs should be used if
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48 271 available.[34] Moreover, the use of second-line anti-TB drugs was listed in the American Thoracic
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50 272 Society (ATS) guidelines as indication for TDM and TDM is also recommended in the European Union
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52 273 Standards for Tuberculosis Prevention and Care.[33,38] Yet, TDM is considered by some to be
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54 274 laborious, expensive and thus unpractical in countries with high TB incidence. Similar injurious
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56 275 arguments of economic rationing of services were applied to second-line drugs for the treatment
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3 276 of MDR-TB in highly endemic settings and such rationing conversely led to amplification of the MDR-
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5 277 TB epidemic.[39] This study will focus on the feasibility of centralized TDM, which could stimulate
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7 278 performing TDM more often as it requires only one qualified laboratory with validated analytical
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9 279 methods and devices in a central location. Other options to facilitate TDM are the implementation of
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11 280 LSS, urine samples, dried-blood spots and saliva-screening methods.[35,40–42] Although
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13 281 incorporating TDM in TB treatment has shown to give high treatment success rates in low endemic
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15 282 countries, like the Netherlands [30], this has not yet been evaluated in well-designed randomized
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17 283 controlled trials.[43] This study will provide a first-ever conclusion on the value of TDM of
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19 284 moxifloxacin and levofloxacin on sputum smear and culture conversion of patients with MDR-TB.
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21 285 It can be considered a limitation that only TDM of fluoroquinolones is performed in this study.
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23 286 However, moxifloxacin and levofloxacin are currently among the core drugs in the MDR-treatment
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25 287 regimen together with linezolid and bedaquiline.[3] Based on TDM criteria [44], we have selected
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27 288 moxifloxacin and levofloxacin, because they show large inter-individual pharmacokinetic variability,
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29 289 which emphasizes the need for personalized dosing.[9,10] Moreover, fluoroquinolone resistance is
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31 290 on the rise and can develop during low drug exposure.[8] TDM of fluoroquinolones aims to find the
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33 291 individual patients who have low drug exposure and would benefit from dose adjustment. Therefore,
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35 292 it is expected that TDM of fluoroquinolones will have the largest impact on MDR-TB treatment
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37 293 outcomes. We did not include TDM for linezolid and bedaquiline in this study, because of unclear
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39 294 evidence for TDM of bedaquiline due to the novelty of the drug [45] and TDM of linezolid has
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41 295 focussed more on preventing toxicity.[46–48]
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43 296 Another limitation is that we are only evaluating interim outcomes such as sputum conversion rates
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45 297 at two months and will not assess outcomes at the end of treatment. However, this study is primarily
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47 298 designed to determine the feasibility of centralized TDM. In addition, this is the first study to
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49 299 evaluate the impact of fluoroquinolone TDM. We believe that reporting the results on sputum
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51 300 conversion rates is relevant as bacterial load and risk of acquired resistance are highest in the first
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53 301 months of therapy. Fast sputum culture conversion reduces the risk of transmission of *M.*
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3 302 *tuberculosis* strains which continues to sustain the MDR-TB epidemic.[49] With the results of this
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5 303 study we aim to design a future study to extensively evaluate TDM of all drugs in the regimen
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7 304 including the final treatment outcomes. However, such study would require substantial funding.
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9
10 305 We hope that this study will show that centralized TDM is feasible and that TDM can improve the
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12 306 quality of treatment in terms of faster sputum conversion rates compared to historical experience. If
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14 307 that might be the case, the major hesitations about TDM in TB treatment can be attenuated
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16 308 favouring the improvement of TB management using a personalized approach.[38]
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45 451 Author contributions:

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47 452 SE, MS, DT, GB, JWA designed the major outlines of the study. OA, LB, JB, GE, SH, HH, LK, HK, JK, KM,
48 453 CM, SM, MM, AS, GS, MT, ST, FV, TW, MW, JZ contributed to the study design. OA, LB, JB, GE, SH, HH,
49 454 LK, HK, JK, KM, CM, SM, MM, AS, MT, ST, FV, TW, MW, JZ will include patients in the study. GS
50 455 performed the sample size calculation. SE wrote the first draft of the manuscript together with MS,
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For peer review only

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3 463 **Figure 1. Workflow of study procedures in local hospitals and central laboratory facility.**

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10 466 **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

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469 **Table 2. Target AUC_{0-24}/MIC and AUC_{0-24} for TDM of moxifloxacin and levofloxacin in patients with multidrug-resistant**
 470 **tuberculosis (MDR-TB). Standard disease is defined as non-cavitary and regular disease on radiograph. Severe disease is**
 471 **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

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

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

475 ^c Levofloxacin critical concentration of 7H11 was extrapolated to 7H10.

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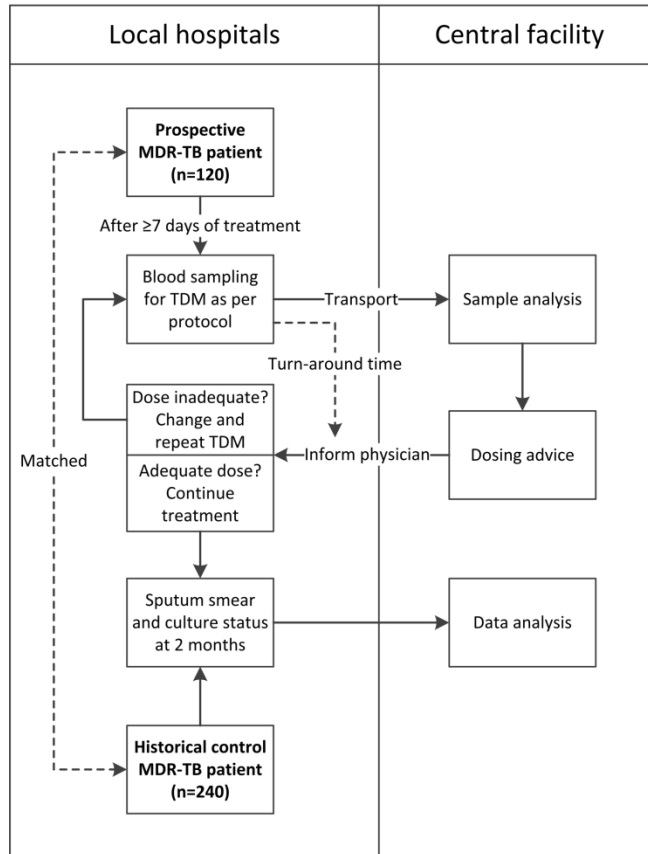


Figure 1. Workflow of study procedures in local hospitals and central laboratory facility.

70x130mm (600 x 600 DPI)

BMJ Open

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3 1 Prospective Evaluation of impROving Fluoroquinolone Exposure using Centralized Therapeutic Drug
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5 2 Monitoring (TDM) in patients with Tuberculosis (PERFECT) – a study protocol of a prospective
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7 3 multicentre cohort study.
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1
2
3 **59 Abstract**
4

5 60 Introduction: Global multidrug-resistant tuberculosis (MDR-TB) treatment success rates remain
6
7 61 suboptimal. Highly active World Health Organization (WHO) Group A drugs moxifloxacin and
8
9 62 levofloxacin show intra- and inter-individual pharmacokinetic variability which can cause low drug
10
11 63 exposure. Therefore, therapeutic drug monitoring (TDM) of fluoroquinolones is recommended to
12
13 64 personalise the drug dosage, aiming to prevent development of drug resistance and optimize
14
15 65 treatment. However, TDM is considered laborious and expensive, and the clinical benefit in MDR-TB
16
17 66 has not been extensively studied. This observational multicentre study aims to determine the
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19 67 feasibility of centralized TDM and to investigate the impact of fluoroquinolone TDM on sputum
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21 68 conversion rates in patients with MDR-TB compared with historical controls.
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28 70 Methods and analysis: Patients aged 18 years or older with sputum smear and culture positive
29
30 71 pulmonary MDR-TB will be eligible for inclusion. Patients receiving TDM using a limited sampling
31
32 72 strategy (t=0 and t=5 hours) will be matched to historical controls without TDM in a 1:2 ratio. Sample
33
34 73 analysis and dosing advice will be performed in a centralized laboratory. Centralized TDM will be
35
36 74 considered feasible if >80% of the dosing recommendations are returned within seven days after
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38 75 sampling and 100% within fourteen days. The number of patients who are sputum smear and culture
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40 76 negative after two months of treatment will be determined in the prospective TDM group and will be
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42 77 compared to the control group without TDM to determine the impact of TDM.
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48 79 Ethics and dissemination: Ethical clearance was obtained by the ethical review committees of the ten
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50 80 participating hospitals according to local procedures or is pending (supplementary file 1). Patients
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52 81 will be included after written informed consent. We aim to publish the study results in a peer-
53
54 82 reviewed journal.
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59 84 Trial registration: This study is registered at clinicaltrials.gov (NCT03409315)
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85

86 Strengths and limitations of this study

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

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99 Introduction

100 Tuberculosis (TB) is one of the major infectious diseases worldwide with an estimated number of
101 10.0 million new cases in 2017.[1] In addition, multidrug-resistant TB (MDR-TB) remains a persistent
102 problem with an estimated 458,000 new patients in 2017.[1] MDR-TB is treated from 9-20 months
103 with a multidrug regimen.[2] The grouping of second-line anti-TB drugs was revised in 2018 by the
104 World Health Organisation (WHO).[3] The fluoroquinolones, specifically moxifloxacin and
105 levofloxacin, are now considered drugs of first choice (Group A drugs), together with bedaquiline and
106 linezolid, in the treatment of MDR-TB.[2,3] The administration of Group A medicines to patients with
107 MDR-TB has been associated with increased treatment success and reduced mortality rates in
108 comparison with other second-line anti-TB drugs.[4] However, the estimated prevalence of
109 fluoroquinolone-resistance among MDR-TB cases is on the rise from 14.5% in 2011 to 22% in
110 2017.[5,6] Mismanagement of MDR-TB treatment, especially the shorter regimen, could amplify the
111 risk of drug resistance even further.[7] Importantly, antibiotic resistance can be acquired due to
112 noncompliance but also insufficient drug exposures (e.g. inter-individual pharmacokinetic variability
113 in patients treated with fluoroquinolones).[8–11] Therapeutic drug monitoring (TDM) can help to
114 prevent acquired resistance by individualising doses based on blood drug concentrations relative to
115 the bacterial susceptibility, ideally measured as the minimal inhibitory concentration (MIC).[7,12]
116 Several studies described the role played by low drug concentrations on treatment outcomes.[13–
117 15] In the light of this evidence, it can be hypothesized that TDM, which aims for adequate dosing
118 and exposure, could improve treatment outcomes. Yet, the added value of TDM in MDR-TB
119 treatment outcomes has not been directly studied.[16,17] One retrospective study reported the
120 effect of TDM on the treatment results of patients with drug-susceptible TB, either with and without
121 diabetes.[18] In the group without diabetes, TDM had a significant beneficial effect with 73%
122 sputum culture conversion at two months amongst patients receiving TDM versus 60% in the control
123 group. The positive effect of TDM was even larger in patients with diabetes and TB. The isoniazid or
124 rifampicin dose was adjusted in 12 out of 17 (71%) of the patients with diabetes based on peak

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3 125 concentration (C_{max}) targets. However, this data is not available for the group without diabetes. To
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5 126 the best of our knowledge, such controlled studies have not yet been performed in people with
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7 127 MDR-TB.
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10 128 The pharmacokinetic-pharmacodynamic parameter of fluoroquinolones is both time- and
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12 129 concentration dependent and therefore uses the ratio of area under the concentration-time curve to
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14 130 minimal inhibitory concentration (AUC_{0-24}/MIC). The target value is $AUC_{0-24}/MIC >146$ for levofloxacin
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16 131 and free or unbound $fAUC_{0-24}/MIC >53$ for moxifloxacin which corresponds to a total (bound and
17
18 132 unbound) $AUC_{0-24}/MIC >106$ assuming a constant protein binding of 50%. [19,20] However, multiple
19
20 133 concentration measurements widely distributed over the dosing interval are required to compute
21
22 134 the area under the concentration-time curve from 0-24 h (AUC_{0-24}). Limited sampling strategies (LSS)
23
24 135 could be adopted to reduce the burden of frequent sampling for both patient and personnel while
25
26 136 providing a reliable estimation of AUC_{0-24} using only two blood samples. [21,22]
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28 137 Unfortunately, TDM is not always easily accessible in high TB burden areas because of practical and
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30 138 financial reasons. Therefore, centralized TDM could be a valuable service. [23] Large laboratories are
31
32 139 generally well organised, have highly trained personnel with adequate performance of analytical
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34 140 methods leading to reliable sample analysis results. [24] In addition, centralizing the TDM procedures
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36 141 will engender more consistent practice from health care practitioners familiar with TDM and the
37
38 142 provision of dosing advice for anti-TB drugs.

39 143 The aim of the present study is, firstly, to investigate the feasibility of centralized TDM of
40
41 144 moxifloxacin and levofloxacin in the treatment of MDR-TB recruited in TB reference centres located
42
43 145 in different continents. Secondly, the impact of TDM on treatment results will be assessed by
44
45 146 comparing two month sputum smear and culture conversion rates among patients who received
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47 147 TDM compared with matched historical controls without TDM.
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55 149 **Methods and analysis**

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3 151 Study design
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5 152 This observational, prospective, multicentre study aims to evaluate the feasibility of centralized TDM
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7 153 of moxifloxacin and levofloxacin as well as the impact of TDM on two month sputum smear and
8
9 154 culture conversion rates of patients with MDR-TB. Study design and procedures are displayed in
10
11 155 Figure 1. The study was registered at clinicaltrials.gov (NCT03409315), recruitment started on 10
12
13 156 February 2018, and is expected to be completed in December 2020.
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19 158 Study location
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21 159 University Medical Center Groningen (UMCG) in Groningen, The Netherlands is the coordinating
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23 160 centre and serves as central laboratory facility for this study. The hospitals that are involved in
24
25 161 patient recruitment are displayed in Table 1.
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30 163 Study population
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32 164 Patients aged 18 years and older are eligible for inclusion if they are diagnosed with pulmonary MDR-
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34 165 TB, have positive sputum smear and culture samples at time of inclusion, are treated with either oral
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36 166 moxifloxacin or levofloxacin, and provide written informed consent. Pregnant or breast feeding
37
38 167 women will be excluded. The decision whether a patient is treated with either moxifloxacin or
39
40 168 levofloxacin is made by the clinician at the start of TB treatment based on local guidelines. Patients
41
42 169 will not be actively assigned to use moxifloxacin or levofloxacin since this is an observational study.
43
44 170 A total number of 120 patients (60 with moxifloxacin, 60 with levofloxacin) will be prospectively
45
46 171 included and compared with 240 matched historical controls (120 with moxifloxacin, 120 with
47
48 172 levofloxacin).
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52 173 The following data will be collected in both groups: sex, age, body weight, height, country of birth,
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54 174 country of residence, comorbidities, corrected QT interval, laboratory values (kidney and liver
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56 175 function, electrolytes), history of previous TB treatment, bacterial susceptibility (including MIC if
57
58 176 available), TB presentation (cavitary or non-cavitary), current MDR-TB regimen (including drug
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3 177 dosages), sputum smear and culture data, treatment outcome (if known), and details on
4
5 178 fluoroquinolone use (duration, possible drug interactions or adverse events).
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8 179 Historical control patients will be matched on age, sex, *Mycobacterium tuberculosis* resistance
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10 180 pattern of the isolate (only regimen core drugs), comorbidities (human immunodeficiency virus [HIV],
11
12 181 diabetes, immunosuppression), presence or absence of cavitary TB on chest radiography, and dosing
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14 182 of the fluoroquinolone (mg/kg body weight, $\pm 10\%$) to prospectively enrolled patients in a 2:1 ratio.
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18 184 Interventions

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21 185 The objective of the feasibility of centralized TDM will be assessed by evaluating the process, by
22
23 186 which a locally collected sample will be analysed in a central laboratory and subsequent dosing
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25 187 advice will be returned to the local physician. In brief, after at least seven days of treatment (steady
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27
28 188 state) two blood samples will be collected for TDM of moxifloxacin or levofloxacin according to a
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30 189 previously developed LSS.[21,22] The first sample will be collected just before drug intake (t=0) and
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32 190 the other at 5 hours after drug intake (t=5). Samples will be transported to the central laboratory for
33
34 191 drug analysis and will be accompanied by a form including key patient characteristics for
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36 192 personalised dosing advice (i.e. sex, age, weight, height, serum creatinine, corrected QT (QTc)
37
38 193 interval, MIC, TB presentation, start of treatment, other anti-TB drugs, and comorbidities). AUC_{0-24}
39
40 194 will be calculated using a population pharmacokinetic model [21,22] and Bayesian dose optimisation
41
42 195 in MWPharm++ (version 1.7.3; Mediware, Groningen, The Netherlands).
43
44
45 196 Dosing is optimised based on AUC_{0-24}/MIC or AUC_{0-24} (in case MIC is unknown), taking into
46
47 197 consideration comorbidities (HIV, diabetes, and immunosuppression), persistence of TB symptoms,
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49 198 and response to treatment so far. The Bayesian dosing software uses sex, age, height, weight, and
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51 199 renal function in addition to drug dose and measured drug concentrations to forecast the drug
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53 200 exposure after a dose change. For patients who are at risk for treatment failure due to the previously
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55 201 mentioned reasons, a higher drug exposure is recommended. This is especially relevant in case of an
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57 202 unknown individual MIC, since the actual MIC might be near the breakpoint, to prevent treatment
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3 203 failure and acquired resistance. The target AUC_{0-24}/MIC and AUC_{0-24} are shown in Table 1. If a dose
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5 204 change is necessary, TDM is to be repeated after at least seven days after the initiation of the new
6
7 205 dose (steady state). Dose increases of moxifloxacin will not be advised in case of a prolonged QTc
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9 206 interval (>450 ms for males, >470 ms for females), because of safety reasons. As levofloxacin may be
10
11 207 less cardiotoxic than moxifloxacin, levofloxacin dose increases are permitted in case of prolonged
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13 208 QTc interval, but only with adequate electrocardiogram monitoring. Patients with prolonged QTc
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15 209 interval will not be excluded from the study, since TDM can still be helpful to verify drug exposure. A
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17 210 closely monitored follow-up including MIC determination can be advised in case of AUC_{0-24} of 25 to
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19 211 $40 \text{ mg}^* \text{h/L}$ in combination with QTc interval prolongation. In case of very low moxifloxacin exposure
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21 212 ($AUC_{0-24} < 20 \text{ mg}^* \text{h/L}$) in combination with a prolonged QTc interval, the physician will be advised to
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23 213 reconsider the anti-TB regimen as moxifloxacin may be less active than expected.
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30 215 Laboratory methods

31 216 Drug analysis:

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33 217 Measurement of moxifloxacin and levofloxacin plasma/serum concentrations will take place at the
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35 218 laboratory of the department of Clinical Pharmacy and Pharmacology in the UMCG, The Netherlands,
36
37 219 and using validated liquid chromatography-mass spectrometry (LC-MS/MS) methods. The method for
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39 220 levofloxacin has an accuracy of 0.1-12.7%, within-run precision of 1.4-2.4%, and between-run
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41 221 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
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43 222 was successfully expanded to 0.20 to 50.0 mg/L in a recent update of the method (data on file).
44
45 223 Accuracy of the moxifloxacin method is 2.7-7.1%, within-run precision 1.4-1.6%, and between-run
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47 224 precision 1.0-1.6%. The calibration curve is linear over a range of 0.05 to 5.00 mg/L.[26] For both
48
49 225 fluoroquinolones only the total concentration (bound and unbound) will be measured. Therefore, the
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51 226 target AUC_{0-24}/MIC values of >150 [19] and >100 [20] will be used for levofloxacin and moxifloxacin,
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53 227 respectively (Table 2).
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3 228 Plasma and serum samples containing levofloxacin are stable for at least ten days at 50 °C and can
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5 229 therefore be transported to the central facility in ambient temperature, without the need of
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7 230 transport on dry ice.[27] The thermal stability of moxifloxacin was also tested according to the
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9 231 method of Ghimire *et al* and showed that moxifloxacin serum and plasma samples are stable for at
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11 232 least ten days at 50 °C as well (data on file).
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234 Microbiology:

16 234 Microbiology:
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18 235 The assessment of sputum smear and culture status after two months of MDR-TB treatment will be
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20 236 performed according to the local procedures, but at least once a month until documented culture
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22 237 conversion. MIC determination is preferred but not mandatory for TDM and will be performed
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24 238 according to local procedures as well. To account for the differences in culture media used in drug
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26 239 susceptibility testing, correction factors based on the critical concentrations in the WHO-document
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28 240 “Technical Report on critical concentrations for drug susceptibility testing of medicines used in the
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30 241 treatment of drug-resistant tuberculosis” will be applied.[28] The target AUC_{0-24}/MIC values for each
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32 242 medium are shown in Table 2. Furthermore, second line molecular drug susceptibility tests will be
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34 243 considered in case MIC data are not available.
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245 Data analysis plan

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41 245 Data analysis plan
42
43 246 The primary outcome to assess the feasibility of centralized TDM will be the turn-around time, which
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45 247 is defined by the time between blood sampling and the peripheral centres receiving the TDM results
46
47 248 including the dosing advice. The procedure is considered feasible if >80% of the collected samples
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49 249 will be reported back to the physician within seven days and 100% within two weeks. Additionally,
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51 250 the feasibility will be evaluated using secondary outcomes of sample quality after shipping and
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53 251 completeness of required information on the sample form.
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55
56 252 Furthermore, we will evaluate the role of TDM on MDR-TB treatment by comparing the percentages
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58 253 of patients with sputum smear and culture conversion at two months in the enrolled groups. In
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3 254 addition, we will evaluate the number of patients with low fluoroquinolone exposure requiring dose
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5 255 changes after TDM to estimate the potential gains.
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10 257 Sample size calculation

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12 258 As the primary endpoint was of descriptive nature and no data were available to perform a well-
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14 259 informed sample size calculation, it was decided to power the study on the clinical impact of TDM.
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16 260 The primary assumption was based on the detection of a proportional difference in sputum smear
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18 261 and culture positivity at two months of treatment in patients with MDR-TB undergoing TDM (35%)
19
20 262 [29] and control patients (60%)[30]. Given an alpha error of 0.05 and statistical power of 80%, we
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22 263 calculated a sample size of 60 per single group is needed (i.e. 60 prospective and 120 historical
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24 264 control patients for moxifloxacin and equally for levofloxacin).
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30 266 **Patient and public involvement**

31
32 267 There has been no patient or public involvement in the design of this study.
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36 269 **Ethics and dissemination**

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39 270 This study will be performed according to the Declaration of Helsinki and Good Clinical Practice.[31]
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41 271 In each recruiting centre ethical clearance has been granted according to local regulations and
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43 272 patient recruitment has begun at most sites (supplementary file 1) . Written informed consent will be
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45 273 obtained from all patients undergoing TDM. The need of new informed consent for historical controls
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47 274 was waived, because of the use of retrospective anonymous data collected for programmatic
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49 275 purposes or previously reported data from studies for which patients had provided informed
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51 276 consent.
52
53
54 277 This study includes historical patients who did not receive TDM as controls instead of prospectively
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56 278 randomising patients to either receive or not receive TDM for ethical reasons. The evidence that
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58 279 TDM actually improves MDR-TB treatment outcomes has not been confirmed in randomised
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3 280 controlled trials, but multiple studies have described treatment failure and risk of antibiotic
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5 281 resistance due to sub therapeutic drug exposure of anti-TB drugs.[8,13,15,19,20] In combination with
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7 282 a large between-patient pharmacokinetic variability [9,10], we hypothesize that TDM is able to
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9 283 improve treatment outcomes by ensuring adequate exposure in individual patients. Moreover, TDM
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11 284 for MDR-TB is recommended in guidelines when it is available.[2,32,33] We therefore considered it
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13 285 unethical to withhold TDM.
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16 286 Study results will be published in a peer-reviewed journal and will be presented at an international
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18 287 conference.
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23 289 **Discussion**

25 290 We present an observational prospective multicentre study which aims to: a) evaluate the feasibility
26
27 291 of centralized TDM in differently resourced settings of varying TB endemicity and geographic region
28
29 292 and b) evaluate the role of TDM of moxifloxacin or levofloxacin on sputum smear and culture
30
31 293 conversion rates in patients with MDR-TB after two months of treatment.
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33

34 294 Presently, TDM is offered as an adjunctive to patients with TB in only a few hospitals worldwide and
35
36 295 is considered to be part of the excellent clinical care.[16,23,34–36] However, general interest in TDM
37
38 296 and MDR-TB treatment optimization has been increasing. A consensus statement on the diagnosis
39
40 297 and treatment of MDR-TB in Europe states that TDM for second-line drugs should be used if
41
42 298 available.[33] Moreover, the use of second-line anti-TB drugs was listed in the American Thoracic
43
44 299 Society (ATS) guidelines as indication for TDM and TDM is also recommended in the European Union
45
46 300 Standards for Tuberculosis Prevention and Care.[32,37] Yet, TDM is considered by some to be
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48 301 laborious, expensive and thus unpractical in countries with high TB incidence. Similar injurious
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50 302 arguments of economic rationing of services were applied to second-line drugs for the treatment
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52 303 of MDR-TB in highly endemic settings and such rationing conversely led to amplification of the MDR-
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54 304 TB epidemic.[38] This study will focus on the feasibility of centralized TDM, which could stimulate
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56 305 performing TDM more often as it requires only one qualified laboratory with validated analytical
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3 306 methods and devices in a central location. Other options to facilitate TDM are the implementation of
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5 307 LSS, urine samples, dried-blood spots and saliva-screening methods.[34,39–41] This study will
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7 308 additionally use LSS to increase feasibility as well as to reduce the burden of TDM. The LSS for
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9 309 moxifloxacin and levofloxacin used in this study (0 and 5 h post-dose samples) were designed to
10
11 310 optimise AUC₀₋₂₄ [21,22], whereas the frequently used sampling schedule at 2 and 6 h post-dose is
12
13 311 more suitable to estimate C_{max} and identify delayed absorption.[42]
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15
16 312 Although incorporating TDM in TB treatment has shown to give high treatment success rates in low
17
18 313 endemic countries, like the Netherlands [29], this has not yet been evaluated in well-designed
19
20 314 randomized controlled trials.[43] This study will provide a first-ever conclusion on the value of TDM
21
22 315 of moxifloxacin and levofloxacin on sputum smear and culture conversion of patients with MDR-TB.
23
24 316 It can be considered a limitation that only TDM of fluoroquinolones is performed in this study.
25
26 317 However, moxifloxacin and levofloxacin are currently among the core drugs in the MDR-treatment
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28 318 regimen together with linezolid and bedaquiline.[3] Based on TDM criteria [44], we have selected
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30 319 moxifloxacin and levofloxacin, because they show large inter-individual pharmacokinetic variability,
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32 320 which emphasizes the need for personalized dosing.[9,10] Moreover, fluoroquinolone resistance is
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34 321 on the rise and can develop during low drug exposure.[8] TDM of fluoroquinolones aims to find the
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36 322 individual patients who have low drug exposure and would benefit from dose adjustment. Therefore,
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38 323 it is expected that TDM of fluoroquinolones will have the largest impact on MDR-TB treatment
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40 324 outcomes. We did not include TDM for linezolid and bedaquiline in this study, because of unclear
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42 325 evidence for TDM of bedaquiline due to the novelty of the drug [45] and TDM of linezolid has
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44 326 focussed more on preventing toxicity.[46–48]
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46 327 Another limitation is that we are only evaluating interim outcomes such as sputum conversion rates
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48 328 at two months and will not assess outcomes at the end of treatment. However, this study is primarily
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50 329 designed to determine the feasibility of centralized TDM. In addition, this is the first study to
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52 330 evaluate the impact of fluoroquinolone TDM. We believe that reporting the results on sputum
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54 331 conversion rates is relevant as bacterial load and risk of acquired resistance are highest in the first
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3 332 months of therapy. Fast sputum culture conversion reduces the risk of transmission of *M.*
4
5 333 *tuberculosis* strains which continues to sustain the MDR-TB epidemic.[49] With the results of this
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7 334 study we aim to design a future study to extensively evaluate TDM of all drugs in the regimen
8
9 335 including the final treatment outcomes. However, such study would require substantial funding.
10
11 336 We hope that this study will show that centralized TDM is feasible and that TDM can improve the
12
13 337 quality of treatment in terms of faster sputum conversion rates compared to historical experience. If
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15 338 that might be the case, the major hesitations about TDM in TB treatment can be attenuated
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17 339 favouring the improvement of TB management using a personalized approach.[37]
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42 480 and Lung Diseases, Tradate, ITA-80, 2017-2020- GBM/RC/LDA.
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48 482 Author contributions:

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50 483 SE, MS, DT, GB, JWA designed the major outlines of the study. OA, LB, JB, GE, SH, HH, LK, HK, JK, KM,
51
52 484 CM, SM, MM, AS, GS, MT, ST, FV, TW, MW, JZ contributed to the study design. OA, LB, JB, GE, SH, HH,
53
54 485 LK, HK, JK, KM, CM, SM, MM, AS, MT, ST, FV, TW, MW, JZ will include patients in the study. GS
55
56 486 performed the sample size calculation. SE wrote the first draft of the manuscript together with MS,
57
58 487 DT, JWA. All authors read and approved the final version of the manuscript.
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491

492 Competing interests: none declared

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For peer review only

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3 494 **Figure 1. Workflow of study procedures in local hospitals and central laboratory facility.**

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10 497 **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

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3 500 **Table 2. Target AUC₀₋₂₄/MIC and AUC₀₋₂₄ for TDM of moxifloxacin and levofloxacin in patients with multidrug-resistant**
4 **tuberculosis (MDR-TB). Standard disease is defined as non-cavitary and regular disease on radiograph. Severe disease is**
5 **501 defined as cavitary or extensive disease on radiograph.**
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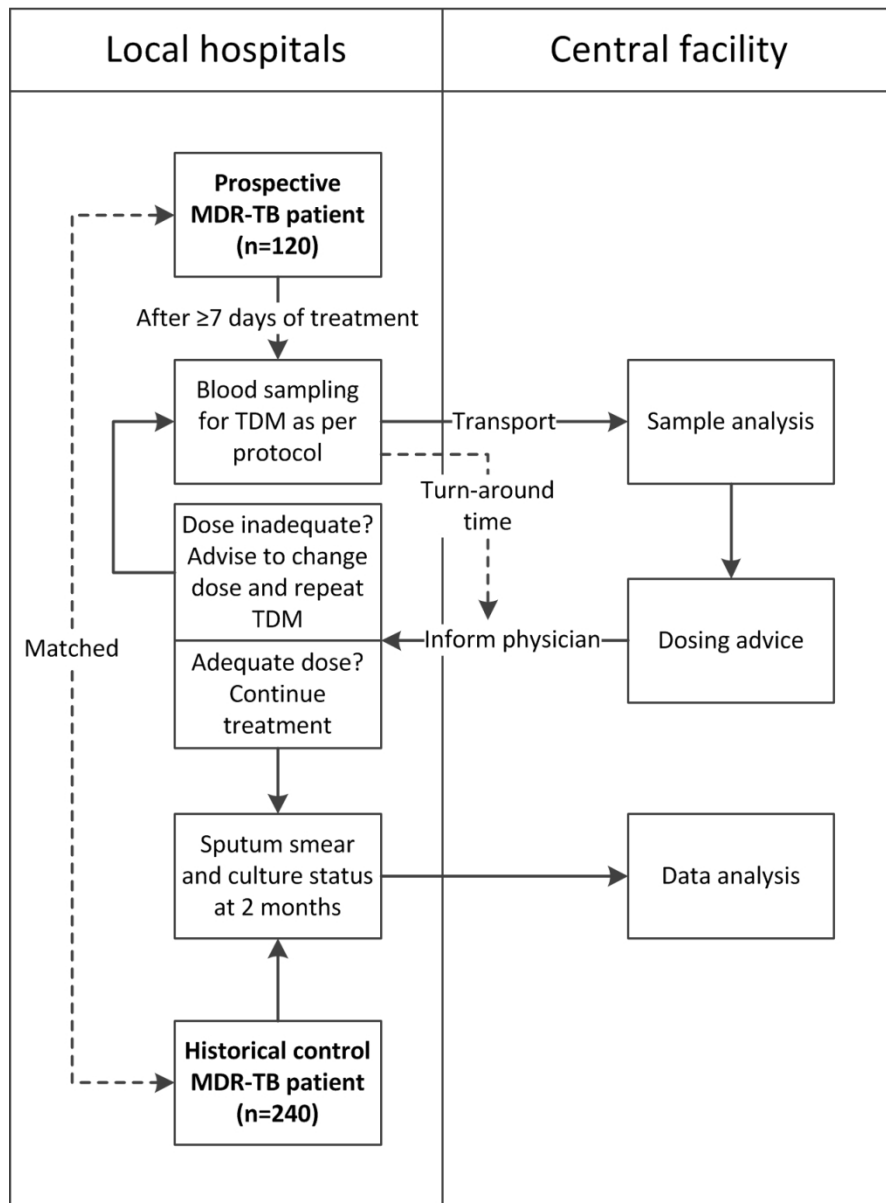
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Fluoroquinolone	Pulmonary MDR-TB	Target AUC ₀₋₂₄ /MIC ^a			Target AUC ₀₋₂₄ (mg*h/L)
		MGIT	7H10/11	LJ	
Moxifloxacin	Standard disease	>100	>50	>25	>40
	Severe disease or comorbidities	>100	>50	>25	>60 ^b
Levofloxacin	Standard disease	>150	>150 ^c	>75	>150
	Severe disease or comorbidities	>150	>150 ^c	>75	>200 ^b

30 503 ^a Minimum inhibitory concentration (MIC) varies depending on growth media; Mycobacteria Growth
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32 504 Indicator Tubes (MGIT), Middlebrook 7H10/7H11, and Lowenstein Jensen (LJ) agar.

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34 505 ^b Target AUC₀₋₂₄/MIC at site of cavity; therefore higher AUC₀₋₂₄ is required.

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36 506 ^c Levofloxacin critical concentration of 7H11 was extrapolated to 7H10.
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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