1   **A double-blinded randomised placebo-controlled phase II trial to evaluate high dose rifampicin for tuberculous**

2   **meningitis: a dose finding study**

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33   **ABSTRACT**

34

35   **Background**

36   High doses of rifampicin may help tuberculous meningitis (TBM) patients to survive. Pharmacokinetic-

37   pharmacodynamic evaluations suggested that rifampicin doses higher than 13 mg/kg intravenously or 20 mg/kg

38   orally (as previously studied) are warranted to maximize treatment response.

39   **Methods**

40   In a double-blinded, randomised, placebo-controlled phase II trial, we assigned 60 adult TBM patients in

41   Bandung, Indonesia, to standard 450 mg, 900 mg or 1350 mg (10, 20 and 30 mg/kg) oral rifampicin combined

42   with other TB drugs for 30 days. Endpoints included pharmacokinetic measures, adverse events and survival.

43   **Results**

44   A double and triple dose of oral rifampicin led to three and five-fold higher geometric mean total exposures in

45   plasma in the critical early days (2 ± 1) of treatment ($AUC_{0-24h:}$ 53·5 mg.h/L vs 170·6 mg.h/L vs. 293·5 mg.h/L,

46   p<0·001), with proportional increases in CSF concentrations and without an increase in the incidence of grade

47   3/4 adverse events. Six-month mortality was 7/20 (35%), 9/20 (45%) and 3/20 (15%) in the 10, 20 and 30 mg/kg

48   groups, respectively (p=0·12).

49   **Conclusions**

50   Tripling the standard dose caused a large increase in rifampicin exposure in plasma and CSF and was safe.

51   Survival benefit with this dose should now be evaluated in a larger phase III clinical trial.

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53   **KEYWORDS:** Tuberculosis, meningeal, RCT, rifampicin, pharmacokinetics, tolerability, survival, Indonesia

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56   **INTRODUCTION**

57   In 2016, the WHO published data on 10.4 million new tuberculosis (TB) cases and 1·3 million deaths caused by

58   this disease worldwide, making it the leading single infectious disease killer.**(1)** In turn, tuberculous meningitis

59   (TBM) is the most devastating form of TB. It occurs in 1-6% of patients with TB,**(2, 3)** leading to death or

60   neurological disability in more than 30% of affected patients.**(2, 4, 5)**

61   Antimicrobial treatment for TBM follows the model for pulmonary TB, with intensive and continuation phases

62   of treatment. It adheres to the same first-line TB drugs and dosing guidelines,**(6)** although it is known that some

63   first-line TB drugs, including rifampicin, achieve suboptimal concentrations beyond the blood-brain and blood-

64   cerebrospinal fluid (CSF) barriers. Rifampicin is a crucial TB drug, evidenced by the high mortality rate in TBM

65   patients with resistance to rifampicin.**(7, 8)** As it takes a long time to develop new drugs to treat TB and TBM, it

66   is important to make the best possible use of existing drugs. We performed a series of studies to evaluate higher

67   doses of rifampicin in Indonesian patients with TBM.

68   A first open-label, randomised phase II clinical trial showed that a 33% higher dose of rifampicin administered

69   intravenously (13 mg/kg iv) for two weeks led to a three-fold higher exposure to rifampicin in plasma and CSF

70   during the first critical days of treatment, and a strong reduction in mortality at six months after the treatment

71   started (adjusted HR 0·42, 95% CI 0·20-0·91).**(9)** We found a clear concentration-effect relationship and

72   derived threshold values for lower mortality, whereas our data suggested that the highest desirable exposures

73   had not been reached yet.**(10)**

74   However, intravenous rifampicin is not widely available in low to middle-income countries, is expensive, and

75   must be administered by healthcare workers, which is impractical over a longer period of time or unfeasible

76   after discharge from the hospital. Our second open-label pharmacokinetic study was conducted; doses of 17 or

77   20 mg/kg of oral rifampicin resulted in average total exposures in plasma (area under the concentration versus

78   time curves, AUC_{0-24h}) that were approximately similar to the values after 13 mg/kg iv, but average peak plasma

79   concentrations (C_{max}) were lower with large interindividual variabilities.**(11)**

80   Both our first and second clinical trial called for the evaluation of doses of rifampicin higher than 13 mg/kg

81   intravenously and 17-20 mg/kg orally. The current study evaluated the pharmacokinetics, safety/tolerability, and

82   efficacy of higher (up to 30 mg/kg) doses of oral rifampicin as TBM treatment.

83

84   **PATIENTS AND METHODS**

85   **Patients**

86   All patients over 14 years old (adults) with clinically suspected meningitis who presented themselves at Hasan

87   Sadikin Hospital, Bandung, Indonesia (the referral hospital for West Java) between December 2014 and

88   November 2016 had an initial screening that included standard CSF tests, blood measurements, and chest

89   radiography.

90   TBM was classified as 'definite' (microbiologically proven) if either CSF microscopy for acid-fast bacilli,

91   Mycobacterium tuberculosis culture, or PCR results were positive. Based on prior evaluation of CSF

92   characteristics of definite and clinically suspected cases in Bandung cohort, patients were classified as having

93   'probable TBM' if they had a CSF/blood glucose ratio <0.5 combined with CSF cells count ≥5cells/µl.**(4)**

94   Patients with the clinical suspicion of TBM were eligible for the study if they fulfilled the inclusion and

95   exclusion criteria listed in Supplement 1. An HIV test was performed in every patient. Microbiological

96   examinations were performed for cryptococci in HIV-infected patients (India ink microscopy and cryptococcal

97   antigen testing), M. tuberculosis (Ziehl-Neelsen microscopy, culture, and GeneXpert®)**(12)**, and bacterial

98   pathogens (Gram staining). The neurological status of patients was classified according to a modification of the

99   British Medical Research Council (BMRC) grading system as 1 (Glasgow Coma Scale (GCS) 15 with no focal

100   neurological signs), 2 (GCS 11-14 or 15 with focal neurological signs), or 3 (GCS <10).

101   Written informed consent to participate in the trial was obtained from all patients or from their relatives if the

102   patient could not provide informed consent. If in the latter case a patient regained the capacity to consider

103   participation, he was consulted and informed consent to continue the study was obtained.

104   **Study design**

105   The overall aim of this study was to find and substantiate the dose of rifampicin to be studied in a larger follow-

106   up trial, based on pharmacokinetic, safety/tolerability and efficacy considerations. The primary objective was to

107   describe the pharmacokinetics of higher doses of rifampicin in TBM patients. The secondary objectives were to

108   evaluate the safety and tolerability and to explore the efficacy of treatment regimens with higher doses of

109   rifampicin.

110   The study was a double-blinded, randomised, placebo-controlled phase II trial with three parallel arms. Eligible

111   patients were assigned a standard (450 mg, ~10 mg/kg, one active and two placebo tablets), double (900 mg,

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112   ~20 mg/kg, two active and one placebo tablets), or triple (1350 mg, ~30 mg/kg, three active tablets) dose of

113   rifampicin for 30 days, in addition to other TB drugs, according to Indonesian national guidelines.

114   Randomisation occurred in variable block sizes and was stratified by BMRC grade. Adjunctive dexamethasone

115   was administered intravenously according to a previous study in Vietnam.(13) After 30 dosages, all the patients

116   continued taking the standard TB regimen up to two months, with subsequent daily doses of rifampicin (450

117   mg) and isoniazid (300 mg) for a minimum of four months. During hospitalisation in the first four weeks of

118   treatment, adherence to the treatment was secured by facility-based Directly Observed Treatment (DOT). After

119   hospitalisation, adherence was monitored through community-based DOT, frequently through close family

120   members, pill counts and the patients' medication intake diary.

121

122   Rifampicin 450 mg (active) tablets and matching placebo tablets were manufactured at PT Kimia Farma,

123   Bandung, Indonesia. The study drugs and other standard TB treatments were administered on an empty

124   stomach. For unconscious patients who could not swallow, the drugs were crushed and delivered through a

125   nasogastric tube.

126

127   The study was approved by the Ethical Review Board of the Medical Faculty of Universitas Padjadjaran,

128   Bandung, Indonesia. External monitoring assessed compliance with the study's protocol and the Data Safety

129   Monitoring Board (DSMB) performed an interim analysis after half of the subjects were included. This trial is

130   registered with ClinicalTrials.gov, number NCT02169882.

131

132   **Bioanalysis and pharmacokinetic (PK) assessments**

133   PK sampling was performed twice, at day 2 (±1) during the first critical days of treatment (PK1) and then at day

134   10 (±1) of treatment (PK2). On each sampling day, serial blood sampling was done just before and at 1, 2, 4, 8

135   and 12 hours after dosing. CSF samples for PK assessment were collected on both PK sampling days, between 3

136   and 9 hours after dosing. Patients had an overnight fast before PK sampling and remained fasted until 2 hours

137   after the administration of the study drugs. Blood samples were centrifuged at 3000 rpm for 15 minutes, and

138   plasma and CSF samples were stored at -80^0C within 30 minutes of being taken. Analysis of the rifampicin

139   concentrations in plasma and CSF was performed as described previously. **[11]** The PK parameters for

5

140   rifampicin in plasma were assessed using standard non-compartmental methods in Phoenix WinNonlin v.6.3

141   (Certara USA Inc., Princeton, NJ) as described previously.(14)

142

143   **Follow-up**

144   Patients were followed-up on until six months after their treatment started. All patients were hospitalised for a

145   minimum of 10 days, enabling real-time surveillance of any TBM-related and drug-related adverse events in

146   that period. Monitoring of liver transaminases and full blood counts occurred on days 3, 7, 10, 14, 30, 45 and

147   60. Any further investigations were performed by indication, e.g. a bilirubin test.

148   Adverse events in these severely ill patients were defined as those possibly/probably related to their treatment

149   (haematological, gastrointestinal and skin disorders). All other adverse events (e.g. new neurological events and

150   respiratory failure) were not incorporated in the assessment of safety and tolerability.

151   The classification and grading of adverse events was based on the Common Terminology Criteria for Adverse

152   Event (CTCAE version 4.03). Treatment was stopped in patients who had grade 4 adverse events.

153

154   Efficacy was assessed by mortality and clinical and neurological responses on days 3, 7, 30, 60 and 180. New

155   neurological events were defined as the occurrence of any of the following: cranial nerve palsy, motor deficits,

156   or seizures. Modified Rankin Scale (MRS) and Glasgow Outcome Scale (GOS) were used to evaluate the

157   clinical and neurological outcomes.

158   After discharge, survival was monitored by phone calls or home visits for patients who could not be reached via

159   phone. Verbal autopsies were performed via phone calls and no post-mortem autopsy was done.

160

161   **Sample size and statistical analysis**

162   Based on a previous clinical trial, the number of participants needed per study arm to estimate the total exposure

163   to rifampicin (AUC_{0-24h}) at a dose of 20 mg/kg, using 95% confidence and a margin of error of 20%, was n=18

164   for days 2±1 and n=16 at day 10±1.(11) Considering dropouts, 20 patients were needed for each study arm. This

165   number of participants was also shown to be sufficient to demonstrate a significant difference in AUC_{0-24h}

166   achieved after 10, 20 and 30 mg/kg of rifampicin.

167   Patient characteristics, rifampicin PK parameters, adverse events were presented descriptively for all patients

168   and by study group. Multiple linear regression analysis was performed to find predictors of PK parameters. The

169   number of patients with plasma AUC_{0-24h} and C_{max} values above the threshold values for lower mortality of 116

170   h*mg/L and 22 mg/L at days 2±1 (as determined previously)(**10**) were assessed for each group. Data for

171   safety/tolerability and survival were analysed by the intention-to-treat principle. Mortality data were presented

172   as proportions in each group at various subsequent time points, and proportions were compared using the Chi-

173   square test. A Cox regression analysis assessed the effect of rifampicin dose to 6-month mortality in all patients

174   and for culture-confirmed TBM patients only. IBM SPSS Statistics (version 22.0) for Windows was used for the

175   statistical analyses; GraphPad Prism Version 5.03 and statistical software R version 3.4.2 (**15**) were used for

176   obtaining graphs. P values of less than 0.05 were judged significant in all analyses.

177

178   **RESULTS**

179   During the study period, out of the 229 patients with suspected TBM, 107 patients were excluded for alternative

180   diagnoses, and the remaining 122 patients were diagnosed with TBM. The 60 TBM patients who fulfilled all

181   eligibility criteria were randomly assigned to each arm (**Figure 1**). Baseline characteristics are depicted in **Table**

182   **1**, showing that the patients' characteristics were equally balanced across the groups, except for fewer males in

183   the 20 mg/kg group (p=0·343) and a higher percentage of HIV positives (p=0·261) and lower CSF protein

184   concentrations (p=0·057) in the 30 mg/kg group. Overall, 53% were male, with a median age of 29·5 years, and

185   10% of patients had HIV infection.

186

187   Fifty-three patients had their first PK assessments (PK1) done, whereas 48 patients had a second PK evaluation

188   (PK2). Patients who failed to undergo a PK assessment were those who died or were withdrawn. The study

189   drugs were administered via a nasogastric tube (NGT) for 33 patients (62%) at the first PK assessment and for

190   nine patients (19%) at the second assessment. In some patients, their eventual plasma AUC_{0-24h} and C_{max} values

191   could not be assessed reliably based on sampling at 1, 2, 4, 8 and 12 hours after the dose (**Table 2**) and in some

192   patients concomitant CSF sampling did not take place during the two PK assessments.

193

194   A higher dose of oral rifampicin resulted in higher geometric mean AUC_{0-24h} values, i.e. three-fold higher for the

195   20 mg/kg group and five-fold higher for the 30 mg/kg group, whereas plasma C_{max} and CSF concentrations

196   showed a more proportional increase upon increasing the rifampicin dose, both in PK1 and PK2 (**Table 2**).

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198   Large interindividual variability was observed in plasma rifampicin AUC_{0-24h} and C_{max} values and in CSF

199   concentrations. For example, the differences between the minimum and maximum plasma AUC_{0-24h} values at

200   the first PK assessment were 9-fold for the 10 mg/kg group, 14-fold for the 20 mg/kg group, and 3-fold for the

201   30 mg/kg group (**Table 2, Figure 2**). In the multivariate analysis, the dose administered was the only predictor

202   of AUC_{0-24h} and C_{max} in plasma and concentrations in CSF, whereas gender, age and BMRC grade were not

203   significant predictors (data not shown). The number of patients with drug administration via an NGT was not

204   significantly different between the three study groups and did not predict AUC_{0-24h}, C_{max} or CSF concentrations

205   either. AUC_{0-24h}, C_{max} values in plasma and CSF concentrations were all highly and positively correlated to each

206   other. For example, plasma AUC_{0-24h} and CSF concentrations correlated with a correlation coefficient of 0·7

207   (Spearman's rho, p= <0·01).

208

209   Comparison of the AUC_{0-24h} values at the first and second PK assessment showed a significant decrease of 33%

210   at the second assessment, p=0·014 (all groups combined), with decreases of 27%, 16%, and 36% in the 10, 20

211   and 30 mg/kg groups respectively (p=0·21, 0·003, and 0·004, paired T-test on log-transformed AUC_{0-24h}

212   values). Similarly, CSF concentrations decreased from day 2 to day 10 of the treatment, but plasma C_{max} values

213   did not (**Table 2**).

214

215   Adverse events during the 30 days after start of study drugs were equally distributed over the groups (**Table 3**).

216   The majority of adverse events was mild. Hepatotoxicity was the most common grade 3 adverse event. All

217   patients with grade 3 adverse events continued treatment and those with grade 3 hepatotoxicity had normal

218   transaminases after a median of 20 days (IQR 7·5-52) of continued treatment without interruption or dose

219   changes of rifampicin. One patient in the 30 mg/kg group had grade 4 hepatotoxicity at day 13 of treatment, as

220   reflected in hyperbilirubinemia of >10 times ULN and transaminases three times the ULN. All TB drugs were

221   interrupted, the dose of rifampicin was unblinded, and the higher rifampicin dose was replaced by a standard

222   dose. The blood total bilirubin had decreased to two times ULN, and transaminases were normalised by day 26

223   of treatment. Rifampicin AUC_{0-24h} and C_{max} values in those with and without grade 3 or 4 adverse events were

224   not significantly different (p= 0·325 and 0·772).

225   The administration of higher doses of rifampicin increased the percentage of patients who achieved AUC_{0-24h}

226   and C_{max} plasma threshold values for decreased mortality, as derived from our first clinical trial(**9**) (**Table 2**).

227   Two out of the 60 patients were withdrawn from the study on day 2, based on the clinician's consideration; one

228 after rifampicin resistance was identified by GenXpert® (20 mg/kg group), and the other because of rapid

229 deterioration (30 mg/kg group) as it was considered unethical to perform multiple blood or CSF sampling in this

230 patient. Only an 'intention to treat' analysis was deemed useful for this study, as most deaths occurred before

231 the patients could complete the full 'per protocol' intervention during one month. This means that the two

232 withdrawn patients were considered to be survivors in our explorative analysis of efficacy.

233 Six-month mortality was 32% (95% Confidence Intervals (CI): 19.5%-48.1%), and the majority (12/19, 63%) of

234 deaths occurred in the first two weeks after admission. Causes of death were suspected respiratory failure (n=7),

235 septic shock (n=3), brain herniation (n=3), sudden cardiac event (n=2), pulmonary embolism (n=2) and head

236 injury (n=1). Six-month mortality was non-significantly lower in the 30 mg/kg group both among all patients

237 (p=0·12) and among the cases with definite TBM (p=0·07) (**Table 4**). Cox regression analysis showed that the

238 Hazard Ratio (HR) for administration of the triple dose versus the standard dose was 0·44 with 95% CI of 0·1-

239 1·9, p=0·27, in all TBM patients, whereas it was 0·23 (95%CI 0·03-2·05, p=0·19) among patients with definite

240 TBM (both adjusted for HIV status and GCS, see also Supplement 2).

241 New neurological events at day 3, 7, 30, 60 and 180 days, as well as functional outcome measured by MRS and

242 GOS were equally distributed among all three groups of patients (data not shown).

243

DISCUSSION

245 This study revealed that tripling the standard dose of oral rifampicin strongly increased the exposure to this

246 pivotal TB drug in plasma and CSF, did not increase the incidence of grade 3 and 4 adverse events, improved

247 the attainment of previously determined exposure threshold values for lower mortality, and showed a trend for a

248 lower six-month mortality rate among patients with microbiologically proven (definite) TBM.

249 The standard dose of rifampicin resulted in low average exposures in CSF, around the Minimum Inhibitory

250 Concentration (MIC) of 0·2-0·4 mg/L of this drug for *M. tuberculosis*.[16, 17] These results are in agreement

251 with data from the literature showing that individual or mean rifampicin CSF concentrations above 1 mg/L are

252 rare.[17-20] Due to its protein binding in plasma,[21] only 15-20% of rifampicin is available to be transported to

253 other tissues, and penetration to the brain is even more limited.

254 Fortunately, our intervention with higher doses of rifampicin was effective from a pharmacokinetic point of

255 view. Tripling the rifampicin dose provided a more than proportional increase in AUC_{0-24h} and a proportional

256 increase in plasma C_{max} and CSF concentrations, which were all correlated to each other. A possible explanation

257   for the non-linear pharmacokinetics is the saturation of hepatic extraction or of the excretion of the drug in bile

258   upon increasing the dose.**(22, 23)** The resulting geometric mean AUC_{0-24h} in the 20 mg/kg and 30 mg/kg group

259   in the first critical days of treatment were higher than that of 600 mg i.v. rifampicin in our previous

260   examination**(11)** (170·6 and 293·5 versus 145·7h*mg/L); the average C_{max} in the 20 mg/kg group was still

261   lower, but the 30 mg/kg group had a similar C_{max} compared to 600 mg i.v. rifampicin (25·5 versus 24·7mg/L).

262   Our data also showed large inter-individual variability in the rifampicin exposure, which is in agreement with

263   the literature and may be enhanced by pharmacokinetic changes in critically ill patients.**(24, 25)** Of note, the

264   lowest observed AUC_{0-24h}, C_{max} and CSF concentrations increased with the higher dose (**Table 2, Figure 2**);

265   these lowest exposures may be associated with more treatment failures and mortality.

266   After the first critical days of treatment, we observed decreased exposures to rifampicin after ten days of

267   treatment, which is explained by the auto-inducing enzyme properties of rifampicin.**(22)**

268

269   Adverse events after the treatment started were equally distributed among the three rifampicin dose groups. The

270   highest 30 mg/kg daily dose of rifampicin was not associated with an increase in the incidence of severe (grade

271   3 or 4) adverse events, although it should be noted that the number of patients in this phase II study was

272   relatively small. Hepatotoxicity, as reflected in ALT and AST increases, was the most common grade 3 adverse

273   event and these increases all resolved without any interruption of the study drugs. Thus, based on our data, grade

274   3 and 4 adverse events and specifically ALT and AST increases did not seem to be dependent on rifampicin

275   dose or exposure. Hepatotoxicity to rifampicin appears to be a hypersensitivity reaction, which is more common

276   with intermittent administration of larger doses.**(26, 27)** Studies of higher doses of rifampicin in pulmonary TB

277   also suggest that hepatotoxicity to rifampicin is idiosyncratic and not dose-related.**(28, 29)**

278   As to efficacy, tripling the dose of oral rifampicin for a period of 30 days led to an increase in the attainment of

279   plasma AUC_{0-24h} and C_{max} thresholds for decreased mortality and highlighted a trend in survival benefit among

280   those with microbiologically proven (definite) TBM. However, this decrease in mortality was not statistically

281   significant, as our study was not designed and powered to detect a difference in mortality.

282   The overall aim of this trial was to find and substantiate the dose of rifampicin to be studied in a larger (phase

283   III) follow-up trial. We propose this dose to be 30 mg/kg orally, as this yielded the highest exposure in plasma

284   and CSF, was found tolerable in severely ill TBM patients, and showed a trend for reduced mortality among

285   patients with definite TBM. A study in Africa with a similar 35 mg/kg oral rifampicin dose was associated with

286   an increase in bactericidal activity **(28, 30)** and the same dose achieved a decrease in time to culture conversion

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287   in patients with pulmonary TB.**(29)** Ideally, a follow-up trial with rifampicin at a 30 mg/kg dose should be

288   performed amongst TBM patients from various continents.

289   The strengths of our study are the double-blinding that we applied and the high proportion of bacteriological-

290   confirmed TBM cases (71%). Our study also has limitations. First, the sample size was limited, inherent to the

291   study's phase II characteristics and objectives, and the study was not powered to compare the efficacy of the

292   three doses of oral rifampicin. As a result, the apparent mortality benefits of high dose oral rifampicin should be

293   interpreted with caution. Second, we could only take single CSF samples for pharmacokinetic purposes, which

294   means that no pharmacokinetic curves could be generated for CSF.

295   In summary, tripling the dose of oral rifampicin to 30 mg/kg resulted in an increased exposure to rifampicin,

296   both in plasma and CSF, which was not associated with an increase in the incidence of grade 3 and 4 adverse

297   events. The higher 30 mg/kg rifampicin dose resulted in an increase in pharmacokinetic target attainment. In

298   terms of efficacy, further investigation in a larger population is needed to confirm our findings.

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408 **Table 1. Patients' characteristics**

Variable	450mg (10 mg/kg) n=20	900mg (20 mg/kg) n=20	1350mg (30 mg/kg) n=20	p value[a]
Sex, male (%)	12 (60)	8 (40)	12 (60)	0.343
Age (median [IQR])	28 [22.3-45.8]	29 [21.3-38.3]	33 [24.3-37.3]	0.976
Body weight (median [IQR])	45 [40-47.3]	45 [38.5-50.5]	48.4 [41.6-54.7]	0.491
BMI (median [IQR])	17.6 [15.4-19]	18.2 [16-20.4]	19.1 [16.2-20.2]	0.396
Temperature (median [IQR])	37.2 [36.5-38.2]	38 [37.2-38.2]	37.6 [37-38]	0.162
Chief complaint, lowered consciousness (%)	17 (85)	16 (80%)	18 (90)	0.594
Headache	19 (33.3%)	19 (33.3)	19 (33.3)	0.558
Neck stiffness (%)	20 (100)	19 (95)	15 (75)	0.401
Cranial nerve palsy (%)	15 (75)	15 (75)	17 (85)	0.614
Motoric abnormality (%)	11 (55)	12 (60)	13 (65)	0.642
TBM grade (%)				0.663
1	0 (0.0)	1 (5.0)	0 (0)	
2	18 (90.0)	18 (90.0)	18 (90)	
3	2 (10.0)	1 (5.0)	2 (10)	
HIV, positive (%)	1 (5.0)	1 (5.0)	4 (20)	0.261
GCS (median [IQR])	13 [12-14]	12.5[11.3—13.8]	13 [11.3-13.8]	0.877
Chest X-ray, TB (%)	13 (86.7)	12 (85.7)	12 (85.7)	0.752
CSF leukocytes baseline (cells/µl) (median [IQR])	188 [60.5-413.8]	245 [98.8-698.8]	240 [128.3-482.5]	0.528
CSF PMN baseline (cells/µl) (median [IQR])	30 [18-46]	29 [13.3-67]	41 [21.3-65.3]	0.625
CSF MN baseline (cells/µl) (median [IQR])	70 [54-82]	71 [33-86.8]	59 [34.8-78.8]	0.580
CSF protein baseline (mg/dl) (median [IQR])	257 [172.7-372.3]	216.5 [158.8-428]	169 [116.5-230.8]	0.057
CSF/Blood glucose ratio baseline (median [IQR])	0.17 [0.09-0.26]	0.2 [0.12-0.21]	0.2 [0.1-0.3]	0.303
Bacteriologically confirmed TBM (%)	14 (70)	14 (70)	15 (75)	0.921
Culture positive (%)	13 (65)	13 (65)	14 (70)	0.928
ZN staining positive (%)	8 (40)	4 (20)	6 (30)	0.386
GenXpert positive (%)	9 (45)	8 (40)	10 (50)	0.582
Drug administration through NGT on PK I (%)	10 (58.8)	12 (60.0)	13 (65)	0.917
Drug administration through NGT on PK II (%)	1 (6.7)	3 (21.4)	5 (26.3)	0.33

Isoniazid dose in mg/kg (median [IQR])	6.7 [6.4-.7.5]	6.7 [6-7.9]	6.2 [5.5-7.2]	0.308
Pyrazinamide dose in mg/kg (median [IQR])	33.7 [33.3-37.5]	33.3 [31.6-39.5]	31.06 [27.4-36]	0.203
Ethambutol dose in mg/kg (median [IQR])	16.8 [16.7-20.1]	16.7 [15-21.1]	15.5 [14-18.5]	0.244
Rifampicin dose in mg/kg (median [IQR])	10 [9.5-11.3]	20 [17.8-23.4]	28 [24.7-32.4]	0.000

409

410 **Abbreviations and explanation**: TB= Tuberculosis; IQR= Interquartile Range; BMI= Body Mass Index; TBM=tuberculous

411 meningitis; HIV= Human Immunodeficiency Virus; GCS=Glasgow Coma Scale; CSF= cerebrospinal fluid; ZN=Ziehl-

412 Neelsen; NGT= nasogastric tube; PK= pharmacokinetic(s); PMN=polymorphonuclear cells; MN= mononuclear cells;

413 mg=milligram; kg=kilogram.

414 [a] Kruskall Wallis test on continuous variables. The $\chi 2$ test was used to compare the proportion of categorical variables.

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418 **Table 2. Pharmacokinetic data of rifampicin**

	450 mg (10 mg/kg) GM [range]	900 mg (20 mg/kg) GM [range]	1350 mg (30 mg/kg) GM [range]	p value[a]
First PK assessment				
Day (Median [range])	1 (1-3)	1 (1-3)	1 (1-3)	
AUC_{0-24h} (mg.h/L)	53.5 [13.4-116.5]	170.6 [26.4-377.1]	293.5 [190.1-533.3]	0.000
$T_{1/2}$ (h) [median(range)]	4.9 [2.1-20]	6.3 [3.7-10.6]	8.8 [3.7-25.6]	0.022
C_{max} (mg/L)	7.2 [2.2-14.1]	18.1 [2-43.6]	25.5 [11.9-55.5]	0.000
Vd/F (L)	59.2 [26.0-217.1]	47.9 [22.4-516.3]	58.1 [24.8-146.3]	0.644
Cl/F (L/h)	8.4 [3.9-33.6]	5.3 [2.4-34.1]	4.6 [2.5-7.1]	0.020
T_{max} (h) (Median [range])	3.6 [1-9]	3.9 [0.9-12]	3.9 [1-12]	0.882[b]
CSF_{3-9h} (mg/L)	0.28 [0.13-0.48]	0.59 [0.13-1.7]	0.74 [0.24-1.20]	0.000
Time to LP (h:m) (median [range])	04:02 [03:02-05:45][c]	04:19 [03:02-06:13][c]	03:57 [03:15-05:29][c]	
AUC0-24h >116 mg*h/L	1/11 (9.1%)	13/15 (86.7)	17/17 (100%)	0.000[d]
Cmax >22 mg/L	0/14	7/18 (39%)	14/19 (74%)	0.000[d]
Second PK assessment				
Day (Median [range])	10 (9-11)	10 (9-11)	10 (9-11)	
AUC_{0-24h} (mg.h/L)	39.23 [17.41-66.53]	144.15 [86.25-241.67]	187.89 [41.61-392.14]	0.000
$T_{1/2}$ (h)	2.09 [1.27-7.75]	3.12 [2.02-6.10]	3.27 [1.65-7.39]	0.008
Cmax (mg/L)	7.55 [4.45-14.3]	21.89 [13.28-32]	25.14 [9.9-54.78]	0.000
Vd/F (L)	34.57 [16.30-80.66]	28.21 [17.20-66.77]	34.47 [19.27-77.37]	0.464
Cl/F (L/h)	11.47 [6.72-25.85]	6.24 [3.72-10.44]	7.19 [3.44-32.44]	0.004
T_{max} (h) (Median [range])	2.72 [1-4]	2.92 [1-8]	3.59 [1-12]	0.768[b]
CSF_{3-9h} (mg/L).	0.23 [0.13-0.63]	0.39 [0.13-0.82]	0.51 [0.13-1.18]	0.008
Time to LP (h:m) (median [range])	03:41 [03:02-06:00][c]	03:51[03:28-05:52][c]	04:02 [03:17-05:57][C]	

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420 **Abbreviations and explanation**: AUC_{0-24h}= area under the time-concentration up to 24 h after dose; GM= Geometric Mean; C_{max}=

421 maximum plasma concentration; T_{max}= time to C_{max}; CSF= cerebrospinal fluid; CSF_{3-9h} = concentration in cerebrospinal fluid between 3h

422 and 9h after drug administration; Vd/F= apparent volume of distribution; Cl/F= apparent total clearance; $T_{1/2}$= Half-life; h= hour;

423 m=minutes; L=litre; mg=milligram; kg=kilogram; LP= lumbar puncture.

424 Data are number (%) or geometric mean (range).

425 [a] One-way ANOVA (analysis of variance) on log-transformed PK data, with Tukey's HSD as post-hoc test.

426 [b] Kruskall Wallis test.

427 [c] CSF samples had to be taken between 3 and 9 h after the dose and were mostly taken around 4 h after the dose.

428 [d] Threshold values for reduced mortality based on previous work.(10)

429 PK1 was at day 2 ±1, PK2 was at day 10±1. AUC_{0-24h} values were estimated in 43 patients both in PK1 and PK2. The rest could not be

430 estimated because the elimination rate constant could not be assessed reliably with sampling at 1, 2, 4, 8 and 12h after dosing, preventing

431 extrapolation of exposures beyond the last measurable concentration. C_{max} could not be assessed in 7 patients.

432

433   **Table 3. Safety and tolerability**

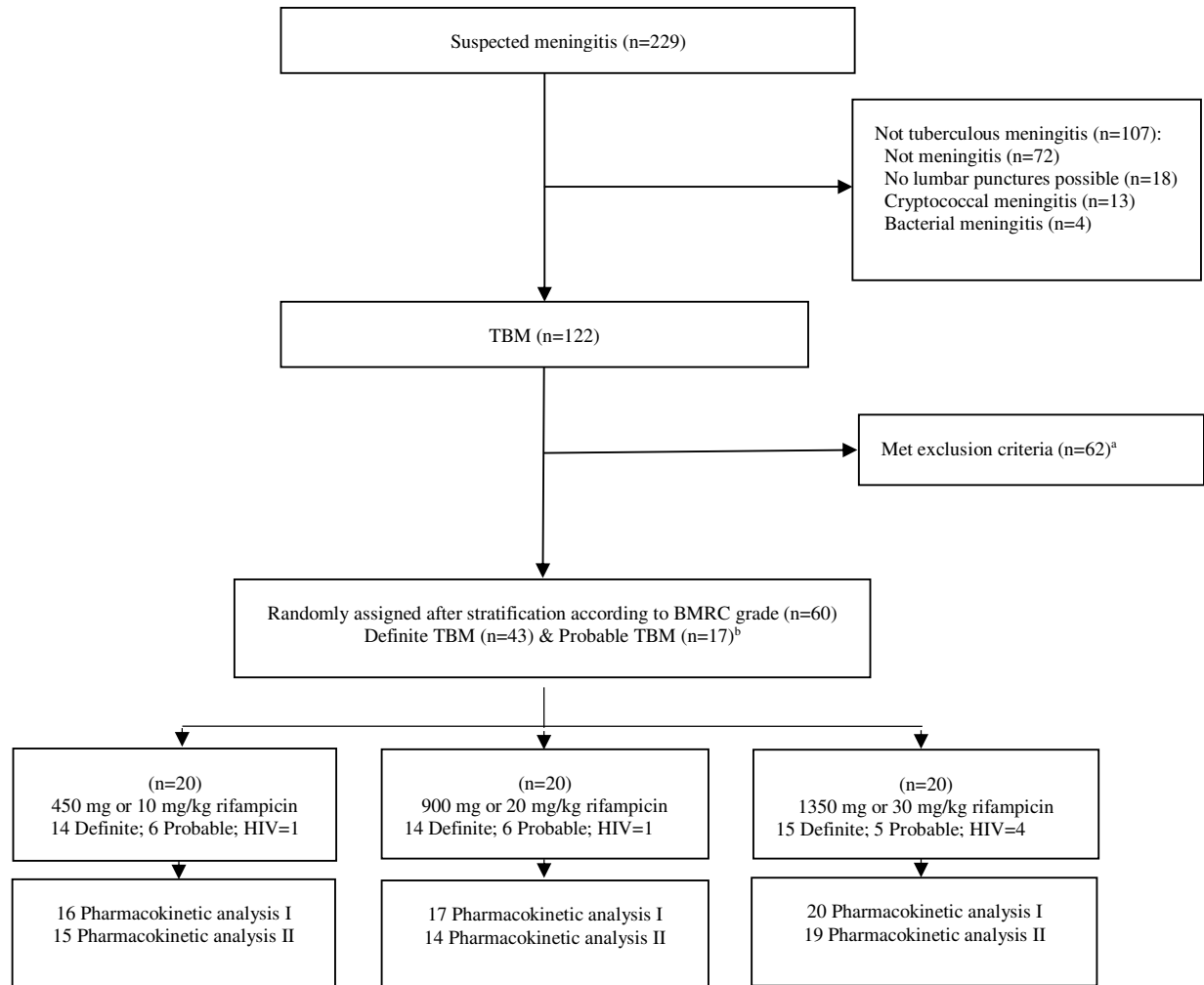|	All	450 mg (10 mg/kg) n=20	900 mg (20 mg/kg) n=20	1350 mg (30 mg/kg) n=20	p
			N (%)		
All AE	51 (85)	17 (85)	16 (80)	18 (90)	0.676
Grade I-II AE	51 (85)	17 (85)	16 (80)	18 (90)	0.676
Grade III-IV AE	15 (25)	3 (15)	8 (40)	4 (20)	0.503
Specific adverse effects					
Purpura					
Grade I-II	1 (1.7)	0	0	1 (5)	0.608
Thrombocytopenia					
Grade I-II	8 (13.3)	2 (10)	4 (20)	2 (10)	0.686
Leukopenia					
Grade I-II	3 (5)	0	1 (5)	2 (10)	0.593
Anemia					
Grade I-II	20 (33.3)	11 (55)	6 (30)	3 (15)	0.107
Grade III	1 (1.7)	0	1 (5)	0	
Hepatotoxicity					
Grade I-II	26 (43)	9 (45)	7 (35)	10 (50)	0.824
Grade III/IV	12 (20)	3 (15)	5 (25)	4 (20)	
Nausea					
Grade I-II	27 (45)	9 (45)	8 (40)	10 (50)	0.444
Vomitus					
Grade I-II	21 (35)	8 (40)	6 (30)	7 (35)	0.359
Abdominal discomfort					
Grade I-II	15 (25)	4 (20)	6 (30)	5 (25)	0.189
Diarrhea					
Grade I-II	8 (13.3)	1 (5)	3 (15)	4 (20)	0.602
Pruritus					
Grade I-II	27 (43.3)	9 (45)	6 (30)	12 (60)	0.442
Grade III	1 (1.7)	0	1 (5)	0	
Rash					
Grade I-II	21 (35)	5 (25)	5 (25)	11 (55)	0.410
Grade III	1 (1.7)	0	1 (5)	0	

434   **Abbreviations and explanation:** Data in number (%) of patients. Classification of adverse events was based on the US National Institutes of Health Common

435   Terminology Criteria for Adverse Event (CTCAE) version 4.03.

18

436   **Table 4. Patients' cumulative mortality per time point**

	All TBM patients				Bacteriologically confirmed (definite) TBM[a]			
n (%)				p				p
	450 mg (10 mg/kg)	900 mg (20 mg/kg)	1350 mg (30 mg/kg)		450 mg (10 mg/kg)	900 mg (20 mg/kg)	1350 mg (30 mg/kg)	
	All (n=60)	n=20	n=20	n=20		n=14	n=14	n=15
Mortality until discharge	13 (22)	5 (25)	5 (25)	3 (15)	0.675	3 (21)	4 (29)	1 (7)	0.301
Mortality until 30 days	14 (23)	5 (25)	6 (30)	3 (15)	0.521	3 (21)	4 (29)	1 (7)	0.301
Mortality until 45 days	15 (25)	5 (25)	7 (35)	3 (15)	0.344	3 (21)	5 (36)	1 (7)	0.158
Mortality until 60 days	15 (25)	5 (25)	7 (35)	3 (15)	0.344	3 (21)	5 (36)	1 (7)	0.158
Mortality until 180 days	19 (32)	7 (35)	9 (45)	3 (15)	0.116	5 (36)	6 (43)	1 (7)	0.069

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438

439   [a] TBM was classified as 'definite' (microbiologically proven) if either CSF microscopy for acid-fast bacilli, *Mycobacterium*

440   *tuberculosis* culture, or PCR results were positive.

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Figure1. Trial profile

Suspected meningitis (n=229)

Not tuberculous meningitis (n=107):
  Not meningitis (n=72)
  No lumbar punctures possible (n=18)
  Cryptococcal meningitis (n=13)
  Bacterial meningitis (n=4)

TBM (n=122)

Met exclusion criteria (n=62)[a]

Randomly assigned after stratification according to BMRC grade (n=60)
Definite TBM (n=43) & Probable TBM (n=17)[b]

| (n=20) 450 mg or 10 mg/kg rifampicin 14 Definite; 6 Probable; HIV=1	(n=20) 900 mg or 20 mg/kg rifampicin 14 Definite; 6 Probable; HIV=1	(n=20) 1350 mg or 30 mg/kg rifampicin 15 Definite; 5 Probable; HIV=4
16 Pharmacokinetic analysis I 15 Pharmacokinetic analysis II	17 Pharmacokinetic analysis I 14 Pharmacokinetic analysis II	20 Pharmacokinetic analysis I 19 Pharmacokinetic analysis II

Abbreviations and explanation: TBM= Tuberculous Meningitis; ICF= Informed Consent Form; HIV=Human Immunodeficiency Virus infection; BMRC=British Medical Research Council; mg=milligram; kg=kilogram.

[a] Sixty two patients were excluded due to: 10 had CSF/blood glucose ratio >0.5, 11 had a history of TB treatment within the last 3 days, 5 had eGFR < 50, 1 had ALT >5x ULN, 1 had a history of rifampicin hypersensitivity, 22 had rapid clinical deterioration, 3 had severe mental psychosis, 1 was pregnant, 3 were younger than 15 years, and 5 declined to signed the ICF. See Supplement 1 for the list of inclusion- and exclusion criteria.

[b] TBM was classified as 'definite' (microbiologically proven) if either CSF microscopy for acid-fast bacilli, *Mycobacterium tuberculosis* culture, or PCR results were positive. Based on prior evaluation of CSF characteristics of definite and clinically suspected cases in Bandung cohort, patients were classified as having 'probable TBM' if they had a CSF/blood glucose ratio <0.5 combined with CSF cells count ≥5 cells/µl.

Patients with rapid clinical deterioration were excluded due to ethical considerations related to blood and CSF PK sampling in these patients. Patients 0-14 years old were excluded as they are considered as paediatric TB patients according to the Ministry of Health Republic of Indonesia regulation for age limitation for paediatric TB management.**(31)**
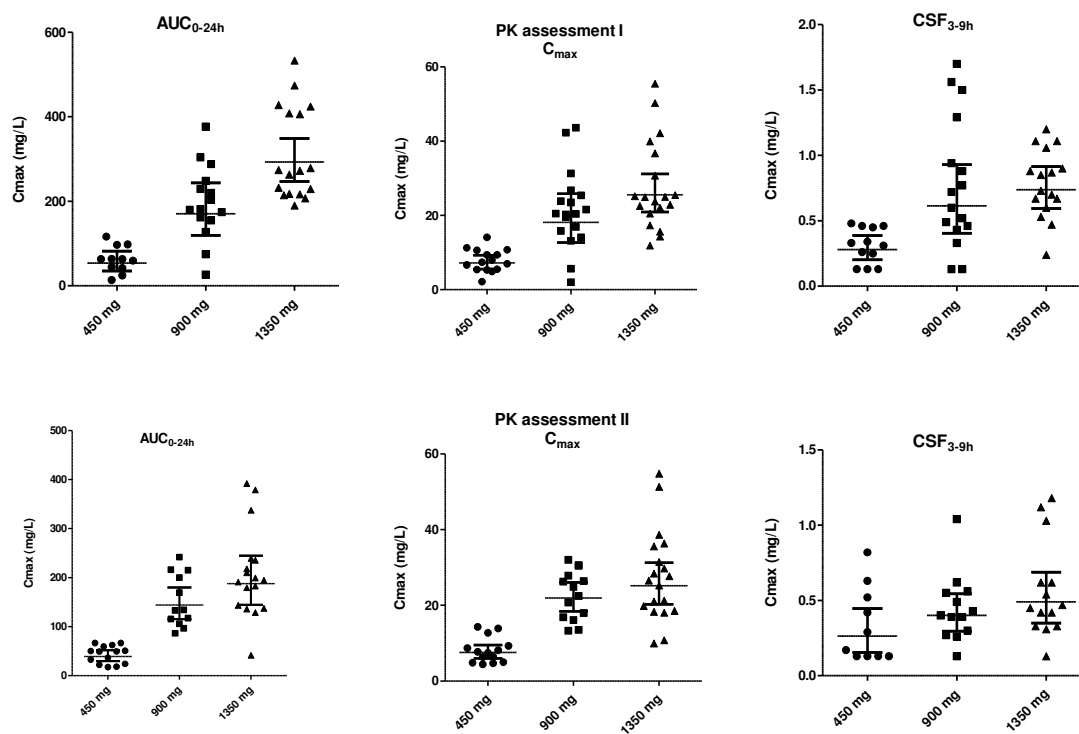
Figure 2. Distribution of rifampicin area under the time-concentration curve up to 24 h after the dose (AUC_{0-24h}) and maximum concentration (C_{max}) in plasma, and concentration in CSF at 3-9h after the dose, at the first and second pharmacokinetic assessments. Lines represent Geometric Mean (GM) and 95% Confidence Interval (CI).

Supplement 2. Survival according to rifampicin treatment in all patients and in patients with mycobacteriologically proven (definite) TBM
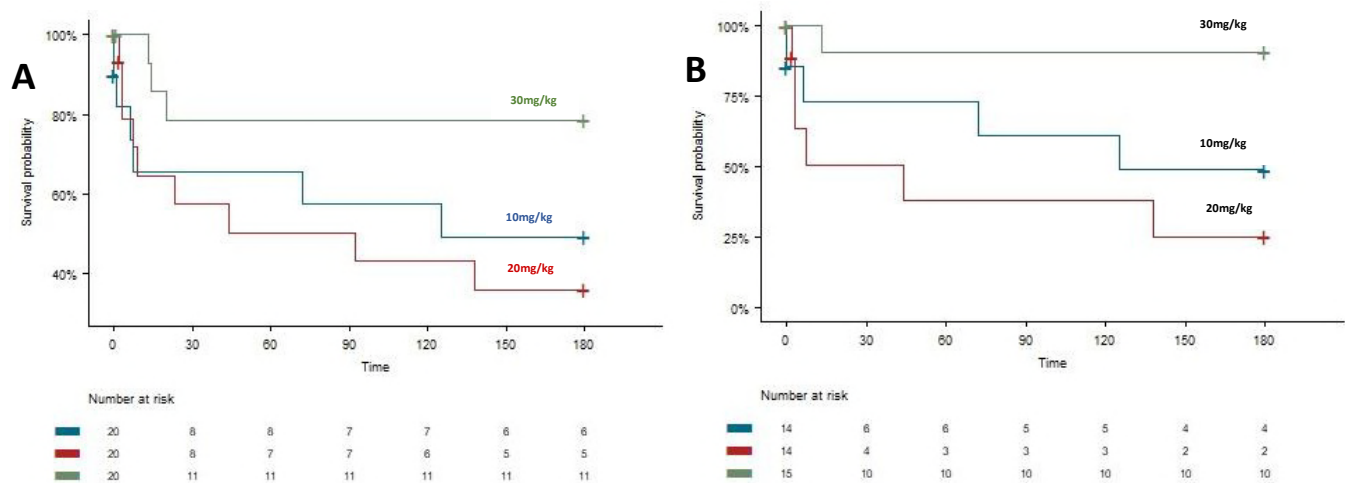
Explanation: Rifampicin dose as predictor for 6-month mortality due to tuberculous meningitis. Kaplan-Meier curves with survival tables for numbers at risk for all patients (**A**) and for patients with mycobacteriologically-proven (definite) tuberculous meningitis (**B**). Hazard ratio (HR) adjusted for HIV status and GCS: (all patients) 0·44 (95% CI 0·1-1·9, p=0·27) and (patients with definite tuberculous meningitis) 0·23 (95% CI 0·03-2·05, p=0·19).