## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | The Home-based Anti-Tuberculosis Treatment Adverse Reactions (HATTAR) study: a protocol for a prospective observational study |
| **AUTHORS** | Yang, Miaomiao; Pan, Hongqiu; Lu, Lihuan; He, Xiaomin; Chen, Hongbo; Tao, Bilin; Liu, Wenpei; Yi, Honggang; Tang, Shaowen |

## VERSION 1 - REVIEW

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| **REVIEWER** | Jorge E. Machado-Alba<br>Universidad Tecnologica de Pereira. Colombia |
| **REVIEW RETURNED** | 19-Nov-2018 |

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| **GENERAL COMMENTS** | The authors treat a topic really important with information of real evidence |

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| **REVIEWER** | Dr Star Khoza<br>University of the Western Cape, South Africa |
| **REVIEW RETURNED** | 05-Dec-2018 |

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| **GENERAL COMMENTS** | General comments<br>This is an important study which attempts to provide answers regarding the occurrence of ADRs in a home-based setting. The strength of the design is the 'natural setting' and the incorporation of genetic testing, which has lacked in many published studies.<br><br>Specific comments<br>1. In the Introduction, page 4, line 26-28: please revise the sentence to read in part ' …, arthralgia, and neurological disorders.' Delete "and so on".<br><br>2. study design: It is stated that a sample size of 3200 will be targeted.However, the methods section also states that all eligible patients will be recruited. This sounds contradictory. The authors can consider providing a little bit more details on the study setting of the four institutions in terms of how many TB patients are treated in these institutions: how many TB patients are treated at any one point in time, and how many new patients are initiated per year. This will contextualize and reconcile the sample size calculation and the statement about recruiting all eligible patients in the study<br><br>3. Methods, page 5, line 53-55: The withdrawal of 'patients who develop diseases that meet exclusion criteria after enrollment" needs clarification. At what stage after enrollment will these patients be withdrawn? I assume it is before patients start receiving TB treatment. Withdrawal of patients who develop |

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| | psychiatric disorders or liver disorders after they initiate treatment will introduce bias. Therefore, it is important to state the specific time at which development of the diseases that meet the exclusion criteria will lead to patient withdrawal from the study.<br>4. Page 5, line 1: The withdrawal of patients whose death is not caused by anti-TB drug-induced ADRs needs clarification. The first objective includes clinical outcomes of TB treatment. Will patients who die from TB be withdrawn? This may bias the outcomes related to treatment since deaths could indicate a poor response to the drugs, which could be due to genetic factors or other factors that the protocol is trying to elucidate.<br>5. It is stated that a matched nested case-control study will be used to evaluate the risk factors for the development of ADRs. What are the variables that will be used to match the cases and the controls? These need to be specified in the protocol. The exact definitions for cases and controls should also be provided. The ratio of the cases and controls should also be specified in the protocol before the analysis is conducted.<br>6. Page 8; All statements in the data analysis plan should be in future tense. |

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| **REVIEWER** | Lina Davies Forsman, MD, PhD, Consultant Infectious Diseases Karolinska Institutet, Sweden |
| **REVIEW RETURNED** | 22-Jan-2019 |

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| **GENERAL COMMENTS** | 1. Please define ADRs and ADEs and the difference between the two concepts. As I understand: (do not cite, find appropriate references please) an “Adverse Drug Reaction is a reaction which is mentioned for specific drug in the prescription explanation given by drug manufacturer, in other words it is an objective adverse reaction evidence-based on the findings from the clinical trials”. “Adverse Drug Event is a side effect which was revealed after usage the drug and is reported by the patient or the doctor who faced with this event in his personal experience”<br>2. Please change to British English (multicentre, not multicentre, for example).<br>3. Including patients from the “floating population” is a strength of the study, as selection bias would be introduced otherwise. However, the term needs to be defined and explained since not all readers are familiar with this concept, that is common in China.<br>4. Perhaps the first exclusion criteria could be explained a bit more thorough? “having a psychiatric illness” is clear, but “requiring the incorporation of a questionnaire investigation” is not so clear. Does it have to do with the severity of the psychiatric illness? Please clarify.<br>5. Regarding the second exclusion criteria, I suggest …with a life-expectancy shorter than 6 months” for more appropriate English.<br>6. “Treatment adherence” is preferable to “treatment compliance”, since it’s regarded less derogatory. According to the 2013 WHO definitions, treatment interruption for more than 2 months is defined as “Loss to follow-up”.<br>7. Are patients excluded if they miss one or all the scheduled laboratory tests in the first two months? A suggestion is to provide more details.<br>8. Suggest “ADR classification” instead of “ADR judgement”. |

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| | 9. Will Bedaquiline be used for MDR-TB? Following the recently changed WHO recommendation, perhaps regular ECGs and cardiac ADRs might need to be added?<br>10. “When all TB patients finish treatment, the local supervising doctors will comprehensively judge the patient's treatment outcomes according to their symptoms and signs, various clinical examinations, drug use, etc., and record them on the management card of every patient.” Please make sure that definitions of treatment outcome follow the latest definitions by the World Health Organization, to be able to compare results with other international studies.<br>11. Regarding the number of included patients, perhaps information about how many TB patients are normally treated in the four included hospitals could be added. How much attrition due to patients not being willing to join have you assumed? Important information to judge whether the study is likely to be able to include enough patients during the study period.<br>12. Statistical analysis. Consider performing Cox regression analysis to also investigate time to event data, interesting information for ADRs.<br>13. The genetic analyses are not described in detail. It is Important that all genetic analyses are prespecified and included in the informed consent so patients know what kind of genetic analysis are being performed and why.<br>14. “Most ADRs induced by anti-TB drugs occur within the first two months of treatment [6, 42], including MDR-TB treatment [43].” This might not be true for all therapies of MDR-TB, since nephrotoxicity of kanamycin are increasing over time (cumulative dose dependent) for example.<br>15. Will the informed consent be in Mandarin or also in other languages if needed? What is the procedure if the patient is illiterate?<br>16. In genetic studies, it is often applicable to correct for the issue of multiple comparison. Will Bonferroni corrections or other methods be used? |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer: 1

Reviewer Name: Jorge E. Machado-Alba

Institution and Country: Universidad Tecnologica de Pereira. Colombia

Please state any competing interests or state ‘None declared’: I declare no conflict of interest.

Please leave your comments for the authors below

Q1. The authors treat a topic really important with information of real evidence

A1. We would like to thank the referee for the compliment.

Reviewer: 2

Reviewer Name: Dr Star Khoza

Institution and Country: University of the Western Cape, South Africa

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

Please leave your comments for the authors below

General comments

This is an important study which attempts to provide answers regarding the occurrence of ADRs in a home-based setting. The strength of the design is the 'natural setting' and the incorporation of genetic testing, which has lacked in many published studies.

Specific comments

Q1. In the Introduction, page 4, line 26-28: please revise the sentence to read in part ' …, arthralgia, and neurological disorders.' Delete "and so on".

A1: We would like to thank the referee for the useful comments. We have revised the manuscript.

Q2. study design: It is stated that a sample size of 3200 will be targeted. However, the methods section also states that all eligible patients will be recruited. This sounds contradictory. The authors can consider providing a little bit more details on the study setting of the four institutions in terms of how many TB patients are treated in these institutions: how many TB patients are treated at any one point in time, and how many new patients are initiated per year. This will contextualize and reconcile the sample size calculation and the statement about recruiting all eligible patients in the study

A2: We would like to thank the referee for the useful comments. Because of our negligence, we did not express clearly. As we described in the manuscript, only newly diagnosed TB patients between January 2019 and December 2020 will be included in present study. We set the target sample size to 3200 newly diagnosed TB patients. Based on our previous ADACS cohort[1], and setting the exclusion rate to 2.4% and the participation rate of eligible subjects to 71.2%[2], at least 4600 newly diagnosed TB patients will be needed within two years. According to the number of newly diagnosed TB patients in each hospital per year (600 patients per hospital), the total number of TB patients in four hospitals is almost 2400 per year, which fully meets the sample size requirement in two years. So, the subjects recruited should be potential eligible patients, not all patients, because 71.2% of patients were unwilling to participate in the study[2]. We have modified this error here. Additionally, we also modified the effect size of odds ratio (OR) to make it more reasonable (the original value is a bit large). We have revised the description in Sample size calculation section.

References

[1] Xia YY, Hu DY, Liu FY, et al. Design of the anti-tuberculosis drugs induced adverse reactions in China National Tuberculosis Prevention and Control Scheme Study (ADACS). BMC public health. 2010;10:267.

[2] Wu S, Xia Y, Lv X, et al. Preventive use of hepatoprotectors yields limited efficacy on the liver toxicity of anti-tuberculosis agents in a large cohort of Chinese patients. J Gastroenterol Hepatol. 2015 Mar;30(3):540-5.

Q3. Methods, page 5, line 53-55: The withdrawal of 'patients who develop diseases that meet exclusion criteria after enrollment" needs clarification. At what stage after enrollment will these patients be withdrawn? I assume it is before patients start receiving TB treatment. Withdrawal of patients who develop psychiatric disorders or liver disorders after they initiate treatment will introduce bias. Therefore, it is important to state the specific time at which development of the diseases that meet the exclusion criteria will lead to patient withdrawal from the study.

A3: We would like to thank the referee for the useful comments. Because of our negligence, we did not express clearly. This refers to the withdrawal of the study after the patient received anti-TB

treatment. These patients are mainly suffering from serious diseases that prevent them from continuing anti-TB treatment, and not subjectively unwilling to continue treatment. However, it does not include patients who are unable to continue treatment because of adverse drug reactions. Otherwise, it will introduce bias. We have revised the Withdrawal criteria to make it clearer ((3) developing serious diseases that prevent them from continuing anti-TB treatment).

Q4. Page 5, line 1: The withdrawal of patients whose death is not caused by anti-TB drug-induced ADRs needs clarification. The first objective includes clinical outcomes of TB treatment. Will patients who die from TB be withdrawn? This may bias the outcomes related to treatment since deaths could indicate a poor response to the drugs, which could be due to genetic factors or other factors that the protocol is trying to elucidate.

A4: We would like to thank the referee for the useful comments. Indeed, the withdrawal of patients who die from TB would bias the outcomes related to treatment. In China, unless there are other exact causes of death, sometimes it is difficult to distinguish whether the patient died of TB or other combined diseases. If these TB patients were not cured, they would be presumed to die of TB when they died. However, in reality, they may also die of other diseases. According to your suggestion, we will include patients who died of TB. Sensitivity analysis (with and without this part of patients) could be performed when the outcomes related to treatment are analyzed. We have revised the Withdrawal criteria to make it clearer ((5) death that is not caused by TB or anti-TB drug-induced ADRs).

Q5. It is stated that a matched nested case-control study will be used to evaluate the risk factors for the development of ADRs. What are the variables that will be used to match the cases and the controls? These need to be specified in the protocol. The exact definitions for cases and controls should also be provided. The ratio of the cases and controls should also be specified in the protocol before the analysis is conducted.

A5: We would like to thank the referee for the useful comments. Because of our negligence, we did not express clearly. Unmatched nested case-control study will be used to evaluate general the risk factors for the development of ADRs (including anti-TB drug-induced hepatotoxicity (ATDH)). Patients who fulfilled the ATDH criteria will be assigned to the case group, whereas controls will be selected from those with sustained normal liver function through the whole therapy. Furthermore, matched nested case-control study will be used to explore the role of genetic polymorphisms in susceptibility to ATDH. For each ATDH case, two controls will be selected randomly and matched for the place of sample collection, age (within 5 years), sex and treatment history. We have revised the Data analysis plan to make it clearer.

Q6. Page 8; All statements in the data analysis plan should be in future tense.

A6: We would like to thank the referee for the useful comments and have revised the manuscript.

Reviewer: 3

Reviewer Name: Lina Davies Forsman, MD, PhD, Consultant Infectious Diseases

Institution and Country: Karolinska Institutet, Sweden

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

Please leave your comments for the authors below

See attached file for comments.

Q1. Please define ADRs and ADEs and the difference between the two concepts. As I understand: (do not cite, find appropriate references please) an “Adverse Drug Reaction is a reaction which is mentioned for specific drug in the prescription explanation given by drug manufacturer, in other words it is an objective adverse reaction evidence-based on the findings from the clinical trials”. “Adverse Drug Event is a side effect which was revealed after usage the drug and is reported by the patient or the doctor who faced with this event in his personal experience”.

A1: We would like to thank the referee for the useful comments. Because of our negligence, we did not give a clear definition. According to WHO, adverse drug event (ADE) is defined as any untoward medical occurrence that may appear during treatment with a pharmaceutical product but which does not necessarily have a causal relationship with the treatment[1], and an adverse drug reaction (ADR) is any response to a drug that is noxious and unintended and that occurs at doses normally used in humans for prophylaxis, diagnosis, or therapy of disease, or for the modification of physiological function[2]. We have revised the manuscript and added the definitions.

References

[1] Nebeker JR, Barach P, Samore MH. Clarifying adverse drug events: a clinician's guide to terminology, documentation, and reporting. Ann Intern Med. 2004 May 18;140(10):795-801.

[2] Kalaiselvan V, Kumar P, Mishra P, et al. System of adverse drug reactions reporting: What, where, how, and whom to report? Indian J Crit Care Med. 2015 Sep;19(9):564-6.

Q2. Please change to British English (multicentre, not multicentre, for example).

A2: We would like to thank the referee for the useful comments and have revised the manuscript.

Q3. Including patients from the “floating population” is a strength of the study, as selection bias would be introduced otherwise. However, the term needs to be defined and explained since not all readers are familiar with this concept, that is common in China.

A3: We would like to thank the referee for the useful comments and have added the definition in the manuscript (people who engage in partial temporary relocation, whose registration of legal residence remains in their original place of habitation and who are ineligible for permanent residence in the locale into which they moved[1]).

References

[1] Chang S. The floating population: an informal process of urbanisation in China. Int J Popul Geogr. 1996 Sep;2(3):197-214.

Q4. Perhaps the first exclusion criteria could be explained a bit more thorough? “having a psychiatric illness” is clear, but “requiring the incorporation of a questionnaire investigation” is not so clear. Does it have to do with the severity of the psychiatric illness? Please clarify.

A4: We would like to thank the referee for the useful comments. Because of our negligence, we did not express clearly. In fact, this mainly means that patients with psychiatric illness cannot self-record the signs and/or symptoms of adverse drug reactions during the anti-tuberculosis treatment. So, we have revised the first exclusion criteria ((1) having a psychiatric disease and unable to fill out the self-recorded diaries during the anti-TB treatment).

Q5. Regarding the second exclusion criteria, I suggest …with a life-expectancy shorter than 6 months” for more appropriate English.

A5: We would like to thank the referee for the useful comments and have revised the manuscript.

Q6. “Treatment adherence” is preferable to “treatment compliance”, since it’s regarded less derogatory. According to the 2013 WHO definitions, treatment interruption for more than 2 months is defined as “Loss to follow-up”.

A6: We would like to thank the referee for the useful comments and have revised the manuscript.

Q7. Are patients excluded if they miss one or all the scheduled laboratory tests in the first two months? A suggestion is to provide more details.

A7: We would like to thank the referee for the useful comments. This refers to patients without all the scheduled laboratory tests in the first two months, which makes it impossible to judge the occurrence of liver injury. We have revised the manuscript.

Q8. Suggest “ADR classification” instead of “ADR judgement”.

A8: We would like to thank the referee for the useful comments and have revised the manuscript.

Q9. Will Bedaquiline be used for MDR-TB? Following the recently changed WHO recommendation, perhaps regular ECGs and cardiac ADRs might need to be added?

A9: We would like to thank the referee for the useful comments. Based on the WHO treatment guidelines for drug-resistant tuberculosis[1], Bedaquiline and Delamanid have now been assigned to a specific subgroup (Group D2) of add-on agents used to treat MDR/RR-TB, and Bedaquiline is still only recommended for adults. The main and rare but serious adverse effects caused by Bedaquiline are QT prolongation, hepatitis, gastrointestinal toxicity, and others. We have revised the manuscript according to your advice.

References

[1] World Health Organization, WHO treatment guidelines for drug-resistant tuberculosis, 2016 update. 2016.

Q10. “When all TB patients finish treatment, the local supervising doctors will comprehensively judge the patient's treatment outcomes according to their symptoms and signs, various clinical examinations, drug use, etc., and record them on the management card of every patient.” Please make sure that definitions of treatment outcome follow the latest definitions by the World Health Organization, to be able to compare results with other international studies.

A10: We would like to thank the referee for the useful comments. We have revised the treatment outcome for TB patients (cured, treatment completed, treatment failed, died, lost to follow-up, not evaluated, treatment success) according to Definitions and reporting framework for tuberculosis - 2013 revision (updated December 2014).

Q11. Regarding the number of included patients, perhaps information about how many TB patients are normally treated in the four included hospitals could be added. How much attrition due to patients not being willing to join have you assumed? Important information to judge whether the study is likely to be able to include enough patients during the study period.

A11: We would like to thank the referee for the useful comments. Because of our negligence, we did not express clearly. As we described in the manuscript, 3200 newly diagnosed TB patients between January 2019 and December 2020 will be recruited from four hospitals. Based on our previous ADACS cohort[1], and setting the exclusion rate to 2.4% and the participation rate of eligible subjects to 71.2%[2], at least 4600 newly diagnosed TB patients will be needed within two years. According to the number of newly diagnosed TB patients in each hospital per year (600 patients per hospital), the total number of newly diagnosed TB patients in four hospitals is almost 2400 per year, which fully meets the sample size requirement in two years. We have modified this description in the manuscript.

Additionally, we also modified the effect size of odds ratio (OR) to make it more reasonable (the original value is a bit large). We have revised this description in Sample size calculation section.

References

[1] Xia YY, Hu DY, Liu FY, et al. Design of the anti-tuberculosis drugs induced adverse reactions in China National Tuberculosis Prevention and Control Scheme Study (ADACS). BMC public health. 2010;10:267.

[2] Wu S, Xia Y, Lv X, et al. Preventive use of hepatoprotectors yields limited efficacy on the liver toxicity of anti-tuberculosis agents in a large cohort of Chinese patients. J Gastroenterol Hepatol. 2015 Mar;30(3):540-5.

Q12. Statistical analysis. Consider performing Cox regression analysis to also investigate time to event data, interesting information for ADRs.

A12: We would like to thank the referee for the useful comments. We have added this in the Statistical analysis (The Cox proportional-hazards regression model will be used in the analysis of time-to-event data).

Q13. The genetic analyses are not described in detail. It is Important that all genetic analyses are prespecified and included in the informed consent so patients know what kind of genetic analysis are being performed and why.

A13: We would like to thank the referee for the useful comments. Because of our negligence, we did not express clearly. Based on our previous studies, matched nested case-control study will be used to explore the role of genetic variations (single nucleotide polymorphisms, SNPs) in susceptibility to ATDH. In recent years, genetic polymorphisms of drug-metabolizing enzymes have been widely studied, but the results have been inconsistent[1]. We are interested in exploring the role of genetic polymorphisms in protoporphyrin IX (PPIX) biosynthesis and disposition pathway related genes in the risk of anti-TB drug-induced hepatotoxicity (ATDH). PPIX is ubiquitously present in all living cells in small amounts as a precursor of heme[2]. High concentrations of PPIX in the liver are known to cause liver injury[3,4]. Isoniazid (INH) and rifampicin (RIF) co-therapy caused accumulation of the endogenous hepatotoxin PPIX through pregnant X receptor (PXR)-mediated transcriptional activations of both cytochromes P450 (CYP450) and aminolevulinic synthase-1 (ALAS1) genes[5]. A PXR-humanized mouse model also further illustrated that co-therapy with RIF and INH targets porphyrin biosynthesis and results in hepatic PPIX accumulation and liver injury[6], which offered a new paradigm for understanding the mechanism of liver injury that is associated with RIF and INH co-therapy[7]. Therefore, it is not difficult to speculate that genetic polymorphisms in PPIX biosynthesis and disposition pathway related genes would affect the activity of enzymes and influence subsequent PPIX biosynthesis and disposition. The genes we want to genotype will include RXR, ALAS1, FECH (Ferrochelatase), HSP90 (Heat shock protein 90), BCRP (Breast cancer resistance protein), ABCG10 (ATP-binding cassette subfamily G member 10) and ABCB6 (ATP-binding cassette subfamily B member 6). Due to space limitations, we only added the information of SNPs detection in the revised manuscript.

References

[1] Chen R, Wang J, Zhang Y, et al. Key factors of susceptibility to anti-tuberculosis drug-induced hepatotoxicity. Arch Toxicol. 2015; 89(6): 883-897.

[2] Sachar M, Anderson KE1, Ma X. Protoporphyrin IX: the Good, the Bad, and the Ugly. J Pharmacol Exp Ther. 2016 Feb;356(2):267-75.

[3] Anstey AV, Hift RJ. Liver disease in erythropoietic protoporphyria: insights and implications for management. Gut. 2007 Jul;56(7):1009-18. Epub 2007 Mar 14.

[4] Casanova-González MJ, Trapero-Marugán M, Jones EA, et al. Liver disease and erythropoietic protoporphyria: a concise review. World J Gastroenterol. 2010 Sep 28;16(36):4526-31.

[5] Maglich JM, Stoltz CM, Goodwin B, et al. Nuclear pregnane x receptor and constitutive androstane receptor regulate overlapping but distinct sets of genes involved in xenobiotic detoxification. Mol Pharmacol, 2002;62:638-46.

[6] Li F, Lu J, Cheng J, et al. Human PXR modulates hepatotoxicity associated with rifampicin and isoniazid co-therapy[J]. Nat Med. 2013;19(4): 418-420.

[7] Lyoumi S, Lefebvre T, Karim Z, et al. PXR-ALAS1: a key regulatory pathway in liver toxicity induced by isoniazid-rifampicin antituberculosis treatment[J]. Clin Res Hepatol Gastroenterol. 2013; 37(5): 439-441.

Q14. “Most ADRs induced by anti-TB drugs occur within the first two months of treatment [6, 42], including MDR-TB treatment [43].” This might not be true for all therapies of MDR-TB, since nephrotoxicity of kanamycin are increasing over time (cumulative dose dependent) for example.

A14: We would like to thank the referee for the useful comments. Indeed, some ADRs would occur after the first two months of treatment or increase over time. But most ADRs would occur within the first two months. Based on our previous anti-TB treatment cohort, the patient's compliance in the first two months is relatively high, especially in self-recording the diaries. We have deleted those imprecise words in the revised manuscript.

Q15. Will the informed consent be in Mandarin or also in other languages if needed? What is the procedure if the patient is illiterate?

A15: We would like to thank the referee for the useful comments. The informed consent will be printed in Simplified Chinese. Because patients surveyed in the four regions are mainly the Han Chinese. If there is a problem, the local doctor will communicate with the patient, and explain the study to the patients. If the patient is illiterate, informed consent will be signed by his/her surrogate. Furthermore, illiteracy rate in China is relatively low, especially in the developed province of Jiangsu.

Q16. In genetic studies, it is often applicable to correct for the issue of multiple comparison. Will Bonferroni corrections or other methods be used?

A16: We would like to thank the referee for the useful comments. Because of our negligence, we did not consider corrections for multiple comparisons. Bonferroni correction method was applied to adjust the P value for multiple comparisons. We have revised the manuscript.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Dr Star Khoza<br>University of the Western Cape, South Africa |
| **REVIEW RETURNED** | 21-Feb-2019 |

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| **GENERAL COMMENTS** | The revised manuscript has adequately addressed all the comments I raised during initial review. I believe the manuscript is now acceptable for publication. |

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| **REVIEWER** | Lina Davies Forsman<br>Unit of Infectious Diseases, Department of Medicine, Karolinska Institutet Stockholm Sweden |
| **REVIEW RETURNED** | 12-Feb-2019 |

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| **GENERAL COMMENTS** | Thanks for the revised manuscript. It's a pity that the treatment outcome results were deleted, although you explained the rationale. The manuscript still contains interesting and new information. Especially the discussion section where you have practical suggestions for minimising attrition is an enjoyable read. Please add reference to STROBE in the manuscript (if not yet done). |