PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Long-term outcomes following antenatal exposure to low-dose aspirin: Study protocol for the 4-year follow-up of the APRIL randomised controlled trial
AUTHORS	Landman, Anadeijda; van Limburg Stirum, Emilie; van 't Hooft, Janneke; van Wassenaer-Leemhuis, Aleid; Finken, Martijn; van Baar, Anneloes; Roseboom, Tessa; Ravelli, Anita; van Wely, Madelon; Oosterlaan, Jaap; Painter, Rebecca; Pajkrt, Eva; Oudijk, Martijn; de Boer, Marjon

VERSION 1 – REVIEW

REVIEWER	Matthew Hoffman Christiana Care Health System, obgyn
REVIEW RETURNED	27-Jan-2022

GENERAL COMMENTS	This thoughtfully written protocol paper provides follow up of children whose mothers participated in the April trial. There is a dearth of objective data on this subject and the available data is largely from a follow up of the CLASP trial done at extremely early child ages (12 and 18months) using instruments that are not viewed as the gold standard today. The protocol is thoughtfully written and covers the depth and breadth of what is needed in this type of study and uniquely evaluates both child health and neurocognitive development with accepted and validated instruments. As the protocol has already been initiated and I have no major criticisms, my comments are limited to the following: 1. The study claims primacy in followup on page 3 line 10. The authors should be aware that a similar effort is ongoing with participants in the ASPIRIN trial published in the Lancet. It is a good thing to have this issue being looked at by two different groups but the claim of being first is not correct. 2. It is a bit unclear about how the authors are thinking about their analysis plan. Is this a non-inferiority trial or a superiority trial. There is statistical nuance in this and would encourage them to more overtly state their intent. Similarly, it affects how one would approach the statistical analysis. Finally, it is worth noting that there is substantial literature around maternal inflammation and delays in neurodevelopment. Both the smaller studies by Marrett and Parazinnin suggest benefit. It would be important to suggest not only the lack of harm but potential benefit. 3. Though the primary trial was not statistically different in changing the rate of preterm birth there was a suggestion of trend. It is unclear how this will be handled in this protocol. 4. Though smoking is addressed as a covariate, there is no discussion of breastfeeding and poverty. These are tow potential things to consider.

REVIEWER	Richard Derman Thomas Jefferson University, Office of Global Affairs
REVIEW RETURNED	14-Feb-2022

GENERAL COMMENTS	This paper presents the protocol now being utilized that follow infants/children of mothers who participated in the APRIL Trial. The study utilized (low-dose) 80mg of aspirin compared to placebo which was begun in early pregnancy through 36 weeks gestation. The primary outcomes are changes in neurodevelopment and behavior. The authors importantly point out that aspirin crosses the placental barrier, and the longer-term effects of low-dose aspirin given to mothers could have the potential to cause neurodevelopmental or behavioral changes in their offspring. Prior data is inconclusive as to whether a mother's use of low-dose aspirin among selective women had a negative, positive, or no impact onthe outcomes to be measured. Since this follow-up study has already begun, editorial comments are limited to the description of the protocol and proposed statistical analyses, although a few comments/questions are directed to the authors and can perhaps be included in the next revision. The paper is clearly written and most adequately describes the components of the follow-up trial. The statistical analysis section is appropriate to assess the two primary outcomes at 1 through 4 years of a child's age. The authors appropriately make note of the limitations of relying on questionnaire-generated data and the confounders associated with the excessive length of time since maternal Aspirin use. The exploratory (secondary outcomes) which provides data on women who experienced either a preterm birth or a baby with SGA will be of particular interest given the increasednumber of such births reported from low and middle-income countries. The data is solely derived from those residing in a single, highly developed country (the Netherlands), thus generalizability to low/middle-income countries where multiple confounders linked to nutrition, inflammation, infestation, and other factors (despite study randomization) may produce different results. The papers of Kutlesic V. et al. (Pediatrics, 2017) and Suchdev, P.S. et al. (Pediatrics, 2017) should be added to the bibliography. Surprisingly, the work of Hoffman, M. et al., funded by the NIH which employed low-dose aspirin to reduce the risk of preterm birth (Lancet, 2020) was also not included in the bibliography. The paper is publishable with minimal edits.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Dr. Matthew Hoffman, Christiana Care Health System

Comments to the Author: This thoughtfully written protocol paper provides follow up of children whose mothers participated in the April trial. There is a dearth of objective data on this subject and the available data is largely from a follow up of the CLASP trial done at extremely early child ages (12 and 18months) using instruments that are not viewed as the gold standard today. The protocol is

thoughtfully written and covers the depth and breadth of what is needed in this type of study and uniquely evaluates both child health and neurocognitive development with accepted and validated instruments. As the protocol has already been initiated and I have no major criticisms, my comments are limited to the following:

1. The study claims primacy in followup on page 3 line 10. The authors should be aware that a similar effort is ongoing with participants in the ASPIRIN trial published in the Lancet. It is a good thing to have this issue being looked at by two different groups but the claim of being first is not correct.

Response from the authors: Dear dr. Hoffman, we would like to thank you for your comments on our manuscript. Our apologies that we were not aware of the fact that the ASPIRIN trial is performing follow-up as well. We think follow-up is of outmost importance to ensure the safety of aspirin use in pregnancy and encourage the follow-up of the ASPIRIN trial. We removed our claim for being the first to perform long-term follow-up on this subject (*please see line 71-73*) and referred to the ASPIRIN follow-up in the Introduction (*please see line 83*) and Discussion (*please see line 386-389*).

2. It is a bit unclear about how the authors are thinking about their analysis plan. Is this a non-inferiority trial or a superiority trial. There is statistical nuance in this and would encourage them to more overtly state their intent. Similarly, it affects how one would approach the statistical analysis.

Response from the authors: In line with the original APRIL study, this follow-up has a superiority design. To make this more clear, we emphasized the design throughout the manuscript (*please see line 56-57, line 244 and line 262*).

3. Finally, it is worth noting that there is substantial literature around maternal inflammation and delays in neurodevelopment. Both the smaller studies by Marrett and Parazinni suggest benefit. It would be important to suggest not only the lack of harm but potential benefit.

Response from the authors: Based on the literature there are possible beneficial effects of the use of aspirin in pregnancy on child development. However, we believe that potential harmful effects should be excluded as well. We revised the sentence in *line 114-115* in the Introduction:
"In light of the increasing use of prophylactic low-dose aspirin during pregnancy it is crucial to obtain more long-term data to exclude potential harm and determine possible benefits in the long term."

Thereby, we included a sentence about the potential benefit of aspirin on fetal brain development in case of maternal inflammation in the Introduction (*please see line 92-93*): Furthermore, aspirin has anti-inflammatory properties, which may reduce fetal brain injury caused by maternal inflammation.

4. Though the primary trial was not statistically different in changing the rate of preterm birth there was a suggestion of trend. It is unclear how this will be handled in this protocol.

Response from the authors: We will compare aspirin to placebo in the primary analysis. Potential confounders will be identified, of which preterm birth is a likely a confounder, for which regression analyses will be performed, as described from line 277. In case of a trend (e.g. for preterm birth), we will discuss the potential effect size and clinical relevance. This study will likely not have enough power to determine whether such observations are true or chance findings. Future IPD meta-analyses in collaboration with other aspirin follow-up trials, might provide a more solid answer.

5. Though smoking is addressed as a covariate, there is no discussion of breastfeeding and poverty. These are two potential things to consider.

Response from the authors: We agree that these variables, among others, are important for child development. We therefore ask about breastfeeding in the general health questionnaire and we will include this in the baseline characteristics table. Unfortunately, the general health questionnaire does not include financial income. However, we will gather data on the women's educational level, that can be used as an indicator of socio-economic status. Potential confounding from these variables will be addressed by a directed acyclic graph analysis.

Reviewer: 2
Dr. Richard Derman, Thomas Jefferson University
Comments to the Author: This paper presents the protocol now being utilized that follow infants/children of mothers who participated in the APRIL Trial. The study utilized (low-dose) 80mg of aspirin compared to placebo which was begun in early pregnancy through 36 weeks gestation. The primary outcomes are changes in neurodevelopment and behavior.

The authors importantly point out that aspirin crosses the placental barrier, and the longer-term effects of low-dose aspirin given to mothers could have the potential to cause neurodevelopmental or behavioral changes in their offspring.

Prior data is inconclusive as to whether a mother's use of low-dose aspirin among selective women had a negative, positive, or no impact on the outcomes to be measured.

Since this follow-up study has already begun, editorial comments are limited to the description of the protocol and proposed statistical analyses, although a few comments/questions are directed to the authors and can perhaps be included in the next revision.

The paper is clearly written and most adequately describes the components of the follow-up trial. The statistical analysis section is appropriate to assess the two primary outcomes at 1 through 4 years of a child's age.

The authors appropriately make note of the limitations of relying on questionnaire-generated data and the confounders associated with the excessive length of time since maternal Aspirin use.

The exploratory (secondary outcomes) which provides data on women who experienced either a preterm birth or a baby with SGA will be of particular interest given the increased number of such births reported from low and middle-income countries.

The data is solely derived from those residing in a single, highly developed country (the Netherlands), thus generalizability to low/middle-income countries where multiple confounders linked to nutrition, inflammation, infestation, and other factors (despite study randomization) may produce different results. The papers of Kutlesic V. et al. (Pediatrics, 2017) and Suchdev, P.S. et al. (Pediatrics, 2017) should be added to the bibliography.

Surprisingly, the work of Hoffman, M. et al., funded by the NIH which employed low-dose aspirin to reduce the risk of preterm birth (Lancet, 2020) was also not included in the bibliography.

The paper is publishable with minimal edits

Response from the authors:
Dear dr. Derman, we truly appreciate your thorough comments on our manuscript and agree that the work of Hoffman et al. should be included in the bibliography (*please see line 83 and 386-389)*.

We also agree that the generalizability of the results of this follow-up study is not yet described in our manuscript. Since this information is required to correctly interpret the results of the follow-up, we added a paragraph in the Discussion (*please see line 384-392*).

VERSION 2 – REVIEW

REVIEWER	Matthew Hoffman Christiana Care Health System, obgyn
REVIEW RETURNED	29-Apr-2022

GENERAL COMMENTS	Thanks you for making changes in response to the comments.