## PEER REVIEW HISTORY

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## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | A study protocol for the Randomised Control Trial: Combined multi-marker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention (ASPRE) |
| **AUTHORS** | O'Gorman, Neil; Wright, David; Rolnik, Daniel; Nicolaides, Kypros; Poon, Liona |

## VERSION 1 - REVIEW

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| **REVIEWER** | Emmanuel Bujold<br>Université Laval, Québec, Qc, Canada |
| **REVIEW RETURNED** | 22-Mar-2016 |

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| **GENERAL COMMENTS** | Large trial that evaluates the impact of low-dose aspirin started in early pregnancy in women at high-risk for preeclampsia is urgently required.<br><br>The trial addressed most of the concerns regarding previous publications regarding the use of aspirin during pregnancy for prevention of PE.<br><br>1) Identification of women at high-risk, including nulliparous women using a highly studied screening program with high sensitivity and high specificity.<br>2) Use of optimal dosage (>100 mg)<br>3) Aspirin taken at bedtime and not a random moment during the day.<br>4) Appropriate primary outcome (preterm PE) that is a much more homogeneous disease than any type of PE that are less predictable and less important clinically.<br>5) Adequate power (90%) to detect a reasonable effect (50%) while most meta-analyses suggest that aspirin started in early pregnancy reduces >50% of preterm PE.<br>6) Appropriate secondary outcomes with an alpha-error established at 1% instead of 5%.<br>7) Multiple visits to reach optimal follow-up<br>8) Adequate evaluation of compliance<br><br>The only small limitation that it is almost impossible to avoid is the potential access and use of other forms of PE screening in the community (such as the NICE guidelines) that could be provided to the participants by their healthcare providers in parallel and that could influence compliance. Compliance follow-up and the highly powered design of the trial should be able to address that very specific limitation at the end.<br><br>Other strengths and limitations are well addressed in the manuscript. |

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| **REVIEWER** | Gaea Moore<br>Stanford School of Medicine<br>Department of Obstetrics and Gynecology<br>United States |
| **REVIEW RETURNED** | 11-Apr-2016 |

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| **GENERAL COMMENTS** | **ASPRE: Combined multi-marker screening and randomized patient treatment with Aspirin for evidence-based PREeclampsia prevention.**<br><br>Summary:<br>- The authors propose an RCT in which a combination of findings at 11-13 weeks of pregnancy are used identify women at increased risk for early preeclampsia (< 37 weeks) and to determine whether these findings may predict responsiveness to low-dose aspirin for improvement of pregnancy outcome. The authors plan to estimate the risk of early preeclampsia using a combination of maternal historical factors and characteristics, maternal uterine artery pulsatility index (PI), mean arterial pressure (MAP), and both maternal serum pregnancy associated plasma protein A (PAPP-A) and placental growth factor (PlGF), in part based on modeling performed by the authors published earlier this year.[1]<br><br>Comments:<br><br>- The authors pose an important question with a proposal which is reasonable and feasible.<br>- I recommend further clarification of the screening algorithm proposed for this trial and clarification of the primary outcome.<br><br>Background<br><br>- Page 4, Line 31: To describe the effect of low-dose aspirin in reducing the risk of preeclampsia, I recommend using Bujold's 2010 meta-analysis[2] (with relative risk of 0.47 for the development of preeclampsia) instead of Askie's meta-analysis ("10% decrease in the rate of preeclampsia") as Bujold's study is used in the power calculation within the current proposal.<br>- Page 4, Line 16 (prediction of preeclampsia): The authors refer to their preliminary study published earlier this year[1] in which a combination of maternal characteristics and biomarkers were utilized to develop a predictive model for preeclampsia. The authors report that a combination of uterine artery PI, MAP, and serum PLGF at 11-13 weeks gestation can predict 75% of preterm-preeclampsia and 47% of term-preeclampsia, at an FPR of 10%. In the conclusion of this modeling study the authors state "Although serum PAPP-A improves the performance of screening by maternal factors or biophysical markers, we found no evidence of improvement to any combination of biomarkers that include serum PLGF." Why is PAPP-A included as a component of modeling in the current RCT ? |

- Page 4, Line 56 (“Aspirin resistance” and discussion of aspirin dosing). Within this RCT a dose of Aspirin of 150mg/day is planned. Currently within the United States, aspirin is dosed at 81mg/day for preeclampsia prevention.
    - The majority of studies of aspirin for the prevention of preeclampsia use a dose of aspirin of 100mg or less. Among the 12 studies included in Bujold’s 2010 meta-analysis of early aspirin (< 16 weeks) for the prevention of preeclampsia, only one used a dose higher than 100mg/day.[2] Bujold’s meta-analysis was referenced by O’Gorman et al as support for the use of aspirin for prevention of preeclampsia (with a 50% reduction in prevalence when aspirin is initiated prior to 16 weeks). In the second meta-analysis cited by the RCT proposal evaluating the effect of early initiation low-dose aspirin for prevention of preeclampsia,[3] all included studies used aspirin at a dose of 75-100mg/day.
    - To justify the higher dose of aspirin the authors cite laboratory work published by Caron[4] describing pregnant women (n=87) as responders or non-responders to aspirin at various doses based on a laboratory test (PFA-100). Within Caron’s work, at doses of 81mg/day, 71% of women were considered to be “responders” to aspirin. Incremental dose adjustments to 162mg/day led to a total of 84% of women being considered “responders.” A subsequent study which utilized the PFA-100 test to guide ASA dosing in pregnant women at increased risk for preeclampsia found that women who required higher doses of aspirin had higher rates of preeclampsia.[5] While this work is intriguing, the PFA-100 test has not been validated in a large randomized clinical trial, and the higher dose of aspirin may introduce greater maternal and or fetal risk.
    - The Cochrane review of antiplatelet agents for preventing pre-eclampsia states “Doses up to 75 mg appear to be safe. There is promising evidence that higher doses of aspirin may be more effective, but this will require careful evaluation… as adverse effects may also increase.[6]
    - I would encourage the authors to consider using Aspirin at a dose of 75-100mg/day
- Page 5, Line 3: The authors state that low dose aspirin is defined as < 300mg/day. Please provide a reference for this statement. Typically low-dose aspirin is 150mg/day or less.[6]
- Please add a comment that reflects that there are multiple processes which lead to the development of preeclampsia among different risk groups, and we do not know which risk factors or pathologic processes may be responsive to early initiation of low-dose aspirin.
- Also consider a statement that reflects the dearth of knowledge regarding the interaction between PlGF levels and low-dose aspirin.

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| | ○ While women with low PlGF early in pregnancy may be at greater risk for development of preeclampsia, it is unclear whether early PlGF levels will predict responsiveness to aspirin.<br>○ In a biomarker analysis of findings from a study of aspirin for the prevention of preeclampsia in high-risk women,[7] those with higher PlGF at 13-16w appeared to have a greater response to aspirin than those with lower PlGF. Although the findings were non-significant and analysis limited by small sample size, women with higher PlGF were more likely to have chronic hypertension and to be African-American, which speaks to fundamental pathophysiologic differences among different risk groups and responsiveness to aspirin.<br>Hypothesis<br>- Page 5, line 38: Recommend adding “preterm preeclampsia” to your instead of “preeclampsia”<br>Aim<br>- Page 5, line 45: Recommend adding “preterm preeclampsia” to your instead of “preeclampsia”<br>Primary outcome – incidence of preterm preeclampsia with delivery < 37w<br>- Within this proposal, preeclampsia is defined simply as the combination of elevated blood pressure (either > 140 sBP or > 90 dBP) and proteinuria<br>- In order to maximize generalizability and relevance of your findings internationally, it would be helpful to define criteria for delivery < 37w and standardize this practice among treatment sites. Within the United States, general practice follows the American College of Obstetricians and Gynecologists guidelines which recommend delivery before 37w when specific criteria, or “severe features” are met (severe range blood pressures, transaminitis, etc).[8] Based on ACOG guidelines, women without these severe features of preeclampsia are not delivered prior to 37w.<br>- The spectrum of hypertensive disorders of pregnancy includes “gestational hypertension,” or new onset hypertension +/- other features of preeclampsia minus proteinuria. This condition may result in delivery prior to 37 weeks.<br>○ Page 11, Line 4: The obstetric record will be reviewed to determine if the condition was gestational hypertension.<br>○ How will this condition be classified according to your study? Recommend that this definition be clearly stated and generalized among your study sites.<br>○ I encourage you to consider adding gestational hypertension as a secondary outcome. |

- The spectrum of hypertensive disorders of pregnancy includes “superimposed preeclampsia,” with the development of worsening hypertension and increased proteinuria in a women with chronic hypertension. This population of women often has baseline proteinuria > 300mg/24 hours.
    - Page 11, Line 4: The obstetric record will be reviewed to determine if the condition was chronic hypertension.
    - How will superimposed preeclampsia be defined? Recommend that this definition be stated in the proposal and generalized among your study sites.

Secondary outcomes

- Recommend addition of gestational hypertension at each gestational age strata.
- Recommend an assessment of effect of ASA based on baseline PlGF levels.

Inclusion criteria

- Page 8, line 34: The authors describe an “algorithm” combining maternal history and characteristics, MAP, uterine artery PI, and biochemical factors (PAPP-A and PlGF).
    - Please outline this algorithm
    - What maternal history and characteristics will be included in this model?

Power calculation

- Page 11, line 46: Sample size calculation is based on a 76% detection rate of the first-trimester combined screening for preterm preeclampsia.
    - Within O’Gorman’s screening study, the detection rate was quoted as 75%.[1] How was the 76% value derived and does the affect your sample size calculation?
- Page 11, line 48: Regarding the prevalence of preterm preeclampsia in the placebo group, the authors quote an estimate of 7.6%.
    - Within the O’Gorman screening study (table 6), the prevalence of preterm preeclampsia among women screened positive was 5.4% (with a model which includes uterine artery PI, MAP, and PlGF but not PAPP-A).[1] It is not clear how the 7.6% estimate was derived – is this the estimate when PAPP-A is included in the predictive model? Does using a prevalence of 5.4% instead of 7.6% change your sample size calculation?

References

1. O'Gorman N, Wright D, Syngelaki A, et al. Competing risks model in screening for preeclampsia by maternal factors and

biomarkers at 11-13 weeks gestation. American journal of obstetrics and gynecology 2016;214:103 e1- e12.
2. Bujold E, Roberge S, Lacasse Y, et al. Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. Obstetrics and gynecology 2010;116:402-14.
3. Roberge S, Villa P, Nicolaides K, et al. Early administration of low-dose aspirin for the prevention of preterm and term preeclampsia: a systematic review and meta-analysis. Fetal diagnosis and therapy 2012;31:141-6.
4. Caron N, Rivard GE, Michon N, et al. Low-dose ASA response using the PFA-100 in women with high-risk pregnancy. J Obstet Gynaecol Can 2009;31:1022-7.
5. Rey E, Rivard GE. Is testing for aspirin response worthwhile in high-risk pregnancy? Eur J Obstet Gynecol Reprod Biol 2011;157:38-42.
6. Duley L, Henderson-Smart DJ, Meher S, King JF. Antiplatelet agents for preventing pre-eclampsia and its complications. Cochrane Database Syst Rev 2007:CD004659.
7. Moore GS, Allshouse AA, Winn VD, Galan HL, Heyborne KD. Baseline placental growth factor levels for the prediction of benefit from early aspirin prophylaxis for preeclampsia prevention. Pregnancy Hypertens 2015;5:280-6.
8. American College of O, Gynecologists, Task Force on Hypertension in P. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstetrics and gynecology 2013;122:1122-31.

**VERSION 1 – AUTHOR RESPONSE**

Dr Bujold

The only small limitation that it is almost impossible to avoid is the potential access and use of other forms of PE screening in the community (such as the NICE guidelines) that could be provided to the participants by their healthcare providers in parallel and that could influence compliance.

Prior to commencing the study at each site, it had been agreed via the local PIs (and in discussion with their colleagues) that we would try to limit other form of PE screening for the duration of the trial. Meetings where held where the local GPs were invited to attend to understand the protocol and rationale for the study. Of course, quite correctly it is impossible to completely avoid other forms of PE screening, however we feel we have kept this small limitation to a minimum.

Dr Moore

Page 4, Line 31: To describe the effect of low-dose aspirin in reducing the risk of preeclampsia, I recommend using Bujold's 2010 meta-analysis2 (with relative risk of 0.47 for the development of preeclampsia) instead of Askie's meta-analysis ("10% decrease in the rate of preeclampsia") as Bujold's study is used in the power calculation within the current proposal.

Done.

Why is PAPP-A included as a component of modeling in the current RCT ?

The reason PAPP-A has been included is that it is already part of the combined test for aneuploidy screening and while its addition does not significantly improve the detection rate of preeclampsia, it does have some contribution.

I would encourage the authors to consider using Aspirin at a dose of 75-100mg/day
Thank you for the recommendation. We have chosen the dose of 150mg carefully based on existing literature. We can no longer change the dosage as firstly aspirin 150mg tablets have been manufactured and secondly the trial has just completed recruitment.

Page 5, Line 3: The authors state that low dose aspirin is defined as < 300mg/day. Please provide a reference for this statement. Typically low-dose aspirin is 150mg/day or less.

I have removed this sentence from the paragraph. There are a number of different doses and even ranges that have been referred to as LDA.I could not find any specific paper that actually states LDA is defined as <300mg.

Please add a comment that reflects that there are multiple processes which lead to the development of preeclampsia among different risk groups, and we do not know which risk factors or pathologic processes may be responsive to early initiation of low-dose aspirin.
done

Also consider a statement that reflects the dearth of knowledge regarding the interaction between PlGF levels and low-dose aspirin.
There is limited data on this subject presently but we will be well placed to address this and contribute to this issue in due course as we will have serial measurements of PlGF at specific points during the pregnancy in high risk patients on aspirin or a placebo.

Page 5, line 38: Recommend adding “preterm preeclampsia” to your instead of “preeclampsia”
done

Page 5, line 45: Recommend adding “preterm preeclampsia” to your instead of “preeclampsia”
done

In order to maximize generalizability and relevance of your findings internationally, it would be helpful to define criteria for delivery < 37w and standardize this practice among treatment sites.

Our sites will be adhering to the NICE Guideline on Hypertensive disorders in pregnancy, which recommend:

Manage pregnancy in women with preeclampsia conservatively (that is, do not plan same day delivery of the baby) until 34 weeks.

Consultant obstetric staff should document in the woman’s notes the maternal (biochemical, haematological and clinical) and fetal thresholds for elective birth before 34 weeks in women with preeclampsia.

Consultant obstetric staff should write a plan for antenatal fetal monitoring during birth.

Offer birth to women with preeclampsia before 34 weeks, after discussion with neonatal and anaesthetic teams and a course of corticosteroids has been given if:

severe hypertension develops refractory to treatment
maternal or fetal indications develop as specified in the consultant plan.

Recommend birth for women who have preeclampsia with severe hypertension after 34 weeks when their blood pressure has been controlled and a course of corticosteroids has been completed (if appropriate).

Offer birth to women who have preeclampsia with mild or moderate hypertension at 34+0 to 36+6 weeks depending on maternal and fetal condition, risk factors and availability of neonatal intensive care.

Recommend birth within 24-48 hours for women who have preeclampsia with mild or moderate hypertension after 37+0 weeks

How will gestational hypertension be classified according to your study? Recommend that this definition be clearly stated and generalized among your study sites.
done.
Gestational hypertension for the study be classified as BP >140 (systolic) or 90 (diastolic) mmHg without proteinuria that occurs after 20 weeks gestation.

I encourage you to consider adding gestational hypertension as a secondary outcome .
Yes I agree. We will not add it formally to the protocol but will certainly be looking at incidence of preexisting hypertension and gestational hypertension.

How will superimposed preeclampsia be defined? Recommend that this definition be stated in the proposal and generalized among your study sites.
Defined in the paper. This has been agreed already between sites.
In preeclampsia superimposed on chronic hypertension, significant proteinuria (as defined earlier) should develop after 20 weeks' gestation in women with known chronic hypertension (history of hypertension before conception or the presence of hypertension at the booking visit at <20 weeks' gestation in the absence of trophoblastic disease).

Recommend addition of gestational hypertension at each gestational age strata.
Recommend an assessment of effect of ASA based on baseline PlGF levels.

We intend to assess both of these recommendations. Thank you.

Please outline this algorithm

The algorithm is referenced in the paper. Reference 6 in the statistical analysis section of the methods.

What maternal history and characteristics will be included in this model?
Patients were asked to complete a questionnaire on maternal age, racial origin (Caucasian, Afro-Caribbean, South Asian, East
Asian and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs), cigarette smoking during pregnancy (yes or no), history of chronic hypertension (yes or no), history of type 1 or 2 diabetes mellitus (yes or no), history of systemic lupus erythematosus or antiphospholipid syndrome (yes or no), family history of PE in the mother of the patient (yes or no),

and obstetric history including parity (parous or nulliparous if no previous pregnancies at or after 24 weeks) and previous pregnancy with PE (yes or no).

Within O'Gorman's screening study, the detection rate was quoted as 75%. How was the 76% value derived and does the affect your sample size calculation?

Quite correctly stated. Apologies for any confusion caused.The 76% came from our group's previous competing risks model published in 2012.
Akolekar R, Syngelaki A, Poon L, Wright D, Nicolaides KH. Competing risks model in early screening for
preeclampsia by biophysical and biochemical markers. Fetal Diagn Ther 2012. DOI: 10.1159/000341264.

I have replaced the O'Gorman paper with this reference as the O'Gorman paper was published after the trial had commenced.

The same is true for the 7.6% (10% screen positive rate) prevalence of preterm preeclampsia and this estimate was derived with PAPP-A in the predictive model. Akolekar et al 2012