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- Comments from the reviewers and editors (email to author requesting revisions)
- Response from the author (cover letter submitted with revised manuscript)*

*The corresponding author has opted to make this information publicly available.

Personal or nonessential information may be redacted at the editor’s discretion.

Questions about these materials may be directed to the Obstetrics & Gynecology editorial office: obgyn@greenjournal.org.
RE: Manuscript Number ONG-19-1002

Urinary Placental Growth Factor Profile In High Risk Women Receiving Low-Molecular-Weight Heparin Therapy During Pregnancy

Dear Dr. Lecarpentier:

Your manuscript has been reviewed by the Editorial Board and by special expert referees. Although it is judged not acceptable for publication in Obstetrics & Gynecology in its present form, we would be willing to give further consideration to a revised version.

If you wish to consider revising your manuscript, you will first need to study carefully the enclosed reports submitted by the referees and editors. Each point raised requires a response, by either revising your manuscript or making a clear and convincing argument as to why no revision is needed. To facilitate our review, we prefer that the cover letter include the comments made by the reviewers and the editor followed by your response. The revised manuscript should indicate the position of all changes made. We suggest that you use the "track changes" feature in your word processing software to do so (rather than strikethrough or underline formatting).

Your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Aug 02, 2019, we will assume you wish to withdraw the manuscript from further consideration.

REVIEWER COMMENTS:

Reviewer #1: In this study, the author measured PlGF and creatinine levels in urinary samples collected from pregnant women with history of severe preeclampsia (PE) in subjects recruited in the Heparin-Preeclampsia trial. Urine samples were collected in every 4wk of gestational windows: 10-13, 14-17, 18-21, 22-25, 26-29, 30-33, and 34-38. Results showed that low urine PlGF levels between 22-25wks are strongly associated with subsequent development of PE related adverse outcomes (PE, FGR, placental abruption and perinatal death). Results of this study provide further evidence and suggest that low urine PlGF/creatinine levels in the mid-gestation could be used at a biomarker to predict high-risk pregnancy population associated with PE and related adverse outcomes.

Following concerns need to be addressed:

1. The volume of urine samples used for PlGF and creatinine assays needs to be included in the M&M. Were samples diluted for the assays?
2. The numbers in Table 2 (n=118 (urinary PlGF/Creat) in composite adverse outcome <34wks and n=143 (urinary PlGF/Creat) in preeclampsia <34wks) do not match those given in Table 1. Please explain?
3. Line 179-180: --- not statistically different ---- (Table 3 Supplementary data). Table 1 and Table supplementary data are missing in the Result Section.
4. Line 195: what are the two adverse outcomes?
5. Lines 271-273: It is stated" The analysis were not performed between 18 and 22 weeks because of very few samples at this gestational window", how many samples were actually measured in the 18-22wks of gestational window?
6. Proteinuria status should be included in Table 1, Maternal Characteristics.
7. Were sFlt-1 concentrations measured in these urinary specimens?
8. References should be listed according to the journal format.
9. Typo errors:
   Line 70: Change "is" to "are".
   Line 150: Delete "for"
   Line 281: Change "duiting" to "during".
Reviewer #2: Lecarpentier and colleagues have submitted the report of a retrospective cohort study designed to characterize the relationship between urinary PIGF and the subsequent development of a composite outcome: preeclampsia, fetal growth restriction, placental abruption and perinatal death. In order to strengthen the report, the authors may wish to consider the following:

1. Introduction, line 83: Consider changing the word "induces" to "is associated with." I'm pretty sure causation has not been demonstrated yet.

2. Introduction, lines 111-113 and Methods, lines 132-135: First, you include "maternal death" in your composite primary outcome in the Methods section, but not in the introduction. Can you please clarify the entities that make up the composite outcome?

3. Introduction and Methods, general comment: Given that most of the work regarding serum and urinary markers in this family (sFLT 1 and PIGF) surrounds predicting preeclampsia, why did you choose to include maternal death, fetal growth restriction, perinatal death and abruption in your composite? All of these can be associated with preeclampsia, but none are only caused by preeclampsia. Please clarify.

4. Methods, general comment: Was your composite outcome prespecified? If so, please state so.

5. Methods, lines 138 to 141: you collected urine samples at 7 different times across gestation. If your null hypothesis was that there is no relationship between urinary PIGF and the subsequent development of your composite outcome; that seems like 7 separate experiments to me. Please clarify if and how you addressed the statistical consequences imparted by multiple testing. Did you apply any adjustments to your significance level?

6. General comment/concern: My main reservation is your inclusion of outcomes (e.g. your composite) not always linked to perturbations in sFLT-1 or PIGF. This would be a more credible report if you limited it to preeclampsia.

Reviewer #3:

1. Title. Why is heparin in the title? Half of the women didn't receive it, and it wasn't a factor in any of the outcomes. If anything, might add low-dose aspirin to the title instead.

2. Abstract.
   a. The abstract (and title and precis) are about urine PIGF, but the study was also about serum sFlt-1 and PIGF. Would clarify this.
   b. Lines 58-60. This sentence is the only one with Ns and percentages, but it reflects the composite at any gestational age, whereas study outcome is development of a composite before 34 weeks (71 women rather than 25 women). Might revise.

3. Introduction.
   Lines 96-107. Would consider that when women are known to be at increased risk for preeclampsia (as in this study), providers may not be looking for another screening test "to predict the onset." These mothers and their fetuses are already being followed more closely. Here or in the discussion, would consider how high the PPV or NPV would have to be to safely deviate from this management. Similarly, in lines 109-113, doesn't predictive performance imply PPV or NPY?

4. Methods. This section is well-written and clearly presented.
   Lines 137-138. Would list the number enrolled and participating in the results rather than methods.

5. Results.
   a. Lines 176-178. The authors write that the adverse outcome composite was not different between the 2 groups (37.6% vs. 38.3%). Those percentages are for the overall composite, not for the study objective of births <34 weeks. The relevant percentages are 15.1% vs. 11.7%.
   b. The frequency of preeclampsia < 34 weeks is a primary study outcome. Would include it in table 1 (there are quite a few outcomes listed in table 1, but this is not).
   c. Please report the individual outcomes that make up the <34-week composite. Might include this information in table 1 and in table 2.
   d. Lines 196-199. In this study of 187 pregnancies, 25 had the outcome composite. The authors write that for a false positive rate of 10%, the sensitivity was 77.7%. Does this mean that 19 women screened positive and developed the composite <34 weeks, and that 19 screened positive but did not, such that the PPV was 50%? Are the authors saying that 6 pregnancies were missed by the screening method? Please clarify this information and report PPV and NPV.

6. Discussion.
a. Please address clinical utility more fully. How would a positive result be used in practice, and would that be cost-effective, considering the false positive rate? How would a negative result be used, considering that the false negative rate was > 20%? Suggest discussing (or referring to) ACOG PB 202 from December 2018. Rather than AUC or likelihood ratios, positive predictive value data are needed. ACOG concluded that based on prior data, biomarker use should remain investigational because of low accuracy.

b. Lines 258-261. The lag time to development of preeclampsia or a related morbidity was 6 weeks +/- 2 weeks. Based on when the test was performed, complications developed between 26 and 34 weeks (considering just 1 SD from the mean). How would this "allow to develop appropriate monitoring"? Please be more specific in lines 278-280 as well.

STATISTICAL EDITOR COMMENTS:

The Statistical Editor makes the following points that need to be addressed:

Table 1: Since the sample size for each cohort was < 100, should round the %s to nearest integer, rather than to nearest 0.1%.

Table 2: Should round the AUC to nearest 0.01 and include CIs. Should include CIs for the sensitivity and LHR(+). Should include on-line data set for readers interested in the full relationship of sensitivity vs specificity for the data.

lines 201-202, 205-206: The use of NPV and PPV is only useful for a particular prevalence of the adverse outcome, so not applicable to other series. Should only cite sens, spec, LHR(+) and LHR(-), since those are independent of prevalence of the adverse outcomes.

lines 209-212: The differences cited ("slightly lower", "higher") are only numerical differences. Should support with statistical comparisons and if NS different, should so state.

EDITOR COMMENTS:

1. Thank you for your submission to Obstetrics & Gynecology. In addition to the comments from the reviewers above, you are being sent a notated PDF that contains the Editor's specific comments. Please review and consider the comments in this file prior to submitting your revised manuscript. These comments should be included in your point-by-point response cover letter.

***The notated PDF is uploaded to this submission's record in Editorial Manager. If you cannot locate the file, contact Randi Zung and she will send it by email - rzung@greenjournal.org.***

- levels are associated
- was this a preplanned study?
- Did you include all of the participant of the parent study and if not, how did you select your groups?
- In objective you state you were looking at outcomes < 34 weeks. Why did you include those at 34 weeks and above?
- are there units here or are both urine PIGF and creatinine measured in same units so this is a simple ratio? Is this urinary creatinine or serum creatinine?
- Please clarify In results you report a ratio between urinary PIGF/Creatinine not the absolute urinary PIGF levels.
- When you write that a study occurred between date 1 and date 2, it literally excludes those boundary dates. For instance, "This study was performed between Feb 2018 and Jan 2019" would mean it was performed from March 2018 to Dec 2018. Do you instead mean that the study was performed from date 1 to date 2? If so, please edit.
- Here in methods, please describe normalization of urinary PIGF by using creatinine. Please note if this is serum or urinary creatinine being used. As you are positing that low urinary PIGF is a useful marker AND you studied urinary levels to make it less invasive than blood levels every month, this is an important distinction since the ratio you report--if comparing urinary PIGF/SERUM creatinininen levels--fails to avoid the blood draw
- limit p values to 3 decimals throughout; please note Statistical editor’s comments regarding other limits.
2. Title: as some of your patients did not receive LMWH please remove this from the title. Consider, if you wish, adding that they were on Aspirin.

3. Precis, Abstract-Conclusion, and elsewhere: please temper this as your participants were all high risk so the "strong association" was in that group only. Please do so here, and throughout your manuscript.

4. The Editors of Obstetrics & Gynecology are seeking to increase transparency around its peer-review process, in line with efforts to do so in international biomedical peer review publishing. If your article is accepted, we will be posting this revision letter as supplemental digital content to the published article online. Additionally, unless you choose to opt out, we will also be including your point-by-point response to the revision letter. If you opt out of including your response, only the revision letter will be posted. Please reply to this letter with one of two responses:
   A. OPT-IN: Yes, please publish my point-by-point response letter.
   B. OPT-OUT: No, please do not publish my point-by-point response letter.

5. As of December 17, 2018, Obstetrics & Gynecology has implemented an "electronic Copyright Transfer Agreement" (eCTA) and will no longer be collecting author agreement forms. When you are ready to revise your manuscript, you will be prompted in Editorial Manager (EM) to click on "Revise Submission." Doing so will launch the resubmission process, and you will be walked through the various questions that comprise the eCTA. Each of your coauthors will receive an email from the system requesting that they review and electronically sign the eCTA.

Any author agreement forms previously submitted will be superseded by the eCTA. During the resubmission process, you are welcome to remove these PDFs from EM. However, if you prefer, we can remove them for you after submission.

6. Our journal requires that all evidence-based research submissions be accompanied by a transparency declaration statement from the manuscript's lead author. The statement is as follows: "The lead author* affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained." *The manuscript's guarantor.

If you are the lead author, please include this statement in your cover letter. If the lead author is a different person, please ask him/her to submit the signed transparency declaration to you. This document may be uploaded with your submission in Editorial Manager.

7. Responsible reporting of research studies, which includes a complete, transparent, accurate and timely account of what was done and what was found during a research study, is an integral part of good research and publication practice and not an optional extra. Obstetrics & Gynecology supports initiatives aimed at improving the reporting of health research, and we ask authors to follow specific guidelines for reporting randomized controlled trials (ie, CONSORT), observational studies (ie, STROBE), meta-analyses and systematic reviews of randomized controlled trials (ie, PRISMA), harms in systematic reviews (ie, PRISMA for harms), studies of diagnostic accuracy (ie, STARD), meta-analyses and systematic reviews of observational studies (ie, MOOSE), economic evaluations of health interventions (ie, CHEERS), quality improvement in health care studies (ie, SQUIRE 2.0), and studies reporting results of Internet e-surveys (CHERRIES). Include the appropriate checklist for your manuscript type upon submission. Please write or insert the page numbers where each item appears in the margin of the checklist. Further information and links to the checklists are available at http://ong.editorialmanager.com.

Please submit a completed STROBE checklist with your submission.

8. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric and gynecology data definitions at https://www.acog.org/About-ACOG/ACOG-Departments/Patient-Safety-and-Quality-Improvement/reVITALize. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

9. Because of space limitations, it is important that your revised manuscript adhere to the following length restrictions by manuscript type: Original Research reports should not exceed 22 typed, double-spaced pages (5,500 words). Stated page limits include all numbered pages in a manuscript (i.e., title page, précis, abstract, text, tables, boxes, figure legends, and print appendixes) but exclude references.

10. Titles in Obstetrics & Gynecology are limited to 100 characters (including spaces). Do not structure the title as a declarative statement or a question. Introductory phrases such as "A study of..." or "Comprehensive investigations into..." or "A discussion of..." should be avoided in titles. Abbreviations, jargon, trade names, formulas, and obsolete terminology also should not be used in the title. Titles should include "A Randomized Controlled Trial," "A Meta-Analysis," or "A Systematic Review," as appropriate, in a subtitle. Otherwise, do not specify the type of manuscript in the title.

11. Specific rules govern the use of acknowledgments in the journal. Please note the following guidelines:

* All financial support of the study must be acknowledged.
* Any and all manuscript preparation assistance, including but not limited to topic development, data collection, analysis, writing, or editorial assistance, must be disclosed in the acknowledgments. Such acknowledgments must identify the entities that provided and paid for this assistance, whether directly or indirectly.

* All persons who contributed to the work reported in the manuscript, but not sufficiently to be authors, must be acknowledged. Written permission must be obtained from all individuals named in the acknowledgments, as readers may infer their endorsement of the data and conclusions. Please note that your response in the journal's electronic author form verifies that permission has been obtained from all named persons.

* If all or part of the paper was presented at the Annual Clinical and Scientific Meeting of the American College of Obstetricians and Gynecologists or at any other organizational meeting, that presentation should be noted (include the exact dates and location of the meeting).

12. The most common deficiency in revised manuscripts involves the abstract. Be sure there are no inconsistencies between the Abstract and the manuscript, and that the Abstract has a clear conclusion statement based on the results found in the paper. Make sure that the abstract does not contain information that does not appear in the body text. If you submit a revision, please check the abstract carefully.

In addition, the abstract length should follow journal guidelines. The word limits for different article types are as follows: Original Research articles, 300 words. Please provide a word count.

13. Only standard abbreviations and acronyms are allowed. A selected list is available online at http://edmgr.ovid.com/ong/accounts/abbreviations.pdf. Abbreviations and acronyms cannot be used in the title or précis. Abbreviations and acronyms must be spelled out the first time they are used in the abstract and again in the body of the manuscript.

14. The journal does not use the virgule symbol (/) in sentences with words. Please rephrase your text to avoid using "and/or," or similar constructions throughout the text. You may retain this symbol if you are using it to express data or a measurement.

15. Please review the journal's Table Checklist to make sure that your tables conform to journal style. The Table Checklist is available online here: http://edmgr.ovid.com/ong/accounts/table_checklist.pdf.

16. Figures:

Figure 1: Please upload high-res versions of these in their original format (eps, tiff, jpeg, etc.). Copying and pasting into Word often results in a lower resolution.

Figure 2: Please upload high-res versions of these in their original format (eps, tiff, jpeg, etc.). Copying and pasting into Word often results in a lower resolution.

17. Authors whose manuscripts have been accepted for publication have the option to pay an article processing charge and publish open access. With this choice, articles are made freely available online immediately upon publication. An information sheet is available at http://links.lww.com/LWW-ES/A48. The cost for publishing an article as open access can be found at http://edmgr.ovid.com/acd/accounts/ifauth.htm.

Please note that if your article is accepted, you will receive an email from the editorial office asking you to choose a publication route (traditional or open access). Please keep an eye out for that future email and be sure to respond to it promptly.

18. If you choose to revise your manuscript, please submit your revision via Editorial Manager for Obstetrics & Gynecology at http://ong.editorialmanager.com. It is essential that your cover letter list point-by-point the changes made in response to each criticism. Also, please save and submit your manuscript in a word processing format such as Microsoft Word.

If you submit a revision, we will assume that it has been developed in consultation with your co-authors and that each author has given approval to the final form of the revision.

Again, your paper will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by Aug 02, 2019, we will assume you wish to withdraw the manuscript from further consideration.

Sincerely,

Nancy C. Chescheir, MD
Editor-in-Chief

2018 IMPACT FACTOR: 4.965
2018 IMPACT FACTOR RANKING: 7th out of 83 ob/gyn journals

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/ong/login.asp?a=r). Please contact the publication office
if you have any questions.
July 30, 2019

To Nancy C. Chescheir
Editor-in-Chief
Obstetrics and Gynecology

Re Manuscript ONG-19-1002

Dear Dr Chescheir,

We thank the reviewers for their constructive comments. We have now included a point-by-point responses as suggested by the reviewers and statistical editor (see below). Our study has been reviewed by our ethics committee (Comité de Protection des Personnes, Ile de France IX, CHU Henri Mondor) and the manuscript respects the STROBE guidelines.

We are grateful to you for consideration of this manuscript.

Sincerely,

Bassam Haddad for all authors.
In this study, the author measured PlGF and creatinine levels in urinary samples collected from pregnant women with history of severe preeclampsia (PE) in subjects recruited in the Heparin-Preeclampsia trial. Urine samples were collected in every 4wk of gestational windows: 10-13, 14-17, 18-21, 22-25, 26-29, 30-33, and 34-38. Results showed that low urine PlGF levels between 22-25wks are strongly associated with subsequent development of PE related adverse outcomes (PE, FGR, placental abruption and perinatal death). Results of this study provide further evidence and suggest that low urine PlGF/creatinine levels in the mid-gestation could be used at a biomarker to predict high-risk pregnancy population associated with PE and related adverse outcomes.

Following concerns need to be addressed:

1. The volume of urine samples used for PlGF and creatinine assays needs to be included in the M&M. Were samples diluted for the assays?

Response: Samples were used undiluted for both PlGF assay and creatinine assay. These are now included in the methods section.

2. The numbers in Table 2 (n=118 (urinary PlGF/Creat) in composite adverse outcome <34wks and n=143 (urinary PlGF/Creat) in preeclampsia <34wks) do not match those given in Table 1. Please explain?

Response: For the prediction studies shown in table 2 and figure 2, we only used samples in the gestational windows 22-26 weeks. Not all patients contributed to urine specimens in this gestational window and the numbers are therefore different. For the prediction studies, we used samples from all patients within 22-26 weeks who developed either composite adverse outcomes <34 weeks or preeclampsia <34 weeks and compared them with all patients who did not develop composite adverse outcomes <34 weeks or preeclampsia <34 weeks respectively. Revised methods and table 2 legend clarify the updated analysis.

3. Line 179-180: --- not statistically different ---- (Table 3 Supplementary data). Table 1 and Table supplementary data are missing in the Result Section.

Response: We have now included Table 1 in the results section. Supplemental data are now included in the results section.

4. Line 195: what are the two adverse outcomes?

Response: We have now clarified the 2 outcomes that were examined in this study. These were composite adverse outcomes <34 weeks or preeclampsia <34 weeks. We have now clarified the text.

5. Lines 271-273: It is stated" The analysis were not performed between 18 and 22 weeks because of very few samples at this gestational window", how many samples were actually measured in the 18-22wks of gestational window?

Response: Only 27 urine samples are available in the 18-22 wks of gestational window.
window. This has been added in the text.

6. Proteinuria status should be included in Table 1, Maternal Characteristics.

Response: This is now included in the table1.

7. Were sFlt-1 concentrations measured in these urinary specimens?

Results: Yes, we did measure urinary sFlt-1 levels and like prior descriptions (Levine RJ et al, JAMA, 2005), urinary sFlt-1 levels were very low (1000 fold lower in urine than in serum). This is consistent with previous descriptions that the molecular weight of sFlt-1 protein is 130 kDA and is too large to be filtered by the intact glomerulus. Since, majority of the study was using urine specimens prior to proteinuria urinary sFlt-1 is uninterpretable. We have clarified this point in the introduction as well.

8. References should be listed according to the journal format.

Response: We have now corrected this.

9. Typo errors:
   Line 70: Change "is" to "are".
   Line 150: Delete "for"
   Line 281: Change "duting" to "during".

Response: The authors thank the reviewers for these corrections. The corrections were performed in the manuscript.

Reviewer #2: Lecarpentier and colleagues have submitted the report of a retrospective cohort study designed to characterize the relationship between urinary PIGF and the subsequent development of a composite outcome: preeclampsia, fetal growth restriction, placental abruption and perinatal death. In order to strengthen the report, the authors may wish to consider the following:

1. Introduction, line 83: Consider changing the word "induces" to "is associated with." I'm pretty sure causation has not been demonstrated yet.

Response: The authors thank the reviewers for this comment. We have now corrected this.

2. Introduction, lines 111-113 and Methods, lines 132-135: First, you include "maternal death" in your composite primary outcome in the Methods section, but not in the introduction. Can you please clarify the entities that make up the composite outcome?

Response: The authors thank the reviewers for this comment. In the HEPEPE trial the composite adverse outcome criteria included the maternal death. However, in the HEPEPE trial, there was no maternal death. We have now added "maternal death" throughout the manuscript where appropriate.
3. Introduction and Methods, general comment: Given that most of the work regarding serum and urinary markers in this family (sFLT 1 and PIGF) surrounds predicting preeclampsia, why did you choose to include maternal death, fetal growth restriction, perinatal death and abruption in your composite? All of these can be associated with preeclampsia, but none are only caused by preeclampsia. Please clarify.

**Response:** The primary outcome (composite adverse outcomes) was already pre-specified in our clinical trial design and hence we are unable to change this. However, as the reviewer correctly points out, since many of the adverse outcomes may not be related to preeclampsia, we have also shown gestational pattern of urinary PIGF and prediction data for preeclampsia <34 week alone (Figure 1C, Figure 2B).

4. Methods, general comment: Was your composite outcome prespecified? If so, please state so.

**Response:** Yes, the composite adverse outcomes were pre-specified and the trial outcomes previously published (Haddad B et al, Obstetrics Gynecology 2015).

5. Methods, lines 138 to 141: you collected urine samples at 7 different times across gestation. If your null hypothesis was that there is no relationship between urinary PIGF and the subsequent development of your composite outcome; that seems like 7 separate experiments to me. Please clarify if and how you addressed the statistical consequences imparted by multiple testing. Did you apply any adjustments to your significance level?

**Response:** Urine samples were collected at 7 different time points so we could examine the longitudinal changes in the urinary biomarker changes throughout pregnancy to help understand pathogenesis. With regards to prediction studies, shown in figure 2 and table 2, our goal was to evaluate whether at a single time point (between 22-26 weeks), will these urinary biomarkers be useful as a prediction test. Table 2 data includes included estimates of effects and confidence intervals which is now becoming the standard for statistical reporting for secondary or exploratory analyses (Harrington D, NEJM 2018).

6. General comment/concern: My main reservation is your inclusion of outcomes (e.g. your composite) not always linked to perturbations in sFLT-1 or PIGF. This would be a more credible report if you limited it to preeclampsia.

**Response:** We are unable to change the composite adverse outcomes as this was already pre-specified in the clinical trial protocol. However, as the reviewer correctly points out, since many of the adverse outcomes may not be related to preeclampsia, we have also shown gestational pattern of urinary PIGF and prediction data for preeclampsia <34 week alone (Figure 1C, Figure 2B). In addition we would like to point out that angiogenic factors are also implicated in other placental insufficiency outcomes such as IUGR, fetal deaths and abruption (Gaccioli F et al, Lancet Child Adolesc Health. 2018; Chaiwarapongsa T, Am J Obstet Gynecol, 2017, Signore C et
Reviewer #3:

1. Title. Why is heparin in the title? Half of the women didn't receive it, and it wasn't a factor in any of the outcomes. If anything, might add low-dose aspirin to the title instead.

Response: The authors thank the reviewers for this comment. We have now changed the title to « Urinary Placental Growth Factor for Prediction of Placental Adverse Outcomes in High Risk Pregnancies »

2. Abstract.

a. The abstract (and title and precis) are about urine PIGF, but the study was also about serum sFlt-1 and PIGF. Would clarify this.

Response: In this study, we only performed urinary biomarker studies. We merely use the serum data from our previous publication (Obstetrics Gynecology, 2018) for comparison only. We have now clarified this in the methods (Line 155).

b. Lines 58-60. This sentence is the only one with Ns and percentages, but it reflects the composite at any gestational age, whereas study outcome is development of a composite before 34 weeks (71 women rather than 25 women). Might revise.

Response: The authors thank the reviewers for this comment. The corrections were performed in the manuscript (Lines 178-179) : « The two groups had comparable baseline characteristics and had similar adverse composite outcomes < 34 weeks (14/93 [15.1%] vs. 11/94 [11.7%]; p=0.50) ».

3. Introduction.

Lines 96-107. Would consider that when women are known to be at increased risk for preeclampsia (as in this study), providers may not be looking for another screening test "to predict the onset." These mothers and their fetuses are already being followed more closely. Here or in the discussion, would consider how high the PPV or NPV would have to be to safely deviate from this management. Similarly, in lines 109-113, doesn't predictive performance imply PPV or NPY?

Response: The authors thank the reviewers for this comment. Predictive performance implies mostly the area under the ROC curve. We agree that patients with a history of early onset and severe pre-eclampsia are monitored more closely. However if the test had robust negative predictive values, perhaps the follow-up could be less frequent and lower health care costs. To truly demonstrate the utility of a biomarker test, one needs a clinical trial to show that knowledge of test will lead to improved pregnancy outcomes similar to the recently published work on use of serum PIGF by Duhig KE and PARROT investigators in Lancet 2019. This is of course beyond the scope of the current manuscript.

4. Methods. This section is well-written and clearly presented.

Lines 137-138. Would list the number enrolled and participating in the results rather
than methods.

Response: The correction was made in the text.

5. Results.

a. Lines 176-178. The authors write that the adverse outcome composite was not different between the 2 groups (37.6% vs. 38.3%). Those percentages are for the overall composite, not for the study objective of births <34 weeks. The relevant percentages are 15.1% vs. 11.7%.

Response: The correction was made in the text.

b. The frequency of preeclampsia < 34 weeks is a primary study outcome. Would include it in table 1 (there are quite a few outcomes listed in table 1, but this is not).

Response: This outcome was added in the table 1 (9.7% vs 7.4%)

c. Please report the individual outcomes that make up the <34-week composite. Might include this information in table 1 and in table 2.

Response: The individual outcomes were added in Table 1 and included in the legend of Table 2

d. Lines 196-199. In this study of 187 pregnancies, 25 had the outcome composite. The authors write that for a false positive rate of 10%, the sensitivity was 77.7%. Does this mean that 19 women screened positive and developed the composite <34 weeks, and that 19 screened positive but did not, such that the PPV was 50%? Are the authors saying that 6 pregnancies were missed by the screening method? Please clarify this information and report PPV and NPV.

Response: We now report NPV and PPV. In the results section, we include the following. «At a cut-off value of urine PI GF concentrations at 114 pg/mg measured during mid-gestation, positive predictive value was 33% and negative predictive value was 98% with a prevalence of 13.4% for composite adverse outcomes. For the subjects that developed composite adverse outcomes, the average time between the urine collection and delivery was 41±14 days”.

6. Discussion.

a. Please address clinical utility more fully. How would a positive result be used in practice, and would that be cost-effective, considering the false positive rate? How would a negative result be used, considering that the false negative rate was > 20%? Suggest discussing (or referring to) ACOG PB 202 from December 2018. Rather than AUC or likelihood ratios, positive predictive value data are needed. ACOG concluded that based on prior data, biomarker use should remain investigational because of low accuracy.

Response: We agree with the reviewer. In the absence of true therapeutic intervention, utility of positive predictive value is limited. However if the test had robust negative predictive values, perhaps the follow-up of these patients could be
less frequent which in turn could lower health care costs. To truly demonstrate the utility of a biomarker test, one needs a clinical trial to show that knowledge of test will lead to improved pregnancy outcomes similar to the recently published work on use of serum PI GF by Duhig KE and PARROT investigators in Lancet 2019. This is now discussed.

b. Lines 258-261. The lag time to development of preeclampsia or a related morbidity was 6 weeks +/- 2 weeks. Based on when the test was performed, complications developed between 26 and 34 weeks (considering just 1 SD from the mean). How would this “allow to develop appropriate monitoring”? Please be more specific in lines 278-280 as well.

Response: We now discuss the following. Urinary PI GF could be used as non-invasive biomarker during mid-pregnancy in high risk women for risk-stratification and tailored management of patients. Among the women with low urinary PI GF levels, blood measurement of sFlt-1 and PI GF could then be used to confirm or rule-out women at high risk to develop adverse outcomes. This would allow to develop appropriate monitoring. However to demonstrate that biomarker related monitoring would be clinical useful, one needs a clinical trial to demonstrate improved outcomes in the group with the knowledge of the test similar to recently conducted PARROT trial.

STATISTICAL EDITOR COMMENTS:

The Statistical Editor makes the following points that need to be addressed:

Table 1: Since the sample size for each cohort was < 100, should round the %s to nearest integer, rather than to nearest 0.1%.

Response: Thank you for this remark. We made the corrections in Table 1.

Table 2: Should round the AUC to nearest 0.01 and include CIs. Should include CIs for the sensitivity and LHR(+). Should include on-line data set for readers interested in the full relationship of sensitivity vs specificity for the data.

Response: Thank you for this remark. We made the corrections in Table 2.

lines 201-202, 205-206: The use of NPV and PPV is only useful for a particular prevalence of the adverse outcome, so not applicable to other series. Should only cite sens, spec, LHR(+) and LHR(-), since those are independent of prevalence of the adverse outcomes.

Response: We have now included the PPV and NPV for urine PI GF biomarker measured during mid-gestation. «At a cut-off value of urine PI GF concentrations at 114 pg/mg measured during mid-gestation, positive predictive value was 33% and negative predictive value was 98% with a prevalence of 13.4% for composite adverse outcomes. For the subjects that developed composite adverse outcomes, the average time between the urine collection and delivery was 41±14 days”.

lines 209-212: The differences cited (“slightly lower”, “higher”) are only numerical
differences. Should support with statistical comparisons and if NS different, should so state.

Response: We made these corrections in the manuscript.

EDITOR COMMENTS:

1. Thank you for your submission to Obstetrics & Gynecology. In addition to the comments from the reviewers above, you are being sent a notated PDF that contains the Editor’s specific comments. Please review and consider the comments in this file prior to submitting your revised manuscript. These comments should be included in your point-by-point response cover letter.

***The notated PDF is uploaded to this submission's record in Editorial Manager. If you cannot locate the file, contact Randi Zung and she will send it by email - rzung@greenjournal.org.***

- levels are associated

- was this a preplanned study?

Response: The Heparin- Preeclampsia trial (HEPEPE) was a randomized clinical trial initiated across 16 secondary or tertiary care centers in France conducted between November 14, 2009, to February 21, 2015, evaluated whether daily LMW heparin, added to low-dose aspirin, started before 14 weeks of gestation reduces placenta-mediated complications in pregnant women with previous severe preeclampsia diagnosed before 34 weeks of gestation. The primary endpoint was a composite outcome that included any of the following events: maternal death, perinatal death, preeclampsia, placental abruption, SGA (birth weight less than the 10th percentile for gestational age). The study was designed with 80% power to detect a primary outcome reduction of 33% relative risk in the enoxaparin–aspirin group compared with the aspirin group alone with a two-sided type I error rate of 5%. The present study is a planned ancillary study of the Heparin- Preeclampsia trial (HEPEPE), consequently, the composite adverse outcome was prespecified.

- Did you include all of the participant of the parent study and if not, how did you select your groups?

Response: We included all the participants from the parent study from whom we had at least one urine sample during pregnancy.

In objective you state you were looking at outcomes < 34 weeks. Why did you include those at 34 weeks and above?

Response: Outcomes <34 weeks were pre-specified. From a clinical standpoint, the outcomes occurring <34 weeks are more meaningful as this is the group with the greatest morbidity. This does not mean that these markers may not be useful beyond 34 weeks. However, the utility of these markers in term disease needs other studies.
- are there units here or are both urine PIGF and creatinine measured in same units so this is a simple ratio? Is this urinary creatinine or serum creatinine?

Response: The urinary PIGF concentrations (pg/ml) are normalized with urinary creatinine measurements (mg/ml) and expressed as pg of PIGF per mg of creatinine.

- Please clarify In results you report a ratio between urinary PIGF/Creatinine not the absolute urinary PIGF levels.

Response: We have now clarified this. The sentence « The urinary PIGF concentrations are normalized with urinary creatinine measurements (pg/mg) and expressed as median (interquartile range, quartile 1-3) » has been added in the results section.

- When you write that a study occurred between date 1 and date 2, it literally excludes those boundary dates. For instance, “This study was performed between Feb 2018 and Jan 2019” would mean it was performed from March 2018 to Dec 2018. Do you instead mean that the study was performed from date 1 to date 2? If so, please edit.

Response: We made these corrections in the manuscript, « This study is a planned ancillary study of the Heparin- Preeclampsia trial (HEPEPE), which was a randomized clinical trial initiated across 16 secondary or tertiary care centers in France conducted from November 14, 2009 to February 21, 2015, that evaluated whether daily LMW heparin, added to low-dose aspirin, started before 14 weeks of gestation reduces placenta-mediated complications in pregnant women with previous severe preeclampsia diagnosed before 34 weeks of gestation”.

- Here in methods, please describe normalization of urinary PIGF by using creatinine. Please note if this is serum or urinary creatinine being used. As you are positing that low urinary PIGF is a useful marker AND you studied urinary levels to make it less invasive than blood levels every month, this is an important distinction since the ratio you report--if comparing urinary PIGF/SERUM creatinininen levels--fails to avoid the blood draw.

Response: We have clarified that our biomarker studies reported here are all in urine specimens (urinary PIGF and urinary creatinine). The urinary PIGF concentrations (pg/ml) are normalized with urinary creatinine measurements (mg/ml) and expressed as pg of PIGF per mg of creatinine.

limit p values to 3 decimals throughout; please note Statistical editor’s comments regarding other limits.

DONE.
2. Title: as some of your patients did not receive LMWH please remove this from the title. Consider, if you wish, adding that they were on Aspirin.

Response: The authors thank the reviewers for this comment. We have now changed the title to « Urinary Placental Growth Factor for Prediction of Placental Adverse Outcomes in High Risk Pregnancies »

3. Precis, Abstract-Conclusion, and elsewhere: please temper this as your participants were all high risk so the "strong association" was in that group only, Please do so here, and throughout your manuscript.

Response: The words "strong" and "strongly" were removed from the manuscript.

4. The Editors of Obstetrics & Gynecology are seeking to increase transparency around its peer-review process, in line with efforts to do so in international biomedical peer review publishing. If your article is accepted, we will be posting this revision letter as supplemental digital content to the published article online. Additionally, unless you choose to opt out, we will also be including your point-by-point response to the revision letter. If you opt out of including your response, only the revision letter will be posted. Please reply to this letter with one of two responses: A. OPT-IN: Yes, please publish my point-by-point response letter. B. OPT-OUT: No, please do not publish my point-by-point response letter.

A. Please publish our point-by-point response letter.

5. As of December 17, 2018, Obstetrics & Gynecology has implemented an "electronic Copyright Transfer Agreement" (eCTA) and will no longer be collecting author agreement forms. When you are ready to revise your manuscript, you will be prompted in Editorial Manager (EM) to click on "Revise Submission." Doing so will launch the resubmission process, and you will be walked through the various questions that comprise the eCTA. Each of your coauthors will receive an email from the system requesting that they review and electronically sign the eCTA. Any author agreement forms previously submitted will be superseded by the eCTA. During the resubmission process, you are welcome to remove these PDFs from EM. However, if you prefer, we can remove them for you after submission.

Agree

6. Our journal requires that all evidence-based research submissions be accompanied by a transparency declaration statement from the manuscript's lead author. The statement is as follows: "The lead author* affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained." *The manuscript's guarantor.

If you are the lead author, please include this statement in your cover letter. If the lead author is a different person, please ask him/her to submit the signed transparency declaration to you. This document may be uploaded with your
submission in Editorial Manager.

Response: Edouard Lecarpentier is the lead author. We included this information in the cover letter.

7. Responsible reporting of research studies, which includes a complete, transparent, accurate and timely account of what was done and what was found during a research study, is an integral part of good research and publication practice and not an optional extra. Obstetrics & Gynecology supports initiatives aimed at improving the reporting of health research, and we ask authors to follow specific guidelines for reporting randomized controlled trials (ie, CONSORT), observational studies (ie, STROBE), meta-analyses and systematic reviews of randomized controlled trials (ie, PRISMA), harms in systematic reviews (ie, PRISMA for harms), studies of diagnostic accuracy (ie, STARD), meta-analyses and systematic reviews of observational studies (ie, MOOSE), economic evaluations of health interventions (ie, CHEERS), quality improvement in health care studies (ie, SQUIRE 2.0), and studies reporting results of Internet e-surveys (CHERRIES). Include the appropriate checklist for your manuscript type upon submission. Please write or insert the page numbers where each item appears in the margin of the checklist. Further information and links to the checklists are available at http://ong.editorialmanager.com.

Please submit a completed STROBE checklist with your submission.

Done

8. Standard obstetric and gynecology data definitions have been developed through the reVITALize initiative, which was convened by the American College of Obstetricians and Gynecologists and the members of the Women's Health Registry Alliance. Obstetrics & Gynecology has adopted the use of the reVITALize definitions. Please access the obstetric and gynecology data definitions at https://www.acog.org/About-ACOG/ACOG-Departments/Patient-Safety-and-Quality-Improvement/reVITALize. If use of the reVITALize definitions is problematic, please discuss this in your point-by-point response to this letter.

Done

9. Because of space limitations, it is important that your revised manuscript adhere to the following length restrictions by manuscript type: Original Research reports should not exceed 22 typed, double-spaced pages (5,500 words). Stated page limits include all numbered pages in a manuscript (i.e., title page, précis, abstract, text, tables, boxes, figure legends, and print appendixes) but exclude references.

Done

10. Titles in Obstetrics & Gynecology are limited to 100 characters (including spaces). Do not structure the title as a declarative statement or a question. Introductory phrases such as "A study of..." or "Comprehensive investigations into..." or "A discussion of..." should be avoided in titles. Abbreviations, jargon, trade names, formulas, and obsolete terminology also should not be used in the title. Titles should include "A Randomized Controlled Trial," "A Meta-Analysis," or "A Systematic Review," as appropriate, in a subtitle. Otherwise, do not specify the type of manuscript in the title.
11. Specific rules govern the use of acknowledgments in the journal. Please note the following guidelines:

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* Any and all manuscript preparation assistance, including but not limited to topic development, data collection, analysis, writing, or editorial assistance, must be disclosed in the acknowledgments. Such acknowledgments must identify the entities that provided and paid for this assistance, whether directly or indirectly.
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* If all or part of the paper was presented at the Annual Clinical and Scientific Meeting of the American College of Obstetricians and Gynecologists or at any other organizational meeting, that presentation should be noted (include the exact dates and location of the meeting).

12. The most common deficiency in revised manuscripts involves the abstract. Be sure there are no inconsistencies between the Abstract and the manuscript, and that the Abstract has a clear conclusion statement based on the results found in the paper. Make sure that the abstract does not contain information that does not appear in the body text. If you submit a revision, please check the abstract carefully.

In addition, the abstract length should follow journal guidelines. The word limits for different article types are as follows: Original Research articles, 300 words. Please provide a word count.

13. Only standard abbreviations and acronyms are allowed. A selected list is available online at http://edmgr.ovid.com/ong/accounts/abbreviations.pdf. Abbreviations and acronyms cannot be used in the title or précis. Abbreviations and acronyms must be spelled out the first time they are used in the abstract and again in the body of the manuscript.

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Figure 1: Please upload high-res versions of these in their original format (eps, tiff, jpeg, etc.). Copying and pasting into Word often results in a lower resolution.
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