Author's response to reviews

**Title:** First Trimester PAPP-A Levels Correlate with sFlt-1 Levels Longitudinally in Pregnant Women with and without Preeclampsia

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**Author's response to reviews:** see over
Dr. Juan Kusanovic  
Editor-in-Chief, *BMC Pregnancy and Childbirth*  

January 8, 2013

Dear Dr. Kusanovic:

We would like to submit our revised manuscript, “First Trimester PAPP-A Levels Correlate with sFlt-1 Levels Longitudinally in Pregnant Women with and without Preeclampsia” (MS: 5448282782266535) for publication as an Original Research Publication in *BMC Pregnancy and Childbirth*. Our paper studied women with preeclampsia and women with normotensive, uncomplicated pregnancies in all three trimesters in pregnancy. We show, for the first time, that first trimester PAPP-A levels correlate with sFlt-1 and sFlt-1/PlGF ratio throughout pregnancy. We also demonstrate that low first trimester PAPP-A levels suggest angiogenic imbalance that is associated with preeclampsia.

We have addressed each of the comments of Reviewers 1 and 2, in addition to the comments listed by the Editorial Office, below. We look forward to hearing from you.

Sincerely,

Aditi R. Saxena, MD, MMSc

**Response to Editorial Office:**

1. **Comment:** What is the importance of correlating PAPP-A with sFlt-1?
   **Response:** PAPP-A is a marker used in routine obstetric care as part of an aneuploidy screen, however, research also suggests that low first trimester levels may indicate risk for preeclampsia later in pregnancy. sFlt-1 is a pathogenic marker of preeclampsia, and research has also shown that sflt-1 levels are markedly elevated in the second and third trimesters in preeclamptic pregnancies, although sflt-1 is not currently used in the clinical setting for the prediction of preeclampsia. This paper shows for the first time the relationship between PAPP-A, a clinical parameter used in routine obstetric practice, with sflt-1 levels obtained both during the first trimester and throughout the pregnancy to determine if PAPP-A correlates with sflt-1 in normal and preeclamptic pregnancies. The background section has been updated to clarify the significance of the relationship of PAPP-A with sFlt-1 (Background, page 3).

2. **Comment:** Soluble endoglin was not measured.
   **Response:** Soluble endoglin was not measured in this study. Instead, PlGF was measured and the relationship of PAPP-A to PlGF and to the sFlt-1/PlGF ratio was explored. There is a significant body of literature demonstrating the significance of sFlt-1 and PlGF in the development of preeclampsia and alterations of these levels prior to the clinical onset of preeclampsia. As a result, the relationship of PAPP-A with sFlt-1 was the focus of this study, with additional exploration of the relationship of PAPP-A with PlGF and sFlt-1/PlGF ratio (Background, page 3).

3. **Comment:** I was surprised that PlGF concentrations in the first trimester were significantly higher in patients with normal pregnancy than in women who later developed preeclampsia.
   **Response:** First trimester PlGF exhibited a different pattern in the first trimester, compared with second and third trimesters, but the difference between normal and preeclamptic pregnancies was not significant (Table 3, page 24).
4. **Comment:** There is a study (Kusanovic et al. JMFNM 2009; 22: 1021) that showed lower sVEGFR-1 as early as 6-15 weeks in women who later developed preeclampsia.

**Response:** We appreciate this comment and have added this citation to our discussion section (Discussion, page 14). It is important to note that although some subjects in the Kusanovic paper were studied early in the first trimester, similar to our study, the timeframe reported was 6-15 weeks, which is a broader time span compared with our study.

5. **Comment:** I recommend that the results section be re-organized because it is very confusing.

**Response:** The results section has been reorganized to improve clarity (Results, pages 7-9).

6. **Comment:** What is the diagnostic performance of low PAPP-A in the first trimester for preeclampsia?

**Response:** This paper demonstrates that low PAPP-A levels in the first trimester may indicate risk for preeclampsia later in the pregnancy. However, since many women with low PAPP-A levels go on to have normal pregnancies, it should not be used for risk stratification because it lacks specificity.

**Response to Reviewer 1:**

1. **Comment:** The study appears to have a nested case-control design.

**Response:** We thank the reviewer for clarifying this issue. The study is indeed a nested case-control study. We have clarified the methods section (Methods, page 4).

2. **Comment:** What was the basis for the number of patients included in the study? Was there a power calculation? The study appears to be underpowered for early-onset preeclampsia. A sub-analysis of angiogenic levels in early vs. late-onset preeclampsia would be of value.

**Response:** We did not perform a power calculation for this study, as there were no relevant preliminary or published data with which to base the power calculation. One other study examined the relationship of PAPP-A with sFlt-1. However, this study measured both parameters later in pregnancy, and their relationship was different than what we observed in our study, which made it difficult to use for a power calculation in our study. The number of cases of early-onset preeclampsia in our study was small, so we were underpowered to analyze early-onset and later-onset preeclampsia as separate groups, although we agree that this would be of interest in a larger study.

3. **Comment:** Did low PAPP-A have an additive value to angiogenic factors in identifying increased risk of preeclampsia? Was PAPP-A independent from other angiogenic factors?

**Response:** PAPP-A levels were independent from other angiogenic factors and appeared to be better than first trimester sFlt-1 in predicting preeclampsia and at least as good as the first trimester sFlt-1/PlGF ratio. This has been clarified in the discussion (page 12). Based on these results, it appears that measuring first trimester sFlt-1 and/or PlGF would not improve the ability to predict development of preeclampsia beyond information obtained from first trimester PAPP-A levels.

**Response to Reviewer 2:**

**Major revisions:**

1. **Comment:** The authors should define how they selected cases and state whether this is a cohort study or a case-control study.

**Response:** The study is indeed a nested case-control study. We have clarified the methods section (Methods, page 4). Selection of cases is described on pages 4 and 5 (Methods).

2. **Comment:** The authors should comment on storage duration of samples and whether they were previously thawed and re-frozen.

**Response:** Specimens were processed within 4 hours of venipuncture in a refrigerated centrifuge and stored at -80°C until analysis (now added Methods, page 5). Samples were not previously thawed and re-frozen prior to analysis.

3. **Comment:** What is the clinical significance of this study? Why was PAPP-A not measured in second and third trimester samples too?

**Response:** PAPP-A is a marker used in routine obstetric care as part of an aneuploidy screen, however, research also suggests that low first trimester levels may indicate risk for preeclampsia later in pregnancy. sFlt-1 is a pathogenic marker of preeclampsia, and research has also shown that sflt-1 levels are markedly elevated in the second and third trimesters in preeclamptic pregnancies, although sflt-1 is not currently used in the clinical setting for the prediction of preeclampsia. This paper shows for the first time the relationship between PAPP-A, a clinical parameter used in routine obstetric practice, with sflt-1 levels obtained both during the first trimester and throughout the pregnancy to...
determine if PAPP-A correlates with sFlt-1 in normal and preeclamptic pregnancies. Based on these results, it appears that measuring first trimester sFlt-1 and/or PlGF would not improve the ability to predict development of preeclampsia beyond information obtained from first trimester PAPP-A levels. The background section has been updated to clarify the significance of the relationship of PAPP-A with sFlt-1 (Background, page 3). PAPP-A levels were obtained from the subjects’ clinical records performed for aneuploidy screening and were not performed for research purposes (in contrast to the angiogenic markers, which were performed for research purposes). As a result, PAPP-A levels were not measured in the second and third trimesters, as the question we wanted to examine was whether first trimester PAPP-A levels (already being obtained as part of aneuploidy screening) predicted preeclampsia, or whether they were associated with an angiogenic profile predictive of preeclampsia.

4. Comment: Did the authors conduct a power calculation?
Response: As stated in the response to Reviewer 1, we did not perform a power calculation for this study, as there were no relevant preliminary or published data with which to base the power calculation. One other study examined the relationship of PAPP-A with sFlt-1. However, this study measured both parameters later in pregnancy, and their relationship was different than what we observed in our study, which made it difficult to use for a power calculation in our study.

Minor revisions:
5. Comment: Please state the actual p values, instead of NS.
Response: The manuscript has been updated and all tables list actual p values.
6. Comment: Pg 11, line 252, the word “with” is missing.
Response: The manuscript was corrected.

Discretionary revisions
7. Comment: What is the clinical relevance of the findings?
Response: This was addressed in the response to comment 3 above (Background, page 3).
8. Comment: Is there another possible explanation for lower first trimester sFlt-1 levels in women who later develop preeclampsia?
Response: sFlt-1 may be regulated by multiple factors, including the renin-angiotensin system. In addition, although sFlt-1 antagonizes VEGF, it may also be regulated in response to levels of VEGF. As a result, it may serve as marker of poor placentation in early pregnancy, which then leads to preeclampsia later in the pregnancy.
9. Comment: What are possible mechanisms for the unexpected finding of higher PlGF levels in women who later develop preeclampsia?
Response: First trimester PlGF exhibited a different pattern in the first trimester, compared with second and third trimesters, but the difference between normal and preeclamptic pregnancies was not significant between the two groups (Table 3, page 24).