Author's response to reviews

**Title:** Copeptin concentration in cord blood in infants with early-onset sepsis, chorioamnionitis and perinatal asphyxia

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**Author's response to reviews:** see over
Bern, March 28th, 2010

Regarding: Revision of MS 4167578704827259, point-by-point reply to the reviewer’s comments

Dear Professor Norton,

We greatly appreciate the possibility to submit our revised manuscript entitled “Copeptin concentration in cord blood in infants with early-onset sepsis, chorioamnionitis and perinatal asphyxia”, which we submit to *BMC Pediatrics* for consideration for publication as a *Regular Article*. We thank the reviewers for their critical comments which have allowed us to improve the quality of the manuscript. We believe we have been able to fully respond to these. Please find below a point-by-point reply to each of the reviewer’s specific comments.

Attached you will find the revised manuscript, which has been approved by all authors. The manuscript has not been published previously and is not under consideration for publication elsewhere. None of the authors has declared a conflict of interest. This study has been approved by the institutional review board. All authors have contributed significantly to the entire work, have seen the final version and approved the submission of the manuscript, and take responsibility for the entire manuscript.

Thank you for your consideration of this manuscript and we look forward to your comments.

Yours sincerely,

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Copeptin concentration in cord blood as a marker of perinatal stress
Luregn J Schlapbach, Stefanie Frey, Susanna Bigler, Chiem Manh-Nhi, Christoph Aebi, Mathias Nelle and Jean-Marc Nuoffer

Editorial requests:

1) Please include the name of the committee which provided ethical approval for your study.
   - Response: We have included the name and address of the Ethical Board in the revised manuscript, as required.

2) "Contributer's statement" should be renamed "Authors' contributions".
   - Response: We have renamed the words as required.
Reviewer 1 (Daniele Trevisanuto):

In this study, Schlapbach et Coll measured umbilical cord copeptin concentrations in a large sample (n. 243) of neonates. Copeptin concentrations were evaluated dividing the study population in 3 main groups of patients based on their disease: sepsis, chorionamnionitis and asphyxia.

The results show that copeptin concentrations were higher in asphyxiated infants, in neonates born by vaginal route and in those with acidosis. In contrast, infection did not influence copeptin concentrations.

The article is original because it measures, for the first time, the copeptin concentrations in asphyxiated and septic neonates.

I have the following criticism and suggestions:

1. Abstract Adequate.

2. Background

In this section, the authors seems to be concentrated on the sepsis (Paragraph 3), and not on the asphyxia aspects. It seems that the objective of “original manuscript” was evaluation of copeptin concentrations in neonates with infection (sepsis and chorionamnionitis). I suggest to add a paragraph on the asphyxia in neonates and the rationale for measurements of copeptin in course of this clinical situation.

- Response: We thank the reviewer for this comment. We have rephrased the introduction section and have added a short section on asphyxia.

3. Methods

Page 6. Chorionamnionitis group. Was histological evaluation of the placenta done in all study population?.

- Response: Histological examinations of the placenta were performed in 70% of the study population.

Asphyxia Group. The definition of asphyxia is arbitrary and is not in agreement with that used in Ref. 19 (Azzopardi et al. NEJM 2009).

- Response: We thank the reviewer for pointing out this important aspect. The definition of asphyxia used in the present study is based on our institutional criteria for asphyxia, which allowed us to identify infants with the same pathology in the database. This asphyxia definition allowed to include milder forms of asphyxia, in comparison to trials on therapeutic hypothermia (Azzopardi et al. NEJM 2009). This - in our opinion - represents an advantage, since we could thereby assess copeptin concentrations in infants covering a range of asphyxia severities. We have stated this more clearly in the revised manuscript, Methods section, page 6.
4. Results
Table I. Characteristics of patients groups: There is a large percentage of SGA infants in the asphyxiated group (28%). As stress hormone cortisol is elevated in SGA infants, could this condition have influenced copeptin concentrations in the asphyxiated neonates?
- Response: The reviewer states correctly that much more infants were SGA in the asphyxia group compared to other groups. Chronic placental insufficiency can result in SGA which may lead to perinatal asphyxia. In fact, Copeptin concentrations in infants with SGA were trendwise higher compared to AGA infants (median 42 vs 15 pmol/l; Man-Whitney p=0.055). In agreement with the suggestion by the reviewer, we therefore included SGA as a covariable in multivariate analyses throughout the revised manuscript. Notably, when adjusting the multivariate analyses as well for the covariable SGA, the analyses result relatively similar (asphyxia vs controls Beta coefficient 1.09, 95%-CI 0.41 – 1.76, p=0.002).

How many patients were enclosed in the chorionamnionitis group on clinical diagnosis and how many on histological diagnosis?
- Response: In the chorioamnionitis group, histological examination showing chorioamnionitis was available in 22/33 (67%) infants. The clinical diagnosis was present in 23/33 (70%) infants in this group. In ten infants, placental histology showed clear chorioamnionitis, while the clinical reports were inconclusive regarding chorioamnionitis.

I was surprised from the high number of patients with arterial hypotension. Which was your definition of hypotension? How many hypotensive infants received inotrops?
- Response: Arterial hypotension was defined as mean arterial blood pressure which was below the gestational age limit at two consecutive measurements and that was treated with either a volume bolus administration or intravenous corticosteroids. This definition is now given in the Methods section, first paragraph, page 5. This explains, why the frequency of arterial hypotension is quite high, especially in the groups that contain predominantly preterm infants (EOS and chorioamnionitis). In the total cohort, only 10 (4%) infants required inotropic support, of which 5 in the EOS group, 3 with asphyxia, 1 with chorioamnionitis, and an extremely preterm infant in the control group

5. Discussion & Conclusions They are well balanced and adequately supported by the data.

6. References

- Response: We thank the reviewer for these helpful comments. We have included the reference by Bidegain et al (J Pediatr 2010) in the Introduction, first paragraph, and have updated the reference by Wellmann S et al which has now been published.

7. Table Are the data expressed as median (range or IQ range)? Please, specify it.
- Response: Data are expressed as medians (interquartile range), or numbers (percentage), where appropriate. We have added a legend to Table 1 specifying data presentation and abbreviations.

8. Figures Adequate.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.
Reviewer 2: Eleftheria Hatzidaki

1. Is the question posed by the authors well defined?
This study aims to establish copeptin concentrations in neonates of different gestational ages and to assess the influence of sepsis, chorioamnionitis and asphyxia on copeptin concentrations.

The authors claim in the results that copeptin concentrations correlate significantly with birth weight and gestational age. However, is there any statically significant difference in terms of birth weight and gestational age between the groups of early onset sepsis and controls, and chorioamnionitis and controls? (Minor Essential Revisions)
- Response: Copeptin concentrations correlated significantly with birth weight (Spearman’s Rho 0.29, p<0.0001) and with gestational age (Rho 0.30, p<0.0001) in the whole cohort (n=243, see manuscript). The correlation was highly similar when only the control group was considered (n=155, birth weight Rho 0.40, gestational age Rho 0.41, p<0.0001 for both). The control group consists of both term and preterm infants (thereby covering a broad range of gestational ages). The early-onset sepsis and the chorioamnionitis groups consists predominantly of preterm infants. Therefore, infants with EOS and with chorioamnionitis have significantly lower birth weights compared to the control groups (see Table I), the Man-Whitney test results at p<0.01 (we did not show the statistical test results on Table 1, since this would entail multiple comparisons that may be more difficult to read for the average reader. We of course are happy to do so if the reviewer wishes). This is why we adjusted the multivariate analyses for birth weight and gestational age.

2. Are the methods appropriate and well described?
The authors have to clarify the criteria for the definition of early onset sepsis. How many clinical signs have to be present? (Minor Essential Revisions)
- Response: We thank the reviewer for this comment. The definition of EOS required presence of AT LEAST TWO clinical signs of infection, PLUS elevated CRP >20mg/l, PLUS treatment for at least 7 days with iv antibiotics, PLUS positive blood cultures. In infants with negative blood cultures but clinical diagnosis of EOS, all first three criteria mentioned above were required to be present. We have stated this more clearly in the revised version.

3. Are the data sound? Yes
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes
5. Are the discussion and conclusions well balanced and adequately supported by the data? Yes
6. Are limitations of the work clearly stated? Yes

7. Do the authors clearly acknowledge any work upon which they are building? both published and unpublished? Yes

8. Do the title and abstract accurately convey what has been found? The term “Perinatal Stress” in the title is not a proper one.

- Response: The reviewer states correctly that while the term „perinatal stress“ is widely used, it is not defined. We therefore suggest a more neutral title „Copeptin concentration in cord blood in infants with early-onset sepsis, chorioamnionitis, and perinatal asphyxia”

In the results lines of the abstract the authors mention “Receiver-operating-characteristic curve......allowed predicting asphyxia with accuracy...... to diagnose asphyxia.....”. I don’t understand what they mean with the term “predicting asphyxia” since copeptin concentration measurements in cord blood are routinely made after delivery. In the patients and methods section the authors define asphyxia. What do they mean with the term “diagnoseasphyxia”? (Minor Essential Revisions)

- Response: We agree with the reviewer that the terms „predict” and „diagnose“ may not be entirely accurate and suggest to replace these terms with „....were associated with...“ in the Abstract and Result sections. The goal of this pilot study was to investigate differences in copeptin cord blood concentrations between two major, clearly defined pathologies (EOS and asphyxia). The strong association between copeptin concentrations and perinatal asphyxia warrants further studies.

Minor Essential Revisions

1. In the background section the authors refer to the use of exogenous vasopressin as a promising therapeutic agent. Is this reference relevant to their study and their results?

- Response: We believe these references (see as well comment 6 by reviewer 1) should be kept in the manuscript: Vasopressin is already being used as a therapeutic agent in the neonatal population, although very little is known about vasopressin levels in this age group. The present data on copeptin may thus hopefully stimulate further observational and therapeutic studies on vasopressin/copeptin in neonates.

2. It would be better to present results in the abstract section as well as in the main text without starting consecutive sentences with the same words “copeptin concentrations”

- Response: We have rephrased the result section in the abstract and the main text according to the suggestion of the reviewer.
3. The format of the manuscript should follow the instructions for BMC Pediatrics authors (e.g. the “Patients and Methods” should be replaced by “Methods”)
- Response: The heading has been changed, as required.

4. In the “References” the authors should follow the instructions for BMC Pediatrics authors (e.g. citations in the reference list should contain all named authors, regardless of how many there are).
- Response: We apologize for this mistake and have reformatted the reference section according to the BMC Pediatrics guidelines.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests.
Reviewer 3: Victor Kiri

Reviewer's report:

The authors must describe how the 155 controls were selected from the pool of over 3700 potential candidates.

- Response: The control group consists of 155 infants, including 75 premature and 80 term neonates. This sample size is based on the following sample size calculations: case group with n=30, expected mean difference of 75 pmol/l, expected standard deviation of 125 pmol/l, power of at least 80% at a 95%-2-sided confidence interval. These assumptions were based on internal preliminary data (since at time when this study was performed, no data had been available at all on copeptin in neonates), and on published results from adults with sepsis (Morgenthaler NG, et al. Shock 2007, 28(2):219-226). Only neonates that had not had any signs of infection, chorioamnionitis or asphyxia were included as controls. In addition, only neonates where cord blood serum had been taken at birth were included. Infants with congenital malformations were excluded. The control group infants were all born between January 2007 and November 2007.

We have revised the Methods section and have specified more in detail the selection criteria for the control group, see Methods section, page 5 and 6.

The authors stated “we hypothesized that copeptin cord blood concentrations are increased in neonates with different stress situations such as EOS and perinatal asphyxia.” If so, why have the authors conducted two-sided tests? They should either involve 1-sided test or change the hypothesis to one of “association between copeptin cord blood concentrations and ...” as stated in the opening sentence of “Conclusions”. For instance, the statement “EOS infants with septic shock or with positive blood cultures did not have higher copeptin concentrations compared to the rest of EOS infants (p>0.05)” should be based on a 1-sided test.

- Response: Given that the present study is a pilot study investigating copeptin in neonates with major disease, the principal aim was to assess associations between copeptin in cord blood and clinical parameters. We used 2-sided tests since no data were available at all on copeptin in this age group, and we could therefore not be certain that indeed copeptin concentrations would increase in certain groups. We agree with the suggestion by the reviewer and have therefore changed the Background section to „We hypothesized that copeptin cord blood concentrations in neonates may be associated with different stress situations such as sepsis and perinatal asphyxia...” (page 4, first sentence). Accordingly, we have changed the result section, page 9, to “Copeptin concentrations did not significantly differ between EOS infants with septic shock or with positive blood cultures compared to the rest of EOS infants”. 
I also have problem with the statement “Interestingly, neonates with the highest copeptin levels had only minor HIE, but the sample was not powered to detect differences in small subgroups”. It suggested the study was powered to detect any differences. The manuscript has no mention of a required sample size or statistical power. The authors should find that gap by providing the power based on their study sample size or modify the sentence to read “…but the study was not powered to detect differences”
- Response: We thank the reviewer for this critique. We have included information on sample size of the control group in the revised manuscript, Methods section, page 6. We believe that the sentence in the discussion section (page 11, bottom line) referring to HIE grades is too hypothetical and should be removed.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests: I declare that I have no competing interests