Author's response to reviews

Title: Evaluation of procalcitonin for diagnosis of neonatal sepsis of vertical transmission

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Author's response to reviews: see over
Comments to Reviewer's report

First of all, we would like to thank the reviewers for their valuable comments and corrections. We apologize for delaying our responses, but we were trying to recover data about PCT quantification procedure requested by one of the reviewers.

Reviewer: Lucia Pacifico

Major Compulsory Revisions

1. The Youden’s index is not a clinically useful index because it gives equal weight to the two diagnostic areas (false-positive and false-negative). In any case, over a wide range of sensitivity and specificity (Table 5) Youden’s index does not change very much.
   **Response:** We have already addressed this point in the discussion (page 11). If other cutoff points with better sensitivity had acceptable specificity we would have selected them, but with our ROC curves, small improvements in sensitivity implied big reductions in specificity, so we finally used Youden’s index to select the cutoff points.

2. Figure 2. Authors elaborated ROC curves (and cut-offs at the three neonatal ages) considering groups 2A (proven sepsis), 2B (clinical sepsis), and 1 (labelled as non-infected on page 9, lines 10-11, and as group of asymptomatic newborns on page 5, line 20). Why did Authors fail to consider for the ROC curves group 2c (including uninfected newborns with neonatal pathology other than an infectious process and negative blood culture)?
   **Response:** We think we have not enough evidence to finally classify group 2C neither as infected nor as not infected since, as exposed in the discussion, intrapartum antibiotics had been used in 22/79 neonates of this group (that may induce negative blood cultures), and 66/79 were treated with antibiotics after birth (as they were also infected). We have added some words to 2nd paragraph, page 10, which now reads:

   “…Yet it is not possible to assure these patients were really uninfected, since the assertion is based mainly on negative bacterial cultures and they may fail in an appreciable number of cases (in our series, intrapartum antibiotics had been used in 22/79 neonates with respiratory disorders, and 66/79 were treated with antibiotics after birth). This is also the reason why we excluded group 2C from ROC curves.”

   a. It is unclear whether the 169 group 1 subjects were, indeed, healthy neonates. Did all 169 group 1 subjects have a normal postnatal course? When were they discharged home? Did they need any management (other than antimicrobial administration) throughout the follow-up period? How long were they followed-up?
   **Response:** Group 1 subjects were admitted to nursery because of prematurity, low birth weight, or risk factors for sepsis. These newborn infants were not defined as “healthy”, but “asymptomatic”. None required medical treatment nor had symptoms suggestive of infection during their first week of life. Premature neonates that stayed for more than a week at the nursery might have had later other episodes not related to vertical transmission sepsis. That last point has not been expressed adequately in the manuscript, so we have clarified group 1 definition (page 5-6), that now reads:

   “The first population (group 1) included a group of asymptomatic newborn infants admitted during the first 24 h of life to the neonatal unit because of prematurity, low birth weight, or at least two risk factors for infection (table 1). They had no clinical signs of sepsis during their first week of life and had a negative blood culture. Patients in this group did not receive antibiotic treatment. Those who were discharged home before the seventh day of live were followed to assure they did not develop late-onset vertical sepsis.”
b. Which was the clinical and therapeutic outcome of infants included, respectively, in groups 2B and 2C?

Response: Every patient in group 2B and 66/79 in group 2C received antibiotics (usually ampicilin + gentamicin/cefotaxime). There were 2 deaths in group 2A, no deaths in group 2B, and 3 deaths in group 2C. We have added last data to the results (page 8, 1st paragraph), that now reads:

“…There were 2 deaths in group 2A, no deaths in group 2B, and 3 deaths in group 2C. Patients in group 2C had significantly lower birth weights and gestational ages (P<0.0001, analysis of variance) than those in groups 1, 2A and 2B”

3. Which nonparametric test was used to made pairwise comparisons of serum PCT concentrations within group 1 subjects?

Response: Mann-Whitney U test. We have now added this information in the text.

4. Figure 1: Were the observations indicated at level 0.001 real observations or undetected values? Authors, indeed, stated that the detection limit of LUMItest is 0.08 ng/mL.

Response: They were undetected values. We have realized that there is a problem in the figure because it was done with raw data, and one of the laboratories marked undetected data as 0.001 and the other as 0.01. For statistical analyses we considered undetected levels as 0.04 ng/ml, so we have drawn figure 1 again.

5. Which was the PCT limit of quantification (rather than the detection limit)? At low PCT levels, which was the coefficient of variation? Which were the within-run as well as the day-to-day imprecision in measuring PCT?

Response: Intraassay coefficients of variation were 5.9% at 31.0 ng/mL and 13.8% at 0.81 ng/mL; Interassay coefficients of variation were 10.8% at 31.0 ng/mL and 25.9% at 0.81 ng/mL. Unfortunately, we do not have data about limit of quantification available. We have tried to recover them but many data were lost when the main laboratory moved to another building two years ago (blood samples were analysed in 2001).

6. Please give more details about the FIVE values of the percentiles indicated in Figure 1.

Response: Figure 1 legend now reads: “…The boxes are limited by the 75th and 25th percentiles of the data. The middle lines represent the median. Values plotted with cross markers are outside values (more than 1.5 times the interquartile range over 75th or under 25th percentiles). Values plotted with plus markers are far out values (more than 3 times the interquartile range over 75th or under 25th percentiles).

7. What dependent variable was used for the multiple regression analyses of PCT? The crude values and the logarithms are said to be not Normally distributed. The assumptions of Normality and constant variance are crucial in a regression analysis.

Response: The dependant variable was PCT concentration. We did three different analyses (PCT at birth, at 12-24 and 36-48 hours of life). For the regression analyses we used log transformation of PCT values, which were not normally distributed but had an acceptable homoscedasticity. Of course, it implies some limitations to the analysis, and so we used it just to detect possible variable associations but not to quantify them. That is the reason why we did not include regression coefficients. Anyway, we think it is a good idea to detail this point in the discussion (page 11, 1st paragraph) and table 3:

“…Chorioamnionitis has been also independently associated with increased PCT values. These findings in our study must be considered cautiously, due to limitations in multiple regression analysis when normality assumption is violated.”
Table 3 “Includes data of 169 asymptomatic newborns (group 1). P values according to multiple linear regression analyses after log transformation of PCT values.”

8. Authors state (abstract) that significantly higher PCT values were found in infants requiring resuscitation at birth (i.e. birth asphyxia) versus those who did not require it, and in infants whose mothers had a history of clinical chorioamnionitis versus those whose mothers were without such history (Table 3). Was the analysis confined to babies with infection as well as to babies without infection?

Response: These findings are confined to asymptomatic infants (group 1), as commented in methods, results, and discussion. We have revised the abstract to also add the word “asymptomatic” in the results.

Was PCT response independently associated with birth asphyxia at 12-24 h as well as at 36-48 h of life? Was PCT response independently associated with clinical chorioamnionitis at birth as well as at 12-24 h of life?

Response: Yes, there were independently associated.

Were the PCT increases associated with these variables smaller or greater than those observed for the presence of infection?

Response: It is not possible to quantify the magnitude of the association due to limitations of multiple regression analysis, as commented in point 6.

9. Page 4, line 14: only reference #6 is appropriate.

Response: We disagree. Reference #5 (Polin RA, The “ins and outs” of neonatal sepsis) is an editorial that talks about limitations of diagnostic markers for neonatal sepsis.

a. P 4, lines 20-21: Authors state that the results of recent studies suggest the usefulness of PCT for early diagnosis of neonatal sepsis, quoting references #9 to #24. Indeed, references #10, #11, #17 did not show any usefulness of PCT, and therefore should be deleted.

b. P 4, line 16: only reference #8 is appropriate.

c. The sentence (P 4, lines 21-22) “there are conflicting data regarding markedly increased concentrations” should read “there are conflicting data regarding what constitutes an abnormal value”.

Response to a, b, and c: We have made some changes to adjust references. Reference #7 has been suppressed. The last paragraph in page 4 now reads:

“The results of recent studies suggest the usefulness of PCT for early diagnosis of neonatal sepsis[9-21], although other investigators have observed lack of accuracy for this marker[22-24]. Also, since markedly increased concentrations during the first 48 h of life in newborn infants without bacterial infection have been reported[10,11,25], there are conflicting data regarding what constitutes an abnormal value.”

d. As the Authors mention the PCT “physiologic” peak (P 9, lines 22-24), references #25, #26, and #27 are inappropriate, and should be deleted.

Response: Reference #25 (Sachse et al. Clin Chem 1998, 44:1343-1344) included a group of “22 healthy neonates without antibiotic treatment”, reporting that serum procalcitonin always increased in the first 24 h after birth and always decreased in infants more than 36 h of age, and in three infants pronounced increases of procalcitonin concentrations (>5 mg/L) were observed in the first 36 h after birth. Reference #27 (Monneret et al. Acta Paediatr 1997, 86:209-212) included a subgroup of 32 “newborn infants free from neonatal pathology”, admitted for nursing during the first 3 days of life because of potential non infectious risk factors (prematurity, twin pregnancy, etc.). They report significant PCT values increase at day 1 (day 0:
0.45 ± 0.08, day 1: 3.82 ± 0.7, and day 3: 0.65 ± 0.12 mcg/L. We think both studies are cited appropriately as they include a group of healthy neonates. Reference #26 (Laporte et al. Arch Pediatr 1997, 4:915) reports the same fact in 150 non-infected neonates. It is an abstract and data included are not enough to know if they were healthy, asymptomatic or symptomatic infants with non infectious diseases (they had favourable outcome without antibiotics). We have finally decided to delete only this last reference.

e. P 10, lines 4-6. The sentence “This phenomenon might be attributed [...] including rapid bacterial colonization of the skin and mucosa” is useless, and should be deleted.  
**Response:** We have deleted these last words.

f. P 10, line 19. Reference #5 is useless, and should be deleted. Indeed, Authors should comment Monneret’s suggestions pertaining the role of hypoxemia for the increased PCT value by quoting and discussing the contents of the following references: Clin Chem. 2003; vol.49:60-68/ Clin Infect Dis 1998; 27:1560-61(letter:reply).  
**Response:** OK, now he have commented Monneret’s suggestions in detail quoting your letter reply. The paragraph now reads:

…”Yet it is not possible to assure these patients were really uninfected, since evidence of no infection was based on negative bacterial cultures, absence of fever, and a normal CRP value, and these criteria may fail in an appreciable number of cases[quote]. Our study has a similar problem, as intrapartum antibiotics had been used in 22/79 neonates with respiratory disorders, and 66/79 were treated with antibiotics after birth…”

**Reviewer: Greg Hodge**

**General**

*The questions posed by the authors is well defined. Although the method to measure PCT is appropriate, the study suffers from the lack of any comparison with other methods to detect neonatal sepsis such as CRP and haematological indices.*

*Another limitation of the study was also pointed out by the authors is that the control group was asymptomatic infants without evidence of infection, which may lead to an over-estimation of the reliability of PCT as a diagnostic test.*

*The authors found that increased serum PCT levels was not specific for neonatal sepsis but related to different factors such as respiratory stress.*

*Notwithstanding the above limitations, the authors do show a moderate diagnostic value for detection of sepsis of verticle transmission by using operator defined cut-off values for PCT.*

*There is also incomplete references to recent research into new methods to define neonatal sepsis such as measurement of multiple plasma cytokines (using cytometric bead array technology) and measurement of leucocyte activation markers.*

**Response:** We have now included reference to that two methods (background, page 4 1st paragraph):

…”Several leucocyte indices and acute-phase protein levels have been evaluated for the diagnosis of sepsis, and more recently, measurement of multiple plasma cytokines [] and leukocyte activation markers [] have showed promising results…”

**Reviewer: Daynia Elizabeth E Ballot**

- *The question posed by the authors is not completely original, but is current and still under*
evaluation. I therefore feel that their contribution is valuable.

- The following points regarding subjects and methods need to be clarified:
  
  o How was group 1 selected? Were these normal neonates selected as controls? Were they admitted for other reasons, if so, what? Why was a sepsis evaluation done in this group of well infants without clinical signs of sepsis?
    Response: Group 1 subjects were admitted to nursery because of prematurity, low birth weight, or risk factors for sepsis. These newborn infants were not defined as “healthy”, but “asymptomatic”, and their care included a sepsis evaluation. We have clarified group 1 definition (page 5-6), that now reads:

  “The first population (group 1) included a group of asymptomatic newborn infants admitted during the first 24 h of life to the neonatal unit because of prematurity, low birth weight, or at least two risk factors for infection (table 1). They had no clinical signs of sepsis during their first week of life and had a negative blood culture. Patients in this group did not receive antibiotic treatment. Those who were discharged home before the seventh day of live were followed to assure they did not develop late-onset vertical sepsis.”

  o What was the total number of deliveries during the study period?
    Response: Although it would be possible to retrieve the data, we did not include it in the study. It should be noted that our study population was a convenience sample (the main investigator at each hospital had to be present to prospectively record the data), so cases of sepsis or suspicion of sepsis included in the study were only part of the cases that happened for that period of time. So it was not possible to calculate real incidence of sepsis.

  o What is the “neonatal unit” as opposed to the “neonatal intensive care unit”?
    Response: We consider neonatal intensive care units those that supply complete monitorization (including electrocardiographic one) and possibility of mechanical ventilation. It does not mean that every neonates in groups 2A, 2B, or 2C required intensive care, but it was available if necessary. In order to avoid confusions we finally have change the term “neonatal intensive care unit” for “neonatal unit” at page 6.

  o Were all infants in the study term babies?
    Response: No, as seen in table 2 (mean gestational age and standard deviation) there were preterm and term babies in all the groups.

  o In group 2A, it is unclear whether all babies born to GBS mothers were included or only those GBS exposed babies who were symptomatic or with positive surface cultures.
    Response: All neonates in groups 2A, 2B, and 2C were symptomatic. We have rewritten group 2A definition in order to make it clearer:

    “Group 2A: Confirmed vertical neonatal sepsis, defined as at least three clinical signs of infection in association with at least one bacteriological evidence of infection. Evidence of infection included positive blood culture, at least three positive surface swabs in the first 24 h of life for traditional pathogens of vertical transmission –such as group B streptococci (GBS) and Escherichia coli–, or GBS culture-positive mother.”

  o I am unclear why group 2C infants were excluded and not just part of 2B, as respiratory symptoms may be due to sepsis. Is this related to the comments in the discussion that hypoxia due to RDS can cause a rise in PCT? Were other infectious markers such as CRP negative in this group?
    Response: Group 2C infants had clinical or radiological evidence of respiratory diseases
(hyaline membrane disease, transient respiratory distress, meconium aspiration or pneumothorax). CRP could be either positive or negative. Despite negative cultures, it is possible some of them were also infected, and so they were excluded from the diagnosis efficacy analysis. Group 2B had clinical signs and laboratory findings suggestive of infection, not explained by other causes, so it is probable most of them were really infected and we decided to consider them as sepsis for the diagnosis efficacy analysis.

- **What about possible contamination where a completely asymptomatic infant with no markers of infection has a positive blood culture?**
  **Response:** In that case the infant would be excluded of group 1 (and of the study). The study was not designed to address the problem of neonatal asymptomatic bacteraemia.

- **How many parents refused consent for inclusion in the study?**
  **Response:** Unfortunately, we did not record that data.

- **Page 7 line 17: anaysis should be analysis**
  **Response:** Corrected.

- **Page 7 line19: Why were these particular perinatal risk factors chosen? (presumably as there is an increased risk of infection). Why were other risk factors e.g. maternal pyrexia, maternal UTI, foul smelling baby not included?**
  **Response:** We selected variables that might have influence over stress response in the fetus. Just two variables were major risk factors for infection (chorioamnionitis and membrane rupture > 18 h), since choosing more risk factors may cause problems of collinearity in multiple linear regression analysis.

- **Page 9 line 12. Need to explain why group 2C was excluded from the ROC analysis?**
  **Response:** Now we have explained it in the discussion (page 10):

  “…Yet it is not possible to assure these patients were really uninfected, since evidence of no infection was based on negative bacterial cultures, absence of fever, and a normal CRP value, and these criteria may fail in an appreciable number of cases. Our study has a similar problem, as intrapartum antibiotics had been used in 22/79 neonates with respiratory disorders, and 66/79 were treated with antibiotics after birth. This is also the reason why we excluded group 2C from ROC curves.”

- **Throughout the paper, there should be a single space before the square bracket enclosing a reference number.**
  **Response:** Corrected.

Thanks again for your comments that help us to improve the manuscript. We would be pleased to respond if any further response were needed regarding this manuscript.

The Editorial Team ask us for indicating whether informed consent was written or verbal in the manuscript. We have now indicated that it was written informed consent in the Methods section (page 5, 1st paragraph):

”The study was approved by the Ethics Committees of the participating hospitals and the parents gave their written informed consent.”

Sincerely,

José B López Sastre
David Pérez Solís

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