Author's response to reviews

Title: Characteristics of neonates with culture-proven bloodstream infection who have low levels of C-reactive protein (<10 mg/L)

Authors:

Mei-Yin Lai (lmi818@msn.com)
Ming-Horng Tsai (mingmin.tw@yahoo.com.tw)
Ming-Chou Chiang (cmc123@cgmh.org.tw)
Reyin Lien (reynl@cgmh.org.tw)
Ren-Huei Fu (rkenny@cgmh.org.tw)
Hsuan-Rong Huang (gbonbon@cgmh.org.tw)
Shih-Ming Chu (kz6479@cgmh.org.tw)
Jen-Fu Hsu (jeff0724@gmail.com)

Version: 3 Date: 29 May 2015

Author's response to reviews: see over
RE: MS# 5077672091640319
Title: Characteristics of neonates with culture-proven bloodstream infection who have low levels of C-reactive protein (≤10 mg/L)

Dear Editor,

Thank you for your appreciated comments on our manuscript. We had the manuscript revised, mostly according to the reviewers’ suggestions. We will respond in detail to the reviewers’ comments. The replies for the reviewers’ criticisms are as followings. We hope this revised version can be acceptable.

Best regards,

Jen-Fu Hsu, MD and Shih-Ming Chu, MD
Chief, Division of Pediatric Neonatology, Department of Pediatrics
Chang Gung Memorial Hospital at Linkou
Kweishan, Taoyuan, Taiwan

Reviewer #1’s report:
I read with great interest the referenced manuscript. Kindly consider the following comments for corrections or rebuttal:
- Major Compulsory Revisions:
1. Under CONCLUSION - can the conclusion simply answer the goal of the study i.e. to characterize neonates with culture proven BSI and CRP level ≤10 mg/L?

Reply:
Thank you for your instructive advice. I think the reviewer suggested to revise the conclusion of the abstract. I will revise it as “A considerable proportion of neonatal BSIs had a normal or low initial CRP level (<10 mg/L), which tended to occur in low birth weight or extremely preterm infants, earlier onset of sepsis, and those infected with CoNS.”, which characterize neonates with culture proven BSI and CRP level ≤10 mg/L. However, I suggested to keep the statement “Plasma CRP level should not be used to rule out severe culture-proven sepsis or guide the empirical choice of antibiotics.” because we had the statement: “Of the BSIs with initial low CRP, 29.1% were treated with inadequate antibiotics, 13.0% progressed to septic shock, and 5.3% had infectious complications.” in the result section of the abstract, thank you.
- Minor Essential Revisions:

1. Under PATIENTS AND METHODS, 'Definitions' section - you may want to place spp (species) after Corynebacterium, Propionibacterium, Penicillium

   Reply:
   
   Thank you for your instructive advice. I will place spp. after Corynebacterium, Propionibacterium, and Penicillium.

2. Under PATIENTS AND METHODS, 'Definitions' section - consider placing the microbiology assay system utilized in your hospital e.g. Vitek system, MALDI –TOF

   Reply:
   
   Thank you for your instructive advice. The microbiology assay system utilized in my hospital is MALDI-TOF (Bruker’s flagship FLEX series). I will add this information in the Patients and methods section, thank you. (Page 6, last paragraph, in the definition section).

3. Under PATIENTS AND METHODS, 'Data on CRP' section - What's the limit of detection of CRP in your hospital? its coefficient of variation? If available

   Reply:
   
   Thank you for your instructive advice. The limit of detection of CRP in my hospital is > 0.5 mg/dL. If the value is less than 0.5 mg/dL, it is undetectable. There is no upper limit of the CRP value in our hospital. The coefficient of variation is not available. I will add this information in the “Patients and methods” “Data on CRP” section: The low limit of detection of CRP in our hospital is > 0.5 mg/L, and there is no upper limit of CRP. (Page 8, the last three lines).

4. Under PATIENTS AND METHODS, 'Data on CRP' section, last sentence of the last paragraph - There is a typographical error on 'intermediate (CRP 21 -100 mg/L), this should be CRP 11 - 100 mg/L

   Reply:
   
   Thank you for your instructive advice. I will correct it accordingly, thank you.

5. Under PATIENTS AND METHODS, 'Statistical analyses' section - I will leave it up to the journal's designated statistician the review of the appropriate statistical analyses utilized in this manuscript

   Reply:
Thank you for your instructive advice. I have confidence regarding the statistical analyses of this study because I have professional statistical consultation from Dr. Chun-Chun Cheng and Dr. Yu-Jr Lin (Please see Acknowledgement section, thank you).

6. Under RESULTS section - Are there data available in your patients regarding the time CRP was obtained in reference to when clinical sepsis was recognized since the kinetics of CRP suggest it starts increasing by 6 hours and peaks at 48 hours (see INTRODUCTION section) suggesting that if CRP level was obtained too early it may be falsely low?

Reply:
Thank you for your question. In our NICU, all patients (mostly are premature infants or critically ill infants) are critically monitored, and septic work up will be done immediately after clinical septic signs and/or symptoms were observed. Actually the time CRP was obtained in reference to when clinical sepsis was recognized is not available currently. However, it is difficult to define the exact time point of clinical sepsis onset if the initial symptoms are non-specific or subtle. I think it is possible regarding the concern that CRP level was obtained too early and may be falsely low. I have mentioned in the limitation of the discussion section that “The timing of blood sampling for culture and CRP was also not uniform”, I will add another sentence: Furthermore, some CRP levels maybe falsely low because they have been obtained too early before starting to rise around 4-6 hours after sepsis onset. (Page 16, last paragraph, the last forth line).

7. Under RESULTS section - Are there data available in your patients regarding the liver synthetic function e.g. albumin since it may be possible that those premature infants with low CRP level do not have the substrate or with low hepatic reserve to produce CRP ab initio? Also, is it possible that these premature infants have no capacity to mount an immune response i.e. with low IL6 response affecting CRP level to be falsely low?

Reply:
Thank you for your question. The data of hepatic synthetic function (e.g. albumin) are not available in most patients for comparisons. I think it may be possible that those premature infants with low CRP level do not have the substrate or with low hepatic reserve to produce CRP, and low IL6 response with no capacity to mount an immune response is also possible. However, we cannot present these data in the result section because these data were not available in most patients.
8. Under RESULTS section - if the levels of albumin are available in a subset of infants in your study, would it be possible to correlate it to CRP level?

Reply:
Thank you for your question. I think it is not possible to correlate the albumin levels to CRP levels because most premature infants have a relatively low albumin level. Furthermore, the levels of albumin are mostly unavailable in the infants of our study.

9. Under RESULTS section, last paragraph before 'Microbiology' section – the episodes of BSI is significantly higher in the high CRP group versus low CRP group and not the intermediate CRP group?

Reply:
Thank you for your instructive advice. The episodes of BSI are significantly higher in the high CRP group versus low CRP group. If the low CRP group is compared with the intermediate CRP group in these items, the difference is not significant. I will address this issue as “These demographics, clinical features and laboratory findings were not significantly different between neonatal BSIs in the low and intermediate CRP groups by post hoc analyses (data not shown)” (Page 10, last two lines).

10. Under RESULTS, 'Treatment and outcomes' section - instead of stating 'birth body weight' you may want to consider instead 'birthweight'

Reply:
Thank you for your instructive advice. I will use “birthweight” to replace birth body weight, thank you.

11. Under DISCUSSION, 3rd paragraph - can you provide the reference to the last statement

Reply:
Thank you for your instructive advice. Actually this statement is based on our study result. However, I found one previous study also had a similar finding, so I will add reference no. 40 and revise the statement as “There were some cases without CRP response in our cohort who presented with a very fulminant course and hepatic failure, which was similar to a finding in a previous study [40].” accordingly, thank you.

12. Under DISCUSSION, 3rd paragraph - the last statement provide some
evidence why a subset of premature infants with culture proven BSI has falsely low CRP level

Reply:
Thank you for your instructive advice. This statement (now in the fourth paragraph of discussion) does provide some evidence why a subset of premature infants with culture proven BSI has falsely low CRP level, thank you. I will provide the reference (ref. no. 40), thank you.

13. Under DISCUSSION, 4th paragraph - scientific name of organisms should be italicized e.g. Pseudomonas spp.

Reply:
Thank you for your instructive advice. I will italicize these scientific names, thank you.

14. Under DISCUSSION, 4th paragraph - are there further identification of CoNS since some species e.g. S. lugdunensis behaves like S. aureus

Reply:
Thank you for your instructive advice. There are no further identification of CoNS. The reviewer mentioned S. lugdunensis behaves like S. aureus. However, S. lugdunensis is rarely seen in the blood culture of our NICU.

15. Under DISCUSSION, 6th paragraph - can you provide reference for the 3rd statement

Reply:
Thank you for your instructive advice. I will provide references (ref. no.41 and 42) for the 3rd statement, thank you.

16. Under Table 1 - can you define NSD/CS under the table's legend?; in the legend section kindly check the spelling 'presences'

Reply:
Thank you for your instructive advice. I will define NSD/CS in the legend of Table 1, and check the spelling “presences”, thank you.

17. Under Table 2 - kindly correct 'Coagulase-negative Staphylococcus aureus', you may want to drop 'aureus'; under the legend, no need to capitalize the species name e.g. Neisseria meningitides

Reply:
Thank you for your instructive advice. I will correct it to be Coagulase-negative
staphylococci (CoNS) and not capitalize the species name in the legend, thank you.

Reviewer #2's report:
General comment: interesting topic; since CRP has low sensitivity it might be more interesting to look in all blood culture samples and compare CRP in blood culture proven with non-blood culture proven sepsis
Reply:
Thank you for your review and appreciated comments.

Major compulsory revisions: difficult to read; not well structured; not always concise; need for more explanatory analyses (post hoc)
Reply:
Thank you for your instructive advice. I will make the flow improved and better structured. I will do the post hoc analyses and stated them in the result section (page 10, the last two lines and page 11, the first two lines).

TITLE: The research is not solely conducted on neonates with low CRP levels, it is a comparison of groups
Reply:
Thank you for your instructive advice. We compared three different CRP groups in order to characterize those with low CRP group. We characterize these neonates and address this issue in the abstract section (including the results and conclusions), thank you.

ABSTRACT
1. Results: ...tended to have; low CRP group; .....relatively more common: please give numbers
Reply:
Thank you for your instructive advice. I will add numbers on these statement in the result section of the abstract, thank you. (93.4%, 82.9%, 84.0%, and 90.6%, respectively) and (55.9%, \(p < 0.001\))

2. Results: In the methods, distinction is made for three groups, the results only reported on two of them, who are the high CRP group (is not defined in the abstract; abstract needs to be completely self-explanatory)
Reply:
Thank you for your instructive advice. First of all, I will add “three CRP groups
(low, ≤ 10 mg/L; intermediate, 11-100 mg/L; and high, > 100 mg/L)” in the method section of the abstract. Then the result section will be clearer. For the statement “In the low CRP group, patients had lower gestational age and birth weight, and an earlier occurrence of BSI. Patients with underlying gastrointestinal pathology, renal disorders, cholestasis, and pulmonary hypertension tended to have elevated CRP levels at the onset of sepsis”, they are the comparisons of all three CRP groups.

3. Conclusion: more caution in interpretation of the results; ..... to rule out severe sepsis....: add culture-proven sepsis, because this was your study population
Reply: Thank you for your instructive advice. I will add “culture-proven sepsis” in the conclusion section, thank you. Because there were still 29.1% of neonatal BSIs with initial low CRP treated with inadequate antibiotics, 13.0% progressed to septic shock, and 5.3% had infectious complications, so I have this interpretation of the result in the conclusion section.

METHODS
1. I miss a definition of clinical sepsis
Reply: Thank you for your instructive advice. I will add the definition of clinical sepsis in the method section. (Page 6, the last paragraph) “An episode of sepsis was defined if a patient had a positive blood culture treated with antibiotics therapy for 5 or more days or treated for a shorter period if the patient died, and the presence of at least two of the following clinical symptoms of sepsis: fever or hypothermia, hyper-or hypoglycemia, apnea or tachypnea, frequent desaturation with increased requirement of ventilator support, bradycardia and/or cyanosis, feeding intolerance, abdominal distension, seizure, decreased activity, skin mottling and hypotension [1,7,9].

2. I miss data on how many blood cultures are taken per year (any difference in indication practice during the study period?)
Reply: Thank you for your question. I am sorry that this information is not available. I think no difference in indication practice during the study period. Based on the patients in our NICU (around 100 patients admission per day), I think more than 1,000 blood cultures were taken per year.

3. Any specialist service(s) of this university hospital? (important to
know the study population)

Reply:

Thank you for your question. No specialist service(s) of this university hospital exists. I will add the following information in the patients and methods section to let the readers know the study population, thank you. “All babies under 34-35 weeks completed gestation, or birth weight less than 2kg, or > 5Kg, or those with any clinical signs of respiratory distress, cardiovascular, gastrointestinal or neurological problems requiring surgical or intensive treatment were eligible to admission in our NICU.” (Page 5, 2nd paragraph)

4. Time interval of CRP measurement: I think you need to reduce the maximum time interval between measurement and blood culture sampling or better include only CRP measurements preceding sampling; now, if I interpret well, CRP measurements are included closest to blood culture sampling on the same day of sampling, so could be 23h after sampling; CRP rises steadily or quickly already after 6h so including a time interval of 23h might introduce bias.

Reply:

Thank you for your instructive advice. In our NICU, all patients (mostly are premature infants or critically ill infants) are critically monitored, and septic work up will be done immediately after clinical septic signs and/or symptoms were observed. I can guarantee almost all CRP were taken at the same time as the blood culture sampling. There is no CRP data taken at a time interval of 23 hours after blood culture sampling. In some cases, the first blood sampling was not adequate and CRP was not done at the same day, then these cases were excluded (see data on CRP section, 2nd sentence: Episodes of BSI that did not have CRP measured on the date of BSI onset were excluded.)


Reply:

Thank you for your instructive advice. For cox regression modeling, the CRP level is not the independent predictor of final mortality. Besides, the difference in the sepsis-attributable mortality rate between those with CRP ≤10 mg/L and those with CRP of 11-100 mg/L was not significant (see result section, the last paragraph, page 12). Therefore, it is not necessary to use Cox regression modeling. I will add a Kaplan-Meier curve in Figure 1 and information in the treatment and outcomes of the result section (Page 12, first paragraph, the third line) “Neonates in the high CRP
group had a significantly higher sepsis-attributable mortality, compared with those in
the intermediate and low CRP group (p < 0.05 by log rank test).”

RESULTS
1. Highlight the most important results (the ones you discuss) because
you have too much overlap with Table 1 and Table 2.
Reply:

Thank you for your instructive advice. I will try my best to highlight the most
important results and avoid overlap with Table 1 and Table 2, thank you. However, I
rarely repeat the numbers or data in Table 1 and Table 2. It is important to highlight
the most important results.

2. Table 2: other subcategories of Gram-positive pathogens: CoNS; S.
Aureus; Group-D Strep; Group-B Strep; other Strep
Reply:

Thank you for your instructive advice. I will clarify the subcategories of
Gram-positive pathogens. However, there were only 3 other streptococcus
[Streptococcus pneumonia (1), and Group D streptococcus (2)], so I will revise them
as in the new Table 2, thank you.

3. I am interested in the group of neonates (n=82) with an increased CRP
response after antibiotic treatment: inappropriate treatment? Changes in
clinical presentation? Specify median CRP and ‘a few days after’
Reply:

Thank you for your question. Not all of them were inappropriate treatment or
changes in clinical presentation. Some of them were just a slower elevation of plasma
CRP and some of them did have increased severity of clinical presentation. I will
specify the median CRP and a few days after in the revised manuscript as (median
[IQR]: 35.0 mg/L [22.8-52.5 mg/L]) at a median of 2.8 days (range 1-7 days) in the
result section. (page 11, the last paragraph).

4. Last sentence subtopic microbiology: Among 96 episode of CoNS
HABSI... had CoNS in their blood cultures: CHECK!!! Also same
sentence: .... without a CRP response....: CHECK!!!, I think you mean
with a CRP response....
Reply:

Thank you for your instructive advice. I am sorry for the mistake that I had an
error. I revise it as Among the 96 episodes of neonatal BSIs with repeated CRP data
that still had a CRP level \( \leq 10 \text{ mg/L} \). 31 (32.3\%) had CoNS in their blood cultures. Because among 178 who had at least one more CRP measurement during the following days after BSI onset, 82 (46.1\%) developed a CRP response >10 mg/L. Then another 96 episodes were no CRP response.

5. I would like to see the data on regression analysis plus Kaplan-Meier curves and I think it is better to do a post hoc analysis with birth-weight subgroups

Reply:

Thank you for your instructive advice. I will add a Kaplan-Meier curve in Figure 1 and information in the treatment and outcomes of the result section (Page 12, first paragraph, the third line). For regression analysis, CRP is not the independent predictor of sepsis-attributable mortality. We did not conclude this issue, either. The most significant difference is between the low and the high CRP groups. The difference is not significant between the low and intermediate CRP groups. I will add the statement of this issue in the result section (Page 10, the last sentence): These clinical features and laboratory findings were not significantly different between neonatal BSIs in the low and intermediate CRP groups by post hoc analyses (data not shown).

Besides, the topic of this study is to focus on three different CRP groups, it seems unreasonable to do a post hoc analysis with birth-weight subgroups after I consulted my statistical consultants (Dr. Chun-Chun Cheng and Dr. Yu-Jr Lin).

DISCUSSION

Reorganize: start with mentioning your principal results; compare pathogen distribution with others; paragraph 2 is for introduction; flow needs to be improved; include discussion on post hoc analyses for better understanding of the results

Reply:

Thank you for your instructive advice. I will revise the discussion according to reviewer’s suggestion. Start with mentioning my principal results in the first paragraph. Comparisons with pathogen distribution in other studies are addressed in the second paragraph. The reviewer suggested paragraph 3 (previous paragraph 2) to be for introduction. Therefore, I will revise it with relation to our study result and add further implication: Therefore, taken together, using CRP level as guide for empirical choice of antibiotics or to rule out serious infections or withhold antibiotic therapy is not recommended.
I will try my best to make the flow improved in the revised manuscript. For post hoc analyses, most differences were between the high CRP and low CRP group, and there is no significant difference between low and intermediate CRP groups regarding to most parameters. There were no post hoc analyses in the result section. I will mention this issue in the discussion section (page 15, 5th paragraph of discussion, start from This study aimed to characterize the NICU patients with normal or low CRP levels at onset of sepsis and compared them with those with an elevated CRP level. We found the differences were most significant between neonatal BSIs in the low CRP group and those in the high CRP (> 100mg/L) group. The insignificant difference between the low CRP and the intermediate CRP groups by post hoc analyses (data not shown) implied that initial CRP level cannot be the sole predictor of final mortality.)

Discretionary Revisions

INTRODUCTION

1. Add more and the major publication on incidence of late-onset BSI: Verstraete et al. (2014); Stoll et al. (2002)
   Reply: Thank you for your instructive advice. I will add them as reference number 3 and number 4, thank you.

2. Difficult to read: e.g. Although some studies have demonstrated…..
   Reply: Thank you for your instructive advice. I will rephrase it as “Although some studies have demonstrated that at least two CRP levels, both \( \leq 10\text{mg/L} \) and obtained 24 hours apart, are needed to identify infants unlikely to be infected….”

3. Add appropriate references on predictors for late-onset BSI; recently, a meta-analysis on predictive signs for healthcare-associated sepsis is published in Pediatrics: Verstraete E, et al. (2015)
   Reply: Thank you for your instructive advice. I will add this paper “Verstraete E, et al. (2015) as reference number 15.

METHODS

1. Episode of BSI was defined according to clinical criteria: which clinical criteria? Rephrase first sentence
   Reply:
Thank you for your instructive advice. I will rephrase the first sentence as An episode of BSI was defined according to presence of clinical sepsis and identification of pathogens. (Page 6, definition section, first sentence). In the next paragraph, I will define the clinical sepsis as the reviewer also have suggested, thank you.

RESULTS
1. Table 1: add median (Q1-Q3) where appropriate
   Reply:
   Thank you for your instructive advice. I will add median (Q1-Q3) where appropriate in the Table 1, thank you.

DISCUSSION
1. p13 A high CRP level is predictably associated: what do you mean by predictably associated?
   Reply:
   Thank you for your instructive advice. To avoid confusion, I revise it as “We found a high CRP level (>100 mg/L) was significantly associated with more severe clinical symptoms and worse outcomes.” (Page 13, first paragraph, 2nd line).

2. More appropriate comparison with late-onset sepsis studies: search for Modi et al. (2009); Mahieu et al. (2000)
   Reply:
   Thank you for your instructive advice. I will add Mahieu et al. (2000) in the reference (ref. no. 37). However, I can’t find the Modi et al (2009) regarding late-onset sepsis study from pubmed, which can be related to our study aim. Do you mean “Modi N, Dore CJ, Sarawatula A, et al. A case definition for national and international neonatal bloodstream infection surveillance. Arch Dis Child Fetal Neonatal Ed. 2009;94:F8-12.” ?

Minor Essential Revisions
METHODS
1. For CoNS BSI….. blood culture positive for CoNS…: double info
   Reply:
   Thank you for your instructive advice. However, this sentence is definition for CoNS BSI and there is no double information.

2. Last sentence: intermediate group CRP 11 instead of 21
   Reply:
Thank you for your instructive advice. I will correct it accordingly, thank you.

RESULTS
Table 1: insert n (%) in the table for every characteristic group (e.g. perinatal history, n (%); Clinical septic symptoms, n (%))

Reply:
Thank you for your instructive advice. Please see the footnote of Table 1, there is a mark “All data were expressed as number (percentage %), unless indicated otherwise.”. Therefore, I suggest not to insert n (%) in the table for every characteristic group, otherwise it will become too crowded, thank you.