PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Necrotizing enterocolitis suspicion in newborns with duct-dependent congenital heart disease: prognosis and risk factor.
AUTHORS	El Louali, Fedoua Prom, Camille Myriem, Belghiti Alaoui Gran, Celia Fouilloux, Virginie Lenoir, Marien Ligi, Isabelle Ovaert, Caroline Michel, Fabrice

VERSION 1 – REVIEW

REVIEWER	Martin van der heide United Kingdom of Great Britain and Northern Ireland
REVIEW RETURNED	01-Feb-2024

GENERAL COMMENTS	In this interesting manuscript El Louali et al. tried to identify risk factors for necrotizing enterocolitis in patients with duct dependent congenital heart disease. They included 23 infants with NEC stage I-III and matched controls 1:1 based on gestational age, birth weight, antenatal or postnatal diagnosis and type of CHD. They found that a diastolic blood pressure lower than 30mmHg, was independently associated with NEC in newborns with duct-dependent CHD. I think the manuscript is about a very interesting subject. The authors have a clear research question and try to answer this question with several variables. However, I also have some major concerns. First of all, there are some methodological issues which are needed to be addressed such as the inclusion of suspected NEC (Bell stage I). Second, the authors study an impressive amount of variables. At the moment it is not clear why all these variables are studies. Third, fundamental information is lacking to reproduce this study. Especially information is lacking about how the authors collected the data of diastolic blood pressure and NIRS in the manuscript. Finally, I would suggest to critically read the manuscript again on the basis of grammar and formatting/layout. Below some comments about the manuscript: Abstract: - In the results section there are multiple typo's. "were significantly higher (p-0.035; p<0.0001; p<0.0001)" should be p=0.035 etc. - PAD is not a common abbreviation for diastolic blood pressure. I should suggest to use 'DBP'. Introduction: - Line 59 to 61 needs to be: Children with CHD, especially those with

	duct-dependent circulation, also have an increased risk of NEC [4, 6, 7-10] with an incidence and mortality up to 3.7 and 24.4%, respectively. Methods: - Infants with NEC Bell stage I are also included in the study. I would suggest to exclude those infants because Bell stage I is not considered as NEC but suspected of NEC. Did the authors use the final NEC stage? Which authors confirmed NEC diagnosis or was it obtained from the hospital records? - Line 83: when is the blood pressure measured? How is the blood pressure measured? Invasive or non-invasive? Why did the authors only look at the diastolic blood pressure and not at the MAP and systolic blood pressure? Did the authors only select the lowest diastolic blood pressure? If so, why? - Line 84: 'Lowest visceral NIRS value in intensive care (average of the 5 lowest visceral NIRS values)'. When was splanchnic NIRS measured? What were the epochs of the measurement? At what location was the sensor placed? Which device was used? Why did the authors only used the lowest value and not variability or the ratio between cerebral and splanchnic NIRS as this is also associated with NEC in multiple studies? - lines 71-76. I would suggest to present the numbers of infants included in the study in the results section. - line 77 and line 80: I would suggest to replace 'birth term' with 'gestational age'. - line 80: the formatting/layout of the methods section is different regarding the first and second part. - line 81: why do the authors present only minimum and maximum values of the variables? When are these values measured? - line 88: 'before development of NEC for NEC group and equivalent duration for each control in no NEC group'. Do the authors mean an equivalent postnatal age? Which days are used for this analysis? Did the authors looked at type of feeding (i.e. formula or mother's milk/donor milk)? - line 120: I suggest that the authors mention something about multiple testing. Results: - Table 2: Why did the authors present the whole population in Table 2? I would suggest to present both groups (NEC vs matched control). - line 144: Why did the authors use a threshold of <30 mmHg? I guess this is mentioned in the statistical analysis but it's not clear why they used a threshold. - line 144: the abbreviation DBP and DAP are both used. I should suggest to use DBP. - line 149: is this a different diastolic blood pressure measurement than mentioned previously in the results section? Inotropic support is a little vague statement. I should suggest to be precise and mentioned that a larger proportion of infants with NEC required epinephrine compared to matched controls. Same comment about fluid balance. Line 149: the authors mention that DBP, inotropic support and fluid balance is significantly different. Higher or lower? The authors should also present the data or mention the Table where the data is presented. - Table 3: the legenda should be translated to English. - Table 3: sodium is not mentioned in the methods section. Why is this relevant in regarding to risk factors for NEC in duct dependent

	CHD? Discussion: - line 176: the question remains when this was measured. Was this measured on day one after birth? Before surgery or after? Furthermore, in the methods section the authors describe that they used the lowest average of 3 consecutive DBP. In the discussion this is not mentioned. Is this the case? If so, the authors should elaborate about this. - line 185: again, when was this measured? Before surgery? 26% of data was missing. Is this 26% of the measurement or 26% of the included infants? What is already published about splanchnic NIRS and NEC? Is NEC in CHD infants different from preterm NEC? - line 188: the authors conclude that the case study of Stapleton et al. has the same findings as this study. However, they found a lower splanchnic oxygen saturation after NEC diagnosis which restored later on. Did the authors measured NIRS after NEC diagnosis? In that case NIRS would not be a risk factor for NEC. - line 191: after correcting in multivariate analysis epinephrine was not statically associated with NEC. Please explain why. Is this due a confounding effect of lower diastolic blood pressure? - line 194: please use the abbreviation PGE1 or epinephrine consistently. - line 204: same comment as with epinephrine regarding fluid balance. - line 215: same comment as with epinephrine and fluid balance regarding peritoneal dialysis. - the authors discuss the possible risk factors PGE1, fluid balance and PD. However, probably these variables are confounders of diastolic blood pressure which is showed in Table 4. Conclusion: Line 234: the authors state that amines is not associated with an increased occurrence of this complication. However, there are no results about amines in the paper. - I should suggest that the authors revise the conclusion based on the comments mentioned previously.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Martin van der heide
Comments to the Author

Q: First of all, there are some methodological issues which are needed to be addressed such as the inclusion of suspected NEC (Bell stage I).
A: We have indeed noted this important limitation. We decided to keep these patients because we realized that even Bell stage I led to a postponement of surgery or a lengthening of hospitalization, therefore having an impact. We have added this data to the discussion to enlighten the reader. Data are exposed in table 3.

Q: Second, the authors study an impressive amount of variables. At the moment it is not clear why all these variables are studies.
A: in fact, we studied many variables, some of which are not relevant. we tried to clean up the results for less confusion. for example we removed natremia which is certainly not relevant in these situations

Q: Third, fundamental information is lacking to reproduce this study. Especially information is lacking about how the authors collected the data of diastolic blood pressure and NIRS in the manuscript.
A: blood pressure is monitored invasively. The somatic (visceral) NIRS (NIRS, INVOS, Covidien, Ireland) sensor is positioned in the lumbar region. Input out pout in balance is recorded hourly and the balance sheet is calculated daily. all medications are also recorded.
Hourly monitoring of blood pressure and NIRS. The data is recorded on dedicated monitoring sheets which are archived in the patient file. We have added this information to the methodology.

Q: Finally, I would suggest to critically read the manuscript again on the basis of grammar and formatting/layout.
A: We review grammar and formatting/layout.

Abstract:
Q: In the results section there are multiple typo's. "were significantly higher (p-0.035; p<0.0001; p<0.0001)" should be p=0.035 etc.
A: We corrected it

Q: PAD is not a common abbreviation for diastolic blood pressure. I should suggest to use 'DBP'.
A: we corrected it

Introduction:
Q: Line 59 to 61 needs to be: Children with CHD, especially those with duct-dependent circulation, also have an increased risk of NEC [4, 6, 7-10] with an incidence and mortality up to 3.7 and 24.4%, respectively.
A: thank you for the suggestion. We corrected it

Methods:
Q: Infants with NEC Bell stage I are also included in the study. I would suggest to exclude those infants because Bell stage I is not considered as NEC but suspected of NEC. Did the authors use the final NEC stage? Which authors confirmed NEC diagnosis or was it obtained from the hospital records?
A: We have indeed noted this important limitation. We decided to keep these patients because we realized that even Bell stage I led to a postponement of surgery or a lengthening of hospitalization, therefore having an impact. Patients died even in this sub group. We have added this data to the discussion to enlighten the reader. Data are exposed in table3

Q: Line 83: when is the blood pressure measured? How is the blood pressure measured? Invasive or non-invasive? Why did the authors only look at the diastolic blood pressure and not at the MAP and systolic blood pressure? Did the authors only select the lowest diastolic blood pressure? If so, why?
A: blood pressure is monitored invasively. Hourly monitoring of blood pressure. The data is recorded on dedicated monitoring sheets which are archived in the patient file. We have added this information to the methodology. We did not collected systolic and mean blood pressure, as diastolic is more relevant in the presence of systemico to pulmonary shunt (as persistent arterial duct).

Q: Line 84: 'Lowest visceral NIRS value in intensive care (average of the 5 lowest visceral NIRS values)'. When was splanchnic NIRS measured? What were the epochs of the measurement? At what location was the sensor placed? Which device was used? Why did the authors only used the lowest value and not variability or the ratio between cerebral and splanchnic NIRS as this is also associated with NEC in multiple studies?
A: The somatic (visceral) NIRS (NIRS, INVOS, Covidien, Ireland) sensor is positioned in the lumbar region. Hourly monitoring of NIRS. The data is recorded on dedicated monitoring sheets which are archived in the patient file. We have added this information to the methodology. Other information related to NIRS are surely interesting, and need prospective assessment.

Q: lines 71-76. I would suggest to present the numbers of infants included in the study in the results section.
A: We added those information in results section. For methodological reasons we also let some information concerning matching in method section.

Q: line 77 and line 80: I would suggest to replace 'birth term' with 'gestational age'.
A: we corrected it

Q: line 80: the formatting/layout of the methods section is different regarding the first and second part.
A: we corrected it

Q: line 81: why do the authors present only minimum and maximum values of the variables? When are these values measured?
A: In our ICU, data are systematically collected hourly or more depending on the patient's condition and reported on the patient's monitoring sheets. These monitoring sheets were used to collect the most relevant data during ICU period.

Q: line 88: 'before development of NEC for NEC group and equivalent duration for each control in no NEC group'. Do the authors mean an equivalent postnatal age? Which days are used for this analysis? Did the authors looked at type of feeding (i.e. formula or mother's milk/donor milk)?
A: each patient had the same gestational age as their control. The sentence mean postnatal age. Breastfeeding is favored in our institution. The exact composition of milk was not available. But we had information concerning volume of feeding and fasting duration.

Q: line 120: I suggest that the authors mention something about multiple testing.
A: as we compare two group (NEC and No NEC), we used binary logistic regression to perform multivariate analysis. We mention these information in method section.

Q: Table 2: Why did the authors present the whole population in Table 2? I would suggest to present both groups (NEC vs matched control).
A: we corrected table 2, now labelled table 3
Q: line 144: Why did the authors use a threshold of <30 mmHg? I guess this is mentioned in the statistical analysis but it's not clear why they used a threshold.
A: Indeed, as mentioned in statistical analysis, the accuracy of different DBP min values for predicting the occurrence of NEC was assessed by calculating the areas under the curve using the Receiver Operator Characteristics (ROC) curves and the best cutoff value was defined as the one with the greatest sensitivity and specificity. The ROC curve is exposed figure 1.

Q: line 144: the abbreviation DBP and DAP are both used. I should suggest to use DBP.
A: we corrected it

Q: line 149: is this a different diastolic blood pressure measurement than mentioned previously in the results section? Inotropic support is a little vague statement. I should suggest to be precise and mentioned that a larger proportion of infants with NEC required epinephrine compared to matched controls. Same comment about fluid balance.
A: In NEC group, all data (inotropic support, DBP, fluid balance…) were collected for the period before occurrence of NEC. Those data were compared to control patient and allowed us to suggest that those factors contribute directly or indirectly to instable situation leading to NEC.

Q: Line 149: the authors mention that DBP, inotropic support and fluid balance is significantly different. Higher or lower? The authors should also present the data or mention the Table where the data is presented.
A: we added the information and table number for those data.

Q: Table 3: the legenda should be translated to English.
A: we corrected it

Q: Table 3: sodium is not mentioned in the methods section. Why is this relevant in regarding to risk factors for NEC in duct dependent CHD?
A: In fact, it's not relevant, we suppressed it

Discussion:
Q: line 176: the question remains when this was measured. Was this measured on day one after birth? Before surgery or after? Furthermore, in the methods section the authors describe that they used the lowest average of 3 consecutive DBP. In the discussion this is not mentioned. Is this the case? If so, the authors should elaborate about this.
A: It's the lowest consecutive Data before the NEC. The post-operative or pre-operative collection period depends on the occurrence of NEC (post or pre-operative).It's collected invasively. We added this information in the methods section

Q: line 185: again, when was this measured? Before surgery? 26% of data was missing. Is this 26% of the measurement or 26% of the included infants? What is already published about splanchnic NIRS and NEC? Is NEC in CHD infants different from preterm NEC?
A: It's the lowest consecutive Data before the NEC. The post-operative or pre-operative collection period depends on the occurrence of NEC (post or pre-operative). We added information concerning methodology in the methods section. Missing data correspond to periods when NIRS was not yet used.

Q: line 188: the authors conclude that the case study of Stapleton et al. has the same findings as this study. However, they found a lower splanchnic oxygen saturation after NEC diagnosis which restored later on. Did the authors measured NIRS after NEC diagnosis? In that case NIRS would not be a risk factor for NEC.
A: No, our data collection was stopped at NEC occurrence in NEC group, as our aim was to identify risk factors. Our sentence was more along the lines of the decline in NIRS before the NEC.

Q: line 191: after correcting in multivariate analysis epinephrine was not statically associated with NEC. Please explain why. Is this due a confounding effect of lower diastolic blood pressure?
A: Indeed, We think that the major risk factor is DBP and that the role of epinephrine is mediated by blood pressure.

Q: line 194: please use the abbreviation PGE1 or epinephrine consistently.
A: we corrected it and use Prostaglandin E& for PGE1 constantly, and epinephrine for adrenaline evenly

Q: line 204: same comment as with epinephrine regarding fluid balance.
A: we think that the low number of patients probably contributes to the erasure of these parameters. A mechanical action (intra abdominal pressure and tissue edema) seems the main mechanism of fluid balance and PD on the occurrence of NEC
Q: line 215: same comment as with epinephrine and fluid balance regarding peritoneal dialysis.
A: we think that the low number of patients probably contributes to the erasure of these parameters. a more mechanical action (intra abdominal pressure tissue edema) seems the main mechanism of action of fluid balance and PD on the occurrence of NEC.

Q: the authors discuss the possible risk factors PGE1, fluid balance and PD. However, probably these variables are confounders of diastolic blood pressure which is showed in Table 4.
A: it is indeed possible that these factors are confounders. We need prospective studies on a larger population.

VERSION 2 – REVIEW

REVIEWER	Martin van der heide United Kingdom of Great Britain and Northern Ireland
REVIEW RETURNED	28-May-2024

GENERAL COMMENTS	I thank the authors for the answers to my comments. I think the authors have matched the neonates perfectly according to the outcomes of NEC and blood pressure. The results are very intersting and in line with previous research. However, a major limitation of the study is that the authors also consider Bell's stage I as NEC. Bell's stage I can be a very different disease such as CPAP-belly, SIP, sepsis etc. The authors do mention that they do research into NEC but infact they study a group that is suspected of NEC. I do understand that it is not easy to enlarge the dataset with only infants with Bell's stage 2 and 3. Nevertheless, a third of the NEC cases have Bell's stage I. In my opinon, the authors can't state that they do research into NEC. After reviewing the manuscript I have some additional comments. Methods Lines 71-73. The authors included Bell's stage 1 to 3. As mentioned before, Bell's stage I is not the same as NEC. When the authors persist in incuding Bell's stage I, the title of the manuscript has to be rewritten. I would suggest the authors use 'suspected of NEC'. This has also be mentioned in the whole manuscript.

	Lines 90-96. Still not clear what is used for analysis regarding blood pressure and splanchnic NIRS. Authors state that 'input and output balance were hourly recorded and daily calculated'. It is not clear to me when the authors measure these values. Do they us a mean value of an hour or a mean value of the whole day? Values of blood pressure and splanchnic NIRS can vary widely during the first weeks after birth. Are these values measured on the same day/hour in both NEC and controls? Results Table 1. Percentages are not provided. Line 151: group 2 refers to control infants? Line 165-167: what are the odds of developing NEC when DBP min ≤ 30mmHg? How many days before NEC diagnosis was diastolic blood pressure measured? Discussion The authors state that they discuss the inclusion of Bell's stage 1. I can't find this additional information. The authors eleborate about inotropic support, fluid balance, and peritoneal dialysis and relation to NEC. However, in multivariate analysis this was not associated with NEC. I would suggest that the authors eleborate more about diastolic blood pressure and the association with NEC. Why do infants with NEC or suspected of NEC have a lower DBP before and also after surgery? Is there a difference between these groups? Lines 207-210: the authors state that the low diastolic blood pressure may induce ischemia. Why is lactate not different in cases and controls? What about pH? Conclusion Again, the authors state that management of inotropic support and a better control of the fluid balance, including peritoneal dialysis may be important tools to reduce the risk of NEC. However, these variables were not associated with NEC in multivariate analysis.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Comments to the Author

I thank the authors for the answers to my comments. I think the authors have matched the neonates perfectly according to the outcomes of NEC and blood pressure. The results are very intersting and in line with previous research. However, a major limitation of the study is that the authors also consider Bell's stage I as NEC. Bell's stage I can be a very different disease such as CPAP-belly, SIP, sepsis etc. The authors do mention that they do research into NEC but infact they study a group that is suspected of NEC. I do understand that it is not easy to enlarge the dataset with only infants with Bell's stage 2 and 3. Nevertheless, a third of the NEC cases have Bell's stage I. In my opinon, the authors can't state that they do research into NEC.

Response:

Dear Reviewer,

Thank you for your review, We agree with you concerning relevance of Bell's stage I. We modified the paper in order to specify that it concern patients suspected of NEC.

Methods
Q: Lines 71-73. The authors included Bell's stage 1 to 3. As mentioned before, Bell's stage I is not the same as NEC. When the authors persist in incuding Bell's stage I, the title of the manuscript has to be rewritten. I would suggest the authors use 'suspected of NEC'. This has also be mentioned in the whole manuscript.
Response: the manuscript is modified as recommended.

Q: Lines 90-96. Still not clear what is used for analysis regarding blood pressure and splanchnic NIRS. Authors state that 'input and output balance were hourly recorded and daily calculated'. It is not clear to me when the authors measure these values. Do they us a mean value of an hour or a mean value of the whole day? Values of blood pressure and splanchnic NIRS can vary widely during the first weeks after birth. Are these values measured on the same day/hour in both NEC and controls?
Response:
Concerning the input and output balance, we use an intake-output chart implemented hourly and the calculation is performed daily.
For splanchnic NIRS and blood pressure, we used the lowest consecutive values before occurrence of suspected NEC. Similarly, in the control group, we used the lowest values, knowing that the investigation had been continued until the equivalent of the day of occurrence of NEC for their respective case-twin.

Results
Q: Table 1. Percentages are not provided.
Response: we added percentages, thank you

Q: Line 151: group 2 refers to control infants?
Response: Yes, we corrected it, thank you

Q: Line 165-167: what are the odds of developing NEC when DBP min ≤ 30mmHg?
Response: OR is 8.7 and CI 1.4-53.5

Q: How many days before NEC diagnosis was diastolic blood pressure measured?
Response: the lowest values were recorded 48 to 72 hours before the suspicion of NEC.

Discussion
Q: The authors state that they discuss the inclusion of Bell's stage 1. I can't find this additional information.
Response: we added paragraph in limitations of study section

Q: The authors eleborate about inotropic support, fluid balance, and peritoneal dialysis and relation to NEC. However, in multivariate analysis this was not associated with NEC. I would suggest that the authors eleborate more about diastolic blood pressure and the association with NEC. Why do infants with NEC or suspected of NEC have a lower DBP before and also after surgery? Is there a difference between these groups?
Response: Even if those factor were not significant in multivariate analysis, there were significant in univariate analysis. Due to our small size of our population, it seems extremely difficult to not consider them as important factor even if not risk factor.
Sub group analysis of NEC group found no difference between pre-operative (n=7) and postoperative NEC (n=12) concerning DBP (24.8+/-3.6 vs 26.8+/-4.1 mmHg respectively, p=0.432). we added this information.

Q: Lines 207-210: the authors state that the low diastolic blood pressure may induce ischemia. Why is lactate not different in cases and controls? What about pH?
Response: Indeed, there was no difference concerning lactate and pH, but those parameters was extremely abnormal in both population testifying to the seriousness of all these patients. That's why it seems interesting to find other factor simplifying prediction of NEC.

Conclusion
Q: Again, the authors state that management of inotropic support and a better control of the fluid balance, including peritoneal dialysis may be important tools to reduce the risk of NEC. However, these variables were not associated with NEC in multivariate analysis.
Response: Given the small size of the sample and the fact that these parameters were still significant in univariate analysis, we considered that it was entirely relevant to treat them as important. In our modified conclusion, we specify that DBP is risk factor while the other factors seems to be important tools to reduce this risk.