## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Platelet Transfusion for Neonates with Thrombocytopaenia: Protocol for a Systematic Review |
| **AUTHORS** | Liu, Dengjun; Wu, Jinlin; Xiong, tao; Yue, Yan; Tang, Jun |

## VERSION 1 – REVIEW

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| **REVIEWER** | Mangesh Deshmukh<br>Fiona Stanley Hospital, Perth, Western Australia |
| **REVIEW RETURNED** | 11-May-2020 |

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| **GENERAL COMMENTS** | This is a protocol for systematic review and possible meta-analysis of RCts and cohort studies on platelet transfusion in neonates with thrombocytopenia. The study should be of some interest to health professionals as it will provide additional information in this field. The manuscript is well written and organised. The methodology is satisfactory. However, I have some comments:<br><br>Objective section: It should be specific. At the moment part of introduction is repeated in it. The extra information should go in the introduction section.<br><br>As per the PRISMA statement for reporting systematic review, publication bias should be assessed by funnel plot/statistical test. This information is missing. Publication bias is critical to assess quality of evidence as per GRADE. |

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| **REVIEWER** | Susanna Fustolo-Gunnink<br>Sanquin Blood Supply Foundation, Center for Clinical Transfusion Research, Amsterdam, the Netherlands |
| **REVIEW RETURNED** | 08-Jun-2020 |

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| **GENERAL COMMENTS** | Esteemed authors,<br>Thank you for submitting your manuscript with a protocol for a systematic review on platelet transfusions in neonates. This is an important and underresearched topic that deserves to be addressed. In general, I believe your protocol offers a comprehensive and in depth approach to answer the research question. In particular, your choice of multiple clinically relevant outcome measures makes this an interesting undertaking, because to my understanding, this has not been done before. I do have some suggestions that could perhaps improve the review, and have listed them below.<br>Firstly, your statement that this is the first review addressing this issue is not completely correct. In reference 28 you have identified a recent systematic review on platelet transfusions in neonates, of which I happen to be a co-author. We assessed whether platelet |

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| | transfusions reduce risk of major bleeding in preterm neonates. In addition, dr. Lani Lieberman et al published a structured review on platelet transfusions in critically ill patients, in which neonates were also included. (10.1182/blood-2013-02-435693) Perhaps you could clarify the additional value of this review in comparison to the existing reviews? For example, you will include more than one outcome measure, perform more extensive and formal risk of bias analyses, and your search is more recent. It would be good to explicitly mention these items in the manuscript so the reader is able to understand how your review is complementary to the existing ones.<br>Secondly, could you clarify why you have chosen to include cohort studies but not case control studies? In my opinion, a well performed case cohort study can be more informative than a mediocre cohort study. If possible, I would suggest to include both, or at least explain why you have chosen for this approach.<br>Thirdly, the protocol would benefit from a bit more detail on outcome measures. For example, how will you define intraventricular hemorrhage or frank rectal bleeding, NEC or BPD? How do you plan to determine the minimum length of follow up required to assess each of these outcome measures?<br>Fourthly, I would highly recommend to pilot the data extraction form. It looks like it is tailored towards randomized controlled trials, but there might be issues for cohort studies (for example, with >2 groups), or case control studies. Perhaps you could develop data extraction forms for different types of studies? This may save you a lot of time during the data extraction process and will make it easier to summarize the data. There are also additional issues that could arise during data extraction that could be addressed beforehand, such as what to do when studies include neonates with postnatal age < and > 28 days? Or what to do when studies use different definitions of in hospital mortality? And will you present both adjusted and unadjusted results?<br>Fifthtly, in our review (reference 28) we discovered that temporal ambiguity was a major issue when assessing neonatal transfusion studies. We excluded the majority of papers (including two RCTs) based on this criterion. Temporal ambiguity is present when it is unclear from a paper whether the outcome occurred (or was measured) before or after the exposure. For example, whether the baby developed bleeding before or after the transfusion was administered. Since intraventricular bleeding is often asymptomatic and detected during screening, there is a time period in between a normal cerebral ultrasound and an ultrasound with bleeding, where we do not know exactly when the bleed occurred. If a transfusion is given within that time period, this will lead to temporal ambiguity. Or sometimes bleeding status at study entry is unknown, because the baby is enrolled soon after birth, and no scan has been made yet. Or both prophylactic and therapeutic transfusions are included. Temporal ambiguity is an important limitation, though it is not easy to assess, there are no formal tools to do this. I believe your review would benefit from an explicit assessment of temporal ambiguity as part of your decision on whether to include or exclude a paper. It would be interesting to see these results in comparison to our review, to see whether we come to the same conclusions for overlapping papers.<br>Sixthly, I am not a search strategy expert, but the search strategy looks like it could be improved by using additional terms (such as NICU or prematurity) but also features such as tiab, MESH terms, * symbols etc. If you have the possibility to ask a search expert or librarian to assist you in further developing your search, this would |

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| | help improve the quality (and potentially also reduce the number of papers you will need to screen).<br>I have limited statistical experience, so I have not been able to fully assess the statistical analysis plans. I could not identify from the author list whether a statistician is involved. If not, perhaps this would be an asset in case the authors will perform a meta analysis?<br><br>Some additional minor issues:<br>- In the abstract, could you specify that it is unclear whether thrombocytopenia increases bleeding risk for neonates? The same applies to your background section, in which you state 'Theoretically, neonates with thrombocytopenia may develop a high risk of bleeding and mortality.' This is true, but the opposite may also be true, and it would help the reader understand the complexity of this problem if you mention both views.<br>- In your background section you mention that thrombocytopenia can be a sole manifestation. This sentence is not completely clear to me. In my experience, thrombocytopenia always has a cause, though sometimes it is difficult to identify it. Solitary thrombocytopenia in an otherwise stable neonate is often related to immune mediated processses, such as FNAIT. Perhaps you could rephrase or clarify this sentence?<br>- In your background section you do not mention IUGR /SGA as an important cause of thrombocytopenia. Why not?<br>- In the methods section you state: 'Comprehensive searches will be performed by two researchers independently'. This implies that you will perform two searches, but I assume you will perform one and have two researchers screen the results independently? Perhaps you could clarify?<br>- In the data synthesis section you mention you will use either RR or OR for dichotomous data. Both have advantages and disadvantages, perhaps you can prespecify which effect estimate you will use for which outcomes and why?<br>- In your discussion, you state 'We will include RCTs and observational cohort studies in this review to strengthen the statistical power because of the limited number of relevant studies.' It is my conviction that in the absence of (many) randomized controlled trials, observational studies are a great source of potentially high quality data, and therefore should be included in systematic reviews. Plus observational studies sometimes have additional benefits over RCTs that could justify including them as well. At the same time, results of RCTs and observational studies cannot easily be combined, so including observational studies might not help with regards to statistical power. Perhaps you could elaborate a bit on your decision to include observational studies? |

**VERSION 1 – AUTHOR RESPONSE**

Reviewer: 1
Reviewer Name: Mangesh Deshmukh
Institution and Country: Fiona Stanley Hospital, Perth, Western Australia
Please state any competing interests or state 'None declared': None declared
Please leave your comments for the authors below
This is a protocol for systematic review and possible meta-analysis of RCts and cohort studies on

platelet transfusion in neonates with thrombocytopenia. The study should be of some interest to health professionals as it will provide additional information in this field. The manuscript is well written and organised. The methodology is satisfactory. However, I have some comments:

Question 1: Objective section: It should be specific. At the moment part of introduction is repeated in it. The extra information should go in the introduction section.
Response: Dr. Deshmukh, thank you very much for your suggestion. The extra information has been removed from the background section. Please see lines 113-115, page 6.

Question 2: As per the PRISMA statement for reporting systematic review, publication bias should be assessed by funnel plot/statistical test. This information is missing. Publication bias is critical to assess quality of evidence as per GRADE.
Response: We appreciate the reviewer's careful review. Publication bias will be assessed using funnel plots if more than 10 studies are included. Asymmetry may arise as a result of publication bias or of a relationship between the trial size and effect size. Egger's linear regression analysis will be performed to test for funnel plot asymmetry.
Please see lines 266-271, page 11.

Reviewer: 2
Reviewer Name: Susanna Fustolo-Gunnink
Institution and Country: Sanquin Blood Supply Foundation, Center for Clinical Transfusion Research, Amsterdam, the Netherlands
Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below
Esteemed authors,
Thank you for submitting your manuscript with a protocol for a systematic review on platelet transfusions in neonates. This is an important and underresearched topic that deserves to be addressed. In general, I believe your protocol offers a comprehensive and in depth approach to answer the research question. In particular, your choice of multiple clinically relevant outcome measures makes this an interesting undertaking, because to my understanding, this has not been done before. I do have some suggestions that could perhaps improve the review, and have listed them below.

Question 1: Firstly, your statement that this is the first review addressing this issue is not completely correct. In reference 28 you have identified a recent systematic review on platelet transfusions in neonates, of which I happen to be a co-author. We assessed whether platelet transfusions reduce risk of major bleeding in preterm neonates. In addition, dr. Lani Lieberman et al published a structured review on platelet transfusions in critically ill patients, in which neonates were also included. (10.1182/blood-2013-02-435693) Perhaps you could clarify the additional value of this review in comparison to the existing reviews? For example, you will include more than one outcome measure, perform more extensive and formal risk of bias analyses, and your search is more recent. It would be good to explicitly mention these items in the manuscript so the reader is able to understand how your review is complementary to the existing ones.
Response: Dr. Fustolo-Gunnink, thank you for your careful review. We agree with you. We have revised the "Strengths and limitations of this study" and Discussion sections. Please see lines 62-74, page 4 and lines 277-287, pages 11-12.

Question 2: Secondly, could you clarify why you have chosen to include cohort studies but not case control studies? In my opinion, a well performed case cohort study can be more informative than a mediocre cohort study. If possible, I would suggest to include both, or at least explain why you have

chosen for this approach.
Response: We have incorporated your valuable suggestion. The current protocol has included case control studies. Please see line 49, page 2 and line 156, page 7.

Question 3: Thirdly, the protocol would benefit from a bit more detail on outcome measures. For example, how will you define intraventricular hemorrhage or frank rectal bleeding, NEC or BPD? How do you plan to determine the minimum length of follow up required to assess each of these outcome measures?
Response: Thank you for your kind suggestions. We assumed that one outcome measure may have different definitions in different studies. We have provided the definitions of outcomes and the length of the follow-up period in the revised version with a newly added supplemental file "Supplemental Table 2. Definitions of outcome measures" to provide additional details in the protocol. Please see lines 177-179, page 8 and the supplemental file "Supplemental Table 2. Definitions of outcome measures".

Question 4: Fourthly, I would highly recommend to pilot the data extraction form. It looks like it is tailored towards randomized controlled trials, but there might be issues for cohort studies (for example, with >2 groups), or case control studies. Perhaps you could develop data extraction forms for different types of studies? This may save you a lot of time during the data extraction process and will make it easier to summarize the data.
Response: We appreciate the reviewer's careful review. We have developed separate data extraction forms for different types of studies. Three data extraction forms (for RCTs, cohort studies, and case control studies) have been designed according to the information and data needed for a meta-analysis. Please see line 193, page 9 and supplemental tables 3.1-3.3.

Question 5: There are also additional issues that could arise during data extraction that could be addressed beforehand, such as what to do when studies include neonates with postnatal age < and > 28 days?
Response: To our knowledge, the majority of infants with thrombocytopenia were diagnosed within 72 h after birth. Thus, the neonates included in these studies are very likely to have a postnatal age < 28 days. Because the aim of this study is to assess the evidence for PT among neonates with thrombocytopenia, we will only include the infants with an established diagnosis of thrombocytopenia within 28 postnatal days. The follow-up time may extend to a postnatal age > 28 days.
We also clarify this information in the revised version. Please see lines 162-164, pages 7-8.

Question 6: Or what to do when studies use different definitions of in hospital mortality? And will you present both adjusted and unadjusted results?
Response: We will record the different definitions and provide structured reporting of these results in the full review. If possible, we have considered conducting a subgroup analysis of neonates stratified according to the disease definition in the study (for example, death within 7 days of the hospital stay or death during the neonatal period).
If the studies provided both adjusted and unadjusted results, we will only present the adjusted results in the review. Please see lines 180-181, page 8.

Question 7: Fifthtly, in our review (reference 28) we discovered that temporal ambiguity was a major issue when assessing neonatal transfusion studies. We excluded the majority of papers (including two RCTs) based on this criterion. Temporal ambiguity is present when it is unclear from a paper whether the outcome occurred (or was measured) before or after the exposure. For example, whether the baby developed bleeding before or after the transfusion was administered. Since intraventricular bleeding is often asymptomatic and detected during screening, there is a time period in between a normal cerebral ultrasound and an ultrasound with bleeding, where we do not know exactly when the bleed occurred. If a transfusion is given within that time period, this will lead to temporal ambiguity. Or

sometimes bleeding status at study entry is unknown, because the baby is enrolled soon after birth, and no scan has been made yet. Or both prophylactic and therapeutic transfusions are included. Temporal ambiguity is an important limitation, though it is not easy to assess, there are no formal tools to do this. I believe your review would benefit from an explicit assessment of temporal ambiguity as part of your decision on whether to include or exclude a paper. It would be interesting to see these results in comparison to our review, to see whether we come to the same conclusions for overlapping papers.
Response: Thank you for your good suggestions. The temporal ambiguity was indeed a major issue. We will conduct a sensitivity analysis of the temporal ambiguity of the original studies to assess its effect on the outcome. The robust of the outcome will be assessed by including or excluding the studies with temporal ambiguity. Please see lines 246-249, page 10.

Question 8: Sixthly, I am not a search strategy expert, but the search strategy looks like it could be improved by using additional terms (such as NICU or prematurity) but also features such as tiab, MESH terms, * symbols etc. If you have the possibility to ask a search expert or librarian to assist you in further developing your search, this would help improve the quality (and potentially also reduce the number of papers you will need to screen). 1
I have limited statistical experience, so I have not been able to fully assess the statistical analysis plans. I could not identify from the author list whether a statistician is involved. If not, perhaps this would be an asset in case the authors will perform a meta analysis?
Response: A improved search strategy has been developed according to the suggestions you and a search strategy expert provided. Please see the file “Supplemental Table 1. Search strategy”.
Dr. Xiong is the corresponding author with statistical experience. He has published several systematic reviews describing the development of the search strategy.
Ref:
1. Xiong T, Maheshwari A, Neu J, et al. An Overview of Systematic Reviews of Randomized-Controlled Trials for Preventing Necrotizing Enterocolitis in Preterm Infants. Neonatology 2020;117(1):46-56.
2. Xiong T, Chen H, Luo R, et al. Hyperbaric oxygen therapy for people with autism spectrum disorder (ASD). The Cochrane database of systematic reviews 2016;10(10):Cd010922.
3. Qiu X, Xiong T, Su X, et al. Accumulate evidence for IP-10 in diagnosing pulmonary tuberculosis. BMC infectious diseases 2019;19(1):924.
4. Huang J, Zhang L, Tang J, et al. Human milk as a protective factor for bronchopulmonary dysplasia: a systematic review and meta-analysis. Archives of disease in childhood Fetal and neonatal edition 2019;104(2):F128-f36.

Question 9: Some additional minor issues:
- In the abstract, could you specify that it is unclear whether thrombocytopenia increases bleeding risk for neonates? The same applies to your background section, in which you state ‘Theoretically, neonates with thrombocytopenia may develop a high risk of bleeding and mortality.’ This is true, but the opposite may also be true, and it would help the reader understand the complexity of this problem if you mention both views.
Response: We have added more sentences to clarify both views: one view is based on theoretical speculation and the other view is supported by recent trials. Please see lines 86-88 and 93-94, page 5.

Question 10: In your background section you mention that thrombocytopenia can be a sole manifestation. This sentence is not completely clear to me. In my experience, thrombocytopenia always has a cause, though sometimes it is difficult to identify it. Solitary thrombocytopenia in an otherwise stable neonate is often related to immune mediated processses, such as FNAIT. Perhaps you could rephrase or clarify this sentence?
Response: We have rephrased this sentence according to your suggestion: “Thrombocytopenia may

be a sole clinical manifestation of alloimmune thrombocytopenia or a complication of other diseases, such as intrauterine growth restriction, polycythaemia, sepsis or necrotizing enterocolitis."
Please see lines 80-83, page 5.

Question 11: In your background section you do not mention IUGR /SGA as an important cause of thrombocytopenia. Why not?
Response: We have rephrased this sentence: "Thrombocytopenia may be a sole clinical manifestation of alloimmune thrombocytopenia or a complication of other diseases, such as intrauterine growth restriction, polycythaemia, sepsis or necrotizing enterocolitis."
Please see lines 80-83, page 5.

Question 12: In the methods section you state: 'Comprehensive searches will be performed by two researchers independently'. This implies that you will perform two searches, but I assume you will perform one and have two researchers screen the results independently? Perhaps you could clarify?
Response: We have clarified this sentence as follows: "Comprehensive searches will be separately performed by two independent researchers in the PubMed, Cochrane Library, and Embase databases from database inception through October 13, 2020." Please see lines 136-138, page 7.

Question 13: In the data synthesis section you mention you will use either RR or OR for dichotomous data. Both have advantages and disadvantages, perhaps you can prespecify which effect estimate you will use for which outcomes and why?
Response: Thank you for your suggestion. We will calculate the RR for dichotomous data in RCTs and cohort studies, as the RR provides a clearer interpretation of the clinical significance than the OR. In addition, we will calculate the OR for dichotomous data in case control studies. Please see lines 230-232, page 10.

Question 14: In your discussion, you state 'We will include RCTs and observational cohort studies in this review to strengthen the statistical power because of the limited number of relevant studies.' It is my conviction that in the absence of (many) randomized controlled trials, observational studies are a great source of potentially high quality data, and therefore should be included in systematic reviews. Plus observational studies sometimes have additional benefits over RCTs that could justify including them as well. At the same time, results of RCTs and observational studies cannot easily be combined, so including observational studies might not help with regards to statistical power. Perhaps you could elaborate a bit on your decision to include observational studies?
Response: Thank you very much for your comments. We agree with the benefits of including observational studies such as cohort studies and case control studies.
We have included observational studies as follows:
Due to the limited number of RCTs, observational studies are a great source of potentially high-quality data. Furthermore, observational studies have additional benefits that may justify the evidence obtained from RCTs as well. We will include RCTs and observational studies in this review because of the limited number of relevant RCTs examining neonates with thrombocytopenia. We will separately combine the results of RCTs and observational studies.
Please see lines 277-287, pages 11-12.

## VERSION 2 – REVIEW

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| **REVIEWER** | Susanna Fustolo-Gunnink<br>Sanquin Blood Supply Foundation, Center for Clinical Transfusion Research, Amsterdam, the Netherlands |
| **REVIEW RETURNED** | 28-Aug-2020 |

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| **GENERAL COMMENTS** | Dear authors, |

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| | thank you very much for revising this manuscript, I am happy with most of the revisions, but have a few remaining questions.<br><br>1) you have defined the duration of follow up to assess outcomes as at least 7 days, but to my understanding this will be enough for some, but not all outcomes. For example, it may not be enough to diagnose BPD. I realize that you will be looking at many different outcomes, and thus this will be an extensive review, but perhaps you could add specific minimum follow up durations to your definitions of the different outcome measures? That way you can avoid drawing less robust conclusions as a result of insufficient follow up.<br>2) with regards to your search strategy: to my understanding, using an asterix (*) indicates that all different possible endings of a word will be included. Therefore, including both 'thrombocyte adminstrat*' and 'thrombocyte administration' is redundant. If I am correct, this may help simplify your search.<br>3) you have included IVH grade I-IV, will you do a separate analysis including grade III-IV only, as these are generally considered severe bleeds?<br>4) in your data extraction form for case control studies you have defined the groups as exposed and non-exposed. However, cases are defined as those with an outcome, and controls as those without an outcome. I would recommend changing this in the data collection form.<br>5) your definition of PDA seems very broad, though I am not an expert in this area. Is there a more standardized definition available? The same applies to BPD (I think these definitions were recently updated?). And have you considered using the Bell score (or any other grading system) for NEC? Perhaps you could expand this section a bit, since accurate outcome definitions are crucial for the results of your study.<br><br>This is a remark for the editor: am I correct in assuming that the first version of the protocol in the proof is the clean version with track changes accepted, and the second is the version with track changes? If so, it seems that some of the changes have not been tracked. For example, in the first version, there was mention of this being the 1st systematic review to address this topic, which I have addressed in one of my comments, but this change is not visible in the document as it now seems that this was never stated. Not sure if this is important, but I thought I would document this here. |

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| **REVIEWER** | Mangesh Deshmukh<br>Fiona Stanley Hospital, Perth, Australia |
| **REVIEW RETURNED** | 03-Sep-2020 |

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| **GENERAL COMMENTS** | The reviewer completed the checklist but made no further comments. |

**VERSION 2 – AUTHOR RESPONSE**

Reviewer: 2
Reviewer Name: Susanna Fustolo-Gunnink
Institution and Country: Sanquin Blood Supply Foundation, Center for Clinical Transfusion Research, Amsterdam, the Netherlands

Competing interests: None declared.

Please leave your comments for the authors below
Dear authors,

thank you very much for revising this manuscript, I am happy with most of the revisions, but have a few remaining questions.

Question 1: You have defined the duration of follow up to assess outcomes as at least 7 days, but to my understanding this will be enough for some, but not all outcomes. For example, it may not be enough to diagnose BPD. I realize that you will be looking at many different outcomes, and thus this will be an extensive review, but perhaps you could add specific minimum follow up durations to your definitions of the different outcome measures? That way you can avoid drawing less robust conclusions as a result of insufficient follow up.
Response: Dr. Fustolo-Gunnink, thank you very much for your suggestion. We have clarified the minimum follow-up durations in the definitions of the different outcome measures, as follows:
The minimum length of follow-up for assessing these outcomes should include the time point for the diagnosis (for example, the follow-up for BPD should extend to 28 postnatal days). If a similar outcome measure had different follow-up times in different original studies, we will try to manage the data according to the timeline.
Please see lines 180-185, page 8, and supplemental file “Supplemental Table 2. Definitions of outcome measures”.

Question 2: With regards to your search strategy: to my understanding, using an asterix (*) indicates that all different possible endings of a word will be included. Therefore, including both 'thrombocyte adminstrat*' and 'thrombocyte administration' is redundant. If I am correct, this may help simplify your search.
Response: Thank you for your kind suggestion. We have simplified the search strategy. Please see the file “Supplemental Table 1. Search strategy”.

Question 3: You have included IVH grade I-IV, will you do a separate analysis including grade III-IV only, as these are generally considered severe bleeds?
Response: We agree with you. We have added the analysis:
If the data are sufficient, we will conduct additional analyses according to the severity of the outcomes (for example, severe PV–IVH (grade III or IV).
Please see lines 178-180, page 8.

Question 4: In your data extraction form for case control studies you have defined the groups as exposed and non-exposed. However, cases are defined as those with an outcome, and controls as those without an outcome. I would recommend changing this in the data collection form.
Response: We have adjusted the data extraction form for case-control studies according to your suggestion. Please see the file “Supplemental Table 3.3 Data extraction sheet for case control studies”.

Question 5: Your definition of PDA seems very broad, though I am not an expert in this area. Is there a more standardized definition available? The same applies to BPD (I think these definitions were recently updated?). And have you considered using the Bell score (or any other grading system) for NEC? Perhaps you could expand this section a bit, since accurate outcome definitions are crucial for the results of your study.
Response: Thank you very much for your suggestion.
The more standardized criteria is used for the definition of PDA.
Although there are updated definitions, BPD is defined by the National Institutes of Health (which is

the most commonly used definition).
We have considered using the Bell score (or any other grading system) for NEC.
We have added additional details about the definitions of outcome measures; please see the supplemental file “Supplemental Table 2. Definitions of outcome measures”.