Peer Review File
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Reviewer A

Comment 1: In general, well written and quite complete review with important information, but it has a little bit "textbook wise" set-up. This reviewer feels that some parts of the review can be shorter such as the section discussing clinical outcome in the era prior TH and the discussion about long-term outcome. Also, the mechanisms underlying HIE is quite lengthy.

Reply 1: We thank and agree with the reviewer. The paragraph entitled: "*Clinical Outcomes in the Era Prior to Therapeutic Hypothermia"* has been shortened and moved to a later part of the manuscript. We have replaced the text describing clinical studies with a new Table (Table 3). Additionally, the mechanisms section has been shortened and more recent findings on the tertiary phase of injury have been incorporated.

Changes in the text: Under the paragraph: "*Clinical Outcomes in the Era Prior to Therapeutic Hypothermia",* previously pages 5-7, lines 103-139, have been deleted. These paragraphs encompassed studies outcomes before the implementation of TH. These data have now been summarized in Table 3. This section has been moved to page 8, lines 168-186.

Comment 2: Although very briefly discussed, there is a clear tendency in American but also European centers to treat "mild" HIE neonates also with TH, despite the often (slightly) different used criteria (Sarnat, modified Sarnat scores, Thompson score). May be this can be mentioned briefly in this review.

Reply 2: Thank you, we have included more information on the use of TH in mild cases of HIE, and discussed ongoing work that are attempting to determine the benefits of TH in mild HIE.

Changes in the text: Previously page 4, line 77-79, revised and now page 4, lines 81-86.

Comment 3: I miss the differentiation in long-term/MRI outcome between the more acute HIE mostly leading to basal ganglia damage and subacute HIE, often related to watershed damage. This should be briefly addressed.

Reply 3: Thank you for the recommendation. We have included MRI patterns of injury in the acute and subacute phases, and the predictive value of MRI at 6-7-year outcomes.

Changes in the text: See changes on page 6, lines 118-131.

Comment 4: The review suggests that oxidative substances and NOS are especially produced during the pro-inflammatory phase, from about 6 hrs onward This is not entirely true: formation of free radicals such as super oxide, non-protein bound iron and (neuronal-NOS induced) NO are also early phenomena. The in this review mentioned current European allopurinol trial is designed

to prevent the production of these early potentially destructive compounds by iv Allopurinol on the resuscitation table before 30 min of life. May be interesting in this respect to mention the results of a fetal allopurinol trial in term fetuses with CTG-diagnosed fetal hypoxia (Kaandorp et al, Arch Dis Childh 2015).

Reply 4: Thank you, I have emphasized the formation of free radicals as part of the acute phase of injury. We appreciate the reviewer pointing us to this interesting and important study that we have now incorporated in our manuscript.

Changes in the text 1: Page 6, line 135.
Changes in the text 2: Starting on page 13, line 297, we have added the following sentences: "*Interestingly, a randomized, double-blind, placebo-controlled multicenter trial reported that maternal allopurinol treatment during suspected fetal hypoxia resulted in significantly lower S100β and neuroketal values, which serve as surrogate measures of brain damage, in the umbilical cord blood of female neonates exclusively. The authors concluded that perinatal maternal treatment with allopurinol may specifically benefit female neonates compared to their male counterparts.*".

Comment 5: The Umbilical cord cell section may be discussed after the pharmacologic part being (partly) a "repair" procedure.

Reply 5: We thank the reviewer for its comment. We have moved this section on umbilical cord blood after the pharmacologic agents, as suggested.

Changes in the text: We have moved the section on umbilical cord blood after the pharmacological agents. The section on umbilical cord blood now starts on page 17, line 373.

Reviewer B

Comment 1: The problem with TH is low-income setting is not just lack of access it may also be ineffective:

Thayyil S et al; HELIX consortium. Hypothermia for moderate or severe neonatal encephalopathy in low-income and middle-income countries (HELIX): a randomised controlled trial in India, Sri Lanka, and Bangladesh. Lancet Glob Health. 2021 Sep;9(9):e1273-e1285. doi: 10.1016/S2214-109X(21)00264-3. Epub 2021 Aug 3.

Reply 1: Thank you for making this insightful point. We have added this study to our introduction and includes a discussion on why TH may have not been effective.

Changes in the text: Page 4, lines: 87-93

Comment 2: Please reword the subtitle "Clinical Outcomes in the Post Therapeutic Hypothermia Era" as what is included is a narrative review of the original studies.

Reply 2: We agree with the reviewer and thank him for pointing this out. We have taken out the word "*Post*" in the subtitle.

Changes in the text: We have accordingly changed the subtitle to "*Clinical Outcomes in the Therapeutic Hypothermia Era*" on Page 10, line 226.

Comment 3: Please check references. There are some repeats: e.g. 45 and 66; 68 and 69

Reply 3: We apologize and have carefully reviewed our references and cleared up these errors. Thank you

Changes in the text: See references

Reviewer C

Comment 1: In the first 14 pages of this review, no new knowledge or understanding is provided. It reads like a history lesson that those in the field already know. It may be useful to clinicians and scientists new to the field. If this is one aim it needs to be stated.

Reply 1: We incorporated new studies earlier in the introduction of our manuscript, and restated out objective to be 1) to revisit the use of TH for HIE and its longitudinal impact on patient outcomes to readers new to the field of HIE, and 2) discuss how emerging therapies address a broader pathophysiology of injury progression in the neonatal brain hours to years after HIE. We have shortened the "historical" section of the manuscript and now summarize many prior findings in tabular form. Thank you.

Changes in the text: See Abstract and Introduction, Page 4, lines: 87-93 we included recent work from the HELIX trial from may 2023, and integrated more recent work thought the body. Also see new Table 3.

Comment 2: On p15 out of a total of 20 pages, a review of new knowledge obtained over the past year from phase 2, or beyond, clinical trials is indicated (lines 320-321).

Reply 2: Correct, and we have now added work from 2022-2023 in the revised manuscript as well.

Changes in the text: See previous comment

Comment 3: The new clinical study cited for EPO alone is reference 118, published in 2022 (lines 337-343). The treatment was not effective.

Reply 3: We agree with the reviewer. This study was indeed already reported in the first version of the manuscript but the results have now been emphasized for clarity. We also added a reference on the lack of effects on biomarkers of neuroinflammation or brain injury.

Changes in the text: Page 15, Lines 325-326

Comment 4: For umbilical cord blood cells, allupurinol, and melatonin, no 2022/2023 studies with results, when combined with hypothermia, are available to report on.

Reply 4: There have been a handful of relevant papers published after our prior submission that we have now added to our manuscript. Below is the list of papers published this year that have been added.

1. We have included the first preclinical multi drug randomized trial for HIE, which was just published in June 2023 (Sabir et al, Sci Rep. 2023 Jun)
2. There is a new pilot study, just published in June 2023, employing mesenchymal stromal cells obtained from allogenic umbilical cord (Cannavò et al, Pediatr Neurol. 2023 Jun)
3. A new paper published in June 2023 reviewed the care strategies known to improve neurological outcomes in neonates with HIE (Cotten et al, Stem Cells Transl Med. 2023 Jun)

Changes in the text: We have added the following sentences to our manuscript.

1. Starting on page 13, line 297: "*Interestingly, Sabir et al., just published the first preclinical multi-drug randomized controlled screening trial for nHIE. The authors investigated 25 potential therapeutic agents and reported caffeine and sonic hedgehog agonist to be the most promising, followed by allopurinol, melatonin, clemastine, ß-hydroxybutyrate, and omegaven.*"
2. Starting on page 13 line 294: "*Aside from adjunctive therapies, other approaches that have shown to improve outcomes in critically ill neonates with HIE are hypocapnia, hypoglycemia, pain control, and functional brain monitoring."*
3. Starting on page 18, line 392: However, the study was prematurely stopped after only 35 out of the planned 160 infants were randomized, due to slow enrollment and *"the logistical complexities of collecting from sick infants, processing, and infusing UCB within the first postnatal hours. The authors performed a pilot phase 1 trial utilizing cryopreserved allogenic mesenchymal stromal cells (MSC) from cord tissue. Six neonates with moderate or severe HIE were enrolled and received one infusion of MSC during TH, 2 infants also received a second dose 2 months later. All infants survived and between the age of 12 and 17 months obtained developmental assessment ranging from average to low-average.*"

Comment 5: Thus, only one new result was presented in 7 lines of text (lines 337-343). The rest of the text is more history prior to 2022.

Reply 5: We have now incorporated new papers published in 2023, as discussed previously. Please note that the primary objective of our manuscript was to provide a comprehensive overview for clinicians involved in the care of HIE patients and scientists engaged in HIE research. We have clarified this in the method section of our manuscript. Indeed, our scope was not to present novel studies on therapeutic approaches for nHIE but rather to focus on the selected studies that had advanced to phase 2 clinical trials and discuss them in more depth. We acknowledge that there are existing reviews published on novel treatments, and we believe that clinicians reading this journal would be most interested in gaining further insights into treatments that have advanced in clinical trials.

As mentioned, although there are numerous reviews on nHIE, including those centered on novel therapeutics, however we have not encountered a comprehensive review that encompasses all essential aspects. In our manuscript, we aim to bridge this gap by providing a comprehensive overview of HIE, including its definition, pathophysiology, the historical implementation of TH as the standard of care, and the imperative for innovative therapeutic approaches.

Changes in the text: No changes to the text were made.

Comment 6: On p18, it is indicated that a recent meta-analysis published in 2022 (reference 147) showed that length of hospitalization was significantly reduced when a neuroprotective therapy was used in combination with hypothermia, compared to hypothermia alone (lines 403-406). The text thereafter is more history.

Reply 6: We agree with the reviewer's perspective, as our purpose was to provide an overview, as discussed above

Changes in the text: No changes to the text were made.

Comment 7: Line 47, 35 weeks is indicated, yet 36 weeks is indicated in Table 2. Which is correct?

Reply 7: 36 weeks is correct; we apologize for the confusion.
Changes in the text: Page 3, Line 57

Comment 8: Lines 63-65, the actual landmark studies should be cited here. The landmark studies by Gluckman et al. (2005, Lancet) and Shankaran et al. (2005, NEJM) should both be cited – these studies currently have reference numbers 77 and 19, respectively, and hence are cited much later than they should be.

Reply 8: We agree with the reviewer and have updated the references.

Changes in the text: Now the above papers are cited on page 4, Line 78-79

Comment 9: After lines 63-65, it is recommended that text is added to indicate that these landmark clinical studies were based on prior preclinical animal research. An example is the research completed by the Gluckman and Gunn lab in Auckland, New Zealand (eg. Gunn et al. 1997, reference 72). This is emphasised in lines 154-156 but needs to be indicated much earlier.

Reply 9: Thank you for this excellent suggestion. The lines have been added.

Changes in the text: Page 4, Line 77

Comment 10: Line 81, need to cite at least one reference that provides the evidence for the data indicated.

Reply 10: We apologize for the oversight; the section has been revised and the statistic no longer used

Changes in the text: Text revised and statistic removed from the Introduction.

Comment 11: Line 93, 'Between the mid-1980s to early 2000s' is indicated as the time window of interest, yet Odd et al (line 120) was published in 2011. Ref 27 (line 131) was published in 2010. Refs 28 and 29 were published in 2006 (line 137 and 139, respectively). The relevant time window needs to be re-considered.

Reply 11: We made changes accordingly.

Changes in the text: Previously line 92, now page 8, line 169-170 reads: "Between the mid-1980s and 20*10s*"

Comment 12: Line 100, need to cite at least one reference that provides the evidence for the data indicated.

Reply 12: Thank you, this section has been thoroughly cited and more information was added to Table 3.
Changes in the text: Page 8, Line 178-181: *Notably, even children with HIE who did not exhibit any obvious neurological deficits and even with mild HIE were found to have a range of long-term cognitive (10,11,72,73,75,76), and neurodevelopmental impairments (10–12,75), such as Attention-Deficit Hyperactivity Disorder and Autism Spectrum Disorder (Table 3).*

Comment 13: Line 102, need to cite at least one reference that provides the evidence for the statement.

Reply 13: Thank you, this section has been thoroughly cited and more information was added to Table 3.
Changes in the text: Page 8, Line 178-181: *Notably, even children with HIE who did not exhibit any obvious neurological deficits and even with mild HIE were found to have a range of long-term cognitive (10,11,72,73,75,76), and neurodevelopmental impairments (10–12,75), such as Attention-Deficit Hyperactivity Disorder and Autism Spectrum Disorder (Table 3).*

Comment 14: Lines 166, 189 and 203 should refer to the Figure.

Reply 14: Thank you, the reference has been added.
Changes in the text: Starting on Page 6, line 133, 146 and 162

Comment 15: Line 218, need to cite the supporting references for the statement made.

Reply 15: Thank you, the reference has been added.

Changes in the text: Page 9, Line 186

Comment 16: Lines 225, 226: Refs 63 and 64 are not original research articles that provide the evidence.

Reply 16: Thank you, the references have been added.
Changes in the text: Page 9, lines 198-200

Comment 17: Line 245: cite the relevant reference for each clinical study.

Reply 17: We thank the reviewer and we have now also added the relevant references at the beginning of the paragraph, as suggested.

Changes in the text: Now line 227 reads as follows: “The NICHD (21), TOBY (34) and the CoolCap (33) trials”

Comment 18: Lines 281-293, Why is the CoolCap study published in 2005 mentioned after the TOBY trial of 2009? Isn’t is better to stay true to chronology?

Reply 18: Initially, we had arranged the discussion of the CoolCap trial last, intending to first cover the NICHD and TOBY studies that utilized whole-body hypothermia. However, we agree with the reviewer's suggestion that it is more appropriate to discuss the studies in chronological order. Therefore, we have revised the manuscript, ensuring a chronological sequence for a better flow of information.

Changes in the text: Now the TOBY trial is discussed after the CoolCap trial

Comment 19: Reference List: The formatting of the title and journal of each paper is extensively inconsistent.

Reply 19: We apologize for the oversight, we have corrected this in accordance with the formatting of the Journal

Changes in the text: See References

Comment 20: Reference List: The year of publication is missing from ref. 25.

Reply 20: We apologize for the oversight, we have corrected this in accordance with the formatting of the Journal

Changes in the text: See References

Reviewer D

Comment 1: Line 80-83 “The use of TH has increased each year and has contributed to the decline of the mortality rate of HIE in the USA from 11.5% in 2010 to 10.6% in 2018. This success can be attributed to the continuous efforts placed in translational research, which have enabled the conversion of basic scientific knowledge into clinical applications”.

This success can also be attributed to increased recognition of moderate to severe infants with HIE among providers which was mentioned on the same paper.

Reply 1: Thank you for this insight. We have revised the introduction so that this statistic is no longer referenced. However, we have added a segment about the importance of correctly identifying HIE.

Changes in the text: Page 4, lines 71-75

Comment 2: The authors missed to add the references in the text, e.g.;

- Line 99-102

- Line 200-202

Reply 2: Thank you, this section has been revised and thoroughly cited with more information in Table 3. The references have been added.

Changes in the text: Page 8, Line 178-181: *Notably, even children with HIE who did not exhibit any obvious neurological deficits and even with mild HIE were found to have a range of long-term cognitive (10,11,71,72,74,75), and neurodevelopmental impairments (10–12,74), such as Attention-Deficit Hyperactivity Disorder and Autism Spectrum Disorder (Table 3).*

Changes in the text: Page 7, Line 159

Comment 3: Can they clarify the following sentence?

Line 267-268 “However, hypothermia was found to significantly reduce death rates and did not increase rates of severe disability among the surviving children”.

Did they mean TH did not decrease rates of severe disability among the surviving children?

Reply 3: Thank you for asking for clarification on this sentence. In the past, there has been a discussion about whether hypothermia, instead of having a beneficial effect on neurodevelopment, could potentially have a detrimental effect. The authors reported that although there was no statistical significance in terms of IQ, it was clear that, conversely, TH did not show any detrimental effects on neurodevelopment. We agree that the sentence as it stands is confusing, so we have chosen to remove this part.

Changes in the text: Now page 11, line 249 simply reads: “However, hypothermia was found to significantly reduce death rate among the surviving children (22).”

Comment 4: They should briefly discuss about the following two important clinical studies under "Clinical Outcomes in the Post Therapeutic Hypothermia Era".

-Effect of Therapeutic Hypothermia Initiated After 6 Hours of Age on Death or Disability Among Newborns with Hypoxic-Ischemic Encephalopathy: A Randomized Clinical Trial. Laptook, and et al, JAMA 2017

-Effect of Depth and Duration of Cooling on Death or Disability at Age 18 Months Among Neonates With Hypoxic-Ischemic Encephalopathy: A Randomized Clinical Trial. Shankaran S, JAMA 2017.

Reply 4: Thank you, the references have been added.
Changes in the text: Page 4, line 82

Comment 5: Dr. Juul SC's new paper; secondary analysis of HEAL trial just published in J Ped Apr 2023. The authors should add this paper to at the end of line 343.

Reply 5: We thank the reviewer for his suggestion and have now added this relevant paper.

Changes in the text: Page 15, 327-330

Reviewer E

Comment 1: The main problem with this review is that the discussion on the potential therapeutic role of ketone bodies, in particular of beta-hydroxybutyrate, and thiamine and its derivatives in neonatal HIE was almost completely overlooked. I suggest to discuss and quote in the manuscript the following recent reports:

1. Sechi G. et al., Thiamine as a possible neuroprotective strategy in neonatal hypoxic-ischemic encephalopathy. Antioxidants 2022.

2. Sechi G, Sechi MM. New therapeutic paradigms in neonatal hypoxic-ischemic encephalopathy. ACS Chem Neurosci 2023.

3. Islas-Fabila P et al., Effect of thiamine pyrophosphate on the characteristics of farrowing and piglet vitality. Theriogenology 2023.

Reply 1: We very much appreciate the reviewer's input. We have incorporated the citation of the recent and significant papers into our manuscript, as suggested. We recognize the importance of delving into further details on each treatment strategy listed, an opinion shared by Reviewers E and G. However, we agree that providing a comprehensive and thorough exploration of all the treatment strategies would require an additional separate manuscript. In this context, we initially selected treatments that had advanced to stage 2 clinical trials, as they are closer to potential clinical application and thus most relevant to our manuscript's scope. We genuinely value the reviewer's insights and are grateful for their understanding of the constraints we faced in terms of manuscript length and focus.

Changes in the text: We have added the recommended papers page 13, lines 293-298

Reviewer F

Comment 1: This article is missing an objective for the review.

Reply 1: We restated out objective to be 1) to revisit the use of TH for HIE and its longitudinal impact on patient outcomes to readers new to the field of HIE, and 2) discuss how emerging therapies address a broader pathophysiology of injury progression in the neonatal brain hours to years after HIE.

Changes in the text: See "Abstract."

Comment 2: The method section is completely missing, a very abbreviated method section is presented in the abstract but leaves out significant amounts of information (such as number of articles identified, how articles were chosen to be included in this article and why others were not) and the article search is incomplete. The authors refer to "table 1" as methods.

Reply 2: We elaborated our Methods and utilized it as a way to outline the boundaries of our review.

Changes in the text: See "Methods."

Comment 3: Abstract: No new information is provided in the abstract that has not already been published and established in the literature.

Reply 3: We acknowledge that there are existing reviews published on novel treatments, and we believe that clinicians reading this journal would be most interested in gaining further insights into treatments that have advanced in clinical trials. Additionally, we included more about the sex differences in HIE, which is often neglected as a biological variable in both pre-clinical and clinical trials.

Changes in the text: See "Abstract."

Comment 4: Key content and findings, the 3rd sentence – it is unclear what "hypoxic ischemic stress at the primary phase" etc is.

Reply 4: This section has been revised and the confusing line removed.

Changes in the text: See Abstract "Key Content and Findings"

Comment 5: The phase the authors call the prolonged phase is in general referred to as tertiary phase.

Reply 5: We agree and have revised the text to consistently use “tertiary phase”

Changes in the text: All “prolonged” phases were replaced with “tertiary” phase for consistency.

Comment 6: HIE affects all gestational ages not just infants > 35 weeks, and the incidence in preterm infants is even higher than in term infants.

Reply 6: Thank you, we recognize our language was misleading. We have clarified and added additional information on HIE in preterm infants

Changes in the text: See introduction, page 3 lines 48-57

Comment 7: The incidence in high resource settings is thought to be 1-3:1000 and as high as 40:1000 in low resource settings. Several publications are available and should be included here as this is a review.

Reply 7: Thank you, we have added more references to reflect the broader range of studies reporting the incidence of HIE in low-resource settings.

Changes in the text: Page 3, Line 51-53

Comment 8: The authors attempt to align the neonatal mortality secondary to birth asphyxia with HIE when in fact these are two very different conditions with very different definitions. The authors should take this into account and be very thoughtful about the terminology used.

Reply 8: We clarified the difference in the definitions and provided appropriate references.

Changes in the text: Page 3, Line 69-75

Comment 9: The mortality and morbidity of 60% is very generalized and warrants particularly in the context of a review further discussion and include the differences in morbidity and mortality for mild/moderate/severe HIE.

Reply 9: Thank you, we agree and clarified that “60%” is in the context of severe HIE. Furthermore, Table 3 details a broader range of mortality and morbidity organized by severity.

Changes in the text: Page 3, Line 52-53 and Table 3

Comment 10: The authors interchange animal with human studies, interchange different species of animals (in general animals have different core temperatures, therefor data can’t just be extrapolated and applied to humans. If the authors truly wish to review this broad topic, they should probably attempt to go systematically through the different species.

Reply 10: The reviewer makes an excellent point. We addressed this by emphasizing that species have different ranges for hypothermia and serve to guide human research. We also clarify the species used when reporting results from various studies.

Changes in the text: Pages 9-10, Lines 193-226

Comment 11: The optimized cooling trial was examining longer and deeper cooling and that was 120 vs 72 hours and 32C vs 33.5C a difference of 1.5C not 5C.

Reply 11: The section has been revised and this reference is no longer used.

Changes in the text: Page 10, Lines 193-226

Comment 12: Furthermore, treatment for mild HIE is available, it is just not as well studied, therefore the effectiveness remains uncertain and further characterization whom of the neonates with mild HIE will benefit from cooling and who doesn't needs to be further studied.

Reply 12: Thank you, we have included more information on the drift of TH usage in mild cases of HIE, and discussed ongoing work on determining the benefits of TH

Changes in the text: Previously page 4, line 77-79, revised and now page 4, lines 81-86.

Comment 13: TH is also available in low resource settings, however as shown in the HELIX trial, identification of the appropriate population has been challenging and therefore treatment success is not clear.

Reply 13: Thank you for making this insightful point. We have added this study to our introduction and include a discussion on why TH may have not been ineffective.

Changes in the text: Page 4, lines: 87-93

Comment 14: The first paragraph is missing all references. This reviewer suggest that the authors summarize the outcome findings of all the studies in a table instead of listing them in a narrative and include the references.

Reply 14: We thank and agree with the reviewer. As outlined in our replay to Reviewer A, the paragraph entitled: "*Clinical Outcomes in the Era Prior to Therapeutic Hypothermia*" have now been shortened. We have replaced the text describing the studies with Table 3, providing a clear overview of the data available. The remaining text summarizes the findings of these studies and their relevance.

Changes in the text: In the Introduction and Table 3.

Comment 15: The authors go on describing some biochemical and physiological mechanism in words, others are simply listed as “explained by XX et al”.

Reply 15: We wrote to emphasize mechanisms of injury that parallel the mechanisms of TH and other therapeutics. To avoid making this section overly dense, we opted to draw attention to reviews that thoroughly address topics that we could not fully address in our allotted space.

Changes in the text: No changes made

Comment 16: The ideal phase for neuroprotection depends on the target of the neuroprotective strategy. One cannot refer to the latent phase as the only phase for neuroprotection. The authors even contradict that in their own Figure 1.

Reply 16: We agree, and one of our objectives is to highlight how therapies that act in the later phases of injury may lead to significant improvements in morbidity after HIE. We only intend to draw attention to how this is where emerging therapies predominantly act. Our aim is to feature studies that explore beyond this, as supported by our Figure 1

Changes in the text: See Abstract.

Comment 17: There are many incorrect statements in this manuscript which makes this reviewer question of the articles were actually read by the authors. As an example, page 11 line 230: the authors quote Marianne Thoresen’s analysis of a subgroup of babies from the TOBY trial, while the article says earlier cooling might be beneficial it also acknowledges the limitations of such statement as the study wasn’t powered adequately. In the meantime, other publications are available who further discuss this subject and have controversial conclusions, none of which are included in the proposed manuscript.

Reply 17: Thank you for the opportunity to improve our manuscript. We referenced Dr. Thoresen’s work (now page 10, lines 212-213) to emphasize the importance of time in initiating TH. While her study had limitations, the results on outcomes after initiating TH before 3 hours of age was appropriate to reference in this context. However, the phrasing wasn’t meant to imply that there is an absolute consensus about earlier cooling and improved long-term outcomes, or to suggest that cooling after 6hrs is not worth investigating. We rephrased this segment, and added a study investigating outcomes after TH initiated after 6hrs. We also re-emphasized the mechanisms behind why the timing is significant.

Changes in the text: Page 10, lines 212-213

Comment 18: In the last paragraph of the “mechanism of action of therapeutic hypothermia” the authors only quote animal studies but leave out the optimized cooling trial.

Reply 18: The references have been included.

Changes in the text: Page 10, Lines: 222-223

Comment 19: In the next section, the authors summarize at length several trials and outcome, but unfortunately leave out the fact that across trials, babies with moderate HIE have a better response to cooling than those with severe HIE.

Reply 19: We appreciate the reviewer's comment. The NICHD, CoolCap, and TOBY focused on comparing the outcomes of infants who received therapeutic hypothermia versus those who received standard care, they did not specifically analyze the outcomes of infants with moderate versus severe HIE separately within the trial results. To the best of our knowledge, large, randomized studies comparing outcomes of neonates with moderate versus severe HIE after cooling have not been published yet. We greatly value the expertise of the reviewer and welcome their insights. We appreciate the opportunity to learn and would be delighted to include any relevant citations in our paper.

Changes in the text: No changes were made to the text

Comment 20: When it comes to HIE, neuroprotection and outcome, other factors such as MRI, seizures etc. are a significant component of extensive research that has resulted in better understanding of the disease and prognostication, all of which have influence on long-term outcome.

Reply 20: Thank you for the recommendation. We have included MRI patterns of injury in the acute and subacute phases, and the predictive value of MRI at 6-7-year outcomes.

Changes in the text: See changes on page 6, lines 118-131.

Comment 21: Furthermore, newer studies have also shown that many underlying conditions can mimic HIE and at a bare minimum the authors should probably comment on the role of genetic testing in future neuroprotective strategies.

Reply 21: We elaborate on the terminology and mimics surrounding HIE, the importance to determine the cause of NE and added two references on the relevance of genetic testing.

Changes in the text: Page 4, Lines 72-75 "A minority of neonatal encephalopathy cases in term infants stem from non-hypoxic/ischemic causes, which can include intracranial infections, intracranial hemorrhage, hypoglycemia, kernicterus, metabolic disorders, inborn error of metabolism and malignant epilepsy syndromes (25,26). Given that these conditions require different treatments than HIE, accurate diagnosis becomes essential (27-29).

Comment 22: When it comes to neuroprotective agents, the only newer information included are the results of the HEAL trial, and the authors speculate about toxicity of the dose without any reference to back up their statement; personal opinions or speculations are not part of a review in contrast a discussion of objective data should be presented. The authors also leave out xenon. And this article is missing review of data about mild HIE.

Reply 22: We have now added as a reference the secondary analysis of the HEAL trial (Juul et al, J Ped, 2023) on line 327-329. Xenon was mentioned on line 292 and referenced (Yin et al, Frontiers, 2022)

Changes in the text: Please see above.

Comment 23: To Figure 1, while the parts "stages of injury" and "therapeutic interventions" are explaining what is shown in the graph, the middle section of the figure is not self-explanatory, and it remains unclear if this is decorative or supposed to serve a purpose.

Reply 23: We added the title "Mechanism" to the middle section of Figure 1. The purpose of the figure is to provide an illustrative timeline of the mechanisms of injury with HIE and where various therapies fall on this timeline. We have added in reference to Figure 1 in the body of "Mechanisms underlying Hypoxic Ischemic Encephalopathy."

Changes in the text: Page 6, line 133, 146 and 162

Comment 24: To Table 2: The authors present "criteria for therapeutic hypothermia" – the purpose of this table is unclear, if this is specific to the authors institution or if the authors try to summarize cooling criteria across the literature, if the latter is the case, all available criteria should be included (EEG background, sentinel events, need for resuscitation, Thompson score etc.).

Reply 24: "Criteria for Therapeutic Hypothermia" is a summary of criteria used for the landmark trials discussed in the previous sentences. More details have been added as recommended in what is now "Table 1."
Changes in the text: Page 4, Lines 77-81

Reviewer G

Comment 1: Authors present several potential adjunctive treatments to TH; in my opinion, it would be interesting to expand a bit the discussion about the role of each neuroprotective treatment described in lines 405-406.

Reply 1: Please see above.
Changes in the text: Please see above.
