Author's response to reviews

Title: "Early identification of brain injury in infants with hypoxic ischemic encephalopathy at high risk for severe impairments: accuracy of MRI performed in the first days of life"

Authors:

Thais Agut (tagut@hsjdbcn.org)
Marisol León (marisol2270@yahoo.es)
Mónica Rebollo (mrebollo@hsjdbcn.org)
Jordi Muchart (jmuchart@hsjdbcn.org)
Gemma Arca (gemmarca@yahoo.es)
Alfredo García-Alix (agarciaalix@hsjdbcn.org)

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Author's response to reviews: see over
Dear Sir

Thank you very much for your comments. We thank to all reviewers for their valuable comments. Definitely their suggestions have improved the final text of this manuscript. We have directed the focus of the paper in the serial aspect of the MR studies.

Below we comment point by point the main concerns exposed by the reviewers:

**Reviewer 1**

1. There is an overemphasis throughout the paper from the abstract through to the discussion on the timing of the MRI and its role in end of life decisions/redirection of care. The timing of the MRI and its accuracy is a relevant question primarily, for clinical prognostication and parental discussions, for the majority of infants who survive. Time critical decision making regarding redirecting care are relatively uncommon, even in western societies. I would broaden the discussion to reflect this.

   We totally agree that early MR studies are not only relevant for helping in redirection of care but also for clinical prognostication and parental discussion. Because they are difficult to perform in sick and unstable babies, in clinical practice we only perform them when there are doubts about prognosis.

2) Although a picture sometimes tells a thousand words, an MRI is not necessarily essential in order to redirect care. The clinical assessment I believe is still useful despite discussions in the literature about how hypothermia may have influenced its role. The paper mentions that all infants had aEEG monitoring but do not discuss the role of aEEG / EEG in time critical redirection of care decisions. The current consensus is that failure of the aEEG / EEG to improve by 48 hours or more is indicative of a poor prognosis. The sensitivities and specificities of aEEG / EEG are equal to if not superior to MRI in numerous papers and summarised in Pediatrics 2013 van Laerhoven (reference 9). Whilst EEG not the topic of the paper perhaps this could be briefly discussed and again pointing to my concern re the message that MRI not always essential although usually performed, this point is relevant to units where MRI may not be as immediately accessible on site.

Thank you very much for the comments. We completely agree that MR is not essential for redirecting care and that neurological examination are two other most important prognostic tools, especially a-EEG traces. Please notice that we have added this point in the first paragraph in page 10:

“*In clinical practice if there are doubts about prognosis, MR performed in the first days of life may be very valuable in infants with significant HIE. Although it is not essential and not always available, it is especially useful if there are inconsistencies with the other prognostic tools as neurological examination or aEEG studies*"
3) Is the topic of this paper a primary aim of this prospective observational study or a sub study of a larger project? I ask this as only 15 of the 40 infants with moderate to severe encephalopathy had 2 scans; if it was the primary question then I would have expected a better 2 scan result even allowing for deaths and drop outs due to transfers to other hospitals.

You are absolutely right, we didn’t mention it but this is in fact a sub study of a larger prospective study. We have specified this point in the methodology section.

We have also added, in the result section, the reason why an MR scan was not performed in 7 of the 40 infants in our study. As it happens in clinical practice, 5 infants in our study with HIE were clinically too unstable and sick during the first days of life to be transferred to the MR scan.

4) The ratio of moderate to severe encephalopathy cases seems to be reversed, is this correct and if so why, does it reflect local cooling practice. You allude to this in discussion but I would expand the sentence.

This is a very relevant issue. Our hospital is a reference center for this pathology. This study was conducted when cooling was starting in our country. We are convinced that these two are the reasons why the ratio of severe to moderate encephalopathy is higher than previously published. Our study does not reflect epidemiological issues about HIE, as it is an individual cohort of cooled infants with HIE from one institution. As the reviewer suggests we have expanded the sentence in the discussion section as a limitation of our study (last paragraph of page 11):

The ratio between infants with severe and moderate HIE may represent a selection bias. Two thirds of our patients were referred from other centers and this study was conducted during the period when implementation of cooling for treatment of HIE infants was started at our country. Most of the infants with severe HIE were outborn.

5) Numbers in the study are still relatively small, but is it possible to comment on the few scans where the MR scores did change from scan 1 to scan 2 in relation to region of injury, this may not be possible within the word limits and small numbers

We totally agree that this is a very important issue as the main results of the study are the little differences between the early and late MR images. There were no important changes between MR images in early and late scans except for one patient. Description of regional changes in signal intensity between the first and second scan have been added in the first paragraph of page 11:

“It is important to highlight that we didn't find any false negative in the patients with serial MR studies. In the 6 patients with normal early scans, the second MR study didn't show any pathological findings. Findings between the first and second conventional MR scans were similar in 9 cases. In the 6 newborns left in which differences were noted in scores between the two sequential scans, there were no serious abnormalities of the first scans that normalized in the second
week. In 3 of these patients differences in scores were because PLIC was score as doubtful in the first scan and absent in the late scan (patient number 1, 7 and 14). Two patients with central pattern (patients 2 and 5) scored higher because mild abnormal signal intensity in the cortex was seen in the late scan that was missed in the early scan. One patient (patient 2) was classified as having a moderate central pattern in the first scan at 74 h (score of 5) and as having severe central pattern (score of 10). Except this infant, no serious abnormalities in the early scans that would have change the prognosis were missed."

Minor revisions and misspellings have been corrected. As suggested, Table 3 has been changed and reordered according first to the grade of encephalopathy and then to the pattern, The p-values in Table 1 have been recalculated.

Reviewer 2

Background

1. The remaining adverse outcome of a significant part of neonates treated with hypothermia are not only seen in major trials, but also in out-of–trial situations (PLoS One. 2013 Dec 31;8(12):e83742; and Neonatology. 2013;104(1):15-21). This could be added to the manuscript.

Very early MRI/proton MRS has also been performed in a small study almost a decade ago (Biol Neonate. 2005;88(4):306-12).

Please note that these three references have been added to the manuscript. Bibliography section as:


2. The validity of MRI after perinatal asphyxia has been published in detail by Rutherford et al.

We completely agree with this point. Please notice that Rutherford's papers are included in the bibliography (references number 9,12,16,17,27).

3. The importance of the present study is the repeated MRI in 15 cases. The authors should focus on this novel and important aspect.
Notice that this point is mentioned in the background section at the end of the first paragraph of page 4:

“As far as we know there is only one study comparing images in early MR studies with the ones in scans performed in the second week of life [13].”

We have also added a sentence at the end of the second paragraph of page 4:

“Two sequential MR studies were performed to test this hypothesis.”

Methods

4. Reference 13 deals with a method of classifying encephalopathy which has not been used by many authors. How does this method relate to more commonly used systems as the Sarnat or Thompson scores?

This Classification system is a modification of the grading system described by Sarnat that focuses in the level of alertness. It sub classifies moderate HIE in A or B if seizures are present or absent respectively and, severe HIE in A or B if brain function is preserved or abnormal respectively.

Our group has been using this method for the last 20 years.


MR imaging

5. In general, optimal use of diffusion-weighted imaging is beyond the first 48 hours of life, but before 8 days of life. Could the authors make a graph or table of the exact scanning times during the first week of life. This time could be added easily to table 3, e.g.

We have included the scanning ages of the early and late scans as suggested in table 3.

6. How many patients were examined during hypothermia, and how was the temperature maintained and monitored?

Four patients in the study were examined during hypothermia (HT). We have also added this information to table 3. Central (rectal) temperature was monitored during the procedure but we haven’t include this information because we think is not relevant for the purpose of the study.
7. Page 6: In the online version of reference 14 the page numbers and classification system cannot be found. Please provide an alternative reference, or give the classification as an (on-line) appendix.

Although Rutherford in her book describes the hypoxic-ischemic lesions in the different brain areas we have change the reference for the classification (on-line appendix in Martinez-Biarge M, Diez-Sebastian J, Kapellou O, Gindner D, Allsop JM, Rutherford MA, Cowan FM. Predicting motor outcome and death in term hypoxic-ischemic encephalopathy. Neurology 2011, 76: 2055-61)

Results

8. Of 40 infants 33 had an MRI. Six died without an MRI. What happened to the seventh without MRI?

Of the seven patients left, six were too unstable, as is usual in clinical practice, to be transported to MR unit and in one patient the MR study was not available for evaluation. This information has been added in the second paragraph of page 7.

9. The p-values of Inborn, and Emergency CS do not seem to be correct!

We have recalculated p-values in table 1 and corrected them.

10. One neonate with severe HIE did not show (any) abnormalities. How did this patient do, and at what time was this scan with unexpected findings performed?

This patient was overcooled when he was referred to our hospital (he was 31ºC on admission). Scans were performed at 99h and 213h of age. He has been followed up and at 26 month of age Bayley Index were 85 for cognitive, 86 for language and 94 for motor evaluation.

11. “Early versus late MR imaging” –this is the most important aspect of the present study, and the authors should focus more on this part. Correlation is not the most informative aspect.

We totally agree with this point and have added some information about this aspect in the Discussion section (third paragraph in page 10):

“A novel and interesting aspect of our study is the two sequential MR scans performed in 15 newborns for hypoxic-ischemic brain injury assessment including DWI in the early examinations.”

12. In table 3 findings between 1st and 2nd scan are similar in 9 cases (mainly without abnormalities), increased in 4 cases and decreased in 2 cases. It would be very interesting to hear in detail about these changes. Were serious abnormalities missed on the 1st scan? Did serious abnormalities of the 1st scan normalize in the second week? This is of utmost clinical importance.
Thank you very much for the comments. We completely agree that this is a very interesting point. We didn’t have any false negative on the early scans and except for one patient no serious abnormalities were missed in the scans performed in the first days of life.

Details about the changes between early and late scans have been added in page 11:

…”It is important to highlight that we didn’t find any false negative in the patients with serial MR studies. In the 6 patients with normal early scans, the second MR study didn’t show any pathological findings. Findings between the first and second conventional MR scans were similar in 9 cases. In the 6 newborns left in which differences were noted in scores between the two sequential scans, there were no serious abnormalities of the first scans that normalized in the second week. In 3 of these patients differences in scores were because PLIC was score as doubtful in the first scan and absent in the late scan (patient number 1, 7 and 14). Two patients with central pattern (patients 2 and 5) scored higher because mild abnormal signal intensity in the cortex was seen in the late scan that was missed in the early scan. One patient (patient 2) was classified as having a moderate central pattern in the first scan at 74 h (score of 5) and as having severe central pattern (score of 10). Except this infant, no serious abnormalities in the early scans that would have change the prognosis were missed” …

Discussion:
Some relevant and/or recent references are missing (Biol Neonate. 2005;88(4):306-12 on very early MRI; Radiology. 2011 Oct;261(1):235-42 on Diffusion Weighted MRI and proton MRS).

These two references have been added (references number 24 and 26)

Page 10, top paragraph: this statement is only important, when redirection of care is considered. In all other cases MRI can be postponed until the full injury has developed.

We totally agree with is comment and have changed the drafting of this paragraph (now the second paragraph of page 10):

“In clinical practice if there are doubts about prognosis or redirection of care is considered, MR performed in the first days of life may be very valuable in infants with significant HIE. Although it is not essential and not always available, it is especially useful if there are inconsistencies with the other prognostic tools as neurological examination or neurophysiological studies.”

Figures 1 and 2 can be omitted (information in table 3), and be replaced by an example of an MRI with diffusion-weighted changes on 1st MRI and abnormalities in T1/T2 on the 2nd MRI.

We have replaced Figures 1 and 2 by an example of MR images on the first and on the second scan

Details: All details and minor revisions have been corrected as suggested.
Reviewer 3

Language errors: all language errors suggested have been corrected [p4: during the entire process; p7: punctate lesions; p8: as pointed out by Wilkinson; p9: prognosis is based on …neuroimaging findings; p9: Thoresen et al.; p9: although it is still unclear; p9: moreover; p10: it is essential]

Major revisions

The originality of this finding lies in the serial aspect of the MRI study.

As commented before we have focus the manuscript in the serial aspects of the MRI study in this cohort. See point 5 from Reviewer 1 and points 3,11,12 from Reviewer 2.

It is indeed relevant that in a center with emphasis on clinical interpretation of HIE, the finding of relevant MRI support for care is important. For that reason the authors should rewrite the discussion focusing on papers who have done that and describe their findings. This list should perhaps focus on those papers where a scoring system was used (according to Hammersmith, Barkovich or Swarte et al. (not in the list of references)). The findings of serial MRI should be rewritten with in mind primary versus secondary injury (network injury in tracts and nuclei connected to sites of primary injury like thalamus and cortex). The second week diffusion changes should be compared to the first week ones just to learn about such network injury. It would also be useful to compare EEG findings with the reported patterns of MRI injury.

This is a sub study form a larger observational prospective study that has also examined the association between MR findings and clinical assessment during the first 6 hours of life. We have also analyzed neurophysiological studies as all the babies had an EEG monitoring and their correlation with the site and extension of hypoxic-ischemic lesions and patterns of MRI injury. Although different scores have been used for grading the Hypoxic-ischemic injury we have chosen the one designed by the group in Hammersmith as it is the one we apply in clinical practice.

Personally I would also like to correlate the lesion pattern with the presence of sentinel events (clear events pointing to an intrapartum cause of the asphyxial moment).

This is a very interesting point and has actually been analyzed (data not shown). We have not found correlation between the patterns of injury and perinatal variables such as the presence of sentinel events.

The relevance of serial ultrasound imaging to depict deep grey matter injury may also need to be mentioned in the discussion and perhaps in the personal ultrasound findings of this cohort.

We consider that serial Doppler-ultrasound is very useful. We performed on admission and then every 24 hours during the first 3-5 days. Data on ultrasound
from this cohort has been collected and we expect to analyze it in the next months.