Subject: BMJ - Decision on Manuscript ID BMJ.2017.038478

Body: 17-Apr-2017

Dear Dr. Petribu,

Manuscript ID BMJ.2017.038478 entitled "Congenital Zika Syndrome: reduction of cerebral calcifications and cerebral volume on follow-up head CT scans. Is there still viral activity?"

Thank you for sending us your paper. I apologise for the slight delay in getting back to you. These findings are intriguing but we are concerned about comments from the reviewers about the possibility they might be due to artifact or positioning changes. We would like you to respond to the comments of the reviewers, and then send the paper to additional pediatric neuroradiologists for evaluation. I am sure you can appreciate that the findings will be scrutinised extensively if published. We want to make sure the interpretation is solid. Let me know if you have any concerns.

Very truly yours,

Elisabeth Loder
eloder@bmj.com

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Report from The BMJ's manuscript committee meeting

These comments are an attempt to summarise the discussions at the manuscript meeting. They are not an exact transcript.

Present: Elizabeth Loder (chair); Angie Wade (statistician); Tiago Villanueva; Georg Roeggla; John Fletcher; Rubin Minhas

Decision: Put points decision: revision is to be seen by our statistician for a full stats report upon return; we will also ask new pediatric radiologists to look at the paper

* We need more information about how you chose these 37 children.

* Several editors were concerned about the ethical aspects of the study. Were there clinical justifications for doing these scans or were they done only for purposes of the study? If the latter, is it ethical to do additional CT scans and expose these children to radiation? Were parents asked to provide informed consent? Were the babies sedated for the scans? If so, was the additional risk of sedation or anesthesia justifiable?

* The title should not contain a question. We suggest something such as "Followup head CT scans of a series of 37 children with congenital Zika Syndrome"

* No analysis plan is given in the methods and there is no abstract. Please include these things in the revision.

* Table 1 should present the paired data to show of those with initial problems, how many of these improved, and how many of those without initial problems developed them. The statistical analyses performed should retain this within person pairing of the observations (McNemar test).

* Fishers exact test of table 2 is not particularly helpful. The null hypothesis is that the evolution group % are the same between the patients initially classified as mild, moderate or severe, which does not make sense as these groups have differing capacity to move in specified directions regardless of any real association. (For example, those in the severe group cannot increase.)

* In relation to table 2 data, the authors state that the greater the reduction of cerebral volume at initial CT scan, the greater the parenchyma reduction on follow up. However, of the severe initial group 13/15 (87%) reduce compared to 10/18 (56%) of the others, which is a non-significant difference (31% diff with 95% ci (-0.5, 55%)). Severe vs moderate is a difference of 17.4% (-13.1, 46%). Hence the p-value of 0.022 is misleading.
The 2 with no initial reduction should also be shown in the table.

* Similarly for tables 3 and 4 and their associated analyses.

* One limitation noted is the subjective quantification of calcifications. Information should be given re the agreement between the 4 assessors pre-consensus.

* We agree with reviewers who are worried about whether there is enough standardization between initial and followup scans to justify the conclusions. Specifically, we are worried about head movement or positioning differences. The other thing is that equipment varied across patients for initial scans although all followup scans were done at a single tertiary hospital. The judgement of calcification appears to rest on seeing white spots. We have all seen how pictures can change quite a bit as you adjust the contrast so we need to be reassured that the images being compared are like for like images. Here many of the were taken at different hospitals using different machines. Although you try and address this problem by using consensus among radiologists we think it takes more to ensure the images are comparable. This is amplified upon by ref2. These things make us worry about the validity of the findings.

* The judgement of cerebral volume appears even more subjective and rests on 2D cross sectional scans. However, if there has been growth in the head then the thickness of grey matter on scans cannot be used as a measure of volume since an equivalent amount of grey matter will be spread more thinly. The categorisation of volume appears to be just mild moderate or severe with subjective cut offs beteen categories. Subjective comparisons of brain volume need to be placed alongside objective measures of head size and cortical thickness.

* The quantification of calcifications is subjective. We need information about how often the radiologists agreed (consider kappa statistics), how long it took them to reach consensus, whether they knew what the previous scans showed when they were doing this.

* In Table 1 you have the paired data and that should be shown as paired and you should use paired tests. * Comparisons should be made within children, then if appropriate between groups.

* On other tables you've done fishers exact test and that is not useful at all. For example, in Table 2 we have evolution – unchanged and increased. If you use fishers your null is that the distribution of unchanged and increased is the same in mild/ mod/ severe but that can't be because if you are severe you can't get worse and if you are mild you are more likely to get better.

* Then there is the matter of reduction of cerebral volume. If you look at the severe initial group you have some reducing but that is nonsignificant when you compare and do a confidence interval. The p values here are misleading and this is the wrong analysis.

* Despite our worries about these things, we agree this may be useful because the information is probably the best that's available at this time. The pictures are fairly dramatic. You see things almost completely clearing up. Could it be the virus is gone completely, or is this artifactual?

* Could you provide some information about the clinical picture in these children? The scans got better but are they clinically better as well? Do these go together? Clinicians will want to know.

* This paper will need a patient involvement statement that tells whether parents were involved in designing the study or not. Can you please also state whether parents were thanked? Is there any plan to distribute materials to patients or advocacy groups?

In your response please provide, point by point, your replies to the comments made by the reviewers and the editors, explaining how you have dealt with them in the paper.

Comments from Reviewers

Reviewer: 1

Recommendation:

Comments:
Comments for Editor and Author:
A) Originality: This research on Congenital Zika Syndrome (CZS) makes a major contribution to the literature. The authors are nearly all from the first epicenter in Recife, Pernambuco, northeast Brazil. Thus, they have the longest followup on the many children born with CZS and understandably have performed follow-up head CT scans for the 37 infants in this study and compared them to the original head CT scans. This comparison of the CT scans, and their interpretation of the diagnostic value based on this comparison, would be a major original contribution to what is already known.

(B) Importance: Yes, this work would matter to general readers including clinicians, teachers, policymakers. The two main reasons are: (1) the diagnostic value of the follow-up CT scans with few or no calcifications despite confirmed diagnosis (in 29 of 37 cases) of CZS, and (2) the documentation in nearly all cases of continued decrease in brain volume (what the authors also refer to as "brain parenchyma"). One or more of the authors from Recife have published in BMJ previously on CZS.

(C) Scientific Reliability: The research question is clearly defined and answered. The overall design of the study is adequate and participants studied are adequately described. The Methods are adequately described. Of note, however, are several suggestions: (1) more emphasis could be placed on the fact that these 37 patients were "convenience sampling" (p.2 of 33) and the repeat CT scans were indicated by "clinical evolution, which is highly variable" (p. 1 of 33). (2) Thus, the reader could ask whether the nearly uniform follow-up

CT scan findings (decreased calcifications and decreased cerebral volume) reported in these 37 patients would occur in infants without clinical evolution.

D) Results: Do answer the research question, are credible, and well-documented in the text, tables, and figures. Several suggestions can be offered: (1) Possibility Table 4 could be omitted because the results are not statistically significant ($p = 0.421$) (2) Also, the Figure 9 flowchart is almost illegible as submitted. Figure 9 does provide a very useful summary (when enlarged). (3) The authors could comment specifically whether "hydrocephalus" was observed in the initial or follow-up CT scans. If so, can they comment on the potential significance and type, either communicating or non-communicating hydrocephalus. (4) The authors could emphasize even more whether results differed between "confirmed" and "probable" cases.

E) Interpretations and Conclusions: The significance of two findings of decrease or "vanished" calcifications and the ongoing decrease in cerebral volume are appropriately emphasized as having MAJOR diagnostic value in evaluating infants approximately one year of age for possible CZS. However, the further speculative (or better stated "hypothetical") interpretation by the authors that these two changes represent that there is "still viral activity" (as stated in the title, and in the conclusion and abstract) can be seen as potential over-interpretation or speculation. The reason is no data are provided in terms of testing for the Zika virus or the immune response, or inflammatory response, to the virus in the infants at or near the time of their follow-up head CT scans. The lack of calcifications on the followup CT scans means the absence of such calcifications does not rule out the diagnosis of CZS. This finding alone is extremely important and a major contribution to our understanding of CZS and to the literature of CZS. Are the authors additionally implying that there is a chronic Zika virus infection in the brain of these infants? This would be very important to determine, but beyond the scope of this study. Finally, the discussion could be shortened in terms of the discussion about microglia and calcifications.

(F) The references are up-to-date and without glaring omissions.

Additional Questions:
Please enter your name: Daniel Lucey

Job Title: Senior Scholar and Infectious Disease Adjunct Professor

Institution: Georgetown University Medical Center, USA

Reimbursement for attending a symposium?: No

A fee for speaking?: No

A fee for organising education?: No

Funds for research?: No

Funds for a member of staff?: No

Fees for consulting?: No

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Reviewer: 2

Recommendation:

Comments:
Thank you for asking me to review this very interesting manuscript in which the authors present following cross-sectional CT imaging of infants with probable or confirmed congenital ZIKA infection. They report that follow-up imaging demonstrated vanishing or decreasing number of subcortical calcifications in the brains of these infants. There is also increased ventriculomegaly that they conclude may represent continued or ongoing loss of brain matter.
Strength:
I think that the manuscript is well written and presents an interesting and surprising finding, if true. The authors offer an intriguing hypothesis as to the underlying histologic basis for the "vanishing" calcifications that is grounded in prior published work that point to the mechanism of brain injury following ZIKA infection, namely that the infection triggers cell death via apoptosis with increased macrophage and microglial activity that clears out the dead cells. The authors hypothesize that this mechanism of increased microglial activity may explain the apparent decrease in calcifications noted on the follow-up scans in these children who have probable and confirmed congenital ZIKA infection. This would make the behavior of congenital ZIKA infection vastly different from all of the other congenital TORCH infections where the calcifications are permanent and stable over multiple years of imaging.
Because of the importance of these implications, I would like to ensure that the imaging unequivocally support the authors' claims. Currently, the authors provide no detailed information regarding the imaging acquisition techniques used to acquire the initial and more importantly the follow-up scans. The make a general statement on pg 2 lines 51, 53 that the "The initial CT scans were acquired in different radiology centers and with different CT equipment, but these scanners had similar technical capabilities".
WEAKNESSES:
More information about the Vendor, acquisition parameters, coregistration of images, Signal to noise and contrast to noise ratios, whether or not there was contrast administration are needed and needs to be included in the METHODS section as differences in these metrics can alter the presentation of imaging findings.
Acquisition Parameters missing:
1. What are the specific imaging acquisition parameters used in acquiring these images?
2. What was the mAS, the kVP, the slice thickness?
3. Were the images overlapping? How many slice detectors did the scanner(s) have?
4. Did the use iterative reconstruction kernels (such as in Dual energy CT scanners).
5. Was the gantry angled? If so, what was the degree of angulation?
6. Were the same acquisition parameters used for the subsequent scan for each patient.
7. Were the patients scanned on the same scanners used for the follow-up scan.
8. Different Vendor CT scanners produce images with different degrees of signal to noise ratios and contrast to noise ratios. Were these controlled in initial and follow-up scans (see figure 5 initial and follow-up scans)

Patient positioning missing:
Differences in patient head positioning can alter the presentation of lesions on head CT. Were patients' follow-up scan co-registered to the initial scan in order to ensure same alignment of the brain on initial and follow-up images. Several of the images included in the manuscript demonstrate that they may not be aligned. Therefore, the slice position is not the

same. This can affect the appearance of findings on initial and subsequent scans (sometimes dramatically).
This issue is best noted on page 26 figure 5 (C vs F). We see in image F the inferior margins of the ethmoidal air cells and we do not see the nasopharynx whereas in C the nasopharynx is very clearly seen, suggesting either that the head position is different or that the slice selection is different, may be due to difference in slice thickness etc, in the two images being compared.
The authors argue that in Table 2 that in comparison the initial scan 1 infant actually had an increase in brain matter on subsequent imaging . This would be quite usual for there to be an increase in brain matter that did not exist on the initial scan. However, this apparent finding can magically occur if the two scans being compared were acquired with different head positioning angulation. Again, this points out the importance of co-registering the initial and follow-up scans.
Contrast Administration:
This needs to be clarified.
Was contrast administered for these scans? Some of the images appear to be contrast enhanced since there is opacification in the posterior margins of the superior sagittal sinus in A, the inter-cerebral veins in B and right sigmoid sinus in C. These vascular structures are not opacified in the follow-up scans (see Figure 4 on page 25 images A, B, C versus follow-up scans D,E and F). A similar findings of vascular structure enhancement is noted for figure 5 on pg 26. This technical difference in initial and follow-up scan contrast administration introduces a very important confounding variable here : 1) lesions that are present on contrast enhancement but that are not visualized on noncontrast enhanced scan usually indicate the presence of an enhancing lesion as opposed to a calcific one. 2) If both scans were acquired following contrast administration, then there is a significant difference in bolus timing which can alter the presentation of lesions. This needs to be clarified.
Differences in Ventricular size:
See figure 5, there is marked increased ventriculomegaly on the follow-up scans compared to the initial scans. Are the patients developing communicating hydrocephalus or is this increased degree of ventriculomegaly the result of continued loss of brain matter .

CONCLUSION:
As I mentioned above, I think that if the findings are true then the findings described in this manuscript would be very important. However, I need more information in order to mitigate some of the very important confounding imaging variables described above. For this reason, I think that the manuscript should be resubmitted with major revision.

Additional Questions:
Please enter your name: Nadia Biassou MD, PhD

Job Title: Neuroradiologist

Institution: National Institutes of Health

Reimbursement for attending a symposium?: No

A fee for speaking?: No

A fee for organising education?: No

Funds for research?: No

Funds for a member of staff?: No

Fees for consulting?:

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Reviewer: 3

Recommendation:

Comments:
Manuscript number: BMJ.2017.03478
Title: "Congenital Zika syndrome: reduction if cerebral calcifications and cerebral volume on follow-up CT scans. Is there still viral activity"
Article type: Research
Reviewer: Patrick Gérardin, CHU Réunion.

General comment

The authors propose to the BMJ as a Research article the long-term follow-up study of CT scans of a cases series of 37 children with congenital Zika syndrome issued from the Recife's cohort, one of the best documented cohort of children affected by the consequences of congenital Zika virus infection. The data are original and expected given the great interest of scientific community on the long-term outcomes of these children. Consequently, this submission has urged editors to hasten the peer-review process through fast track. The paper is well-written and deserve publication. These things being said, I propose however major and minor revisions among which the critical point to revise is the statistical analysis which could be appropriate for independent observations but this is not the case here. Longitudinal data must be treated with statistics for paired series whenever possible (For example, in table 1, Mac Nemar tests on discordant pairs rather than Chi square tests) allowing to account for intra-individual correlations.

Major compulsory revisions
Methods.
Page 3. At the end of the method section, specify the appropriate statistic tests be used to account for intra-individual correlations observed with longitudinal data (the outcome status depends of baseline status, these are not independent observations for using statistical tests for independent data).
The statistics are flawed and must be repaired with appropriate test for paired data.
Results.
Information should be given on the timing of maternal infection (feta gestational age at exposure).
Page 5. Table 1. Chi square and Fisher tests for independent data are inappropriate, use Mac Nemar tests on discordant pairs (it is mandatory if discordant pairs are > 10).
Table 1.

Cerebral lobes Computed tomography (CT) Chi-square or
or Fisher tests
P valuesMac Nemar
tests
P values

	Initial n (%)	Follow-up n (%)		
Frontal				
Yes	35 (100)	33 (94.3)	0,493 *	Use Mac Nemar Test
No	0 (0)	2 (5.7)		
Parietal				
Yes	28 (80.0)	19 (54.3)	0,022 **	Use Mac Nemar Test
No	7 (20.0)	16 (45.7)		
Temporal				
Yes	22 (62.9)	21 (60.0)	0,806 **	Use Mac Nemar Test
No	13 (37.1)	14 (40.0)		
Occipital				
Yes	21 (60.0)	12 (34.3)	0,031 **	Use Mac Nemar Test
No	14 (40.0)	23 (65.7)		

(*) Fisher's exact test (**) Chi square test

Page 5. Lines 47 to 54. "The greater the reduction of cerebral volume in the initial CT scan, the greater the parenchyma reduction on follow-up CT scan" may be fair and expected. I will temper this statement given it is based on subjective assessment without adequate parenchyma brain volume evaluation. I would support this finding with appropriate data on head circumference evolution.

Page 6. Lines 23 to 31. It is written page 4 lines 12 to 14 that microcephaly was observed at birth in 35 out of 37 children, but it is also written here that 4 patients were "with normal cerebral volume on initial CT scan". How 2 children can have normal cerebral volume on initial CT scan and primary microcephaly ?

Discussion.

Page 8. Calcifications are deemed an indicator of viral infection of the CNS. Recapitulate in the first paragraph for non-specialists a few references demonstrating that calcifications are regularly of viral origin as well as their other aetiologies.

Page 8. Lines 36 to 41. It is written that cortico-subcortical calcifications vanished in one child and diminished in 34 other children, while calcifications persisted in the basal ganglia cerebellum. Discuss the possibility of non-viral origin for persistent calcifications (thrombo-vascular origin?)

Minor compulsory revisions

Methods.

Page 2. Lines 6 to 8. Recall definition of congenital Zika syndrome (CZS) used by the Brazilian Ministry of Health to allow further comparisons with consensual definition (Moore CA et al, JAMA Pediatr 2017).

Page 2. Lines 17 to 20. Explain in few words for non-Portuguese readers in what consist the protocol for the routine investigation of microcephaly in Brazil.

Page 3. Line 25. Specify if follow-up CT scans were all performed in the same centres than those used for the initial CT scan. Specify in what consists the qualitative analysis. In the absence of MRI-based volume assessment, it is reasonable to give the criteria on which qualitative assessment of brain volume was based.

Results.

Decimals in page 4 and tables must be written with point instead commas.

Page 4. Line 14. The definition of severe microcephaly using the Intergrowth-21st belongs to the method section.

Page 4. Lines 12 to 14. Two children were born without primary microcephaly. Did they experience microcephaly of postnatal onset or significant slowing of head circumference growth in early lifetime?

Figure 9 title. Flowchart demonstrates the frequency of cerebral volume reduction on initial CT scan.

Discussion.

Page 10. Lines 36 to 54. Zika virus neuropathogenesis results from apoptosis and clastic cytolysis of brain parenchymal cells. Apoptosis does not imply inflammation. Discuss the postnatal course of calcifications in other STORCH infections.
In the limitation section, discuss that the absence of MRI scan in this series does not allow to provide insights on brain reduction by demyelination.
Minor discretionary revisions
Introduction.
Turn the sentences to the past or the preterit. For instance, "the aim of the study was instead "is".
Page 1. Line 38. Explain in a few words for non-specialists and the BMJ broad readership what is a "colpocephaly".
Methods.
Turn the sentences to the past or the preterit. For instance, "Initial head CT scan showed instead "shows".
Discussion.
Given the locations of calcifications, discuss the pathogenesis of neuro-invasiveness.
Also discuss the possibility of microcephaly of postnatal onset and how it differs from parenchymal brain reduction by demyelination (as seen in Chikungunya virus-associated severe neonatal encephalitis).

Additional Questions:
Please enter your name: Gérardin Patrick

Job Title: Paediatrician and Epidemiologist

Institution: CHU Reunion

Reimbursement for attending a symposium?: No

A fee for speaking?: No

A fee for organising education?: No

Funds for research?: Yes

Funds for a member of staff?: No

Fees for consulting?: No

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