PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Fetal intracranial haemorrhages caused by fetal and neonatal alloimmune thrombocytopenia: An observational cohort study of 43 cases from an international multicentre registry
AUTHORS	Tiller, Heidi; Kamphuis, Marije; Flodmark, Olof; Papadogiannakis, Nikos; David, Anna; Sainio, Susanna; Koskinen, Sinikka; Javela, Kaija; Wikman, Agneta; Kekomaki, Riitta; Kanhai, Humphrey; Oepkes, Dick; Husebekk, Anne; Westgren, Magnus

VERSION 1 - REVIEW

REVIEWER	Professor M F Murphy Professor of Blood Transfusion Medicine, University of Oxford Consultant Haematologist, NHS Blood and Transplant and Oxford University Hospitals NHS Blood and Transplant John Radcliffe Hospital Headley Way Oxford, OX3 9BQ UK No conflicts of interest.
REVIEW RETURNED	14-Dec-2012

GENERAL COMMENTS	This is an important observational study characterising the occurrence of intracranial haemorrhage (ICH) in fetus/neonates affected by fetal and neonatal alloimmune thrombocytopenia (FNAIT). The key findings are the early occurrence of ICH, often before 28 weeks gestation, in the first born child. The severe clinical outcomes of ICH were also documented, as was the apparent relative ineffectiveness of antenatal management in subsequent pregnancies. Specific Comments 1. It is surprising that the Kamphuis systematic review is not used for the reference for the incidence of ICH in FNAIT. 2. Is there a reference to a publication of the NOICH study to provide further information for readers? 3. The case definition is clumsily written. The 3 sections have different ways of diagnosing FNAIT, but only the second refers to ICH. Surely, the case definition should be 1) a case of FNAIT, however defined, and 2) a documented ICH. 4. UK spelling should be used e.g. haemorrhage. 5. Was there really a case with both anti-HPA-5a and anti-HPA-5b?

	6. It might seem very basic but further details about the distinction between the primigravidae women (only 10) and those with a first-born child affected (27) by ICH due to FNAIT. Does that mean that 17 of those with a first-born child with ICH due to FNAIT were not primigravidae? Details of their previous obstetric history would be useful to present. 7. The presentation of data on the outcome of the subsequent pregnancies is inadequate. It would be helpful to have more details of the antenatal management, which apparently varied from no treatment to different schedules of intravenous immunoglobulin. It would be interesting to know if the failures occurred in pregnancies managed conservatively compared to say the schedules suggested by Pacheco et al. 8. It would also have been interesting to have more information on the HPA-1a antibody levels. Data only on the highest levels are provided in 15 (34%) of the FNAIT pregnancies affected by ICH. No information is provided about the timing of the measurements or the pattern of the levels in individual patients with multiple measurements, ideally related to the occurrence of ICH.

REVIEWER	James B. Bussel, MD Professor of Pediatrics and Professor of Pediatrics in Obstetrics and Gynecology and in Medicine Director, Platelet Research & Treatment Program Division of Pediatric Hematology Oncology Weill Cornell Medical College , USA
REVIEW RETURNED	04-Jan-2013

GENERAL COMMENTS	This is an interesting report of cases of antenatal hemorrhage secondary to alloimmune thrombocytopenia. Some of the findings are remarkable including the fact that a majority of the cases occur by the end of the second trimester; this is a completely novel finding. In addition, the paper is reasonably well written and is from groups that have a long history of active involvement in top notch serologic testing in this field such that there would be no doubt about the diagnosis in these cases. Nonetheless there are several issues that should be considered. First, the authors ignore a paper describing 37 antenatal hemorrhages in 33 women that was published in 2010 or 2011. That paper apparently describes the timing of the hemorrhages in the previous fetuses and thus is a retrospective study with much less detail than the current one. This study should obviously be referenced and included in the current report that the authors are submitting. Second, it would help to have some form of flow diagram that would give a better clue as to where the authors' patients have come from in that it would make it clearer as to what is the flow in terms of the timing of pickup and the perhaps antigen typing and overall outcome. This would definitely make things much easier. In general, some of the results are a little hard to follow. In the discussion it would be important to include the fact that one of

	the reasons for the predominant number of cases in the first affected fetus is that once it happens, many mothers do not want to become pregnant again and the ones that do are likely at the current time to get some form of antenatal management. As indicated, figure 1 is hard to follow and understand what is going on. Figure 2 provides interesting illustrations if slides are going to be made from it but I am not exactly sure what it adds to the manuscript per se.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Professor M F Murphy
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No conflicts of interest.

Comments to the Authors
This is an important observational study characterising the occurrence of intracranial haemorrhage (ICH) in fetus/neonates affected by fetal and neonatal alloimmune thrombocytopenia (FNAIT). The key findings are the early occurrence of ICH, often before 28 weeks gestation, in the first born child. The severe clinical outcomes of ICH were also documented, as was the apparent relative ineffectiveness of antenatal management in subsequent pregnancies.

Specific Comments
1. It is surprising that the Kamphuis systematic review is not used for the reference for the incidence of ICH in FNAIT.
Response: The Kamphuis systematic review has now been added as reference in the Introduction.

2. Is there a reference to a publication of the NOICH study to provide further information for readers?
Response: No, this is the first data to be published from the NOICH registry study, therefore only the homepage is given as reference. A manuscript describing the whole NOICH FNAIT study population is in preparation.

3. The case definition is clumsily written. The 3 sections have different ways of diagnosing FNAIT, but only the second refers to ICH. Surely, the case definition should be 1) a case of FNAIT, however defined, and 2) a documented ICH.
Response: We agree, and 2 subheadings have been removed and now both FNAIT case definitions and ICH definitions are written under the same subheading “Study design and inclusion criteria”. Also, the sentence “Cases where an ICH could not be confirmed were not included in the study” has been added to avoid misunderstanding.

4. UK spelling should be used e.g. haemorrhage.
Response: Ok

5. Was there really a case with both anti-HPA-5a and anti-HPA-5b?
Response: Since we do not have detailed information about the genotype of the mother (she could have a null genotype), we have changed the text and refer to anti-gpIa/IIa antibodies: “In one woman with records of two ICH cases, incompatibility in the gpIa/IIa system was confirmed and in one of her ICH pregnancies anti-HPA-5 antibodies were detected.”

6. It might seem very basic but further details about the distinction between the primigravidae women (only 10) and those with a first-born child affected (27) by ICH due to FNAIT. Does that mean that 17 of those with a first-born child with ICH due to FNAIT were not primigravidae? Details of their previous obstetric history would be useful to present.
Response: We have elaborated this section and it now reads: “However, most mothers had been pregnant before they had their first child: Eight women had one or more 1st trimester miscarriages and six women had one or more 2nd trimester losses. For two women, we lack data on gravida status. The mothers were primigravidae in only 10/37 (27%) index cases. In total, ten mothers (23%) experienced one or more 2nd trimester miscarriages (altogether 20) before or after the ICH case.”

7. The presentation of data on the outcome of the subsequent pregnancies is inadequate. It would be helpful to have more details of the antenatal management, which apparently varied from no treatment to different schedules of intravenous immunoglobulin. It would be interesting to know if the failures occurred in pregnancies managed conservatively compared to say the schedules suggested by Pacheco et al.
Response: We have elaborated on the ananatal management strategies for the subsequent pregnancies in the results section and added the following text:
“The intravenous immunoglobulin schedules varied greatly, with a median starting time 18 weeks (range 16-35 weeks).(…)
These two treatment failures were from the same woman: She received IVIG treatment from gestational week 17 in both pregnancies. In the first ICH recurrence pregnancy, fetal hydrops was detected by US at 19 weeks. Five intrauterine platelet transfusions were given before fetal death occurred at 26 weeks. In the second ICH recurrence pregnancy, an ICH was detected at 27 weeks. She received five intrauterine platelet transfusions before a live boy was delivered at 31 weeks. The boy had a large ICH and is mentally retarded and has CP.”

8. It would also have been interesting to have more information on the HPA-1a antibody levels. Data only on the highest levels are provided in 15 (34%) of the FNAIT pregnancies affected by ICH. No information is provided about the timing of the measurements or the pattern of the levels in individual patients with multiple measurements, ideally related to the occurrence of ICH.
Response: We agree that this is very interesting, and are happy to provide more details on anti-HPA-1a antibody levels. The following text has been added to the manuscript:
“In two pregnancies we have serial anti-HPA-1a antibody level measurements starting in 1st trimester, and in both these pregnancies the anti-HPA-1a antibody levels fell from the 1st to 3rd trimester. The ICH occurred between 28 and 34 weeks in these pregnancies. The median anti-HPA-1a antibody levels measured during 1st trimester, 2nd trimester, 3rd trimester and post partum did not differ significantly from each other or from the overall median highest antibody level (data not shown).”

Reviewer: James B. Bussel, MD
Professor of Pediatrics and
Professor of Pediatrics in Obstetrics and Gynecology and in Medicine Director, Platelet Research & Treatment Program Division of Pediatric Hematology Oncology Weill Cornell Medical College , USA

This is an interesting report of cases of antenatal hemorrhage secondary to alloimmune thrombocytopenia. Some of the findings are remarkable including the fact that a majority of the cases occur by the end of the second trimester; this is a completely novel finding. In addition, the paper is reasonably well written and is from groups that have a long history of active involvement in top notch serologic testing in this field such that there would be no doubt about the diagnosis in these cases.

Nonetheless there are several issues that should be considered.

First, the authors ignore a paper describing 37 antenatal hemorrhages in 33 women that was published in 2010 or 2011. That paper apparently describes the timing of the hemorrhages in the previous fetuses and thus is a retrospective study with much less detail than the current one. This study should obviously be referenced and included in the current report that the authors are submitting.
Response: The mentioned reference is now included and commented in the manuscript. We have added the following text to the discussion:
“In a recent study by Bussel et al,(13) antenatal management to prevent recurrence of ICH caused by FNAIT was studied. Gestational age at the time of ICH is reported in this study, but without any data with regards to how the timing of ICH was assessed. These data are therefore difficult to assess, but in support of our data they report that as many as 8/37 (22%) of ICH cases in their study population occurred before 28 gestational weeks.” (…) “(The present study suggests that IVIG treatment during pregnancy is protective in regard to ICH in most cases,) which is in accordance with previous studies (ref Bussel 2010)”

Second, it would help to have some form of flow diagram that would give a better clue as to where the authors’ patients have come from in that it would make it clearer as to what is the flow in terms of the timing of pickup and the perhaps antigen typing and overall outcome. This would definitely make things much easier.
Response: We have included a flow diagram as suggested (Figure 1)

In general, some of the results are a little hard to follow.
Response: Corrections in the text have been done which has made the result section easier to follow (se response to reviewer 1). Also the suggested flow chart considerable improved the presentation and we hope that this fulfill the reviewer’s requirement.

In the discussion it would be important to include the fact that one of the reasons for the predominant number of cases in the first affected fetus is that once it happens, many mothers do not want to become pregnant again and the ones that do are likely at the current time to get some form of antenatal management.
Response: We agree, and these comments have been included in the discussion as following: “The high number of first-born children in the study population may not necessarily mean that the risk of ICH caused by FNAIT is genuinely higher in the first-born child. The distribution could be skewed towards nulliparous women since these women may choose not to have more children due to high recurrence risk. Further, most of these women received antenatal treatment during the subsequent pregnancy thereby reducing the incidence of ICH in the younger siblings. Nevertheless, this finding challenges the current management strategy where antenatal treatment is given in subsequent pregnancies after FNAIT has been diagnosed in the first child.”

As indicated, figure 1 is hard to follow and understand what is going on.
Response: We acknowledge your concern, and we have discussed the complexity of this figure among the authors. We believe it is important to show the details of how the timing of bleeding onset

was evaluated for each case, since this is our main focus and also a major novel result.
Figure 2 provides interesting illustrations if slides are going to be made from it but I am not exactly sure what it adds to the manuscript per se.
Response: We have removed Figure 2 from the manuscript as suggested.