Chapter 6

IVIG for pregnancies at risk for FNAIT: an uncompleted randomised trial comparing 0.5 and 1.0 g/kg bodyweight

Van den Akker ESA, Westgren M, Husebekk A, Kanhai HHH, Oepkes D, for the NOICH-study group. Intravenous immunoglobulin for pregnancies at risk for fetal and neonatal alloimmune thrombocytopenia: an uncompleted randomised trial comparing 0.5 and 1.0 g/kg bodyweight. Submitted for publication.
ABSTRACT

Objective To test the hypothesis that Intravenous immunoglobulin (IVIG) in a low dose of 0.5 g/kg/wk is at least as effective as the standard dose of 1.0 g/kg/wk in preventing intracranial haemorrhage (ICH) and severe fetal thrombocytopenia in pregnancies at risk for fetal and neonatal alloimmune thrombocytopenia (FNAIT).

Design Two dose open-label randomised international multicentre trial.

Setting Four tertiary care centres in Sweden, The Netherlands and Australia.

Population Pregnant women with alloantibodies against human platelet antigen (HPA) and a previous child with FNAIT, without an ICH.

Methods Pregnant women were randomised to low dose (n=12) or standard dose (n=11) IVIG.

Main outcome measures Primary outcome was ICH in the fetus or neonate, detected by cranial ultrasound. Secondary outcomes included fetal platelet count in cord blood at birth, the nadir of the platelet count, the IgG levels during pregnancy, type of neonatal treatment needed and signs of bleeding other than ICH.

Results The Trial Steering Committee recommended the study to be stopped early. After two years of recruitment, only 23 patients of the calculated sample size of two arms of 106 patients had been randomised. For this rare disease, the participation of at least ten other referral centres was needed and anticipated, but could not be realised.

All but one of the patients received the allocated dose of IVIG. Survival was 100%. None of the neonates had an ICH, a difference of 0% (95% CI: -25.2 to 23.6%). The upper limit of the 95% CI of the difference was not less than the prespecified 5%, therefore it remains uncertain if there is equivalence. The median platelet count at birth was $81 \times 10^9/L$ (range 8-269) in the low dose group and $110 \times 10^9/L$ (range 11-279) in the standard dose group ($P = 0.644$).

Conclusion Whether the effectiveness of a low dose of IVIG is equivalent to the standard dose in the treatment of FNAIT remains uncertain. The trial can be
regarded as a successful pilot, showing feasibility and acceptability of the study design both for patients and clinicians, a possible basis for a larger randomised trial. We regard the use of 0.5 g/kg/wk IVIG in pregnant women with FNAIT and a previous child without ICH still an option, only advised however in the setting of prospective studies.

INTRODUCTION

Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is caused by platelet destruction due to specific maternal IgG antiplatelet antibodies crossing the placenta. These antibodies are produced after exposure of the antigen-negative mother to the paternally inherited antigens on the fetal platelets during pregnancy. In Caucasians, FNAIT is most often caused by human platelet antigen HPA-1a. Two percent of the Caucasian population is HPA-1a negative (HPA-1bb). Sensitisation occurs in 6-12% of HPA-1bb mothers. Unlike Rhesus disease, the first pregnancy is frequently affected.

Prospective studies have shown that HPA-1a immunisation occurs in 1 in 365 pregnancies. The major complication of the resulting thrombocytopenia is intracranial hemorrhage (ICH) in the fetus or newborn, with an estimated incidence of 1 in 10,000 to 20,000 pregnancies1-3.

Until recently, repeated fetal blood sampling and intrauterine platelet transfusions were the first choice of treatment of fetuses with alloimmune thrombocytopenia. Bussel et al. were the first to report the use of maternal administration of IVIG in the treatment of FNAIT4. In all seven cases reported, the fetal platelet count increased substantially after a weekly dose of 1 gram per kg maternal body-weight. Several recent studies have shown that non-invasive management using IVIG alone is the safest and most effective option currently available5,6.

The mechanism of action of IVIG in FNAIT is still unclear. Possible explanations are dilution of anti-HPA antibodies in maternal serum, blocking of the placenta receptor (Fc-R) and blocking of the Fc-receptors on the fetal macrophages7.

IVIG is an immunomodulatory drug, produced from multiple donor blood transfusions. Potential risks therefore are transmission of viral diseases such as hepatitis B, C and HIV, although donor screening and viral inactivation measures make these risks in Western countries low. Headaches and fever, as well as renal and cardiovascular dysfunction have been described8. The long-term side effects for mother and child are still unclear. One study on short term follow-up, found
an increase of IgE in children after maternal IVIG administration. At present, no clinically apparent adverse effects in early childhood have been demonstrated. As with any drug, especially in pregnancy, the lowest effective dose should be used since long-term safety is difficult to prove. Apart from the safety issue, IVIG is and will remain an expensive drug.

The empiric dose of 1.0 g/kg/wk has been commonly used since the first publication of Bussel et al. In FNAIT, no dose-effect studies have been done. Results of a recent study suggest that placental IgG is not further increased despite high IgG concentrations in the mother as a result from IVIG treatment. This suggests a saturation of the placental Fc-receptor and consequently a reduction in passage of antibodies to the fetus. Based on these data, a lower dose of IVIG is possibly as effective, likely to be safer and certainly less expensive.

The aim of our study was to determine whether 0.5 g/kg/wk of IVIG is as effective as 1.0 g/kg/wk, in the prevention of ICH in FNAIT.

METHODS

Study design
This was a prospective, open label randomised study of two treatment arms, performed in four tertiary care centres in three countries.

Patients
Women with a singleton pregnancy who previously gave birth to a child with a platelet count < 150 x 10^9/L in the first week of life due to HPA alloimmunisation. HPA alloimmunisation was confirmed by the presence of maternal anti-HPA antibodies and the offending HPA antigen in the fetus or homozygous father. In the case of a heterozygous father the platelet antigen genotype of the fetus was proven positive by testing amniocytes before 28 weeks. Gestational age at inclusion was between 12 and 28 weeks. The study was performed according to the principles described in the Declaration of Helsinki, and each patient gave written informed consent to participate in the study. Each local Institutional Review Board (IRB) approved the protocol.

Exclusion criteria included: women with autoimmune thrombocytopenia, multiple pregnancies, fetuses and neonates with major congenital anomalies or chromosomal abnormalities and women with a previous children with FNAIT and an ICH. Patients with immunoglobulin-A deficiency were only excluded if
they had a severe allergic constitution and patients who ever had an allergic reaction to blood products due to anti-IgA antibodies.

**Randomisation**
Randomisation was performed between 26 and 28 weeks’ gestation, after stratification for centre and for HPA-1a and non-HPA-1a, by the Central Internet Database Service (www.medscinet.net), through the study website (www.noich.org). The patient was randomised to either the low dose group, IVIG 0.5 gram per kg maternal bodyweight at the time of injection per week, or the standard dose group, IVIG 1.0 g/kg/wk.

**Used medication**
The IVIG used in this study was immunoglobulin from the company the clinicians were used to work with was given weekly, in a day-care setting, starting at 28 weeks and continued until delivery. Products used were Freeze-dried Immunoglobulin IV (CLB Sanquin Amsterdam) and Gammagard (Baxter). The IVIG infusions were administered over a period of 3 to 6 hours, according to the tolerance. Side effects and complications were recorded in the MedSciNet database.

**Management protocol**
Before the first IVIG treatment, and every two weeks thereafter, fetal ultrasound examination was done to rule out ICH. The total IgG levels were measured pre-delivery in maternal serum and postpartum in the umbilical cord blood.

The choice for timing and mode of delivery, elective Caesarean section or intended vaginal birth was left to the discretion of the obstetrician obviously with consent from the patient. Standard recommendations at vaginal delivery were not to use fetal scalp electrodes or scalp blood samplings and to refrain from ventouse or forceps application.

Immediately after birth the platelet count in the umbilical cord blood was measured in the local laboratory, first automatically and, in case of a platelet count ≤ 100 x 10⁹/L, a manual count. In all centers where the deliveries took place, HPA compatible platelets were required to be available within 12 hours after delivery. A neonatologist examined all neonates directly after birth. Within the first days after birth, an ultrasound of the neonatal cerebrum was performed. Signs of minor and major bleedings were recorded. Neonatal management was left to the discretion of the neonatologist. The course of the neonatal platelet count was noted, together with any form of treatment for thrombocytopenia.
Laboratory investigations
After centrifugation, maternal and cord serum samples were stored at -80 degrees. Maternal and cord blood sera were assayed for their total IgG levels by nephelometry (Behring Diagnostics Ltd, Ubridge, Middlesex, UK).

Outcomes
The primary outcome measure was fetal or neonatal ICH. Secondary outcome measures were platelet count in the umbilical cord blood at birth, the total IgG levels in maternal serum, the total IgG levels in cord blood, the occurrence of other signs of bleeding in the neonate and type of neonatal treatment. Any maternal and neonatal adverse event possibly associated with IVIG treatment was recorded.

Sample size calculation and statistical analysis
The required number of patients to prove that low dose IVIG is not associated with a worse outcome as compared to high dose, depends on the expected frequencies of adverse outcome in both groups. The study was set up as an equivalence trial. The null hypothesis was that the standard dose (1.0 g) is superior. We wanted to test if the lower dose, (0.5 g), was not inferior. We assumed that the probability of failure (occurrence of ICH) was 1%, if both groups were equal. For sample size calculation, we assumed that the low and the standard doses had the same risk of failure. We estimated a 5% specified maximal difference, meaning that the lower dose is inferior if the risk of failure is 5% higher than in the standard dose group. For a power of 80% and a one-sided 5% significance level, this means that 106 patients in each group were needed to reject the null hypothesis. For the primary end point, the treatments are considered equivalent if the upper limit of the confidence interval of the difference is less than 5%\(^\text{12}\). The confidence interval for the difference in proportion was calculated using the quasi-exact method by Chen\(^\text{13}\).

Analysis was performed on the intention-to-treat principle, which comprised all randomised patients who received at least one dose of study medication. The Kruskal-Wallis test was used to compare continuous variables. Categorical variables were assessed with Fisher’s exact test. All data are expressed as mean (SD) or median (range), and a \( P \) value < 0.05 was considered significant. Calculations were performed using SPSS 15.0 for Windows statistical package (SPSS Inc., Chicago, IL, USA).
RESULTS

Recruitment for the study started on January 1, 2005. The first patient was randomised on January 10. At the start of the study, a total of 15 centers in eight countries agreed to the protocol and gave verbal assurance to their participation. We estimated that the inclusion of the required 212 patients would take three years. We had estimated that the specialised centers in The Netherlands, Germany, England and Canada, should be able to include 30 patients each, Sweden, Scotland and Switzerland were expected to include 20 patients each and Norway and Denmark 15 patients each.

Despite regular contact between the trial steering committee and the responsible investigators-to-be in these centres, only four centers, in the Netherlands, Sweden and Australia, managed to actually recruit patients for the study. In September 2007 a total of only 23 pregnancies had been randomised, which led to the decision by the steering committee to advise on prematurely ending the recruitment. Some of the reasons mentioned for not participating were inability to obtain IRB approval, an even lower number of eligible patients seen leading to a loss of interest in the study, and being too busy with projects of higher priority.

The last patient that was randomised delivered in December 2007. A total of 26 patients had been eligible for the study in the four participating centers. All but one of the randomised patients received the assigned treatment. One patient requested to change from 0.5 g to 1.0 gram per kg per week in week 34. The number of patients in each group with details of the flow through the stages of the trial is given in figure 1.

The baseline characteristics of all randomised patients are given in table 1, showing no statistically significant differences between the two groups.

<table>
<thead>
<tr>
<th>Table 1: Patient characteristics</th>
<th>IVIG 0.5 g/kg/wk n=12</th>
<th>IVIG 1.0 g/kg/wk n=11</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>31 (29-39)</td>
<td>32 (24-43)</td>
<td>0.597</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (1-2)</td>
<td>1 (1-3)</td>
<td>0.810</td>
</tr>
<tr>
<td>Number of doses IVIG</td>
<td>10 (7-11)</td>
<td>11 (7-12)</td>
<td>0.436</td>
</tr>
<tr>
<td>Caucasian</td>
<td>12</td>
<td>11</td>
<td>1.0</td>
</tr>
<tr>
<td>HPA-1a</td>
<td>11</td>
<td>11</td>
<td>0.338</td>
</tr>
<tr>
<td>Platelet count (nadir) of sibling</td>
<td>17 (5-70)</td>
<td>11 (2-45)</td>
<td>0.532</td>
</tr>
</tbody>
</table>

Data shown as number or median (range)

IVIG: Intravenous Immunoglobulin
**Figure 1:** Flow diagram, showing the progress through the study of pregnancies at risk of FNAIT, treated with low or standard dose IVIG.

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Assessed for eligibility (n=26)

Enrollment
Randomised

Excluded (n=3)
Not meeting inclusion criteria (n=1)
Refused to participate (n=2)

Allocated to standard dose IVIG (1.0 g/kg/wk) (n=11)
Received allocated intervention (n=11)

Allocated to low dose IVIG (0.5 g/kg/wk) (n=12)
Received allocated intervention (n=11)

Lost to follow-up (n=0)
Discontinued intervention (n=0)

Follow-Up
Lost to follow-up (n=0)
Discontinued intervention (n=1)

Analysed (n=11)
Excluded from analysis (n=0)

Analysis
Analysed (n=12)
Excluded from analysis (n=0)
```
In Table 2 the primary and secondary outcomes (ICH, platelet count at birth and nadir in first week, neonatal survival, neonatal treatment, maternal and fetal side effects and mode of delivery) are given. Perinatal survival was 100%, no fetal or neonatal intracranial haemorrhages were observed. The difference in primary outcome between the two groups was therefore 0%, with a 95% confidence interval of –25.2% to 23.6%.

Table 2: Outcome of pregnancies with FNAIT after low or standard dose IVIG treatment

<table>
<thead>
<tr>
<th>Outcome</th>
<th>IVIG 0.5 g/kg/wk</th>
<th>IVIG 1.0 g/kg/wk</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal ICH</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Neonatal ICH</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Platelet count at birth</td>
<td>81 (8-269)</td>
<td>110 (11-279)</td>
<td>0.644</td>
</tr>
<tr>
<td>Nadir of platelet count in 1st week</td>
<td>71 (8-266)</td>
<td>110 (9-202)</td>
<td>0.943</td>
</tr>
<tr>
<td>Signs of bleeding (non-ICH)</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Perinatal survival</td>
<td>12 (100%)</td>
<td>11 (100%)</td>
<td>1.0</td>
</tr>
<tr>
<td>IVIG in neonatal period</td>
<td>1 (8%)</td>
<td>0</td>
<td>0.338</td>
</tr>
<tr>
<td>Platelet transfusions in neonatal period</td>
<td>2 (17%)</td>
<td>3 (27%)</td>
<td>0.547</td>
</tr>
<tr>
<td>Maternal side effects</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Fetal or neonatal adverse events</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Gestational age at birth</td>
<td>38+6 (34+3-39+4)</td>
<td>38+6 (34+3-38+5)</td>
<td>0.665</td>
</tr>
<tr>
<td>Vaginal birth</td>
<td>7 (58%)</td>
<td>10 (91%)</td>
<td>0.037</td>
</tr>
<tr>
<td>Planned caesarean section</td>
<td>4 (33%)</td>
<td>1 (9%)</td>
<td>0.168</td>
</tr>
<tr>
<td>Emergency caesarean section</td>
<td>1 (8%)</td>
<td>0</td>
<td>0.338</td>
</tr>
<tr>
<td>Birth weight (gram)</td>
<td>3087 (1940-3650)</td>
<td>3420 (2605-3750)</td>
<td>0.049</td>
</tr>
<tr>
<td>Platelet count &lt; 30 x 10^9/L</td>
<td>1 (8%)</td>
<td>2 (18%)</td>
<td>0.493</td>
</tr>
<tr>
<td>Platelet count &lt; 50 x 10^9/L</td>
<td>3 (25%)</td>
<td>4 (36%)</td>
<td>0.563</td>
</tr>
<tr>
<td>Platelet count &lt; 150 x 10^9/L</td>
<td>9 (75%)</td>
<td>7 (64%)</td>
<td>0.563</td>
</tr>
</tbody>
</table>

Data shown as number (%), or median (range)
ICH: intracranial hemorrhage; IVIG: Intravenous Immunoglobulin
Table 3 shows the maternal and cord blood IgG concentrations at birth for the two groups, compared to the levels in the normal population. Maternal IgG levels, as expected, were higher than in untreated pregnant women, no differences were observed between the two treatment regimens. Cord blood IgG levels were similar in the three groups.

Table 3: IgG concentrations (medians and ranges, g/L) at delivery in maternal and cord blood sera, in pregnancies with FNAIT treated with low (0.5 g/kg/wk) or standard dose (1.0 g/kg/wk) IVIG, compared with published reference ranges in normal pregnancies.

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Total IgG concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low dose IVIG</td>
</tr>
<tr>
<td>Cord blood</td>
<td>16.0 (14.4-21.1)</td>
</tr>
<tr>
<td>Maternal serum</td>
<td>19.4 (17.8-24.1)</td>
</tr>
<tr>
<td>Cordblood / Maternal serum</td>
<td>0.82 (0.63-1.08)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The purpose of our study was to compare the effectiveness of a low dose of intravenous immunoglobulin, 0.5 g/kg maternal weight per week with the commonly used dose of 1.0 g/kg/wk, to prevent fetal or neonatal ICH in pregnant women with HPA antibodies. An equally effective lower dose of this expensive multido- nor blood product in pregnancy would likely be safer and certainly less costly.

The trial however, had to be ended prematurely after three years, in which we managed to randomise only 23 of the planned 206 pregnant women. The main reason was a much lower than anticipated number of participating centres. Since the decision to end the trial prematurely was taken purely for administrative reasons, we agree with Kahn and Hills that the results have to be taken as they stand, and need to be shared honestly with the scientific community.

The trial design and workflow seemed both feasible and acceptable to all collaborating clinicians in the participating centres. Almost all eligible patients agreed to participate and completed the assigned intervention. These facts, together with the absence of any difference in primary or secondary outcomes of the study, lead us to suggest that this study might be regarded as a successful pilot study, a possible basis for a larger randomised trial. We regard the use of...
0.5 g/kg/wk IVIG in pregnant women with FNAIT and a previous child without ICH still an option, only advised however in the setting of prospective studies. We obviously will refrain from any speculation on possible outcome of the trial in case of completion.

None of the children in our study suffered from ICH. Only one other trial has been published using this clinically most relevant parameter as the primary outcome measure. They randomised 73 patients in five years, in 36 different centres, comparing IVIG 2.0 g/kg/wk to IVIG 1.0 g/kg/wk plus prednisone. Power calculations were not given. One child in each group had a mild ICH, apparently unrelated to FNAIT since both children had a platelet count > 100 x 10^9/L at birth. Apart from more maternal side-effects in the prednisone group, no significant differences between the treatment regimens were found.

The commonly quoted increased severity of FNAIT in subsequent pregnancies is based on a few small case-series. The true risk for ICH in case of an previous pregnancy with FNAIT but without a fetal or neonatal ICH is unknown, and possibly very low. Since only fetuses with platelet counts < 50 x 10^9/L or < 30 x 10^9/L are supposed to be at risk for ICH, the use of the fetal or cord blood platelet count as surrogate outcome measure seems a logical choice. In our study, 8% in the low dose group and 18% in the standard dose group had platelet counts < 30 x 10^9/L at birth, a nonsignificant difference. The first published randomised trial comparing different treatment protocols for FNAIT used platelet counts as primary outcome. They compared IVIG 1.0 g/kg per week with or without dexamethasone in 54 patients randomised in three years in 18 different centres. Dexamethasone appeared not to add to the effect of IVIG, with 5/26 versus 6/28 patients with cord platelet counts < 30 x 10^9/L. A Cochrane review concluded that their sample size was inappropriately calculated, leading to insufficient power to determine any significance in difference between the groups.

The same group published a second study describing two parallel trials, one with 40 patients and one with 39 patients, taking seven years and participation of 42 centres to complete. Sample size calculations were not given. In the first trial, IVIG plus prednisone appeared to result in a higher platelet count at second sampling than IVIG alone. In the second trial, with patients comparable to our group, no differences in platelet counts between the treatment groups were found, with a total of 6/39 patients with cord platelet counts < 50 x 10^9/L.
We found that the maternal IgG levels were twice as high after IVIG treatment, independent of the dose, compared to published IgG levels in normal pregnancies. The IgG levels in cord blood were also similar in both treatment groups, however they were within the same range as published IgG levels in untreated controls. These results support the assumption that fetal IgG levels do not increase with higher IgG levels in the mother. The proposed working mechanism of IVIG by saturating the placental Fc-receptor as described by Urbaniak might already work at the low dose of 0.5 g/kg/wk23.

Several lessons can be learned from the attempts, including our own, to perform randomised trials in this rare disease. Firstly, the only clinically relevant outcome measure is fetal or neonatal ICH, for which the risk in subsequent, treated pregnancies is not well known and possibly much lower than generally assumed. The few and consistently underpowered published trials all suggested absence of difference between the various compared treatments. No studies incorporating a placebo group have been published. Therefore in our view, none of the currently recommended management protocols are based on adequate evidence.

Secondly, although the true incidence of ICH in untreated pregnancies with a history of FNAIT but without a sibling with ICH is unknown, the use of IVIG seems very effective in preventing ICH. Therefore, although a dose-finding trial should ideally include a placebo-arm, it is unlikely that this would be acceptable for both patients and clinicians. Given the rarity of the occurrence of ICH in this group of patients, the use of this parameter as primary outcome measure results in sample sizes that seem very difficult to obtain. The surrogate outcome of a low platelet count may be more practical, with an incidence of around 20% in most studies published thusfar. Such a design however cannot address the suggested protective effect of IVIG on fetal endothelium, reducing the likelihood of ICH even in case of very low platelet counts, as suggested by Radder et al24.

Thirdly, international multicentre studies running for long periods of time are likely the only option to complete adequately powered trials. This requires commitment from colleagues who may only deal with a handful of FNAIT pregnancies annually, and for whom this particular disease is not a main research subject. These and other large multicentre trials on the management of rare fetal disorders may benefit from international organisations such as the International Society for Fetal Medicine and Surgery (IFMSS), the North-American Fetal Therapy Network (NAFTNET) and the Eurofoetus initiative. Such networks may arrange or support web-based data-sharing facilities and funding to be used for jointly
by researchers studying rare diseases, helping each other to complete large scale projects.

In conclusion, our trial failed to show equivalence of the two doses according to our prespecified criteria, thus it provides no support for the recommendation to lower the standard dose of IVIG of 1.0 g/kg/wk. However, there is also a lack of evidence for using the 1.0 g/kg/wk. Both based on previous in vitro studies and the current trial results, it is certainly possible that 0.5 g/kg/wk is as effective as 1.0 g/kg/wk. Taking safety and costs into account, treating new patients at risk for FNAIT without a sibling with an ICH with the low dose may be an option, although we would strongly recommend to restrict this to patients who give informed consent to participate in a formal prospective study.

REFERENCES


Kahn KS, Hills R. Can we trust the results of trials that are stopped early? BJOG 2006; 113: 766-768.


FETAL THROMBOCYTOPENIA: PREVENTIVE STRATEGIES