Fetal alloimmune thrombocytopenia and maternal intravenous immunoglobulin infusion

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background
Different therapeutic approaches have been used in fetal-neonatal alloimmune thrombocytopenia, but many centers administer immunoglobulin G infusions to the pregnant woman. We studied the effect of maternal antenatal immunoglobulin infusions on fetal platelet counts in pregnancies with fetal alloimmune thrombocytopenia.

Design and Methods
We retrospectively analyzed the clinical courses of fetuses with fetal alloimmune thrombocytopenia whose mothers were treated with immunoglobulin G infusions in a single center between 1999 and 2005. In a center-specific protocol, weekly maternal immunoglobulin G infusions were given to 25 pregnant women with previously affected neonates and four women with strong platelet antibodies, but no previous history of fetal alloimmune thrombocytopenia; before each infusion diagnostic fetal blood sampling was performed to determine fetal platelet counts and immunoglobulin G levels.

Results
There were 30 fetuses with fetal alloimmune thrombocytopenia, confirmed by initial fetal blood sampling showing fetal platelet counts between 4×10^9/L and 130×10^9/L and antibody-coated fetal platelets using a glycoprotein specific assay. Despite weekly antenatal maternal immunoglobulin G infusions fetal platelet counts did not change significantly. Maternal and fetal immunoglobulin G levels, measured before every infusion, increased significantly with the number of maternal immunoglobulin G infusions.

Conclusions
In this group of fetuses with fetal alloimmune thrombocytopenia no consistent increase of fetal platelets was achieved as a result of regular maternal immunoglobulin G infusions.

Key words: fetal alloimmune thrombocytopenia, maternal IgG infusion, intrauterine therapy.

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Introduction

In fetal alloimmune thrombocytopenia fetal platelets are destroyed due to maternal allo-antibodies directed at paternally inherited human platelet antigens (HPA). Neonates with severe thrombocytopenia suffer from skin and mucous membrane petechiae and 10-20% develop intracranial hemorrhage.[1-3] Besides the more invasive therapeutic approach of repeated intrauterine transfusions of compatible platelets, intravenous (i.v.) administration of high-dose (non-specific) immunoglobulin G to the pregnant mother has been used.[1,4-14] In this retrospective observational study we analyzed fetal and maternal immunoglobulin levels and fetal platelet counts under such a regime.

Design and Methods

Patients

Data from 29 pregnant women (28 singletons and one twin pregnancy), treated between 1999 and 2005, were analyzed retrospectively. These women, who were otherwise healthy, comprised 25 referred because they had previously delivered neonates with fetal alloimmune thrombocytopenia (defined as a platelet count below 100×10^9/L and antibody-coated platelets, as measured by a platelet glycoprotein-specific assay; molecular analyses not consistently done) as well as hematoma or skin bleeding at birth, and four women with prior spontaneous abortions found to carry high titers of platelet antibodies. The 25 women with a previous history of fetal alloimmune thrombocytopenia had delivered between one and four affected neonates, but none with intracranial hemorrhage. All pregnant women had normal platelet counts, normal immunoglobulin G levels and no clinical signs or history of bleeding. All parents had been genotyped for HPA.

Serological and molecular testing

EDTA-anticoagulated and natural blood samples were obtained from all pregnant women and fetuses for a full blood count including platelet count, assays of overall immunoglobulin levels and anti-HPA antibody levels and, at the initial sampling, antigen testing. The paternal genotype was also determined at the beginning of each treatment course.

Platelets were counted using an automated platelet counter (Sysmex model E 5000, Digitana, Hamburg, Germany). In addition, blood smears with Pappenheim staining were performed in each case in order to determine whether platelet agglutinates were present or not.

At each maternal blood sampling the previously diagnosed maternal HPA antibodies were confirmed by immunofluorescence and monoclonal antibody-specific immobilization of platelet antigen (MAIPA) assay.[15] The titer of HPA antibodies was considered "high" for MAIPA photometric extinction units at 492-690 nm from 0.81 – 2.0, "medium" for an extinction between 0.41–0.8 and "weak" for extinction between 0.2–0.4.

The fetal origin of the intrauterine blood samples was confirmed by measuring mean corpuscular volume[16] and by the Kleihauer-Betke test (acid elution test[17]). In addition to serological typing, at each first fetal blood sampling the fetal HPA genotype was determined.[18]

Treatment protocol

Once the diagnosis of fetal alloimmune thrombocytopenia had been confirmed by invasive testing, the pregnant women received weekly infusions of intravenous immunoglobulin (Endobulin® Immuno GmbH, Heidelberg, Germany) at a dose of 1g/kg body weight. All cases were managed identically in a specific protocol by one highly experienced team. In order to measure fetal platelet counts (and to administer an intrauterine transfusion only at the last fetal blood sampling immediately before Cesarean delivery), a 22-gauge spinal needle was inserted through the anterior abdominal wall of the woman into the umbilical vein under sonographic guidance. From 1 to 3 mL of blood was then removed for the measurement of fetal platelet count, determination of antibody status by indirect MAIPA assay (only at the first and last fetal blood samplings) and assay of immunoglobulin G level. The fetuses did not receive direct sedation, but the pregnant women were given 7.5 mg midazolam intravenously prior to the fetal blood sampling.

No more than 4 hours before delivery, which was by Cesarean section in all cases, each fetus received one intrauterine platelet transfusion. These transfusions were done with platelets prepared from either maternal (n=22) or group O, HPA compatible platelets (n=7). All neonates were examined clinically for isolated or confluent petechiae, hematoma, mucous bleeds, including sonographic examination of the abdomen and brain.

Treatment was performed according to the institutional standard protocols in effect at the time. Informed consent was obtained from all pregnant women, and the study was approved by the Institutional Review Board.

Platelet preparations for intrauterine transfusions

Maternal and donor platelets were selected and prepared as described previously.[19,20] Unrelated donors had group O red blood cells and were negative for cytomegalovirus antibodies. They were HPA-typed and matched and lacked the antigen corresponding to the individual maternal platelet antibodies as well as platelet, HLA and cytomegalovirus antibodies. The donor platelets were collected using an automated hemapheresis procedure (MCS+, Haemonetics, Braintree, Massachusetts, USA) and leukocyte-depleted.[20]

Statistical methods

The individual courses of fetal platelet counts and maternal and fetal immunoglobulin G levels as well as the ratio between maternal and fetal immunoglobulin levels were first analyzed visually in XY plots over gestation.

For detailed statistical analysis, the maximum likelihood method was used to estimate maternal and fetal immunoglobulin G levels and fetal platelet counts longitudinally for gestational ages for which there were sufficient numbers of cases. This method assumes a missing value mechanism ("missing at random") which is independent of the non-measured data instead of simple assumption of non-randomness.[21-23] The missing-at-random method was empirically validated by Cox regression results. Because fetal platelets counts were not normally distributed, a logarithmic transformation was applied prior to the analysis. All statistical calculations were performed using a commercial statistics package.[24]

Results

Patients' characteristics

All 29 women included in this study had been pregnant before (between two and seven times, median three deliveries). Twenty-five women had previously given birth to between one and four neonates with neonatal alloimmune thrombocytopenia. Four women did not have a positive history of neonatal alloimmune thrombo-

cytopenia; these women had had between two and four previous pregnancies ending in spontaneous abortions and showed high levels of HPA antibodies.

Maternal antibody specificities were anti-HPA-1a in 25 women, anti-HPA-3a in two and anti-HPA-5b in another two women; the HPA antibody were directed towards paternally inherited platelet antigens in all cases. Maternal HPA-1a antibodies scores were higher (extinction mean=1.48; range=1.02-1.93) than those for HPA-3a (n=2: extinctions 0.65 and 1.0) and HPA-5b (n=3; extinctions 1.24, 0.5, 1.11). Maternal HPA antibody levels, assessed at almost every fetal blood sampling, did not change significantly ($P<0.05$; *Online Supplementary Figure S1*) despite repeated fetal blood sampling.

Clinical courses

Treatment was begun promptly when the patient first presented (between 20 and 33 weeks' gestation, median 27 weeks) and continued until delivery, which occurred at a median gestational age of 35.3 weeks (range, 35.0-37.1 weeks).

Overall, 219 fetal blood samples were taken, with between two and 14 samples per fetus (median, seven). Only at the final fetal blood sampling did each fetus receive an intrauterine platelet transfusion which raised the fetal platelet count at delivery to between 40×10^9/L and 376×10^9/L (median 178×10^9/L). There were no procedure-related fetal complications. None of the fetuses or neonates had bleeding complications (major or minor) at birth, and there were no fetal or neonatal losses. No adverse events occurred during or after the immunoglobulin infusions.

Fetal platelet counts

At the time of the first blood sampling, at a mean gestational age of 27.0 weeks (range, 20.0-33.0 weeks), the mean fetal platelet count was 56.3×10^9/L (range, 4×10^9/L – 130×10^9/L; median, 43.5×10^9/L). The mean minimal fetal platelet count during gestation was 21.5×10^9/L (range, 4×10^9/L – 60×10^9/L; median, 19.5×10^9/L). The average platelet count before birth (and before the single intrauterine platelet transfusion immediately prior to

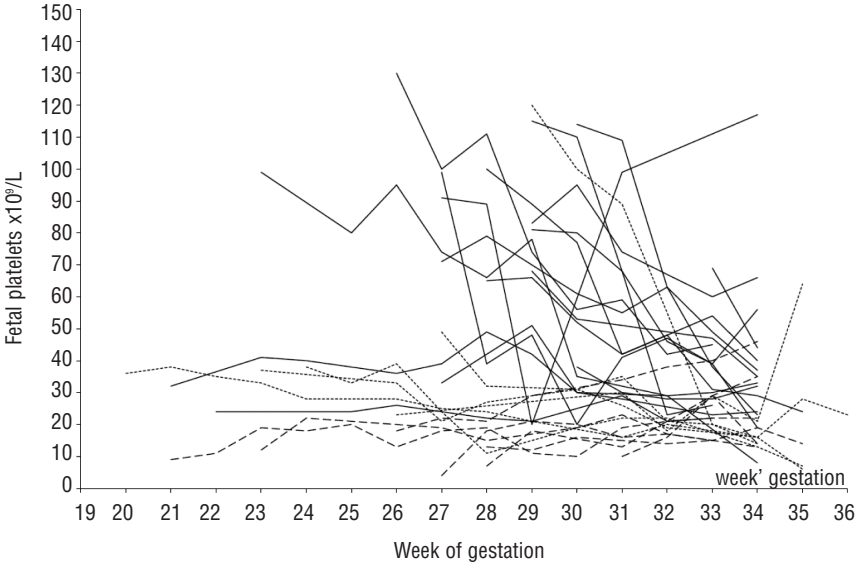

Figure 1. Platelet counts in 30 fetuses with fetal alloimmune thrombocytopenia whose mothers received weekly intravenous immunoglobulin G. There was a trend towards a decrease in fetal platelet counts during weekly maternal immunoglobulin G infusions. The dashed lines indicate fetuses with an initial platelet count ≤20x10⁹/L, the dotted lines fetuses with higher starting values which then fell to ≤20x10⁹/L; solid lines indicate all other fetuses.

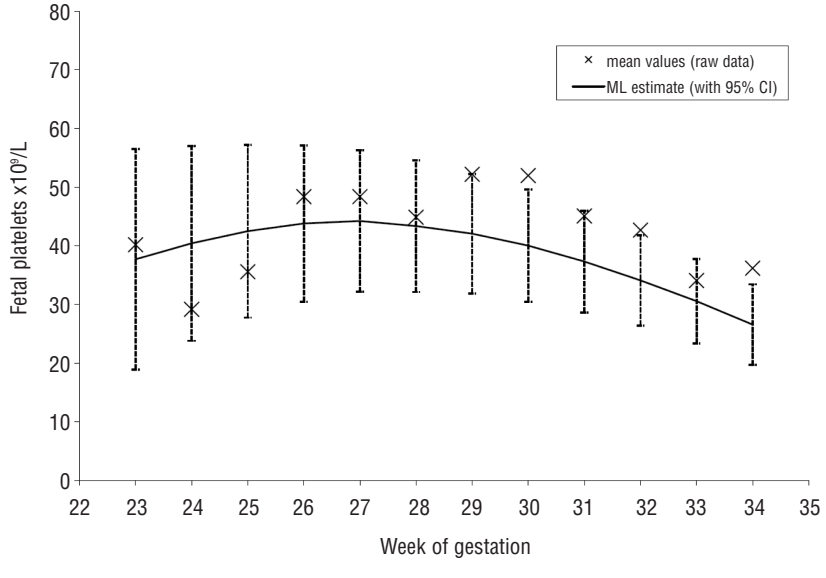

Figure 2. Mean platelet counts (actual values and statistical model) in fetuses with alloimmune thrombocytopenia, whose mothers were treated with intravenous immunoglobulin G, between 23 and 34 weeks' gestation. The X symbols indicate mean values of the raw data, the solid line indicates the mean values from the maximum likelihood estimation and the vertical bars indicate the 95% confidence intervals for each week's estimate.

delivery) was 31.3×10⁹/L (range, 6×10⁹/L -117×10⁹/L; median, 24×10⁹/L). Throughout gestation, 17/30 (57%) of the fetuses had minimum intrauterine platelet counts less than or equal to 20×10⁹/L. Only four fetuses (13%) had a final platelet count above 50×10⁹/L immediately before their pre-delivery intrauterine platelet transfusion. The individual fetal platelet counts during weekly maternal intravenous immunoglobulin G infusions are shown in Figure 1 and the statistical model for these mean fetal platelet counts in Figure 2.

There was no consistent increase of fetal platelet counts despite the maternal immunoglobulin G infusions, but a trend towards decreasing fetal platelet counts was noted from 26 to 34 weeks of gestation. Longitudinal analysis of the log-transformed data confirmed that there was a decrease in fetal platelets between the first measurements at 26 weeks and the last at 34 weeks despite maternal immunoglobulin G infusions (Figure 2). The numerical data for the statistical analysis of fetal platelet counts between 26 and 34 weeks are presented in *Online Supplementary Table S1*.

Levels of maternal and fetal immunoglobulin G and human platelet antigen antibodies

Both fetal and maternal immunoglobulin G levels increased in women receiving weekly maternal intravenous immunoglobulin infusion for fetal alloimmune thrombocytopenia (*Online Supplementary Figures S2* and *S3*; *Online Supplementary Tables S2* and *S3*). There were no significant changes in maternal HPA antibody levels, as determined by indirect MAIPA (*Online Supplementary Figure S1*) or fetal HPA antibody levels (*data not shown*). The ratio between maternal and fetal immunoglobulin levels remained constant in the majority of mother-fetus pairs with maternal levels being about three times higher than fetal levels (*data not shown*).

Discussion

Treatment options for fetal alloimmune thrombocytopenia include: (i) very preterm delivery at 32 weeks, accepting the risk of intracranial hemorrhage known to occur as early as 14-16 weeks' gestation; (ii) repeated maternal immunoglobulin G infusions;[25,26] (iii) repeated fetal platelet transfusions with a cumulative procedure-related risk; and (iv) serial scans with the questionable option of late termination.[27,28] In order to assess efficacy of any treatment and to balance the risks and benefits of non-invasive and invasive approaches, the natural course of the disease should be known. However, truly longitudinal data on the natural course of fetal platelets in fetal alloimmune thrombocytopenia are lacking.

The initial treatment proposed for fetal alloimmune thrombocytopenia was intrauterine platelet transfusions,[5,29] but other approaches without the risk of fetal cordocentesis were soon explored. Maternal (i.e. transplacental) administration of steroids[30] or maternal non-specific immunoglobulin G infusions without or with additional steroids[4] produced responses in 30-85% of cases.[31] However, as the intention of these less invasive treatments was to reduce or ultimately avoid cordocentesis,[32] longitudinal data from individual fetuses were not available to judge the therapeutic effect of the maternal treatments. For example, in a summary of three multicenter trials with data from 107 fetuses with fetal alloimmune thrombocytopenia treated with maternal immunoglobulin infusion with or without additional steroids,[1] the 107 fetuses had a mean initial platelet count of 38×10⁹/L (median, 23×10⁹/L) at a mean gestational age of 25 weeks (range, 17-36 weeks). The pregnant women received repeated administrations of intravenous immunoglobulin G, but there was no follow-up measurement of fetal platelet counts. At birth, the average platelet count, for the 98/107 neonates for whom data were available, was 25×10⁹/L (median, 20×10⁹/L). One of the treated fetuses had an intracranial hemorrhage.

Berkowitz reported the results of a multicenter trial conducted in 42 institutions, contributing data from a total of 79 fetuses whose mothers were treated with intravenous immunoglobulin G and, some, also with maternally administered steroids.[9] In the "standard" and "high risk" subgroups "(platelets <20×10⁹/L or affected older sibling) initial platelet counts (at an average of 24 weeks' gestation) were 56×10⁹/L and 8.5×10⁹/L, respectively. Some, but not all fetuses had follow-up platelet counts, and the actual number of intrauterine transfusions for platelet counts below 50×10⁹/L, as per protocol, was not given.

Kaplan *et al.* analyzed 37 cases of fetal alloimmune thrombocytopenia, excluding "more seriously affected cases", treated with intravenous immunoglobulin G or steroids with initial and follow-up fetal blood sampling.[33] They stratified the fetuses according to outcome and platelet counts. Maternal immunoglobulin G infusion was considered successful in only 7/27 cases (26%) and steroids in only 1/10 (10%).

Birchall *et al.* reported the effects of different forms of treatment for fetal alloimmune thrombocytopenia, stratifying 56 fetuses according to whether the mother was administered intravenous immunoglobulin G, steroids or both.[34] Treatment with maternal intravenous immunoglobulin G was considered successful in 12/18 (67%) fetuses (10 treated with intravenous immunoglobulin alone, and 2 with additional steroids).

In the current study, we analyzed data from 30 fetuses treated by one team only and with longitudinal data on fetal platelet counts (up to 14 fetal blood samples per fetus), representing the largest data set of patients and fetal platelet counts from a single centrer with a high level of expertise in fetal procedures.[9,10] To the best of our knowledge, ours is the only cohort with such a rich fetal follow-up. Platelet counts at first sampling ranged from 4×10⁹/L to 130×10⁹/L with a mean of 44×10⁹/L and a median of 56×10⁹/L. Almost one quarter (23.3%) of fetuses with fetal alloimmune thrombocytopenia had an initial platelet count of 20×10⁹/L or less at the first blood sampling between 20 and 33 weeks, but 17/30 (57%) had a minimal platelet count of 20×10⁹/L at some time during their intrauterine life, making the severity of the disease of the current group comparable with that of the cohorts in the other studies mentioned above. Applying the criteria of Birchall *et al.*,[34] for example, in our cohort, intravenous immunoglobulin G, using the same therapeutic regime, was successful in only 4/30 (13%) patients.

Weekly maternal intravenous immunoglobulin G infusions raised maternal and fetal immunoglobulin G levels; however, repeated diagnostic fetal blood sampling (median 7, ranging from 2 to 14 per fetus) failed to show an increase in fetal platelet counts, in contrast to the findings in other sufficiently large series with less intense fol-

low-up. In fact, there was significant statistical evidence for an absence of an increase of fetal platelet counts between 26 and 34 weeks of gestation despite maternal intravenous immunoglobulin G therapy. In addition, the cost for weekly maternal intravenous immunoglobulin G (estimated €1500 per week) may be in the same range as weekly fetal blood sampling with platelet transfusions.

Limitations of the current study

We did not study the predictive value of previous affected pregnancies, their severity or time of onset of disease or the association with antibody levels and the degree of fetal thrombocytopenia. Nor was the therapeutic effect of steroids, alone or in combination with intravenous immunoglobulin G, part of our study. These aspects have been used to stratify cases by risk and to tailor antenatal treatment.[9,13,34,35]

Multiple invasive procedures (between two and 14 fetal blood sampling procedures per fetus; median, seven) carry a cumulative risk. Overton *et al.* combined personal data from 84 intrauterine platelet transfusions for fetal alloimmune thrombocytopenia together with data from three other studies and estimated a pooled pregnancy loss rate of about 6% per pregnancy.[3] In their report the procedural risk was calculated from three studies each comprising 12-15 cases per center, but the numbers of operators per center were not given. In two larger studies multiple centers contributed patients, with some centers providing data on only one case.[9,10] In our study all fetal procedures were performed by the same highly experienced team (a single operator performing all the fetal procedures). Probably due to this, there were no fetal or neonatal losses or emergency deliveries because of complications and it may not be appropriate to generalize our results to all centers. Given the retrospective nature of our analysis it is possible that we missed mild or moderate complications which may have occurred longer after the treatment and which were not recorded in the standard documentation.

There is, so far, no standard for the treatment of fetal alloimmune thrombocytopenia,[26] but a tendency to avoid serial fetal blood sampling and to follow these high-risk cases in specialized centers.[36] A recent study showed the value of a standardized assessment of maternal antibody levels at least for the prediction of the severity of fetal alloimmune thrombocytopenia, which may ultimately aid in selecting and timing the appropriate therapy.[35]

Published evidence suggests that maternally infused immunoglobulin may be effective at least in some cases, but the largest multicenter studies, aiming to reduce invasive testing, had only limited numbers of blood sampling procedures per fetus with fetal alloimmune thrombocytopenia. Others have failed to find consistent success with maternal immunoglobulin G infusions or even reported fetal complications (including intrauterine death and intracranial hemorrhage) in a significant number of treated fetuses.[29]

We studied a cohort of 30 fetuses with fetal alloimmune thrombocytopenia whose mothers received weekly immunoglobulin G infusions and who underwent repeated diagnostic fetal blood samplings. The increases in maternal and fetal immunoglobulin levels documented at these multiple occasions contrast with the lack of significant increase of fetal platelet counts. Since we do not have data on the true natural course of fetal platelet counts in fetal alloimmune thrombocytopenia, we cannot exclude that an even more pronounced reduction would have occurred without maternal immunoglobulin G infusions. However, an increase to safe platelet levels could not consistently be achieved by maternal intravenous immunoglobulin G alone. The natural course of fetal platelet counts in fetal alloimmune thrombocytopenia in the absence of any treatment is - and for ethical reasons will most likely remain - unknown.

Authorship and Disclosures

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