morphisms and that these genotypes were associated with an increased risk of the development of DALD.[14] As demonstrated in the work by Boggio *et al.*, elevated osteopontin levels could mediate TIMP-1 overexpression, and both molecules could thus impair Fas-induced cell death and activation-induced cell death. It is not yet clear whether this is the causal event accounting for the apoptosis defect observed in DALD or whether it worsens a defect of apoptosis as a consequence of another genetic defect that remains to be identified. Other unanswered questions are what is the signaling pathway of this inhibiting mechanism and what is the trigger of the TIMP-1 over-expression in ALPS . It could be either a consequence of the proliferation itself, or a consequence of an additional genetic defect. In any case this might be considered as a "modifier" which could potentiate the partial Fas-induced cell death defect observed for some Fas mutations with low clinical penetrance.

Finally, if the pathological role of TIMP-1 is confirmed *in vivo*, these findings could point to a central role of apoptosis in key check-points of self-tolerance. Additionally they could constitute a step forward in the understanding of the pathophysiological mechanisms involved in ALPS and DALD, and could pave the way to the understanding of other autoimmune diseases.

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## Antenatal treatment of fetal alloimmune thrombocytopenia: a current perspective

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Fetal alloimmune thrombocytopenia is caused by maternal sensitization to paternally-derived antigens on fetal platelets, most commonly HPA-1a.[1] It occurs in approximately 1 in 1000 live births and is the commonest cause of severe fetal and neonatal thrombocytopenia, and of intracranial hemorrhage in neonates born at term.[2] Since there is currently no routine screening, first-time cases of fetal alloimmune thrombocytopenia are generally identified following the birth of a markedly thrombocytopenic neonate. Antenatal management is thus only possible in subsequent pregnancies.

Intracranial hemorrhage is the most devastating complication of fetal alloimmune thrombocytopenia and often occurs antenatally. Assessment of projected clinical severity is thus based on the development of intracranial hemorrhage in a previous sibling. If there is such a history of intracranial hemorrhage, the chance of this complication occurring again in the next pregnancy is extremely high in

an untreated, antigen-positive sibling.[3]

Administration of intravenous immunoglobulin (IVIG) to the mother, initially given in conjunction with dexamethasone, was first used to prevent recurrence of antenatal intracranial hemorrhage in 1988.[4] This approach of providing IVIG-based medical therapy administered to the mother to increase the fetal platelet count has since been extensively investigated in hundreds of maternal-fetal pairs.[5] The efficacy of IVIG-based therapy has been supported by numerous studies[6-16] (Table 1A) but not by others[17-19] (Table 1B). The studies presented in Tables 1A and 1B surprisingly report virtually identical percentages of cases of intracranial hemorrhage: 2.7% *versus* 2.9%, respectively. However, overall mean birth platelet counts differed markedly between the two groups. While platelet counts are considered to be surrogate markers of intracranial hemorrhage, fortunately, the likelihood of fetal and neonatal intracranial hemorrhage, in the absence of this complication having occurred in a previous sibling, is relatively low.

Over time, a number of considerations have emerged concerning the assessment of the efficacy of maternal IVIG-based therapy in fetal alloimmune thrombocytopenia (Table 2) in addition to the lack of a universally-accepted response criterion.[20] A recent randomized study demonstrated that IVIG 1 g/kg/week alone does not work well in more severely affected patients (i.e., those with previous intracranial hemorrhage or whose initial fetal blood platelet count is $\leq 20\times10^9$/L).[12] Among patients treated in the standard-risk arm (platelet count at pre-treatment fetal blood sampling > $20\times10^9$/L and no history of intracranial hemorrhage in a previous sibling), less intensive therapy was appropriate and prednisone 0.5 mg/kg/day was as good as IVIG. However, among high-risk fetuses, a satisfactory increase in platelets was seen in only 18% of cases treated with maternal IVIG 1 g/kg/week alone *versus* 82% of those treated with maternal IVIG 1 g/kg/week plus prednisone 1 mg/kg/day. Thus, IVIG 1 g/kg/week alone does not appear to be sufficiently effective for the approximately 50% of severely affected fetuses whose initial fetal platelet count is less than $20\times10^9$/L.[21] Further studies suggested that IVIG x 2 infusions of 1 g/kg/week combined with 0.5-1.0 mg/kg/day prednisone is the most effective medical regimen for use in the most severely affected fetuses.[16] These comparisons demonstrated that risk stratification was essential and that the severity and response to therapy of fetal alloimmune thrombocytopenia was not the same in all cases.

Another management strategy is the use of weekly fetal blood sampling associated with intra-uterine platelet transfusion. Since fetuses with alloimmune thrombocytopenia are vulnerable to compromised hemostasis due to severe thrombocytopenia, impaired platelet function, and endothelial dysfunction; the risks of fetal blood sampling are considerable and well-documented.[10-12,14,15,17,18,22,23] Birchall and colleagues from Europe reported a number of procedure-related complications, including exsanguination and emergency Cesarean deliveries attributed to infection, needle dislodgement, severe fetal bradycardia, cord spasm and thrombosis.[11] For these reasons, fetal blood sampling is generally coupled with intra-uterine platelet transfusion if platelet counts are low (e.g., < $50\times10^9$/L).[7,22] In addition, serial fetal blood sampling and intra-uterine platelet transfusions may further sensitize the mother.[20,24] Finally, if maternal platelets are used in an intra-uterine transfusion, antiplatelet antibodies may be transfused into the fetus with the platelets.[20,25] Overall, as treatment results with maternal IVIG-based therapy have improved substantially[6,7,12,14,16] (Table 3), while the morbidity and mortality from fetal blood sampling remain considerable, the consensus at many centers at present is to minimize its use, and, if possible, to avoid fetal blood sampling completely.[9,13,15,23] This requires the use of "blind" treatment and, therefore, therapy that will be effective in fetuses with all degrees of severity since; for example, it is unknown whether the fetal platelet count is below or above $20\times10^9$/L without pre-treatment fetal blood sampling.

The study by Giers *et al.*[19] in this issue of the journal introduces an entirely novel approach to management in which serial weekly fetal blood samples are taken and intra-uterine platelet transfusions are given only at the final sampling. Twenty-nine pregnancies were studied, 25 of which were from mothers with one to four previously affected children; sibling history was negative for intracranial hemorrhage in all cases. No intra-uterine platelet transfusions were performed except immediately prior to birth, despite the fact that the mean minimal fetal platelet count during pregnancy was $21.5\times10^9$/L (range, 4-$60\times10^9$/L). Maternal therapy was weekly IVIG, 1 g/kg of Endobulin, without steroids (or intra-uterine platelet transfusion). Giers *et al.* concluded that their data support very limited efficacy of IVIG (mean fetal platelet count at initial intra-uterine platelet transfusion, $56.3\times10^9$/L [range, 4-$130\times10^9$/L]; mean fetal platelet count at final sampling prior to intra-uterine platelet transfusion, $31.3\times10^9$/L [range 6-$117\times10^9$/L]). This 6-year retrospective study included 219 fetal blood sampling procedures (median, 7 per fetus; range, 2-14). Not one procedure-related complication occurred in this study. Because the lack of adverse events associated with fetal blood sampling was attributed to one highly-skilled operator performing all procedures, as in a series of more than 5000 such procedures in France,[26] the reproducibility of such a complication-free outcome may not be generalizable to other centers.[19] Finally, the overall poor response to IVIG may stem from additional factors beyond the use of a dose of only 1 g/kg/week. These include the brand of IVIG (Endobulin) and the large number of fetal blood sampling procedures performed, as serial sampling may contribute to maternal sensitization, thus masking the therapeutic effects of maternal IVIG.[20,24]

## Perspectives

The management of fetal alloimmune thrombocytopenia has progressed in recent years. Examples of this include implementation of non-invasive approaches, in which fetal blood sampling and intra-uterine platelet transfusion are minimized or eliminated completely, and treatment stratified according to estimated risk.

### Non-invasive interventions

The current focus on non-invasive approaches[9,13,15,23] serves both as a response to the high complication rate of

invasive strategies[10-12,14,15,17,18,22,23] and as a testament to the efficacy of maternal IVIG-based treatment. In one study Yinon *et al.* suggested that IVIG without fetal blood sampling is safe and effective in women who, like the subjects featured in the study conducted by Giers *et al.*,[19] lacked a history of fetal or neonatal intracranial hemorrhage in a previous child. The 24 fetuses who underwent this treatment had significantly higher platelet counts at birth com-

**Table 1.** Maternal IVIG response success and failure overview.

**A. Maternal IVIG Response Success Overview***

| Study | N. of fetuses treated with maternal IVIG (dose) | IVIG type | Steroid** type and dose | ICH (N.) | Mean BPC x10⁹/L | Median BPC x10⁹/L | BPC >50x10⁹/L |
|---|---|---|---|---|---|---|---|
| Lynch 1992 | 18 (1 g/kg/wk (n=17), 0.5 g/kg/wk (n=1)) | Sandoglobulin; Sandoz, (East Hanover, NJ) | Dex: 1.5-5 mg/d; Prednisone: 10 mg/d | None | 107.1 | 60 | 11/18 (61%) |
| Bussel 1996 | 54 (1 g/kg/wk) | IVIG from Sandoz (East Hanover, NJ), Polygam (American Red Cross, Bethesda MD) Veinoglobulin-I (Alpha Therapeutics, Los Angeles) | Dex: 1.5 mg/d; Predn: 60 mg/d | None | 102.8 | 76 | 37/54 (64%) |
| Dawkins 1999 | 9 (1 g/kg/wk) | Intragam (CSL Ltd, Broadmeadows) | Steroid for rash on mother (n=3) | None | 191.8 | - | - |
| Mackenzie 1999 | 11(1 g/kg/wk) | Not stated | - | None | 132.1 | 144 | 7/11 (63.6%) |
| Silver 2000 | 8 (1g/kg/wk) | Not stated | - | None | - | 61.5 | 5/8 (62.5%) |
| Birchall 2003 (maternal therapy arm) | 18 (1 g/kg/wk (n=17), 0.8 g/kg/wk (n=1)) | Not stated | Prednisolone: 0.5 mg/kg/d | Yes (1) | - | 69.5 | 12/18 (66.7%) |
| Berkowitz 2006 | 79 (1 or 2 g/kg/wk) | Not stated | Predn 0.5 or 1 mg/kg/d | Yes (3); 2 with Grade 1; 1 with Grade 3 | High Risk: 82.3 (n=40) | - | Standard Risk: 33/38 (86.8%) |
| Yinon 2006 | 14 (1g/kg/wk) | Not stated | - | None | 118 | - | - |
| Berkowitz 2007 | 77 (1 or 2 g/kg/wk) | Not stated | Predn 0.5 mg/kg/d | Yes(2) | 152 | - | 68/77 (88.3%) |
| van den Akker 2007 | 86 (1 g/kg/wk) Group 2 (sibling with ICH) n=11, (sibling without ICH): n=22 Group 3 (sibling with ICH): n=5, (sibling without ICH): n=48 | Not stated | - | None | - | Group 2: only results after IUT reported Group 3: (sibling with ICH): 15, (sibling without ICH) 137 | Group 2 (sibling with ICH): 11/11 (100%); (sibling without ICH): 22/22 (100%) Group 3 (sibling with ICH): 1/5 (20%) (sibling without ICH): (42/48) (87.5%) |
| Bussel 2010 | 37 (1 or 2 g/kg/wk) | Not stated | Predn1 mg/kg/d | Yes (5) | 107.3 (n=33) | 87 (n=33) | 27/33 (82%) |
| Total | 411 | | | 11/411 (2.7%) | 120.3 (n=260) | | 276/343 (80.5%) |

**B. Maternal IVIG Response Failure Overview***

| Study | N. of fetuses treated with maternal IVIG (dose) | IVIG Type | Steroid** type (dose) | ICH (N.) | Mean BPC x10⁹/L | Median BPC x10⁹/L | BPC>50 >50x10⁹/L |
|---|---|---|---|---|---|---|---|
| Kaplan 1998 | 27 (1 g/kg/wk) | - | - | Yes (2) | "Success" (n=7): 152 "Plateau" (n=11): 52 "Failure" (n=9): 25 | - | - |
| Sainio 1999 | 11 (1 g/kg/wk) | Sandoglobulin; Novartis | Prednisone (20 and 30 mg/d) | None | 109.3 | 123 | 7/11(64%) |
| Giers 2010 | 30 (1 g/kg/wk) | Endobulin (Immuno GmbH, Heidelberg, Germany) | - | None | 31 | - | 4/30 (13.3%) |
| Total | 68 | | | 2/68 (2.9%) | 58.7(n=68) | | 11/41 (26.8%) |

*IVIG: intravenous immunoglobulin; Dex: dexamethasone; Predn: prednisone; BPC: birth (or final) platelet count (x10⁹/L) of those treated with maternal IVIG-based therapy; wk: week; ICH: intracranial hemorrhage. *data included refer to fetuses treated with maternal IVIG-based therapy; inclusion criteria for this table: n>5 fetuses and treatment with maternal IVIG-based therapy; this table does not include the studies by Radder et al. (2001)[23] and Bussel et al. (1988)[4], as their findings have been incorporated in other studies mentioned. **in conjunction with IVIG.*

**Table 2.** Possible reasons for observed differences in the results of antenatal management of alloimmune thrombocytopenia.

| | |
|---|---|
| 1. | Worse disease in European patients possibly caused in part by larger family size |
| 2. | In Europe, more PUBS procedures were performed with the possibility of maternal sensitization based on transplacental hemorrhage with the outcome of more severe fetal thrombocytopenia. |
| 3. | Different criteria for what constitutes a "response" |
| 4. | Different preparations of gammaglobulin with potentially different properties pertinent to this indication for treatment |
| 5. | Variable transfusion practices for *in utero* provision of platelets, with potential effects on the presence of antiplatelet IgG in platelet alpha granules |
| 6. | Possible role of HLA or ABO blood group incompatibility |
| 7. | Treatment without risk stratification or intensification (other than provision of intra-uterina transfusion) |

*PUBS: percutaneous umbilical blood sampling, IVIG: intravenous immunoglobulin, HLA: human leukocyte antigen.*

**Table 3.** Outcomes of maternal therapy: platelet counts; multicenter US experience.

| Study | IVIG dose (g/kg/week)* | Steroid type, dose (mg/day)*, if given | Mean BPC $x10^9$/L | Median BPC $x10^9$/L | N. with platelet count >$50x10^9$/L |
|---|---|---|---|---|---|
| Lynch 1992 | 1** | Dexamethasone, 1.5, 3, or 5<br>Prednisone, 10 | 107.1 (n=18) | 60 (n=18) | 11/18 (61%) |
| Bussel 1996 | 1 | Dexamethasone, 1.5<br>Prednisone, 60 | 102.8 (n=54) | 76 (n=54) | 37/54 (64%) |
| Berkowitz 2006 | 1 or 2 | Prednisone, 0.5 or 1 | 82.3 (n=40)*** | - | 33/38 (86%)**** |
| Berkowitz 2007 | 1 or 2 | Prednisone, 0.5 | 152 (n=77) | - | 68/77 (88.3%) |
| Bussel 2010 | 1 or 2 | Prednisone, 1 | 107.3 (n=36) | 87 (n=33) | 27/33 (82%)***** |
| All studies | | | 117.1 (n=225) | | 176/220 (80%) |

*IVIG: intravenous immunoglobulin, BPC: birth platelet count ($x10^9$/L) *including salvage therapy, **one patient received 0.5 g/kg/week IVIG, ***high risk arm only, ****standard risk arm only, *****data unavailable for 3 patients*

pared to their affected siblings and fetuses of mothers who refused treatment ($118\times10^9$/L *versus* $25\times10^9$/L and $24\times10^9$/L, respectively; $P<0.05$).[13] Furthermore, in a group treated with "blind" IVIG, preterm births were significantly less frequent than in groups treated with invasive approaches.[23] While avoiding fetal blood sampling entirely is not without its own pitfalls (inability to assess the need for transfusion, determine aspects of severity, etc.), this approach may help to avoid fetal complications ranging from exsanguination to enhanced maternal sensitization and seems feasible in many cases.[20,24,25]

### Risk stratification

Recent studies have demonstrated the need to acknowledge variations in severity in treatment protocols.[12,16] Severity may be affected by a myriad of factors whose roles in fetal alloimmune thrombocytopenia are not yet understood, such as HLA and, possibly, ABO incompatibility.[27,28] It has become clear that fetuses at different risk levels should not be treated identically, and that IVIG 1 g/kg/week alone is not sufficient to treat high-risk cases. Finally, there is now compelling support for the role of a risk-based approach to maternal IVIG-based therapy in the prevention of recurrent intracranial hemorrhage among affected fetuses, again indicating that treatment stratification based on sibling history is appropriate.[16]

### Screening and biomarkers

Screening all pregnancies for fetal alloimmune thrombocytopenia is also a novel approach whose discussion is beyond the scope of this perspective review. Its appropriate implementation remains to be clarified.

Finally, development of biomarkers of severity would be extremely useful. Analogy could be made to the use of middle cerebral artery Doppler studies to predict the severity of fetal anemia.

### Summary

Non-invasive approaches and the implementation of risk stratification (including combination therapy of IVIG with steroids and/or the use of more than 1 g/kg/week of IVIG) are appropriate in the management of fetal alloimmune thrombocytopenia. The data are equally compelling that fetal blood sampling cannot be considered routine in most centers and that Giers *et al.*[19] were exceptional and fortunate in their skilled performance of this technically-demanding procedure.

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## B-cell-directed therapy for chronic graft-*versus*-host disease

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Although acute morbidity and mortality associated with allogeneic hematopoietic stem cell transplantation have steadily decreased over the past 20 years, chronic graft-*versus*-host disease (GVHD) remains a common complication and few new treatment approaches have been identified during this period. Unfortunately, therefore, chronic GVHD remains a frequent long-term toxicity that often affects patients who might otherwise be cured of their primary disease. Our current knowledge of the immune pathophysiology of chronic GVHD is limited in part because few animal models have been developed that mimic the varied clinical manifestations of this disease in humans.[1] The effective prevention of chronic GVHD by the depletion of T cells from the stem cell graft demonstrates that donor T cells play a critical role in this disease. When patients and donors are HLA-matched, minor histocompatability antigens expressed in normal tissues of the recipient have been shown to elicit both $CD4^+$ and $CD8^+$ T-cell responses which result in either direct cell killing or cytokine-induced tissue injury. Thus, current therapies for chronic GVHD are targeted primarily and non-specifically against donor T-cell activity.

Although donor T cells play a central role in the development of chronic GVHD, there is emerging evidence that donor B cells also contribute to the clinical manifestations of this disease.[2] In a mouse model of major histocompatability complex-mismatched transplantation, donor B cells were found to be necessary for the development of chronic GVHD.[3] In humans, at least some of these B cells produce known autoantibodies.[4] Our laboratory has previously shown that Y chromosome-encoded minor histocompatability antigens elicit specific antibody responses following sex-mismatched hematopoietic stem cell transplantation, and the presence of these allo-antibodies correlates with the development of chronic GVHD.[5,6] Patients with minor histocompatability antigen-specific antibodies have also been found to have alloreactive $CD4^+$ T cells directed against different epitopes derived from the same protein[7] but the pathogenicity of these antibodies remains unproven. Several mechanisms whereby donor B cells can interact