Immune Hemolytic Anemia—Selected Topics

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Autoimmune hemolytic anemia (AIHA) is most often idiopathic. However, in recent years, AIHA has been noted with increased incidence in patients receiving purine nucleoside analogues for hematologic malignancies; it has also been described as a complication of blood transfusion in patients who have also had alloimmunization. As the technology of hematopoietic stem cell transplantation has become more widespread, immune hemolysis in the recipients of ABO-mismatched products has become better recognized. The syndrome is caused by passenger lymphocytes transferred from the donor, and although transient, can be quite severe. A similar syndrome has been observed in recipients of solid organ transplants when there is ABO-incompatibility between donor and recipient.

Unusual Aspects of the Epidemiology of AIHA

Association with therapy with purine nucleoside analogues

Because of the association of AIHA with chronic lymphocytic leukemia, many people with AIHA can be expected to have been treated with purine nucleoside analogues such as fludarabine and cladribine. In 1995, Myint et al reported on 52 patients with AIHA treated with fludarabine, in whom severe AIHA occurred in 12 (23%) after a median of four courses. Nine of these 12 had no prior evidence of AIHA. Eight were retreated with fludarabine at a later time, and severe AIHA recurred in 6.1 The authors opined that a disturbance of immunoregulatory T cells was responsible for the problem, in that T-cell lymphopenia is a recognized effect of fludarabine. Weiss et al reviewed this subject, and reported on 24 patients with AIHA following fludarabine therapy for chronic lymphocytic leukemia.2 Most of the patients developed the AIHA after one to three courses of drug, and seven (29%) died of complications related to the AIHA. Of 8 patients rechallenged with fludarabine at a later time, 7 had recurrent AIHA, and 3 died. The authors propose as a mechanism the release of a suppressed autoantibody to a native erythrocyte antigen.

This problem has also been reported after the use of cladribine. Fleischman and Croy reported a patient with severe AIHA that developed a few weeks after treatment of chronic lymphocytic leukemia with cladribine.3 More recently, Aslan et al reported a case of a patient with Waldenström’s macroglobulinemia who developed warm antibody AIHA a few months after therapy with cladribine.4 The authors bolster the causative assumption by noting that cold-mediated IgM antibody is more likely to occur in macroglobulinemia rather than a warm antibody. Byrd et al noted a case of fludarabine-associated AIHA that recurred fatally upon treatment with pentostatin.5

Clinicians need to be aware of the risk that use of purine nucleoside analogues in treating malignant lymphoproliferative disorders may induce AIHA, and that this may
be severe or even fatal at times. There may be a role for rituximab in the treatment of such episodes.\textsuperscript{6}

\textbf{Association with blood transfusion}

While alloantibody formation is a recognized and reasonably common complication of blood transfusion, the possibility of autoantibody formation has not been well recognized. Young et al analyzed over 2600 patients with a positive direct antiglobulin test or indirect antiglobulin test, and identified 41 patients who had both an autoantibody and an alloantibody. About a third of them developed their autoantibody in close temporal association with alloimmunization following recent transfusion.\textsuperscript{7} Therefore, AIHA developed either concurrently or shortly after alloimmunization from blood transfusion. The authors conclude that AIHA is a potential complication of allogeneic red blood cell transfusions, and recommended supportive treatment with iron and erythropoietin analogues, avoiding multiple transfusions.\textsuperscript{8-10}

\textbf{Association of immune hemolysis with allogeneic hematopoietic cell transplantation}

Immune hemolysis may be a complication of hematopoietic stem cell transplantation when there is a minor ABO blood group incompatibility between the donor and recipient. It occurs most often when the donor is group O and the patient group A, and may occur in up to 10-15\% of patients.\textsuperscript{11,12} The resultant hemolysis begins within the first 2 weeks after infusion, and may be abrupt in onset and severe, sometimes with accompanying intravascular hemolysis and renal failure.\textsuperscript{14}

The problem has been attributed to the “passenger lymphocyte syndrome,” caused by production of antibody by rapidly proliferating passively-transferred lymphocytes transfused with the stem cell product. Passive transfer of antibody in plasma accompanying the product does not appear to be important, as the hemolysis does not occur immediately. The antibody production and subsequent hemolysis occur while the patient is still pancytopenic from the conditioning regimen, before immune reconstitution occurs. It is thought to be related to the fact that IgG-type anti-A and anti-B are more common in group O persons than in group A and B persons. Hemolysis may also be a result of anti-D, anti-E, anti-s, anti-Jkb and anti-Jka. The hemolysis lasts 5-10 days and gradually subsides as the recipient’s residual incompatible red cells are lysed and replaced by transfused group O cells, or by red cells of donor type produced by engrafting stem cells. Further, antibody production by passenger lymphocytes that do not engraft dissipates.

Several factors appear to predispose to passenger lymphocyte syndrome: use of cyclosporine alone without methotrexate for graft-vs-host disease prophylaxis; use of peripheral blood stem cell product rather than bone marrow; use of reduced-intensity preparative regimens; use of a non-genotypically HLA-matched donor; and possibly, use of a female donor. The problem has not been noted with umbilical cord blood stem cell transplantation.

Patients develop rapid onset of hemolysis, associated with a positive direct antiglobulin test and the presence of anti-A or anti-B antibodies in their serum. Evidence of intravascular hemolysis with accompanying hemoglobinuria and renal failure may ensue.

Management strategies include reduction of the plasma volume of stem cell product, to reduce the amount of anti-A and anti-B infused. While passive transfer of antibody is not thought to be the main problem, large volumes of anti-A or anti-B in donor plasma can sometimes cause hemolysis, as well.\textsuperscript{15} Red cell transfusions should be with compatible cells or group O cells (if patient is type AB, and donor is type A or B, donor cells may be used). Corticosteroids are commonly used. Platelet transfusions and other plasma-containing products should be of recipient type, to minimize infusing anti-A and anti-B, and renal function should be assured. If significant hemolysis occurs, exchange transfusion may be required to replace the patient’s antigen-positive red blood cells with group O red blood cells. Prophylactic red blood cell exchange transfusion has been proposed in some cases.\textsuperscript{13,16}

When there is a major ABO-incompatibility, such as when the donor is type A or type B, and the recipient type O, hemolysis may be prevented by removing red cells from the donor product. Persistence of recipient ABO antibodies may lead to persistent hemolysis for a few months, and patients may need to be supported with transfusion of type O blood. Recommendations for blood product support in minor and major ABO-mismatched hematopoietic stem cell transplantation have been reviewed in detail recently by Petz.\textsuperscript{14}

In some reported instances of hemolytic anemia post-hematopoietic stem cell transplantation, the degree of hemolysis exceeded the total erythrocyte volume of the recipient, suggesting that transfused group O cells, in addition to the recipient’s native cells, were also being lysed—a phenomenon called “bystander” hemolysis. This hemolysis of cells that are negative for the antigen against which the antibody is directed is poorly understood and controversial.\textsuperscript{13,16,17}

Autoimmune hemolytic anemia has also been reported following hematopoietic stem cell transplantation. This is thought to be due to antibody being produced by the donor immune system against antigens on red cells of donor origin, hence AIHA. In a number of cases studied, no evidence of residual recipient cells was found, leading to the conclusion that the cause was an autoimmune reaction of the graft against its own product. A report in a pediatric population noted an incidence of 6\%, with a median time of onset of 4 months after transplant; mortality was quite high, perhaps worsened by the additional immunosuppressive therapy required to manage the AIHA.\textsuperscript{18} It was more
Evans syndrome. There have been reports of successful lowing cord blood transplants, with resultant AIHA and nins. Additional reports have included several cases fol-
18 months, versus 2-8 months for those with cold aggluti-
Patients with warm AIHA developed it later, between 6 and 
warm antibody AIHA, and 4 had cold agglutinin disease. 
offered by Petz. 

cipient and donor red cells, as well as the donor organ tis-
should be used that are ABO-compatible with both the re-
recipient serum, irrespective of donor organ type. If the 
cell exchange is necessary to decrease the volume of in-
ance of adequate renal function. Sometimes red blood 
assistance of ABO-incompatible plasma products, and mainte-
patients includes transfusion of group O red cells, avoid-
larly anti-D. Onset is between 3 and 24 days. In one study of 
be increased more than 4-fold, particularly in the presence 
them to disruption and exposure of phosphatidyrl serine, and a subsequent surface for formation of tenase and prothrombinase complexes. Other factors im-
prophylactic anticoagulation in such patients. However, 
point out that thrombosis also occurred in 15% of 
patients who did not have a lupus anticoagulant, so 
other factors are likely at work.
Kokori et al, in a review of AIHA in patients with sys-
temic lupus erythematosus, found the risk of thrombosis to 
be increased more than 4-fold, particularly in the presence of IgG anticardiolipin antibody. The association of sero-
logic indications of lupus, namely a false-positive test for 
syphilis, has been noted in the past in patients with AIHA 
by Conley and Savarese.

Hendrick has reviewed this issue recently and con-
cluded that patients with AIHA are indeed at high risk for 
thermoembolism. In an audit of 23 patients with warm 
antibody AIHA and 5 with cold agglutinin hemolysis, 
venous thromboembolism was noted in 6, of which 4 cases 
were fatal. These patients did not have detectable anti-
phospholipid antibodies. In an analysis of 36 hemolytic 
episodes, venous thromboembolism occurred in 5 of 15 
without anticoagulant prophylaxis, but in only 1 of 21 in 
which prophylaxis was used.

Although it is premature to recommend anticoagulant 
prophylaxis in general for patients with hemolytic episodes 
from AIHA, consideration might be given to those at par-
icularly high risk, such as those with evidence of coexist-
antiphospholipid antibodies.

**Lymphoproliferative disorders**

Patients with lymphoproliferative disorders are well known to have a higher risk for development of AIHA; this is par-
ticularly true of chronic lymphocytic leukemia. Interest-
ingly, there may also be an increased risk for future de-
velopment of lymphoproliferative disorders in patients with 
AIHA. Sallah et al reported on 107 patients with AIHA, of

**Complications of AIHA**

**Thromboembolism**

In an early review of AIHA, the most common cause of 
death was pulmonary embolism (4 of 47 patients). All of 
these patients had had a splenectomy, and all were receiv-
ing corticosteroid therapy. In a more recent review by 
Pullarkat et al, 8 of 30 patients (27%) suffered from an 
episode of venous thromboembolism. A total of 9 had a 
detectable lupus anticoagulant and 17 had anticardiolipin 
antibodies detected. Among the 8 with thrombosis, 5 had a 
lupus anticoagulant and 4 had anticardiolipin antibodies.
The authors attributed the thrombosis to disruption and 
loss of red cell membranes, resulting in exposure of phos-
phatidyrl serine, and a subsequent surface for formation of 
tenase and prothrombinase complexes. Other factors im-
licated in the thrombotic tendency in patients with AIHA 
include cytokine-induced expression of monocyte or en-
dotheial tissue factors. The authors postulated that the 
detection of a lupus anticoagulant identifies patients with 
AIHA at particularly high risk for venous thromboembo-
lism and suggested that serious consideration be given to 
prophylactic anticoagulation in such patients. However, 
they point out that thrombosis also occurred in 15% of 
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AIHA. Sallah et al reported on 107 patients with AIHA, of
whose 67 had idiopathic AIHA, and 40 had an associated
immune disorder (e.g., rheumatoid arthritis, temporal ar-
teritis, Crohn’s disease, lupus, thyroiditis, SJögren’s syn-
drome). Nineteen of the 107 (18%) subsequently devel-
oped a malignant lymphoproliferative disorder, at a me-
dian of 26 months after onset of the AIHA.36 Risk factors for
development of such a disorder were age, the presence of an
underlying autoimmune disease, and a coexistent serum
gammopathy. None of the patients had underlying HIV in-
fecion. The authors postulate that the development of a ma-
lignant lymphoid disorder is likely a multistep process, with
an earlier proliferative phase involving chronic antigenic
stimulation prior to a mutation leading to malignant change.

**Therapy of Refractory Cases of AIHA**

The standard therapeutic approaches to treatment of AIHA
close corticosteroids, splenectomy and immunosuppres-
sive drugs. In the past several years, certain newer therapies
have become available, and have shown evidence of suc-
cess. These are primarily used in patients who are not can-
didates for or fail to respond to splenectomy, those who
relapse after splenectomy, and those who cannot maintain
stable hemoglobin levels without unacceptably high doses of
corticosteroids.

**Intravenous immune globulin (IVIG)**

Flores et al reviewed the cases of 73 patients treated with
IVIG, and found responses in 29 (40%).37 Children were
more likely to respond, as were patients with initial hepatomegaly and lower initial hemoglobin levels.

**Danazol**

Danazol, which has been used more in refractory cases of
immune thrombocytopenia, has also been used in AIHA.
Ahn reported good to excellent results in the majority of
patients treated.38 In another series of 17 patients treated with
the combination of prednisone and danazol, excellent
responses were noted in 80% who received the combination
as first-line therapy; treatment was less effective in patients
who had relapsed and in those with Evans’ syndrome.39

**Newer immunosuppressives**

Howard et al reported on the use of mycophenolate mofetil
in 4 patients with refractory AIHA.40 Patients were treated
with 500 mg per day initially, then 1000 mg per day. All 4
had a complete or good response.

**Monoclonal antibodies**

There has been considerable interest in the past several
years in the use of the monoclonal antibodies widely used in
the treatment of B-cell lymphoid neoplasms, namely
rituximab (Rituxan®), and to a lesser extent alemtuzumab
(Campath-1H®). Zecca et al first reported on a child with
pure red cell aplasia and AIHA treated successfully with
rituximab and IVIG.41 Another report, in 5 children with
AIHA, described excellent responses, but with a resultant
not-unexpected prolonged B-cell deficiency.42 Shanafelt
et al reported on 5 patients, of whom 2 had a complete
response. In an additional 4 patients with Evans’ syndrome,
complete responses occurred in either the immune thromb-
ocytopenia or the AIHA, but not both.43 Trape et al noted
the benefits of rituximab for residual AIHA in 5 patients
following chemotherapy of a lymphoproliferative disor-
der.44 Mantadakis et al offered a case report of a patient
with refractory Evans’ syndrome who responded for 7
months to rituximab, and then responded a second time
after relapse.45 Ramanathan et al noted 2 patients with re-
fractory disease who demonstrated prolonged remissions
with rituximab.46 Not all reports have been favorable, how-
ever: Zaja et al noted no response to rituximab in 2 patients
with AIHA, though a patient with cold agglutinin disease
responded well.47

Gupta et al reported on the combined use of rituximab,
cyclophosphamide and dexamethasone in 8 patients with
refractory AIHA in the setting of chronic lymphocytic leuke-
demia. The results were excellent, including in relapsed pa-
ients, with 5 patients converting to negative DAT status.48

There has been only limited experience with alemtuzumab in AIHA, with one report noting responses in
3 of 4 patients treated.49

The role of monoclonal antibodies in the therapy of
autoimmune cytopenias has been reviewed in detail re-
cently.50 It is reasonable to conclude that monoclonal anti-
body therapy, specifically rituximab, is a safe and effective
therapy for AIHA. It is likely that as our experience with
the drug evolves, it will be used at an earlier point in therapy,before more toxic immunosuppressives, rather than only in
refractory cases.

**References**

1. Myint H, Copplestone JA, Orchard J, et al. Fludarabine-
related autoimmune haemolytic anaemia in patients with
344.
2. Weiss RB, Freiman J, Kweder SL, Diehl LF, Byrd JC.
Hemolytic anemia after fludarabine therapy for chronic
3. Fleischman RA, Croy D. Acute onset of severe autoimmune
hemolytic anemia after treatment with 2-
cholorodeoxyadenosine for chronic lymphocytic leukemia.
4. Aslan DL, Peterson BA, Long-Tsai M, Eastlund T. Early-
onset autoimmune hemolytic anemia after cladribine therapy
for Waldenstrom’s macroglobulinemia. Transfusion.
2006;46:90-94.
5. Byrd JC, Hertler AA, Weiss RB, Freiman J, Kweder SL,
Diehl LF. Fatal recurrence of autoimmune hemolytic anemia
following pentostatin therapy in a patient with a history of
fludarabine-associated hemolytic anemia. Ann Oncol.
1995;6:300-301.
6. Paydas S. Fludarabine-induced hemolytic anemia: success-
7. Young PP, Uzielbo A, Trulock E, Lublin DM, Goodnough LT.
Autoantibody formation after alloimmunization: are blood
transfusions a risk factor for autoimmune hemolytic anemia?


