Autoimmune Hemolytic Anemia in Pediatric Liver or Combined Liver and Small Bowel Transplant Patients – A Case Series and Review of the Literature

Short Title: AIHA in Pediatric Solid Organ Transplantation

ABSTRACT

Background:

Autoimmune hemolytic anemia (AIHA) occurring after solid organ transplantation is an infrequently reported entity. We describe in this report 6 cases of AIHA in pediatric liver or combined liver and small bowel transplant patients.

Study Design and Methods:

We restrospectively identified and reviewed the records of pediatric liver or combined liver and small bowel transplant patients with both serological and clinical evidence of AIHA. We also performed an English language literature review for prior publications of AIHA occurring after solid organ transplantation.

Results:

We identified 6 patients presenting with severe hemolysis 9 months to 14 years after transplantation. All 6 developed warm AIHA, and two had concomitant cold agglutinins. All except one patient received various therapeutic combinations including steroids, IVIG, rituximab, plasmapheresis, native splenectomy and vincristine. Five patients achieved remission two weeks to three months following presentation. Although tacrolimus has been speculated to play a causative role in the development of AIHA after organ transplantation, our case series demonstrated slightly better outcomes despite continuing tacrolimus as compared to published cases where most patients either received significantly reduced doses of tacrolimus or were switched to a different immunosuppressant (83% vs. 76% cumulative literature remission rate).

Conclusion:

AIHA may occur in solid organ transplant patients at a much higher frequency than previously believed. Hemolysis is often severe and resistant to steroid treatment alone. Thus early diagnosis and institution of aggressive multi-modality treatment, including the use of rituximab, may be needed to achieve remission.

Key Words: Autoimmune hemolytic anemia, rituximab, transplantation-solid organ

INTRODUCTION

Autoimmune hemolytic anemia (AIHA) is defined as the destruction of red blood cells (RBC) mediated by autoantibodies. Primary or idiopathic AIHA has no clear association with an underlying condition and is the most common form of this disease. In secondary AIHA, there is a clearly identifiable co-existing condition including autoimmune diseases (e.g. systemic lupus erythematosus), lymphoproliferative disorders, viral infections, stem cell or solid organ transplant, or the use of certain medications. The overall incidence of AIHA in the general population is approximately 1 in 80,000. AIHA cases are usually mediated by warm-reactive (IgG) or cold-reactive (IgM) autoantibodies. Warm AIHA (WAIHA) accounts for 70% of acquired immune hemolytic anemia cases, whereas cold AIHA or cold agglutinin syndrome (CAS) makes up only 15.6% of cases. Another 7% of patients develop mixed warm and cold AIHA (mixed AIHA).[1] It is important to make the clinical distinction between WAIHA and CAS since the treatment and prognosis vary greatly.

Transplant-associated immune hemolytic anemia is known to occur in patients following stem cell transplantation. Frequently this is mediated by ABO antibodies due to major or minor incompatibility (passenger lymphocyte syndrome), leading to hemolysis with a relatively acute presentation noted within days to weeks after transplant.[2] In contrast, AIHA mediated by autoantibodies can also develop in this clinical setting, but with a much lower observed frequency and occurring months to years after transplant. Reported incidences of AIHA in pediatric and adult stem cell transplant patients are 6%[3] and 3 to 5%,[4,5] respectively. Most published cases of AIHA presenting after stem cell transplantation occurred in patients who received T cell depleted stem cells. The mechanism of autoantibody formation has been

speculated to involve dysregulated immune reconstitution after transplant, which may lead to the polyclonal proliferation of B lymphocytes that can produce self-reacting antibodies.[4-7]

Until recently, there had been only a handful of reports in the literature of AIHA in the setting of solid organ transplantation and none of these publications were sufficiently powered to report the incidence.[8-14] However, the retrospective study published in 2009 by Botija et al. found that AIHA occurred at an incidence of 12.2% in 49 pediatric intestinal transplant patients.[15] Surprisingly, CAS appeared to be the most common type of AIHA, followed by WAIHA and mixed AIHA in all of these reported cases. It remains difficult to determine the true incidence of AIHA following solid organ transplantation based on current publications. However, we found it fascinating that within the last three years we have encountered 6 cases of well-documented AIHA occurring in liver or combined liver and small bowel pediatric transplant patients at our center. This may corroborate the relatively high incidence that was reported by Botija and coworkers. The details of our cases are described below.

MATERIALS AND METHODS

Between April 2007 and October 2010, immunohematology work-ups (antibody screen and direct antiglobulin test (DAT)) for pediatric liver or combined liver and small bowel transplant patients were reviewed for serologic evidence of AIHA. If serologic evidence was present, clinical evidence of hemolysis was sought in each patient and response to various treatment modalities was evaluated through retrospective chart reviews.

We performed an English language literature search on Pubmed using various combinations of the search terms “liver transplant”, “small bowel transplant”, “multivisceral transplant”, “autoimmune hemolytic anemia” and “Evans syndrome”. We found 17 similar cases of transplant-associated AIHA reported in 8 publications, and an additional three case reports of transplant-associated Evans syndrome in both pediatric and adult populations.

RESULTS

We identified 7 patients with serologic evidence of warm or cold autoantibodies between April 2007 and October 2010. During this same time period, 72 pediatric liver and 32 combined liver and small bowel transplantations were performed. Six of these patients were included in our review after clinical evidence of hemolysis was verified. There were four males and two females, ranging in age from five to 24 months at the time of first transplant. Two patients required re-transplantation for acute or chronic rejection. Only one patient received an organ with minor ABO incompatibility (recipient blood type: A+; donor blood type: O+). Primary immunosuppression included tacrolimus for every patient; five patients also received either mycophenolate mofetil and/or prednisone (See Table 1).

Five of the 6 patients presented with clinically overt hemolysis between 9 to 41 months after transplant, and the remaining patient presented 14 years after the first transplant. Patients ranged in age from 15 months to 15 years of age at the time of AIHA presentation. All 6 patients developed a positive DAT for anti-IgG, ranging from microscopically positive to 3+ in reactivity strength. Warm autoantibodies were identified in all 6 patients; in 5 patients, the warm autoantibodies were present both in the plasma and eluate, in one patient they were only evident

in an eluate prepared from the patient's RBC. Two of the 6 patients also developed a positive DAT for anti-C3d with 1+ reactivity strength. Cold autoantibodies were identified in both of these cases: one with a titer of 16 at 4ºC and a thermal amplitude up to 30ºC, while the other reacted up to 37ºC but the titer was unknown. All 6 patients required transfusion support and had laboratory evidence of hemolysis, including low hemoglobin (nadirs between 3.7 to 6.8 g/dL), depleted haptoglobin, and elevated reticulocyte count, indirect bilirubin and lactate dehydrogenase. On peripheral smear, microspherocytes were observed in all cases. Concurrent with hemolysis, four patients demonstrated significant falls in their platelet counts from baseline all with platelet counts < 50,000/µL. Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) surveillance studies were performed in all of the patients by serology or PCR. One patient developed evidence of CMV and another of EBV infection, although neither of them developed evidence of cold autoantibodies (See Table 2).

Initial treatment of AIHA included high dose steroids alone or in combination with IVIG for all patients. In four patients, rituximab was subsequently added for persistent hemolysis despite first line treatment. Other therapies utilized included native splenectomy, plasmapheresis and vincristine. The two patients with concomitant cold agglutinins were also kept warm as part of the therapy for hemolysis. Time to first remission in five patients ranged from two weeks to three months. One of the five patients relapsed twice, but has since achieved remission. The last patient still has persistent hemolysis following three months of treatment. All patients' primary transplant immunosuppressive regimens including tacrolimus were maintained with only minor dose adjustments during the acute episode of hemolysis in an effort to avoid organ rejection.

DISCUSSION

AIHA occurring in the setting of transplantation is not well described. In stem cell transplant, the reported incidence ranges from 3 to 5%[4,5] in adult patients, and was reported to be slightly higher at 6%[3] in pediatric patients. In solid organ transplant patients, the literature on AIHA until recently consisted of only single case reports or small case series. Therefore incidence of this entity was thought to be very low. However, Botija et al. recently performed the largest retrospective study to date in a cohort of 49 pediatric intestinal transplant patients and found an incidence of 12.2%.[15] At our center, we observed 6 cases of well documented AIHA in pediatric liver or combined liver and small bowel transplant patients within the last three years. This suggests that the incidence of AIHA in this population may be significantly higher than previously believed. Because it is not well-described, recognition of this entity remains challenging in clinical practice, and it is likely that many such cases were unrecognized in the past. However, the true incidence cannot be established without a prospective surveillance study of the solid organ transplant patient population consisting of frequent monitoring of hemolysis laboratory parameters and serologic findings.

In our case series, we identified 6 pediatric patients following liver or combined liver and small bowel transplants with clinically evident AIHA. There were no mortalities in this patient cohort. Two patients developed severe hemolysis with hemoglobin ranging from 3.7 to 3.9 g/dL at nadir, while the four remaining patients had hemoglobin nadirs between 6.1 to 6.8 g/dL. The observed severity of anemia in our case series is consistent with published data, where most patients also had hemoglobin nadirs ranging between 2.9 to 6.7 g/dL. Only one of our patients received a minor ABO mismatched organ. This raised a concern for hemolysis due to ABO antibodies

produced by passenger lymphocytes directed against recipient RBC. Passenger lymphocyte syndrome-associated hemolysis is typically noted between five to 17 days after transplant.[16] In this patient, the time to develop hemolysis occurred 11 months after transplant and there was never evidence of anti-A on this patient's RBC. The combination of delayed hemolysis without evidence of anti-A isohemagglutinins makes passenger lymphocyte syndrome a very unlikely mechanism for hemolysis.

We identified 8 published reports of AIHA occurring in the setting of liver or small bowel transplantation (See Tables 3 & 4).[8-15] In all, 17 patients were diagnosed and treated for AIHA, 13 of whom were pediatric patients and four were adults. Of the 17 reported patients, one died prior to initiation of treatment and one was treated with steroids only. The remaining 15 patients received in addition to steroids, other therapeutic modalities including IVIG, rituximab, plasmapheresis, native or graft splenectomy, alemtuzumab and cyclophosphamide. Thirteen patients achieved remission, three died (one from complications of anemia and two from infectious complications), and one continued to require low dose steroid maintenance for ongoing hemolysis.

In our case series, all 6 patients survived without significant morbidities due to hemolytic or infectious complications. Five patients reached clinical remission between two weeks to three months, three of whom had protracted clinical courses taking at least two months to reach clinical remission. Similar to previously reported cases, refractoriness to conventional steroid treatment was common, with only one of the five patients responding to steroid treatment alone. All other patients required various combinations of steroids, IVIG, rituximab, plasmapheresis,

native splenectomy and vincristine. One patient has ongoing hemolysis, despite triple therapy with steroids, IVIG and rituximab and is currently on a steroid taper. In total, four of the 6 patients received rituximab treatment and three out of the four are currently in remission. Rituximab, a monoclonal anti-CD20, is known to exert its effect on B cells and has been reported to be beneficial in the treatment of WAIHA and CAS. The drawbacks associated with the use of rituximab include increased immunosupression, up to 6 weeks of delay in therapeutic effects on hemolysis in some patients, six months of IVIG repletion therapy and higher cost. Nevertheless, it is possible that the early use of rituximab contributed to the relatively good clinical outcome observed in our patient population.

Interestingly, many of the patients in the previously reported cases were diagnosed with CAS (7/17 patients).[9-11,13,15] This is different from our experience since all 6 of our patients developed WAIHA, including two with concomitant cold agglutinins, and none developed CAS alone. CAS is usually diagnosed in the clinical setting of a patient presenting with signs and symptoms of hemolysis and acrocyanosis. They will usually have significantly elevated cold agglutinin titers of greater than 1,000 at 4ºC. Thermal amplitude is considered to be a better indicator of a clinically significant cold agglutinin since most healthy individuals may have cold autoantibodies, but they do not react above room temperature. Further reading revealed that these previously published reports often did not provide detailed serologic results, and most made the assumption that the cold autoantibodies found were clinically significant even though titers at 4ºC were less than 64 or not performed. Furthermore, the thermal amplitude test was either not performed or poorly documented in these reports. It should be noted that Petz and Garratty found strongly reactive concomitant cold agglutinins in 35% of patients with WAIHA, which when

appropriately tested, usually displayed normal titers at 4º C and restricted thermal amplitude, indicating the lack of clinical significance of such cold agglutinins.[1] Similarly, the cold agglutinins in those previously reported post-solid organ transplant AIHA cases may not be in themselves pathogenic either. However, the pathogenic, warm autoantibodies might have been missed. Three of the patients in these reports appeared to have developed mixed AIHA.[12,15] We also observed this relatively rare form of immune hemolysis in two of our patients. One developed a cold agglutinin with a titer of 16 at 4ºC that was reactive up to 30ºC; and the other patient's cold agglutinin titer was unknown, however, it was reactive up to 37ºC. The first patient responded excellently to steroid therapy alone while being kept warm and reached remission in two weeks, but the other patient relapsed twice and required steroids, IVIG and rituximab triple therapy for a month before achieving remission.

Evans syndrome (ES) is a rare autoimmune disorder defined by the combination of immune thrombocytopenia and AIHA. Interestingly, there are three case reports in the literature of ES presenting after liver transplant (See Tables 3 & 4).[17-19] In our case series, four of the 6 patients developed concurrent severe thrombocytopenia with platelet counts falling below 50,000/µL, and one required multiple platelet transfusions for active bleeding. Although none of them was given the formal diagnosis of ES, the temporal relationship between AIHA and the onset of severe thrombocytopenia raises the possibility that some of these cases represented ES. Although there have been only three such reported cases in both the pediatric and adult liver transplant population, our observation suggests ES may be much more common than previously thought.

Since the early 1990's, tacrolimus has been an integral part of the primary immunosuppressive regimen in wide use for liver transplant patients.[20] Consequently the role of tacrolimus as the causative agent in the relatively rare entity of AIHA following transplantation is difficult to assess. Calcineurin inhibitors such as tacrolimus have been postulated to cause an imbalance in the T and B cell immunity, leading to a more pronounced B cell response and formation of autoantibodies.[9,21] This theory bears similarity to the mechanism of pathogenesis proposed for the observed relatively higher incidence of AIHA in patients who receive T cell depleted stem cells transplants. Furthermore, there are differences reported in the absorption, bioavailability, distribution and metabolism of tacrolimus in pediatric versus adult patients.[22] This raises the possibility that these biological differences may underlie the observed higher incidence of AIHA in pediatric patients as compared to their adult counterparts.

Because tacrolimus was suspected to have contributed to the development or possibly exacerbate the clinical course of AIHA, the treating physicians in most of the previously reported cases chose to significantly reduce the dose of tacrolimus, or switch to another immunosuppressive agent. However, in our cohort, all patients were maintained on tacrolimus with only minor dose adjustments to stay within the therapeutic range while they were treated for AIHA. Compared to the previously reported cases in which tacrolimus was discontinued or given at a significantly reduced dose ,[8-11,13,15] our patients had slightly better outcomes despite continuing tacrolimus treatment, with five of the 6 patients (83% versus the 76% cumulative remission rate reported in the literature) reaching remission. Therefore, our findings suggest that maintaining tacrolimus during an acute hemolytic episode may be an acceptable alternative. This is an important finding because tacrolimus reduction or withdrawal shortly after transplantation, especially in small

bowel transplant recipients, is potentially very harmful given the increased chances for organ rejection.

In summary, AIHA occurring in solid organ transplant patients is not well-understood and the exact incidence is not yet known. Early clinical suspicion and diagnosis will facilitate prompt and effective therapy to minimize complications related to hemolysis or treatment associated infection. In the majority of published studies and our own experience, most patients were resistant to conventional steroid treatment for AIHA. It appears that early institution of aggressive multi-drug treatment regimens, especially the use of rituximab, may be needed to achieve remission.

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Table 1. Patient demographics and transplant characteristics

Patients	Gender	Initial diagnosis	Age at transplant	Graft Type	Primary immunosuppression
1	M	Gastroschisis	11 months & 14 months	OLT + SBT x2	T/S/MMF
2	F	Gastroschisis	20 months	OLT + SBT	T/MMF
3	M	Biliary atresia	5 months	OLT	T/S
4	M	Biliary atresia	1 year & 15 years	OLT x 2	T
5	M	Intestinal atresia	10 months	OLT + SBT	T/MMF
*6	F	Citrullinemia	6 months	OLT	T/S

* This patient received a liver with minor ABO incompatibility (recipient: A+ / donor: O+)
M: male
F: female
T: tacrolimus
S: steroid
MMF: mycophenolate mofetil
OLT: orthotopic liver transplant
OLT + SBT: combined small bowel and liver transplant
All donor splenectomies were performed during transplant

Table 2. Immunohematology findings, clinical characteristics and outcomes

Patients	Age at time of AIHA diagnosis	DAT (direct antiglobulin test)		Antibodies identified	Interval between transplant and onset of hemolysis	Time to remission following treatment for hemolysis	Nadir hemoglobin level (g/dL)	Concurrent severe thrombocytopenia (<50,000/µL)	Treatment	Outcome
		IgG	C3d							
1	3.5 years	1+	1+	WAA & CAA (4ºC titer: 16; reactive up to 30ºC)	29 months	2 weeks	6.6	Yes	S/Keep warm	Remission
2	4.5 years	3+	Negative	WAA	32 months	3 months	3.7	Yes	S/IVIG/R/PE/ native splenectomy	Remission
3	15 months	3+	Negative	WAA	9 months	3 months	6.1	No	S/IVIG/R/V	Remission
4	15 years	3+	Negative	WAA	14 years	2 months	6.1	Yes	S/IVIG/PE/ native splenectomy	Remission
5	5.5 years	Microscopically positive	Negative	WAA*	41 months	Ongoing	6.8	No	S/IVIG/R	Ongoing treatment
6	17 months	1+	1+	WAA & CAA (Titer at 4ºC unknown, reactive up to 37ºC)	11 months	1 month	3.9	Yes	S/IVIG/R/ keep warm	2 relapses, currently in remission

* WAA is present only in the eluate
S: steroids
R: rituximab
PE: plasmapheresis
V: vincristine
WAA: warm autoantibody
CAA: cold autoantibody

Table 3. Literature review of reported cases of autoimmune hemolytic anemia or Evans syndrome in pediatric solid organ transplant patients (liver, liver/small bowel, multivisceral)

Type of publication	Age or Age range at time of AIHA diagnosis	Gender	Type of transplant	Primary Immuno-suppression	Type of autoimmune condition	Time to develop hemolysis after transplant	Time to remission following treatment for hemolysis	Nadir hemoglobin level (g/dL)	Treatment	Primary Immuno-suppression modification	Outcome	Reference
Case series (6)	NP	M (4) F (2)	Liver; liver/small bowel; multivisceral	T/S/B/A/TM	WAIHA (3); CAS (1); Mixed AIHA (2)	1-21 months (average 10 months)	NP	NP	S/IVIG/R/PE/A/graft splenectomy/keep warm	Tacrolimus switched to sirolimus (4)	Remission (5); Died of infection (1)	Botija[15]
Case report	16 months	M	Liver	T	Warm acting IgG and IgM AIHA	8 months	10 weeks	3.9	S/IVIG/R	Tacrolimus switched to cyclosporine	Remission	Schappi[8]
Case series (3)	13 months; 2 year 5 months; 4 year 1 month	F (3)	Liver	T	CAS (3)	6-26 months	3 weeks-1 month	3.6-5.3	S/PE/keep warm	Tacrolimus switched to cyclosporine	Remission (3)	Wong[9]
Case report	3 years	F	Multivisceral	T	CAS	18 months	7 months	5.8	S/R/C/ exchange blood transfusion	Tacrolimus dose reduced	Remission	Brunner[10]
Case report	9 years	F	Liver	T/S	CAS	4 months	NP	3	S/keep warm	Tacrolimus switched to cyclosporine	Remission	Kitamura[11]
Case report	7 years	M	Liver	T	Mixed AIHA	6 years	NA	2.9	None – patient died prior to initiation of treatment	None	Died of complications of anemia	DiGiuseppe[12]
Case report	8 years	M	Liver	T/MMF	Evans syndrome	22 months	20 months	5.2 Platelet nadir: < 10,000/μL	S/IVIG/R/C/F/native splenectomy	Tacrolimus switched to cyclosporine	Remission	Domenech[17]
Case report	5 months	M	Liver	T	Evans syndrome	3 months	1 month	5.4 Platelet nadir: 44,000/μL	S/IVIG	Tacrolimus dose reduced	Remission	Yokoyama[18]

M: male F: female
T: tacrolimus S: steroid MMF: mycophenolate mofetil B: basiliximab A: alemtuzumab TM: thymoglobulin R: rituximab C: cyclophosphamide
PE: plasmapheresis F: fludarabine AIHA: autommune hemolytic anemia WAIHA: warm autoimmune hemolytic anemia

CAS: cold agglutinin syndrome Mixed AIHA: mixed warm & cold autoimmune hemolytic anemia NP: not provided NA: not applicable

Table 4. Literature review of reported cases of autoimmune hemolytic anemia or Evans syndrome in adult solid organ transplant patients (liver, liver/small bowel, multivisceral)

Type of publication	Age or age range at time of AIHA diagnosis	Gender	Type of transplant	Primary Immuno-suppression	Type of autoimmune condition	Time to develop hemolysis after transplant	Time to remission following treatment for hemolysis	Nadir hemoglobin level (g/dL)	Treatment	Primary Immuno-suppression modification	Outcome	Reference
Case report	NP	M	Liver	T/S/MMF	CAS	12 months	7 months	6	S/IVIG/R	Tacrolimus dose reduced	Remission	Guitard[13]
Case series (3)	59, 59 and 66 years	M (2) F (1)	Liver	T/MMF	DAT positive AIHA: IgG (1); IgG & C3d (1); DAT negative AIHA (1)	3-6 years	NP	4.7- 6.7 (schistocytes seen in one patient)	S/IVIG/R/ native splenectomy	Mycophenolate mofitil discontinued (1)	Remission (1); Low dose steroid maintenance (1); Died of infection (1)	Retana[14]
Case report	38 years	M	Liver	T (discontinued due to recurrent sepsis)/S	Evans syndrome	7 months	NP	8 Platelet nadir: 3,000/µL	S/IVIG	None	Died of infection	Au[19]

T: tacrolimus
S: steroid
MMF: mycophenolate mofetil
R: rituximab
DAT: direct antiglobulin test
AIHA: autoimmune hemolytic anemia
CAS: cold agglutinin syndrome
NP: not provided