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Treatment and Outcomes of Immune Cytopenias Following Solid Organ Transplant in Children

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Abstract

Background—Immune cytopenias are a recognized life-threatening complication following pediatric solid organ transplants (SOT), but treatment responses and overall outcome are not well described. The aim of this study was to evaluate the demographic characteristics, response to treatments, and outcomes of a cohort of patients who developed immune cytopenias following SOT.

Methods—In this single center retrospective review, patients with immune cytopenias after SOT were identified by electronic medical record (EMR) search and transplant databases from 1995-2012.

Results—Of 764 SOT patients, 19 (2.4%) developed immune cytopenias. Incidence varied widely by transplant type from 1.2% (renal) to 23.5% (multivisceral). Autoimmune hemolytic anemia (AIHA) was the most common immune cytopenia. Overall median time from transplant to immune cytopenia was 8 m and varied by transplant type from 3 m (liver) to 74 m (heart). Standard therapies for immune cytopenias were often used and ineffective. The most effective therapy for the immune cytopenia was changing immunosuppression from tacrolimus to another agent. Three of 19 patients died; none directly attributed to the immune cytopenia.

Conclusions—Immune cytopenias are not rare after SOT, and patients usually do not respond well to traditional first line therapies. Provided that the risk of organ rejection is otherwise

manageable, temporary cessation of tacrolimus could be more widely explored in this challenging clinical context.

Keywords

autoimmune hemolytic anemia; immune thrombocytopenia; autoimmune neutropenia; solid organ transplant; tacrolimus

Introduction

Cytopenias following solid organ transplantation (SOT) in children are frequent and often multifactorial. In addition to common causes of cytopenias, including bone marrow suppression related to medications or infections, rare etiologies occur more commonly in the post-transplant setting. These include passenger lymphocyte syndrome, thrombotic microangiopathy, post-transplant lymphoproliferative disease (PTLD), graft versus host disease, hemophagocytic syndrome, and immune mediated cytopenias [1-5]. Autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), and autoimmune neutropenia (AIN) are reported in the literature following liver, intestinal, heart, pancreas, and kidney transplants [5-8]. Based on single center reviews, the prevalence of immune cytopenias following liver and intestinal transplantation ranges from 3-12% [3,9]. In non-ABO matched transplants, AIHA has been noted in 9% of kidney transplants, 29% of liver transplants, and 70% of heart-lung transplants [10].

There are multiple known triggers for immune cytopenias after transplant, including ABO incompatibility, passenger lymphocyte syndrome, infections, and immune dysregulation related to immunosuppression. Case reports suggest that the treatment response of immune cytopenias following transplant may be different from primary immune cytopenias, and different treatments approaches may be necessary [7,11-15]. Reported strategies include standard immune cytopenia treatments, including steroids, IV immunoglobulin, rituximab, and splenectomy, as well as other strategies, such as plasmapheresis, vincristine, and converting from one immunosuppression agent to another [7,8,11,13,15]. We report the demographics, incidence rates, response to treatments, and outcomes of pediatric patients who developed immune cytopenia following SOT at a large pediatric center.

Methods

In this IRB approved, single center retrospective review, patients with immune cytopenias after SOT were identified by EMR search, ICD-9 billing codes, and transplant program databases from January 1995 through June 2012. Transplant types included: heart, lung, kidney, liver, and multivisceral transplants. Medical records were reviewed for all SOT patients with anemia, AIHA, thrombocytopenia, ITP, and/or neutropenia. Laboratory evidence of immune cytopenia was defined as a positive direct coombs test, highly sensitive direct coomb test, GIIb-IIIa antibody, direct anti-neutrophil antibody, and/or bone marrow aspirates consistent with AIHA, ITP, or AIN. Bone marrow findings consistent with immune cytopenias had increased progenitor cells, hypercellularity, and/or large and increased number of megakaryocytes. Three patients included in this analysis were previously reported [8].

ITP response to therapy was defined by using the International consensus group criteria [16]. Response to AIHA treatment included a 2 g/dL increase in hemoglobin or achieving transfusion independency in a previously transfusion dependent patient. An increase in the absolute neutrophil count (ANC) to 500 cells/ul was considered a response in AIN. Any other outcome was considered a non-response to treatment. If patients had multiple cytopenias, time to presentation was the time from the transplant to the first immune cytopenia.

The Buchanan and Adix score was used to grade bleeding with severe bleeding defined as grade 3 [17]. AIHA severity was determined by red cell transfusion requirements, with >10 transfusions over the course of their illness considered severe. Severe AIN was defined as an ANC < 500 cells/ul. The data were collected using REDCap and analyzed in SAS 9.4 [18].

Results

General Demographics

During a 17 year period, 2.4% (19/764) of patients following SOT developed an immune cytopenia (Table I). 68% (13/19) were males. Transplant types included: liver (6/141), heart (5/199), multivisceral (4/17), kidney (4/340), and lung (1/55). The median age at organ transplantation was <3 years for all transplant types. Two cardiac transplant patients received an ABO mismatched organ. Two patients developed immune cytopenias after receiving a second transplant; one patient received a second liver transplant after graft rejection and another patient received a second kidney transplant after renal vein thrombosis. A single patient received both liver and kidney transplants and is counted in both totals. One patient had concurrent autoimmune hepatitis; no other patients had additional autoimmune diagnoses.

Demographics of Immune Cytopenias

Of the 19 patients with immune cytopenias, 18 saw a hematologist through an inpatient consultation or in the outpatient clinic. A bone marrow aspirate and biopsy was obtained in 42% (18/19) patients. An infectious evaluation was completed at the onset of immune cytopenia in many patients: CMV (14), EBV (15), adenovirus (2), and parvovirus (2) with one positive test for adenovirus and one for EBV.

Warm IgG-mediated AIHA was the most common immune cytopenia (12/19). Six patients (32%) were diagnosed with Evans syndrome. The incidence of immune cytopenias varied significantly by transplant type (Table I). ITP was seen more frequently in liver and multivisceral transplants (7/10) than in other SOT types ($p<.05$). Overall median time from transplant to immune cytopenia was 8 months. The median time from transplant to immune cytopenia varied by transplant type from 3 months after liver transplantation to 74 months after cardiac transplantation (Figure 2).

The transplant type associated with the highest incidence of immune cytopenias was multivisceral transplants (23.5%). Median time to presentation was 8 months (range 3-12 months). Multivisceral transplants included liver, small intestine, pancreas, stomach, and duodenum, but varied whether spleen was included. Fifty-three percent (9/17) of patients

who received multivisceral transplants included a spleen transplant. While 75% of patients with multivisceral transplant and immune cytopenias received a spleen transplant, there was no significant difference in the development of immune cytopenias in the patients that received a spleen transplant and those that did not ($p=0.29$).

Signs and Symptoms of Immune Cytopenias

The most common presentation of an immune cytopenia was an asymptomatic lab finding (8/19, 42%). The most common symptoms experienced at any point during the course of immune cytopenias included fatigue ($n=11$), bruising ($n=8$), pallor ($n=8$), bleeding ($n=7$) and infection ($n=4$). Patients with ITP tended to have more bleeding than is typical in a primary ITP pediatric patient; five (5/10) ITP patients experienced grade 3 bleeding, necessitating ITP-directed therapy. In AIHA, the median number of transfusions was 3 (range 0 to >40); only 2/12 patients with AIHA had severe disease. All 3 patients with AIN had severe neutropenia, and 2 developed serious bacterial infections. Patients with multivisceral transplants and immune cytopenias had a more severe course, as defined as requiring an ICU admission for complications of immune cytopenias, than those with other transplant types ($p<0.01$).

Treatments of Immune Cytopenias

Most (15/19) SOT patients received pharmacologic treatment for an immune cytopenia (Table II). Figure 1 shows the different treatments administered to an individual post-transplant patient with immune cytopenias. Many patients received multiple therapies (median 3, range 0-5). Most frequent treatments for immune cytopenias included steroids (12/19), IVIG (12/19), and rituximab (6/19). Treatment definitions are outlined in the Methods. The most effective therapies for AIHA were: switching from tacrolimus to sirolimus or cyclosporine (100% response rate (RR)) and rituximab (75% RR). Treatment with IVIG had only a 43% RR in AIHA. Switching tacrolimus to an alternate agent also gave the best ITP response rate (RR 100%), in comparison with IVIG (RR 50%) and steroids (RR 50%). For AIN, switching from tacrolimus to sirolimus or cyclosporine had a 100% RR. Treatment with rituximab had a 0% RR in ITP, and steroids had a 50% RR or less in all three types of immune cytopenias. The median time from first dose to response for each treatment type was: IVIG (7 days (d), range 2-16 d), steroids (4 d, range 2-19 d), rituximab (28 d, 26-112 d), and switching from tacrolimus to sirolimus or cyclosporine (12 d, range 8-21 d).

Sixteen (16/19) patients were on tacrolimus at the time of onset of the immune cytopenia. Immunosuppressant agents to prevent graft rejection were changed in 5 (26%) patients; in all 5, tacrolimus was stopped. Two patients were switched to sirolimus, two were switched to cyclosporine, and one patient switched initially to cyclosporine, and when the immune cytopenia did not improve, ultimately to sirolimus. AIHA, ITP and AIN resolved in 100% (5/5) of these patients. In 4 of 5 patients, the timing of response could not be attributed to other treatments. In one patient, IVIG was given 2 days after the immunosuppressant switch, and thus the treatment response could be attributable to this treatment as well. Due to transplant team preference (2/5) and acute rejection (1/5), tacrolimus was restarted in 3 of

the 5 patients. Immune cytopenias did not recur in any of these patients when tacrolimus was restarted (median time off tacrolimus 13 months).

Complications During Therapy for Immune Cytopenias

Of the 19 patients, 11 had no complications during therapies given for their immune cytopenias. Complications which occurred while on steroid therapy included: obesity (n=3), hypertension (n=1), behavior changes (n=1), and insulin-dependent diabetes (n=1). Complications after rituximab included: infection with varicella zoster (n=1) and parainfluenza (n=1) and acquired transient hypogammaglobulinemia (n=2). One patient who switched immunosuppression agents to treat the immune cytopenia developed liver graft rejection.

Outcomes

The majority of patients (17/19) were hospitalized at least once for reasons related to their immune cytopenia, including: urgent transfusion requirement (n=7), neutropenic fever (n=2), significant bleeding (n=3), diagnostic work up (n=1), infection (n=2), severe thrombocytopenia (n=1), and for pharmacologic treatment of the immune cytopenia (n=2). Transfusional hemosiderosis occurred in 1 patient with AIHA who was transfusion dependent for several weeks with a peak serum ferritin 10,642 ng/mL. ICU care was required in 37% (7/19) of patients, and 3 patients died. The cause of deaths were: respiratory failure with parainfluenza infection 3 months after diagnosis of AIHA; cardiac failure two years after diagnosis of ITP; and cardiac failure 18 months after diagnosis of ITP. No deaths were attributed to the immune cytopenia. One patient developed post-transplant lymphoproliferative disorder (PTLD) prior to development of the immune cytopenia, and one developed PTLN after the development of the immune cytopenia. The median duration of follow up for the 19 patients was 25 months (IQR 6-62 months) after diagnosis of immune cytopenia.

Discussion

Although the predisposition to the development of immune cytopenias after transplant is poorly understood, the relationship between immunodeficiency and immune dysregulation resulting in immune cytopenias is well described. In this single center study, we found that 2.4% (19/764) of patients had potentially life-threatening immune cytopenias following SOT. The predisposition of certain types of organ transplants, such as multivisceral transplants, towards a higher rate of immune cytopenias suggests that certain features, such as a higher quantity exposure to donor antigens, are risk factors for predisposing patients to immune cytopenias. Transplantation of the spleen has previously been reported as a risk factor for the development of immune cytopenia but this association was not seen in this patient population [9].

In this study, we found that the duration of time from transplant to the development of immune cytopenia was quite variable. Certain transplant types, such as liver and multivisceral, had a shorter median time to immune cytopenias. The variability in timing by transplant type suggests a pathophysiologic difference in the underlying mechanism of the

development of immune cytopenias in different transplant types; for example, early immune cytopenias may be related to a large exposure to donor antigens and later development may be related to medications or immunosuppression. Patients with immune cytopenias after SOT were more likely to develop Evans syndrome similarly to patients with other secondary immune cytopenias. Thus, patients with a newly diagnosed immune cytopenia after SOT should be screened for additional immune cytopenias.

In this pediatric patient population, ITP had a more severe course than a typical pediatric population, with 5/10 (50%) patients experiencing Grade 3 bleeding [19]. The tendency towards bleeding in this ITP population may be due to non-immune platelet factors; such as other organ dysfunction tending towards uremia or synthetic coagulopathy; a higher tendency towards antibody associated qualitative platelet dysfunction; or other associated conditions, such as capillary fragility related to immunosuppression.

Most notably, patients with immune cytopenias following SOT did not respond well to traditional first line therapies. Steroids and IVIG had a response rate of 50% or less in all immune cytopenias. Thus, although these agents are often initially used as treatment, other therapeutic options should be discussed, anticipated, and considered early when these patients present. In our case series, the timing of switching from tacrolimus to sirolimus or cyclosporine appeared to result in remission of all affected cell lines. Tacrolimus disrupts negative thymic selection while down-regulating T regulatory cells, leading to an increased number of self-reactive T cells and increasing the risk of autoimmunity [20,21]. While tacrolimus has been thought to contribute to the development of immune cytopenias after SOT, this drug is a standard immune suppressive agent in transplant medicine for preventing organ rejection. Although switching immunosuppression in this patient population needs careful consideration by the transplant team due to the risk of organ rejection, switching from tacrolimus to another agent should be considered early in SOT patients with immune cytopenias refractory to other therapies. Importantly, those who restarted tacrolimus did not experience recurrence of the immune cytopenias, indicating that permanent discontinuation of tacrolimus may not be necessary and several months off the medication may be sufficient to reverse the immune cytopenia.

This case series has a number of limitations. The identification of this patient population was difficult as patients commonly develop *non-immune* cytopenias after SOT. Due to this limitation, this record review may have only identified the most severely affected patients, and the actual incidence, particularly of mild immune cytopenias, may be higher. The requirement of necessary laboratory evaluation also may have reduced the overall number of included patients. The retrospective nature clearly limited the available information to that which could be found in the hospital medical record. Lastly, true overall treatment response rate was confounded by the fact that most patients received multiple therapies and the timing and order of these treatments varied between patients. In addition, some patients with immune cytopenias may have improved regardless of pharmacologic intervention over time.

In this single center study, we found that the development of potentially life-threatening immune cytopenia following SOT is not uncommon. Given the high rate after multivisceral transplant in particular, physicians should have a heightened suspicion for immune cytopenia

when any cytopenia develops in this population. After an initial attempt using typical first-line treatments, switching immunosuppression, particularly from tacrolimus to another agent, should be considered early in refractory patients.

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Figure 1.

One patient's immune cytopenia course after kidney transplant: (1) initial diagnosis of immune cytopenias, (2) stopped azathioprine, (3) started steroids, (4) switched tacrolimus to cyclosporine, (5) given IVIG, and (6) switched from cyclosporine to sirolimus. ANC: absolute neutrophil count, hb: hemoglobin, plt: platelet count.

Figure 2. Median duration between solid organ transplant and diagnosis of immune cytopenia in months. Liver (n=6), Multivisceral (MV, n=4), Kidney (n=4), Heart (n=5), Lung (n=1).

Table I
Demographics of Immune Cytopenias by Type of Solid Organ Transplant

Transplant Type	Incidence by type of transplant *	Median Age at transplant (months)	Time to Immune Cytopenia presentation (months)	Type of Immune Cytopenia **
Liver	4.2% (6/141)	21 (7-59)	3 (1-46)	AIHA 2 ITP 4 AIN 1
MV	23.5% (4/17)	11 (4-26)	8 (3-12)	AIHA 3 ITP 3
Heart	2.5% (5/199)	12 (2-56)	74 (13-112)	AIHA 3 ITP 2 AIN 1 GT 1
Renal	1.2% (4/340)	27 (14-110)	10 (1-90)	AIHA 3 ITP 2 AIN 1
Lung	1.8% (1/55)	21	24	AIHA 1

AIHA autoimmune hemolytic anemia, ITP immune thrombocytopenia, AIN autoimmune neutropenia, GT Glanzmann's Thrombocythemia;

* 1 patient had 2 different transplants,

** 6 patients had Evans syndrome with multiple immune cytopenias

Table II
Responses of Immune Cytopenias to Individual Treatments

Immune Cytopenia	Treatment	n *	Response Rate
Autoimmune Hemolytic Anemia	Steroids	7	43%
	IVIG	7	43%
	Rituximab	4	75%
	Tacrolimus to Sirolimus	3	100%
	Tacrolimus to Cyclosporine	1	100%
Immune Thrombocytopenia	Steroids	4	50%
	IVIG	6	50%
	Rituximab	2	0%
	Tacrolimus to Sirolimus	2	100%
	Tacrolimus to Cyclosporine	1	100%
Immune Neutropenia	Steroids	2	0%
	IVIG	2	50%
	Rituximab	0	n/a
	Tacrolimus to Sirolimus	1	100%

* 6 patients had Evans Syndrome with more than one immune cytopenia