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ORIGINAL ARTICLE

Autoimmune cytopaenia after paediatric intestinal transplantation: a case series

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Summary

Autoimmune cytopaenia is a rare, but severe complication after solid organ transplantation. We retrospectively analysed 57 paediatric intestinal transplants performed in 49 patients between 1999 and 2009. Autoimmune cytopaenia was observed in six patients; it appeared after an average of 10 months post-transplant. Warm autoimmune haemolytic anaemia was developed in three patients, cold autoimmune haemolytic anaemia in one and two presented a mixed type. Incidence and causes for haematological cytopaenia such as the following were investigated: immunosuppression, major blood mismatch, viral infection, malignancy, passenger lymphocyte syndrome and lymphoproliferative disorders. Initial treatment included high-dose steroids, intravenous immunoglobulin, plasmapheresis and maintenance of body temperature above 37 °C in those with cold autoantibodies. Inclusion of the spleen in multivisceral transplants seems to be an important risk factor. All patients, except one, relapsed after classic therapy, requiring additional treatments. Sirolimus conversion was performed in four patients. One died after infection. The immunosuppressive therapies associated with other concomitant factors, such as viral infections, lymphoproliferative disorders, graft-versus-host disease, passenger lymphocyte syndrome and the inclusion of the spleen as part of multivisceral graft seem to play an important part in the development of autoimmune processes after intestinal transplantation. Therapy is not well established, especially in those resistant to first-line treatment.

Introduction

Autoimmune cytopaenia is a rare, but severe complication of solid organ transplantation in children. This autoimmune disorder has been described previously. However, there is no consensus on the role of immunosuppressive therapy as inductor of haemolysis or on its treatment, especially in those resistant to first-line treatment [1–3].

Post-transplant haemolysis can be caused by both immune- and non-immune-mediated mechanisms. In case of immune haemolytic anaemia, these mechanisms can be classified as autoimmune, alloimmune or drug-induced. Autoimmune haemolytic anaemia (AIHA) is characterized by the production of IgG and/or IgM antibodies directed against red blood cells. Depending on the temperature at which autoantibodies act, AIHA can be classified as warm or cold type. Warm antibodies are typically IgG, may or may not fix complement and have their greatest affinity near 37 °C. On the other hand, cold antibodies are typically IgM, fix complement and react most strongly near 0 °C. Occasionally, patients may have a mixed-type AIHA, with both warm and cold autoantibodies [4]. Immune anaemia can be associated with immune thrombocytopaenic purpura, known as Evans Syndrome [5].

The aim of this was to characterize the clinical presentation, diagnosis, treatment and outcome of children with autoimmune cytopaenia after intestinal transplantation.

Patients and methods

Between October 1999 and October 2009, 49 paediatric patients received 57 intestinal transplants, of which 22 were small bowel transplants, 19 combined small bowel and liver, 14 multivisceral and 2 modified multivisceral transplants. Ten of the multivisceral grafts had the spleen included. All organs came from deceased donors and had identical ABO blood type. Before transplant surgery, the presence of irregular haemagglutinins and Coombs test were systematically ruled out.

Indications included necrotizing enterocolitis ($n = 6$), volvulus ($n = 9$), chronic intestinal pseudo-obstruction ($n = 8$), intestinal ischaemia ($n = 6$) gastroschisis ($n = 6$), intestinal atresia ($n = 5$), microvillus inclusion disease ($n = 3$), intestinal epithelial dysplasia ($n = 2$) and other indication's ($n = 4$). Retransplants were required in eight patients, six because of acute rejection and two because of chronic rejection.

From the beginning of this study, the immunosuppressive protocol was periodically reviewed and modified. Until 2004, first line immunosuppression consisted of azatioprine–basiliximab for preconditioning and tacrolimus–corticosteroids for maintenance therapy. Between 2004 and 2005, induction was based on thymoglobulin. After 2005, this procedure included basiliximab and tacrolimus–prednisone therapy. In certain specific cases, individual immunosuppressive protocols were used, such as in patients who received three transplants, who were treated with alemtuzumab as induction and tacrolimus as maintenance. HLA-matching, crossmatch or donor-specific anti-HLA antibodies were not systematically performed. Statistical analysis was performed using the Fisher exact test. A P-value <0.05 was considered significant.

Results

Autoimmune cytopaenia was observed in six of our patients (12.2%): two females and four males. Prednisone, basiliximab and tacrolimus were used as the primary immunosuppression except in two patients. One received thymoglobulin as induction treatment followed by tacrolimus. The other received alemtuzumab and tacrolimus therapy at the third transplant. This was because of an acute rejection of the first graft and a chronic rejection of the second. Acute rejection was observed in two patients and was controlled in both cases with high doses of steroids (Table 1). Immune haematological cytopaenia appeared with an average of 10 months post-transplant (range: 1–21 months). At that time, all of them had a trough blood level of tacrolimus between 5 and 10 ng/ml.

Incidence of autoimmune cytopaenia by risk factor is shown in Table 2. In patients with multivisceral transplants, the inclusion of spleen as a part of the graft resulted as a risk factor for the development of autoimmune cytopaenia. Gender and immunosuppressive protocol were not statistically significant risk factors.

Causes for autoimmune haematological cytopaenia were investigated: immunosuppression, major blood group mismatch, viral infection, malignancy, passenger lymphocyte syndrome and lymphoproliferative disorder. PCR for cytomegalovirus (CMV) was positive in patient 3 and PCR for Epstein–Barr virus (EBV) in patient 1. After further studies, patient 1 was diagnosed with EBV-associated post-transplant lymphoproliferative disorder. IgM for Parvovirus B19 was positive in patient 5. There was no clinical or laboratory evidence for malignancy or viral infection in patients 2 and 4. Concomitant graft-versus-host disease, with severe skin and intestinal affectation, was diagnosed in patient 6 and it was resistant to high doses of steroids, basiliximab and splenectomy, requiring four weekly doses of alefacept (15 mg i.m.) for remission.

Treatment for anaemia included high doses of steroids (5 mg/kg/day for 3 days), intravenous immunoglobulin (1 g/kg/day for 5 days) in all of our patients and mainte-

Table 1. Patient and transplant characteristics.

Patient number	Initial diagnosis	Gender	Graft type	Spleen	Primary immunosuppression	Acute rejection
1	Omphalocele	M	MV	Yes	FK+CS+BS	Yes
2	Gastroschisis	M	SB	No	FK+CS+BS	No
3	Volvulus	M	MV	Yes	FK+AL	No
4	Mesenteric teratoma	F	MV	Yes	FK+CS+BS	No
5	Gastroschisis	M	SB+L	No	FK+TM	No
6	Sd. Gardner	F	MVM	Yes	FK+CS+BS	Yes

M, male; F, female; MV, multivisceral; MVM, modified multivisceral; SB, small bowel; SB+L, small bowel + liver; FK, tacrolimus; CS, corticosteroids; BS, basiliximab; AL, alemtuzumab; TM, thymoglobulin.

Table 2. Risk factors for autoimmune cytopaenia.

Risk factor	Incidence of autoimmune cytopaenia	%	*P*-value
Graft type			
SB/SB+L	2/41	4.8	0.03
MV-S	4/10	40	
MV-NS	0/6	0	
Immunosuppression			
Steroid-free	2/14	14.3	0.71
Steroid-containing	4/39	9.3	
Gender			
Male	4/26	5.3	0.47
Female	2/23	8.7	

SB, small bowel; SB+L, small bowel + liver; MV-S, multivisceral with spleen; MV-NS, multivisceral without spleen.

nance of body temperature over 37 °C in those patients who presented cold haemagglutinins. All patients, except patient 3, relapsed after standard therapy. The donor spleen had to be removed in patients 5 and 6 and they were treated with plasmapheresis after first-line treatment. All of them (except patient 3) were ultimately treated with four infusions of anti CD 20 monoclonal antibody (375 mg/m^2 once a week). Substitution of tacrolimus for sirolimus was attempted in patients 1, 4, 5 and 6 (Table 3) to minimize thymic dysfunction, the risk of developing tacrolimus-related microangiopathic haemolytic anaemia and renal damage. Finally, clinical remission was achieved and autoantibodies remained undetectable in all of them except patient 5 who died before remission (*Pneumocystis carinii* infection), and patient 6 who required alternative therapies. In this latter case, remission occurred after treatment with alemtuzumab in four doses of 5–10–15–30 mg i.v.

The overall patient survival for our series is 63% (31/49) and 55% (31/57) for the graft. Graft survival and patient survival in patients with haematological complications was 83% (5/6).

Discussion

Haemolysis, which is caused by a variety of immune and nonimmune mechanisms, is a well-recognized complication of solid organ transplant surgery. Immune-mediated haemolytic anaemia after solid organ transplant surgery may be alloimmune or autoimmune. Alloimmune haemolytic anaemia tends to occur within the first weeks after transplant surgery. It is the result of red blood cell antibody production from passenger B lymphocytes accompanying the allograft [6–8]. The late onset of the anaemia in our patients and the ABO identical nature of the donor made this aetiology unlikely.

Microangiopathic haemolytic anaemia associated with tacrolimus is a well-known disorder. It is a severe microvascular occlusive thrombotic microangiopathy with red cells fragmentation and platelet aggregation; but, it can be also an abnormal immune response. There is some evidence that calcineurin-dependent agents impair thymic functions resulting in escape and clonal proliferation of potentially autoreactive T cells.

Autoimmune haemolytic anaemia is a rare cause of haemolytic anaemia after transplant surgery. Autoimmune-mediated haemolysis typically occurs from 2 to 25 months (median 10 months) after transplant surgery, as in our series. Among both types, warm type is more common. Red blood cells are usually coated with IgG with or without C3. Cold type autoimmune haemolysis, as in our patient, occurs later after transplant surgery. Only complement C3 is usually found on the red blood cell [9]. It is rare to find patients with mixed-type warm acting IgM and IgG and it seems to have poor prognosis. Patient 5 and 6 had a severe clinical course compared with the other patients. In both of them, warm IgG and IgM were found.

In addition to being able to diagnose this type of anaemia (clinical course, laboratory findings and immunohaematological studies), it is important to recognize its aetiology to decide the best treatment. Causes of AIHA are generally viral infections. For this reason, a complete screening of recent CMV, EBV, herpesvirus 6, parvovirus

Table 3. Clinical characteristics and outcome of patients with autoimmune cytopaenia.

Patient number	Delay transplant cytopaenia (months)	Cytopaenia	Antibodies	Coombs	Treatment	Sirolimus conversion	Outcome
1	12	A	IgG	+	CS/IVIG/RT	Yes	Remission
2	21	A	IgM	+	CS/IVIG/RT	No	Remission
3	2	A	IgG	+	CS/IVIG	No	Remission
4	13	A+T	IgG +AP	–	CS/IVIG/RT	Yes	Remission
5	1	A+T	IgM+IgG	–	CS/IVIG/PF/SP/RT	Yes	Died
6	8	A+T	IgM+IgG	–	CS/IVIG/PF/SP/RT/Alemtuzumab	Yes	Remission

A, anaemia; T, thrombocytopaenia; AP, antiplatelets; +, positive; –, negative; CS, corticosteroids; IVIG, intravenous immunoglobulin; RT, rituximab; PF, plasmapheresis; SP, graft splenectomy.

B19 infection must be performed [10]. Other agents, like *Mycoplasma pneumoniae* [11], have also been reported as responsible for haematological disorders. Lymphoproliferative disorders could be another possibility, as in patient 1. This patient developed a grade II lymphoproliferative disorder with polyclonal plasmocytosis, tonsillar and paratracheal node affectation. This explains the importance of PCR-EBV controls after transplant surgery. One last possibility we would like to emphasize is the production of alloantibodies by lymphocytes from the donor (graft-versus-host disease) as in patient 6. In this case, immunosuppression must be carefully managed [12].

This leads us to several hypotheses that have been discussed [13]. There is increasing evidence that supports the importance of intact T-cell immunity for proper B-cell regulations. There is some evidence that calcineurin-dependent agents impair thymic functions, resulting in escape and clonal proliferation of potentially autoreactive T cells. Based on this, some cases of clinical remission in autoimmune cytopaenia have been reported after conversion of immunosuppressive therapy with tacrolimus into sirolimus or cyclosporine. These autoimmune manifestations should not be confused with haemolytic anaemia, which is secondary to a microangiopathic process, as part of the haemolytic uremic syndrome. This has been associated with the use of calcineurin inhibitors and it is secondary to endothelial injury [14,15].

Composite multivisceral grafts containing spleen have more donor passenger leucocytes and this appears to be an important risk factor for the development of autoimmune cytopaenia. On the other hand, its inclusion seems to show some protective effect on small bowel rejection and can decrease the risk of sepsis from encapsulated organisms associated with asplenic state. This makes us think carefully about the inclusion of the spleen in the multivisceral transplants considering possible benefits and risks [16].

Autoimmune haemolytic anaemia treatment varies depending on whether the patient has warm autoantibodies, cold agglutinins or if there is an underlying disorder that must be treated. Cold AIHA therapy includes maintaining an adequate body temperature, with or without plasmapheresis, which can offer a temporary benefit in some patients. Corticosteroids are less effective than in warm type, but, in some cases, can be useful. Initial treatment for warm AIHA should be high-dose corticosteroids, with 1–3 weeks' maintenance [17]. Resistance to corticosteroids or dependence on high doses are common findings; in these cases, intravenous immunoglobulin can be used as second-line therapy for its decreased morbidity. Splenectomy should be considered in those patients in whom the spleen is included as part of the graft. Although there is a limited experience in the use of Rituximab in children, our results for refractory autoimmune cytopaenia are similar to those observed by other authors [18–20]. Five of our patients received four infusions of Rituximab (375 mg/m^2 once a week). No infusion side effects were seen and tolerance to treatment was good. Potentially dangerous infectious complications can occur, but have not been seen in our series [21]. To reduce the risk of infection, intravenous immunoglobulin must be recommended until hypogammaglobulinaemia resolves.

Patients with no response to rituximab are candidates for new line therapies, such as alemtuzumab, a humanized IgG monoclonal antibody specific for the CD52 antigen, which induced clinical and immunological remission in our patient with mixed-type refractory AIHA, as described by others [22,23].

On the basis of the importance of T-cell immunity for proper B-cell regulation, we must consider the immunosuppressive regimen of a transplant recipient with AIHA [24,25] and sirolimus could be an alternative immunosuppressive agent in resistant patients.

Conclusions

Autoimmune cytopaenia is a rare, but severe complication after solid organ transplantation. The immunosuppressive therapies used, associated with other concomitant factors such as viral infections, lymphoproliferative disorders, graft-versus-host disease or passenger lymphocyte syndrome, play an important part in the development of autoimmune processes after intestinal transplantation and should be tested systematically. This is another difficulty in managing the immunosuppressive treatment for these patients, as they suffer from immunological dysregulation. The inclusion of the spleen as part of the multivisceral graft, which appears to be an important risk factor for the development of autoimmune cytopaenia, on the other hand, should have a protective effect on rejection. For these reasons until their immunological and haematological effects are clarified, its inclusion in multivisceral transplants should be carefully discussed.

Few patients respond to classic therapies. Treatment with rituximab has been proven to be effective in some of these cases. In our series, as in others described in the literature, mixed type of AIHA, with both warm and cold autoantibodies, should have a worse prognosis and poorer treatment response, requiring alternative therapies for remission.

Authorship

GB, MY and ER: participated in writing the paper. MM, JS, EM-O, AMA and ML-S: participated in the research. GP: participated in the research design.

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