Graft-versus-host disease in paediatric liver transplantation: A review of the literature

A Kakotrichi, MD; **J Hind**, BMedSci, BMBS, FRCPCH

Paediatric Liver, GI and Nutrition Centre, King's College Hospital, London, UK

Corresponding author: *J Hind (jhind@nhs.net)*

Background. Graft versus host disease (GvHD) is a major complication after haematopoietic stem-cell transplantation but much less common after solid organ transplant and rare after liver transplantation. Early symptoms can resemble viral infections or drug-reactions and thus it may be under-investigated. There are no standard treatment guidelines for GvHD after paediatric liver transplantation.
Objectives. To review all cases of GvHD reported to date after paediatric liver transplant.
Methods. Literature review of all English-language full-text articles published between 1990 and 2017
Results. Case series articles and case reports were reviewed and collated. Symptoms, diagnostic investigations, treatments and outcomes were described for all reported cases.
Conclusion. GvHD in paediatric liver transplantation is difficult to treat and has a high mortality, but better recognition and understanding with novel therapies available offer hope for the future.

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Graft-versus-host disease (GvHD) was recognised as early as the 1960s by Gowans[1] as an immunological attack by the lymphoid cells of a graft against tissues of the host. It was subsequently described by Billingham[2] in 1966 in his historic Harvey Lecture, who described the conditions for its development that stand to date: (*i*) the graft should contain immunologically competent cells; (*ii*) the host antigens produced should not be present in the transplant donor; and (*iii*) the recipient should be incapable of generating an efficient response to destroy the transplanted cells.

GvHD became well known to the scientific community as a major complication of haematopoietic stem-cell transplantation (HSCT); donor T-cells react to host antigens on antigen-presenting cells (APCs,) causing further activation of donor cytotoxic T-cells, natural killer cells (NKCs) and macrophages, which target tissue-specific cells in the host, ending in organ damage. It is a multiorgan disease that can also complicate solid-organ transplantation and in rare cases, liver transplantation.

The clinical presentation of GvHD has been extensively described after allogeneic bone-marrow transplantation; however, it can also complicate solid-organ transplantation in adults and in children.[3-7] GvHD after solid-organ transplant may be under-reported and under-investigated owing to the difficulty in its recognition. Early symptoms are nonspecific and resemble common viral infections or drug reactions, and may therefore be easily attributed to other causes.

Currently, there are no universal treatment guidelines for GvHD after paediatric liver transplantation; steroids and other immunosuppression agents have been tried, but are only described in individual case reports and case series. The purpose of this review was to review all reported cases of GvHD reported in paediatric liver transplantation to date.

Methods

We searched PubMed for English-language full-text articles published between 1990 and 2017 for eligible studies. The following search terms were used both alone and in combination: 'GvHD', 'aGvHD' [acute graft-versus-host disease], 'graft-versus-host-disease', 'cGvHD' [chronic graft-versus-host disease], 'paediatric', 'pediatric', 'liver', 'transplantation', 'solid organ', 'graft', 'multivisceral', 'kidney', 'pancreas', 'spleen', 'prevention', 'treatment', 'steroids', 'refractory', 'cyclosporine', 'FK', 'tacrolimus', 'sirolimus', 'MMF' [mycophenolate mofetil], 'ATG', 'anti-thymocyte globulin', 'alemtuzumab', 'rituximab', 'MSCs' [mesenchymal stem cells], 'infliximab', 'etanercept', 'basiliximab', 'ECP' [extracorporeal photopheresis]. The references of the articles were also searched for relevant publications.

The search found 1 case series of paediatric GvHD after isolated liver transplantation, 2 case series containing paediatric cases of GvHD after liver transplantation in mixed adult and paediatric populations, and 5 case reports of paediatric GvHD after liver transplantation that were individually published.[8-17] Studies that described GvHD after combined liver/intestinal, pancreatic, spleen, multivisceral transplantation or combinations of the above, are described briefly.

Pathophysiology

The immunobiology and pathogenesis of acute GvHD can be summarised in three steps.

Step 1: (occurs pre-GvHD, beginning before the donor cells are implanted). Prior disease, comorbidities, infection and the conditioning regimen damage the host tissues, resulting in activation of the host immune cells and the secretion of pro-inflammatory cytokines: tumour necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6 and interferon gamma (IFN-γ).[18] These cytokines upregulate cell surface adhesion molecules and major histocompatibility complex antigens on host APCs, which trigger a response by mature donor T-cells.[19] At the same time, the donor tissue is exposed to a foreign environment.[20]

Step 2: Donor $CD4^+$ and $CD8^+$ T-cells are activated by host and donor antigen-presenting cells (APCs) and by pro-inflammatory cytokines, resulting in a Th1 response. This response activates further T-cells and natural killer cells (NKCs), and induces macrophages to

release TNF-α and IL-1. The most potent APCs are the dendritic cells (DCs), which are activated by inflammatory cytokines, lipopolysaccharides (LPS) and necrotic cells damaged by the host conditioning.[18] Naive T-cells proliferate and differentiate following activation. IFN-γ is released from activated T-cells and upregulates adhesion molecules and chemokines, which in turn mediate damage to the skin and gastrointestinal (GI) tract through nitric oxide (NO) induction and Fas.[21]

Step 3: Damage to the target tissues is specific and is caused primarily by activated T-cells' cytotoxic damage against host cells through Fas-Fas ligand-mediated apoptosis in the liver, and perforin-granzyme-B-mediated cytolysis in the GI tract and the skin.[19] The mechanism of action depends also on positive feedback of inflammatory molecules adding to the pre-existing cytokine cascade, thus increasing the T-cell activity.[6]

Clinical manifestations of GvHD

GvHD after paediatric solid-organ transplantation occurs infrequently, and GvHD after paediatric liver transplantation has been rarely reported. It has been described more commonly in adult patients in the literature, with a varying incidence of 0.1 - 2%, and is related to high mortality and morbidity for those adults affected.[3,5,22]

The most common manifestations of GvHD have been described after HSCT, and involve the skin, the GI tract, the liver and rarely the eyes and the oral mucosa.[19,20,23]

The most common manifestations after liver transplantation are fever and a skin rash. They usually occur within 1 - 8 weeks after the transplantation. The rash is maculopapular and it can progress to erythroderma or bullous desquamation in more severe stages. GvHD can also become a multisystem disease with involvement of the GI tract and the bone marrow. GI symptoms include diarrhoea, emesis, blood in the stools or anorexia and abdominal pain. The symptoms are attributed to the lymphocyte infiltration and subsequent loss of absorption of the intestinal mucosa. The bone marrow, when affected, usually manifests with thrombocytopenia, neutropenia, lymphopenia, and haemolytic anaemia (HA).

The main difference in GvHD after liver transplantation to that after HSCT lies within the function of the transplanted liver; in GvHD after liver transplantation, this remains normal. The immunocompetent donor B-lymphocytes ('passenger lymphocytes') are transferred in or with the liver (in the lymphatics or lymph nodes that accompany the donor liver), and they are activated on host APCs; as the lymphocytes and liver are both of donor origin, the liver function remains intact.

Another type of GvHD has been described after solid-organ transplantation that demonstrates features of a humoral rather than a cellular response; evidence of haemolysis and fever have been demonstrated in ABO-unmatched transplants, with circulating red-cell bound isohaemagglutinins found in the peripheral blood of such patients, against their A or B antigens.[24] These antibodies are thought to originate from B-passenger lymphocytes, and this response leads to haemolysis.

GvHD after paediatric small-bowel, combined liver/intestinal, multivisceral and other solid-organ transplantation

GvHD has been described in paediatric liver/intestinal transplantation (LITx), small-bowel transplantation (SBTx), multivisceral transplantation (MVTx), modified multivisceral transplantation (MMVTx) and other solid-organ transplantation, such as pancreatic.

Intestinal transplantation

Mazariegos *et al.*[25] reported the incidence of GvHD in intestinal transplantation to be around 6.5%. They presented 8/122 paediatric patients (3 after SBTx and 5 after MVTx) with GvHD, diagnosed with histology and blood chimerism studies. Andres *et al.*[26] also reported 10% of intestinal transplantation patients (post-SBTx, LITx and MVTx) who subsequently developed GvHD.

Multivisceral transplantation

The Spanish group of Feito-Rodrıguez *et al.*[27] reported that GvHD occurred in higher rates in MVTx and MMVTx compared with solid-organ transplant (e.g. liver grafts), and also compared with SBTx or combined liver/SBTx. This may be due to the higher amount of lymphoid tissue in the multivisceral grafts. In SBTx and MVTx, the incidence varies from 9 - 29.4%, and the clinical symptoms are similar to those of GvHD after HSCT, involving the skin, GI tract, liver and bone marrow.[28]

Severity of GvHD

GvHD can be categorised as either acute graft-versus-host disease (aGvHD) or chronic graft-versus-host disease (cGvHD), depending on the presentation of symptoms in relation to the transplantation. Acute GvHD occurs when symptoms evolve in the first 100 days of transplant, and cGvHD after the 100th day of the surgery. There is also another classification, according to the American Society for Blood and Marrow Transplantation[29,30] issued in 2006, of late-onset aGvHD (symptoms occurring after day 100), and an overlapping entity of aGvHD and cGvHD occurring any time after transplant.

To quantify the severity of aGvHD after HSCT, the modified Seattle-Glucksberg score is commonly used. The four grades of aGvHD are based on the extent of the involvement of the skin, the GI tract and the liver (Table 1). For cGvHD, another classification is utilised (Table 2).

To date, there is no known grade system/index to accurately evaluate the severity of GvHD after liver transplantation as a multiorgan entity involving the skin, the gut, and possibly the bone marrow. In contrast to the scales described in Tables 1 and 2, in GvHD post-liver transplant the liver is spared; therefore, to quantify its severity with the Glucksberg grade would be a misconception.

Presentation

The case reports and case series are summarised in Table 3. The data add up to a total of 13 patients who developed GvHD after liver transplantation (left lateral segment orthotopic liver transplantation (LLS OLT) or living-donor liver transplantation (LDLT)) in the literature.

Time of presentation

In the presented studies, the earliest presentation of aGvHD after liver transplantation was on day 11 in the case report of Cattral *et al.*,[8] and the latest presentation of aGvHD was on day 95 in a case report by Comenzo *et al.*,[10] although no skin biopsy was obtained in the latter case, and the diagnosis of aGvHD was based only on clinical findings and response to treatment.

In one case report by Dunn *et al.*,[12] a patient presented with features of aGVHD and cGvHD, confirmed by chimerism and a positive skin biopsy, 6 years after liver transplantation. Three more patients in other case series and case reports presented with late-onset aGvHD at 140, 160 and 480 days after transplantation.[9,15,16]

Presenting symptoms

The most common symptom identified was skin manifestations, affecting 11 out of the 13 patients (84.6%). The rash was predominantly

described as maculopapular, which in 4/11 (36.4%) cases affected more than 75% of the patient's body surface and involved desquamation; in 2 cases, it was described as a vesiculopapular rash or a generalised erythema, respectively.

Gastrointestinal symptoms were described in 9/13 (69.2%) cases, with diarrhoea being the principal symptom in 8 cases. Vomiting and blood in the stools accounted for 3/9 (33.3%) and 2/9 (22.2%) of the cases, respectively.

Fever (not associated with intercurrent infections) was reported in 8/13 (61.5%) patients, although the incidence may have been higher as this information was not available for 3 patients.

Haematological or BM involvement was reported in 10/13 (76.9%) of patients. Eight patients (61.5%) developed pancytopenia, 1/8 presenting with HA and subsequently pancytopenia, while another developed HA and lymphopenia. In 1 patient anaemia was mentioned, but no other information was provided.

Of the 3 patients who developed HA, Cattral *et al.*[8] described HA preceding the GvHD symptoms (as possible humoral GvHD), whereas Pinna *et al.*[9] reported a transient HA that was self-limiting and associated with the formation of isoagglutinins formed by the donor against the host red blood cells.

Diagnosis

Several techniques and tests have been used to identify GvHD in the skin, the GI or the BM, with skin biopsy predominantly used for the skin manifestations; endoscopy and GI histology for the intestinal symptoms; and in recent years, fluorescence *in situ* hybridisation (FISH) and immunostaining for donor HLA markers in peripheral blood.[26,31,32]

GvHD symptoms are usually nonspecific, and might be missed or misinterpreted as a drug reaction or an infection; therefore, biopsies of all involved tissues tend to be supportive in establishing the diagnosis, but their sensitivity and specificity are low.[19,33-35] Skin biopsy may reveal apoptotic bodies, lymphocytic infiltration of the epithelium, lymphocytes out of the vessels (exocytosis) and vacuolisation of the basal epithelial layer in the native involved organs – all suggestive but not pathognomonic of GvHD (Fig. 1).[26,27,34-36]

Histopathological grades of aGvHD have been suggested for GvHD after HSCT since 1974. A four-point grading system was introduced by Lerner *et al.*,[37] where grade I changes consisted of basal-cell vacuolation, grade II of cell necrosis, grade III dyskeratosis and spongiosis, and grade IV epidermal sloughing.Research showed that it is almost impossible to distinguish drug-induced dermatitis from aGvHD, as the lesions described in histopathology are almost identical.[38] Furthermore, there was no correlation between the histopathological findings and the degree of severity of aGvHD (Fig. 2).[39,40]

Donor chimerism studies or immuno-staining for donor HLA markers are methods used to detect the donor immunocompetent cells in the recipient's peripheral blood. In cases of sex-mismatched donor/recipient, the donor cells can be identified by FISH for X and Y chromosomes; however, the FISH analysis is quantitative and it does not distinguish between T or B lymphocytes.[31,32]

From the total of 13 patients with GvHD presented in Table 1, 5 had a positive skin biopsy (38.5%), and another 30.8% had a positive GI biopsy, with 7 patients presenting with symptoms of both GI and skin GvHD, but biopsy confirmation in only one organ. Eight (61.5%) patients had positive donor HLA markers in peripheral blood and one (7.7%) had a positive bone marrow aspirate. There were 2 patients in the same case series by Kamei *et al.*[13] for which there are no data on the diagnostic tests used to establish the diagnosis.

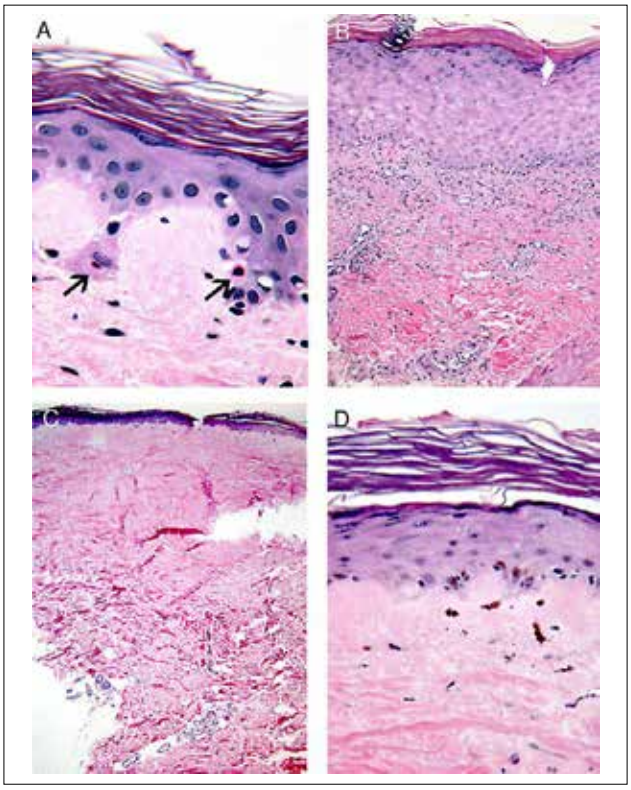

Fig. 1. Skin biopsy. Progression of histologic changes from acute to chronic cutaneous GvHD. (A) Screening skin biopsy, day 85. A focal apoptotic body formation is present at the tips of rete ridges (arrow) with focal surrounding lymphocytic satellitosis (original magnification ×400). (B) Lichen planus-like chronic GvHD, day 426. The thickened epidermis displays orthokeratosis, hypergranulosis, and acanthosis. The striking lichenoid reaction along the damaged basal layer includes a prominent lymphocytic inflammation and infiltration, apoptotic changes, loss of rete ridges, and prominence of the superficial vascularity (original magnification ×100). (C) Progression of GVHD from panel A into a sclerotic stage, day 382. A zone of dense, relatively avascular homogenized collagen has replaced the papillary and upper reticular dermis (original magnification ×63). (D) High-power view shows a hyperkeratotic epidermis with flattening of the rete ridges, vacuolar changes, and lymphocytic infiltration along the basal layer, with disruption of the epidermal melanin unit and with coarse clumps of melanin in the epidermis and incontinent melanin pigment in the sclerotic papillary dermis (original magnification ×160). Reproduced from Shulman et al.[36] *with permission from Elsevier Inc. under the terms of the Creative Commons Attribution-NonCommercial-No Derivatives License (CC BY NC ND).*

Risk factors

The literature on risk factors for GvHD post liver transplantation is limited. Most studies aiming to identify potential risk factors for GvHD have been reported after MVTx or combined LITx in the literature, but not in liver grafts alone. Wu *et al.*[41] reported a series of 241 patients (both children and adults), in which the younger children, especially those younger than 5 years old, presented with a higher incidence of GvHD; furthermore, recipients of MVTx (with or without a liver graft) also had a higher incidence of GvHD compared with isolated SBTx recipients, and the inclusion of spleen in the MVTx grafts, or recipient splenectomy, was also associated with GvHD.

Past studies have shown that an HLA-homogenous graft with one-way donor-recipient HLA matching may lead to a higher incidence of GvHD, putting living-related liver transplantations

from a parental donor at higher risk of this complication. The mechanism of action lies in the fact that T-cells from donors homozygous to all HLA loci in the recipient present no HLA antigens that can be recognised as foreign and therefore they cannot lead to rejection. However, the donor's passenger circulating immune cells can identify the recipients' HLA as foreign, as half of them derive from the other parent. This could also lead to chimerism and GvHD. Whitington *et al*.,[11] reported one such case that led to cGvHD.[11] The group of Kamei *et al*.[13] (Table 1) analysed 8 cases of fatal GvHD and also 906 living-donor liver transplantations, and suggested that the risk for fatal GvHD could depend on the number of loci with donor-dominant one-way HLA matching; if the donor is homogenous with one-way HLA matching at three loci, the risk is significantly higher.

Another risk factor mentioned in the literature is the underlying diagnosis, especially a pre-existing immunodeficiency. Smith *et al*.[42] described one paediatric patient with a pre-existing undiagnosed unclassified combined immunodeficiency, which led to bone-marrow aplasia, higher susceptibility to infections and, finally, multiorgan failure and death.

Pinna *et al*.[9] (Table 1) analysed a series of 124 paediatric liver transplantations, and of those, only 3 developed GvHD (2.4%). However, cytomegalovirus infection is suggested to have played a role, as the infection itself, in combination with the use of immunocompromising drugs, may have led to an increased 'cytokine storm' response of donor T-cells to host antigens, thereby increasing the risk of GvHD.

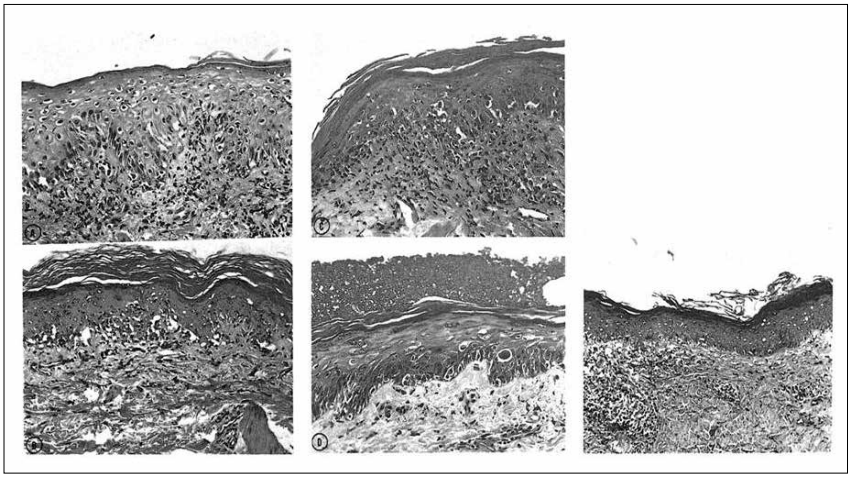

Fig. 2. Cutaneous grade 2 GvHD. Note vacuolar change of basal cell layer and dyskeratotic cells in epidermis in all biopsies. The amount of infiltrate varies markedly. All of these biopsies were taken between day 2l and day 25 post transplantation, and all patients had clinical GvHD and comparable peripheral white blood cell counts. (A) Prominent mononuclear-cell infiltrate in the upper part of the dermis. (Haematoxylin-eosin stain, original magnification ×250.) (B) Prominent exocytosis of mononuclear ceils into the epidermis. (Haematoxylin-eosin stain, original magnification ×260.) (C) Prominent mononuclearcell infiltrate in dermis and prominent exocytosis into epidermis. (Haematoxylin-eosin stain, original magnification ×250.) (D) Minimal infiltrate in both epidermis and dermis. (Haematoxylin-eosin stain, original magnification ×250.) (E) Focal distribution of infiltrate is evident on left side of photomicrograph. (Haematoxylin-eosin stain, original magnification ×160.). Reproduced from Hymes et al.[39] *with permission from Elsevier Inc.*

Treatment

The most common treatment of GvHD after liver transplantation is steroids. Methylprednisolone (MEP) or prednisolone (PSL) have been used widely in treating GvHD after HSCT, after MVTx, and after isolated liver transplantation. Steroids successfully disrupt the three steps of the pathophysiology of GvHD. The success rate of steroids in treating GvHD was found to be higher in patients after a liver transplantation (83%) compared with those who also received an intestinal graft (46%).[6]

A second approach is the discontinuation of the immunosuppression to treat GvHD; in 4/13 (30.8%) patients described above, trials of weaning or completely stopping immunosuppression were reported. Out of the 4 cases, 2 continued to have uncontrolled GvHD, whereas in the other 2, the GvHD resolved.

Table 1. Acute graft-versus-host disease grading: Modified Glucksberg grade

	Skin (affected surface)	Liver (bilirubin mg/dL)	GI tract (fluid loss; mL/kg/day)
Stage			
1	<25%	2.0 - 3.0	>30 mL/kg
2	25 - 50%	3.1 - 6.0	>60 mL/kg
3	>50% or erythroderma	6.1 - 15.0	>90 mL/kg
4	Bullae desquamation	>15	>90 mL/kg or severe abdominal pain +/- ileus
Glucksberg grade	Skin stage	Liver stage	GI stage
I	1 - 2	0	0
II	3	1	1
III	-	2 - 3	2 - 4
IV	4	4	-

GI = gastrointestinal.

Table 2. Chronic graft-versus-host disease classification

Grade	Criteria
Mild	1 or 2 organs mildly affected AND no pulmonary involvement
Moderate	3 organs mildly affected OR 1 organ moderately affected OR mild pulmonary involvement
Severe	1 organ severely affected OR moderate or severe pulmonary involvement

Table 3. Case studies of graft-versus-host disease (GvHD) presentation after liver transplantation

Study	Age, gender	Transplant type	Time of onset POD (days)	Presentation					Diagnosis	Therapy			Outcome
				Skin	GI	Liver	Fever	Other		Induction	Maintenance therapy	Therapy after GvHD Dg	
Cattral *et al.*[8]	7 y, M	OLT	11	+	-	NA	+	HA, Pancyt	Skin Bx +, chimer +	-	MEP	IV MEP twice, + GM-CSF in relapse	Resolution
Pinna *et al.*[9]	9 m, F	LLS OLT	35	+	+	NA	+	A, ARDS	GI Bx +	-	FK + steroids	Stopped Ix	Uncontrolled
										-			
	8 m, F	LLS OLT	94	+	+	NA	–	-	GI Bx + Chim 3.20%	-	FK + Steroids	Stopped steroids + MMF, 2nd episode CsA + steroids + MMF	Resolution
	8 m, M	LLS OLT	140	–	+	NA	+	HA + L	GI Bx +, Chim 4.9%, then 3% in 2nd episode		FK + steroids + MMF + donor BMT	No change (FK+PSL+MMF), only RBC Tx	Resolution
Comenzo *et al.*[10]	5 y, M	OLT	95	+	-	NA	+	Pancyt	Chimer +	Plasmapheresis	CsA	Reduced CsA + GM-CSF	Resolution
Whitington *et al.*[11]	9 m, F	LDLT	50	+	-	NA	–	Pancyt	BM Bx+, chimer 100%	-	CsA+ AZA+ MEP	CsA + ATG + splenectomy	Uncontrolled - cGvHD
Dunn *et al.*[12]	10 m,*	LLS OLT	1 825	+	+	NA	-	-	Skin Bx+ cGvHD microchim 1%	-	Steroids + CsA + AZA	MEP + decreased Ix	Uncontrolled with MEP, reduced Ix trials -resolution
Kamei *et al.*[13]	8 m,*	LDLT	14	+	+	NA	+	Pancyt	*	*	*	*	Uncontrolled
	6 m,*	LDLT	23	+	+	NA	+	-	*	*	*	*	
Schäppi *et al.*[14]	16 m, M	LDLT	22	+	+	NA	?	Pancyt	Skin Bx +	Basiliximab	FK + steroids + MMF	Steroids + DZB + ATG + Alemtuzumab	Uncontrolled
Sharma *et al.*[15]	3 w, M	LDLT	480	-	+	NA	+	Pancyt	GI Bx +	-	FK + steroids	Steroids	Resolution
Sheu *et al.*[16]	2 y 8 m, M	OLT	160	+	+	NA	+	Pancyt	Skin Bx + Chim 99%	Chemotherapy	FK + chemotherapy	Topical Tac +MEP	Resolution
Yuksekkaya *et al.*[17]	12 m, F	OLT	86	+	–	NA	-	Pancyt	Skin Bx+ Chim 100%	Chemotherapy	FK + steroids + chemotherapy	MEP + Basiliximab + ATG	Uncontrolled

y = years; m = months; w = weeks; POD = postoperative day; GI = gastrointestinal; GvHD = graft-versus-host disease; Dg = diagnosis; OLT = orthotopic liver transplantation; - = negative; NA = not applicable; Pancyt = pancytopenia; chimer = chimerism; + = positive; CsA = cyclosporine A; GM-CSF = granulocyte macrophage-colony stimulating factor; HA = haemolytic anaemia; Bx = biopsy; MEP = methylprednisolone; IV = intravenous; LDLT = living-donor liver transplantation; BM = bone marrow ; AZA = azathioprine; ATG = anti-thymocyte globulin; cGvHD = chronic graft-versus-host disease.; LLS = left lateral segment; A = anaemia; ARDS = acute respiratory distress syndrome; FK = FK506 (tacrolimus); DZB = daclizumab; Ix = immunosuppression; MMF = mycophenolate mofetil; PSL = prednisolone; RBC = red blood cell; Tx = transfusion; L = leukopenia; Microchim = microchimerism; Tac = tacrolimus.
*No information available.

Pinna *et al.*[9] reported 1 case of weaning immunosuppression that was followed by the development of acute cellular rejection of the graft, so that immunocompromising drugs had to be restarted. The patient had a second episode of GvHD, and was treated with cyclosporine A, steroids and MMF, and his GvHD subsequently resolved.

Dunn *et al.*[12] used high doses of intravenous steroids for GvHD of the skin and GI tract, with no response. On the second trial of weaning immunosuppression, the GvHD resolved, although acute rejection of the graft occurred and it was managed by adding tacrolimus to the treatment regimen.

Other examples of withdrawing immunosuppression ended in resolution of the GvHD without rejection of the graft, but in these case reports the patients were adults.[43]

In conclusion, GvHD has been treated first-line either by augmentation of immunosuppression (i.e intravenous MEP) or by reduction of immunosuppression (i.e. stopping ciclosporin in cases), with variable and unpredictable results.

Novel therapies

For recipients who developed aGvHD and did not respond to first-line treatment with steroids, several immunosuppressive agents have been proposed, but studies derive mainly from the experience of GvHD cases after HSCT, especially those with cutaneous manifestations. There is currently no consensus on a single therapy of immunosuppression for these cases.[44] Novel therapies include MSC therapy and ECP.

Bone-marrow-derived CD34 fibroblast-like MSCs are known to differentiate into numerous types of tissues (muscle, bone, fat, etc.) and have the property of migrating to damaged tissues; they can produce chemokines and nitric oxide (NO). NO plays a role in macrophage function, cytokine receptor expression and T-cell receptor signalling, and regulating T-cell immunity pathways. MSCs are thought to respond to pro-inflammatory cytokines such as IFN-γ, IL-1α, IL-1β and TNF-α, and in turn (*i*) upregulate the inducible NO synthase gene, leading to NO production, and (*ii*) produce leukocyte chemokines which bring immune cells (T-cells, B-cells and APCs) close to the NO production and lead to immune-cell suppression. By these mechanisms of action, MSCs constitute a new immunosuppressive and immunomodulatory drug that ameliorated GvHD symptoms in mouse experiments.[45] Two paediatric patients have been described where MSCs were given with good results.[6]

ECP is now being used as a novel secondary therapy for GvHD. The patient's blood is leukophoresed and centrifuged to remove the leukocyte-enriched buffy coat. This layer of buffy coat is photosensitised and irradiated with UVA light, then reinfused to the patient. This causes an immunomodulatory effect, upregulating anti-inflammatory cytokines and down-regulating pro-inflammatory cytokines. It has been described in a child after MVTx; however, there are no data regarding its use in patients post liver transplantation.[46,47]

Other monoclonal antibodies have also been used for GvHD, especially after HSCT. Infliximab (anti-TNF-α) and etanercept have been used, especially in steroid-resistant (SR) gastrointestinal GvHD after HSCT, but their use is limited owing to the increased incidence of fungal infections.[39] These drugs, as well as anti-interferon-gamma (anti-IFN-γ) agents, can impede and block the action of the cytokine storm, ameliorating the symptoms of GvHD.

Polyclonal anti-thymocyte globulin (ATG), humanised or chimeric monoclonal antibodies have been used for severe visceral manifestations of GvHD.[48,49] In the reviewed literature, three case studies were found where ATG was used in uncontrolled GvHD: in the first, it had a positive short-term effect; in the second it did not have any effect; and in the third, it improved the skin but not the bone-marrow manifestations, and the recipient died due to sepsis.

Alemtuzumab is another immunosuppressive drug used in GvHD after HSCT, and in some cases, has been used as a second-line treatment in GvHD after liver transplantation. It is a humanised monoclonal antibody targeting the CD52 antigen expressed on T-cells, B-lymphocytes, macrophages and dentritic cells, all of which are involved in the pathophysiology of aGvHD. In a study in Grenoble in 2014, it was used in 24 adult patients with SR GvHD after HSCT. The response rate was 62.4%.[50]

Imatinib mesylate has also been proposed as a novel treatment for cGvHD, but its mechanism of action is as an antifibrotic agent rather than an immunosuppressive drug. It inhibits kinases (i.e. platelet-derived growth factor receptor). It has been used in cases of fibrotic cutaneous cGvHD with good response.[51]

Lastly, a new suggested treatment for GvHD after HSCT is human serum-derived alpha-1-antitrypsin (A1AT), as it is known to downgrade the production of pro-inflammatory cytokines and upregulate anti-inflammatory cytokines. It has been found that A1AT (*i*) inhibits proteases (i.e. proteinase-3 (PR-3)), and at the same time (*ii*) facilitates the production of anti-inflammatory agents, such as IL-1 receptor antagonists and IL-10. By decreasing the production of TNF-α and IL-1β and inhibiting the release of IL-8, it is a promising therapy. It has been described in mouse models of HSCT in recent years, and has been proposed in other review studies as a potentially safe treatment for a broad spectrum of immune-mediated conditions, including GvHD.[52,53]

Prevention

Several studies have used different immunosuppression protocols (induction and/or maintenance) and studied parameters possibly associated with the prevention of GvHD. Andres *et al.*[26] suggested using ATG or basiliximab (chimeric monoclonal antibody against IL-2 receptor) on top of tacrolimus and steroids in intestinal transplantation, but failed to show a significant difference in reduction of GvHD between the group of patients that received the ATG/basiliximab and the one that did not.

Whitington *et al.*,[11] in their case report, attempted a course of ATG for GvHD of the bone marrow that had a positive transient effect on the blood counts; however, after bone-marrow recovery the patient continued to suffer from chronic GvHD due to chimerism, leading to recurrence of donor-derived circulating cells.

Schäppi *et al.*,[14] in a case report, suggested giving alemtuzumab (for T-cell suppression) in a patient with severe uncontrollable GVHD after LDLT; however, it did not help.

Outcomes

GvHD is a serious multiorgan complication with high morbidity and mortality although the symptoms may show partial or complete resolution.[42] Amplified immunosuppression with PSL or MEP or other drugs was reported in a total of 8/13 patients, 3 of whom developed uncontrolled GvHD. In 2 cases, there were no available data on the treatment they received after GvHD presentation. For 3 patients, management suggested withholding immunosuppression.

In the reviewed literature, 7/13 (53.8%) of the cases were reported as having complete resolution of GvHD, whereas 46.2% ended in uncontrolled GvHD (multiorgan involvement, cGvHD or death). Of the 7 patients whose GvHD resolved, 5 received treatment with MEP or PSL, or augmentation of immunosuppression. The remaining 2 patients whose GvHD resolved were treated with discontinuation of immunosuppression.

Conclusion

GvHD after liver transplantation is a rare complication, but has a high mortality rate. Prompt consideration of symptoms is desirable to initiate treatment early.

A maculopapular skin rash, especially accompanied by fever, should always be considered as a sign of GvHD, and should prompt early investigation and modification of the treatment.

Biopsies from all the tissues involved can help to differentiate between GvHD staging and monitoring, and although the histolopathology may not be specific for GvHD, it may help to establish the diagnosis.

Early diagnosis, early immune-therapy modification and the promise of novel therapies may improve the outlook of GvHD after liver transplantation in the future.

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1. Gowans JL. The fate of parental strain small lymphocytes in F1 hybrid rats. Ann N Y Acad Sci 1962;99(3):432-455. https://doi.org/10.1111/j.1749-6632.1962.tb45326.x
2. Billingham RE. The biology of graft-versus-host reactions. Harvey Lect 1966;62:21-78.
3. Taylor AL, Gibbs P, Bradley JA. Acute graft versus host disease following liver transplantation: The enemy within. Am J Transplant 2004;4(4):466-474. https://doi.org/10.1111/j.1600-6143.2004.00406.x
4. Roberts JP, Ascher NL, Lake J, et al. Graft vs. host disease after liver transplantation in humans: A report of four cases. Hepatology 1991;14:274-281.
5. Akbulut S, Yilmaz M, Yilmaz S. Graft-versus-host disease after liver transplantation: A comprehensive literature review. World J Gastroenterol 2012;18(37):5240-5248. https://doi.org/10.3748/wjg.v18.i37.5240
6. Green T, Hind J. Graft-versus-host disease in paediatric solid organ transplantation: A review of the literature. Pediatr Transplant 2016;20(5):607-618. https://doi.org/10.1111/petr.12721
7. Soejima Y, Shimada M, Suehiro T, et al. Graft-versus-host disease following living donor liver transplantation. Liver Transpl 2004;10(3):460-464. https://doi.org/10.1002/lt.20101
8. Cattral MS, Langnas AN, Wisecarver JL, et al. Survival of graft-versus-host disease in a liver transplant recipient. Transplantation 1994;57(8):1271-1274.
9. Pinna AD, Weppler D, Berho M, et al. Unusual presentation of graft-versus-host disease in pediatric liver transplant recipients: Evidence of late and recurrent disease. Pediatr Transplant 1999;3(3):236 -242.
10. Comenzo RL, Malachowski ME, Rohrer RJ, Freeman RB, Rabson A, Berkman EM. Anomalous ABO phenotype in a child after an ABO-incompatible liver transplantation. N Engl J Med 1992;326(13):867-870. https://doi.org/10.1056/NEJM199203263261305
11. Whitington PF, Rubin CM, Alonso EM, et al. Complete lymphoid chimerism and chronic graft-versus-host disease in an infant recipient of a hepatic allograft from an HLA-homozygous parental living donor. Transplantation 1996;62(10):1516-1519.
12. Dunn SP, Krueger LJ, Butani L, Punnett H. Late onset of severe graft-versus-host disease in a pediatric liver transplant recipient. Transplantation 2001;71(10):1483-1485.
13. Kamei H, Oike F, Fujimoto Y, Yamamoto H, Tanaka K, Kiuchi T. Fatal graft-versus-host disease after living donor liver transplantation: Differential impact of donor-dominant one-way HLA matching. Liver Transpl 2006;12(1):140-145. https://doi.org/10.1002/lt.20573
14. Schäppi MG, Belli DC, Rimensberger PC, et al. Fatal GvHD as a complication of liver transplantation for undetermined fulminant hepatic failure and associated aplastic anemia. Liver Transpl 2006;12(11):1693-1697. https://doi.org/10.1002/lt.20916
15. Sharma A, Armstrong AE, Posner MP, et al. Graft-versus-host disease after solid organ transplantation: A single center experience and review of literature. Ann Transplant 2012;17(4):133-139.
16. Sheu J, Saavedra AP, Degar BA, et al. Bone marrow engraftment and associated dermatologic sequelae in a three-yr-old after liver transplantation. Pediatr Transplant 2015;19(2): E41-E46. https://doi.org/10.1111/petr.12412
17. Yuksekkaya HA, Arikan C, Tumgor G, Aksoylar S, Kilic M, Aydogdu S. Late-onset graft-versus-host disease after pediatric living-related liver transplantation for Langerhans cell histiocytosis. Pediatr Transplant 2011;15(6):E105-E109. https://doi.org/10.1111/j.1399-3046.2008.00899.x
18. Reddy P, Ferrara JLM. Immunobiology of acute graft-versus-host disease. Blood Rev 2003;17(4):187-194.
19. Ball LM, Egeler RM, EBMT Paediatric Working Party. Acute GvHD: Pathogenesis and classification. Bone Marrow Transplant 2008;41(SUPPL 1):S58-S64. https://doi.org/10.1038/bmt.2008.56
20. Ferrara JL, Deeg HJ. Graft-versus-host disease. N Engl J Med 1991;324:667-674. https://doi.org/10.1056/NEJM199103073241005
21. Hill GR, Ferrara JL. The primacy of the gastrointestinal tract as a target organ of acute graft-versus-host disease: Rationale for the use of cytokine shields in allogeneic bone marrow transplantation. Blood 2000;95(9):2754-2759.
22. Jeanmonod P, Hubbuch M, Grünhage F, et al. Graft-versus-host disease or toxic epidermal necrolysis: Diagnostic dilemma after liver transplantation. Transpl Infect Dis 2012;14(4):422-426. https://doi.org/10.1111/j.1399-3062.2012.00746.x
23. Dustin ML. Role of adhesion molecules in activation signaling in T lymphocytes. J Clin Immunol 2001;21(4):258-263.
24. Ramsey G, Nusbacher J, Starzl TE, Lindsay GD. Isohemagglutinins of graft origin after ABO-unmatched liver transplantation. N Engl J Med 1984;311(18):1167-1170. https://doi.org/10.1056/NEJM198411013111807
25. Mazariegos GV, Abu-Elmagd K, Jaffe R, et al. Graft versus host disease in intestinal transplantation. Am J Transplant 2004;4(9):1459-1465. https://doi.org/10.1111/j.1600-6143.2004.00524.x
26. Andres AM, Santamaría ML, Ramos E, et al. Graft-vs-host disease after small bowel transplantation in children. J Pediatric Surg 2010;45(2):333-336. https://doi.org/10.1016/j.jpedsurg.2009.10.071
27. Feito-Rodríguez M, de Lucas-Laguna R, Gómez-Fernández C, et al. Cutaneous graft versus host disease in pediatric multivisceral transplantation. Pediatr Dermatol 2013;30(3):335-341. https://doi.org/10.1111/j.1525-1470.2012.01839.x
28. Mangus RS, Tector AJ, Kubal CA, Fridell JA, Vianna RM. Multivisceral transplantation: Expanding indications and improving outcomes. J Gastrointest Surg 2013;17(1):179-186. https://doi.org/10.1007/s11605-012-2047-7
29. Martin PJ, Schoch G, Fisher L, et al. A retrospective analysis of therapy for acute graft-versus-host disease: Initial treatment. Blood 1990;76(8):1464-1472.
30. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005;11(12):945-956. https://doi.org/10.1016/j.bbmt.2005.09.004
31. Van Tol MJ, Langlois van den Bergh R, Mesker W, et al. Simultaneous detection of X and Y chromosomes by two-colour fluorescence *in situ* hybridization in combination with immunophenotyping of single cells to document chimaerism after sex-mismatched bone marrow transplantation. Bone Marrow Transplant 1998;21(5):497-503. https://doi.org/10.1038/sj.bmt.1701122
32. Jonsson JR, Hogan PG, Thomas R, et al. Peripheral blood chimerism following human liver transplantation. Hepatology 1997;25(5):1233-1236. https://doi.org/10.1002/hep.510250528
33. Couriel D, Caldera H, Champlin R, Komanduri K. Acute graft-versus-host disease: Pathophysiology, clinical manifestations, and management. Cancer 2004;101(9):1936-1946. https://doi.org/10.1002/cncr.20613
34. Kuykendall TD, Smoller BR. Lack of specificity in skin biopsy specimens to assess for acute graft-versus-host disease in initial 3 weeks after bone-marrow transplantation. J Am Acad Dermatol 2003;49(6):1081-1085. https://doi.org/10.1016/S0190
35. Socie G, Mary JY, Lemann M, et al. Prognostic value of apoptotic cells and infiltrating neutrophils in graft-versus-host disease of the gastrointestinal tract in humans: TNF and Fas expression. Blood 2004;103(1):50-57. https://doi.org/10.1182/blood-2003-03-0909
36. Shulman HM, Kleiner D, Lee SJ, et al. Histopathologic diagnosis of chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: II. Pathology Working Group Report. Biol Blood Marrow Transplant 2006;12(1):31-47. https://doi.org/10.1016/j.bbmt.2005.10.023
37. Lerner KG, Kao GF, Storb R, Buckner CD, Clift RA, Thomas ED. Histopathology of graft-vs.-host reaction (GvHR) in human recipients of marrow from HL-A-matched sibling donors. Transplant Proc 1974;6(4):367-371.
38. Drijkoningen M, De Wolf-Peeters C, Tricot G, Degreef H, Desmet V. Drug-induced skin reactions and acute cutaneous graft-versus-host reaction: A comparative immunohistochemical study. Blut 1988;56(2):69-73.
39. Hymes SR, Farmer ER, Lewis PG, Tutschka PJ, Santos GW. Cutaneous graft-versus-host reaction: Prognostic features seen by light microscopy. J Am Acad Dermatol 1985;12(3):468-474.
40. Elliott CJ, Sloane JP, Sanderson KV, Vincent M, Shepherd V, Powles R. The histological diagnosis of cutaneous graft versus host disease: Relationship of skin changes to marrow purging and other clinical variables. Histopathology 1987;11(2):145-155.
41. Wu G, Selvaggi G, Nishida S, et al. Graft-versus-host disease after intestinal and multivisceral transplantation. Transplantation 2011;91(2):219-224. https://doi.org/10.1097/TP.0b013e3181ff86ec
42. Smith DM, Agura E, Netto G, et al. Liver transplant-associated graft-versus-host disease. Transplantation 2003;75(1):118-126. https://doi.org/10.1097/01.TP.0000039845.31111.B1
43. Wang B, Lu Y, Yu L, Liu C, Wu Z, Liu X. Diagnosis and treatment for graft-versus-host disease after liver transplantation: Two case reports. Transplant Proc 2007;39(5):1696-1698. https://doi.org/10.1016/j.transproceed.2007.02.078
44. Hymes SR, Alousi AM, Cowen EW. Graft-versus-host disease: Part II. Management of cutaneous graft-versus-host disease. J Am Acad Dermatol 2012;66(4):535.e1-16. https://doi.org/10.1016/j.jaad.2011.11.961
45. Ren G, Zhang L, Zhao X, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2008;2(2):141-150. https://doi.org/10.1016/j.stem.2007.11.014
46. Hart JW, Shiue LH, Shpall EJ, Alousi AM. Extracorporeal photopheresis in the treatment of graft-versus-host disease: Evidence and opinion. Ther Adv Hematol 2013;4(5):320-334. https://doi.org/10.1177/2040620713490316
47. Maeda A, Schwarz A, Kernebeck K, et al. Intravenous infusion of syngeneic apoptotic cells by photopheresis induces antigen-specific regulatory T-cells. J Immunol 2005;174(10):5968-5976.
48. Carpenter PA, Macmillan ML. Management of acute graft-versus-host disease in children. Pediatr Clin North Am 2010;57(1):273-295. https://doi.org/10.1016/j.pcl.2009.11.007
49. Kröger N, Solano C, Wolschke C, et al. Antilymphocyte globulin for prevention of chronic graft-versus-host disease. N Engl J Med 2016;374(1):43-53. https://doi.org/10.1056/NEJMoa1506002
50. Meunier M, Bulabois CE, Thiebaut-Bertrand A, et al. Alemtuzumab for severe steroid-refractory gastrointestinal acute graft-versus-host disease. Biol Blood Marrow Transplant 2014;20(9):1451-1454. https://doi.org/10.1016/j.bbmt.2014.05.031
51. Olivieri A, Locatelli F, Zecca M, et al. Imatinib for refractory chronic graft-versus-host disease with fibrotic features. Blood 2009;114(3):709-718. https://doi.org/10.1182/blood-2009-02-204156
52. Tawara I, Sun Y, Lewis EC, et al. Alpha-1-antitrypsin monotherapy reduces graft-versus-host disease after experimental allogeneic bone marrow transplantation. Proc Natl Acad Sci USA 2012;109:564-569. https://doi.org/10.1073/pnas.1117665109
53. Lewis EC. Expanding the clinical indications for α(1)-antitrypsin therapy. Mol Med 2012;18:957-970. https://doi.org/10.2119/molmed.2011.00196

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