Successful Sequential Liver and Hematopoietic Stem Cell Transplantation in a Child with CD40 Ligand Deficiency and Cryptosporidium -Induced Liver Cirrhosis

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AUTHORSHIP PAGE

QP, DOD and CPL had the patient under their care and conceived, designed and wrote the article. TF, RR, SM planned and performed liver transplant and critically reviewed the paper. DE performed liver histological analysis. CF, VE, FF performed haematopoietic stem cell transplantation and critically reviewed the paper. PM followed the patient after liver transplant and critically reviewed the paper. All authors listed contributed to writing the manuscript and are responsible for the content of the paper. The authors declare no conflicts of interest.

ABBREVIATIONS PAGE

CD40L, CD40 ligand (CD154);

GVHD, graft-versus-host disease;

CMV, Cytomegalovirus;

HCV, hepatitis C virus;

HSCT, hematopoietic stem cell transplantation;

Ig, immunoglobulins;

IVIG, intravenous immunoglobulin;

LFT, liver function test;

LT, liver transplant;

MMF, mycophenolate mofetil;

PCR, polymerase chain reaction;

PLT, platelet;

SOT, solid organ transplantation;

VOD, veno-occlusive disease;

WBC, white blood cell;

XHIGM, X-linked hyper-IgM syndrome.

ABSTRACT

BACKGROUND

Hematopoietic stem cell transplantation (HSCT) is curative in patients with primary immunodeficiencies. However, pre-HSCT conditioning entails unacceptably high risks if the liver is compromised. The presence of a recurrent opportunistic infection affecting the biliary tree and determining liver cirrhosis with portal hypertension posed particular decisional difficulties in a seven-year-old child with X-linked CD40-ligand deficiency. We aim at adding to the scanty experience available on such rare cases, as successful management with sequential liver transplantation (LT) and HSCT has been reported in detail only in one young adult to date.

METHODS

a closely sequential strategy, with a surgical complication-free LT, followed by reduced-intensity conditioning, allowed HSCT to be performed only one month after LT, preventing *Cryptosporidium parvum* recolonization of the liver graft.

RESULTS

combined sequential LT and HSCT resolved the cirrhotic evolution and corrected the immunodeficiency so that the infection responsible for the progressive sclerosing cholangitis did not recur.

CONCLUSIONS

hopefully this report of the successful resolution of a potentially fatal combination of immunodeficiency and chronic opportunistic infection with end-stage organ damage in a child, will encourage others to adopt a sequential transplant approach to this highly complex pathology. However, caution is to be exercised to carefully balance the risks intrinsic to transplant surgery and immunosuppression in primary immunodeficiencies.

INTRODUCTION

X-linked hyper-IgM syndrome (XHIGM) [1,2] is a primary combined immunodeficiency disorder due to mutations in the *CD40LG* gene encoding CD40 ligand (CD40L) [3]. CD40L is specifically expressed by activated CD4+ follicular helper T cells on antigen recognition and its interaction with the cognate receptor CD40, constitutively expressed on B cells, leads to B cell proliferation, somatic hypermutation in the immunoglobulin (Ig) variable region and Ig class switch. Mature IgM/IgD-producing B cells then switch to secreting high-affinity antibodies of different classes (IgG, IgA, IgE) and mount an effective response against specific pathogens [4]. As T cell interactions with CD40-bearing monocytes, macrophages and dendritic cells are essential for their activation, cellular immunity is also compromised [5]. Neutropenia is common in XHIGM patients and may contribute to infections [6].

Most XHIGM patients present recurrent infections in infancy. Usually laboratory studies show high serum IgM with low or absent IgG, IgA and IgE, even if IgM may, at times, be normal or even low. Encapsulated bacteria are the most common cause of XHIGM infections, responsible for recurrent pneumonia, sinus infections, otitis media, Fungal (*Candida*, *Cryptococcus*, *Histoplasma*) and opportunistic infections, e.g. *Pneumocystis jiroveci* pneumonia and *Cryptosporidium parvum* diarrhea. [7]. *C. parvum* is a common enteric parasite in immunocompromised individuals [8] and C40/CD40L signaling is essential to develop resistance to this infection and to avoid its evolution into recurrent cholangitis, that leads to biliary cirrhosis [9,10]. XHIGM has a poor long-term outcome and current curative treatment is haematopoietic stem cell transplantation (HSCT) [11,12]. However, post-HSCT prognosis still has a median survival from diagnosis of 25 years. A young age at HSCT and normal liver histology are predictors of significantly better overall survival rates [13]. Therefore, if the liver is already seriously compromised, a liver transplant (LT) ought to be considered before HSCT [14].

CLINICAL CASE

A 7-year-old Venezuelan boy presented with a diagnosis of XHIGM and liver cirrhosis, without history of affected relatives. He had suffered from abdominal distension, associated with loose stools and failure to thrive as from the 8th month of life. He had a jejunal biopsy at 3 years of age that evidenced grade-one mucosal dystrophy. Serology was positive for HLA-DR2 but negative for celiac disease. Low gamma-globulin levels were observed, but erroneously attributed to a transitory hypogammaglobulinemia. No recurrent infections were reported. Altered liver enzyme levels (liver function tests, LFTs) were noted for the first time. As his symptoms did not improve on a gluten-free diet, he was put on a milk-protein-free diet, which improved both stools and growth. At 5, MR-cholangiography and liver biopsy had been performed due to persisting liver dysfunction and sclerosing cholangitis and cirrhosis had been diagnosed. Repeated stool examinations demonstrated *C. parvum* colonization.

The association of *Cryptosporidium* cholangitis and IgG and IgA deficiency, with normal IgM levels, raised the suspicion of XHIGM. *CD40LG* sequencing confirmed the diagnosis, evidencing a nonsense mutation (c.773T>G, p.L258X) with complete loss of CD40L expression. Although his healthy sister was heterozygous for the same mutation, their mother was genotypically normal, implying a germinal mosaicism. Three weekly intravenous immunoglobulin (IVIG) infusions, prophylactic oral co-trimoxazole, paromomycin and ursodeoxycholic acid were started and he drunk only boiled water.

Upon admission to our department, he had a 3rd-10th centile for height and weight and a palpable liver 3 cm below the costal margin and a palpable spleen at the umbilical level. Laboratory tests showed a mild reduction in total white blood cell (WBC, 2950/mL) and

platelet (PLT, 57,000/mm^3) counts. LFTs were abnormal, but synthetic liver functions were maintained. Low IgG and IgA with normal IgM levels were confirmed (see **Table 1**). There was no evidence of *Cryptosporidium* infection: stools were normal and repeated fecal tests for *C. parvum* were negative.

Abdominal ultrasonography evidenced an enlarged, hyperechoic liver with nodular margins, a patent portal vein and massive splenomegaly (>20 cm) without biliary dilatation or focal hepatic lesions. MR-cholangiogram confirmed sclerosing cholangitis. Upper gastrointestinal endoscopy evidenced engorged tortuous oesophageal varices with cherry-red spots, which were banded.

A multidisciplinary decision was made to proceed to LT followed by HSCT from a matched unrelated donor as the liver disease was progressing and conditioning for HSCT entailed the risk of liver failure. Liver and HSCT donation by the same living related donor was explored, but his parents were not eligible. He was listed for LT and transplanted 5 months later with a whole liver graft from a deceased donor. The native liver was cirrhotic, with sparse areas of regeneration and porto-central bridging (**Figure 1A**). Histological examination revealed advanced ductopenia and identified *Cryptosporidia* in the bile ducts (**Figure 1B**). Immunosuppression was basiliximab, given on days 0 and 4 and tacrolimus was titrated to achieve trough levels of 8-10 μg/L. Prophylactic antimicrobial treatment included broad-spectrum antibiotics, paramomycin, liposomal amphotericin, aciclovir and nebulized pentamidine. IVIG (0.5 mg/Kg) were infused once per week.

His early course was complicated by an episode of mild/moderate rejection on postoperative day 9, with fever and raised LFTs, which responded to steroids. At day 14 post-LT a Cytomegalovirus (CMV) reactivation was observed through CMV PCR assays (1500 copies/ml) and treated with iv ganciclovir (5 mg/kg twice daily) until complete negativization of CMV PCR after 2 weeks of treatment.

Conditioning for HSCT was started 26 days after LT with normal LFTs. Hepatotoxicity was minimized by the administration of treosulfan 14 g/m^2/day for 3 days, fludarabine 30 mg/m^2/day for 5 days and thiotepa 10 mg/kg for 1 day. He received HSCT from a fully-matched, unrelated donor on day 33 after LT, matched for patient blood group (0, Rh positive versus A, Rh positive). He received a total of 5 x 10^8 cells/Kg (5.1 x 10^6/kg CD34+ cells).

HSCT infection prophylaxis included intravenous antivirals (ganciclovir until day -3 and acyclovir from day -2), antifungals (liposomal amphotericine B) and anti-*Cryptosporidium* treatment with oral paromomycin and azithromycin. Defibrotide was administered intravenously until day +27 to reduce the risk of veno-occlusive disease (VOD). Graft-versus-host disease (GVHD) prophylaxis was anti-thymocyte globulin (1 mg/kg on day -3, 3 mg/kg on days -2, -1 and 0), mycophenolate mofetil (MMF) from day 0 until day + 30 and tacrolimus.

Neutrophil engraftment was achieved on day +15 and PLT engraftment on day + 20.

A full donor chimerism (>97% donor) was observed on day +75 and is still stable to date. The post HSCT period was complicated by cutaneous GVHD from day +12, which was successfully treated with steroids only, administered for 45 days including a slow tapering. CMV reactivation was observed at day +21 and was initially treated with intravenous ganciclovir. This was discontinued and replaced by foscarnet, according to internal protocols, when CMV PCR detected an increased viremia. *C. parvum* was not isolated.

He was discharged in good clinical condition on day +56. However, there was a sub-acute painless bilateral visual loss from day +95, which was diagnosed as bilateral optic neuritis with abnormal visual evoked potentials. Because of the risk of optic neuropathy associated with tacrolimus, it was replaced by cyclosporine A (target levels: 100-150 ng/ml) with partial recovery of the visual function.

At one year post-LT, a protocol liver biopsy performed with normal LFTs showed features of minimal focal injury of the bile ducts (grade 1), significant inflammation of the portal tracts

(grade 2) and minimal endothelitiis (grade 1) (**Figure 2A**). Alternative explanations for the histological features were mild acute rejection, GVHD or early biliary changes of cholangitis. MMF was added at 600 mg/m^2/day, but the dose was halved after one month due to myelosuppression. Three months later, a repeated liver biopsy showed an improved liver histology. A skin biopsy, performed at the same time because of marked xerosis with peri-follicular papules, showed a mild GVHD. Thus, the combined immunosuppression with cyclosporine A and MMF was continued. At 2 years post LT, liver biopsy was repeated and evidenced a complete normalization of liver histology (**Figure 2B**).

He is currently 10 years old, in his 30th month after HSCT and is still on immunosuppressive treatment with cyclosporine A and MMF. He has normal and stable blood cell counts, normal liver enzyme levels and function tests and has had normal stable serum Ig levels as from 7 months post-HSCT. He has returned to a free diet without recurrence of either diarrhea or dermatitis. No further infections or clinical evidence of liver dysfunction have been observed, even if a stable sight impairment is still present. His scholastic progress is normal for his age.

DISCUSSION

XHIGM allowed the *Cryptosporidium* infection to progress to chronic cholangitis and liver cirrhosis with portal hypertension in this patient. However, a sequential strategy, with LT, closely followed by HSCT, successfully dealt both with primary immunodeficiency and advanced liver disease. Although supportive management of XHIGM includes IVIG, granulocyte colony-stimulating factor for neutropenia, antibiotic prophylaxis against opportunistic infections and precautions to avoid *Cryptosporidium* exposure, it does have a high cumulative morbidity and mortality [(15)]. To date, the only curative treatment for XHIGM is HSCT ([11,12]), ideally performed before the onset of life-threatening complications and organ damage [(9,10,13)].

Could HSCT alone have been successfully performed in our patient? (**Table 2**). Although 2 school-aged brothers with XHIGM and sclerosing cholangitis cleared *Cryptosporidium*

infection and normalized their LFTs after immune reconstitution by HSCT, both of them had only mild fibrosis at liver biopsy [16]. Two other XHIGM children with liver dysfunction were successfully treated by HSCT [17], but neither of them had cirrhosis. Another XHIGM child with deranged LFTs and portal fibrosis with bridging at biopsy, without evidence of sclerosing cholangitis or cryptosporidial infection, was given isolated HSCT [18]. His LFTs normalized 2-years post-transplant, even if ultrasound showed an enlarged granular liver with irregular margins, suggestive of progressive parenchymal disease. However, liver disease had not yet reached the stage of established cirrhosis in any of these children treated by an isolated HSCT and the last child, with the most serious liver damage, had no evidence of opportunistic infection. Therefore, the potential of HSCT alone to reverse established chronic liver disease in XHIGM remains questionable [14].

Indeed, the advanced sclerosing cholangitis with cirrhosis and overt portal hypertension in our case, discouraged such an approach. Furthermore, myeloablative conditioning carries a high risk of liver toxicity in the presence of cholangiopathy [19] and post-HSCT hepatic complications may lead to significant morbidity with high transplant-related mortality [20]. Moreover, patients with Child A cirrhosis are at risk for decompensation after HSCT, even if they are on a reduced-intensity conditioning regimen [21] and are not deemed suitable candidates for HSCT alone.

Could, on the other hand, an isolated LT have been considered?

In a retrospective analysis of a large international cohort [13], 3 adult patients underwent LT and 2 also received HSCT. Both patients with combined transplant survived, whilst, the third, who underwent LT alone at 38, died shortly afterwards. Liver biliary involvement was identified as the only significant negative predictor of survival in 176 XHIGM patients [13]. Isolated LT in adult XHIGM patients with hepatitis-C virus (HCV) liver disease has been reported [22,23]. Their HCV infection did not respond to conventional PEG-INF and ribavirin treatment, progressing to end-stage liver disease. After LT, they were given low-level

tacrolimus immunosuppression and HCV relapse was treated with direct acting antivirals. They were thriving after LT, on a lifelong regimen of IVIG infusions and *Pneumocystis*, fungal and CMV prophylaxis.

Although LT alone might have been a possibility in our case, lifelong immunosuppression in a patient with immunodeficiency carries a high risk of life-threatening infections. Moreover, *Cryptosporidium* may well be dormant in niches that cannot be reached by treatment [24,25,26] (**Figure 1B**). *Cryptosporidium* re-infection might have lead to a recurrence of sclerosing cholangitis, graft failure and a fatal outcome, despite optimal supportive treatment [27]. Consequently, it was decided to adopt a sequential approach that involved as short an interval as possible between the two transplants [14] to minimize the risk of graft infection.

Allogeneic HSCT in young SOT patients still remains a high-risk procedure. Literature on sequential SOT and HSCT in children with primary immunodeficiencies is scanty and most data come from case reports on successful transplantations which may well represent a positive reporting bias [28]. Since LT may be followed by

medical and surgical complications, which, in our case, could have compromised a timely HSCT, it was decided to wait until a whole liver graft from a deceased donor became available [29]. Liver donation from his parents had been taken into consideration because immune tolerance would have ensured liver graft survival after HSCT and tolerance could have been achieved by mixed-donor T cell chimerism. Reconstituted host T and B cells develop tolerance to the transplanted organ as they mature, permitting graft survival without immunosuppression [30]. In a series of 10 children who had received liver and HSC from identical living donors, immunosuppression was withdrawn 2 months after HSCT and stable allograft tolerance was demonstrated [31]. Unfortunately, neither parents qualified as a donor in our case.

Moreover, apart from the toxicity inherent to HSCT conditioning, there were concerns that GVHD after allogeneic HSCT might have put the liver graft at risk, or that alloimmunity to

the liver graft might have increased the risk of rejection of the haematopoietic graft [32]. The decision was not an easy one and was debated at length within the multidisciplinary team. A report of a liver and bone marrow transplantation in 2000 from an unrelated donor in an 18-year-old with end-stage liver disease, associated with XHIGM, encouraged us [14]. The fact that no other similar report has been published to date is justified by the rarity of this association, the complexity of the procedure and the risks involved.

Since standard HSCT using high-dose chemoprophylaxis may lead to serious hepatic problems [21], a reduced intensity conditioning regimen was chosen. Treosulfan is an immunosuppressive and myeloablative agent with a low-toxicity profile and has very rarely been associated to severe VOD [33]. Treosulfan-containing regimens allow for a high rate of stable donor engraftment, with a reduced regimen-related toxicity and a low GVHD rate. This makes it particularly suitable for patients with inherited disorders, like immunodeficiencies, who often arrive at the transplant stage with organ damage and co-morbidities [34,35]. Prophylactic defibrotide was used to further reduce the VOD risk [36].

A report on XHIGM HCV-positive patients who were given an isolated LT [22,23] showed that one of them had no evidence of rejection [23]. The authors attributed this to the CD40L deficiency. The CD40L/CD40 pathway is one of several co-stimulatory systems of alloimmunity. Antibodies that block co-stimulation through the CD40–CD40L pathway may offer benefits in preventing transplant rejection [37]. Despite this, in a CD40L-deficient animal model, these antibodies did not prolong the allograft survival, probably due to activation of alternative pathways of co-stimulation [38].

Our patient had no major liver complication after the combined sequential transplantation. A mild-moderate episode of rejection 9 days after LT, before the HSCT, confirms that CD40L deficiency does not suffice to prevent acute cellular rejection. A 12-month protocol liver biopsy showed focal injury of the bile ducts, with minimal edema of the portal tracts, suggesting a mild GVHD (**Figure 2A**). The histological improvement observed three months

later and confirmed at a follow-up biopsy (**Figure 2B**), made a cholangitis relapse highly improbable, while the concomitant occurrence of cutaneous GVHD added credibility to it having been the underlying cause of the liver changes.

In conclusion, in a pediatric patient with primary immunodeficiency and cirrhosis with portal hypertension, liver transplantation, followed at a short interval by HSCT, resolved both pathologies and provided a satisfactory quality of life. More data on the long-term outcomes of combined transplantation are essential so as to improve our decisional capacity in such complex patients.

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FIGURE LEGENDS

Figure 1

A_The explanted liver was cirrhotic, with mild stromal inflammation (H&E, 25x).

B_*Crytposporidium parvum*: 2-5 micron, acid-fast, spherical structures in the lumen of a bile duct of the explanted liver. The stool specimens were negative (Grocott stain, 400x).

Figure 2

A_Follow-up liver biopsy at 1 year: moderately expanded portal tract with mild portal lymphocytic infiltration and subendothelial venular infiltration (PAS-D, 400x).

B_Follow-up liver biopsy at 2 years: normal portal tract; some ballooning of hepatocytes (PAS-D, 400x).

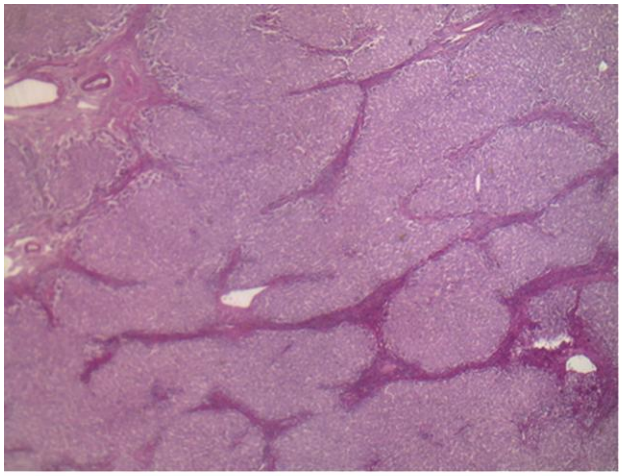
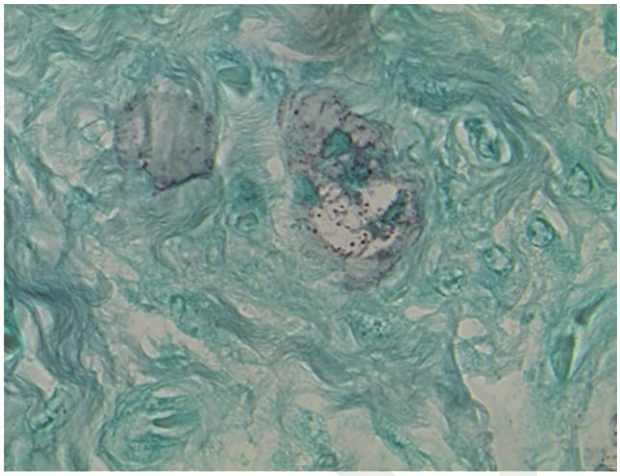


Figure 1A.



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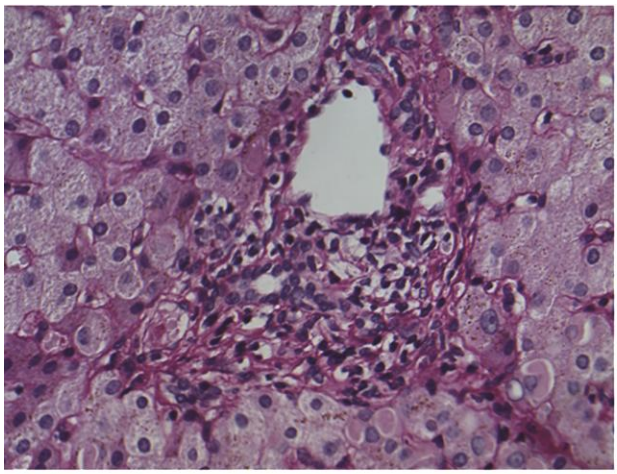

Figure 2A.

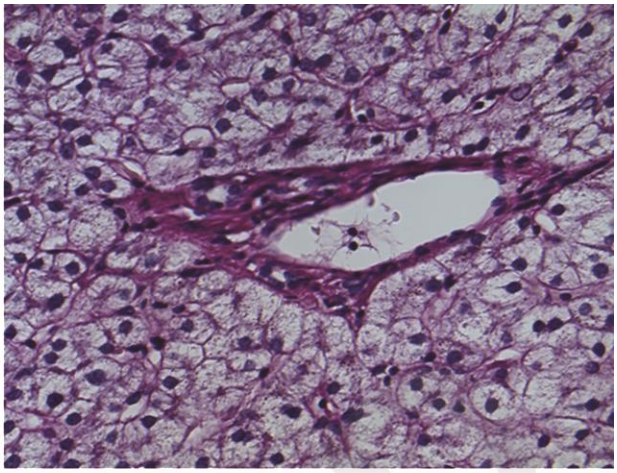

Figure 2B.

TABLES

Table 1. Evolution of liver function tests and immunoglobulin levels before and after liver transplantation.

	AST (IU/L)	ALT (IU/L)	γ-GT (IU/L)	Total Bilirubin (mg/dL)	Conjugate Bilirubin (mg/dL)	INR	Albumin (g/dL)	IgA (mg/dL)	IgM (mg/dL)	IgG (mg/dL)
At referral	79	60	159	1.65	1.13	1.11	3.25	10	82	303 §
At LT	60	40	97	1.87	1.12	1.22	3.55	10	80	416 §
At HSCT	26	25	32	0.3	0.15	1.16	3.87	<4	43	618 §
At 3-month FU #	21	31	18	0.68	0.23	0.99	4.1	10	50	580 §
At 12-month FU #	28	21	7	0.6	0.3	1.05	3.9	220	121	1314 §§
At 2-year FU #	26	21	8	0.7	0.3	1.08	4	265	107	1236 §§
Reference range	**3-35**	**3-35**	**7-30**	**0.3-1.1**	**< 0.25**	**0.8-1.2**	**3.9-5.2**	**30-198**	**45-200**	**605-1230**

#,interval from HSCT; §, on regular IVIG infusions; §§, on no IVIG infusion from 7 months after HSCT

Abbreviations: FU, follow-up; HSCT, haematopoietic stem cell transplantation; IVIG, intravenous immunoglobulins; LT, liver transplantation

TABLE 2. Haematopoietic stem cell transplant alone in XHIGM with liver involvement

Reference & Case	Age at transplant (years)	Sex	Presentation		BM/HSCT	BM/HSCT conditioning	GVHD prophylaxis	Post-HSCT course
			Infections	**Liver disease**				
Ref. 16								
Brother A	6	M	*C.parvum* diarrhea & cholangitis	LFTs > 7x ULN Sclerosing cholangitis	Matched sibling BM	BU & CY	MTX & CyA	PMN>500/mL on D+17; no GVHD/VOD; Sinusitis, resolved; C.parvum neg. on D +60; LFTs < ULN on D +60
Brother B	8	M	Sinus infection; C.parvum	LFTs > 5x ULN Non-	Matched sibling BM	BU & CY	MTX & CyA	PMN >500/mL on D +15; VOD on D +13;

			cholangitis	concentric portal fibrosis				GVHD on D+49; Sinusitis, resolved; C.parvum neg. on D >16; LFTs < ULN on D +60
Ref. 17								
Sibling 1	9	M	Strept. Gingivitis; Otitis media; Pneumonia; *C.parvum* cholangitis at ERCP	LFTs > 4x ULN Cholangitis Portal & bridging fibrosis	Matched sibling PBSC with boost at + 10 months	Flu, BU, hATG	CyA	PMN count normal on D +100; no GVHD; no infections; LFTs improved
Sibling 2	5	M	Oral ulcers; Otitis media; Sinusitis; *C.parvum* positive in stools	LFTs > 5x ULN Cholangitis Portal fibrosis	Matched sibling PBSC	Flu, BU, hATG	CyA	PMN count normal at 2 years;full chimerism at 14 months; no GVHD; no infections;

								LFTs improved
Ref. 18								
Case	3.5	M	Oral ulcers; Upper and lower airway infections; *C. parvum* negative in stools	LFTs abnormal; Chronic cholestasis; Portal fibrosis	Matched sibling PBSC	TRS & CY	CyA	PMN>500/ml on D13; GVHD mucositis (tongue); CMV; LFTs < ULN at 12 months

Abbreviations: HSCT, haematopoietic stem cell transplant; XHIGM, X-linked hyper-IgM immunodeficiency; LFTs, liver function tests; ULN, upper limit of normal; BM, bone marrow; PBSC, peripheral blood stem cells; BU, busulfan; CY, cyclophosphamide; MTX, methotrexate; CyA, cyclosporine A; ERCP, endoscopic retrograde cholangio-pancreatography; Flu, fludarabine; hATG, horse-derived anti-thymocyte globulin; TRS, treosulfan; GVHD, graft-versus-host disease; VOD, veno-occlusive disease; PMN, polymorphonuclear neutrofils; CMV, cytomegalovirus.