Chronic Cholangiopathy Associated with Primary Immune Deficiencies Can Be Resolved by Effective Hematopoietic Stem Cell Transplantation

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Objectives To investigate effects and outcome of hematopoietic stem cell transplantation (HSCT) on sclerosing cholangitis, in pediatric patients with different primary immunodeficiencies (PIDs).

Study design From databases in 2 tertiary centers for immunodeficiencies and liver disease, we have identified children with PIDs and sclerosing cholangitis, who have paired clinical, radiologic, and histologic information before and after HSCT and studied their clinical progress and outcome.

Results Seven of 13 children (53.8%) died at a median interval of 4 months (range, 3 months-5 years) after HSCT. However, 6 surviving children (46.2%) with different PIDs and less severe cholangiopathies showed an improvement in markers of liver injury within months of successful unrelated reduced intensity conditioning HSCT. The repeated native liver biopsy, performed in 4 patients at a median of 96 (range, 4-144) months post-HSCT, showed a considerable improvement. Biochemical markers of liver function in the survivors completely normalized after a median of 13 months (range, 2-48). All patients continue to have a mildly dilated extrahepatic biliary system on ultrasonography with no intrahepatic ductal changes on magnetic resonance cholangiography after a follow-up of median 18 years (range, 2-20).

Conclusions Effective HSCT has the potential to improve biochemical and histologic features of cholangiopathy in children with PIDs, presumably by clearance of chronic infection following establishment of immune competence. However, careful patient selection is critical as advanced liver injury is often associated with serious complications and mortality. *(J Pediatr 2019;209:97-106)*.

Primary immunodeficiencies (PIDs) are rare monogenic conditions where different components of the innate or adaptive immune response are genetically impaired, leading to various clinical phenotypes that include a combination of severe or recurrent infections, autoimmune phenomena, and/or lymphoid malignancies.[1] Their prognosis has been significantly ameliorated by progress in their molecular and genetic characterization, earlier detection and anti-infectious prophylaxis with immunoglobulin supplementation, and the long-term use of antibacterials and antifungals. The most impressive breakthrough in the management of the severe PID disorders, however, has been the advent of allogeneic hematopoietic stem cell transplantation (HSCT), which has become a standard of care for life-threatening PIDs.[2]

Liver involvement occurs in ~25% of patients with PIDs; of those, some 60% are histologically and radiologically diagnosed with sclerosing cholangitis (SC).[3] In many of them, SC is associated with chronic infection with *Cryptosporidium* species, in some progressing further to biliary cirrhosis, end-stage liver disease,[4] and even cholangiocarcinoma.[5] In a rodent model, the interaction between the antigen presenting cell surface molecule CD40 and its ligand CD40L on T cells has been shown to be critical for the control of *Cryptosporidium* infection.[6] Children with CD40 ligand deficiency, the most common form of hyper-IgM syndrome, are, therefore, particularly prone to *Cryptosporidium* infection and the development of chronic cholangiopathy.[7]

The management of chronic liver disease in patient with PIDs is complex due to its dependence on a degree of immune competence, its progressive nature, and the negative impact of advanced liver damage on success of HSCT. To avoid observed prompt recurrence of the liver disease in the graft caused by ongoing immune defect and added antirejection treatment, a combined sequential liver and HSCT has been described in cases.[8,9]

ALT	Alanine aminotransferase	MRCP	Magnetic resonance cholangiopancreatography
AST	Aspartate-aminotransferase		
DOCK-8	Dedicator of cytokinesis 8	MUD	Matched unrelated donor
ERCP	Endoscopic retrograde cholangiopancreatography	PCR	Polymerase chain reaction
		PID	Primary immunodeficiency
GGT	Gamma-glutamyl transpeptidase	RIC	Reduced intensity conditioning
GvHD	Graft-vs-host disease	SC	Sclerosing cholangitis
HSCT	Hematopoietic stem cell transplantation		

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The authors declare no conflicts of interest.

0022-3476/$ - see front matter.
https://doi.org/10.1016/j.jpeds.2019.01.015

We present here our experience of the effects of isolated HSCT on liver disease in children with different PIDs and associated liver involvement of variable severity.

Methods

The patients were identified from the database of the Pediatric Liver Center, King's College Hospital, London, where they had been referred for investigation of liver disease between 1990 and 2015. They had been followed up jointly by hepatologists at King's College Hospital and immunologists at the Great Ormond Street Hospital for Sick Children in London. Hepatologic work up, as previously described,[3] included exclusion of other causes of chronic liver disease of childhood, radiologic investigations (ultrasonography, endoscopic retrograde cholangiopancreatography [ERCP] and/or magnetic resonance cholangiopancreatography [MRCP]). *Cryptosporidium* surveillance of the stools was undertaken regularly using both microscopy and polymerase chain reaction (PCR) testing[10]; the bile and liver biopsy material was also tested for the organism where available. In this report, we focus on those patients with PIDs who had liver disease not severe enough to warrant consideration for combined liver and HSCT transplantation.[8] All patients received HSCT as previously described.[11]

Of the available surviving 6 patients, 4 of them (and/or their parents) agreed to follow-up liver biopsies and provided informed written consent. The study was regarded as an audit of clinical practice and did not require a formal ethical approval. A retrospective histologic review of the available liver biopsy samples was performed by a liver histopathologist blinded to the clinical background. The Nakanuma system, recently described as the preferential histologic scoring system for cholestatic conditions[12] was used to assess disease grade in the form of cholangitis and hepatitis activity and disease stage in the form of fibrosis, bile duct loss, and deposition of copper-binding protein granules. The biopsy samples were also assessed for other pathology such as the presence of infective organisms and cholestasis. Where more than a single pre-HSCT biopsy was present, grading and staging were undertaken on the biopsy sample immediately prior to transplant.

Statistical methods involved a logistic regression analysis of the variables that can influence survival after HSCT. The variables included in the model were the type of conditioning, presence of acute graft-vs-host disease (GvHD), post-transplant infection, and days to neutrophil recovery. Histologic changes were also compared with the outcomes. Statistical analysis was performed by Cox proportional hazard regression models with a backward stepwise selection and using R 2.5.0 software package (http://www.R-project.org).

Results

We identified 13 patients with PIDs and SC over the observation period. They were all boys; 7 (61.5%) had CD40-ligand deficiency, 2 (15.4%) dedicator of cytokinesis 8 (DOCK-8) deficiency, and 1 each (7.7%) had Wiskott-Aldrich syndrome, MHC class I deficiency, cartilage-hair hypoplasia syndrome, and undefined combined immunodeficiency. Two patients were south Asians (patient 9 and patient 11) and the remaining 11 were Caucasians (1 Irish, 1 middle-Eastern background). Their pre-HSCT clinical data are presented in **Table I**.

One child required an emergency liver transplant after developing worsening jaundice, encephalopathy, and coagulopathy post-HSCT, but died of multiorgan failure (patient 1). Another patient (patient 13) received sequential liver and HSCT 5 weeks later, as previously described[8] and remains well after 20 years follow-up. Overall, 6 of the 13 (46.2%) patients survived. Of those, 5 of 6 received matched unrelated donor (MUD) HSCT. Among 7 children who died, 2 received MUD, 4 mismatched MUD (three 9/10 mismatch, one 5/6 mismatch from a cord donor), and 1 matched sibling donor (MSD) HSCT. At HSCT, the median age of the survivor group was 10.5 years (range, 3-18 years), and of the nonsurvivor group the median age was 16 years (range, 8-17 years). The survivors received reduced intensity conditioning (RIC) with fludarabine (150 mg/m^2) and melphalan (140 mg/m^2) in 5, or treosulfan (42 g/m^2) in 1 patient. In addition, 4 received antithymocyte globulin (12.5 mg/kg) and 2 alemtuzumab (anti-CD52 antibody, Campath) (1 mg/kg). GvHD prophylaxis was with cyclosporine A, aiming for trough levels 150-250 μmol/L, mycophenolate mofetil (40 mg/kg/d), or tacrolimus, aiming for levels 8-10 μmol/L (**Table II**). Neutrophil engraftment was achieved at a median 12 days (range, 8-47 days) in the survivors compared with a median 10 days (range, 5-17 days) in nonsurvivors. Platelet engraftment was observed at a median 10 days for both groups with a range of 6-48 days in survivors and 9-15 days in nonsurvivors. Post-HSCT T-lymphocyte immune reconstitution (defined as CD3+ count >300 cells/μL) was seen at a median 60 days (range, 39-90 days) in the survivors compared with a median of 90 days (range, 35-300 days) in nonsurvivors. GvHD was seen in 8 patients (4 patients from each group) with combined skin (in 6), liver (in 3), and/or gut (in 2) involvement. The surviving patients had only a mild skin, gut, or liver GvHD. Two patients had graft rejection, both from the nonsurvivor group, and the remaining 10 patients had a donor T cell lymphocyte engraftment on the whole blood between 90% and 100% and one at 71%.

Seven patients died at median 4 months (range, 3 months-5 years) post-HSCT. The causes of the death were disseminated cryptosporidiosis (4), severe GvHD (2), pneumonitis (3), encephalitis (2), and multiorgan failure (4) (**Table II**). None of the variables included in the logistic regression analysis had an influence on survival (P = ns).

The survivor group was followed up for a median of 18 years (range, 2-20 years) post-HSCT. They are all off immunosuppression and all but one remains off immunoglobulin supplementation therapy (patient 11). Three of them continue to receive antibiotic prophylaxis.

Table I. Clinical features

Patients	Diagnosis	Age at presentation	Presentation	Imaging pre-HSCT	Laboratory markers pre/post-HSCT						
					Bil (μmol/L)	Alb (g/L)	AP (IU/L)	ALT (IU/L)	GGT (IU/L)	PT	APTT
1	CD40 Ligand def	18 mo	Tuberculous osteomyelitis, FTT, SC developing from 7 y of age, *Cryptosporidium* positive	Not available	12/42	43/28	242/767	147/48	362/934	13.5/16	28/38.5
2	CD40 Ligand def	4 mo	Chest infection, FTT, SC, varices and GI bleeding, splenectomy	Chronic cholangiopathy	29/427	39/20	773/125	112/46	693/179	9.7/15.6	33/68
3	MHC Class II def	6 mo	Diarrhoea, SC, FTT, *Cryptosporidium* positive, hearing loss, meningoencephalitis, and VP shunt	Chronic cholangiopathy	10/270	38/28	215/310	46/149	ND	15/14.5	33/36.5
4	DOCK-8 def	7 mo	Food allergy, pneumonia, SC	Dilated bile ducts	6/10	39/45	253/183	14/49	15/14	11.4/12	29/34
5	CD40 Ligand def	6 weeks	Recurrent chest infections, SC, *Cryptosporidium* positive	Dilated intrahepatic ducts	14/8	40/45	530/164	186/26	282/20	12/11	28/34
6	Wiskott Aldrich syndrome	3.5 years	Food allergies, FTT, *Molluscum contagiosum*, splenectomy, SC, alopecia	Generalized duct dilatation with a common bile duct of 11.2 mm, absent spleen	4/10	35/40	1043/298	213/46	908/110	12/12	32/31
7	CD40 Ligand def	6 mo	Chest infections, diarrhoea, hepatomegaly	Chronic cholangiopathy	7/80	47/43	166/437	42/119	60/151	11.3/11	28/33
8	CHH	4 mo	Recurrent chest infections, T cell def, low Igs, hepatomegaly, SC diagnosed by ERCP, short stature	Chronic cholangiopathy	6/9	32/44	424/181	56/13	104/17	14/11	43/35
9	CD40 Ligand def	6 mo	Bronchiectasis, FTT, recurrent severe chest infections, SC	Moderate SC	35/92	39/29	1046/1967	279/332	214/507	9.4/10.7	38.5/36
10	DOCK-8 def	3 years	Sore lips and eczema, bronchiectasis, dilated descending aorta, CMV retinal dystrophy, SC, *Cryptosporidium* positive	Chronic cholangiopathy	8/206	32/38	131/1413	244/926	113/198	10.8/23	27/180
11	CD40 Ligand def	15 mo	*Cryptosporidium* positive, adenovirus, diarrhoea, otitis, hepatomegaly	Mild SC	7/3	35/41	1344/240	361/70	507/44	10/9.7	30/12
12	Undefined combined immune deficiency	6 mo	Bronchiectasis, SC, chronic fungal oropharyngitis, FTT, RML resection	Chronic cholangiopathy	14/136	45/36	657/260	295/75	131/281	12/13.3	35/38.6
13	CD40 Ligand def	3 years	Diagnosis following sibling death from PJP, by 5-y abnormal LFTs, hematemesis requiring sclerotherapy and banding of oesophageal varices, liver Tx at 18 y, D+35 post liver Tx - HSCT	ERCP: extrahepatic and intrahepatic cholangiopathy and grade 2 esophageal varices, USS: heterogeneous nodular liver	144/11	34/39	810/141	67/23	166/38	22/11	38.5/22

Alb, albumin; *Bil*, bilirubin; *Bx*, biopsy; *CHH*, cartilage hair hypoplasia; *CMV*, cytomegalovirus; *FTT*: failure to thrive; *Ig*, immunoglobulin; *LFT*, liver function test; *ND*, not done; *PJP*, poneumocystis *Jirovecii pneumonitis*; *RML*, right middle lobe; *Tx*, transplant; *USS*, ultrasound scan.

Table II. HSCTs

Patients (y of HSCT)	Age at HSCT (y)	Donor	Conditioning	GvHD prophylaxis	Engraftment Neut/PLT/CD3 >300 (D+)	Chimerism at D+100	GvHD	Outcome
1 (1998)	16	MMUD 9/10 BM	Cycl/TBI/Campath	Tacro/MP	11/10/90	0% WB	Yes Skin	Death/Graft failure/*Cryptosporidium*, pulmonary bleed, renal failure
2 (2005)	17	MMUD 5/6 cord × 3	Flu/Cycl/TBI 2 Gy ATG	CSA/MMF then Tacro	9/*/NA	100% WB	Yes Skin/Liver/GI	Death/Pneumonitis/*Cryptosporidium*
3 (1998)	16	MSD BM	Bu/Cycl	CSA/MTX post	10/9/NA	99% WB	No	Death/CMV/adenovirus/pneumonitis
4 (2000)	10	MMUD (1Cmm) 9/10 BM	Flu/Mel ATG	CSA/MP	11/10/60	100% WB	No	Well Off IS/Ig
5 (1998)	11	MUD BM	Flu/Mel ATG	CSA	13/33/40	100% WB	Yes Skin G II	Well Off IS/Ig
6 (2001)	17	MUD 10/10 BM	Flu/Mel Campath	CSA	12/6/75	100% WB	No	Well Off IS/Ig
7 (2001)	16	MMUD 9/10 BM	Flu/Mel ATG	CSA/MP	17/†/120	100% WB	Yes Liver (mild)	Death/*Cryptosporidium*, EBV, renal failure
8 (1998)	10	MUD BM	Flu/Mel ATG	CSA/MP	8/10/39	53% WB CD15 41% CD3 71%	Yes skin/liver	Well Off IS/Ig
9 (2006)	8	MUD PBSC	Flu/Cycl Campath/YTH24/54 Anti-CD45	CSA/MMF	5/†/35	NA (graft rejection)	No	Death/Disease progression/rejected graft
10 (2014)	14	MMUD 9/10 PBSC	Flu/Mel Campath	CSA/MMF	10/15/90	91% WB	Yes gut	Death/MOF - CMV encephalitis, cryptosporidiosis
11 (2016)	3	MUD 10/10 PBSC then T cells	Flu/Treo Campath	CSA/MMF	13/15/90	100% WB	Yes skin G II	Well Off IS
12 (2003)	17	MUD BM	Flu/Mel Campath	CSA	14/12/300	90% WB	No	Autoimmune hemolysis, Death/mumps encephalitis
13 (1998)	18	MUD BM	Flu/Mel	CSA, ATG 1 vial/kg (D32-37), MP 1(D37)	47/48/?	100% WB	Yes skin/gut	Well/On Tacro for liver graft

ATG, antithymocyte globulin; *BM*, bone marrow; *Bu*, busulphan; *Cycl*, cyclophosphamide; *CSA*, cyclosporin A; *EBV*, Epstein-Barr virus; *Flu*, fludarabine; *G I*, grade I; *G II*, grade II; *IS*, immunosuppression; *Mel*, melphalan; mm, mismatch; *MMF*, mycophenolate mofetil; *MMUD*, mismatched MUD; *MP*, methylprednisolone; *MOF*, multiorgan failure; *MTX*, methotrexate; *Neut*, neutrophil; *PBSC*, peripheral blood stem cell; *PLT*, platelet; *Tacro*, tacrolimus; *TBI*, total body irradiation; *WB*, whole blood.
Neutrophil engraftment defined as neutrophil count above 0.5 × 10^9/L for 3 consecutive days post-HSCT.
PLT engraftment defined as platelet count above 20 × 10^9/L without support post-HSCT.
CD3 >300: CD3 count > 300 cells/μL.
*Patient platelet count was kept above 50 × 10^9/L.
†Patient platelet count was always above 20 × 10^9/L.

Histopathologic Findings

The histopathologic analysis included 11 liver biopsy samples pre-HSCT (6 who died and 5 survivors) and 9 available paired tissue samples. Patient 13, who had a liver allograft biopsy performed after liver transplant, was excluded from this analysis. Patient 2 had pretreatment liver biopsy performed 8 years before HSCT. Overall, there were 4 paired biopsy samples from the patients who died and 4 from the survivors, consented for the elective biopsies, performed at median age of 96 months (range, 4-144) post-HSCT (**Table III**).

Two out of 11 children (18.2 %), who underwent pre-HSCT biopsy procedures had Nakanuma staging of >3 and both died post-HSCT. The remaining 4 nonsurviving patients (36.3%) had Nakanuma stage 2 or below. Among the 5 survivors, all (45.5%) had Nakanuma stages 2 or below, suggesting that higher Nakanuma stage pre-HSCT may be associated with adverse prognosis.

Post-HSCT biopsy samples available from the 4 survivors showed that there was an improvement in Nakanuma grade in 3 (75 %) and in Nakanuma stage in 1 (25 %) in comparison with pre-HSCT assessment. Among the children who died, there was a progression of Nakanuma stage and grade in 2/4 (50 %), and remaining 2 biopsy samples (50%) showed no progressive features.

Survivors' Case Reports

Patient 4. A diagnosis of an undefined combined immunodeficiency syndrome was made in a 2-year-old boy, following a history of recurrent pneumonias, including one caused by *Pneumocystis Jirovecii,* bronchiectasis, and chronic *Cryptosporidium* enteritis. He was found to have compound heterozygous mutations in the *DOCK8* gene (940G>T; 1963C>T), suggesting that he and his affected sister have an autosomal recessive variant of hyper IgE syndrome (DOCK8 deficiency). At the age of 8 years, he was shown to have intra- and extrahepatic cholangiopathy on ultrasound scan and ERCP. His liver tests were mildly abnormal: alanine aminotransferase (ALT) 50 IU/L (nr <35 IU/L), aspartate aminotransferase (AST) 69 IU/L (nr <55 IU/L), and serum gamma-glutamyl transferase (GGT) was normal. A pre-HSCT liver biopsy, performed at 8 years of age, showed portal fibrosis with minimal biliary features. Two years later, he received a successful RIC HSCT from a 9/10 antigen matched unrelated donor. His post-HSCT recovery was uneventful. Seven years after HSCT, a follow-up liver biopsy was performed and reported as normal. He remains well, off all medications and with normal biochemical markers, but with persisting moderate dilatation of extrahepatic bile ducts on ultrasonography 13 years after HSCT.

Patient 5. A diagnosis of CD40 ligand deficiency was made at the age of 6 weeks following *Pneumocystis Jirovecii* pneumonitis. He received co-trimoxazole prophylaxis and regular intravenous immunoglobulin supplements every 3 weeks and remained relatively infection-free, but developed moderate to severe SC, confirmed on liver biopsy performed at the age of 11 years (**Figure 1**, A). ERCP showed severe intra- and extrahepatic cholangiopathy (**Figure 2**, A; available at www.jpeds.com). He had chronic *Cryptosporidium* enteritis, despite repeated attempts of eradication with paromomycin and azithromycin. His liver tests showed persistent elevation of ALT: 181 IU/L, AST: 193 IU/L, and GGT: 365 IU/L (normal <55 IU/L) with normal bilirubin, prothrombin time, and serum albumin. At age 11.5 years. he received an unrelated matched RIC HSCT. Following the HSCT he had worsening with a total bilirubin of 206 μmol/L (normal <20 μmol/L), and GGT 660 IU/L. In addition, he developed fever and grade 1 skin GvHD with hepatosplenomegaly, but no ascites. Liver biopsy could not be performed due to thrombocytopenia, but on steroid, ursodeoxycholic acid, antibiotic, and symptomatic treatment, his liver function gradually improved and normalized completely 5 months post-HSCT. Engraftment studies demonstrated a full donor chimerism 2 weeks after HSCT. He remained *Cryptosporidium* negative in the stools. Ten years after HSCT, an MRCP showed a normal intrahepatic biliary tree (**Figure 2**, B), although on ultrasound monitoring his extrahepatic bile ducts remained mildly dilated. Twelve years post-HSCT, a percutaneous liver biopsy was performed and showed only focal bile duct changes in essentially a normal liver parenchyma (**Figure 1**, B). His only medical problems are conductive deafness (presumably paromomycin related), requiring hearing aids, and mild arterial hypertension, fully controlled by lisinopril.

Patient 6. He was diagnosed with Wiskott-Aldrich syndrome in infancy, following history of bleeding from severe eczematoid skin rash because of thrombocytopenia and recurrent respiratory bacterial infections. His subsequent course was complicated by severe enteropathy with multiple food allergies and extremely severe widespread *Molluscum contagiosum*, which was successfully treated with topical cidofovir as previously reported.[13] His stools were chronically *Cryptosporidium* positive. To control the recurrent bleeding, he underwent splenectomy at age 9 years. One year later, he was noted to have abnormal liver tests (AST: 102 IU/L, GGT: 406 IU/L, bilirubin: 4 μmol/L) and dilated bile ducts on abdominal ultrasound scan (USS). An ERCP showed findings consistent with SC, which was confirmed on the liver biopsy (**Figure 3**, A; available at www.jpeds.com). At the age of 17 years, he underwent matched unrelated RIC HSCT achieving >95% donor engraftment at 1 month post-HSCT. Four months later a liver biopsy was performed, prompted by a rise in biliary enzymes, while still on gastrostomy feeding, showing that the inflammatory component had considerably improved, while fibrosis persisted (**Figure 3**, B; available at www.jpeds.com). Four years after the HSCT his liver tests improved (bilirubin: 9 μmol/L AST: 35 IU/L, GGT: 80 IU/L). His allergic manifestations had improved, his stools became *Cryptosporidium* negative, and he stopped having serious infections. Twelve years after HSCT his abdominal ultrasound scan showed that the pre-HSCT dilatation of the common, right, and left bile ducts remains unchanged.

Table III. Histopathologic features of the liver tissue

Patients	Pre-HSCT liver biopsy - final Nakanuma stage 1-4	Pre-HSCT Nakanuma grade CA 0-3 and HA 0-3	Other pathology	Post-HSCT liver biopsy - final Nakanuma stage 1-4	Post-HSCT Nakanuma grade CA 0-3 and HA 0-3	Other pathology
1	3 = Moderate progression	N/A		Allograft	CA = 3 HA = 1	*Cryptosporidium* present, Canalicular cholestasis, Cholatestasis, Reticuloendothelial and hepatocellular siderosis (grade 2)
2	4 = Advanced progression	CA = 3 HA = 1	*Cryptosporidium* not identified, Canalicular cholestasis, Cholatestasis, No siderosis	4 = Advanced progression	CA = 2 HA = 0	Cryptosporidium not *identified*, Canalicular cholestasis and cholatestasis, Grade 1 siderosis, GvHD
3	1 = No or minimal progression	CA = 0 HA = 0	*Cryptosporidium* not identified	2 = Mild progression	CA = 1 HA = 0	*Cryptosporidium* identified
4	1 = No or minimal progression	CA = 0 HA = 0	*Cryptosporidium* not identified	1 = No or minimal progression	CA = 0 HA = 0	*Cryptosporidium* not identified
5	2 = Mild progression	CA = 2 HA = 1	*Cryptosporidium* not identified	2 = Mild progression	CA = 0 HA = 0	*Cryptosporidium* not identified
6	1 = No or minimal progression	CA = 2 HA = 0	*Cryptosporidium* not identified	2 = Mild progression	CA = 0 HA = 0	Peri-ductal sclerosis, *Cryptosporidium* not identified, no siderosis
7	2 = Mild progression	CA = 2 HA = 0	*Cryptosporidium* not identified	3 = Moderate progression	CA = 0 HA = 1	*Cryptosporidium* not identified, Canalicular cholestasis, GvHD
8	2 = Mild progression	CA = 0 HA = 1	*Cryptosporidium* not identified	1 = No or minimal progression	CA = 0 HA = 0	*Cryptosporidium* not identified, moderate macrovesicular steatosis
9	3 = Moderate progression	CA = 2 HA = 0	*Cryptosporidium* not identified	N/A	N/A	N/A
10	2 = Mild progression	CA = 0 HA = 0	*Cryptosporidium* not identified	N/A	N/A	N/A
11	2 = Mild progression	CA = 2 HA = 0	*Cryptosporidium* present	N/A	N/A	N/A
12	1 = No or minimal progression	CA = 1 HA = 1	*Cryptosporidium* not identified	1 = No or minimal progression	CA = 0 HA = 1	Porto-lobular granulomas, mild macrovesicular steatosis, *Cryptosporidium* not identified
13	4 = Advanced progression	CA = 3 HA = 0	*Cryptosporidium* not identified, Canalicular cholestasis and cholatestasis	Allograft	N/A	N/A

CA, cholangitis activity; *HA*, hepatitis activity.

Biopsies and explants (patients 1 and 13) were assessed using the Nakanuma grading system[12] to assess both cholangitis activity (0 = no activity, 1 = mild activity, 2 = moderate activity, 3 = marked activity) and hepatitis activity (0 = no activity, 1 = mild activity, 2 = moderate activity, 3 = marked activity). Nakanuma staging was scored using fibrosis, bile duct loss, and as deposition of copper binding protein as follows: stage 1 (scores of 0, no progression), stage 2 (scores of 1-3, mild progression), stage 3 (scores of 4-6, moderate progression), and stage 4 (scores of 7-9, advanced progression).

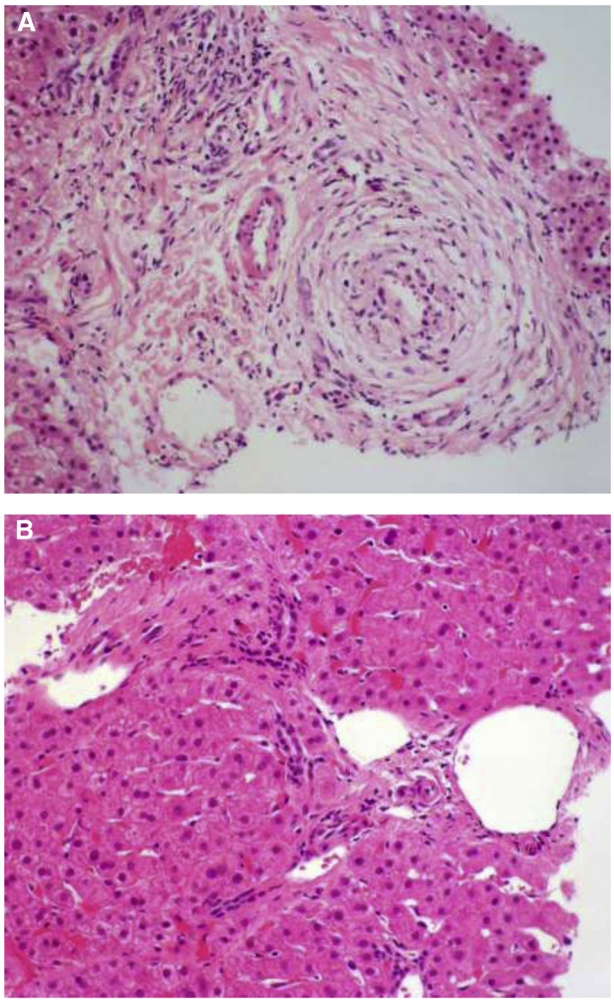

Figure 1. Liver biopsy findings of patient 5. **A,** The biopsy before HSCT shows an expanded portal tract with periductal concentric fibrosis and a minimal inflammatory infiltrate. The bile duct is damaged with the exfoliation of the biliary epithelium. **B,** In the biopsy 12 years post-HSCT, changes in the portal tracts have improved with only mild periportal fibrosis and a slight architectural irregularity of bile ducts. The lining epithelium is well-preserved. (hematoxylin and eosin staining, ×200).

Patient 8. He was diagnosed with undefined combined immunodeficiency in infancy, later identified as cartilage-hair hypoplasia syndrome with compound heterozygous mutations in the RNA component of mitochondrial RNA-processing endoribonuclease gene (146G>A; 242A>G). His initial clinical problems included chronic enteropathy, short stature, and bronchiectasis. Liver dysfunction with mild hepatosplenomegaly was noted at the age of 9 years. He was diagnosed with SC following ERCP and liver biopsy (**Figure 4**, A; available at www.jpeds.com). Conventional microscopy could not identify *Cryptosporidium* in his stools, but PCR testing was positive. At the age of 10 years, he underwent a RIC HSCT from a matched unrelated donor. Four months post-HSCT engraftment studies showed a stable donor chimerism at 90%. His liver function tests deteriorated temporarily after HSCT, but became completely normal 12 months post-HSCT. During a 9-year follow-up, his extrahepatic bile ducts remain dilated on USS; his chest problems and chronic diarrhea have resolved, but he required growth hormone treatment for short stature for 2 years. Percutaneous liver biopsy performed 9 years post-HSCT demonstrated a complete resolution of cholangiopathy with some centrilobular steatosis, which was interpreted as secondary to alcohol abuse (**Figure 4**, B).

Patient 11. This boy presented at the age of 15 months with recurrent otitis media, *Cryptosporidium* diarrhea, and failure to thrive. He went on to develop liver dysfunction and was diagnosed with SC. During immunologic work up, he was diagnosed with CD40 ligand deficiency [mutation c.688G>T; p.(Glu230*)]. At the age of 36 months, he received 10/10 matched MUD HSCT, complicated by grade II skin GvHD, adenoviremia, and liver dysfunction, but eventually recovered over 2 months and stopped all immune suppression 12 months after HSCT. He remains clinically very well with mild residual dilatation of the biliary tract on ultrasound and normal liver function 30 months after HSCT. No follow-up liver biopsy was performed.

Patient 13. This patient has been previously described,[8] but no follow-up liver biopsy was performed.

Discussion

This case series spans over more than 30 years and reflects our learning curve in the management of these rare immunodeficiency conditions. There is a considerable mortality (>50%) associated with advanced chronic liver disease when HSCT is performed late. However, our experience also indicates that chronic cholangiopathy associated with PIDs can considerably improve and even histologically revert after successful HSCT. We show resolution or amelioration of the histologic signs of liver disease in all surviving children with different types of serious PIDs, who remain in good health and off immune suppression after a follow-up of nearly 20 years.

The mechanism of liver disease in PIDs is incompletely understood[14]; in some cases the liver damage derives from a chronic colonization of the gastrointestinal and biliary tracts by *Cryptosporidium* species,[15] despite regular immunoglobulin supplementation and antibiotic/antifungal prophylaxis. A significant problem in the clinical management of the cholangiopathy in these patients is the poor sensitivity of conventional microbiological methods for protozoan detection. When more sensitive laboratory techniques, such as PCR-based assays, are used, the detection rate increases considerably.[10] The largest series reported so far on hyper-IgM syndrome identified *Cryptosporidium* in 81% of the patients with the liver disease at presentation.[16] Successful HSCT restores immunologic competence in children with CD40

ligand deficiency,[17-20] including eradication of *Cryptosporidium* infection[21,22] and it is conceivable that the observed improvement in SC in our patients post-HSCT is a consequence of established immune competence. However, during the immediate pre- and post-HSCT stages, the requirement for additional immunosuppression can lead to the exacerbation of *Cryptosporidium* infection with ensuing worsening of the liver damage, and even fatal systemic cryptosporidiosis, despite aggressive anti-protozoan treatment with azithromycin and paromomycin.[17,23] In this series, almost all children had a biochemical deterioration of the liver disease shortly after HSCT. In 7 patients, this worsening contributed to death, but only in 4 of them were we able to confirm a disseminated cryptosporidiosis. These children have also developed additional problems including GvHD, pneumonitis, and multiorgan failure.

A significant obstacle in performing HSCT is the periinterventional hepatotoxicity, attributed to conditioning regimen, sinusoidal obstruction syndrome, drug toxicity, and/or GvHD. Sequential HSCT and liver replacement have been done successfully in the presence of severe liver damage,[8,9] but the strategy to follow for cases with less severe liver involvement is unclear. The liver disease is the most important predictor of mortality in all age patients with PIDs.[16,24] Azzu et al reported overall mortality of 38% in the liver disease group compared with 6% in no liver disease group among all age patients with hyper IgM syndrome.[24] Similar to our present experience, they have also noted a considerable positive effect of earlier HSCT on the outcome. Ideally, elective isolated HSCT should be performed before the liver damage reaches an advanced stage to acquire immune competence essential for infection control, particularly in PIDs with a high risk of microbial invasion of the biliary system, such as hyper IgM syndrome.[4,16] In CD40 ligand deficiency, the most common form of hyper-IgM syndrome, HSCT has been reported as curative in 72% of patients without hepatic disease, but only in 58% of those with severe liver involvement, although this difference did not reach statistical significance.[18] Routine use of ursodeoxycholic acid[25] and defibrotide[26] post-HSCT is recommended to reduce the risk of serious hepatic complications.

All surviving patients from our series continue to have mild extrahepatic bile duct dilatation on USS, but no features of intrahepatic cholangiopathy on ultrasound scan or MRCP. It is possible that ERCP, a technique more sensitive than MRCP, could have shown the subtler intrahepatic bile duct changes post-HSCT, but this invasive test was not clinically justified. SC in PIDs is clinically and histologically indistinguishable from other pediatric forms of SC, such as neonatal, autoimmune, Langerhans cell histiocytosis-associated, and primary SC, but radiologic features are often more advanced due to its accelerated nature.[3] Our present experience is similar to that of Dimicoli et al, who reported 2 brothers with CD40 ligand deficiency who cleared *Cryptosporidium* from the stools and lost clinical features of cholangiopathy following HLA-identical myeloablative HSCT from sibling donors.[21] Jacobsohn et al described the improvement of liver disease in 2 patients after nonmyeloablative HSCT from unaffected siblings.[22] In contrast to our series, their patients did not have a residual dilatation of biliary system on USS. However, these authors have not demonstrated a histologic amelioration of the liver disease. The evolution toward normality of the histologic appearances documented in the 4 survivors with biopsy results from our series is exceptional in other forms of chronic cholangiopathies of childhood and clearly highlights the key role of an effective immune system in the recovery. In our series, the higher pre-HSCT histopathology cholangiopathy scores, indicative of more advanced liver disease, were observed in 50% of children who died. Similarly, the liver disease was progressive on post-HSCT biopsy in 2 out of 4 nonsurvivors in a strong contrast to the surviving children, where results from all follow-up biopsies showed a clear histologic improvement. This is consistent with a positive prognostic value of the milder histologic changes pre-HSCT, although our sample size was not large enough to confer a statistical significance. Our management recommendations are intuitive and only partly evidence-based because of the considerable individual differences in a limited number of patients, but we would suggest that patients with PIDs with liver involvement, but no signs of decompensated chronic liver disease such as gastrointestinal bleeding, ascites, coagulopathy, or hypoalbuminemia, be considered for an isolated HSCT, if appropriate donor is available. A more severe liver disease is likely to affect the success of even well-matched HSCT and would require sequential liver and HSC replacement.

In the previously reported studies, a complete donor engraftment was not required to achieve clinical immune competence.[21,22] Our experience seems also to support this observation. Even a partial chimerism appears to be sufficient for immune reconstitution and subsequent clearance of infection implicated in chronic cholangiopathy. Presence of mild GvHD had no effect on the post-HSCT outcome in our series. Although our sample size is relatively small and post-HSCT complications are difficult to predict, our impression is that both the extent of liver injury before HSCT and early immune reconstitution were the critical determinants of a positive outcome. The converse appears to be the case with the development of systemic infection, multiorgan failure, or poor engraftment.

Several groups have investigated genotypic/phenotypic correlation in PIDs, including hyper IgM syndrome, but the results were largely inconclusive.[16,27-29] The lack of genotypic correlation with clinical complications makes the decision-making process even more difficult in those children with PIDs who have incipient liver disease. It appears that the availability of a good, preferably related HLA-matched donor, a nonmyeloablative (RIC) option, and a lack of demonstrable *Cryptosporidium* infection justify the risks associated with HSCT. Separate to this series, we have followed a cohort of 20 patients with different PIDs, including hyper IgM syndrome, who had no matched donors and were kept on strict *Cryptosporidium* avoidance program (boiling drinking water, standard anti-infectious prophylaxis

[immunoglobulin supplements and co-trimoxazole], and oral paromomycin or azithromycin). None of them developed biochemical or radiological signs of liver disease after a median follow-up of 12 years. This suggests that a robust and early anti-protozoan regimen could delay or perhaps even prevent development of cholangiopathy in some PIDs.

Our study has several weaknesses. It has spanned over more than 30 years, with a higher mortality at the beginning of the observation period, most probably because of our limited experience in the clinical management and possible underappreciation of the role of a pre-existing liver injury. In recent years, we have noted a significant decline in the number of children with PIDs referred to us with advanced liver disease, probably reflecting the fact that *Cryptosporidium* avoidance protocols are implemented for those PID conditions at high risk and also that HSCT is now performed electively at an earlier stage, particularly when a good match is available. Finally, some of the follow-up information is incomplete due to the difficulties in obtaining comprehensive data on geographically widely scattered patients over a long period of time spanning well into adulthood.

In conclusion, our experience confirms that, despite high mortality, in selected children with mild to moderate liver involvement secondary to PID, an early well-matched HSCT represents a valuable therapeutic option, with a particular potential for complete reversal of established liver disease once the immune competence is acquired. HSCT may not be required in all patients with PID; continuous careful hepatologic surveillance is of paramount importance in order to offer the optimal selection and timing for HSCT. ■

Submitted for publication Oct 29, 2018; last revision received Dec 14, 2018; accepted Jan 8, 2019.

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50 Years Ago in *THE JOURNAL OF PEDIATRICS*

Parents of the Mentally Retarded Child: Emotionally Overwhelmed or Informationally Deprived?

Matheny AP Jr, Vernick J. J Pediatr 1969;74:953-9.

Matheny and Vernick describe an intervention within a mental retardation clinic aimed at changing parental expectations about their child's prognosis based on focused communications. The research goals were to assess concordance between clinician and parent expectation scores, and to determine the number of families who agreed to advance the staff's recommendations for the child's educational or institutional placement. Notably, the staff proposed that 7 (18%) of these 40 children with mental retardation be institutionalized and 24 (60%) be placed in specialized schools, isolated from mainstream education. The functional abilities of the children within each family context were not described nor were the degrees of mental retardation specified other than the cohort average IQ of 65.1.

Over the past 50 years, our terminology, laws, attitudes, and expectations have changed substantially, as seen in evolving medical practice and societal treatment of persons with disabilities. The term intellectual disability is now used instead of mental retardation ensuring that these individuals are included in the legal protections afforded those with disabilities with greater focus on their attendant civil rights including requirements to minimize the barriers that prevent participation in mainstream society.[1] In fact, within 6 years of this article's publication, federal law mandated that children with disabilities be educated in the least restrictive environment, which often means accommodations in regular classrooms.[2] Although we have made significant strides in reducing restrictive living and educational settings, highly restrictive settings persist, particularly for those with the severest forms of disabilities.[3] Because healthcare professionals tend to heavily emphasize cognitive ability in quality-of-life assessments and treatment recommendations,[4] they need greater exposure to, and training about intellectual disability to appropriately advocate for the accommodations and services needed to help these children achieve maximum independence in school and community settings.

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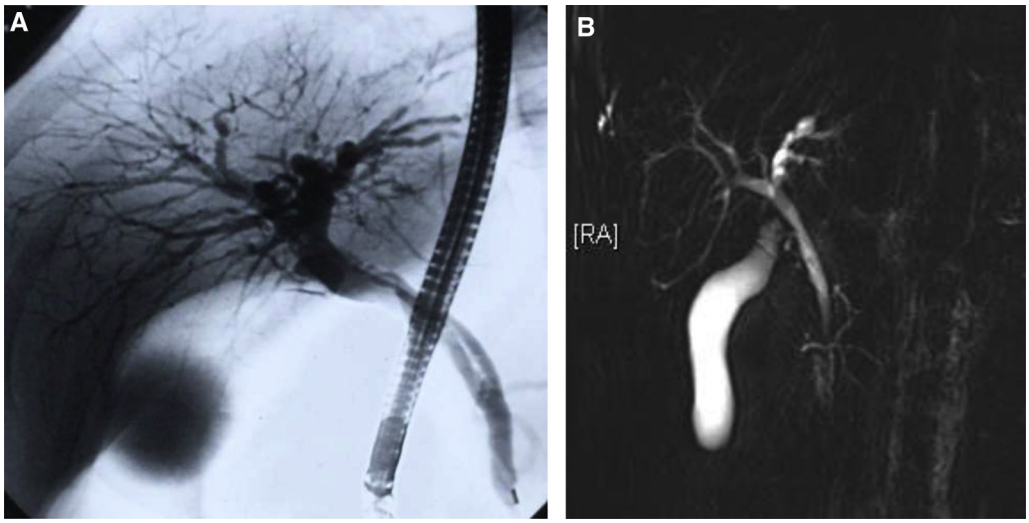

Figure 2. Radiologic findings in patient 5. **A,** ERCP before HSCT shows advanced intrahepatic and extrahepatic changes, suggestive of moderate SC, with no distal stricture. **B,** Post-HSCT MRCP demonstrates normal appearances of extrahepatic bile ducts with residual stricture of the left duct.

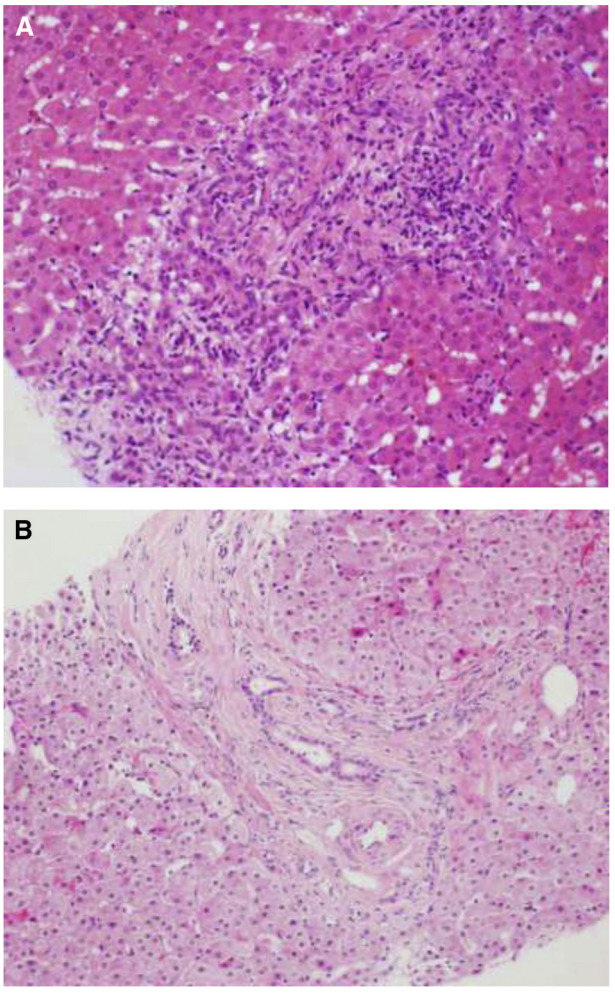

Figure 3. Liver biopsy findings of patient 6. **A,** Pre-HSCT biopsy shows an expanded portal tract with bile ductular reaction and cholangiolitis. **B,** In liver biopsy 2 months post-HSCT, periportal fibrosis and irregularity in bile duct contour remain, but no inflammation is noted. (hematoxylin and eosin staining; A, ×200; B, ×100).

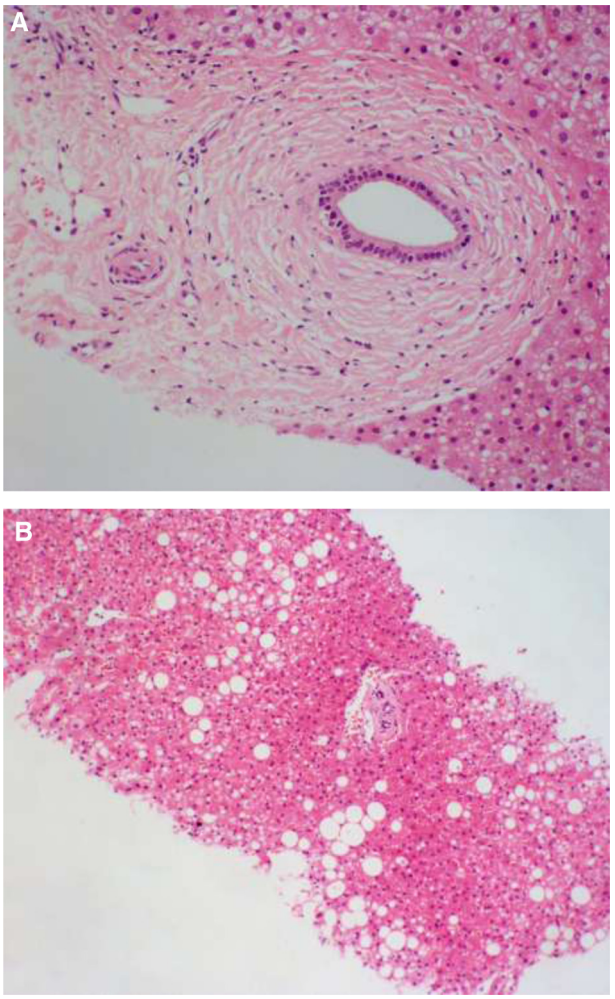

Figure 4. Liver biopsy findings of patient 8. **A,** Pre-HSCT biopsy reveals a dilated bile duct, surrounded by slight periductal fibrosis. **B,** Liver biopsy 9 years post-HSCT shows macrovesicular steatosis in the pericentral and midzonal area. The portal tract is unremarkable with no cholangiopathic features (hematoxylin and eosin staining; A, ×200; B, ×100).