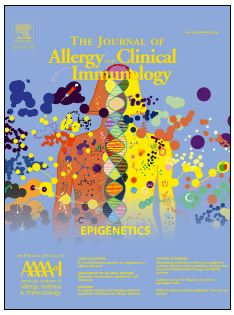
Accepted Manuscript



Combined liver and hematopoietic stem cell transplantation in X-linked hyper IgM syndrome

Giorgia Bucciol, MD, Sarah K. Nicholas, MD, Pier Luigi Calvo, MD, Andrew Cant, MD, J.David M. Edgar, MBChB BAO, FRCP FRCPath, Teresa Español, MD, PhD, Francesca Ferrua, MD, Miguel Galicchio, MD, Andrew R. Gennery, MBChB, MD, Nedim Hadzic, MD, PhD, I.Celine Hanson, MD, Gustavo Kusminsky, MD, Andrzej Lange, MD, Dr. med. Sci., FRCP, Fanny Lanternier, MD, PhD, Nizar Mahlaoui, MD, Despina Moshous, MD, PhD, Zohreh Nademi, MD, PhD, Benedicte Neven, MD, PhD, Matias Oleastro, MD, Fulvio Porta, MD, Paola Quarello, MD, Marcelo Silva, MD, Mary A. Slatter, MBChB, Elena Soncini, MD, Marek Stefanowicz, MD, Francesco Tandoi, MD, Mikołaj Teisseyre, MD, PhD, Troy R. Torgerson, MD, PhD, Paul Veys, MD, PhD, Katja G. Weinacht, MD, PhD, Beata Wolska-Kuśnierz, PhD, Jacques Pirenne, MD, PhD, M. Teresa de la Morena, MD, Isabelle Meyts, MD, PhD

PII: S0091-6749(19)30077-6

DOI: https://doi.org/10.1016/j.jaci.2018.12.1013

Reference: YMAI 13842

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 4 October 2018

Revised Date: 20 December 2018

Accepted Date: 31 December 2018

Please cite this article as: Bucciol G, Nicholas SK, Calvo PL, Cant A, Edgar JDM, Español T, Ferrua F, Galicchio M, Gennery AR, Hadzic N, Hanson IC, Kusminsky G, Lange A, Lanternier F, Mahlaoui N, Moshous D, Nademi Z, Neven B, Oleastro M, Porta F, Quarello P, Silva M, Slatter MA, Soncini E, Stefanowicz M, Tandoi F, Teisseyre M, Torgerson TR, Veys P, Weinacht KG, Wolska-Kuśnierz B, Pirenne J, de la Morena MT, Meyts I, Combined liver and hematopoietic stem cell transplantation in X-linked hyper IgM syndrome, *Journal of Allergy and Clinical Immunology* (2019), doi: https://doi.org/10.1016/j.jaci.2018.12.1013.

Combined liver and hematopoietic stem cell transplantation in X-linked hyper IgM syndrome.

Giorgia Bucciol, MD[a], Sarah K. Nicholas, MD[b], Pier Luigi Calvo, MD[c], Andrew Cant, MD[d], J. David M. Edgar, MBChB BAO, FRCP FRCPath[e], Teresa Español, MD, PhD[f], Francesca Ferrua, MD[g,h], Miguel Galicchio, MD[i], Andrew R. Gennery, MBChB, MD[d], Nedim Hadzic, MD, PhD[j], I. Celine Hanson, MD[k], Gustavo Kusminsky, MD[l], Andrzej Lange, MD, Dr. med. Sci., FRCP[m], Fanny Lanternier, MD, PhD[n,o,p], Nizar Mahlaoui, MD[o,p,q], Despina Moshous, MD, PhD[q], Zohreh Nademi, MD, PhD[r], Benedicte Neven, MD, PhD[p,q], Matias Oleastro, MD[s], Fulvio Porta, MD[t], Paola Quarello, MD[u], Marcelo Silva, MD[v], Mary A. Slatter, MBChB[d], Elena Soncini, MD[t], Marek Stefanowicz, MD[w], Francesco Tandoi, MD[x], Mikołaj Teisseyre, MD, PhD[y], Troy R. Torgerson, MD, PhD[z], Paul Veys, MD, PhD[aa], Katja G. Weinacht, MD, PhD[bb], Beata Wolska-Kuśnierz, PhD[cc], Jacques Pirenne, MD, PhD[dd], M. Teresa de la Morena, MD[z], Isabelle Meyts, MD, PhD[a]

[a]Laboratory of Childhood Immunology, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium, and Department of Pediatrics, University Hospitals Leuven, Leuven, Belgium

[b] Solid Organ Transplant Immunology, Section of Immunology, Allergy, and Rheumatology, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas

[c]Pediatric Gastroenterology, Department of Pediatrics, Regina Margherita Children's Hospital, AOU Città della Salute e della Scienza, Turin, Italy

[d]Great North Children's Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK, and The Primary Immunodeficiency Group, Institute of Cellular Medicine, Newcastle upon Tyne University, Newcastle upon Tyne, UK

[e]Regional immunology Service, The Royal Hospitals, Belfast, UK

[f]Immunology Unit, Vall d'Hebron University Hospital, Barcelona, Spain

[g]Department of Pediatric Immunology and HSCT, Great North Children's Hospital, Newcastle upon Tyne, UK.

[h]San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Pediatric Immunohematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, and Vita-Salute San Raffaele University Milan, Italy.

[i]Allergy and Immnunology Service, Hospital de Niños VJ Vilela, Rosario, Argentina

[j]Pediatric Center for Hepatology, Gastroenterology and Nutrition, King's College Hospital, London, UK

[k]Immunology, Allergy and Rheumatology Section, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas

[l]Stem Cell Transplantation Unit, Austral University Hospital, Buenos Aires, Argentina

[m]L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland, and Lower Silesian Center for Cellular Transplantation with National Bone Marrow Donor Registry, Wroclaw, Poland

[n]Service of Infectious and Tropical Diseases, Necker-Enfants Malades University Hospital, Infectiology Center Necker Pasteur, Paris, France

[o]French National Reference Center for Hereditary Immune Deficits (CEREDIH), Necker-Enfants Malades University Hospital, Paris, France

[p]Paris Descartes University, Sorbonne Paris Cité, Institut Imagine, Paris, France

[q]Pediatric Hematology-Immunology and Rheumatology Unit, Necker-Enfants Malades University Hospital, Paris, France

[r]Immunology Department, Great Ormond Street Hospital for Children, London, UK

[s]Rheumathology and Immunology Service, Hospital Nacional de Pediatría JP Garrahan, Buenos Aires, Argentina

[t]Pediatric Hematology Oncology and HSCT Unit, Spedali Civili, Brescia, Italy

[u]Pediatric Oncology-Hematology, Stem Cell Transplantation and Cellular Therapy Division, Regina Margherita Children's Hospital, AOU Città della Salute e della Scienza, Turin, Italy

[v]Hepatology Unit, Austral Hospital, Buenos Aires, Argentina

[w]Department of Pediatric Surgery and Organ Transplantation, Children's Memorial Health Institute, Warsaw, Poland

[x]Liver Transplantation Centre, General Surgery 2U, Department of Surgical Sciences, Molinette Hospital, AOU Città della Salute e della Scienza, Turin, Italy

[y]Department of Gastroenterology, Hepatology, Feeding Disorders and Pediatrics, Children's Memorial Health Institute, Warsaw, Poland

[z]Department of Pediatrics/Immunology, University of Washington and Seattle Children's Research Institute, Seattle, Washington

[aa]Bone Marrow Transplant Unit, Great Ormond Street Hospital for Children, London, UK

[bb]Stanford School of Medicine, Department of Pediatrics, Division of Stem Cell Transplantation and Regenerative Medicine, Stanford, California

[cc]Immunology Department, Children's Memorial Health Institute (CMHI), Warsaw, Poland

[dd]Abdominal Transplantation Surgery, Laboratory of Abdominal Transplantation, University Hospitals Leuven and KU Leuven, Leuven, Belgium

Corresponding author: Isabelle Meyts, Department of Pediatrics, University Hospitals Leuven

Herestraat 49, 3000 Leuven, Belgium; Tel +32 16 343841; Fax +32 16 343842

Isabelle.Meyts@uzleuven.be

Funding: GB is supported by the Research Foundation - Flanders (project G0C8517N). IM is supported by the Jeffrey Modell Foundation and by the Research Foundation - Flanders (project G0C8517N).

Disclosures: Dr. Edgar reports personal fees from Shire UK Ltd, personal fees from Octapharma, non-financial support from Grifols, personal fees and non-financial support from ALK Abello, outside the submitted work. Dr. Meyts reports a CSL Behring Unrestricted Research Grant 2017-2019. The remaining authors have nothing to disclose.

Capsule summary: Liver disease in X-linked hyper IgM syndrome (XHIGM) is an important predictor of mortality. In case liver transplantation (LT) is required, a survival benefit is observed when LT is combined with HSCT.

Key words: X-linked hyper IgM syndrome, HIGM, liver transplant, HSCT, Cryptosporidium, sclerosing cholangitis, primary immunodeficiency

To the Editor,

X-linked hyper IgM syndrome (XHIGM or HIGM1) is a combined immunodeficiency caused by mutations in the CD40 ligand encoding gene (*CD40LG*), leading to an impairment of both cellular and humoral immunity (1). Lack of CD40L and hence of CD40L/CD40 interaction results in defective T-cell function and co-stimulation, with ensuing impaired class switch recombination and somatic hypermutation in B cells, hindering an effective secondary antibody response. Moreover, NK cell, dendritic cell and monocyte activation are impaired, which results in impaired inflammatory responses (1). Around 40% of patients present with *Pneumocystis jirovecii* interstitial pneumonia, and recurrent respiratory tract infections are present in 80% of cases. Other pathogens include mainly bacteria, mycobacteria, fungi, such as *Histoplasma*, *Cryptococcus* and *Candida*, and viruses, especially Cytomegalovirus (CMV) (1–3). Gastrointestinal manifestations are described in 40% of patients and include diarrhea with failure to thrive, inflammatory bowel disease, and oral ulcers; neuroendocrine tumors of the gastrointestinal tract have been reported (1,2,4,5). Liver involvement can manifest as elevation of liver enzymes, infectious hepatitis and sclerosing cholangitis. *Cryptosporidium* infection has been reported in as many as half of the cases of sclerosing cholangitis, often leading to cirrhosis and liver failure (1–5). Central nervous system (CNS) disease can be present in up to 11% of patients at presentation, in the form of CNS infections or neurodegenerative disease (1–5). Hematological abnormalities such as neutropenia and anemia are described in over 60% and 15-20% of patients, respectively (1,3–5). Despite immunoglobulin substitution and antimicrobial prophylaxis, the overall prognosis is poor, with a median survival time from diagnosis of 25 years (6). Both European and US registries report mortality of 10-20% before the age of 30 years in their cohorts of patients (1,3–6).

A recent retrospective observational study analyzed the survival rate of 176 patients diagnosed with XHIGM between 1964 and 2013 and compared outcomes for those patients treated with or without hematopoietic stem cell transplantation (HSCT) (6). Liver or biliary involvement at diagnosis represented the only significant predictor of mortality, and survival was not influenced by HSCT (6). However, a survival benefit for transplanted patients was noted starting from 1987, suggesting improvements in transplant practice over the last four decades. Also, patients treated with HSCT demonstrated improvement of scales of daily living (Lansky or Karnofsky) when compared to the non-transplanted group (6). A recent retrospective study from the United Kingdom of 24 XHIGM patients reported a crude mortality of 38% in patients with liver disease, compared with 6% in patients without liver disease (7). Mortality as high as 80% was reported for XHIGM patients with liver disease treated with HSCT,

compared to 10% for those treated with HSCT without liver pathology (6). It is therefore generally recommended that XHIGM patients receive HSCT upfront, before the onset of liver disease (6,7).

Liver transplantation (LT) has been performed in small numbers of patients with XHIGM who have end-stage liver disease, with or without concomitant HSCT. The mortality in XHIGM patients only receiving LT is high. This is likely because the underlying immune defect is not corrected and could further be aggravated by the immunosuppression needed for LT, as previously reported (6,8). Improved outcome is noted when both LT and HSCT are performed (6,7). The aim of this study was to systematically report and compile the data on published and unpublished patients with XHIGM who received LT with or without HSCT. Patients were included from the previous study by de la Morena *et al*. (6), as well as through contacting corresponding authors of previously reported cases (the relevant bibliographic references can be found in this article's online repository at www.jacionline.org). Additionally, a query was sent to the Primary Immune Deficiency Treatment Consortium (PIDTC), the Inborn Errors Working Party of the European Group for Blood and Marrow Transplantation (IEWP-EBMT) and the Stem Cell Transplant for primary Immune Deficiencies in Europe registry (SCETIDE). For review of the questionnaire utilized, please see this article's online repository at www.jacionline.org.

We report the clinical manifestations, clinical course and outcome of thirteen patients with XHIGM from three continents and eight countries, who underwent LT for sclerosing cholangitis. Five of these patients (P1, P4, P5, P7, P11) were previously reported as cases in the literature (the relevant bibliographic references can be found in this article's online repository at www.jacionline.org); three patients (P1, P2, P8) were included from the previous study and one (P7) in the study by Azzu *et al* (7). LT and HSCT characteristics are summarized in Table 1. Except for one patient (P8, diagnosed in adulthood after onset of sclerosing cholangitis), all patients were diagnosed with XHIGM in infancy or childhood, with a median age at diagnosis of XHIGM of 3.5 years (range: 5 months-36 years). Additional clinical and genetic data are summarized in supplemental Table E1 and can be found in this article's online repository at www.jacionline.org. A genetic diagnosis of XHIGM was confirmed in ten of the 13 patients; in three, diagnosis was based on clinical and immunological criteria (hyper IgM and absent CD40L expression). Of the nine reported mutations, one is a missense in the promoter region of *CD40L*, one is a splice site missense mutation downstream of exon 1, one is a nonsense mutation in exon 5, five are frameshift mutations (one in exon 1, one in exon 2 and three in exon 5), and one is a partial deletion of the *CD40L* gene. Familial cases were found in five patients. Six patients suffered from recurrent respiratory tract infections and four patients had chronic lung disease, but only two presented with confirmed

Pneumocystis jirovecii pneumonia. One patient suffered from pulmonary *Mycobacterium avium* infection and aspergillosis. One patient developed tuberculosis of the shoulder and knee and septic arthritis of the hip, and another patient had an episode of cryptococcal meningitis. Seven patients manifested neutropenia, and one suffered from pancytopenia due to hypersplenism. Eight patients had gastrointestinal manifestations such as chronic diarrhea, and six patients failed to thrive. In all but one patient (P3), liver disease was diagnosed as sclerosing cholangitis, and *Cryptosporidium spp* infection was reported in eleven cases. P3 manifested liver disease after HSCT; his liver histology was compatible with chronic graft-versus-host disease (GvHD), but he also suffered from *Cryptosporidium* infection since before HSCT (microscopically identified), not responding to antimicrobial treatment. All patients were receiving immunoglobulin substitution treatment at the time of LT; *Pneumocystis jirovecii* pneumonia prophylaxis with trimethoprim-sulfamethoxazole was used in eleven out of thirteen patients. Azithromycin, nitazoxanide and paromomycin were used alone or in combination as prophylaxis or treatment of *Cryptosporidium* infection in seven patients. Two patients were receiving ursodeoxycholic acid for sclerosing cholangitis.

A total of 14 LT and 10 HSCT were performed on 13 patients (P1 received two LT and two HSCT; Table I). The median age at LT was 14 years (range: 8-38 years). The average time lap between XHIGM diagnosis and onset of liver disease in this cohort was 4.5 years (range 0,1-17.5 years). Nine patients underwent LT and HSCT. Four patients received LT alone. Amongst those patients treated with both liver and HSCT, in five patients the LT followed HSCT by an average of 15 months (range: 1.6 months-3 years), while in four patients LT preceded HSCT by an average of 1 month (range: 1-2 months). All patients received liver allografts from deceased unrelated donors. Living related donor LT were not utilized for patients in whom a family member was the HSCT donor. One LT was performed as emergency transplant in the context of liver failure after HSCT (P9). In the patients for whom the information is available, standard ABO matching was used for the LT. Immunosuppression regimens for LT consisted of standard therapies according to the center performing the LT. These included tacrolimus for 11 patients (P1-P7, P9, P12, P13), which was combined with steroids in 8 patients. Two patients (P5, P11) received cyclosporine, and mycophenolate mofetil (MMF) was added to dual calcineurin inhibitor and steroids in 3 patients (P1, P4, P13). Basiliximab was added as induction in two patients (P1, P5).

Nine patients received ten HSCT. In five patients, the HSCT preceded the LT. Donor characteristics included five patients treated with matched unrelated donors (MUD, three bone marrow, two unknown), three patients with mismatched unrelated donors (bone marrow), and two patients with matched siblings (bone marrow). Conditioning was myeloablative in two cases and reduced intensity in

seven. The conditioning regimen used in the patients who received HSCT first was not limited due to liver dysfunction in any of the cases. Out of ten HSCT performed, five resulted in full donor chimerism, one in mixed chimerism (64% on granulocytes and 80% on lymphocytes), and two in HSC graft failure (P1, P9). Both patients who lost donor chimerism manifested a recurrence of cryptosporidiosis and liver failure. P1 showed loss of chimerism 6 months after a MUD HSCT with non-myeloablative conditioning, followed by relapse of *Cryptosporidium* infection, sclerosing cholangitis and cirrhosis of the previously transplanted liver (LT was performed 2 months before HSCT). He was rescued by a second HSCT from a different unrelated donor, after reduced intensity conditioning, closely followed by a second LT, both successful. P9 had early loss of donor chimerism after a mismatched unrelated donor (MMUD) HSCT with myeloablative conditioning. He experienced acute liver failure and died from disseminated *Cryptosporidium* infection and hemophagocytic lymphohistiocytosis (HLH) after emergency LT. It is possible that the graft failure in P1 was facilitated by the presence of allogeneic T-cells from the recently transplanted liver. Seven patients experienced GvHD. Two of those who received HSCT after LT manifested acute liver GvHD, grade II (P5, P10). We can speculate that the occurrence of GvHD on the transplanted liver was more likely to happen due to the HLA mismatch between the liver donor and the HSC donor, although also two patients treated with HSCT before LT manifested acute GvHD on the native liver (P3, P4).

Six of 13 patients died, four of whom had received LT alone without HSCT (4/4), one in whom LT preceded HSCT (1/4) and one in whom LT occurred after HSCT (1/5) (Fig. 1). In patients who received HSCT, survival was better if HSCT was performed in more recent years, probably due to improved transplant practices (Supplemental Fig. S1). In five of the six patients who died, *Cryptosporidium* infection was present before LT/HSCT and a persistence/relapse of cryptosporidiosis with or without liver disease was considered the cause of death. The sixth patient died of early LT-related complications. Only one patient (P6) who received LT followed by HSCT developed a relapse of *Cryptosporidium* infection without sign of liver disease two years post-LT. Except in one case where HSC engraftment failed (P9), *Cryptosporidium* was not identified after LT in those patients who had received successful HSCT prior to LT.

This case series describes the current published data available on liver transplantation in patients with XHIGM. Liver involvement in XHIGM is commonly described as sclerosing cholangitis, which is reported in 6-20% of patients and is frequently associated with *Cryptosporidium* infection (3,5–7). Sclerosing cholangitis is responsible for one third of the cases of cirrhosis and liver failure in XHIGM patients, while

it represents only 4% of causes of LT in the general population. It has also been reported in association with liver carcinoma and cholangiocarcinoma (1,3). The mortality noted herein (6/13 patients; 46%) is in line with previously published data showing how liver disease represents a significant negative predictor of survival for XHIGM patients, who otherwise have better survival if not affected by hepatic dysfunction (6,7,9). Moreover, the survival rate of pediatric LT recipients has increased over the last few decades, and is set now between 70 and 80% at 20 years after transplant (E7 - Online Repository), while in our cohort overall crude survival was noted to be less than 55% with an average follow-up of 6.8 years. However, survival is improved in those XHIGM patients who underwent both LT and HSCT, regardless of the order of transplantation (80% survival with an average follow-up of 6 years, fig. 1). GvHD on the transplanted livers after allogeneic HSCT was found in two out of nine patients but was never the reason of liver graft failure or death. Also, only one patient experienced HSC graft failure when HSCT was performed after LT, possibly influenced by the presence of allogeneic T-cells in the liver graft. The need for LT also in patients who underwent HSCT before the onset of liver failure can be explained by the irreversibility of an already established biliary disease, mostly diagnosed many years before HSCT was performed. In two cases, moreover, the occurrence of liver GvHD post-HSCT likely worsened the course of disease in the already compromised liver. Relapse of *Cryptosporidium* and liver disease was associated with fatal outcome in all LT patients who did not receive a HSCT, highlighting the role of this pathogen in this PID (7). HSCT represents the only currently available definitive treatment of XHIGM. When performed at an early age, before major complications such as liver disease, HSCT can improve a patient's quality of life as long as appropriate immunologic reconstitution is achieved and there is absence of long term HSCT-related complications, such as GVHD (6). This report highlights the importance of HSCT when XHIGM patients require a LT for survival (6). Despite the underlying immune deficiency, XHIM patients who received both LT and HSCT tolerated the associated immunosuppressive regimens commensurate to the organ transplanted, suggesting that such dual therapies are feasible and associated with potential success in these patients. Transplant related CMV status of donor liver allografts was not addressed in this study due to limited information. Given the risk associated with CMV for XHIGM patients, procurement of CMV-negative liver allografts should be carefully considered. Although there is an important time lapse between the first and last patient to receive a LT (1993 and 2016), we do not believe this may have contributed significantly to the outcomes. Instead, *Cryptosporidium parvum* remains a major factor influencing outcomes, perhaps given the limited effective therapeutic options for these patients. Solid organ transplantation (SOT) for patients with primary immunodeficiencies can be difficult. The limited availability of organs precludes candidates in

whom there is high concern for allograft failure. In the case of PID patients, concern for infectious complications and potential GvHD, induced by T-lymphocytes present in the allograft, are real considerations. However, the data reported herein emphasize the potential cure of combined LT and HSCT in those patients with XHIGM in need for LT. Therefore, we argue that the diagnosis of XHIGM should not be an *a priori* reason to exclude XHIGM patients from LT, as long as HSCT is included in the treatment strategy. Our recommendation for these patients is to perform HSCT before LT. Our case series demonstrates the fundamental importance of immune reconstitution to control infection and allow a successful LT, as shown by the 100% rate of Cryptosporidium recurrence in patients who received only LT, or who experienced HSC graft failure. In case of fulminant liver failure and urgent LT, we recommend to plan a HSCT as soon as possible after the LT, preferably after healing of the surgical wounds. As for conditioning and support therapy, we suggest to prefer reduced intensity regimens, to avoid cyclophosphamide if possible, and to implement fluid restriction and ursodeoxycholic acid therapy during conditioning to reduce the risk of veno-occlusive disease.

We thank Virginie Courteille (SCETIDE registry) and Natalia Winciorek for the assistance in data input.

Giorgia Bucciol, MD[a]
Sarah K. Nicholas, MD[b]
Pier Luigi Calvo, MD[c]
Andrew Cant, MD[d]
J. David M. Edgar, MBChB BAO, FRCP FRCPath[e]
Teresa Español, MD, PhD[f]
Francesca Ferrua, MD[g,h]
Miguel Galicchio, MD[i]
Andrew R. Gennery, MBChB, MD[d]
Nedim Hadzic, MD, PhD[j]
I. Celine Hanson, MD[k]
Gustavo Kusminsky, MD[l]
Andrzej Lange, MD, , Dr. med. Sci., FRCP[m]
Fanny Lanternier, MD, PhD[n,o,p]
Nizar Mahlaoui, MD[o,p,q]
Despina Moshous, MD, PhD[q]

Zohreh Nademi, MD, PhD[r]

Benedicte Neven, MD, PhD[p,q]

Matias Oleastro, MD[s]

Fulvio Porta, MD[t]

Paola Quarello, MD[u]

Marcelo Silva, MD[v]

Mary A. Slatter, MBChB[d]

Elena Soncini, MD[t]

Marek Stefanowicz, MD[w]

Francesco Tandoi, MD[x]

Mikołaj Teisseyre, MD[y]

Troy R. Torgerson, MD, PhD[z]

Paul Veys, MD, PhD[aa]

Katja G. Weinacht, MD, PhD[bb]

Beata Wolska-Kuśnierz, PhD[cc]

Jacques Pirenne, MD, PhD[dd]

M. Teresa de la Morena, MD[z]

Isabelle Meyts, MD, PhD[a]

[a]Laboratory of Childhood Immunology, Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium, and Department of Pediatrics, University Hospitals Leuven, Leuven, Belgium

[b] Solid Organ Transplant Immunology, Section of Immunology, Allergy, and Rheumatology, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas

[c]Pediatric Gastroenterology, Department of Pediatrics, Regina Margherita Children's Hospital, AOU Città della Salute e della Scienza, Turin, Italy

[d]Great North Children's Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK, and The Primary Immunodeficiency Group, Institute of Cellular Medicine, Newcastle upon Tyne University, Newcastle upon Tyne, UK

[e]Regional immunology Service, The Royal Hospitals, Belfast, UK

[f]Immunology Unit, Vall d'Hebron University Hospital, Barcelona, Spain

[g]Department of Pediatric Immunology and HSCT, Great North Children's Hospital, Newcastle upon Tyne, UK.

[h]San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Pediatric Immunohematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, and Vita-Salute San Raffaele University Milan, Italy.

[i]Allergy and Immnunology Service, Hospital de Niños VJ Vilela, Rosario, Argentina

[j]Pediatric Center for Hepatology, Gastroenterology and Nutrition, King's College Hospital, London, UK

[k]Immunology, Allergy and Rheumatology Section, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas

[l]Stem Cell Transplantation Unit, Austral University Hospital, Buenos Aires, Argentina
[m]L. Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland, and Lower Silesian Center for Cellular Transplantation with National Bone Marrow Donor Registry, Wroclaw, Poland
[n]Service of Infectious and Tropical Diseases, Necker-Enfants Malades University Hospital, Infectiology Center Necker Pasteur, Paris, France
[o]French National Reference Center for Hereditary Immune Deficits (CEREDIH), Necker-Enfants Malades University Hospital, Paris, France
[p]Paris Descartes University, Sorbonne Paris Cité, Institut Imagine, Paris, France
[q]Pediatric Hematology-Immunology and Rheumatology Unit, Necker-Enfants Malades University Hospital, Paris, France
[r]Immunology Department, Great Ormond Street Hospital for Children, London, UK
[s]Rheumathology and Immunology Service, Hospital Nacional de Pediatría JP Garrahan, Buenos Aires, Argentina
[t]Pediatric Hematology Oncology and HSCT Unit, Spedali Civili, Brescia, Italy
[u]Pediatric Oncology-Hematology, Stem Cell Transplantation and Cellular Therapy Division, Regina Margherita Children's Hospital, AOU Città della Salute e della Scienza, Turin, Italy
[v]Hepatology Unit, Austral Hospital, Buenos Aires, Argentina
[w]Department of Pediatric Surgery and Organ Transplantation, Children's Memorial Health Institute, Warsaw, Poland
[x]Liver Transplantation Centre, General Surgery 2U, Department of Surgical Sciences, Molinette Hospital, AOU Città della Salute e della Scienza, Turin, Italy
[y]Department of Gastroenterology, Hepatology, Feeding Disorders and Pediatrics, Children's Memorial Health Institute, Warsaw, Poland
[z]Department of Pediatrics/Immunology, University of Washington and Seattle Children's Research Institute, Seattle, Washington
[aa]Bone Marrow Transplant Unit, Great Ormond Street Hospital for Children, London, UK
[bb]Stanford School of Medicine, Department of Pediatrics, Division of Stem Cell Transplantation and Regenerative Medicine, Stanford, California
[cc]Immunology Department, Children's Memorial Health Institute (CMHI), Warsaw, Poland
[dd]Abdominal Transplantation Surgery, Laboratory of Abdominal Transplantation, University Hospitals

Bibliography

1. Qamar N, Fuleihan RL. The Hyper IgM Syndromes. Clin Rev Allergy Immunol. 2014 Apr 1;46(2):120–30.

2. de la Morena MT. Clinical Phenotypes of Hyper-IgM Syndromes. J Allergy Clin Immunol Pract. 2016 Nov 1;4(6):1023–36.

3. Levy J, Espanol-Boren T, Thomas C, Fischer A, Tovo P, Bordigoni P, et al. Clinical spectrum of X-linked hyper-IgM syndrome. J Pediatr. 1997 Jul;131(1 Pt 1):47–54.

4. Winkelstein JA, Marino MC, Ochs H, Fuleihan R, Scholl PR, Geha R, et al. The X-Linked Hyper-IgM Syndrome: Clinical and Immunologic Features of 79 Patients. Medicine (Baltimore). 2003 Nov;82(6):373.

5. Leven EA, Maffucci P, Ochs HD, Scholl PR, Buckley RH, Fuleihan RL, et al. Hyper IgM Syndrome: a Report from the USIDNET Registry. J Clin Immunol. 2016 Jul 1;36(5):490–501.

6. Morena MT de la, Leonard D, Torgerson TR, Cabral-Marques O, Slatter M, Aghamohammadi A, et al. Long-term outcomes of 176 patients with X-linked hyper-IgM syndrome treated with or without hematopoietic cell transplantation. J Allergy Clin Immunol. 2017 Apr 1;139(4):1282–92.

7. Azzu V, Kennard L, Morillo-Gutierrez B, Slatter M, Edgar JDM, Kumararatne DS, et al. Liver disease predicts mortality in patients with X-linked immunodeficiency with hyper-IgM but can be prevented by early hematopoietic stem cell transplantation. J Allergy Clin Immunol. 2018 Jan 1;141(1):405-408.e7.

8. Lanternier F, Amazzough K, Favennec L, Mamzer-Bruneel M-F, Abdoul H, Tourret J, et al. Cryptosporidium spp. Infection in Solid Organ Transplantation: The Nationwide “TRANSCRYPTO” Study. Transplantation. 2017 Apr;101(4):826.

9. Mitsui-Sekinaka K, Imai K, Sato H, Tomizawa D, Kajiwara M, Nagasawa M, et al. Clinical features and hematopoietic stem cell transplantations for CD40 ligand deficiency in Japan. J Allergy Clin Immunol. 2015 Oct 1;136(4):1018–24.

Figure 1. Kaplan Meier curve of survival of 13 patients with XHIGM undergoing LT with/without associated HSCT.

Table I. Transplant characteristics of 13 patients with XHIGM who underwent liver transplantation with or without HSCT.

Patient	Age	Age at diagnosis of liver disease	SC	*Cryptosporidium* infection	Order of transplantations	Time elapsed between HSCT/LT	Year of and age at LT	IS	Year of and age at HSCT	Type of HSCT	Conditioning	GvHD	Rx	Complications	Donor chimerism at last FU	Survival after LT	Outcome
P1	27 y	9.5 y	Yes	Yes	LT - HSCT	2 m	2009 - 17 y	C/S Tacro MMF	2009 - 17 y	MUD	NM Flu ATG	No	-	HSC graft failure, relapse of Cryptosporidiosis and sclerosing cholangitis after LT	Lost	8 y	A/W
					HSCT - LT	7 w	2010 - 19 y	C/S Tacro MMF Basilix	2010 - 19 y	MUD	RIC Flu Treo ATG	Acute, gut	C/S	-	Full		
P2	25 y	10 y	Yes	Yes	HSCT - LT	2 y	2009 - 16 y	C/S Tacro	2007 - 14 y	Matched cord from sibling	RIC Flu Mel Alem	No	-	-	NA	9 y	A/W
P3	13 y	7 y	No	Yes	HSCT - LT	3 y	2015 - 10 y	C/S Tacro	2012 - 7 y	Mismatched MUD	RIC Flu Treo Alem	Acute, gut and liver (grade IV)	C/S Inflix ATG ECP	Catheter-related sepsis, *Klebsiella* UTI, Adenovirus infection after HSCT	Mixed	2 y	A/W
P4	18 y	5 y	Yes	Yes	HSCT - LT	1 y	2008 - 8 y	C/S Tacro MMF	2007 - 7 y	MUD	M Bu Cy ATG	Acute and chronic, skin, gut and liver (grade III)	C/S Tacro MMF Etanercept	HHV-6 infection, hypertension after HSCT. Transient renal and hepatic insufficiency after LT	Full	10 y	A/W
P5	11 y	3 y	Yes	Yes	LT - HSCT	4 w	2015 - 8y	Tacro CSA Basilix	2015 - 8 y	MUD	RIC Flu Treo Thio	Acute, skin and liver (grade II)	C/S MMF	Mild/moderate acute LT rejection, CMV reactivation, bilateral optic neuritis	Full	3 y	A/W
P6	23 y	18 y	Yes	Yes	LT - HSCT	4 w	2016 - 21 y	C/S Tacro	2016 - 21 y	Matched sibling	RIC Flu Mel	No	-	-	Full	2 y	Alive, relapse of *Cryptosporidium* infection (asymptomatic)
P7	38 y	5 y	Yes	No	LT - HSCT	5 w	1998 - 18 y	C/S Tacro	1998 - 18 y	MUD	RIC Flu Mel ATG	Acute, skin and gut (mild)	C/S	-	NA	20 y	A/W
P8	†38 y	33 y	Yes	No	LT	-	1995 - 38 y	NA	-	-	-	-	-	NA	-	Deceased soon after LT	Cause of death: LT-related complications
P9	†16 y	11y	Yes	Yes	HSCT - LT	2 m	1999 - 16 y	Tacro	1999 - 16 y	Mismatched MUD	M Cy Alem TBI	Acute, skin	NA	HSC graft failure and liver failure after HSCT. Pulmonary hemorrhage and renal failure after emergency LT	Lost	Deceased soon after LT	Cause of death: disseminated cryptosporidiosis and HLH
P10	†12 y	7 y	Yes	Yes	LT - HSCT	2 m	2005 -	NA	2005 -	Mismatc	RIC	Acute, gut	-	Pleuric effusion,	Full	Deceased	Cause of death:

							12 y		12 y	hed MUD	Flu Mel Thio ATG	and liver (grade II)		renal failure, relapse of Cryptosporidiosis and sclerosing cholangitis after LT		4 m after LT	relapse of *Cryptosporidium* infection and sclerosing cholangitis, renal failure
P11	†13 y	6 y	Yes	Yes	LT	-	1993 - 10 y	C/S CSA	-	-	-	-	-	-	-	Deceased 3 y after LT	Cause of death: relapse of *Cryptosporidium* infection and sclerosing cholangitis
P12	†15 y	13 y	Yes	Yes	LT	-	2016 - 14 y	Tacro	-	-	-	-	-	Hepatic artery stenosis, relapse of cryptosporidiosis, chronic rejection	-	Deceased 1 y after LT	Cause of death: relapse of *Cryptosporidium* infection, fulminant liver failure
P13	†25 y	NA	Yes	Yes	LT	-	2008 - 24 y	C/S Tacro MMF	-	-	-	-	-	Hemorrhagic shock during biopsy, *Enterococcus* bacteremia, CMV reactivation, pericarditis, relapse of Cryptosporidiosis	-	Deceased 1 y after LT	Cause of death: relapse of *Cryptosporidium* infection, sepsis

†deceased; A/W: alive and well; Alem: alemtuzumab; ATG: anti-thymocyte globulin; Basilix: basiliximab; Bu: busulfan; CMV: Cytomegalovirus; C/S: cortico-steroids; CSA: cyclosporine A; Cy: cyclophosphamide; Flu: fludarabine; FU: follow-up; GvHD: graft-versus-host disease; HHV-6: Human Herpesvirus 6; HLH: hemophagocytic lymphohistiocytosis; HSCT: hematopoietic stem cell transplantation; LT: liver transplant; m: months; M: myeloablative; Mel: melphalan; MMF: mycophenolate mofetil; MUD: matched unrelated donor; NA: not available; NM: non-myeloablative; RIC: reduced intensity conditioning; Rx: therapy; SC: sclerosing cholangitis; Tacro: tacrolimus; TBI: total body irradiation; Thio: thiotepa; Treo: treosulfan; UTI: urinary tract infection; w: weeks; XHIGM: X-linked hyper IgM syndrome; y: years.

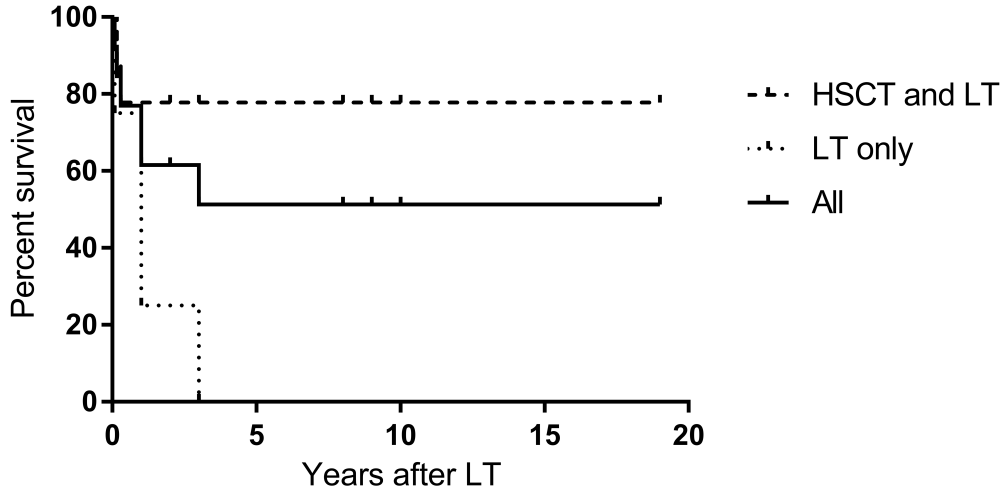


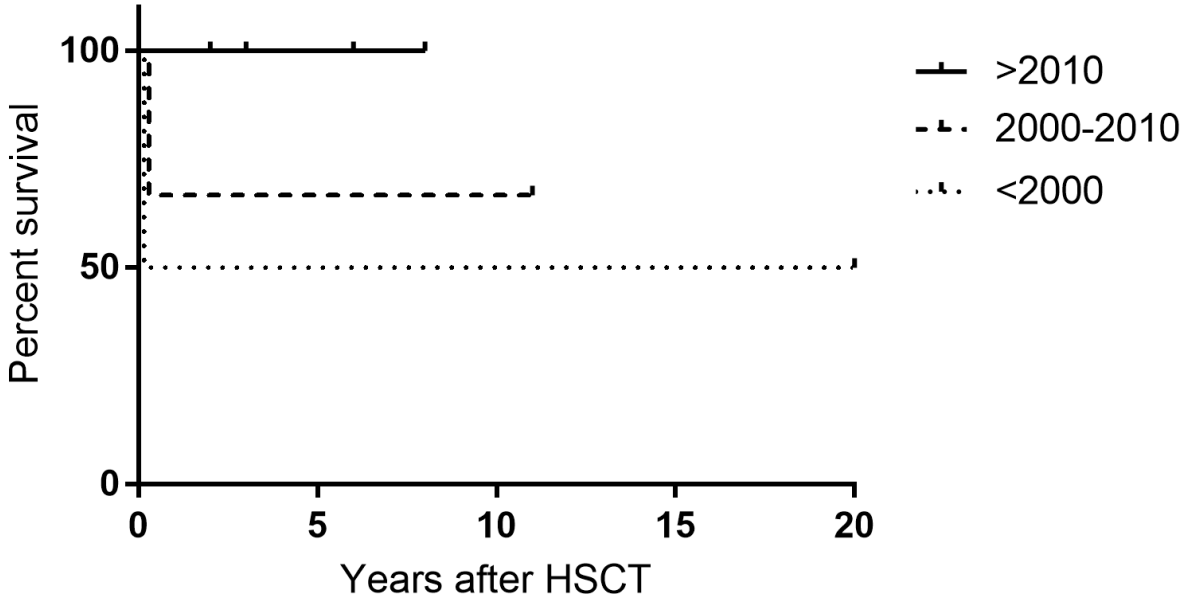
Table E1. Clinical, genetic and immunological characteristics of 13 HIGM patients treated with liver transplantation.

Patient	Age	Age at diagnosis of XHIGM	Familial cases	Genetic diagnosis	Lack of CD40L expression	Ig levels	Neutropenia	Anemia	PJP	Chronic LD	Other infections	Chronic diarrhea	FTT	Rx	HSCT	Current Rx	Outcome
P1	27 y	5 m	No	Yes c.-191A>C	Yes	IgM 1.85 g/L IgG 1.98 g/L IgA <0.04 g/L	No	No	Yes	No	HHV-6	Yes	No	Ig Cotrim Nitaz	Yes	Tacro	A/W
P2	25 y	7 y	No	No	Yes	IgM ↑ IgG ↓ IgA ↓	No	No	No	No	Staphylococcus aureus	Yes	Yes	Ig Cotrim	Yes	Ig	A/W
P3	13 y	6 y	Yes Brother	No	Yes	IgM ↑ IgG NA* IgA ↓	No	No	Suspected	Yes	-	Yes	Yes	Ig Cotrim Azithro Nitaz Paromo High dose CS	Yes	Tacro CS	A/W
P4	18 y	2 y	No	Yes c.120delA	Yes	IgM 2.7 g/L IgG 2.7 g/L IgA <0.06 g/L	Yes	No	No	No	Respiratory infections	No	No	Ig Cotrim	Yes	Tacro MMF	A/W
P5	11 y	5 y	No	Yes c.773T>G	Yes	IgM 0.8 g/L IgG 3 g/L* IgA 0.1 g/L	No	No	No	No	-	Yes	Yes	Ig Cotrim Paromo	Yes	Cyclo MMF	A/W
P6	23 y	6 m	Yes Brother	Yes c.500delG	Yes	IgM 0.48 g/L IgG 0.99 g/L IgA 0.01 g/L	Yes	No	No	No	Sinusitis, Criptococcal meningoencephalitis	No	No	Ig Cotrim Paromo Nitaz G-CSF Thalido	Yes	Tacro Thalido	A/W
P7	38 y	3 y	Yes Brother	Yes g.11880_11884 delGAGCA	Yes	IgM ↑ IgG ↓ IgA ↓	Yes	Yes	No	No	*Pseudomonas* and *Streptococcus mitis* biliary infection	No	No	Ig Cotrim Paromo UDCA Diuretics	Yes	Tacro	A/W
P8	†38 y	36 y	No	Yes c.156+1G>T	NA	IgM ↑ IgG ↓ IgA ↓	Yes	No	No	No	Respiratory infections	No	No	Ig	No	-	Deceased aged 38 y
P9	†16 y	8 m	No	Yes	Yes	IgM ↑ IgG ↓ IgA ↓	Yes	No	No	Yes	Respiratory infection, septic arthritis of the hip, joint tuberculosis of shoulder and knee	No	Yes	Ig Cotrim Crude thymic preparation FFP	Yes	-	Deceased aged 16 y
P10	†12 y	7 y	No	Yes c.676-679del	NA	IgM ↑ IgG ↓ IgA ↓	NA	NA	NA	NA	-	Yes	Yes	Ig Cotrim Azithro UDCA	Yes	-	Deceased aged 13 y
P11	†13 y	6 m	Yes Brother Cousin	Yes c.231_232insT	Yes	IgM 1.12 g/L IgG 1.38 g/L IgA <0.05 g/L	No	No	No	No	Respiratory infections, Parvovirus and Leishmania	Yes	Yes	Ig	No	-	Deceased aged 13 y

											donovani infection						
P12	†15 y	1 y	Yes Brother	Yes Xq26.3 del(135728471_ 135730458)	NA	IgM ↑ IgG ↓ IgA ↓	Yes	No	No	Yes	Respiratory infections, pulmonary mycobacterium avium and aspergillosis	Yes	Yes	Ig Cotrim Penta Azithro Rifabutin Rifampin Ethamb Vorico L-AMB Nitaz	No	-	Deceased aged 15 y
P13	†25 y	11 y	NA	NA	NA	NA	Yes	No	Yes	Yes	-	Yes	Yes	Ig Cotrim	No	-	Deceased aged 25 y

†deceased; *during Ig supplementation

A/W: alive and well; Azithro: azithromycin; Cotrim: cotrimoxazole (trimethoprim-sulfamethoxazole); C/S: cortico-steroids; Ethamb: ethambutol; FFP: fresh frozen plasma; FFT: failure to thrive; G-CSF: granulocyte colony stimulating factor; HHV-6: Human Herpesvirus 6; HSCT: hematopoietic stem cell transplantation; m: months; Ig: immunoglobulin; L-AMB: liposomal amphotericin B; LD: lung disease; MMF: mycophenolate mofetil; NA: not available; Nitaz: nitazoxanide; Paromo: paromomycin; Penta: pentamidine; PJP: *Pneumocystis jirovecii* pneumonia; Rx: therapy; Tacro: tacrolimus; Thalido: thalidomide; UDCA: ursodeoxycholic acid; Vorico: Voriconazole; XHIGM: X-linked hyper IgM syndrome; y: years.



Case reports

P1 presented in 1991 at the age of five months with *Pneumocystis jirovecii* pneumonia requiring prolonged mechanical ventilation. A diagnosis of XHIGM was made and he was started on substitutive immunoglobulins (Ig) and trimethoprim-sulfamethoxazole prophylaxis. A mutation was later identified in the promotor region of the *CD40LG* gene (c.123A>C – c.-192A>C adapted to current reference genome (E1)). At the age of 9 years he developed sclerosing cholangitis, with documented *Cryptosporidium* infection not responding to nitazoxanide treatment, gradually evolving into liver cirrhosis. At the age of 17 years he underwent a deceased-donor orthotopic LT, followed three months afterwards by a matched unrelated donor (MUD) hematopoietic stem cell transplantation (HSCT) from peripheral stem cells, after ~~reduced intensity~~ non-myeloablative conditioning regimen with fludarabine and anti-thymocyte globulin (ATG). Immunosuppressive treatment with tacrolimus, mycophenolate mofetil (MMF) and steroids was used during LT. In the first few months after the transplants, he experienced loss of HSCT engraftment and a relapse of *Cryptosporidium* infection and sclerosing cholangitis despite anti-parasitic prophylaxis with nitazoxanide. A second peripheral stem cell-HSCT from a different MUD and a second LT were subsequently performed 14 months after the first HSCT. He received reduced intensity conditioning with fludarabine, treosulfan and ATG, and engraftment was successful. The immediate post-HSCT course was complicated by hepatic failure, renal failure and pneumonia with *Candida spp.* and *Cryptosporidium parvum* growth on sputum culture. 50 days after the HSCT he underwent a second deceased-donor orthotopic LT, with an immunosuppressive regimen consisting of basiliximab, tacrolimus, MMF and steroids. He then experienced acute intestinal graft-versus-host disease (GvHD) that responded well to steroid treatment. At 26 years of age, he is now 7 years after the second HSCT and LT, has a donor chimerism of 100%, a good quality of life with a score of 90 as measured by Karnofsky scales, and has recently stopped Ig substitutive therapy.

P2 was diagnosed with XHIGM in 2000, at 7 years of age, based on absent CD40L expression on peripheral lymphocytes. He presented with respiratory infections, chronic diarrhea and failure to thrive, and at the age of 10 years he developed sclerosing cholangitis associated with *Cryptosporidium* infection. He was treated with substitutive Ig and trimethoprim-sulfamethoxazole prophylaxis, and at the age of 14 years he received a matched sibling HSCT from bone marrow, after reduced intensity conditioning with fludarabine, melphalan and Campath. The course of transplant was uncomplicated. Two years after the HSCT, he underwent a deceased-donor orthotopic LT, without major complications. He is now 24 years of age and has a good quality of life, scoring 90 on the Karnofsky scale.

P3 was started on Ig therapy for a non-specific hypogammaglobulinemia at the age of 1 year, in 2006. At the age of six years he and his brother were diagnosed with XHIGM, based on absent CD40L expression on peripheral lymphocytes. He suffered from severe interstitial pneumonitis, requiring intensive care and likely caused by *Pneumocystis jirovecii*, and was therefore treated with trimethoprim-sulfamethoxazole. A lung biopsy showed signs of lymphocytic interstitial pneumonitis. He also manifested protracted diarrhea and failure to thrive, and *Cryptosporidium* was detected in the stools by PCR. Treatment with azithromycine, paromomycin and nitazoxanide was attempted for the *Cryptosporidium* infection, without effect. He underwent a mMUD HSCT from bone marrow (match 9/10) at the age of seven years, after reduced intensity conditioning with fludarabine, treosulfan and ATG. The dose of CD34+ cells was $5.8*10^6$/kg. Engraftment was successful, but he suffered from grade IV GvHD involving gut and liver. Other post-HSCT complications included *Adenovirus* infection, *Klebsiella* urinary tract infection, catheter-related sepsis, chronic esophagitis and feeding problems, and severe liver dysfunction. Liver biopsies showed chronic cholestasis and cellular infiltrate around the bile ducts, that could be attributed to both GvHD or persistent *Cryptosporidium* infection. Significant immunosuppression was attempted with steroids, MMF, Infliximab, sirolimus, and ATG, without results. Extracorporeal photopheresis was then started with very slow beneficial effects, but because of the irreversible liver damage a deceased-donor orthotopic LT with ABO matching was finally performed at the age of 10 years. Steroid and tacrolimus were used as immunosuppressive treatment during transplant, and he experienced no complications. He is now 12 years old, alive and well, with a mixed donor chimerism of 64% on granulocytes (CD15+), 84% on B cells (CD19+), and 80% on T cells (CD3+). He is still immunosuppressed with tacrolimus and steroids, while Ig substitution and antibiotic prophylaxis have recently been stopped.

P4 suffered from recurrent upper and lower respiratory tract infections, hypogammaglobulinemia, neutropenia, and was diagnosed with XHIGM at the age of 2 years. A mutation in *CD40L* was identified (c.120delA) (E2), and Ig substitution and prophylaxis with trimethoprim-sulfamethoxazole were started. At the age of 5 years he developed liver disease in the form of sclerosing cholangitis, and *Cryptosporidium* infection was detected. He underwent a MUD HSCT from peripheral blood stem cells at the age of 7 years, after myeloablative conditioning with busulfan, cyclophosphamide and ATG. The CD34+ cell dosis was $5.5*10^6$/kg. After transplant he developed hypertension and acute mucocutaneous, intestinal and hepatic GvHD (grade III), which continued in chronic form. He was therefore treated with steroids, ATG, cyclosporine A and MMF. A year later, he received a deceased-donor orthotopic LT. The

immunosuppressive treatment consisted of steroids, tacrolimus and MMF. Etanercept was added 14 months after LT because of persistent skin and oral chronic GVHD. The course of LT was complicated by transient renal and liver insufficiency. Immunoglobulin substitution could be suspended soon after HSCT. P4 is now 18 years old, alive and well, still immunosuppressed with tacrolimus and MMF.

P5 presented with diarrhea and failure to thrive since the age of eight months in 2008. At the age of three years, hypogammaglobulinemia and elevation of liver enzymes were noted for the first time. He was diagnosed with sclerosing cholangitis and liver cirrhosis at 5 years of age. *Cryptosporidium parvum* was repeatedly isolated in the stools and didn't respond to treatment with paromomycin. CD40L expression was absent on peripheral lymphocytes and genetic analysis confirmed the diagnosis of XHIGM due to a mutation in *CD40LG* (c.773T>G, p.L258X (E3)). His healthy sister was heterozygous for the same mutation, while their mother was genotypically normal, implying a germinal mosaicism. He was started on Ig substitution, prophylactic trimethoprim-sulfamethoxazole treatment, paromomycin and ursodeoxycholic acid, and he never suffered from recurrent infections. Due to the progressive liver disease, at the age of 8 years he underwent deceased-donor orthotopic LT with ABO matching, complicated by a reactivation of Cytomegalovirus (CMV) and by an episode of mild/moderate rejection, which responded to steroids. The immunosuppressive regimen used during LT comprised basiliximab and tacrolimus. Four weeks after LT, he received a MUD HSCT from bone marrow after reduced intensity conditioning with treosulfan, fludarabine and thiotepa. The dose of CD34+ cells was $5.1*10^6$/kg. Neutrophil engraftment was seen at day +15 and platelet at day +20. He experienced CMV reactivation, bilateral optic neuritis probably associated with tacrolimus, that was then replaced by cyclosporine A, and acute cutaneous GvHD (grade II), successfully treated with steroids. During treatment with cyclosporine A, he developed hepatic GvHD for which MMF was started. A full donor chimerism (>97% donor) was observed on day +75 and is still stable to date. Finally, he suffered from limited chronic cutaneous GvHD 15 months after HSCT. He is now 11 years of age, alive and well. He has no sign of liver dysfunction or GvHD, and is still immunosuppressed with cyclosporine A and MMF.

P6 was diagnosed with XHIGM in 1995, at the age of six months, after his older brother died because of progressive panencephalitis in the context of XHIGM. CD40L expression was absent on peripheral lymphocytes and genetic analysis identified the causing mutation in *CD40LG* (c.500delG, p.G167fs*24). He was immediately treated with Ig substitution and trimethoprim-sulfamethoxazole prophylaxis, but despite this he suffered from recurrent bacterial sinusitis throughout childhood. He also manifested

recurrent neutropenia requiring granulocyte colony-stimulating factor (G-CSF) treatment, oral aphtosis treated with thalidomide, and he suffered from an episode of criptococcal meningoencephalitis. He was diagnosed with sclerosing cholangitis and *Cryptosporidium* infection at the age of 18 years, not responding to treatment with azithromycin, paromomycin and nitazoxanide. At 21 years of age he underwent deceased-donor orthotopic LT with ABO matching, followed four weeks later by a matched sibling HSCT from bone marrow, after reduced intensity conditioning with fludarabine and melphalan. The dose of CD34+ cells was $4.28*10^6$/kg. Immunosuppressive treatment during LT consisted of steroids and tacrolimus. He did not experience any complications or GvHD and is now 23 years old, alive and well, despite recurrence of *Cryptosporidium* infection (detected in the stools).

P7, suffering from respiratory tract infections, was diagnosed with XHIGM at the age of three years in 1984, based on absent CD40L expression on peripheral lymphocytes. His younger brother was also affected and died of *Pneumocystis jirovecii* pneumonia. Genetic analysis later demonstrated a small deletion causing a frameshift in *CD40LG* (c.465delGAGCAinsC - c.444delGAGCAinsC adapted to current reference genome (E4)). He was treated with Ig replacement therapy, trimethoprim-sulfamethoxazole and paromomycin prophylaxis. Since the age of 5 years he manifested liver involvement, and at 10 years he developed sclerosing cholangitis, without detection of *Cryptosporidium* infection. He then progressed to liver cirrhosis, developing ascites and portal hypertension with esophageal varices, which required sclerotherapy and banding. Therapy with ursodeoxycholic acid and diuretics was started. *Pseudomonas* and *Streptococcus mitis* were isolated from bile culture. At 18 years of age he underwent deceased-donor orthotopic LT with ABO matching, followed five weeks later by a MUD HSCT, after reduced intensity conditioning with fludarabine, melphalan and ATG. The dose of CD34+ cells was $6.55*10^6$/kg. Immunosuppressive treatment during LT consisted of steroids and tacrolimus. Mild skin and gut GvHD developed in the second week after HSCT and was successfully treated with steroids. He did not experience any other significant complications and is now 37 years old, alive and well.

P8 manifested sclerosing cholangitis in 1990, at the age of 33 years, and three years later was diagnosed with XHIGM and started on Ig substitutive therapy. The molecular defect was identified as a splice site mutation downstream of exon 1 in *CD40LG* (c.156+1G>T). Clinically, he only presented with upper respiratory infections and neutropenia; *Cryptosporidium* infection was not detected. Due to the liver disease, he underwent LT at the age of 38 years, but he died shortly thereafter of transplant-related complications.

P9 presented at the age of 8 months in 1983 with recurrent respiratory tract infections, failure to thrive and neutropenia, and was diagnosed with XHIGM. Genetic testing later confirmed the diagnosis (genetic details not available). He was treated with Ig replacement therapy, trimethoprim-sulfamethoxazole prophylaxis, and for a short period of time with crude thymic extract and fresh frozen plasma. He went on to develop chronic lung disease, septic arthritis of the hip and joint tuberculosis of the shoulder and the knee. Sclerosing cholangitis associated with Cryptosporidium infection was diagnosed at the age of 11 years. Cryptosporidiosis was identified by direct microscopy examination on stool and broncho-alveolar lavage. He underwent a mismatched unrelated donor (mMUD) HSCT from bone marrow at the age of 16 years, with the mismatch of one HLA-A antigen, after myeloablative conditioning with cyclophosphamide, Alemtuzumab and total body irradiation at a total dose of 14.4 Gy. Early engraftment of platelets and neutrophils with 100% donor chimerism was complicated by acute cutaneous GvHD and followed by complete loss of chimerism and acute liver failure, requiring LT. LT was performed in an emergency setting and the post-operative course was complicated by pulmonary hemorrhage and renal failure. The patient ultimately died a short time after LT from disseminated cryptosporidiosis and hemophagocytic lymphohistiocytosis.

P10 was diagnosed with XHIGM at the age of 7 years, in 2000. He carried a deletion in *CD40L* (c.676-679del, p.G226fs*15). He suffered from chronic diarrhea and failure to thrive, *Cryptosporidium* infection and sclerosing cholangitis. Further details about his infectious and immunological history are not available. Since the diagnosis he was treated with Ig substitution, prophylaxis with trimethoprim-sulfamethoxazole and azithromycin, and ursodeoxycholic acid. At the age of 12 years he underwent deceased-donor orthotopic LT, followed by a mMUD HSCT two months later. He was conditioned with fludarabine, thiotepa, melphalan and ATG and received $4.24*10^6$/kg CD34+ cells from bone marrow. Engraftment was successful, but the post-transplant course was complicated by a relapse of *Cryptosporidium* infection, sclerosing cholangitis, CMV and Epstein-Barr virus (EBV) reactivation, Candida infection and acute kidney failure. The patient succumbed to complications two months after HSCT.

P11 was diagnosed with XHIGM in 1983, at 6 months of age, after his older brother died in infancy from *Pneumocystis jirovecii* pneumonia. A first cousin was also diagnosed with the disease. CD40L expression was absent on peripheral lymphocytes and genetic testing demonstrated the presence of a novel mutation in this family (c.231_232insT, p.78fsX8) (E5,E6). Clinically, he presented with upper and lower

respiratory tract infections, chronic diarrhea, failure to thrive and *Parvovirus* infection. He was treated with substitutive Ig since the diagnosis. During childhood he developed *Cryptosporidium* infection and sclerosing cholangitis, that finally evolved in end-stage liver disease. He received a liver transplantation (LT) at the age of 10 years, but he died two years later from a relapse of sclerosing cholangitis and persistent *Cryptosporidium* infection.

P12 presented in 2003 at the age of 7 months with staphylococcal scalded skin syndrome and a polymicrobial soft tissue infection. Because of this unusual infection and a family history of a brother who died of pneumococcal sepsis, he underwent an immune deficiency evaluation and was eventually diagnosed with XHIGM due to a deletion in CD40LG (Xq26.3del(135728471_135730458)). He was treated with Ig substitution and trimethoprim-sulfamethoxazole prophylaxis. He suffered from mild respiratory tract infections throughout his life, but experienced severe pulmonary disease with Mycobacterium avium complex and Aspergillus since the age of 13 years, resulting in chronic lung disease and bronchiectasis. He also manifested mild neutropenia, diarrhea, failure to thrive, and at the age of 13 years he was diagnosed with *Cryptosporidium* infection and sclerosing cholangitis. He received treatment with pentamidine, azithromycin, rifabutin, rifampin, ethambutol, voriconazole, liposomal amphotericin and nitazoxanide. At 14 years he underwent deceased-donor orthotopic LT, which was complicated by hepatic artery stenosis and recurrence of cryptosporidium infection, with concern for graft failure due to chronic rejection. He never recovered from the LT well enough to be able to undergo HSCT, and finally passed away one year after liver transplant from fulminant liver failure.

P13 presented with *Pneumocystis jirovecii* pneumonia at the age of two months, and subsequently suffered from chronic interstitial lung disease, diarrhea and failure to thrive. He was diagnosed with XHIGM at the age of 11 years and Ig substitution and prophylaxis with trimethoprim-sulfamethoxazole were started. Immunologically, he manifested neutropenia. He developed sclerosing cholangitis, with documented *Cryptosporidium* infection, which led to a LT at 24 years of age. He received a deceased-donor orthotopic LT, and the immunosuppressive treatment consisted of steroids, tacrolimus and MMF. He experienced several post-transplant complications, among which hemorrhagic shock after a biopsy, Enterococcus bacteremia, CMV reactivation and pericarditis. He developed a relapse of Cryptosporidiosis two months after LT and succumbed to sepsis in the context of evolving *Cryptosporidium* infection in the first year after transplant.

Bibliography

E1. Van Hoeyveld E, Zhang P-X, De Boeck K, Fuleihan R, Bossuyt X. Hyper-immunoglobulin M syndrome caused by a mutation in the promotor for CD40L. Immunology. 2007 Jan 18;120(4):497–501.

E2. Teisseyre M, Teisseyre J, Kalicinski P, Wolska-Kusnierz B, Ismail H, Bernatowska E, et al. Liver Transplantation for Severe Hepatic Graft-Versus-Host Disease in Two Children After Hematopoietic Stem Cell Transplantation. Transplant Proc. 2010 Dec 1;42(10):4608–10.

E3. Quarello P, Tandoi F, Carraro F, Vassallo E, Pinon M, Romagnoli R, et al. Successful sequential liver and haematopoietic stem cell transplantation in a child with CD40 ligand deficiency and Cryptosporidium-induced liver cirrhosis. Transplantation. 2018 Mar 22;102(5):823–8.

E4. Hadžić N, Pagliuca A, Rela M, Portmann B, Jones A, Veys P, et al. Correction of the Hyper-IgM Syndrome after Liver and Bone Marrow Transplantation. N Engl J Med. 2000 Feb 3;342(5):320–4.

E5. Kraakman ME, Weers M, Español T, Schuurman RK, Hendriks RW. Identification of a CD40L gene mutation and genetic counselling in a family with immunodeficiency with hyperimmunoglobulinemia M. Clin Genet. 2008 Jun 28;48(1):46–8.

E6. Martinez Ibanez V, Espanol T, Matamoros N, Iglesias J, Allende H, Lucaya T, et al. Relapse of sclerosing cholangitis after liver transplant in patients with hyper-Ig M syndrome. Transplant Proc. 1997 Mar;29(1–2):432–3.

E7. Venick RS, Farmer DG, Soto JR, Vargas J, Yersiz H, Kaldas FM, et al. One Thousand Pediatric Liver Transplants During Thirty Years: Lessons Learned. J Am Coll Surg. 2018 Apr 1;226(4):355–66.

Supplementary figure S1. Kaplan Meier curve of survival of 9 patients with XHIGM treated with HSCT and LT, based on year of HSCT.