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Liver disease predicts mortality in patients with XHIM but can be prevented by early hematopoietic stem cell transplantation

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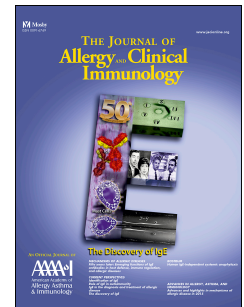
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Title page

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Liver disease predicts mortality in patients with XHIM but can be prevented by early hematopoietic stem cell transplantation

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Key messages

- Liver disease is common in XHIM and is characterized by increased ALT, GGT, bilirubin and PT, but not by ALP or albumin.
- Patients with liver disease tend to have abnormalities on imaging such as hepatomegaly, dilated bile ducts or evidence of cirrhosis.
- Patients should have at least annual monitoring of their liver function tests and liver imaging. Detection of abnormal LFTs or liver imaging should prompt referral to a hepatologist.
- Histological diagnosis is important for characterizing the type of liver disease.
- PCR techniques to detect micro-organisms is preferable to serological techniques given the antibody deficiency in these patients.
- Cytomegalovirus and *Cryptosporidium* should be screened for at least annually, even in asymptomatic patients and should be treated whenever possible.
- Early HSCT prior to establishment of liver disease may prevent significant XHIM-related liver complications and improve survival in this rare immunodeficiency disorder.

Capsule summary

In the largest detailed study of liver disease in X-linked immunodeficiency with hyper-IgM, we show that liver disease is commoner than generally recognized and that early hematopoietic stem cell transplantation may prevent liver disease progression and improve survival.

Keywords: abnormal liver function, sclerosing cholangitis, chronic hepatitis, CD40 ligand deficiency, XHIM, hematopoietic stem cell transplant

Abbreviations

ALP, alkaline phosphatase

ALT, alanine transferase

CMV, cytomegalovirus

GGT, gamma-glutamyl transpeptidase

HSCT, hematopoietic stem cell transplantation

PT, prothrombin time

XHIM, X-linked immunodeficiency with hyper-IgM

Author contributions

WJHG & DSK initiated the investigation of liver disease in XHIM patients. LK, VA & WJHG designed the study. LK, VA, BMG, MS & DE collected the data. LK & VA analysed the data and wrote the manuscript. All authors made intellectual contributions to the manuscript.

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Conflict of interest

The authors have no conflicts of interest.

To the Editor:

X-linked immunodeficiency with hyper-IgM (XHIM) is a severe primary immunodeficiency disorder characterized by low levels of/absence of IgG, IgA, and IgE, with normal to raised serum IgM concentrations (1). XHIM results from mutations in the CD40 ligand (CD40L) gene found on activated CD4 T cells (2); disruption of this gene results in aberrant interaction with CD40 on B cell membranes, thus inhibiting B cell proliferation and immunoglobulin class switching (3). CD40 is also found on other cells such as monocytes, endothelial and epithelial cells. Therefore, XHIM patients have a combined immunodeficiency, resulting in recurrent bacterial and opportunistic infections. Sinopulmonary infections occur in ~80% of patients; gastrointestinal manifestations, including chronic diarrhea, affect ~50% of patients; and hepatic complications which are principally biliary in nature are reported to affect ~20% of XHIM patients (4, 5). The clinical presentation was broadly reflected in our cohort (Table E1, online repository).

An appreciation of the true burden and nature of liver pathology in XHIM patients has been limited, hence we present a detailed characterization of liver pathology in a sizeable cohort of patients with XHIM from several UK centers.

All UK centers submitting XHIM data to the UK Primary Immunodeficiency Network were asked to complete a standardized proforma. We collated data for 24 patients from three centers. Statistical comparisons were performed using Mann-Whitney test for continuous variables, Fisher's exact test for categorical data, and Log-rank test for survival data. Further methodology is included in the online repository.

We found a higher prevalence of liver disease (33%) in XHIM patients compared with most other studies: ~20% in data from a European registry (5) and a multi-national study (6), and only 6% in two United States registries (4, 7). A small case series from Newcastle described liver disease in 3 of 8 XHIM patients (37.5%) and there is likely to be a notable overlap with our patient population from Newcastle (8).

Our study found that XHIM patients without liver disease had normal liver biochemistry, normal imaging and normal histology, where performed. However, XHIM patients with liver disease had i) statistically significant elevations in liver biochemistry excluding albumin (Table 1 & Figure E1, online repository), ii) abnormal liver imaging including splenomegaly, heterogeneous or cirrhotic appearing livers and biliary dilatation or sclerosing cholangitis and iii) abnormal histology showing cholangiopathy/sclerosing cholangitis in 75% of those with liver disease, chronic hepatitis in 50%, and both features in 25% (Table E2, online repository).

Cryptosporidium and cytomegalovirus (CMV) infection have previously been associated with development of cholangitis in patients with XHIM (5, 9). In our case series, 38% of patients with liver disease tested positive for *Cryptosporidium*, and the prevalence was three times higher than in patients without liver disease (13%) (Table 1). However, we did not find a statistically significant association between *Cryptosporidium* infection and development of liver disease, or indeed, sclerosing cholangitis. CMV prevalence was not different between XHIM patients with or without liver disease (Table 1).

Crude mortality was higher in the liver disease group (38%) compared with the non-liver disease group (6%). Although the average age of patients was higher in the liver disease

group (39.1 years versus 18.4 years), the standardized mortality ratio of 94 in those with liver disease versus 16 in those without, supports this conclusion by correcting for this potential confounding factor (Table E3, online repository).

Kaplan-Meier log-rank analysis shows decreased survival in XHIM patients with liver disease, regardless of whether time zero is plotted as birth (hazard ratio 4.5, $p>0.05$) or date of diagnosis of XHIM (hazard ratio 6.9, $p=0.05$) (Figures 1A and 1B respectively). Our data reflects the survival curves in a highly powered recent study (6).

Hematopoietic stem cell transplant (HSCT) was performed in 89% of patients without liver disease (average age of HSCT 3.1 years) compared with 50% in those with liver disease (average age of HSCT 14.5 years). Early HSCT before age 10 was performed in those without liver disease and survival was excellent. All patients with liver disease were transplanted as teenagers and liver disease at the time of HSCT conferred a poor prognosis, with the exception of a patient who was contemporaneously liver transplanted (Figure 1C & discussion in online repository).

A previous study has described the survival benefit in XHIM all-comers (10), but we show that those with early HSCT do not develop liver disease despite an average follow up of 11 years, and this confers a notable survival benefit ($p=0.0022$). Multivariate analysis supports this conclusion and further discussion can be found in the online repository.

To date, this is the most detailed study of liver disease in XHIM. We conclude that liver disease in patients with XHIM is a later-occurring diagnosis, characterized histologically by sclerosing cholangitis and chronic hepatitis, which has a poorer outcome and may be preventable through early HSCT. Patients with XHIM should undergo annual monitoring of LFTs, including GGT and clotting, liver imaging (especially in those without HSCT) and exclusion of pathogens such as *Cryptosporidium* and CMV via PCR even if patients are asymptomatic. The presence of liver disease confers a poor prognosis in these patients and we strongly advocate specialist hepatology input in their management. Early diagnosis is key and a multidisciplinary approach regarding HSCT and liver transplantation should be sought.

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Figure legend

Figure 1. Kaplan Meier survival curves in XHIM patients with or without liver disease. A) Time zero is birth. B) Time zero is diagnosis of XHIM. In both plots, the timings of HSCT are shown as dashed lines above the graph.

Tables

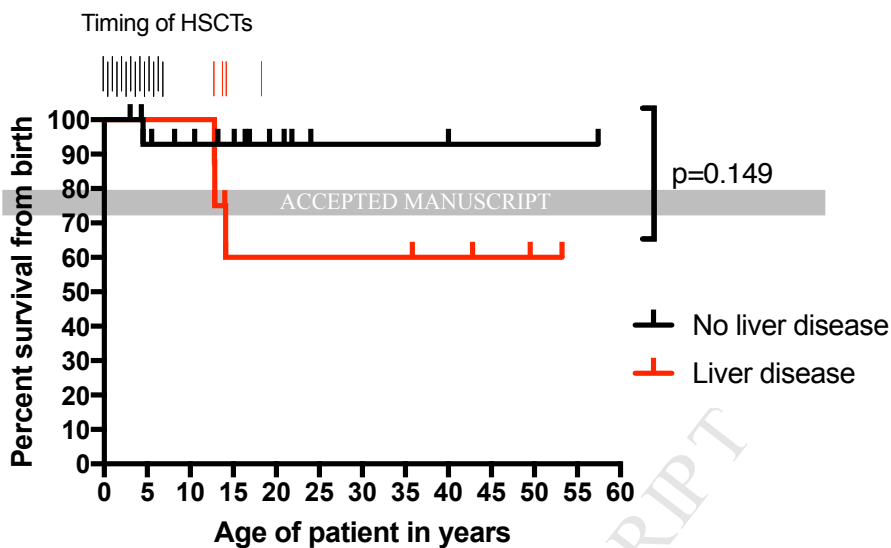
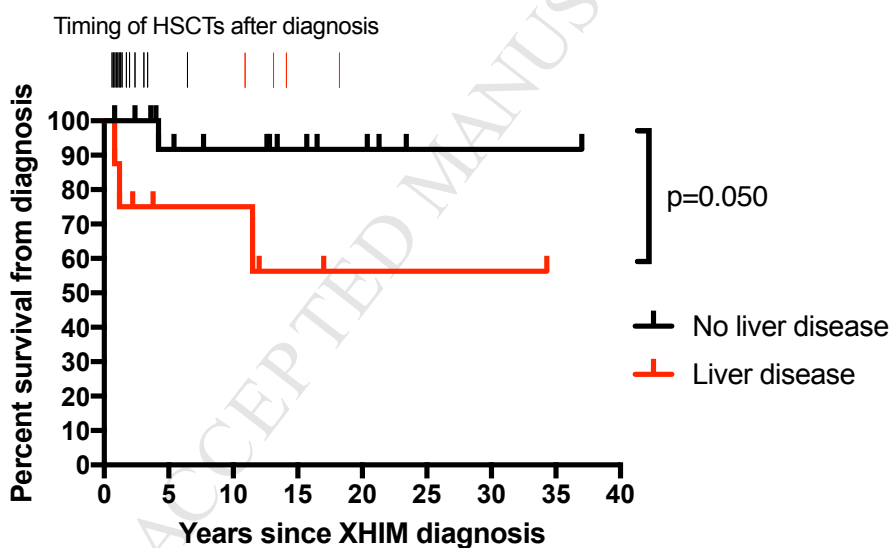
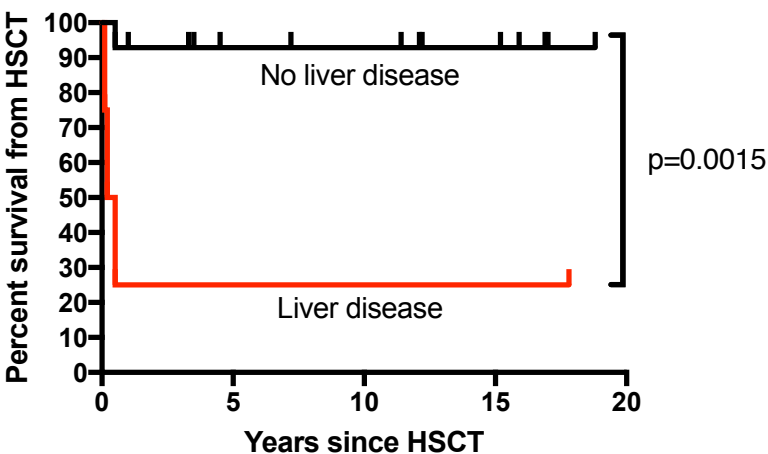
Table 1. Characteristics of XHIM patients with or without liver disease.

	No liver disease	Liver disease	P-value
Number of patients	16 (67%)	8 (33%)	
ALT (7-40 U/L)	39	187	0.0003
GGT (U/L)	15	1238	0.003
ALP (30-130 U/L)	212	460	0.001
Bilirubin (0-20 mg/L)	6	75	0.0018
PT (9.8-12.6s)	11.6	13.8	0.0329
Albumin (35-55 g/L)	41	39	ns
Imaging	Not available (n=7/16, 44%) Normal (n=9/16, 56%)	Sclerosing cholangitis/ cholangiopathy (n=6/8, 75%) Hepatomegaly (n=2/8, 25%) Periportal echogenicity (n=1/8, 13%)	
Histology	Normal (n=2, 12.5%) GVHD post HSCT (n=1, 6.25%)	Sclerosing cholangitis (n=6/8, 75%) Chronic hepatitis (n=4/8, 50%) Both (n=2/8, 25%)	
Max IgM levels (0.23-2.6 g/L)#	1.4	4.1	0.0286
Min IgG levels (2.3-16 g/L)#	4.5	3.9	ns
Min IgA levels (0.7-4 g/L)#	0.18	0.18	ns
CMV infection	3/16 (19%)	1/8 (13%)	ns
Cryptosporidium infection	2/16 (13%)	3/8 (38%)	ns
Chronic diarrhoea	8/16 (50%)	3/8 (38%)	ns
Mean age of alive patients (years)	18.4	39.1	
Mean age at death (years)	4.5	13.2	
Crude mortality ratio	1/16 (6%)	3/8 (38%)	
Number receiving bone marrow transplant	14/16 (88%)	4/8 (50%)	
# Range includes newborn to adult			

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Figure 1

A**B****C**

Online Repository

Abbreviations

ALP, alkaline phosphatase
 ALT, alanine transferase
 CBD, common bile duct
 CMV, cytomegalovirus
 CNS, central nervous system
 CT, computed tomography
 DN, de novo (diagnosis of XHIM)
 ERCP, endoscopic retrograde cholangiopancreatography
 FTT, failure to thrive
 GGT, gamma-glutamyl transpeptidase
 GI, gastrointestinal system
 GVHD, graft versus host disease
 FH, family history (of XHIM)
 HR, hazard ratio
 HSCT, hematopoietic stem cell transplantation
 LFT, liver function test
 MRCP, magnetic resonance cholangiopancreatography
 PSC, primary sclerosing cholangitis
 PT, prothrombin time
 SMR, standardized mortality ratio
 UK, United Kingdom
 US, ultrasound
 XHIM, X-linked immunodeficiency with hyper-IgM

Patients and Methods

All UK centers submitting XHIM data to the UK Primary Immunodeficiency Network were asked to complete a proforma to standardize the data collection (details in Letter to the Editor). Three centers contributed data for 24 patients in total. Criteria for submission of patients included definitive genetic or mutational analysis confirming XHIM, functional analysis showing absent CD40L protein, probable diagnosis of XHIM based on X-linked inheritance, male sex, or clinical presentation diagnosed by participating clinicians.

The proforma data included the patient's age, sex, whether XHIM was a de novo CD40L deficiency or whether there was a family history of the condition, genetic/mutation/molecular analysis, diagnosis date of CD40L deficiency, immunoglobulin maximum and minimum levels, past medical history, allergies, current medications, and values of alanine transaminase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin, albumin and prothrombin time (PT). Data was collected regarding evidence of liver disease from histology or from imaging including ultrasound (US), computerized tomography (CT), magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP). Information regarding formal hepatology review

and hematopoietic stem cell transplantation (HSCT) were recorded. Questions were also asked about presence of hepatotropic viruses including cytomegalovirus (CMV), the presence of *Cryptosporidium* and its management with additional enquiry regarding symptoms of chronic diarrhea.

Data were analyzed in Graphpad Prism using methods described in the main article. P-values less than 0.05 were considered statistically significant. Multivariate analysis was performed in ClustVis (<http://biit.cs.ut.ee/clustvis/>).

Additional results

Mutational analysis, immunological findings and clinical phenotype

All 24 patients were of male sex and about half had an X-linked pattern family history of XHIM. All had missing CD40L expression and 67% had genetics or mutational analysis performed (Table E1). There were no T cell abnormalities in our patient cohort, in keeping with diagnostic guidelines. We documented minimum and maximum immunoglobulin levels, corresponding to pre- and post- intravenous immunoglobulin therapy. Whereas there was a lack of association with IgA and IgG levels, patients with liver disease had statistically higher maximum IgM levels (Table 1). The clinical phenotype of our patients was sinopulmonary disease in 83%, GI disease in 54%, liver disease in 33%, hemaopoietic disorders in 33%, CNS disorders in 13% and skin manifestations in 8% (Table E1).

Liver biochemistry

Eight patients (33% of the XHIM cohort) demonstrated convincing biochemical liver disease with confirmatory radiological and histological evidence. One patient developed graft versus host disease following HSCT. This patient was not considered to have XHIM-related liver disease (see discussion below).

XHIM patients with liver disease had statistically significant elevations in ALP, GGT, ALT, bilirubin and PT when compared to those without liver disease. However, there was no statistical difference in albumin level (Figure E1). Hepatotropic viruses were routinely checked in clinical practice – hepatitis B and C were absent in the current cohort.

Liver imaging

Nine of the 16 patients without liver disease had abdominal imaging available, all of which were reported as normal (Table 1). All patients with liver disease showed imaging abnormalities on various modalities such as ultrasound, CT, MRCP or ERCP. The most common abnormalities were splenomegaly, a heterogeneous or cirrhotic appearing liver and biliary dilatation or sclerosing cholangitis. Typical findings for those with liver disease are listed in Table E2.

Liver histology

All patients with abnormal liver biochemistry and radiology underwent liver biopsy. Histological cholangiopathy/sclerosing cholangitis was the most common liver

abnormality, seen in 6 patients (75%), followed by chronic hepatitis in 4 patients (50%) or both features in 2 patients (25%). Granulomatous hepatitis was also observed in one patient (13%) who also had chronic hepatitis. There was not a secondary cause of granuloma formation in this patient.

All patients with histological sclerosing cholangitis had a dilated biliary tree, cholangiopathy or overt sclerosing cholangitis on their imaging. Histological chronic hepatitis could not be identified from imaging alone (Table E2).

CD40L mutation pattern did not correlate with development of liver disease.

Gastrointestinal infections

Chronic diarrhea was present in 50% of those without liver disease and 38% of those with liver disease (not statistically significant). *Cryptosporidium* infection was identified in 2/16 (13%) of patients without liver disease and 3/8 (38%) of patients with liver disease; this trend did not reach statistical significance (Table 1). Asymptomatic *Cryptosporidium* infection was present only in the liver disease group (25%). *Cryptosporidium* was diagnosed by microscopy in 5 patients with supplementary PCR testing in two patients.

CMV rates of 19% (3/16 patients) in the non-liver disease group were not significantly different from 13% (1/8 patients) in the liver disease group (Table 1). All patients were tested by PCR. Diarrhea manifested in half of affected patients.

Additional discussion

Genetic analysis and clinical phenotype

There was no correlation between mutation type (deletion, frameshift, premature stop codon) or the CD40L domain affected, and the clinical phenotype, consistent with previous findings (1-5).

We noted that maximum IgM levels are higher in the liver disease group, but the clinical significance of this finding is unclear. It has previously been reported that IgM levels may increase with age (5), and our findings may simply reflect an older cohort in the group with liver disease.

Role of liver imaging and histology

Patients with biopsy-proven liver disease had elevated liver function tests (except for albumin) and prolonged PT compared to those without liver disease (Table 1, Figure E1). All patients with liver disease had abnormal liver imaging in the form of ultrasound, MRCP or ERCP (Table 1, Table E2). Sclerosing cholangitis/cholangiopathy was the most common presentation and could be predicted from the biliary dilatation seen on various imaging modalities (US, CT, MRCP, ERCP). Chronic hepatitis was not easily predicted from imaging (Table E2).

We consider it of paramount importance that XHIM patients are screened annually for liver disease with blood tests including liver function and clotting, with a low threshold for consideration of abdominal imaging if any abnormalities are detected.

Hepatotropic viruses should be excluded in all patients with abnormal LFTs and we recommend early hepatology input to manage liver disease burden.

Detection of cryptosporidium & CMV

Previous studies have suggested a role of persistent *Cryptosporidium* infection leading to biliary disease in this group of patients (2, 5). Studies looking at CD40 agonist antibody use in CD40L deficiency have shown suppression of *Cryptosporidium* oocyst shedding into the stool with this therapy (6). In a previous case series of 79 patients, it was demonstrated that in 5 patients with sclerosing cholangitis, 4 had *Cryptosporidium* infection (7), whereas in a more recent study, *Cryptosporidium* was present in 25% XHIM patients with liver disease and 7% in the overall cohort (1). In our case series, 38% of patients with liver disease tested positive for *Cryptosporidium*, and the prevalence was three times higher than in patients without liver disease (13%) (Table 1). We could not find a statistically significant association between *Cryptosporidium* infection and development of liver disease, or indeed, sclerosing cholangitis. Our methodological preference is to test for *Cryptosporidium* using PCR as previous studies have shown microscopy to be less sensitive and less specific than PCR analysis for *Cryptosporidium* infection (8, 9). Based on our data showing that some patients had asymptomatic *Cryptosporidium* infection, we would also advocate testing for this pathogen in all immunodeficient patients as early treatment (for example with nitazoxanide) may delay/prevent development of cholangiopathy (10). As we do in clinical practice, we recommend advising XHIM patients to boil or filter tap water in order to reduce their risk of infection; there is limited data that rates of endemic *Cryptosporidium* transmission may be higher in immunocompromised individuals (11).

In addition to cryptosporidiosis, CMV has also been implicated in the pathogenesis of cholangiopathy in various immunodeficiencies including human immunodeficiency virus infection (12) and XHIM (2, 13). However, we found no significant difference in CMV prevalence between those with or without liver disease (Table 1).

Interestingly, the pathophysiology of CD40L deficiency and sclerosing cholangitis suggests a role for TNF-alpha. In mouse models, infection of CD40L deficient mice with *Cryptosporidium* leads to chronic infection and cholangitis (14, 15). Furthermore, CD40L knock out mice showed increased mRNA for *Tnfrsf1a* (TNF-alpha) and *Tnfrsf1b* (TNF-beta). In addition TNF-alpha production was seen in areas of inflammation of biliary epithelium on microscopy (15). Triple knock-out mice for CD40L/*Tnfrsf1a*/*Tnfrsf1b* did not develop sclerosing cholangitis of biliary tree or gall bladder in the context of chronic *Cryptosporidium* infection (15). Speculatively, therefore, TNF-alpha may be an important pathway in sclerosing cholangitis in XHIM patients.

Hematopoietic stem cell transplantation

Several studies have reported that curative HSCT improves outcomes and survival of patients with XHIM and that younger age was optimal for the timing of

transplantation because persistent infections and severe organ damage were frequently observed in older patients (16-18).

In our cohort, 75% of patients underwent HSCT, compared to other studies reporting rates from 6-52% (1, 4, 7, 18). Eighty-nine percent (16/18) of patients in the non-liver disease group were transplanted at an average age of 3.1 years, with a mean follow up time of 11 years. One patient developed graft versus host disease (GVHD) in their liver post-HSCT, but underwent successful liver transplantation without further abnormalities in liver function. One patient died of severe GVHD post HSCT (Table E3). No XHIM-related cholangiopathy or hepatitis has occurred in these patients despite an average of 11 years of follow up post-HSCT. By comparison, only 50% (4/8) of patients in the liver disease group underwent HSCT at an average age of 14.5 years. Higher age and liver disease at HSCT conferred a poor prognosis (Figure 1C). The cause of death in three patients of these patients was liver failure (whilst awaiting liver transplantation), multi-organ failure secondary to hemophagocytic syndrome, and severe cryptosporidiosis. HSCT was performed alongside liver transplantation in one patient, who of note, has not had liver disease recurrence despite a follow-up of over 17 years. Two patients with liver disease who have not undergone HSCT have end stage liver disease requiring liver transplantation and are currently undergoing assessment. In clinical practice, having treated XHIM and other immunodeficiency-related liver diseases, we find that those receiving liver transplantation without correction of the immunodeficiency confers an extremely poor prognosis (BG & VA, unpublished data). Indeed in this study and elsewhere, we find that patients undergoing HSCT have far worse outcomes if they have liver disease that remains uncorrected (i.e. without undergoing liver transplantation). Two previous reports support better outcomes in those undergoing both HSCT and liver transplantation. One case study indicates that HSCT shortly after liver transplantation prevented recurrence of liver disease in one patient (19). Another study suggested that two patients who received liver transplantation after HSCT did well, compared with a patient receiving a liver transplant without HSCT, who went on to die (1). We will therefore be approaching the transplant assessment of the aforementioned patients with a view to contemporaneous HCST as this likely confers a survival advantage.

There are several potential variables affecting survival in this dataset (e.g. HSCT timing, liver disease); early HSCT and no liver disease cluster closely to explain survival multivariate analysis (not shown). Although we cannot prove causality, these factors co-vary intimately, thereby hinting that early HSCT may confer a survival benefit by preventing liver disease onset.

The benefits of our study include detailed investigation of liver disease in a complex primary immunodeficiency as well as the inclusion of data from more than one center with a cross-sectional look at practice in the UK. Limitations include data gathered from a single country and less than full recruitment of all UK centers.

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Figure legends

Figure E1. Biochemical liver function tests in XHIM patients with or without liver disease. P-values <0.05 are considered significant.

Tables

Table E1. Hereditary, genetic, functional and clinical phenotypes of patients with XHIM.

ID	FH/ DN	CD40L protein absence	Mutation type	Protein domain affected by mutation	Body system affected
1	FH	Y	-		Sinopulmonary, GI
2	DN	Y	-		Skin, neutropenia
3	DN	Y	c.164 delA	ECU domain	Sinopulmonary, neutropenia, GI, CNS
4	FH	Y	c.289 G>A	ECU domain	Sinopulmonary
5	DN	Y	Gene deletion/ no amplification by PCR	n/a	Sinopulmonary, FTT
6	FH	Y	c.798 del	TNFH domain	Sinopulmonary, neutropenia, skin, FTT
7	DN	Y	-		Sinopulmonary, neutropenia
8	DN	Y	-		Neutropenia, GI
9	FH	Y	Unspecified exon 5 nucleotide deletion	TNFH domain	Sinopulmonary, GI, CNS
10	DN	Y	c.516 T>A	TNFH domain	Sinopulmonary, GI
11	FH	Y	-		Sinopulmonary, GU
12	DN	Y	c.289 G>A	ECU domain	Sinopulmonary, GI, GU
13	DN	Y	c.680 del	TNFH domain	Sinopulmonary, GI, FTT
14	DN	Y	c.540 del	TNFH domain	Sinopulmonary, GI
15	DN	Y	Novel mutation in TM domain*	TM domain	Sinopulmonary, GI, CNS
16	FH	Y	Point mutation affecting amino acid E76	ECU domain	Sinopulmonary, anaemia, GI
17	FH	Y	-		Sinopulmonary, GI, liver
18	FH	Y	-		CNS, liver
19	DN	Y	Gene deletion	n/a	Sinopulmonary, GI, liver
20	FH	Y	Unspecified exon 5 point mutation	TNFH domain	Sinopulmonary, neutropenia, skin, liver
21	FH	Y	-		Liver
22	DN	Y	c.163 T>G	ECU domain	Sinopulmonary, GI, liver
23	DN	Y	M99Cfs1 amino acid point mutation	ECU domain	Sinopulmonary, liver
24	FH	Y	Novel mutation in TM domain*	TM domain	Sinopulmonary, leukemia, GI, CNS, liver

* Unpublished mutation (courtesy of Dr E Staples, Dr DS Kumararatne)

Table E2. Imaging and histological findings in the 8 XHIM patients with liver disease.

Patient ID	Imaging	Histology
17	Sclerosing cholangitis & dilated CBD on ERCP; hepatomegaly & splenomegaly on US	Sclerosing cholangitis, periportal fibrosis
18	Increased periportal echogenicity on US	Chronic hepatitis, interface hepatitis, portal fibrosis
19	Sclerosing cholangitis on ERCP	Sclerosing cholangitis, mild chronic hepatitis,
20	Cholangiopathy & dilated ducts on US & ERCP	Sclerosing cholangitis, CBD inflammation, minimal portal tract inflammation
21	Sclerosing cholangitis & ascites on US	Sclerosing cholangitis
22	Heterogenous liver, cholangiopathy with duct dilatation, splenomegaly on US/CT/MRCP	Cholangiopathy, chronic hepatitis, ductopenia
23	Heterogenous liver, cholangiopathy with duct dilatation, cirrhosis on US/CT/MRCP	Cholangiopathy, perisinusoidal & biliary fibrosis, cirrhosis
24	Heterogenous liver, marked hepatomegaly, normal ducts on US/CT/MRCP	Chronic active hepatitis, fibrosis, granulomatous disease

Table E3. Age-adjusted mortality, crude mortality and standardized mortality ratio in XHIM patients with or without liver disease.

Age	XHIM, No liver disease			XHIM, Liver disease			UK male population
	Death	Total	Death rate	Death	Total	Death rate	Death rate
0-9	1	5	0.2	0	0	0	0.000249
10-19	0	6	0	3	4	0.75	0.000095
20-29	0	3	0	0	0	0	0.000245
30-39	0	0	0	0	1	0	0.000428
40-49	0	1	0	0	2	0	0.000930
50-59	0	1	0	0	1	0	0.002037
Crude death rate			0.0625			0.375	0.003983
SMR			15.7			94.1	
# Cause of death in the non-liver disease group: severe GVHD post-HSCT							
* Causes of death in the liver group: liver failure awaiting liver transplant, multi-organ failure secondary to hemophagocytic syndrome post-HSCT, & severe cryptosporidiosis							

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Figure E1

ACCEPTED MANUSCRIPT

