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Celiac disease autoantibodies in severe autoimmune liver disease and the effect of liver transplantation

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Abstract

Background/aims—Celiac disease (CD) is associated with primary biliary cirrhosis, primary sclerosing cholangitis and autoimmune hepatitis. We investigated the following: (i) the prevalence of tissue transglutaminase antibodies (tTGAs) and endomysial antibodies (EMAs) in end-stage autoimmune liver disease (ESALD), (ii) the correlation among auto-antibodies and the human leucocyte antigen (HLA) haplotype, and (iii) the effect of liver transplantation on antibody kinetics.

Methods—Pretransplantation sera from 488 patients (310 with ESALD, and 178 with non-autoimmune disease) were tested for tTGAs. Positive samples were also tested for EMAs, and retested 6–12 and ≥ 24 months post-transplantation. Results were correlated with the HLA type of the recipient.

Results—Serological evidence of CD was found in 3% (ESALD) vs. 0.6% (non-autoimmune) of the patients (five-fold increased risk in ESALD). The prevalence of tTGAs (14.2 vs. 5.4%, $P = 0.0001$) and EMAs (4.3 vs. 0.78%, $P = 0.01$) was significantly higher in patients with the HLA-DQ2 or HLA-DQ8 haplotypes. tTGAs and EMAs normalized in 94 and 100%, respectively, without gluten exclusion post-transplantation. Post-transplantation, of the five patients with symptoms of ‘classical’ CD, three improved. Intestinal lymphoma was diagnosed in another two cases with clinically ‘silent’ CD.

Conclusions—Patients with ESALD, especially those who are HLA-DQ2 or HLA-DQ8 positive had a high prevalence of CD-associated antibodies. Both tTGAs and EMAs decreased post-transplantation without gluten withdrawal. Immunosuppression may improve symptoms of CD, but might not prevent progression to intestinal lymphoma.

Keywords

autoimmune hepatitis; primary biliary cirrhosis; primary sclerosing cholangitis; serology

Celiac disease (CD), also known as gluten-sensitive enteropathy or celiac sprue, is a common, chronic disease of the small intestine caused by an inappropriate immune response to ingested wheat gluten proteins and related proteins in barley and rye (1).

Celiac disease is strongly associated with the human leucocyte antigen (HLA) molecules DQ2, encoded by DQA1*05/DQB1*02, and DQ8, encoded by DQA1*03/DQB1*0302 (2,3).

The clinical presentations associated with CD are numerous and include classical, atypical, silent and latent forms (4). Most patients with CD remain undiagnosed because symptom-free subjects (latent and silent forms) and those with atypical clinical presentations may remain without a diagnosis (4,5).

The immunoglobulin (Ig) A-class endomysial antibody (EMA) and IgA tissue transglutaminase antibody (tTGA) have high sensitivities (> 93% for both) and specificities (> 99 and > 95% respectively) for the diagnosis of classical CD, and can be used for screening or to determine the prevalence of CD in at-risk populations (5–9). Human tissue transglutaminase performs much better than guinea-pig tissue transglutaminase (7,8). The EMA test has greater specificity than the tTGA tests, whether human umbilicus or monkey oesophagus is used (5–8). Variable rates of false-positive results have been reported by using guinea-pig or human recombinant tissue transglutaminase in chronic liver disease of various aetiologies (10,11). However, the EMA test that is performed by an immunofluorescence technique has a specificity of almost 100% for CD (compared with small bowel biopsy) in patients with chronic liver disease, and the presence on serum of this antibody has a high positive predictive value for CD (12,13).

Liver abnormalities are frequent in patients with CD. Isolated hypertransaminasaemia with non-specific histological changes on the liver biopsy is the commonest 'hepatic' presentation of CD, occurring in 40% of adults and in 54% of children with untreated CD (14,15). Conversely, CD has been found in as many as 9% of patients with chronic unexplained hypertransaminasaemia (16,17). Moreover, the association between CD and autoimmune liver disorders such as primary biliary cirrhosis (PBC) (18–21), primary sclerosing cholangitis (PSC) (22,23) and autoimmune hepatitis (AH) (24,25) is well documented. However, data on the prevalence of CD in end-stage autoimmune liver disease (ESALD) are scarce (26).

The impact of liver transplantation on the kinetics of the tTGAs and EMAs and clinical outcome of those with untreated CD is unknown. It can be hypothesized that the correction of the complex structural/functional abnormalities (i.e. increased intestinal permeability) related to cirrhosis after liver transplantation and the use of immunosuppression may have a synergistic effect towards the induction of a decrease in tTGA and EMA levels on a normal gluten-containing diet. Immunosuppression might control the clinical manifestations of CD.

The aims of this study are (i) to determine the prevalence of tTGAs and EMAs in patients with ESALD and compare with the prevalence found in a group of patients with non-autoimmune end-stage liver disease; (ii) to evaluate the importance of the HLA-class II DQ2 or -DQ8 haplotypes on the prevalence of antibodies associated with CD and (iii) to evaluate the impact of liver transplantation/immunosuppression on the titre of the tTGAs and EMAs and clinical manifestations of those subjects with CD on a normal gluten-containing diet.

Material and methods

Patients

All the patients who underwent orthotopic liver transplantation (OLT) at the Mayo Clinic, Rochester, from March 1991 until August 2001 ($n = 310$) with the diagnosis of PBC, PSC and AH were identified. Clinical data were collected from the medical record. While one exclusion criteria was a prior diagnosis of CD, no patient was found to have a clinical diagnosis of CD before OLT. Maintenance immunosuppressive therapy was defined for purposes of this

manuscript as the immunosuppressive drugs used in the period between 6 months and 1 year after OLT.

Disease controls

The IgA-tTGA was tested in the serum taken and stored before liver transplantation in a group of adult patients with end-stage non-autoimmune liver disease not known to have had CD ($n = 178$). Those cases with positive tTGA were tested for EMA. Clinical data were retrospectively collected from the medical record.

Human leucocyte antigen typing

Human leucocyte antigen-class II typing was performed in all the subjects as part of the evaluation for OLT and obtained for review through the Transplant Center database. HLA-class II was typed by low-resolution polymerase chain reaction (PCR) using sequence-specific primers (One Lambda Inc., Canoga Park, CA, USA) method. This method is a sequence-specific primer test to identify alleles of the HLA-class II gene locus by PCR (27).

Serological testing (prospective study)

Serum Ig A-class tTGAs were determined in the serum collected and stored before liver transplantation. EMA was tested in those patients with positive IgA-tTGA. Patients with positive IgA-tTGA antibodies before liver transplantation were retested for tTGA and EMA in serum taken 6–12 and ≥ 24 months after transplantation. IgG-tTGA was tested in all patients with ESALD and the CD-associated haplotypes HLA-DQ2 or HLA-DQ8 ($n = 182$). In cases with negative IgA-tTGA but positive IgG-tTGA, total IgA levels were determined in sera (normal value = 60–400 mg/dl).

Tissue transglutaminase antibody

Tissue transglutaminase antibodies were determined by a commercial enzyme-linked immunosorbent assay (INOVA Diagnostics Inc., San Diego, CA, USA). This test uses native human tissue transglutaminase antigen isolated from red blood cells. The result was considered positive if the sample demonstrate 20–30 U/ml (weak positive) or > 30 U/ml (moderate to strong positive).

Endomysial antibody

Endomysial antibody was visualized by indirect immunofluorescence on monkey oesophagus (Bindazyme™; Birmingham, UK). The result was considered positive if intense fluorescent spaces were present between smooth muscle fibres at titres $\geq 1:5$.

Histology and celiac disease diagnosis

Intestinal tissue that had been obtained before or after liver transplantation in any of those subjects with both tTGA and EMA positivity was reviewed using light microscopy. The degree of the histological lesion was classified according to Marsh–Oberhuber scale (28). Patients found to have both positive (moderate to strong) tTGAs and positive EMAs were considered to fulfil criteria for a serologically based retrospective diagnosis of CD.

Statistical analysis

Data were summarized using means $\pm$ standard deviation for continuous variables and per cents and counts for categorical variables. The χ^2 test or Fisher's exact test were used as significance tests for comparison among those patients who were HLA-DQ2 or HLA-DQ8 positive vs. those HLA-DQ2/DQ8 negative. The McNemar's exact test was used to compare pre-OLT and

6–12 months post-OLT values of IgA-tTGA. A P value < 0.05 was considered statistically significant.

Ethical issues

This study was approved by the Institutional Review Board of the Mayo Foundation.

Results

Patients

We included 488 patients with end-stage liver disease who underwent liver transplantation, 310 [119 males, mean age (range): 53.2 (21, 71)] with autoimmune aetiology: 155 (50%) with PSC, 112 (36%) with PBC and 43 (14%) with AH; and 178 (115 males, mean age (range): 49.6 (18, 69)] with a variety of non-autoimmune aetiologies.

Two hundred and sixty-six (54%) of the patients (182 and 84 with autoimmune and non-autoimmune aetiology respectively) carried the HLA-DQ2 or HLA-DQ8 haplotypes.

Thirty-three patients (10.6%) with ESALD were positive for IgA-tTGA (26 HLA-DQ2 or HLA-DQ8 positive vs. seven HLA-DQ2/8 negative, $P = 0.0001$) and nine (3%) had a positive EMA (eight HLA-DQ2 or HLA-DQ8 positive vs. one HLA-DQ2/8 negative, $P = 0.01$) (Fig. 1 and Table 1).

Only one (0.6%) of 178 patients with end-stage non-autoimmune liver disease was positive for IgA-tTGA (104.7 U/ml). The EMA was also positive. This patient was HLA-DQ2 positive and had non-alcoholic steatohepatitis (Table 2).

Patients with ESALD were more frequently IgA-tTGA positive than those with non-autoimmune aetiology (10.6 vs. 0.6%, $P = 0.0001$), as well as tend to a higher proportion of EMA positive (3 vs. 0.6%, $P = 0.07$).

Nine patients (3%) with ESALD demonstrated both high serum levels of IgA-tTGA (mean 137.6 U/ml, range 93.5–185.3) and a positive IgA-EMA, which support a serological-based retrospective diagnosis of CD. These patients presented a higher level of IgA-tTGA than those with a positive value for IgA-tTGA, but were IgA-EMA negative (137.6 ± 23.9 vs. 34.7 ± 14.2 U/ml, $P = 0.0001$).

Two (1%) of 182 patients tested with ESALD who carried the HLA-DQ2 or HLA-DQ8 haplotypes were IgG-tTGA positive (28.3 and 76.8 U/ml respectively) but IgA-tTGA negative. Both had normal levels of total IgA in serum (132 and 216 mg/dl respectively).

Clinical characteristics of seropositive patients

Clinical manifestations before liver transplantation—Five of the 10 double-positive (tTGA and EMA) patients reported significant chronic diarrhoea (two with steatorrhoea) before transplantation. Osteomalacia with multiple vertebral fractures was present in one patient. Four patients were free of symptoms related to CD before transplantation. Five patients had ulcerative colitis (patients 3, 5, 6, 7, 9). Patient 3 also had type 1 diabetes mellitus. Four patients with CD autoimmunity did not have any associated diseases (Table 3).

Clinical manifestations after liver transplantation—After transplantation, diarrhoea disappeared in three (patients 1, 7, 10) of the cases with significant chronic diarrhoea before transplantation. Patient 1 developed unexplained chronic iron deficiency anaemia (haemoglobin levels between 9.9 and 11.6 g/dl) after transplantation. Chronic diarrhoea (and steatorrhoea) worsened in patient 4 and was unchanged in patient 3. The last two patients were

clinically tested for CD using the IgA-tTGA; in both cases, the test was negative (tTGA value 6.7 and 3.4 U/ml respectively). No intestinal biopsy was performed in patient 4. Small intestine biopsies were performed in patient 3. None of these patients were clinically diagnosed with CD or received a gluten-free diet. Two patients (cases 3 and 6) with PSC and ulcerative colitis, who underwent total proctocolectomy after liver transplantation owing to dysplasia in the colonic mucosa, developed severe diarrhoea thought to be secondary to pouchitis and cuffitis respectively. Remission of diarrhoea was induced with a combination of steroids, ciprofloxacin and 5-aminosalicylic acid compounds.

Histopathology—Intestinal tissue was available for re-review in two patients. Patient 5 had intestinal tissue before and after transplantation. The small-intestine biopsy taken 1 year before transplantation (because loss of folds on the duodenal mucosa during upper endoscopy indicated for oesophageal varices surveillance) showed total villous atrophy with increased number of intraepithelial lymphocytes (Marsh–Oberhuber type 3c). The patient underwent liver transplantation; a gluten-free diet was not started due to the lack of symptoms suggestive of CD before or after transplantation. Intestinal tissue taken 8 years after transplantation showed intestinal lymphoma and total villous atrophy (Marsh–Oberhuber type 3c) in the non-neoplastic mucosa (Fig. 2A and B). Patient 3 had several small bowel mucosal specimens taken after he underwent liver transplantation. In 1996 (1 year after OLT), a small bowel mucosal biopsy specimen showed partial villous atrophy with crypt hyperplasia with a normal number of intraepithelial lymphocytes (10 IELs/100 enterocytes) (Fig. 3). In 2000, a second small bowel mucosa biopsy showed crypt hyperplasia without atrophy (Marsh–Oberhuber type 2). In 2003, a third small bowel biopsy specimen was normal (Marsh–Oberhuber 0). Finally, in 2004, a small bowel specimen showed partial villous atrophy, and crypt hyperplasia. The number of IELs was normal. Prednisone 10 mg/day, tacrolimus 4 mg/day and azathioprine 150 mg/day were used as immunosuppressive therapy and for maintenance of ulcerative colitis remission in this patient from 1996 to 2004.

Malignant complications—Two patients developed intestinal lymphoma (8 and 9 years after the OLT respectively). The clinical manifestations were intermittent diarrhoea, abdominal pain and unexplained loss of weight. No lymphadenopathy, fever, pruritus or splenomegaly was present. Both malignancies were high-grade B-cell phenotype lymphomas (CD20⁺ with λ or κ light chain restriction respectively). One of the cases showed an aberrant co-expression of CD3. The localization of the lymphoma, in both cases, was on the proximal jejunum. Both neoplasms were negative for Epstein–Barr virus by ‘*in situ*’ hybridization. The bone marrow aspirate was negative for lymphoma in both cases. The treatment included a significant decrease in immunosuppression, followed by surgical resection and chemotherapy. One patient died 2 months after lymphoma diagnosis because intra-abdominal sepsis secondary to leak from the duodenum (with the presence of residual lymphoma at the level of anastomosis). The other patient with lymphoma was still alive through October 2006 without evidence of lymphoma recurrence.

Allograft complications and long-term function—Ischaemic-type biliary strictures with persistent cholangitis led to retransplantation in patient 6, 3 months after the first transplant operation. OLT was complicated with bile leak that requires Roux-en-Y conversion on day 1 in patient 10. Moderate cellular rejection of the transplanted and retransplanted liver occurred in patients 3 and 6 respectively. Rejection was resolved with intravenous methylprednisolone boluses. All patients showed an excellent long-term allograft function as assessed by normal bilirubin, aminotransferases, albumin and prothrombin time (median of follow-up 10.3 years; range 7–17 years).

Non-cancer-related mortality—One patient died 5 years after the OLT as a consequence of massive cerebral haemorrhage secondary to thrombotic thrombocytopenic purpura.

Effect of liver transplantation/immunosuppression on immunoglobulin A tissue transglutaminase and endomysial antibody serology—The levels of IgA-tTG (mean $\pm$ standard deviation) in patients who had a positive IgA-tTG serology before OLT decreased significantly 6 months to 1 year after OLT, as compared with the serum levels before the OLT (62.8 ± 49.5 vs. 10.8 ± 12.7 U/ml, $P < 0.001$) in the absence of gluten exclusion. Thirty-one (94%) of 33 patients who had an abnormal value of IgA-tTG before the OLT demonstrated normalization of the IgA-tTG when retested 6 months to 1 year after the OLT ($P < 0.001$). The titre of IgA-tTG was normal after ≥ 24 months of the OLT in all tested patients ($n = 17$, including one case with positive tTG at 6–12 months after OLT). EMA was negative in all 33 patients at 6 months to 1 year after OLT (including those 9 EMA positive in the pretransplantation sample) and in those patients ($n = 17$) tested after ≥ 24 months of the OLT (including five of nine EMA positive in the pretransplantation sample) (Fig. 4).

Type and dosage of immunosuppressive therapy in those patients with pretransplantation immunoglobulin A tissue transglutaminase antibody positive—Maintenance immunosuppressive therapy was triple immunosuppression in 11 (37%), dual therapy in 11 (37%) and monotherapy in eight (24%). The maintenance immunosuppressive regimen was not available in the clinical record in four cases with positive IgA-tTG but negative IgA-EMA. Tacrolimus was used in 23 (76%), prednisone in 19 (65.5%), azathioprine in 12 (41%), cyclosporine in six (21%) and mycophenolate of mofetil in three (10.3%). The most frequent combination was prednisone and tacrolimus in 13 patients (48%) (Table 4).

Discussion

This study yielded three major findings: firstly, we demonstrated a high prevalence of tTGAs and EMAs (14.2 and 4.3% respectively) in a large cohort of HLA-DQ2- or HLA-DQ8-positive patients with ESALD. Secondly, the serum titre of tTGAs and EMAs showed a significant decrease after liver transplantation, with complete normalization in 94 and 100% of the cases, respectively, in the absence of gluten exclusion. Thirdly, serological evidence of CD was more common in patients with ESALD as compared with either patients with end-stage non-autoimmune liver disease or the prevalence found by screening in the general population in the United States (29).

This study demonstrates that patients with ESALD are a high-risk population for CD, and the importance of HLA-class II molecules for presentation of gluten peptides to gluten-reactive CD4⁺ T cells in CD pathogenesis (30,31). Thus, celiac autoimmunity is common in patients with autoimmune liver disease, especially those that carry the HLA haplotypes that are at risk for CD. Moreover, our observation strongly supports the idea of active serological screening for CD in patients with ESALD in the presence of HLA-DQ2 or HLA-DQ8 molecules. The absence of the HLA susceptibility genotypes, DQ2 and DQ8, makes CD very unlikely (32–34).

Interestingly, the titre of the tTGAs and EMAs decreased to normal in most patients despite a gluten-containing diet after 6 months of the liver transplantation, and this effect on antibody kinetics appears to be sustained long term (≥ 2 years after OLT). The drop in tTG levels might reflect (i) removal of the diseased liver that may have been the target for tTGA antibodies or non-specific humoral response in cirrhosis that normalizes after allograft tolerance (false-positive test), (ii) immunosuppressive drugs used to prevent allograft rejection may have actually suppressed tTGA/EMA levels, especially in those cases who really have CD (higher

titre of tTGA and positive EMA). This post-transplantation suppression on tTGA and EMA is of major relevance to the clinical approach of diarrhoea occurring after liver transplantation. In post-transplant patients, serology may not be sufficient to diagnose/exclude CD. This finding also supports pretransplantation screening in those at-risk patients.

Cirrhosis has been associated with increased levels of tTGAs in the absence of CD (false-positive test); thus, tTGAs alone do not necessarily reflect CD, especially if they are a lower titre or found without the presence of EMA. Indeed, tissue transglutaminase appears to be an antigen that is exposed in end-stage liver disease (11). Conversely, other autoantibodies, such as smooth muscle and anti-actin antibodies, frequently occur in CD in the absence of liver disease; this suggests that there is a common genetic predisposition to CD and the autoimmune liver disorders (35). Thus, the IgA-EMA detected by indirect immunofluorescence may be the preferable test for screening for CD in patients with chronic liver disorders (12,13). Other proposed mechanisms to explain elevated levels of antibodies against tTG in cirrhosis are cross-reaction to antigens present in the tTGA antigen source, immune dysregulation (specially, hypergammaglobulinaemia) associated with the chronic liver disease or the development of antibodies directed against tissue transglutaminase in the diseased liver (11–13).

Immunosuppression may play a role in the suppression of tTGA and EMA levels. Tacrolimus induces the inhibition of calcineurin with the secondary block of the interleukin-2-dependent growth and differentiation of T cells (36). This effect coupled with the potent anti-inflammatory properties of corticosteroids strongly suppresses T-cell-mediated effector mechanisms and T-cell-dependent B-cell activation, including antibody production. The allograft contribution to the decrease in tTGA and EMA must be considered. The correction of the complex structural/functional abnormalities (i.e. increased intestinal permeability) related to cirrhosis after OLT may decrease the antigenic milieu and B-cell activation, leading to the marked decrease of tTGA and EMA. Allograft and immunosuppressive therapy may have a synergistic effect. Immunosuppression may also improve symptoms of CD; however, the late development of lymphoma is a concern. Indeed, clinical and histological response has been reported in refractory CD (type I) with steroids and azathioprine (37,38). The gluten-free diet, however, is effective for both improving symptomatic presentations and decreasing the risk of chronic complications (i.e. malignancies) (39). Two patients with both IgA-tTGA and IgA-EMA developed lymphoma of the jejunum. While the predominant type of lymphoma associated with CD is that of enteropathy-type T-cell lymphoma with an extremely aggressive clinical course, CD is also a risk factor for B-cell phenotype lymphoma (40,41). Thus, whether these blood tissue malignancies observed in our patients are related to late post-transplant lymphoproliferative disorder with Epstein–Barr virus (EBV) negative (42) or a complication of silent CD remains very difficult to determine; the jejunal location and associated villous atrophy (patient 5) may suggest an association with undetected CD.

The major limitation of our study is the lack of confirmation of CD by intestinal biopsy. However, the combination of a high titre of IgA-tTGA and the very high specificity of the IgA-EMA autoantibody makes a diagnosis of CD highly likely. Further support to the diagnosis of CD came from the presence of symptoms associated with CD before and after liver transplantation and compatible histological findings in a small subset of patients. Our serological screening protocol was based on antibodies of the IgA isotype; some cases of CD might be missed if we assumed that the prevalence of selective IgA deficiency has been found to be 2.6–5% in patients with CD (43,44). However, in our study, tTGA of the IgG isotype was abnormal in only 1% of those at-risk individuals with ESALD with normal IgA-tTGA. Selective IgA deficiency was excluded in both of those cases by normal total IgA serum levels; thus, we assumed that the most plausible explanation is a false positive of the IgG-tTGA test.

In summary, this study demonstrated that patients with ESALD who express the HLA-DQ2 or HLA-DQ8 molecules have a high prevalence of CD-associated antibodies. Liver transplantation and/or immunosuppressive drugs used to prevent allograft rejection produced a significant decrease in serum levels of tTGAs and EMAs, but the clinical impact on CD outcome, especially the risk of malignancy, is unclear.

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Glossary

Abbreviations

AH	autoimmune hepatitis
CD	celiac disease
EMA	endomysial antibody
ESALD	end-stage autoimmune liver disease
GFD	gluten-free diet
HLA	human leukocyte antigen
PBC	primary biliary cirrhosis
PSC	primary sclerosing cholangitis
tTG	tissue transglutaminase
tTGA	tissue transglutaminase antibody

Fig. 1. Patient enrollment and summary of the results. tTGA, tissue transglutaminase antibody; EMA, endomysial antibody.

Fig. 2. (A) Pretransplantation duodenal biopsy (patient 5) showed total villous atrophy with increased intra-epithelial lymphocytes (Marsh–Oberhuber type 3c) characteristic of celiac disease (haematoxylin–eosin, original magnification $\times 40$). (B) The post-transplantation biopsy [8 years after orthotopic liver transplantation (OLT)] showed intestinal lymphoma and total villous atrophy in the non-neoplastic mucosa (haematoxylin–eosin, original magnification $\times 40$).

Fig. 3. Partial villous atrophy with crypt hyperplasia (Marsh–Oberhuber type 3a) without intra-epithelial lymphocytosis, consistent with partially treated celiac disease, was observed 1 year after transplantation in patient 3 (haematoxylin–eosin, original magnification $\times 40$).

Fig. 4. Tissue transglutaminase and endomysial antibodies (EMAs) levels before and after orthotopic liver transplantation.

Table 1
Summary of celiac disease serology, aetiology and human leucocyte antigen status in patients with end-stage autoimmune liver disease

Antibodies	HLA-DQ2+/DQ8+			HLA-DQ2-/DQ8-			P value *		
	PBC, n=65	PSC, n=89	AH, n=28	Total, n = 182	PBC, n=47	PSC, n=66		AH, n=15	Total, n = 128
tTGA	6	6	14	26	1	5	1	7	0.0001
EMA	2	4	2	8	0	1	0	1	0.01

* P value for comparison among HLA-DQ2/DQ8-positive vs. HLA-DQ2/DQ8-negative patients.
AH, autoimmune hepatitis; EMA, endomysial antibody; HLA, human leucocyte antigens; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; tTGA, tissue transglutaminase antibody.

Table 2

Aetiology of liver disease and human leucocyte antigens status in 178 liver transplant recipients with end-stage non-autoimmune liver disease

Aetiology	n (%)	Human leucocyte antigen genotyping		
		DQ2 or DQ8 positive	DQ2 and DQ8 negative	Not available
Non-alcoholic fatty liver disease	26 (15%)	14	9	3
Laennec's cirrhosis	24 (13%)	11	12	1
α -1 antitrypsin deficiency	22 (12%)	9	12	1
Systemic amyloidosis*	20 (11%)	11	8	1
Cryptogenic cirrhosis	20 (11%)	6	12	2
Metastatic neuroendocrine tumour	15 (8%)	6	9	0
Chronic viral infection	13 (8%)	9	4	0
Primary hyperoxaluria	12 (8%)	7	5	0
Budd–Chiari syndrome	7 (4%)	5	2	0
Acute liver failure (drug related)	5 (3%)	1	3	1
Haemochromatosis	4 (2%)	1	3	0
Polycystic disease	4 (2%)	1	2	1
Other	6 (3%)	3	3	0
Total	178 (100%)	84	84	10

* All those patients had an extensive work-up to rule out an autoimmune aetiology.

Table 3
Demographical and clinical characteristics in patients with both high levels of immunoglobulin A tissue transglutaminase antibodies and immunoglobulin A endomysial antibody positive

No. of case	Gender/age	Liver disease	tTGA levels (U/ml)		EMA	Symptoms associated with celiac disease before OLT	Immunosuppressive drugs*
			Pre-OLT	Post-OLT			
1	F/57	PBC	136.3	8.3	Pos	Chronic diarrhea	Tacrolimus
2	F/50	PBC	145.6	76.6	Pos	Osteomalacia, vertebral fractures	Prednisone
3	M/47	PSC	137.2	7.7	Pos	Chronic diarrhea, steatorrhea	Mycophenolate mofetil
4	M/42	AH	121.4	6.6	Pos	Chronic diarrhea, steatorrhea, weight loss	Tacrolimus
5	M/39	PSC	137.2	6.8	Pos	None [†]	Cyclosporine
6	M/37	PSC	93.5	5.8	Pos	None	Tacrolimus
7	F/44	PSC	137.1	10.8	Pos	Chronic diarrhea, weight loss	Azathioprine
8	M/39	AH	145.2	9.3	Pos	None [†]	Prednisone
9	F/41	PSC	185.3	10.8	Pos	None	Tacrolimus
10 [‡]	M/51	NASH	104.7	—	Pos	Chronic diarrhea	Azathioprine
							Cyclosporine
							Tacrolimus

* At the time of serum post-OLT was collected.

[†] Cases with intestinal (jejunum) lymphoma at follow-up.

[‡] Case no. 10 is from the end-stage non-autoimmune liver disease cohort.

AH, autoimmune hepatitis; EMA, endomysial antibody; NASH, non-alcoholic steatohepatitis; OLT, orthotopic liver transplantation; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; tTGA, tissue transglutaminase antibodies.

Table 4

Immunosuppressive drugs and dosage used in patients with positive immunoglobulin A tissue transglutaminase antibodies

Drug	# patients	Minimum (mg/day)	Maximum (mg/day)	Mean ($\pm$ SD) (mg/day)
Prednisone	19	5	25	9.5 $\pm$ 6
Tacrolimus	23	2	14	5.8 $\pm$ 3.5
Azathioprine	12	50	200	116 $\pm$ 43
Cyclosporine	6	150	400	267 $\pm$ 87
Mycophenolate mofetil	3	500	2000	1500 $\pm$ 866

SD, standard deviation.