Abstract

Background: The liver and celiac disease (CeD) share a complex relationship. While in some patients, isolated hypertransaminasemia is the only manifestation of CeD, liver diseases (LD) may also be associated with the presence of isolated tissue transglutaminase antibodies IgA (tTG IgA) without histologic evidence of CeD.

Aims: To examine the yield of tTG IgA testing (a) in the workup for chronic liver disease (CLD) or cytolysis and (b) to identify biopsy-confirmed CeD (BxCeD) among patients with concomitant LD.

Methods: Retrospective study including two cohorts. Cohort 1 represented 444 consecutive individuals without known CeD for which liver specialists requested tTG IgA. Incidence of positive tTG and BxCeD was evaluated. Cohort 2 included 212 consecutive individuals with positive tTG IgA and subsequent duodenal biopsies. The frequency and clinical characteristics of individuals without BxCeD were examined, with and without concurrent LD.

Results: The rate of first time positive tTG IgA among the tests requested by a liver specialist (cohort 1) was 2.0% (n = 9). However, 33.0% (n = 3) of these patients did not have BxCeD. Cohort 2 included 33 individuals with coexisting LD, of which 42.4% did not have BxCeD, compared with 16.2% of the patients without LD (P < 0.001). The majority of the patients without BxCeD (65.1%) showed an increase < 3 times upper limit of normal of tTG IgA.

Conclusions: Although there is clinical value in testing for CeD in the context of LD, there could be a high rate of positive CeD serology unaccompanied by histologic signs in patients with coexisting LD.

Keywords: Celiac disease; Liver disease; Potential celiac disease; Tissue transglutaminase antibodies
our aims were to evaluate the yield of tTG IgA testing in the etiological workup for elevated transaminases or CLD and the rate of potential CeD in a population with coexisting LD.

MATERIALS AND METHODS

Study Population
We performed a retrospective study at the Centre Hospitalier de l’Université de Montréal (CHUM). A list of all tTG IgA tests performed between May 2003 and December 2016 was obtained through the Department of Biochemistry database. Two cohorts were formed based on the following criteria. First, we identified consecutive patients with tTG IgA tests performed upon request by a liver specialist between January 2013 and December 2016 (cohort #1). This cohort included individuals with an already defined LD and followed by a liver specialist, as well as patients referred for the evaluation of elevated transaminases or signs of CLD. Individuals with established CeD were excluded, as well as individuals without any hepatic conditions (tTG IgA performed on hospitalized patients without LD or included as healthy controls for a research project). Cohort 2 included consecutive patients with positive tTG IgA performed at our institution between May 2003 and December 2016 that also had duodenal biopsies within 3 months following serology. This cohort included patients with and without LD, and excluded any patients with known CeD, already on a gluten-free diet or with inconclusive histologic results. Cohort 2 was further divided in two groups: biopsies with histologic signs of CeD (BxCeD) and biopsies without any signs of CeD (NoBxCeD) (see Definition section).

Study Procedures
Medical records and histology reports were retrospectively reviewed to find appropriate clinical information. We further reviewed the longitudinal history of individuals with no BxCeD to determine if they initiated a gluten-free diet (GFD) and if they developed any GI symptoms. CeD serologies and duodenal histologic results of these patients were also longitudinally collected from medical record until May 1, 2018. When appropriate and in the presence of missing data, additional questioning and testing (GI symptoms, GFD adherence, repeated tTG, other serologies such as EMA, DGP IgA/IgG, HLA typing) according to current ACG guidelines for the management of CeD (12) were proposed to the patient, either through a clinical or a telephone encounter, with the previous approbation of the patient’s physician. This study was approved by the local Ethics Committee of the CHUM.

Definitions
A new diagnosis of CeD was defined as both positive tTG IgA and compatible histology with no prior history of GFD or established diagnosis of CeD in the medical records. In addition to seropositive individuals with histologic findings compatible with Marsh 3 criteria, we chose to consider seropositive individuals with intraepithelial lymphocytosis, that is, more than 25 intraepithelial lymphocytes per 100 enterocytes in the duodenum that were subsequently put on a GFD by their gastroenterologist due to GI symptoms, anemia or osteopenia as individuals with a CeD diagnosis (BxCeD) (13–16). When we evaluated our population with no BxCeD, we used strict timeframe criteria, excluding any patient with a delay longer than three months between the serologic and histologic evaluations. Etiology and diagnosis of LD were established by our liver specialists according to recommended clinical, serological and/or histologic criteria. The presence of cirrhosis was identified according to clinical presentation, liver ultrasound result, histologic evaluation, and/or transient elastography with liver stiffness measurements according to the etiology of LD. New consults for LD evaluation at our center may be asked an initial standard workup including tTG IgA according to the referral reason prior to meeting with the liver specialist, and thus, the indication of the tTG for these patients was the referral reason (elevated LFTs or CLD evaluation). Elevated transaminases were defined as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels > 31 IU/L for women and AST > 31 IU/L ALT > 39 IU/L for men (17). Anemia was defined as a hemoglobin value < 120 g/L for women and < 140 g/L for men.

tTG Assays
Three different human recombinant tTG assays were used during the study period: Bindazyme Human Anti-Tissue Transglutaminase Enzyme Immunoassay Kit MK038 (The Binding Site, Birmingham, UK), Varelisa Phadia Diagnostic (Phadia, Uppsala, Sweden) and Bioplex 2200 Celiac IgA Kit (Bio-Rad, USA). tTG IgA titers are presented as a ratio of the tTG IgA levels compared to positive threshold of the test (upper limit of normal [ULN]) instead of the absolute value, a strategy also used in previous studies (18–21). Positive criteria for tTG titers were > 4 for Bindazyme (equivocal 4–10) (19), > 5 for Varelisa (equivocal 5–8) and > 15 for Bioplex 2200.

Statistical Analysis
Continuous variables are presented as median and interquartile range (IQR) or mean ± standard deviation (SD). Categorical variables are expressed as frequencies and proportions. Mann–Whitney U tests were performed to compare continuous data, while Chi-square tests were conducted for dichotomous data. In addition, odds ratio and 95% confidence intervals (CIs) were calculated, as well as multivariate logistic regressions when appropriate. Statistical significance was defined as a P-value < 0.05. Statistical analyses were performed using GraphPad Prism, version 7.03 (La Jolla, CA), except for odds ratio and multivariate
logistic regressions that were performed with STATA 15.1IC (College Station, TX).

RESULTS

Cohort 1: Anti-transglutaminase Antibody Tests Performed as Part of Etiological Workup for CLD

A total of 487 consecutive patients had tTG IgA tests performed at the request of a liver specialist, and 444 were included in the cohort #1 (Supplementary Figure 1). The mean age of the cohort was 52.5 (SD 14.5) years, and 53.6% of the patients were female. The main reasons for performing a tTG IgA test were etiological workup for CLD and elevated transaminases (36 and 28% of patients, respectively), while the indication of the test for the remaining of the cohort was related to CeD (other autoimmune disease, GI symptoms, anemia, osteoporosis) (Figure 1). 96.4% of the tests were performed with Bioplex 2200 kit. Of the 444 patients, 9 (2.0%) had positive tTG serologies. The clinical characteristics of cohort 1 are presented in Table 1. The incidence of positive tTG IgA among patients evaluated for elevated transaminases or etiologic investigation of CLD was 1.8% (Table 2). We did not find any significant association between the characteristics presented in Table 2 and positive tTG, except when patients had a combination of anemia, GI symptoms and autoimmune disease (odds ratio [OR] 13.09 95% CI 3.00 to 57.12, \( P = 0.001 \)). Of note, none of the individuals evaluated for CLD but with normal transaminases had positive tTG IgA.

Out of the nine patients with positive tTG IgA in cohort 1, eight had duodenal biopsies performed (Table 3). Every patient had increased transaminases and seven had GI symptoms, anemia and/or coexisting autoimmune disease (Table 3). One patient is suspected of having presented with celiac hepatitis, considering AST and ALT three to five times the ULN, and all other diagnoses being ruled out; there was a resolution of the transaminitis after 15 months on a GFD. Three patients (33%) had normal duodenal biopsies (from four to seven biopsies per exam) with one patient having benefitted from repeated esophagogastroduodenoscopy and duodenal biopsies, while on a gluten-containing diet. Two of these patients had an elevation of tTG IgA < 3× ULN. HLA typing was obtained for one, showing HLA DQ2.5.

Taken collectively, the rate of positive tTG IgA tests performed at our center as part of a workup initiated by a liver specialist was 2%, mostly among individuals with elevated transaminases, anemia, GI signs and symptoms, and/or other autoimmune diseases. However, the incidence of biopsy-confirmed CeD was only 1.1%, since 33% of the positive TTG IgA were not associated with any histologic signs of CeD upon evaluation of duodenal biopsies. In two out of three patients, the increase of tTG IgA was < 3× ULN.

Cohort 2: Potential CeD Among Individuals with Coexisting Liver Disorders

Considering the possible high incidence of positive tTG IgA not associated with any histologic signs of CeD observed among individuals with coexisting LD, we examined the list of positive TTG IgA tests performed at our center since 2003 to determine if that phenomenon was reproducible in a larger cohort (cohort #2). Five hundred and forty-eight consecutive individuals with positive tTG IgA also had a medical record at CHUM. With our inclusion and exclusion criteria, we isolated 212 patients with both positive TTG IgA and duodenal biopsies performed at our center within a period of

Figure 1. Indications for performing tTG IgA assay in the assessment of liver disease. †Autoimmune diseases include primary biliary cholangitis, autoimmune hepatitis, type 1 diabetes, hypothyroidism, scleroderma, lupus, psoriasis, and Sjögren's syndrome. ‡Gastrointestinal signs and symptoms include diarrhea, bloating, dyspepsia, weight loss, and abdominal pain. §Other conditions include unexplained cholestasis, hypoalbuminemia, chronic fatigue syndrome, dermatitis herpetiformis and hepatic veno-occlusive disease.
|                         | Cohort 1 Individuals with tTG performed upon request by a liver specialist | Cohort 2 Individuals with positive tTG undergoing diagnostic evaluation for CeD |
|-------------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| n = 444                 | n = 212                                                                   |
| Female (%)              | 238 (53.6)                                                               | 135 (63.7)                                                                      |
| Age, median, (IQR)      | 54.5 (44.0–63.0)                                                         | 47.0 (35.0–59.8)                                                                |
| GI signs and symptomsa (%) | 130 (29.3)                                                              | 113 (53.3)                                                                      |
| Other autoimmune or inflammatory conditionsb (%) | 170 (38.3)                                                              | 49 (23.1)                                                                      |
| Immunosuppressive drugsc (%) | 108 (24.3)                                                             | 10 (4.7)                                                                        |
| Anemia (%)              | 134 (30.2)                                                               | 88 (41.5)                                                                       |
| Cirrhosis (%)           | 150 (33.8)                                                               | 11 (5.2)                                                                        |
| Liver disease           |                                                                          |                                                                                 |
| PBC (%)                 | 44 (9.9)                                                                 | 6 (2.8)                                                                         |
| AIH (%)                 | 29 (6.5)                                                                 | 4 (1.9)                                                                         |
| PSC (%)                 | 23 (5.2)                                                                 | 2 (0.9)                                                                         |
| Viral hepatitis (%)     | 66 (14.9)                                                                | 2 (0.9)                                                                         |
| NASHd (%)               | 114 (25.7)                                                               | 7 (3.3)                                                                         |
| Alcoholic liver disease (%) | 47 (10.6)                                                              | 7 (3.3)                                                                         |
| Othere (%)              | 70 (15.8)                                                                | 5 (2.4)                                                                         |
| No etiology/unknown     | 51 (11.5)                                                                | 0                                                                                |
| Celiac disease investigation |                                                                      |                                                                                 |
| Type of tTG assay: Bindazyme/Varelisa/Bioplex 2200 (%) | 0/3.6/96.4                                                               | 19.8/48.6/31.6                                                                  |
| Positive tTG (%)        | 9 (2.0)                                                                  | 212 (100)                                                                       |
| <3 times ULN (%)        | 4 (0.9)                                                                  | 53 (25.0)                                                                       |
| 3–10 times ULN (%)      | 3 (0.7)                                                                  | 49 (23.1)                                                                       |
| >10 times ULN (%)       | 2 (0.5)                                                                  | 110 (51.9)                                                                      |
| Antiendomysium: positive/negativef n | 0 / 1 s                                                                 | 7 / 4 s                                                                         |
| DGP IgA/IgG: positive/negativef n | 1/1 h                                                                  | 11/1 i                                                                          |
| Duodenal biopsies (%)   | 71 (16.0)                                                                | 212 (100)                                                                       |
| Biopsies compatible with CeD (%) | 5 (1.1)                                                               | 169 (79.7)                                                                      |
| Marsh 1 (%)             | 0                                                                       | 6 (2.8)                                                                         |
| Marsh 3 (%)             | 5 (1.1)                                                                  | 163 (76.9)                                                                      |
| HLA testingg n         | 1                                                                       | 19                                                                               |
| DQ2/DQ8/DQ2+DQ8/double neg | 1/0/0/0                                                                  | 15/2/1/1                                                                        |

**Notes:**

- AIH, Autoimmune hepatitis; CeD, Celiac disease; DGP, Deamidated gliadin peptides antibodies; GI, Gastrointestinal; IQR, Interquartile range; NASH, Nonalcoholic steatohepatitis; PBC, Primary biliary cholangitis; PSC, Primary sclerosing cholangitis; ULN, Upper limit of normal.
- aGI signs and symptoms include diarrhea, bloating, dyspepsia, weight loss and abdominal pain.
- bAutoimmune and inflammatory conditions include connective tissue diseases, rheumatic diseases, autoimmune liver diseases, pancreatitis, lymphoma (unrelated to coeliac disease), infections, eosinophilic GI disorders, inflammatory bowel diseases, and autoimmune endocrine conditions, such as type 1 diabetes.
- cImmunosuppressive drugs include prednisone, thiopurines, mycophenolate, cyclosporine, tacrolimus and rapamycin.
- dNASH was considered as a diagnosis if there was associated fibrosis or management by a liver specialist. Steatosis only was not considered as liver disease.
- eOther conditions include Caroli disease, possible celiac hepatitis, drug-induced hepatotoxicity, Budd-Chiari, post liver transplant for subacute liver failure, nodular regenerative hyperplasia, hemochromatosis, Fontan-associated liver disease, hepatic sarcoidosis, alpha 1-antitrypsin deficiency, portal vein thrombosis, Kabuki syndrome, hepatic nodules of unknown etiology, IgG4 cholangitis, hepatocellular carcinoma, cardiac cirrhosis, hepatic metastasis, cystic fibrosis, EBV hepatitis, congenital hepatic fibrosis and hepatitis E.
- fTesting performed at the initial investigation (or after initial biopsies results), see Supplementary Tables 1 and 2 for follow-up data on cases with noBxCeD.
- gThe case in cohort 1 and three out of four cases with negative antiendomysium antibodies in cohort 2 had noBxCeD.
- hThe case with negative DGP also had negative tTG.
- iThe case with negative DGP had noBxCeD.

Not representative of the entire cohorts, were performed in cases of no BxCeD despite positive tTG (with the exception of three cases in cohort 2, for which it was performed even with BxCeD [DQ2 n=2, DQ8 n=1]).
Table 2. Incidence of positive tTG according to the indication of the test and clinical characteristic in cohort 1

| Indication of the test | Positive tTG |
|------------------------|--------------|
| n (%)                  |              |
| Entire cohort (n = 444) | 9 (2.0)      |
| **Indication of the test** |              |
| **CeD-related indications (combined)** | 4 (2.6) |
| Autoimmune disease* (n = 60) | 3 (5.0) |
| Gastrointestinal symptoms* (n = 67) | 1 (1.5) |
| Anemia (n = 17) | 0 |
| Osteoporosis (n = 4) | 0 |
| Others (n = 7) | 0 |
| **Liver related indications (combined)** | 5 (1.8) |
| Elevated liver transaminase levels (n = 124) | 4 (3.2) |
| Chronic liver disease evaluation (n = 161) | 1 (0.6) |

**Etiology of liver disease**

| Clinical characteristics | Positive tTG |
|--------------------------|--------------|
| n (%)                    |              |
| Cryptogenic hypertransaminasemia (n = 26) | 1 (3.8) |
| NASH (n = 114) | 3 (2.6) |
| PBC (n = 44) | 0 |
| AIH (n = 29) | 1 (3.4) |
| PSC (n = 23) | 1 (4.3) |
| Alcoholic liver disease (n = 47) | 2 (4.3) |
| Viral hepatitis (n = 66) | 1 (1.5) |
| Drug-induced hepatotoxicity (n = 7) | 0 |
| Methotrexate toxicity (n = 15) | 0 |
| Others* (n = 48) | 0 |
| No etiology/unknown (n = 25) | 0 |

*Autoimmune diseases include primary biliary cholangitis, autoimmune hepatitis, type 1 diabetes, hypothyroidism, scleroderma, lupus, psoriasis and Sjögren’s syndrome.

*GI signs and symptoms include diarrhea, bloating, dyspepsia, weight loss and abdominal pain.

*Other conditions include unexplained cholestasis, hypoalbuminemia, chronic fatigue syndrome, dermatitis herpetiformis and hepatic veno-occlusive disease.

*Other conditions include Budd-Chiari, postliver transplant, nodular regenerative hyperplasia, hemochromatosis, Fontan-associated liver disease, hepatic sarcoidosis, alpha 1-antitrypsin deficiency, portal vein thrombosis, Kabuki syndrome, hepatic nodules of unknown etiology, IgG4 cholangitis, hepatocellular carcinoma, cardiac cirrhosis, hepatic metastasis, cystic fibrosis, EBV hepatitis, cholestasis, congenital hepatic fibrosis and hepatitis E.

*Without associated GI symptoms, anemia or autoimmune disease.

Follow-up data were available from ten of the fourteen patients among the group with coexisting LD who had no BxCeD in cohort 2 (Supplementary Table 1), and the mean follow-up duration was 95.1 (SD 49.7) months. Except for one patient with normalized tTG IgA on a GFD, the nine other patients were on a gluten-containing diet. Three patients with initial mildly elevated tTG titers with Bindazyme or Varelsa assays had repeated positive tTG IgA with Bioplex 2200 assays, with two of them still not presenting histologic findings of CeD on subsequent duodenal biopsies. Two deceased patients also had repeated mildly elevated titers of tTG IgA but normal duodenal histology on subsequent biopsies. Finally, four patients had repeated negative tTG IgA. (Supplementary Table 1). Thus, individuals with coexisting liver disorders tend to present with a higher rate of either false positive or transient elevation of tTG IgA, especially when the tTG titers are < 3× ULN. Considering the presence of HLA DQ alleles predisposing to CeD, subsequent TGG positivity and improvement on GFD (Supplementary Table 1), we estimated the rate of potential CeD in this cohort with coexisting LD at the time of initial assessment as 18.2% (6 out of 33 patients), with one patient showing clinical improvement with on a GFD.

Furthermore, acknowledging that 16.2% of the subgroup without coexisting LD still had no BxCeD despite positive tTG, we also examined the characteristics and evolution of these patients (Supplementary Table 2). The majority had coexisting autoimmune diseases or inflammatory conditions (55.2%). Follow-up data were available for 18 out of 29 patients. Nine patients were initially considered as CeD by the gastroenterologist and recommended GFD. Two patients later presented histologic signs of CeD on subsequent duodenal biopsies. The remaining seven patients stayed on a gluten-containing diet. Four had persistent elevated tTG IgA with the Bioplex 2200...
assay and normal duodenal histology on subsequent biopsies. One presented negative endomysium antibodies (EMA) and coexisting eosinophilic gastritis, allowing us to suspect a production of tTG IgA unrelated to CeD. Finally, one patient presented both negative tTG IgA and EMA on follow-up and another showed mildly elevated tTG IgA with Bioplex 2200 but refused to repeat the duodenal biopsies. The mean follow-up duration was 70.7 (SD 50.4) months. Thus, acknowledging the presence of HLA DQ alleles predisposing to CeD, repeated increase of tTG or improvement on GFD (Supplementary Table 2), the estimated rate of potential CeD in this cohort was 8.9% (16 out of 179 individuals) with two patients showing objective histologic progression toward CeD and four patients still not having developed histologic signs of CeD despite gluten-containing diet.

Collectively, LD, autoimmune diseases and a weak elevation of tTG IgA (<3× ULN) were associated with the absence of histologic signs of CeD. Potential CeD was more frequent in the group with LD compared to the remaining cohort.

**DISCUSSION**

In this retrospective study, we reported a high rate of positive CeD serology unaccompanied by histologic features in a population with coexisting LD. Moreover, we described the experience of a liver transplant referral center for the investigation of CeD. Among patients with CLD or cytolysis, CeD was found to be associated with autoimmune diseases, anemia and GI symptomatology. CLD in investigation without elevated transaminases was not associated with CeD.

Testing for CeD is generally suggested for patients with AILD, or CLD accompanied by symptoms or anemia, and in the presence of cryptogenic transaminitis (4–7). Our results showed positive tTG IgA in 3.8% of the individuals evaluated for cryptogenic transaminitis and 3.4% of the individuals with autoimmune hepatitis, which is in accordance with previous studies (4,7). Although the indications of the test for five patients with positive tTG were “elevated liver enzymes or chronic liver disease”, three of them also had coexisting autoimmune disease, GI symptoms or anemia. Interestingly, seroprevalence of CeD

| Etiology of liver disease | Test | tTG IgA Titors (U/mL) | Duodenal histology | Autoimmune conditions/GI symptoms/anemia | Elevated AST and/or ALT |
|--------------------------|------|-----------------------|--------------------|------------------------------------------|-------------------------|
| Primary sclerosing cholangitis | Bioplex 2200 | 37.3 | Compatible with CeD | Diarrhea | Yes |
| Cryptogenic cirrhosis | Bioplex 2200 | >250 | Normal at two separate occasions | Diarrhea, anemia | Yes |
| NASH cirrhosis | Bioplex 2200 | 20.3 | Normal | Turner syndrome, hypothyroidism, abdominal pain | Yes |
| Alcoholic liver disease | Bioplex 2200 | 42.4 | Not performed. Patient deceased | Lupus, anemia | Yes |

| Etiology of liver disease | Test | tTG IgA Titors (U/mL) | Duodenal histology | Autoimmune conditions/GI symptoms/anemia | Elevated AST and/or ALT |
|--------------------------|------|-----------------------|--------------------|------------------------------------------|-------------------------|
| Alcoholic Liver disease | Bioplex 2200 | 58.8 | Compatible with CeD | Anemia | Yes |
| Suspected Celiac hepatitis | Bioplex 2200 | >250 | Compatible with CeD | Psoriasis, weight loss anemia | Yes |
| NASH | Bioplex 2200 | 80.8 | Compatible with CeD | None | Yes |
| Mixed HCV and alcoholic liver disease | Bioplex 2200 | 86.3 | Compatible with CeD | None | Yes |
| Autoimmune hepatitis | Bioplex 2200 | 37.1 | Normal | Lupus, Sjögren hypothyroidism, abdominal pain | Yes |

ALT, Alanine aminotransferase; AST, aspartate aminotransferase CeD, Celiac disease; GI, Gastrointestinal; HCV, Hepatitis C virus; NASH, Nonalcoholic steatohepatitis.

This patient also had negative antiendomysium antibodies.

This patient was HLA DQ2.5+.
among individuals evaluated for CLD, but without coexisting autoimmune disease, GI symptoms or anemia was still 1.6%. However, diagnostic accuracy of isolated IgA tTG may be low in this population (22–24).

Accordingly, 33 to 42.4% of the patients with coexisting LD and positive tTG IgA had no histologic signs of CeD, but the majority had less than 3× ULN increase of the tTG IgA. Hyperactive and nonspecific immune response due to hypergammaglobulinemia or cross-reactivity with other antigens, increased intestinal permeability, and possibly an immune-mediated reaction toward tissue transglutaminases in the liver are possible explanations (5,11,24,25). It is also important to note that the expression and activity hepatic tissue transglutaminase increase proportionally to the degree of liver fibrosis and could be triggered by any insult that stimulates apoptosis of hepatocytes (11,26). Most of our cohort with coexisting LD and no histologic signs of CeD were initially tested with previous assays that are not of use nowadays, and the majority of these tests should have been considered as equivocal and repeated. However, the threshold chosen for the Bindazyme assay may also have been used by others (19) and we still have observed repeated increase with different assays in three of these patients with LD. Moreover, among the entire cohort tested with Bindazyme or Varelisa, 4 (3.6%) of the patients with BxCeD had equivocal tTG titers and 16 (14.3%) had < 3× ULN increase of tTG. Nonetheless, others have acknowledged the weak or transient nonspecific positivity of tTG IgA.

Table 4. Analyses for the association between clinical characteristics and normal biopsies despite positive tTG in cohort 2

| Entire cohort (n = 212) | Univariate analysis | Multivariate analysis |
|-------------------------|---------------------|----------------------|
|                         | Normal biopsies n (%) | OR 95% CI | P-value | OR 95% CI | P-value |
| GI symptoms<sup>a</sup> | 7 (6.2) | 0.96 | 0.49–1.89 | 0.91 | 0.47 | 0.12–1.81 | 0.27 |
| tTG < 3× ULN | 28 (52.8) | 6.73 | 3.17–14.28 | <0.0001 | 8.34 | 2.53–27.52 | <0.001 |
| Liver disease | 14 (42.4) | 3.81 | 1.72–8.45 | 0.001 | 5.34 | 0.87–32.87 | 0.07 |
| Coexisting autoimmune/inflammatory disorder<sup>b</sup> | 21 (42.9) | 7.22 | 3.49–14.97 | <0.0001 | 5.74 | 1.75–18.79 | 0.004 |

| Immunosuppressive drugs<sup>c</sup> | 8 (80.0) | 19.09 | 3.86–93.76 | <0.0001 | 8.41 | 0.77–91.58 | 0.08 |
| Cytolysis | 7 (14.3) | 0.55 | 0.22–1.37 | 0.2 | 0.08 | 0.01–0.62 | 0.016 |
| Anemia | 13 (14.8) | 0.42 | 0.19–0.90 | 0.026 | 0.39 | 0.11–1.43 | 0.16 |

| Cohort with liver disease (n = 33) | Univariate analysis | Multivariate analysis |
|--------------------------|---------------------|----------------------|
|                         | Normal biopsies n (%) | OR 95% CI | P-value | OR 95% CI | P-value |
| GI symptoms<sup>a</sup> | 3 (27.3) | 0.55 | 0.13–2.40 | 0.43 | 0.09 | 0.01–2.32 | 0.15 |
| tTG < 3× ULN | 10 (71.4) | 9.60 | 1.85–49.88 | 0.007 | 10.06 | 0.40–251.66 | 0.16 |
| Autoimmune liver disease<sup>d</sup> | 7 (58.3) | 2.80 | 0.65–12.09 | 0.17 | 0.66 | 0.04–10.30 | 0.77 |
| Coexisting autoimmune/inflammatory disorder<sup>b</sup> | 5 (71.4) | 7.94 | 1.60–39.42 | 0.011 | 2.58 | 0.14–46.71 | 0.52 |
| Cytolysis | 7 (63.6) | 0.23 | 0.05–1.08 | 0.06 | 0.05 | 0.01–1.02 | 0.052 |
| Cirrhosis | 5 (45.5) | 1.20 | 0.28–5.18 | 0.80 | 1.12 | 0.09–13.73 | 0.26 |
| Anemia | 5 (41.7) | 0.82 | 0.18–3.78 | 0.80 | 0.21 | 0.11–336.53 | 0.37 |

CI, Confidence interval; GI, Gastrointestinal; OR, Odds ratio.
<sup>a</sup>GI signs and symptoms include bloating, dyspepsia, abdominal pain, diarrhea weight loss and nausea.
<sup>b</sup>Autoimmune and inflammatory conditions include connective tissue diseases, rheumatic diseases, pancreatitis, lymphoma (unrelated to coeliac disease), infections, eosinophilic GI disorders, inflammatory bowel diseases and autoimmune endocrine conditions.
<sup>c</sup>Immunosuppressive drugs include prednisone, thiopurines, mycophenolate, cyclosporine and rapamycin.
<sup>d</sup>This group include primary biliary cholangitis, autoimmune hepatitis and primary sclerosing cholangitis.
titers among patients with coexisting LD. Volta and colleagues previously recommended considering CeD in patients with primary biliary cholangitis, but only in the presence of elevated of tTG IgA higher than five times the ULN (8). Rubio-Tapia and colleagues reported the disappearance of previously positive CeD serology after liver transplantation in individuals with AILD who were not following a GFD (25). Among a cohort of 738 patients with CLD, Germenis and colleagues identified 47 patients with positive tTG IgA, but histologic signs of CeD were present in only three patients with concomitant positive EMA (11). Finally, in a cohort of patients with non-alcoholic fatty liver disease (NAFLD) or cryptogenic chronic hepatitis, Iacono and colleagues demonstrated the benefit of performing tests for both tTG IgA and IgG, or EMA before duodenal biopsy, since isolated positive tTG IgA or IgG was not associated with histologic signs of CeD (24). In our study, the rate of potential CeD among individuals with LD (18.2%) was higher than the one observed in the group without coexisting LD (8.9%), the latter being similar the rate reported in an Italian cohort (27). As for this last study, we also observed mild and fluctuating tTG IgA serology among patients with potential CeD not on a GFD (27).

Transient celiac autoimmunity is not exclusive to LD patients; it has recently been reported in 5% of the subjects of a paediatric cohort carrying HLA risk alleles for CeD and followed up for up to 20 years (28). A lower but still high rate of patients without any histologic signs of CeD was observed among the subgroup without coexisting LD. However, this subgroup also included many patients with other autoimmune diseases or inflammatory conditions and a large proportion were still considered as CeD by the gastroenterologist. As for the subgroup with LD, these patients showed a mild elevation (<3 × ULN) of tTG IgA. The low yield of weakly elevated titers of tTG IgA for significant mucosal damage was previously reported (18–21). In a paediatric cohort, only 30.2% of the patients with tTG one to three times ULN and histologic assessment had biopsy-confirmed CeD (18). Also, 20% of the latter only had histologic findings compatible with CeD on bulbar biopsies (18), a possible explanation for some of our patients with normal duodenal histology, but whom eventually developed or were considered as CeD by the gastroenterologist due to other clinical features. Finally, since some patients in our cohort were receiving immunosuppressive medication, it is possible that these drugs may have attenuated the cytotoxic reaction toward enterocytes, only allowing for mild humoral immunity (29,30).

This study presents fifteen years of data from our liver transplant center evaluating the levels of tTG IgA and rate of CeD among individuals with various LD, including a large cohort of patients at the time of CeD diagnosis. We used strict criteria for the assessment of biopsy-proven CeD and potential CeD. However, due to the study’s retrospective design, it has several limitations notably the lack of standardized clinical and histologic evaluation, the variable number of duodenal biopsies performed, the presence of HLA typing or antiendomysium serology for only a minority of patients, and the three consecutive assays used by our laboratory in the study period. Since the purpose of our study was to evaluate the use of tTG in this population, we established our case identification strategy on positive tTG found within the database from the Department of Biochemistry. We acknowledge that some CeD patient may present with negative tTG or IgA deficiency, and thus, we may have missed some diagnoses. In addition, we only considered individuals with both serology and duodenal biopsies conducted at our center, excluding many patients who had their tTG IgA tests performed elsewhere. Taken collectively, we demonstrate the pertinence of measuring tTG IgA during the evaluation for LD, especially in the presence of autoimmune diseases, elevated transaminases, anemia or GI symptoms. However, it is important to note that more than one third of these patients may present with only a mild increase in tTG IgA levels and no duodenal villous atrophy or intraepithelial lymphocytosis. With the increasing incidence of NAFLD and AILD worldwide and the growing awareness of CeD, we may be soon confronted to a new facet of this fascinating disease.

Supplementary data

Supplementary data are available at Journal of the Canadian Association of Gastroenterology online.

Acknowledgments

The authors would like to thank the members of the Division of Hepatology of Centre Hospitalier de l’Université de Montréal for their collaboration with this work, as well as Mrs. Nancy Presse for statistical assistance in the initial phase of the project. This manuscript was edited by Mrs. Christina Tam. This assistance was paid by Centre de recherche du Centre de recherche du Centre Hospitalier de l’Université de Montréal. AT was supported by Phase 1 award from Fonds de Recherche Santé Québec (FRQS) Programme FRQS/MSSS de formation pour médecins résidents en médecine spécialisée visant une carrière en recherche. All authors approved the final version of the manuscript and disclose no conflict of interests. Author contributions: Planning the study: LC and AT; collecting and/or interpreting data: LC, GB, NG, P-O.H, CV, MB and AT; drafting the manuscript: LC, CV, MB and AT.

References

1. Singh P, Arora A, Strand TA, et al. Global prevalence of celiac disease: Systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2018;16(6):823–36.
2. Choung RS, Unalp-Arida A, Ruhl CE, et al. Less hidden celiac disease but increased gluten avoidance without a diagnosis in the United States: Findings from the National Health and Nutrition Examination Surveys From 2009 to 2014. Mayo Clin Proc. 2017;92(1):30–38.
3. Castillo NE, Vanga RR, Thetheria TG, et al. Prevalence of abnormal liver function tests in celiac disease and the effect of a gluten-free diet in the US population. Am J Gastroenterol. 2015;110(8):1216–22.
4. Sainsbury A, Sanders DS, Ford AC. Meta-analysis: Coeliac disease and hypertransaminasemia. Aliment Pharmacol Ther. 2011;34(1):33–40.

5. Rubio-Tapia A, Murray JA. The liver in celiac disease. Hepatology 2007;46(5):1650–8.

6. Majumdar K, Salkha P, Puri A, et al. Coeliac disease and the liver: Spectrum of liver histology, serology and treatment response at a tertiary referral centre. J Clin Pathol. 2018;71(5):412–9.

7. Emami MH, Hashemi M, Kouhestani S, et al. Should we look for celiac disease among all patients with liver function test abnormalities? Int J Prev Med. 2012;3(3):167–72.

8. Volta U, Cao G, Tovali F, et al. Gut liver axis: An immune link between celiac disease and primary biliary cirrhosis. Expert Rev Gastroenterol Hepatol. 2013;7(3):253–61.

9. Bizzaro N, Tampoia M, Villalta D, et al. Low specificity of anti-tissue transglutaminase antibodies in patients with primary biliary cirrhosis. J Clin Lab Anal. 2006;20(5):184–9.

10. Floreani A, Betterle C, Baragiotta A, et al. Prevalence of coeliac disease in primary biliary cirrhosis and of antimitochondrial antibodies in adult coeliac disease patients in Italy. Dig Liver Dis. 2002;34(4):258–61.

11. Germenis AE, Yiannaki EE, Zachou K, et al. Prevalence and clinical significance of immunoglobulin A antibodies against tissue transglutaminase in patients with diverse chronic liver diseases. Clin Diagn Lab Immunol. 2005;12(6):941–8.

12. Rubio-Tapia A, Hill ID, Kelly CP, et al.; American College of Gastroenterology. ACG clinical guidelines: Diagnosis and management of celiac disease. Am J Gastroenterol. 2013;108(5):656–76; quiz 677.

13. Ludvigsson JF, Bai JC, Biagi F, et al.; BSG Coeliac Disease Guidelines Development Group; British Society of Gastroenterology. Diagnosis and management of adult coeliac disease: Guidelines from the British Society of Gastroenterology. Gut 2014;63(8):1230–28.

14. Bieber TJ, Ciacci C. World gastroenterology organisation global guidelines: Celiac disease February 2017. J Clin Gastroenterol. 2017;51(9):755–68.

15. Corazza GR, Villanacci V. Coeliac disease. J Clin Pathol. 2005;58(6):573–4.

16. Singh P, Lauwers GY, Garber JJ. Outcomes of seropositive patients with marsh 1 histology in clinical practice. J Clin Gastroenterol. 2016;50(8):619–23.

17. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. Am J Gastroenterol. 2003;98(5):960–7.

18. Gidrewicz D, Potter K, Teivonen CL, et al. Evaluation of the ESPGHAN celiac guidelines in a North American pediatric population. Am J Gastroenterol. 2015;110(5):760–7.

19. Singh P, Kurray L, Agnihotri A, et al. Titers of anti-tissue transglutaminase antibody correlate well with severity of villous abnormalities in celiac disease. J Clin Gastroenterol. 2015;49(3):212–7.

20. Swallow K, Wild G, Sargur R, et al. Quality not quantity for transglutaminase antibody 2: The performance of an endomysial and tissue transglutaminase test in screening celiac disease remains stable over time. Clin Exp Immunol. 2013;171(1):100–6.

21. Di Tola M, Marino M, Goertze S, et al. Identification of a serum transglutaminase threshold value for the noninvasive diagnosis of symptomatic adult celiac disease patients: A retrospective study. J Gastroenterol. 2016;51(11):1031–9.

22. Bardella MT, Valenti L, Pagliari C, et al. Searching for coeliac disease in patients with non-alcoholic fatty liver disease. Dig Liver Dis. 2004;36(5):333–6.

23. Rubio-Tapia A, Murray JA. Liver involvement in celiac disease. Minerva Med. 2008;99(6):595–604.

24. Lo Iacono O, Petta S, Venezia G, et al. Anti-tissue transglutaminase antibodies in patients with abnormal liver tests: Is it always celiac disease? Am J Gastroenterol. 2005;100(11):2472–7.

25. Rubio-Tapia A, Abdulkarim AS, Wiesner RH, et al. Celiac disease autoantibodies in severe autoimmune liver disease and the effect of liver transplantation. Liver Int. 2008;28(4):467–76.

26. Ellis L, Bergamini CM, Bardella MT, et al. Transglutaminases in inflammation and fibrosis of the gastrointestinal tract and the liver. Dig Liver Dis. 2009;41(8):541–50.

27. Volta U, Cao G, Giancola F, et al. Features and progression of potential celiac disease in adults. Clin Gastroenterol Hepatol. 2016;14(5):686–93.e1.

28. Liu E, Dong F, Barón AE, et al. High incidence of celiac disease in a long-term study of adolescents with susceptibility genotypes. Gastroenterology 2017;152(6):1329–1336.e1.

29. Goeres MS, Meijer JW, Wahab PJ, et al. Azathioprine and prednisone combination therapy in refractory celiac disease. Aliment Pharmacol Ther. 2003;18(5):487–94.

30. Al-Toma A, Verbeek WH, Hadithi M, et al. Survival in refractory coeliac disease and enteropathy-associated T-cell lymphoma: Retrospective evaluation of single-centre experience. Gut 2007;56(10):1373–8.