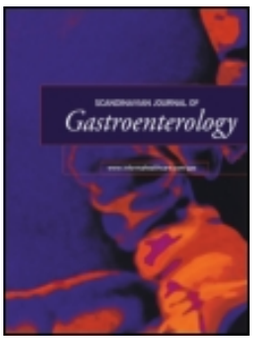

Scandinavian Journal of Gastroenterology

ISSN: 0036-5521 (Print) 1502-7708 (Online) Journal homepage: http://www.tandfonline.com/loi/igas20

Transient elastography in patients with celiac disease: A noninvasive method to detect liver involvement associated with celiac disease

Sara Massironi, Roberta Elisa Rossi, Mirella Fraquelli, Maria Teresa Bardella, Luca Elli, Marco Maggioni, Serena Della Valle, Matilde Pia Spampatti, Massimo Colombo & Dario Conte

To cite this article: Sara Massironi, Roberta Elisa Rossi, Mirella Fraquelli, Maria Teresa Bardella, Luca Elli, Marco Maggioni, Serena Della Valle, Matilde Pia Spampatti, Massimo Colombo & Dario Conte (2012) Transient elastography in patients with celiac disease: A noninvasive method to detect liver involvement associated with celiac disease, Scandinavian Journal of Gastroenterology, 47:6, 640-648, DOI: 10.3109/00365521.2012.679683

To link to this article: https://doi.org/10.3109/00365521.2012.679683

Published online: 19 Apr 2012.

Scandinavian Journal of Gastroenterology. 2012; 47: 640–648

informa
healthcare

ORIGINAL ARTICLE

Transient elastography in patients with celiac disease: A noninvasive method to detect liver involvement associated with celiac disease

SARA MASSIRONI[1], ROBERTA ELISA ROSSI[1,2], MIRELLA FRAQUELLI[1], MARIA TERESA BARDELLA[3], LUCA ELLI[3], MARCO MAGGIONI[4], SERENA DELLA VALLE[1,2], MATILDE PIA SPAMPATTI[1,2], MASSIMO COLOMBO[2,5] & DARIO CONTE[1,2]

[1]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Gastroenterology Unit 2, Milan, Italy,* [2]*Postgraduate School of Gastroenterology, Università degli Studi di Milano, Milan, Italy,* [3]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Center for Prevention and Diagnosis of Celiac Disease, Milan, Italy,* [4]*A.O. San Paolo and Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, U.O.C. Anatomia Patologica, Milan, Italy, and* [5]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and Università degli Studi di Milano, Gastroenterology Unit 1, Milan, Italy*

Abstract

Background. Liver involvement in celiac disease (CD) is clinically relevant and could require specific treatment in addition to gluten-free diet (GFD). Transient elastography (TE), a noninvasive tool for assessing liver stiffness (LS), has widely been reported as an accurate surrogate marker of liver fibrosis. ***Aims.*** To prospectively identify celiac patients with liver involvement by TE and to assess the effect of GFD. ***Material and methods.*** Ninety-five histologically confirmed CD patients (24 newly diagnosed) were consecutively evaluated by TE and compared with 146 patients with chronic hepatitis C (HCV) and 54 healthy subjects. ***Results.*** LS ranged between 2.8 and 6.7 kPa (median 4.9) in healthy subjects, defining 6.9 kPa as the upper reference limit (2 SD above the mean levels). TE was above 6.9 kPa in 10 (10.5%) CD patients. Median TE values resulted significantly higher in CD patients with hypertransaminasemia than those without [6.1 *vs.* 4.2 kPa ($p < 0.01$)]. Among the 24 newly diagnosed patients with CD, median TE values declined from 4.4 to 4 kPa, after 6 months of GFD, resulting below 6.9 kPa in 100% of the patients. ***Conclusions.*** A subset of CD patients with hypertransaminasemia showed liver involvement by TE. Accordingly, based on its accuracy in predicting liver fibrosis, TE could be used to identify those CD patients suitable for liver biopsy.

Key Words: *celiac disease, FibroScan®, liver fibrosis, liver stiffness, transient elastography*

Introduction

Celiac disease (CD) is an immune-mediated enteropathy caused by permanent immunological intolerance to ingested gluten, affecting about 1% of the population in Western countries [1], resulting in small intestine injury, malabsorption, and multiorgan involvement [2–9]. Hepatic injury is signaled by elevated serum transaminases [aspartate- and alanine-aminotransferase (AST/ALT)] and occurs in as many as 50% of untreated CD patients, the condition being reverted upon treatment with a gluten-free diet (GFD) [10–15]. Isolated hypertransaminasemia may be the only sign of early-detected CD [16–19], being the expression of either nonspecific histological changes of the liver or more severe liver disease; in fact only a minority of these patients may harbor autoimmune liver disorders, like primary biliary cirrhosis [20–23], primary sclerosing cholangitis [24], and autoimmune hepatitis [25–28], whose progression

Correspondence: Sara Massironi, MD, PhD, Gastroenterology Unit II, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Via F. Sforza 35, 20122 Milano, Italy. Tel: +39 02 55033445. Fax: +39 02 55033644. E-mail: sara.massironi@libero.it

(Received 31 January 2012; revised 19 March 2012; accepted 21 March 2012)

ISSN 0036-5521 print/ISSN 1502-7708 online
DOI: 10.3109/00365521.2012.679683

is not apparently affected by GFD [10,29]. Though mechanisms underlying liver injury in CD and related complications are still poorly understood, malabsorption and related liver steatosis [30], increased intestinal permeability [31,32], bacterial overgrowth [33], intestinal inflammation [34,35], and genetic predisposition [36–38] are likely cofactors.

The identification of CD-associated hepatic damage may have significant clinical implications, due to possible association with autoimmune liver disorders, which require specific treatment in addition to GFD. Though liver biopsy is the standard of care to diagnose and stage liver disease, its application is limited by risk of bleeding, poor patient compliance and unsatisfactory diagnostic sensitivity due to sampling errors, particularly as far as assessment of hepatic fibrosis is concerned [39–42]. To overcome these constraints, noninvasive assays have been proposed to assess liver disease severity, but only transient elastography (TE, FibroScan®, Echosens, Paris) has gained popularity, since it is a user-friendly and sensitive tool to evaluate liver stiffness (LS), which is considered a reliable surrogate of liver fibrosis [43]. TE has been extensively applied to stage liver disease severity and to stratify therapy patients with chronic hepatitis C (HCV), being identified cut-offs of LS able to discriminate patients with histologically near-normal liver from patients with severe damage, including cirrhosis [44], thus providing an accurate tool to repeatedly monitor the course and response to therapy of HCV patients [45]. With this in mind, we aimed to evaluate by TE consecutively recruited CD patients to identify CD-related liver involvement and its response to GFD, using as a negative comparator a group of healthy individuals and as a positive comparator patients with histologically proven chronic HCV, in whom distinct TE cut-offs predicting minimal changes, severe fibrosis and cirrhosis had been previously identified.

Methods

The study was carried out between June 2010 and June 2011 at the Gastroenterology Units II and I, Fondazione IRCCS Ca' Granda-Ospedale Maggiore Policlinico, Milan, Italy. Patients with CD, chronic HCV, and healthy subjects were investigated. Demographic and clinical characteristics of the study groups are detailed in Table I.

Patients with CD

Ninety-five patients (37 males and 58 females, aged 17–84 years, median 41 years) with CD were consecutively enrolled in the study. The diagnosis of CD was made according to currently accepted criteria [3] and based on both serology and histology. Anti-endomysial antibodies were sought by direct immunofluorescence on monkey esophagus (Bio-Rad, Milan, Italy); positive staining around the smooth muscle was considered positive. Anti-transglutaminase antibodies were assessed by ELISA (Eurospital, Trieste, Italy); titers above 7 arbitrary units were considered positive [46,47]. During upper gastrointestinal tract endoscopy, four biopsies were taken from the second portion of the duodenum and the

Table I. Demographic and clinical characteristics, AST/ALT levels, and liver stiffness (TE) in celiac disease (CD) in patients with chronic hepatitis C (HCV) and controls.

	CD (#95)	HCV (#146)	Controls (#54)
Age (years); median (range)	41 (17–84)	47 (21–67)	40 (21–64)
Male/female	37/58	97/49	22/34
BMI[§]: 25–30, # (%)	4 (4.5)	45 (31)	3 (5.5)
AST (IU/L); median (range)	19 (7–926)	45 (20–559)	19 (12–34)
ALT (IU/L); median (range)	19 (9–1838)	68 (19–855)	18 (8–36)
Platelets (10^9/L); median (range)	345 (130–400)	194 (97–296)	340 (125–390)
INR; median (range)	1.04 (0.9–1.15)	1.07 (0.87–1.28)	1.02 (0.95–1.10)
Bilirubin (mg/dL); median (range)	0.45 (0.12–1.10)	0.7 (0.2–2.5)	0.4 (0.15–1.13)
Albumin (g/dL), median (range)	4.3 (3.8–5.1)	4.3 (3.6–5.2)	4.5 (3.9–5.4)
METAVIR F0–1/2–3/4 (#) (%)		66/55/25 45/38/17	
Steatosis, # (%)*	36 (38)	68 (46.5)	17 (31.5)
Grade 1	29	36	7
Grade 2	5	19	6
Grade 3	2	13	4
TE (kPa); median (range)	4.3 (2.5–14.7)	7.8 (3.4–21.1)	4.9 (2.8–6.7)

[§]BMI (body mass index) in kg/m^2.

*Grade 1 = attenuation in the posterior segments of the liver; Grade 2 = a loss of echoes from the diaphragm; Grade 3 = a loss of echoes from the walls of the portal vein.

histological diagnosis of CD was done according to the Marsh classification modified by Oberhuber et al. [48,49].

Most of the patients (71, i.e. 75%) were already on GFD for a mean period of 3 years (range 1–31). Among them, 10 patients had elevated ALT/AST values (range 40–1838 U/L), including the only one patient with CD and refractory sprue. The remaining 24 patients (25%, 16 women and 8 men) were newly diagnosed, and nine of these (37.5%) presented with hypertransaminasemia (range 41–96 U/L).

Excluded were patients with liver disease of different etiologies (hepatitis B and D virus infection, hemochromatosis, Wilson's disease, drug-induced liver disease, nonalcoholic steato-hepatitis), obesity (body mass index [BMI] >30), metabolic syndrome, and human immunodeficiency virus (HIV) infection.

Patients with HCV

One hundred forty-six patients with chronic HCV (>6 months persistence of HCV RNA) were consecutively referred for a staging liver biopsy. Of these patients, 121 (83%) had histologically proven mild to severe chronic hepatitis, whereas 25 (17%) had established cirrhosis (20 in stage A and 5 in stage B based on the Child-Pugh classification criteria). Liver fibrosis was scored according to METAVIR [50].

Healthy subjects

The control group consisted of 54 healthy volunteers, 21 males and 33 females, with persistently normal transaminases and no liver disease, who were recruited from the medical staff and acquaintances of patients.

Each participant underwent a clinical evaluation, routine laboratory tests and TE measurements. All subjects gave their written informed consent to the study, which was approved by the local ethics committee.

Laboratory investigation

Laboratory tests, including serum transaminase (AST/ALT) levels, albumin, total bilirubin, alkaline phosphatase, γ-glutamyl-transpeptidase, platelet count, glucose and INR test, were measured in all patients at enrollment, by using current standard methods. Antibodies against hepatitis C virus (anti-HCV) were assayed by a second-generation enzyme-linked immunoassay (ELISA, Ortho Diagnostic Systems, Raritan, NJ). Serum HCV RNA was tested by nested reverse transcriptase polymerase chain reaction and typed by a line probe assay (InnoLipa, Innogenetics, Zwijndrecht, Belgium), with approximate sensitivity of 25 IU/mL.

Serum autoantibodies (anti-nuclear antibody, anti-smooth muscle antibody, liver/kidney microsomal antibody, anti-soluble liver antigen, and anti-mitochondrial antibody) were assayed in all patients by indirect immunofluorescence.

Liver biopsies

All patients with chronic HCV underwent an ultrasound-guided liver biopsy (16 G, BioMol, Hospital Service, Pomezia, Italy, Philips iU22, Bothell, WA, USA) to stage severity of hepatitis. All the examinations were carried out by two highly experienced hepatologists (SM and MF). While liver specimens were considered adequate if longer than 2 cm or containing at least 12 portal spaces, patients with a smaller specimen underwent repeated procedure during the same session. Five-micron thick sections of formalin-fixed, paraffin-embedded liver tissue were stained with hematoxylin–eosin and Masson trichrome, and read by a liver pathologist blind to TE and clinical data. Grading and staging were evaluated according to METAVIR [50] (staging F0 = fibrosis absent, F1 = portal fibrosis without septa, F2 = portal fibrosis with few septa, F3 = severe fibrosis, F4 = cirrhosis). Liver steatosis was quantified according to a published score system [51].

Ultrasound examination and transient elastography

All patients underwent a complete upper abdominal ultrasound examination focused on detecting possible liver impairment. The degree of steatosis on ultrasound scan was determined using the decrease in the echo amplitude (e.g. degree of posterior beam attenuation caused by the high reflectivity of the fatty tissue), which shows attenuation in the posterior segments of the liver (grade 1), a loss of echoes from the diaphragm (grade 2) or a loss of echoes from the walls of the portal vein (grade 3). TE assessment was carried out with a FibroScan® (Echosens, Paris, France), which consists of a 5 MHz ultrasound transducer probe mounted on the axis of a vibrator operated by hepatologists with extensive experience with the technique (SM, RER). TE was performed twice by the two operators, who had both previously performed at least 100 examinations and were blind to clinical data. For each examination, a median value of 10 successful examinations (expressed in kPa) was deemed as representative, with a success rate of 60% or greater and an interquartile range lower than 30% [52]. Intraobserver and

interobserver agreement were analyzed using the intraclass correlation coefficient (ICC). Patients with contraindications or limitations to TE examination, such as a BMI of 30 kg/m^2 or greater, pregnancy or cardiac failure [53,54], were excluded from the study. Patients with inadequate LS measurements were also excluded from the analysis. In patients with chronic HCV, TE was carried out at the time of the diagnostic liver biopsy.

In the 24 newly diagnosed CD patients, TE was performed at the enrollment and repeated after 6 months of GFD.

Statistical analysis

Results were expressed as median and range, unless otherwise stated. All data were tested for a normal distribution using the Kolmogoroff–Smirnoff test. Potential differences between groups were evaluated by the Mann–Whitney and the Kruskal–Wallis tests, followed by Dunn's multiple comparison test when appropriate. Correlation between variables was assessed by determination of the Spearman coefficient. Differences between percentages were evaluated by Fisher's exact test. A p-value < 0.05 was considered statistically significant.

Results

LS values obtained in healthy controls ranged between 2.8 and 6.7 kPa (median 4.9). The upper reference limit for TE, defined as 2 SD above the mean levels of healthy subjects, was 6.9 kPa. The overall interobserver agreement expressed as ICC was 0.98 (95% CI 0.96–0.98) and intraobserver agreement ICC was 0.98 (0.95–0.99).

Considering the 6.9 kPa cut-off value, 10 CD patients (10.5%) and 87 HCV patients (60%) exhibited TE values above this cut-off ($p < 0.005$, chi-square test) and, conversely, 85 CD patients (89.5%) and 59 HCV patients (40%) had TE values below this cut-off.

As summarized in Figure 1, TE values in patients with CD [median 4.3 kPa (range 2.5–14.7)] were similar to those observed in controls [median 4.9 KPa (range 2.8–6.7)] and both resulted significantly lower than those in HCV patients [median 7.8 kPa (range 3.4–21.1)] ($p < 0.001$).

Of the 95 patients with CD, 19 (20%) showed an increase in ALT and/or AST values (AST >38 U/L, ALT >40 U/L, in accordance with upper reference values). In this subgroup, TE values were above 6.9 kPa in 6 of 19 (32%). TE values in CD patients with elevated transaminases were significantly higher [median 6.1 kPa (range 3.2–14.7)] than in those with normal ALT/AST [median 4.2 kPa (range 2.5–8.8)] ($p < 0.01$), and similar to those observed in patients with chronic HCV infection [median 7.8 kPa (range 3.4–21.1) ($p = 0.06$)] (Figure 1). Among all the CD patients, ten (10.5%) showed TE above 6.9 kPa, six of whom had concomitant elevated ALT/AST levels, four had a new diagnosis of CD, and six were already on GFD (Figure 2).

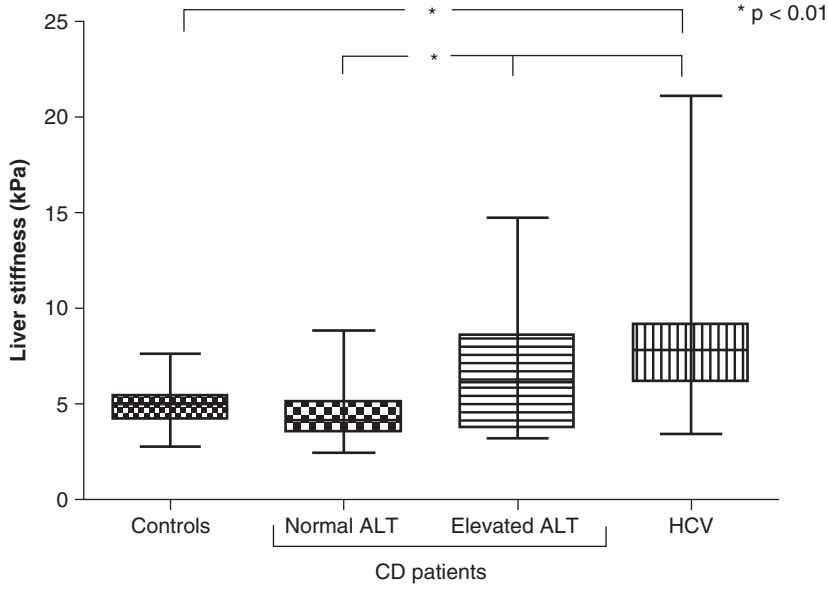

Figure 1. Liver stiffness (kPa) in celiac patients (CD, according to AST/ALT levels) versus patients with chronic hepatitis C (HCV) and controls (*$p < 0.01$).

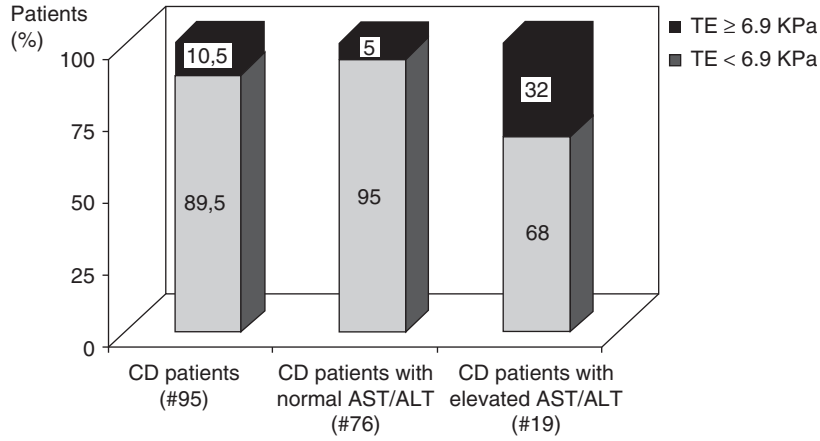

Figure 2. Proportion of CD patients with TE values <6.9 kPa (shaded areas ■) or ≥6.9 kPa (black areas ■), respectively. Data are shown for all celiacs (left column) and according to AST and ALT levels (central and right column, respectively).

A TE value higher than 12.5 kPa, which is consistent with underlying cirrhosis [52], was observed in 15 (10.3%) of 146 HCV patients and in only one CD patient with increased ALT, despite the GFD, who thereafter underwent liver biopsy, showing a picture of autoimmune hepatitis (Figure 3A and B). Among the 146 patients with chronic HCV infection, the cut-off of 12.5 kPa identified METAVIR fibrosis stage F4, with a sensitivity of 60%, yielding a correct identification of cirrhosis in 15 of 25 patients with 10 false-negative results (10 cirrhotic patients had TE values <12.5 kPa), a specificity of 99% (95% CI: 87–100%), and an overall accuracy of 93%.

As regards the 6.9 kPa cut-off value, it predicted the histological diagnosis of significant fibrosis (METAVIR score >2) in 69 of 80 HCV patients, correctly classifying 80% of HCV patients as having or not significant fibrosis, with a sensitivity of 86% and a specificity of 73%. Among HCV patients, LS measurements significantly correlated with BMI, platelet count, AST, ALT, staging, and steatosis ($p < 0.001$).

In CD patients, no direct correlation was found between LS measurements and transaminase levels, age, BMI, age at diagnosis of CD, Marsh classification modified by Oberhuber or degree of steatosis at the time of ultrasound examination ($p = 0.3$).

LS, as determined by TE, did not differ between patients on regular GFD and those still exposed to gluten, although increased ALT/AST levels were more frequently observed in the latter group (37.5% vs. 14%).

As shown in Figure 4, in the group of 24 newly diagnosed CD patients, median TE values declined from 4.4 (range 2.5–9.2) to 4 kPa (range 2.5–4.5) after 6 months of GFD ($p < 0.01$), resulting below 6.9 kPa in 100% of the patients after diet. In addition, transaminase levels normalized after GFD therapy.

Finally, hepatic steatosis was determined by ultrasound to be present in 36 CD patients (38%) and was scored as mild in 29, moderate in 5 and severe in 2 patients, without influence on LS measurements (Table I).

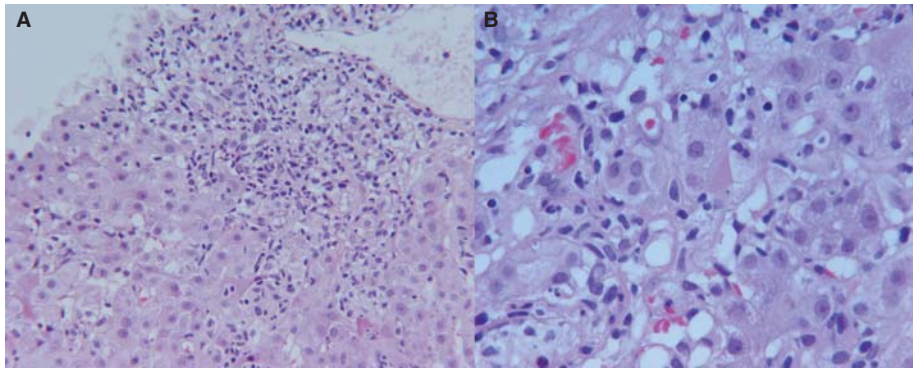

Figure 3. Liver histology of the patient with autoimmune hepatitis. A. Expanded portal tract shows diffuse lympho-plasmacellular and eosinophilic infiltration, with plurifocal interface hepatitis. (HE, 10×). B. Intralobular diffuse necro-inflammatory activity associated with some steatotic vacuoles. (HE, 20×).

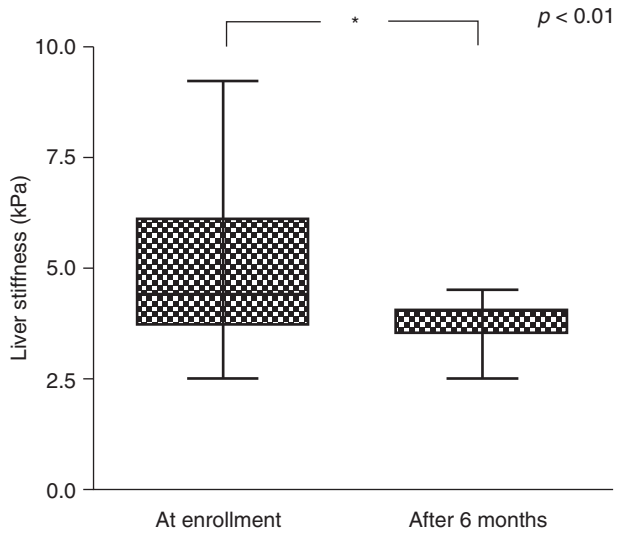

Figure 4. Liver stiffness (kPa) changes during follow-up in 24 newly diagnosed CD patients after 6 months of gluten-free diet.

Discussion

This study clearly demonstrates that TE identifies patients having CD with hepatic involvement, independently on transaminase levels. However, while the prevalence of CD patients with liver disease appears to be small (10.5%), the severity of liver involvement graded by TE appears to be greater in patients with elevated ALT compared to patients with normal ALT values.

Several reports have shown that TE accurately predicts liver fibrosis in patients with chronic liver disease of different etiologies [55–58]. Since the prognosis and management of chronic liver disease largely depends on the extent and progression of liver fibrosis, the accurate staging of hepatic fibrosis plays a pivotal role in predicting the outcome of hepatic damage [52].

TE has gained popularity as a surrogate marker of liver fibrosis, but so far, the accuracy of TE has been validated in the setting of chronic HCV only [52], where distinct TE cut-offs have shown to accurately predict significant fibrosis and cirrhosis [44].

In the specific setting of patients with CD showing hypertransaminasemia, one critical point is the recognition of those who are at high risk of developing serious liver autoimmune complications.

In the present study, 95% of healthy controls had TE values below 6.9 kPa. LS measurements greater than 6.9 kPa were detected in 10 of 95 CD patients (10.5%), independently on ALT/AST values, only one patient having TE above 12.5 kPa, i.e. suggestive of cirrhosis. According to transaminase levels, 19 of 95 patients with CD (20%) showed an increase in ALT and/or AST values that could suggest a liver impairment, whereas an actual hepatic involvement, evaluated by TE, was detected in only 10 CD patients. According to our data, significant or severe liver fibrosis is not a common finding in CD, thus confirming previous studies [54,59,60]. Moreover, from this study, the absolute values of TE observed in patients with CD were within the reference range, considering the group of healthy subjects as controls. However, TE values in celiac patients with hypertransaminasemia were significantly higher than those in patients with normal AST/ALT and as high as in patients with chronic liver disease, suggesting the presence of a possible underlying liver damage. Moreover, the persistence of high LS values after 6 months of GFD is highly suggestive of autoimmune liver disease. In fact, in the current series the only one patient showing TE above 12.5 kPa, despite GFD, had concomitant histologically proven autoimmune hepatitis, therefore suggesting the actual presence of hepatic fibrosis in a proportion of CD patients with hypertransaminasemia not reversible on GFD. In this setting, hepatic fibrosis could be triggered by the presence of transglutaminase type 2 (TG2), a family of calcium-dependent enzymes central to the pathogenesis of CD, implicated in fibrogenesis and in other processes, as wound healing, tissue repair, apoptosis, and inflammation [61]. TG2 seems to be overexpressed in tissues involved by the CD immunological response [35,61] and it could have a role in other fibrotic processes as those triggered by HCV infection and the formation of Mallory bodies in various types of hepatic damage [62,63].

However, we acknowledge that further studies in the setting of CD patients are necessary before TE could be confidently applied with the aim of avoiding liver biopsy. Furthermore, since data reported on TE accuracy and reproducibility have primarily dealt with chronic viral hepatitis [52], TE cut-off values for significant fibrosis need to be specifically determined in a CD setting.

In our series, 20% of CD patients had elevated AST/ALT, a lower percentage than that reported by previous studies, which showed hypertransaminasemia in approximately 50% of untreated celiac patients [10,12]. In this study, when focusing on the 24 newly diagnosed patients, nine of them (37.5%) had elevated aminotransferase levels and, as previously reported [10–18], hypertransaminasemia was the most frequent hepatic alteration, with a higher prevalence in the group of patients not yet receiving GFD (37.5% vs 14%). However, no significant differences in TE values were detected when newly diagnosed patients were compared with patients already receiving GFD.

We acknowledge that this study has some limitations like the inclusion of only 19 CD patients with hypertransaminasemia and the lack of systematic liver biopsy, which did not allow to assess the actual prevalence and degree of liver fibrosis in CD patients. However, the use of liver biopsy in this setting of patients still remains controversial and by many considered unethical.

As previously reported [10–15,19], we observed a normalization of transaminase levels after GFD that was accompanied by a significant decrease in LS values after 6 months of GFD, thus discouraging liver biopsy in CD patients, considering both the reversibility of hepatic abnormalities after GFD and the low risk of severe liver disease. Liver biopsy is therefore recommended in patients with persistent hypertransaminasemia [10] or, accordingly to our study, in case of persistent abnormal TE, despite GFD for at least 6 months.

Overall, clinically relevant hepatic fibrosis represents an infrequent finding in CD patients and seems to occur only in the subgroup with elevated transaminases. The identification of CD-associated hepatic damage may have significant clinical implications, not only to stage possible liver involvement in CD, but also to identify autoimmune liver disorders, which require specific treatment in addition to GFD therapy.

In conclusion, this study demonstrates that TE would be a beneficial diagnostic tool to use at enrollment and during follow-up to identify those patients who are at high risk for liver dysfunction and therefore suitable for liver biopsy, especially considering the excellent patient compliance and the safety of the technique, whereas more studies are needed to validate TE cut-offs predictive of liver involvement.

Acknowledgement

Sara Massironi and Roberta Elisa Rossi designed and wrote the paper and subsequently revised it critically, contributing equally to this work; Mirella Fraquelli and Sara Massironi contributed to acquisition of data; Serena Della Valle and Matilde Pia Spampatti performed literature research; Marco Maggioni contributed to acquisition of data and their interpretation; Maria Teresa Bardella and Luca Elli revised the paper critically for important intellectual contents; Dario Conte and Massimo Colombo corrected the final version of the manuscript. Finally, all the authors approved it.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Tack GJ, Verbeek WH, Schreurs MW, Mulder CJ. The spectrum of celiac disease: epidemiology, clinical aspects and treatment. Nat Rev Gastroenterol Hepatol 2010;7:204–13.
[2] Mirzaagha F, Azali SH, Islami F, Zamani F, Khalilipour E, Khatibian M, et al. Coeliac disease in autoimmune liver disease: a cross-sectional study and a systematic review. Dig Liver Dis 2010;42:620–3.
[3] Rubio-Tapia A, Murray JA. Celiac disease. Curr Opin Gastroenterol 2010;26:116–22.
[4] Schuppan D, Junker Y, Barisani D. Celiac disease: from pathogenesis to novel therapies. Gastroenterology 2009;137: 1912–33.
[5] Di Sabatino A, Corazza GR. Coeliac disease. Lancet 2009; 373:1480–93.
[6] Kagnoff MF. Celiac disease: pathogenesis of a model immunogenetic disease. J Clin Invest 2007;117:41–9.
[7] Rostom A, Murray JA, Kagnoff MF. American gastroenterological association (aga) institute technical review on the diagnosis and management of celiac disease. Gastroenterology 2006;131:1981–2002.
[8] Hernandez L, Green PH. Extraintestinal manifestations of celiac disease. Curr Gastroenterol Rep 2006;8:383–9.
[9] Schuppan D, Ciccocioppo R. Coeliac disease and secondary autoimmunity. Dig Liver Dis 2002;34:13–15.
[10] Rubio-Tapia A, Murray JA. The liver in celiac disease. Hepatology 2007;46:1650–8.
[11] Kinney MO. Mildly abnormal liver tests. Don't forget coeliac disease and drug history. BMJ 2010;341:c4603.
[12] Volta U. Pathogenesis and clinical significance of liver injury in celiac disease. Clinic Rev Allerg Immunol 2009;36:62–70.
[13] Duggan JM, Duggan AE. Systematic review: the liver in celiac disease. Aliment Pharmacol Ther 2005;21:515–18.
[14] Pollock DJ. The liver in celiac disease. Histopathology 1977; 1:421–30.
[15] Maggiore G, Caprai S. The liver in celiac disease. J Pediatr Gastroenterol Nutr 2003;37:117–19.
[16] Bardella MT, Vecchi M, Conte D, Del Ninno E, Fraquelli M, Pacchetti S, et al. Chronic unexplained hypertransaminasemia may be caused by occult celiac disease. Hepatology 1999;29:654–7.
[17] Volta U, De Franceschi L, Lari F, Molinaro N, Zoli M, Bianchi FB. Coeliac disease hidden by cryptogenic hypertransaminasaemia. Lancet 1998;352:26–9.
[18] Lindgren S, Sjöberg K, Eriksson S. Unsuspected coeliac disease in chronic 'cryptogenic' liver disease. Scand J Gastroenterol 1994;29:661–4.
[19] Bardella MT, Fraquelli M, Quatrini M, Molteni N, Bianchi P, Conte D. Prevalence of hypertransaminasemia in adult coeliac patients and effect of gluten-free diet. Hepatology 1995;22:833–6.
[20] Logan RF, Ferguson A, Finlayson ND, Weir DG. Primary biliary cirrhosis and celiac disease: an association? Lancet 1978;1:230–3.
[21] Sorensen HT, Thulstrup AM, Blomqvist P, Nørgaard B, Fonager K, Ekbom A. Risk of primary biliary liver cirrhosis in patients with coeliac disease: Danish and Swedish cohort data. Gut 1999;44:736–8.
[22] Feld JJ, Meddings J, Heathcote EJ. Abnormal intestinal permeability in primary biliary cirrhosis. Dig Dis Sci 2006; 51:1607–13.
[23] Watt FE, James OF, Jones DE. Patterns of autoimmunity in primary biliary cirrhosis patients and their families: a population-based cohort study. QJM 2004;97:397–406.

[24] Ludvigsson JF, Elfström P, Broomé U, Ekbom A, Montgomery SM. Celiac disease and risk of liver disease: a general population-based study. Clin Gastroenterol Hepatol 2007;5:63–9.
[25] Volta U, De Franceschi L, Molinaro N, Cassani F, Muratori L, Lenzi M, et al. Frequency and significance of anti-gliadin and anti-endomysial antibodies in autoimmune hepatitis. Dig Dis Sci 1998;43:2190–5.
[26] Teufel A, Weinmann A, Kahaly GJ, Centner C, Piendl A, Wörns M, et al. Concurrent autoimmune diseases in patients with autoimmune hepatitis. J Clin Gastroenterol 2010;44: 208–13.
[27] Kaukinen K, Halme L, Collin P, Färkkilä M, Mäki M, Vehmanen P, et al. Celiac disease in patients with severe liver disease: gluten-free diet may reverse hepatic failure. Gastroenterology 2002;122:881–8.
[28] Bardella MT, Elli L, De Matteis S, Floriani I, Torri V, Piodi L. Autoimmune disorders in patients affected by celiac sprue and inflammatory bowel disease. Ann Med 2009;41:139–43.
[29] Rubio-Tapia A, Abdulkarim AS, Wiesner RH, Moore SB, Krause PK, Murray JA. Celiac disease autoantibodies in severe autoimmune liver disease and the effect of liver transplantation. Liver Int 2008;28:467–76.
[30] Freeman HJ. Hepatobiliary and pancreatic disorders in celiac disease. World J Gastroenterol 2006;12:1503–8.
[31] Lindberg T, Berg NO, Borulf S, Jakobsson I. Liver damage in coeliac disease or other food intolerance in childhood. Lancet 1978;1:390–1.
[32] Novacek G, Miehsler W, Wrba F, Ferenci P, Penner E, Vogelsang H. Prevalence and clinical importance of hypertransaminasaemia in coeliac disease. Eur J Gastroenterol Hepatol 1999;11:283–8.
[33] Stevens FM, McLoughlin RM. Is coeliac disease a potentially treatable cause of liver failure? Eur J Gastroenterol Hepatol 2005;17:1015–17.
[34] Ventura A, Magazzù G, Greco L. Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease. SIGEP study group for autoimmune disorders in celiac disease. Gastroenterology 1999;117:297–303.
[35] Korponay-Szabó IR, Halttunen T, Szalai Z, Laurila K, Király R, Kovács JB, et al. *In vivo* targeting of intestinal and extraintestinal transglutaminase 2 by coeliac autoantibodies. Gut 2004;53:641–8.
[36] Smyth C, Kelleher D, Keeling PW. Hepatic manifestations of gastrointestinal diseases. inflammatory bowel disease, celiac disease, and Whipple's disease. Clin Liver Dis 2002;6:1013–32.
[37] Cassinotti A, Birindelli S, Clerici M, Trabattoni D, Lazzaroni M, Ardizzone S, et al. HLA and autoimmune digestive disease: a clinically oriented review for gastroenterologists. Am J Gastroenterol 2009;104:195–217.
[38] Boberg KM, Spurkland A, Rocca G, Egeland T, Saarinen S, Mitchell S, et al. The HLA-DR3,DQ2 heterozygous genotype is associated with an accelerated progression of primary sclerosing cholangitis. Scand J Gastroenterol 2001;8:886–90.
[39] Castera L, Pinzani M. Biopsy and non-invasive methods for the diagnosis of liver fibrosis: does it take two to tango? Gut 2010;59:861–6.
[40] Bedossa P, Carrat F. Liver biopsy: the best, not the gold standard. Journal of Hepatology 2009;50:1–3.
[41] Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. American association for the study of liver diseases. Liver Biopsy Hepatology 2009;49:1017–44.
[42] Regev A, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. Am J Gastroenterol 2002;97:2614–18.
[43] Ziol M, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. Hepatology 2005;41:48–54.
[44] Stebbing J, Farouk L, Panos G, Anderson M, Jiao LR, Mandalia S, et al. A meta-analysis of transient elastography for the detection of hepatic fibrosis. J Clin Gastroenterol 2010;44:214–19.
[45] Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, et al. Transient elastography: a new non-invasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 2003;29:1705–13.
[46] Lewis NR, Scott BB. Systematic review: the use of serology to exclude or diagnose coeliac disease (a comparison of the endomysial and tissue transglutaminase antibody tests). Aliment Pharmacol Ther 2006;24:47–54.
[47] Hill ID. What are the sensitivity and specificity of serologic tests for celiac disease? do sensitivity and specificity vary in different populations? Gastroenterology 2005;128(Suppl 1): S25–32.
[48] Marsh MN. Gluten, major histocompatibility complex, and the small intestine. a molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). Gastroenterology 1992;102:330–54.
[49] Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol 1999;11:1185–94.
[50] The French METAVIR Cooperative Study Group. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. Hepatology 1994; 20:15–20.
[51] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21.
[52] Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008;48: 835–47.
[53] Del Poggio P, Colombo S. Is transient elastography a useful tool for screening liver disease? World J Gastroenterol 2009; 15:1409–14.
[54] Roulot D, Czernichow S, Le Clésiau H, Costes JL, Vergnaud AC, Beaugrand M. Liver stiffness values in apparently healthy subjects: influence of gender and metabolic syndrome. J Hepatol 2008;48:606–13.
[55] Stasi C, Arena U, Vizzutti F, Zignego AL, Monti M, Laffi G, et al. Transient elastography for the assessment of liver fibrosis in patients with chronic viral hepatitis: the missing tool? Dig Liver Dis 2009;41:863–6.
[56] Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, et al. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008;134:960–74.
[57] Fraquelli M, Rigamonti C, Casazza G, Conte D, Donato MF, Ronchi G, et al. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. Gut 2007;56:968–73.
[58] Castera L. Invasive and non-invasive methods for the assessment of fibrosis and disease progression in chronic liver disease. Best Pract Res Clin Gastroenterol 2011;25:291–303.
[59] Corpechot C, El Naggar A, Poupon R. Gender and liver: is the liver stiffness weaker in weaker sex? Hepatology 2006;44: 513–14.

[60] Colombo S, Belloli L, Zaccanelli M, Badia E, Jamoletti C, Buonocore M, et al. Normal liver stiffness and its determinants in healthy blood donors. Dig Liver Dis 2011; 43:231–6.

[61] Elli L, Bergamini CM, Bardella MT, Schuppan D. Transglutaminases in inflammation and fibrosis of the gastrointestinal tract and the liver. Dig Liver Dis 2009;41:541–50.

[62] Nardacci R, Ciccosanti F, Falasca L, Lo Iacono O, Amendola A, Antonucci G, et al. Tissue transglutaminase in HCV infection. Cell Death Differ 2003;10:S79–80.

[63] Strnad P, Harada M, Siegel M, Terkeltaub RA, Graham RM, Khosla C, et al. Transglutaminase 2 regulates Mallory body inclusion formation and injury-associated liver enlargement. Gastroenterology 2007;132:1515–26.