tive, cutoff >150 $\mu$g/L) and cocaine metabolite (qualitative, >150 $\mu$g/L). The patient was confronted with the results and continued to deny abuse. She did, however, submit three subsequent urines that were negative for cocaine metabolite: on November 28, 2001; December 12, 2001; and January 8, 2002 (qualitative, <300 $\mu$g/L). She reported that her urine tested negative because she had stopped using the tea. She was referred to addiction medicine for treatment.

With the increased prevalence of alternative medicine in America, clinicians are faced with the difficulty of determining whether a particular herbal product could be responsible for test positivity or whether the patient is truly positive. Although complete interference profiles have not been adequately defined for most immunoassays, the widespread use of herbals would argue against significant cross-reactivity in routinely used immunoassays. This case also emphasizes the need for GC/MS confirmation in some clinical situations where abuse is suspected. Only through GC/MS analysis were we able to definitively establish that the patient's mugwort contained actual cocaine. Although there was no definitive proof that the patient actually contaminated the mugwort with cocaine, the sample she produced and claimed to be the source of her urine positivity was shown to contain both cocaine and cocaine metabolite. Tea brewed from mugwort obtained from an herbalist did not test positive. This case was clearly not an herbal cross-reactivity because the presence of drug was confirmed by GC/MS. Someone added the drug to the patient's mugwort; whether it was a friend, family member, or the patient herself has not been established, but it is unlikely that she purchased this product from a legal distributor with cocaine on it. Clinicians thus should not underestimate the lengths that patients will take to evade detection.

**Reference**
**1.** Hanrahan C. Mugwort. In: Krapp KM, ed. The Gale encyclopedia of alternative medicine. Farmington Hills, MI: Gale Group, 2000.

**Comparative Evaluation of Serologic Tests for Celiac Disease Diagnosis and Follow-Up,** *Silvia Martini,[1] Giulio Mengozzi,[2*] Giuseppe Aimo,[2] Laura Giorda,[2] Roberto Pagni,[2] and Carla Sategna Guidetti[1]* ([1] Dipartimento di Medicina Interna, Università di Torino, 10126 Torino, Italy; [2] UOA Laboratorio Analisi Chimico-Cliniche, Azienda Ospedaliera San Giovanni Battista, 10126 Torino, Italy; * author for correspondence)

Anti-endomysium antibody (EmA) testing is used in the diagnosis of celiac disease (CD). The identification *(1)* of tissue transglutaminase (tTG) as the main antigen of EmA led to the development of commercial ELISAs for serum anti-tTG detection. At first, a guinea pig antigen *(2–7)* yielded both sensitivity and specificity lower than those of EmA; therefore, human recombinant tTG *(8–11)* was

introduced to improve diagnostic accuracy and to overcome problems such as species specificity and cross-reactivity to contaminant proteins. However, the standardization of assays *(12)*, the choice of cutoff value, the clinical relevance of these autoantibodies *(13, 14)*, and the diagnostic accuracy of different commercial tests remain unresolved *(15–18)*. This study aimed to assess the diagnostic accuracy of five commercially available IgA anti-tTG ELISA reagent sets (four using a human recombinant and one a guinea-pig tTG antigen) for pathologically confirmed CD and to evaluate the changes in anti-tTG autoantibody concentrations during treatment of CD with a gluten-free diet (GFD).

This prospective study included 101 consecutive untreated adults (79 women and 22 men; median age, 37 years; range, 21–72 years) referred to University gastroenterologic outpatient clinic between January 2000 and May 2001 in whom CD was subsequently diagnosed by means of the typical appearance of small intestinal mucosa *(19)* (Marsh grade III in 95 patients and grade II in 6 patients) and by a positive clinical response to a GFD. We reported on 34 of these patients (all EmA-positive) previously *(17)*. A duodenal biopsy was performed in all patients on the basis of clinical history and serologic assessment, including EmA testing and nutritional indexes. In all patients, a follow-up biopsy and serologic monitoring were repeated at 1 year ± 1 month after gluten withdrawal. For a control group, we studied 190 individuals (119 women and 71 men; median age, 38 years; range, 20–77 years). These included 89 healthy controls and 101 disease controls (56 with inflammatory bowel disease and 45 patients with other diseases: 12 with malignancies, 10 with autoimmune diseases, 9 with chronic liver disease, and 14 with heart failure). CD was excluded in all on the basis of clinical history, IgA-EmA negativity, or duodenal biopsy, the last having been performed on patients undergoing routine upper diagnostic endoscopy. The study was performed according to the principles of the Helsinki Declaration, and oral informed consent was obtained from each participant.

Serum EmAs were detected by immunofluorescence, using commercial slides of monkey esophagus (The Binding Site Ltd., distributed by Alfa Biotech) *(20)*. Sera were tested, as indicated by the manufacturer, at a 1:10 initial dilution, with the inclusion of positive and negative controls in every batch of tests. CD EmA-negative sera were further tested at a 1:5 dilution, and no false-negative results were obtained.

Both qualitative and quantitative IgA anti-tTG antibody assessments were performed, as described previously *(17)*, without the knowledge of the patients' clinical diagnoses. We used four commercially available sandwich ELISAs that use human recombinant antigen (h-tTG): h-tTG 1 (DRG Diagnostics, distributed by Pantec S.r.l.; intra- and interassay CVs <10% and <15%, respectively); h-tTG 2 (EU-tTG® IgA; Eurospital S.p.A.; within- and between-assay CVs, 5.5% and 8.6%, respectively); h-tTG 3 (Immunodiagnostik, distributed by Li StarFISH s.a.s.; intra- and interassay CVs <11% and <15%, respec-

tively); and h-tTG 4 (CELIKEY™; Pharmacia & Upjohn, which uses human recombinant antigen extracted from eukaryotic cells (within- and between-run CVs, 4.5% and 8.7%, respectively). The same evaluation was also carried out with a sandwich ELISA that uses guinea pig tTG antigen (gp-tTG; GENESIS Diagnostics, distributed by Pantec S.r.l.; within- and between-assay CVs <12%).

All measurements were done in the same laboratory room and by a single operator with the inclusion in every batch of tests of the same positive and negative control sera provided by the Binding Site Ltd. for EmA appraisal. Nine different batches with two reagent lots were used for each tested assay over a 2-month period. Titers were expressed as arbitrary units, calculated according to calibration curves provided by the manufacturers of the h-tTG 1, h-tTG 4, and gp-tTG assays, and as a percentage of one reference calibrator for the h-tTG 2 and h-tTG 3 assays.

The mean (SE) and the 95% confidence interval (CI) of the mean were adopted for descriptive statistics.

The Wilcoxon–Mann–Whitney *U*-test and the Wilcoxon signed-rank analysis were applied for comparisons between groups and for comparing results before and after GFD. ROC analysis was performed both to estimate the performance of each assay and to select the cutoffs that provide the best combination of sensitivity and specificity. For all statistical analyses, a two-tailed $P < 0.05$ was considered significant.

The distributions of antibody concentrations in CD patients and controls for each transglutaminase assay are summarized in Fig. 1. The mean concentrations in healthy controls were different from those in diseased controls for h-tTG 1, h-tTG 3, and gp-tTG, but not for h-tTG 2 and h-tTG 4.

For diagnosis of CD, areas under the ROC curves (95% CIs) were 0.97 (0.95–0.99) for h-tTG 1, 0.97 (0.95–0.99) for h-tTG 2, 0.78 (0.71–0.85) for h-tTG 3, 0.96 (0.93–0.99) for h-tTG 4, and 0.91 (0.90–0.92) for gp-tTG assay.

The sensitivities and specificities of the assays are reported in Table 1. For comparison, the anti-EmA that
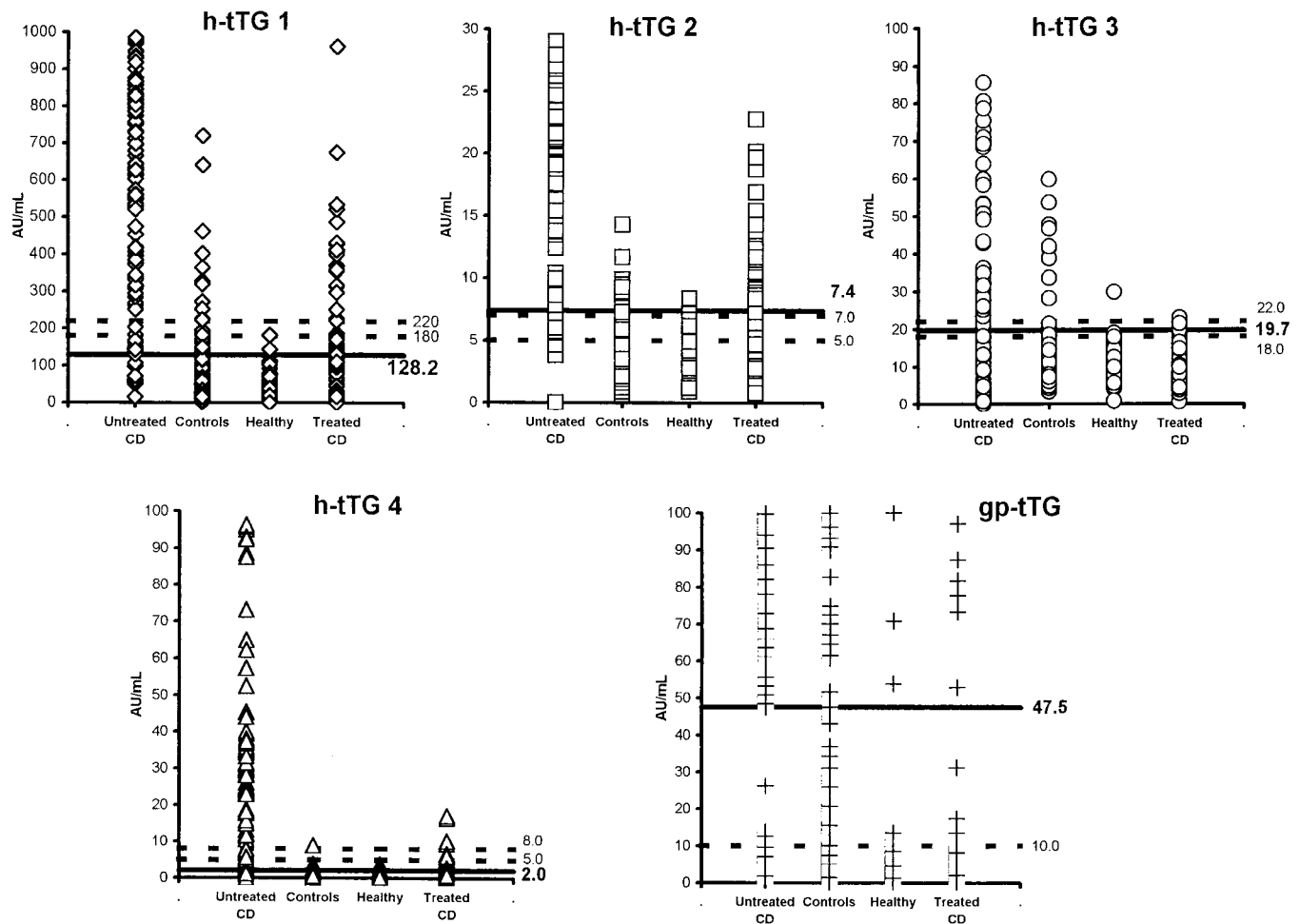


Fig. 1. Individual IgA anti-tTG concentrations detected with different ELISAs in both untreated and treated CD patients and controls.

The *dashed lines* represent limits suggested by the manufacturers, whereas the *solid lines* show the threshold values providing the best combination of sensitivity and specificity according to ROC curve analysis. For clarity reasons, values on the *y axis* were cut at 1000 and 100 arbitrary units (AU)/mL for the h-tTG 1 and gp-tTG assay, respectively.

had contributed to the selection of patients had a sensitivity of 94% (95% CI, 91–97%). Its specificity was not determined because a negative result was a selection criterion for the control group.

After a 1-year GFD, anti-tTG concentrations decreased (Fig. 1) in 96% of patients with h-tTG 1, 89% with h-tTG 2, 83% with h-tTG 3, 90% with h-tTG 4, and 96% with gp-tTG. Mean percentage changes in anti-tTG values were 81% (range, 75–86%) for h-tTG 1, 51% (range, 43–60%) for h-tTG 2, 51% (range, 42–61%) for h-tTG 3, 77% (range, 69–85%) for h-tTG 4, and 75% (range, 67–82%) for gp-tTG.

Duodenal biopsy showed mucosal recovery in 12 patients, a consistent improvement (Marsh grade I) in another 51, and nearly no change (Marsh grade II or III) in the remaining 38 patients. The concordances of the different assays in both positive (persistent histologic impairment and positive serologic markers) and negative (reconstituted mucosa and negative serologic markers) individuals vs histologic score were 29% for h-tTG 1, 65% for h-tTG 2, 14% for h-tTG 3, 16% for h-tTG 4, and 19% for gp-tTG, compared with 48% for EmA testing.

From our results, the assay based on guinea pig antigen cannot be recommended because of a large number of false negatives, which confirms previous reports (2–4, 21, 22). On the other hand, ELISAs using human recombinant antigen provided more encouraging results, notwithstanding great variability among the different assays, likely attributable to a different approach in antigen isolation, e.g., the use of eukaryotic (*Baculovirus*) vs prokaryotic organisms. Furthermore, the four ELISAs differed in technical details: the short incubation time and the limited number of washes render some tests (h-tTG 2 and h-tTG 4) faster and easier to carry out than others, although methodologic differences can be overcome by automation. These and probably other unknown determinants may be responsible for the differences in sensitivity and specificity we found at the usual cutoffs: none of the tests achieved 100% specificity, but some achieved a sensitivity comparable to that of the EmA test. The problem of negative serology in untreated CD patients is becoming increasingly recognized (23–25): EmA-negative CD is probably underestimated. Duodenal biopsy should

always be considered if the clinical picture is suggestive. The h-tTG 2 test, which in spite of its unsuitable specificity identified five of six EmA-negative CD patients, deserves attention. The finding of sera positive for EmA but negative for tTG antibody and vice versa is intriguing and might cause questions about the pathogenetic role of tTG antibody in CD, thus supporting the hypothesis suggesting the presence of autoantibodies directed against antigens other than tTG (26, 27).

EmA seroconversion, which is still used in clinical practice and is still believed to suggest reliably adherence to the diet and indirectly to predict resolution of mucosal histologic abnormalities, does not indicate complete villous recovery and cannot substitute for follow-up biopsy, whereas the persistence of serum EmA is a reliable indicator of both poor diet compliance and mucosal impairment (28–32). Until now, few studies have considered the effect of dietary gluten exclusion on tTG (15, 16, 32), but all used a guinea pig antigen and most included children, whose sensitivities for serologic testing for CD are different from adults (33). None of the above studies compared results on the same series of patients, and outcomes were related to clinical improvement and self-reported dietary compliance. To our knowledge, this is the first prospective study to evaluate objectively, by means of intestinal biopsy in a large group of newly diagnosed adult patients, the effect of a 1-year gluten withdrawal on tTG outcome. Dietary treatment led to a significant decrease in anti-tTG concentrations in the majority of patients. Compared with EmA, the ELISA test using guinea pig tTG did not provide better results; on the contrary, at least one of the reagent sets that use human recombinant antigen seemed to better reflect the mucosal pattern, in agreement with previous reports (15, 16, 31).

In conclusion, in accordance with previous reports showing the superiority of the EmA test because of its better interlaboratory reproducibility (12) and suggesting that ~60% of tTG-positive results would be false positives in a population with a medium risk of CD (14), our data indicate that, at present, ELISAs using human recombinant antigen cannot replace EmA evaluation, but could be used as a first-level investigation for noninvasive testing in the diagnostic panel for CD. To avoid unnecessary

**Table 1. Sensitivity and specificity (95% CIs) of the assays, according both to the cutoffs suggested by manufacturers (considering borderline values either as positive or as negative) and to the threshold value providing the best combination of sensitivity and specificity on the basis of ROC curve analysis.**

| | Assay | | | | |
|---|---|---|---|---|---|
| | h-tTG 1 | h-tTG 2 | h-tTG 3 | h-tTG 4 | gp-tTG |
| Sensitivity, % (n = 101) | | | | | |
|   Borderline values as positive | 83 (79–88) | 96 (94–98) | 66 (61–72) | 80 (76–85) | 79 (74–84) |
|   Borderline values as negative | 83 (79–88) | 91 (88–94) | 62 (57–68) | 72 (67–77) | 79 (74–84) |
|   Calculated cutoff (ROC curves) | 92 (89–95) | 91 (88–94) | 65 (59–70) | 91 (88–94) | 76 (71–81) |
| Specificity, % (n = 190) | | | | | |
|   Borderline values as positive | 92 (89–95) | 82 (78–86) | 91 (88–94) | 99 (99–100) | 96 (94–98) |
|   Borderline values as negative | 94 (91–97) | 93 (90–96) | 94 (91–97) | 99 (99–100) | 96 (94–98) |
|   Calculated cutoff (ROC curves) | 91 (88–94) | 94 (91–97) | 93 (90–96) | 94 (91–97) | 93 (90–96) |

biopsies, positive results, in the absence of suggestive clinical evidence, should be further confirmed by the well-consolidated EmA test, which provides 100% specificity. For follow-up, reliable serologic markers that predict mucosal outcome are not yet available, thus supporting the opinion, at least for the moment, that "It's not time to put the biopsy forceps away" *(34)*.

**References**

1. Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997;3:797–801.
2. Dieterich W, Laag E, Schopper H, Volta U, Ferguson A, Gillett H, et al. Autoantibodies to tissue transglutaminase as predictor of celiac disease. Gastroenterology 1998;115:1317–21.
3. Sulkanen S, Halttunen T, Laurila K, Kolho KL, Korponay-Szabo IR, Sarnesto A, et al. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. Gastroenterology 1998;115:1322–8.
4. Lampasona V, Bazzigaluppi E, Barera G, Bonifacio E. Tissue transglutaminase and combined screening for coeliac disease and type 1 diabetes-associated autoantibodies. Lancet 1998;352:1192–3.
5. Sugai E, Selvaggio G, Vazquez H, Viola M, Mazure R, Pizarro B, et al. Tissue transglutaminase antibodies in celiac disease: assessment of a commercial kit. Am J Gastroenterol 2000;95:2318–22.
6. Leon F, Pena R, Camarero C, Sanchez L, Eiras P, Del Amo A, et al. Limitations of anti-guinea pig liver transglutaminase IgA in screening of celiac disease. Gastroenterology 2001;120:586–7.
7. West J, Lloyd CA, Hill PG, Holmes GKT. IgA-antitissue transglutaminase: sensitivity and specificity for diagnosing coeliac disease. Gut 2001;48(Suppl I):A63.
8. Seissler J, Boms S, Wohlrab U, Morgenthaler NG, Mothes T, Boehm BO, et al. Antibodies to human recombinant tissue transglutaminase measured by radioligand assay: evidence for high diagnostic sensitivity for celiac disease. Horm Metab Res 1999;31:375–9.
9. Sardy M, Uwe O, Karpati S, Paulsson M, Smyth N. Recombinant human tissue transglutaminase ELISA for the diagnosis of gluten-sensitive enteropathy. Clin Chem 1999;45:2142–9.
10. Amin M, Eckhardt TM, Kapitza S, Fleckenstein B, Jung G, Seissler J, et al. Correlation between tissue transglutaminase antibodies and endomysium antibodies as diagnostic markers of coeliac disease. Clin Chim Acta 1999;282:219–25.
11. Sblattero D, Berti I, Trevisiol C, Marzari R, Tommasini A, Bradbury A, et al. Human recombinant tissue transglutaminase ELISA: an innovative diagnostic assay for celiac disease. Am J Gastroenterol 2000;95:1253–7.
12. Stern M for the Working Group on Serologic Screening for Celiac Disease. Comparative evaluation of serologic tests for celiac disease: a European Initiative toward standardization. J Pediatr Gastroenterol Nutr 2000;31:513–9.
13. Clemente MG, Frau F, Musu MP, De Virgiliis S. Antibodies to tissue transglutaminase outside the coeliac disease [Abstract]. Ital J Gastroenterol Hepatol 1999;31:546.
14. Biagi F, Pezzimenti D, Campanella J, Vadacca GB, Corazza GR. Endomysial and tissue transglutaminase antibodies in coeliac sera: a comparison not influenced by previous serological testing. Scand J Gastroenterol 2001;36:955–8.
15. Bardella MT, Trovato C, Cesana BM, Pagliari C, Gebbia C, Peracchi M. Serological markers for coeliac disease: is it time to change? Dig Liver Dis 2001;33:426–31.
16. Basso D, Gallo N, Guariso G, Pittoni M, Piva MG, Plebani M. Role of anti-transglutaminase (Anti-tTG), anti-gliadin, anti-endomysium serum antibodies in diagnosing celiac disease: a comparison of four different commercial kits for anti-tTG determination. J Clin Lab Anal 2001;15:112–5.
17. Martini S, Mengozzi G, Aimo G, Pagni R, Sategna Guidetti C. Diagnostic accuracies for celiac disease of four tissue transglutaminase autoantibody tests using human antigen. Clin Chem 2001;47:1722–5.
18. James MW, Scott BB. Coeliac disease: the cause of the various associated disorders? Eur J Gastroenterol Hepatol 2001;13:1119–21.
19. Marsh MN. Morphology and immunopathology of the jejunal lesion in gluten-sensitivity. Eur J Gastroenterol Hepatol 1991;3:108–14.
20. Chorzelsky TP, Sulej J, Tchorzewka M, Jablonska S, Beutner EH, Kumar V. IgA class endomysium antibodies in dermatitis herpetiformis and coeliac disease. Ann N Y Acad Sci 1983;420:325–34.
21. Biagi F, Ellis HJ, Yiamaku JY, Brusco G, Swift GL, Smith PM, et al. Tissue transglutaminase antibodies in celiac disease. Am J Gastroenterol 1999;94:2187–92.
22. Koop I, Ilchmann R, Izzi L, Adragna A, Koop H, Barthelmes H. Detection of autoantibodies against tissue transglutaminase in patients with celiac disease and dermatitis herpetiformis. Am J Gastroenterol 2000;95:2009–14.
23. Rostami K, Kerkhaert J, Tiemessen R, Mary B, von Blomberg E. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in practice. Am J Gastroenterol 1999;94:888–94.
24. Dickey W, Hughes DF, McMillan SA. Reliance on serum endomysial antibody testing underestimates the true prevalence of coeliac disease by one fifth. Scand J Gastroenterol 2001;35:181–3.
25. Dahele A, Kingstone K, Bode J, Anderson D, Ghost S. Anti-endomysial antibody negative celiac disease. Does additional serological testing help? Dig Dis Sci 2001;46:214–21.
26. Uhlig HH, Lichtenfeld J, Osman AA, Richter T, Mothes T. Evidence for existence of coeliac disease autoantigens apart from tissue transglutaminase. Eur J Gastroenterol Hepatol 2000;12:1017–20.
27. Dickey W, McMillan SA, Hughes DF. Sensitivity of serum tissue transglutaminase antibodies for endomysial antibody positive and negative coeliac disease. Scand J Gastroenterol 2001;36:511–4.
28. Valentini RA, Andreani ML, Corazza GR, Gasbarrini G. IgA endomysium antibody: a valuable tool in the screening of coeliac disease but not its follow-up. Ital J Gastroenterol 1994;26:279–82.
29. Sategna-Guidetti C, Grosso S, Bruno M, Grosso SB. Reliability of immunological markers of coeliac sprue in the assessment of mucosal recovery after gluten withdrawal. J Clin Gastroenterol 1996;23:101–4.
30. Wahab PJ, Meijer JWR, Mulder CJJ. Follow-up small intestinal histology in coeliac disease: incomplete recovery. Eur J Gastroenterol Hepatol 1999;12:A19–20.
31. Dickey W, Hughes DF, Path FRC, McMillan A. Disappearance of endomysium antibodies in treated celiac disease does not indicate histological recovery. Am J Gastroenterol 2000;95:712–4.
32. Fabiani E, Catassi C, and the International Working Group on Eu-tTG. The serum IgA class anti-tissue transglutaminase antibodies the diagnosis and follow-up of coeliac disease. Results of an international multi-centre study. Eur J Gastroenterol Hepatol 2001;13:659–65.
33. Murray JA. Serodiagnosis of celiac disease. Clin Lab Med 1997;17:445–64.
34. Murray JA. It's not time to put away the biopsy forceps. Am J Gastroenterol 1999;94:869–71.