with 50 μg/L OLZ added. As can be seen in Fig. 1D, extracts from protein precipitation and solid-phase methods showed a substantial reduction in instrument response compared to mobile phase. In contrast, the ethyl acetate liquid–liquid preparation used in our assay suppressed the signal of the analytes by <3%.

This assay, because of the high simplicity of the extraction procedure, the short duration of the analysis, the small amount of starting sample needed, and its high selectivity, is being used routinely by our research group to monitor plasma OLZ concentrations in schizophrenic patients. In fact, in a set of 17 smoking and nonsmoking patients, a wide drug concentration range was found [0.8–71 μg/L; mean (SD), 34 (4) μg/L for doses between 5 and 10 mg/24 h], consistent with the large variability of OLZ pharmacokinetics reported previously (16).

The high sensitivity shown by this assay makes it suitable for measuring the extremely low concentrations of OLZ in schizophrenic patients who are heavy smokers. It could also potentially be helpful in clarifying the clinically relevant role played by smoking, through increases in the clearance of OLZ via CYP1A2, and in demonstrating the marked variability of the antipsychotic response in schizophrenic patients (2). Likewise, the method could potentially be very useful for monitoring those patients who are given other drugs capable of inducing OLZ metabolism (e.g., anticonvulsants and barbiturates).

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Anti-Tissue Transglutaminase Antibodies in Arthritic Patients: A Disease-specific Finding? Antonio Picarelli,1 Marco Di Tola,1 Luigi Sabbatella,1 Stefania Vetrano,1 Maria Cristina Anania,1 Antonio Spadaro,2 Maria Laura Sorci,2 and Egisto Tacconi2 (1 Gastroenterological Unit, Department of Clinical Sciences, and 2 Rheumatological Unit, Department of Medical Therapy; University “La Sapienza”, 151-00161 Rome, Italy) * address correspondence to this author at: Department of Clinical Sciences, Policlinico “Umberto I”, University of Rome “La Sapienza”, Viale del Policlinico, 155-00161 Rome, Italy; e-mail a.picarelli@flashnet.it

Tissue transglutaminase (tTG), widely distributed in human organs, is a multifunctional enzyme involved in the cross-linking of extracellular matrix proteins, fibrogenesis, and wound healing (1). Recently, iTG has been proposed as the autoantigen of anti-endomysial antibodies (EMAs) (2), a serologic marker of celiac disease (CD) (3). Use of anti-iTG antibodies has been advocated in the diagnostic work-up of CD (4), although positive results have been reported in patients with other intestinal disorders, such as inflammatory bowel disease (5–7).

The aim of the present investigation was to evaluate the
occurrence of anti-tTG-positive results in a cohort of arthritic patients, in whom the target organ is located at a distance from the intestine. Changes in anti-tTG antibodies were also investigated in patients with rheumatoid arthritis (RA) during treatment with methotrexate (MTX), a drug previously shown to decrease rheumatoid factor, soluble interleukin-2 receptor, and interleukin-6 (8).

In this retrospective study, we enrolled 203 patients [121 males and 82 females; mean (SD) age, 51.4 (14.1) years; range, 17–76 years] attending our Rheumatological Unit in 1998–2000 and presenting without clinical signs and symptoms suggestive of CD. Of these patients, 183 belonged to three different groups: 74 had RA according to the American Rheumatism Association criteria (9); 67 had psoriatic arthritis (PsA) according to the European Spondyloarthropathy Study Group criteria (10); and 42 had ankylosing spondylitis (AS) according to modified New York criteria (11). The remaining 20, having knee, hand, or hip osteoarthritis (OA) according to the American College of Rheumatology criteria (12–14), were considered as disease controls. Sixty untreated CD patients [25 males and 35 females; mean (SD) age, 42.5 (11.2) years; range, 19–70 years], diagnosed according to the current criteria (15), were selected as disease controls. Fifty-four blood donors [26 males and 28 females; mean (SD) age, 40.2 (12.8) years; range, 20–67 years] were enrolled as healthy controls.

Serum was collected from all patients, and synovial fluid (SF) was obtained by knee aspiration from 68 arthritic patients (33 with RA, 26 with PsA, 9 with OA) for diagnostic proposes. All procedures followed in this study were in accordance with the ethical standards of the responsible institutional committee on human experimentation.

IgA anti-tTG antibodies were measured by an enzyme immunoassay (EIA) in which microtiter plate wells were coated with recombinant human (rh) tTG (Eurospital). According to the manufacturer’s instructions, serum was diluted 1:26, and SF was diluted 1:5. Absorbance was measured at 450 nm (A450 nm). For the intraassay variation, 10 sera were tested four times in the same run; the CV (range) was 0.9–3.6%. For the interassay variation, 10 sera were tested in three different runs, and the CV (range) was 2.2–7.7%. The mean anti-tTG value of the healthy controls + 3 SD (A450 nm = 0.2) was used as the cutoff to identify positive results. Positive anti-tTG concentrations were classified as high (A450 nm >0.57), observed only in CD patients, and low (A450 nm = 0.2–0.57), observed in both CD and arthritic patients. In 40 randomly selected arthritic patients (10 with RA, 10 with PsA, 10 with AS, and 10 with OA), anti-tTG antibodies were also measured by another EIA, in which wells were coated with native human (nh) tTG isolated from erythrocytes (INOVA Diagnostics Inc.).

IgA EMAs were identified by indirect immunofluorescence on sections of monkey esophagus (Eurospital). According to the manufacturer’s instructions, serum was diluted 1:5 and incubated for 30 min, whereas undiluted SF was incubated for 1 h. The presence of EMAs, evidenced by reticulin-like staining of smooth-muscle bundles, was evaluated by two observers (agreement rate, 98.8%) unaware of the patients’ conditions.

Forty-five of the 74 RA patients were treated with MTX, administered in three oral doses of 2.5 mg for a total of 7.5 mg/week. If after each month of treatment clinical improvement was not satisfactory, an additional dose of 2.5 mg was prescribed, up to a maximum dose of 15 mg/week (8). After 6 months of treatment, anti-tTG antibodies were again evaluated.

The statistical significance of differences was determined by the Mann–Whitney test for independent data and by the Wilcoxon test for paired data. Spearman rank correlation was used to evaluate correlations. P values ≤0.05 were considered significant. All statistical evaluations were carried out using Statgraphic (STSC Inc.) and GraphPad Prism (GraphPad Inc.) software.

We found markedly increased anti-tTG antibody concentrations in 40 of 60 CD patients (67%). Low-positive values were detectable in 16 of 60 CD (27%), 31 of 74 RA (42%), 23 of 67 PsA (34%), and 14 of 42 AS patients (33%). Low-positive values were also detectable in 2 of 20 OA patients (10%) and 1 of 54 healthy controls (2%). We found no anti-tTG antibodies in four CD patients (6%; Fig. 1). Anti-tTG antibodies were significantly higher in CD, RA, PsA, AS, and OA patients (P <0.001 for each) than in healthy controls. In CD patients, anti-tTG antibodies were also significantly higher (P <0.001 for each) than in RA, PsA, AS, and OA patients. Furthermore, in OA patients, anti-tTG antibodies were significantly lower than in RA (P = 0.009), PsA (P = 0.018), and AS patients (P = 0.044), whereas we found no significant differences among RA,
PsA, and AS patients. Serum EMAs were detectable in all 60 CD patients, whereas in contrast, we found no EMAs in any of the arthritic patients or healthy controls.

Anti-tTG antibody concentrations in the sera and SF of the 68 arthritic patients who underwent knee aspiration were significantly correlated ($r = 0.69; P < 0.001$). No EMAs were found in any of the SF samples examined.

After 6 months of MTX treatment, serum anti-tTG antibodies decreased significantly in the 45 RA patients who underwent this procedure ($P < 0.001$).

All above-mentioned anti-tTG antibody concentrations were measured by use of microtiter plate wells coated with rhtTG. Wells coated with nhtTG were also used for serum samples from 40 randomly selected arthritic patients, and the results were compared. Antibody concentrations measured with rhtTG and nhtTG were significantly correlated ($r = 0.917; P < 0.0001$; Fig. 2 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol49/issue12/).

Although anti-tTG antibodies are currently used in the diagnostic work-up of CD, some authors have demonstrated the presence of anti-tTG-positive results in patients with inflammatory bowel disease (5–7), chronic liver disease (5, 7, 16), insulin-dependent diabetes mellitus (5), and heart failure (17), suggesting a limited specificity. Other authors have suggested that use of rhtTG as the EIA substrate, instead of the guinea pig tTG, could reduce the rate of false positives in patients with chronic liver disease and insulin-dependent diabetes mellitus (18, 19). Some of the above-mentioned studies, however, have been performed with rhtTG (7, 16, 17).

In the present investigation, we found increased anti-tTG antibodies in ~40% of patients with RA, PsA, and AS. In OA patients, anti-tTG antibody concentrations were halfway between those of arthritic patients and healthy controls, whereas in contrast, EMAs were not found in any of the above-mentioned patients. We also identified a suspicious zone that included low-positive anti-tTG values from both arthritic and CD patients, suggesting that only high concentrations are strongly associated with CD, whereas lower titers may not be disease specific. It is unclear whether EIA positivity corresponds to the real presence of anti-tTG antibodies in arthritic patients. However, because anti-tTG values decreased after 6 months of MTX treatment and serum/SF antibody concentrations correlated between them, positive anti-tTG results appear to be an arthritic disease-associated event.

On the other hand, increased tTG has been demonstrated in the intestines of patients with CD and Crohn disease (6, 20). This antigenic overexpression could explain, at least in part, the anti-tTG induction observed in these patients. Likewise, Rosenthal et al. (21) have demonstrated a relationship between tTG and pathologic mineralization in articular chondrocytes, which in turn leads to the development of destructive arthritis. Thus a mechanism similar to those in the intestine could occur in synovial tissue.

If anti-tTG antibodies are present in EMA-negative arthritic patients, it is possible that tTG is not the only antigen of the EMAs. Lock et al. (22) have demonstrated that although tTG immunoabsorption is enough to abrogate anti-tTG EIA reactivity, it failed to completely abolish EMA binding activity. Another study showed the existence of EMA antigenic epitopes not related to tTG (23). Furthermore, using a proteomic approach, Stulik et al. (24) recently identified new CD autoantigen, such as ATP-synthase β-chain and enolase-α, whereas in contrast, other authors have demonstrated that EMAs are unable to bind tTG-knockout tissues (25). Although the rhtTG and nhtTG used in the present study are species specific, EIA-positive results could also subtend antibodies directed against impurities of the substrate used for coating plate wells. To verify whether EIA-positive results correspond to the real presence of anti-tTG antibodies in arthritic patients, other molecular investigations are necessary.

In conclusion, our data demonstrate that both the serum and SF of arthritic patients can give anti-tTG-positive results. This feature should be considered before anti-tTG antibody tests are used; furthermore, EMAs should always be evaluated when CD is suspected, especially in patients with known arthritis.

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Reliability of IFCC Method for Lactate Dehydrogenase Measurement in Lithium-Heparin Plasma Samples, Ilonna Herzum, Robert Büberl, Harald Renz, and Hans Günther Wahl* (Klinikum der Philipps-Universität Marburg, Department of Clinical Chemistry and Molecular Diagnostics, 35033 Marburg, Germany; * author for correspondence: fax 49-6421-2865994, e-mail hg.wahl@med.uni-marburg.de)

In a recent Technical Brief on the IFCC-recommended method for lactate dehydrogenase (LD) measurement, Bakker et al. (1) questioned the reliability of this method for heparin samples. According to their findings, the IFCC method produced an excessive frequency of significantly discordant duplicate measurements (17.8%; 27 of 152 samples) compared with the formerly applied French Society (SFBC)-recommended method (1.4%; 2 of 140 samples). Neither increased centrifugation time and speed or the use of plasma separators to reduce the plasma contamination with blood cells, nor LD measurements without concurrent measurements of other analytes to eliminate possible interferences lowered the reported high rate of duplicate errors. The problem was also not attributable to the clinical chemistry analyzer because measurements on both Hitachi 911 and 717 analyzers (Roche Diagnostics GmbH) showed results similar to those obtained with the previously used Modular analyzer (Roche Diagnostics GmbH). Only when serum samples or secondary tubes for centrifuged plasma samples were used was there a significantly lower frequency of duplicate errors: 2.6% (3 of 114) for serum and 1.1% (1 of 94) for secondary plasma tubes. The authors pointed out that the differences in buffer composition of the IFCC (pH 9.40 ± 0.05) (2) and SFBC (pH 7.4–7.8) methods influenced the integrity of platelets and erythrocytes. In this case, the presence of cells might play a role in the high frequency of duplicate errors.

We have been measuring LD activity on three Roche Hitachi 917 analyzers according to the recommendations of the IFCC (2) in our laboratory since February 2003. The within-run imprecisions (CV) for a heparin-plasma sample (n = 20) were 0.8% (197.4 U/L) and 0.7% (321.3 U/L), and the between-run CV were 1.4% for the Roche Precipath U (178.3 U/L; n = 28) and 1.1% for the Roche Precinorm U calibrators (241.0 U/L; n = 28). During this period of time we observed no obviously erratic LD measurements for controls or patients when several samples from the same patient were analyzed within a few hours for follow-up. All measurements were routinely performed with plasma samples from plastic lithium-heparin-gel tubes (Monovette prod. no. 03.1631.001; Sarstedt). Blood sample collection was done without vacuum drawing. After centrifugation for 10 min at 3000g, the primary tubes were used for analysis. To reevaluate the reliability of the IFCC-recommended LD method in our laboratory with special regard to the reported high frequency of duplicate errors, we performed 140 LD duplicate measurements and studied the possible interferences from platelet contamination, cuvette and reagent probe/stirrer carryover effects, and different primary plasma tubes. To avoid long time delays between duplicate measurements, the analyses of the 140 samples were performed in the same run in groups of 5 with reruns.

The statistical analysis of the results from the duplicate measurements was calculated according to the Bland–Altman procedure (3–5). Fig. 1 shows the Bland–Altman plot with the means of the duplicate LD measurements (U/L) plotted against the absolute differences between duplicate measurements (U/L). For the differences the values of the second measurements were subtracted from the values of the first. The limits of agreement [mean (2 SD), 0.3 (26.6) U/L] (3–5) are shown as solid lines in Fig. 1, and the mean of the differences as a dashed line. The frequency of duplicate errors [differences exceeding mean (2 SD)] under these conditions was 3.6% (5 of 140). This is more than the reported 1.4% for the SFBC method but less than the 17.8% reported for the IFCC method (1). The 95% confidence interval (−1.95 to 2.54 U/L; mean ± 2 SE) includes 0, so there is no evidence of systematic bias (3–5). When comparing our data with the results published by...