Autoantibodies to Tissue Transglutaminase in Sjögren's Syndrome and Related Rheumatic Diseases

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ABSTRACT. ***Objective.*** Sjögren's syndrome (SS) has been reported in up to 15% of patients with biopsy proven celiac disease (CD). The diagnosis of CD in the setting of SS and other systemic rheumatic diseases can be difficult because they are often associated with a number of gastrointestinal symptoms and diseases. Although the diagnosis of CD is often confirmed by a small bowel biopsy, marker autoantibodies directed against the endomysium of transitional epithelium (EMA) and tissue transglutaminase (tTG) are highly correlated with biopsy-proven disease and serve as a valuable screening test. We used an IgA-anti-tissue transglutaminase antibody (anti-tTG) ELISA to assess the prevalence of anti-tTG in an unselected cohort of patients with SS and other systemic rheumatic diseases.

Methods. Sera from 50 patients with SS, 50 with systemic lupus erythematosus (SLE), 50 with rheumatoid arthritis (RA), 30 with systemic sclerosis (SSc), and 50 healthy controls were tested for autoantibodies to tTG. A comparison group of 40 sera from patients with biopsy-confirmed CD was also included. IgA anti-tTG was measured by a commercially available ELISA kit (Inova, San Diego, CA) that employs purified tTG.

Results. Six of the 50 (12%) IgA sufficient SS patients had anti-tTG compared to 2 (4%) normal sera, 3 (6%) SLE, 2 (7%) SSc, and 1 (2%) RA. By comparison, in the CD cohort, 33 (83%) had anti-tTG. Five of 6 SS patients with anti-tTG had symptoms, signs, or small bowel biopsy findings consistent with a diagnosis of CD. IgA anti-tTG and EMA were accompanied by other IgA autoantibodies in SS sera.

Conclusion. Anti-tTG ELISA is a reliable method to indicate a coexisting diagnosis of CD in patients with SS. Interestingly, the frequency of false positive tTG tests in any of the systemic rheumatic diseases is not significantly greater than in controls. Further, our study shows that anti-tTG is more prevalent in SS than in other systemic rheumatic diseases. The tTG ELISA may be used as a screening test to identify patients with SS who are at risk and require further evaluation for the presence of CD. (J Rheumatol 2003;30:2613–9)

Key Indexing Terms:
SJÖGREN'S SYNDROME TISSUE TRANSGLUTAMINASE ENDOMYSIUM ANTIBODY
DIAGNOSIS CELIAC DISEASE

Sjögren's syndrome (SS) is an autoimmune exocrinopathy characterized by xerophthalmia, xerostomia, and circulating autoantibodies to intracellular autoantigens[1,2]. Autoantibodies commonly seen in SS include those directed against a 52 kDa SSA/Ro, 60 kDa SSA/Ro, SSB/La, and α-fodrin antigens[2-5]. Of particular interest is the coexistence of GI diseases in SS that include dysphagia due to decreased saliva production, impaired pancreatic function, gastric antral inflammation and atrophy, and autoimmune liver diseases[6]. In 1965, Pittman and Holub described the association of celiac disease (CD) and SS[7]. Since then, a few reports have confirmed this association and have suggested the frequency of SS in CD to be as high as 15%[8-11].

Although a definitive diagnosis of CD relies on intestinal biopsy, the IgA anti-endomysial antibody (EMA) assay is a useful serological screening test. A number of studies have found IgA EMA detection to be highly sensitive (85-100%) and specific (90-100%) in the general population. Nevertheless, determination of a positive or negative IgA EMA by indirect immunofluorescence (IIF) can be difficult in the presence of other IgA autoantibodies. False positive and negative interpretations may result in sensitivities as low as 60% because SS sera commonly show reactivity with a variety of other intra- and extra-cellular antigens[8]. In addition, IIF assays are notably labor intensive, semi-quantitative, and reliant upon subjective interpretation, introducing the possibility of inter-observer variability[12].

The identification of tissue transglutaminase (tTG) as the predominant autoantigen targeted by EMA[13] has led to the

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development of alternative assays that include a quantitative enzyme-linked immunosorbent assay (ELISA) that measures IgA antibodies to tTG (anti-tTG) and theoretically overcomes some of the problems encountered with EMA. A number of studies of patients with known CD have shown that IgA anti-tTG ELISA have a sensitivity of 85-100% and specificity of 90-100%, and have shown good quantitative correlation (r = 0.86) as well as strong concordance (95%)[14–16]. Since CD and other gastrointestinal (GI) diseases are associated with SS, it is important to make an accurate diagnosis. In this setting, a sensitive and specific screening test might preclude more invasive and costly tests such as small bowel biopsy. We conducted a cross-sectional study of the frequency of anti-tTG antibodies in SS and other systemic rheumatic diseases and their association with CD.

MATERIALS AND METHODS

Subjects. Sera were obtained from 50 patients with primary SS that fulfilled diagnostic criteria for the disease[17]. For comparison, unselected sera were obtained from patients meeting classification criteria for systemic lupus erythematosus (SLE, n = 50)[18], rheumatoid arthritis (RA, n = 50)[19], or systemic sclerosis (SSc, n = 30), as well as 50 healthy volunteers, and 40 patients with biopsy-confirmed CD. All SS, SLE, SSc, and RA controls were IgA sufficient. A retrospective chart review was conducted for patients with positive anti-tTG results to assess whether these individuals had a previous diagnosis of CD.

Autoantibody detection. Sera were tested for a broad spectrum of autoantibodies relevant to systemic rheumatic diseases using indirect immunofluorescence (IIF) on commercial HEp-2 cells (HEp-2000, Immuno Concepts, Sacramento, CA, USA), immunodiffusion, and ELISA[20,21]. The detection of IgA EMA employed a commercially available kit (Inova Diagnostics Inc., San Diego, CA, USA) that was used as described[22,23]. Two experienced technicians who were blinded to the patient's history or results of the small bowel biopsy examined the IIF slides. As established by the manufacturer, typical staining of the endomysium at titers of 1:5 or greater was considered positive.

IgA anti-tTG antibody detection employed a commercial anti-tTG ELISA kit (QuantaLite: Inova) based on antigen purified from guinea pig liver[22]. Standardized units of ≥ 20 were considered positive for IgA anti-tTG antibodies, as established by the manufacturer. Anti-EMA tests were performed for all patients with CD and controls, but only sera from patients with rheumatic disease with a positive tTG were tested further for EMA antibodies by conventional IIF[22]. IgG antibodies were detected on the commercial EMA substrate using a rhodamine conjugated goat anti-human IgG secondary antibody (Jackson ImmunoResearch, West Grove, PA, USA).

Intestinal biopsy and histopathological analysis. Patients with CD underwent clinical evaluation and small intestinal biopsy as described[23]. A diagnosis of CD was made when there was an increased number of intraepithelial lymphocytes with associated subtotal or total villus atrophy[24].

Analyses. The proportion of subjects with positive anti-tTG results and the associated 95% confidence interval (95% CI), was calculated for SS patients and non-SS controls. Proportions for each group were compared statistically using binomial methods. The sensitivity, specificity, positive and negative predictive values of the anti-tTG ELISA for the diagnosis of CD were estimated for SS patients. Sensitivity was defined as the proportion of patients with known CD who had a positive anti-tTG result. Specificity was calculated as the proportion of patients without CD who tested negative for anti-tTG. Positive predictive value was defined as the proportion of anti-tTG positive patients with CD, and the negative predictive value as the proportion of anti-tTG negative patients without CD. The agreement between anti-tTG and anti-EMA results was evaluated among patients with CD and results were corrected for agreement occurring due to chance alone using the kappa statistic.

RESULTS

The frequency of anti-tTG antibodies in the SS group was 12% (95% CI: 0.05, 0.24) as compared to 4% (95% CI: 0.02, 0.09) in the combined group of non-SS samples, including SLE, RA, SSc, and controls (Table 1). Thus, anti-tTG antibodies were 3 times more likely to be positive in SS compared to non-SS controls (p = 0.05). By comparison, in the CD cohort, 33 (83%) had anti-tTG and 35 (88%) had anti-EMA. The percent agreement for the 2 assays in patients with CD was 95%, and the chance-corrected proportional agreement (kappa statistic) was 0.80. Anti-tTG levels varied greatly in the CD group as compared to rheumatic disease patients and healthy controls (Figure 1).

Retrospective chart review showed that 5/6 SS patients with anti-tTG had a concurrently positive EMA and biopsy-

Table 1. Proportion of patients with positive IgA anti-tTG result (≥ 20 units).

Group	n	Anti-tTG Positive Proportion	(95% CI)
CD	33	0.83	(0.67, 0.93)
NS	2	0.04	(0.005, 0.14)
RA	1	0.02	(0.001, 0.11)
SLE	3	0.06	(0.01, 0.16)
SSc	2	0.07	(0.008, 0.22)
SS	6	0.12	(0.05, 0.24)

tTG: tissue transglutaminase; CD: celiac disease; NS: normal sera; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; SS: Sjögren's syndrome.

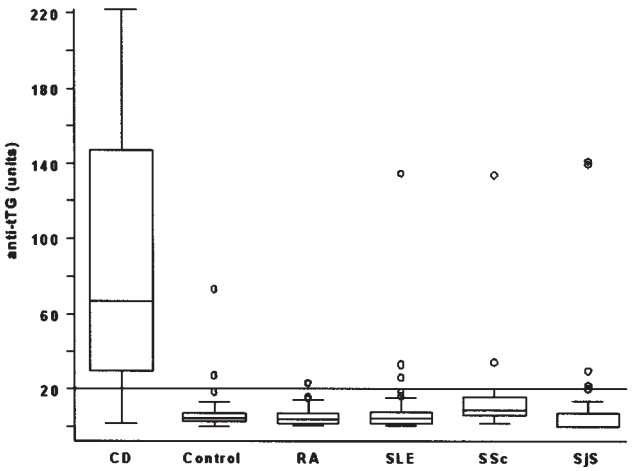

Figure 1. Box plot of anti-tTG levels in Sjögren's syndrome, celiac disease, and controls. Results ≥ 20 units are considered positive. Upper and lower bounds of each box represent the 25th and 75th percentiles, and the center line is the median. tTG: tissue transglutaminase; CD: celiac disease; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; SS: Sjögren's syndrome.

proven CD with typical GI symptoms (Table 2). Thus, the positive predictive value of anti-tTG for CD among patients with SS was 83%, with an estimated sensitivity of 100%, specificity 97%, and negative predictive value 100%. It is interesting to note that the one SS patient with a positive anti-tTG test but no clinical evidence of CD had a negative EMA by the IIF assay. The EMA assay of sera from SS patients showed both strong IgA staining of the endomysium of transitional epithelium and intense speckled cytoplasmic staining of the transitional epithelium (endomysial staining) as well as adjacent tissue layers (i.e. smooth muscle, adventitia, and epithelium) (Figure 2a, c). Of interest, the IgG staining was very weak even at serum dilutions of 1:10 (Figure 2c). By comparison, the serum from a CD patient with high titers of tTG showed intense staining that was restricted to the endomysial layer (Figure 2c). At the recommended serum dilutions of 1:5 or 1:10 for the screening EMA test, the presence of IgA autoantibodies to other intra- and extra-cellular autoantigens was quite remarkable and tended to obscure the endomysial staining pattern. The speckled cytoplasmic staining persisted even at serum dilutions as high as 1:640 and when the EMA pattern was absent (Figure 2c). There were no obvious associations between anti-tTG and the severity of symptoms or histological grade of the biopsy or with other autoantibodies such as 52- and 60-kDa SSA/Ro or SSB/La (Table 2). Neither was the presence of the speckled cytoplasmic pattern correlated with the presence of SSA/Ro or SSB/La antibodies.

Followup of the 2 control sera with positive anti-tTG results showed that one (a laboratory technologist with the high positive) subsequently sought medical attention, had a positive EMA and clinical and small bowel biopsy features of CD (Table 2). The other individual did not have symptoms of CD and serological testing for EMA was negative. The single RA patient with low positive results (23 units) has been lost to followup and it is unknown whether this patient has clinical features of CD. One patient with SLE and a high positive anti-tTG (135 units) had a history of biopsy proven CD, and has subsequently developed seropositive erosive RA. Another SLE patient with moderate levels of anti-tTG, negative EMA, and long-standing bowel symptoms was being investigated for possible Crohn's disease. Her gastroenterologist was notified of the result and is evaluating the patient for the presence of CD. The other SLE patient with a positive result had low anti-tTG levels (26 units). Both SSc patients with positive anti-tTG had symptoms attributed to sclerodermatous involvement of the GI tract but had not been evaluated for CD. Retrospective analysis of these SSc serum showed that both had a positive EMA test.

DISCUSSION

The prevalence of CD in the general population is reported to be 0.3-0.5%[8,25,26]. Therefore, we were interested in determining the frequency of anti-tTG, a widely-used serological screening test for CD, in SS and other systemic rheumatic diseases. The rationale for this study was 2-fold. First, patients with systemic rheumatic diseases such as SS, often have GI symptoms, and other studies have shown that up to 15% of SS patients have CD[8]. Thus, it is important to consider the diagnosis of CD in this setting because it can be an unrecognized or latent clinical problem[27]. Second, it is important to evaluate the performance of screening tests for CD in patients with coexisting autoimmune diseases[26,28]. Since SS and other systemic rheumatic diseases often have associated hypergammaglobulinemia, we postulated that the anti-tTG ELISA would have a high false positive rate in these conditions. Our results show a 3-fold higher preva-

Table 2. Features of individuals with a positive anti-tTG result.

Sample	Age	Gender	Anti-tTG (units)	Other Autoantibodies	Clinical Features
SS 1	44	F	21	52 + 60 kDa SSA/Ro, SSB/La, EMA	GI symptoms, CD, SB biopsy
SS 2	51	F	29	60 kDa SSA/Ro, SSB/La, EMA	GI symptoms, CD, SB biopsy
SS 3	48	F	139	52 + 60 kDa SSA/Ro, SSB/La, EMA	CD, SB biopsy
SS 4	41	F	139	EMA	CD, SB biopsy
SS 5	44	F	141	52 + 60 kDa SSA/Ro, SSB/La, EMA	CD, SB biopsy
SS 6	31	F	21	60 kDa SSA/Ro	Asymptomatic
SLE 1	38	F	26	dsDNA, histone	Deceased, CNS disease
SLE 2	40	F	33	Histone, centrosome	Autoimmune hepatitis, oral & genital ulcers, possible Crohn's
SLE 3	36	F	135	SSA/Ro, RF	Erosive arthritis, SB biopsy, SD
SSc 1	42	F	135	CENP, EMA	CREST, Polycystic kidneys
SSc 2	39	M	34	Scl-70 (topo 1), EMA	DS, ER, gastritis
RA 1	70	F	23	RF	Raynaud's, hypothyroidism
NS 1	26	F	73	EMA	CD, SB biopsy
NS 2	51	M	27	None	Asymptomatic

CD: celiac disease; CENP: centromere protein antibodies; CREST: limited form of systemic sclerosis marked by calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia; DS: diffuse scleroderma; EMA: endomysial autoantibodies; ER: esophageal reflux; RF: rheumatoid factor; SB: small bowel; SS: Sjögren's syndrome; topo 1: topoisomerase 1; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; RA: rheumatoid arthritis; NHS: normal serum.

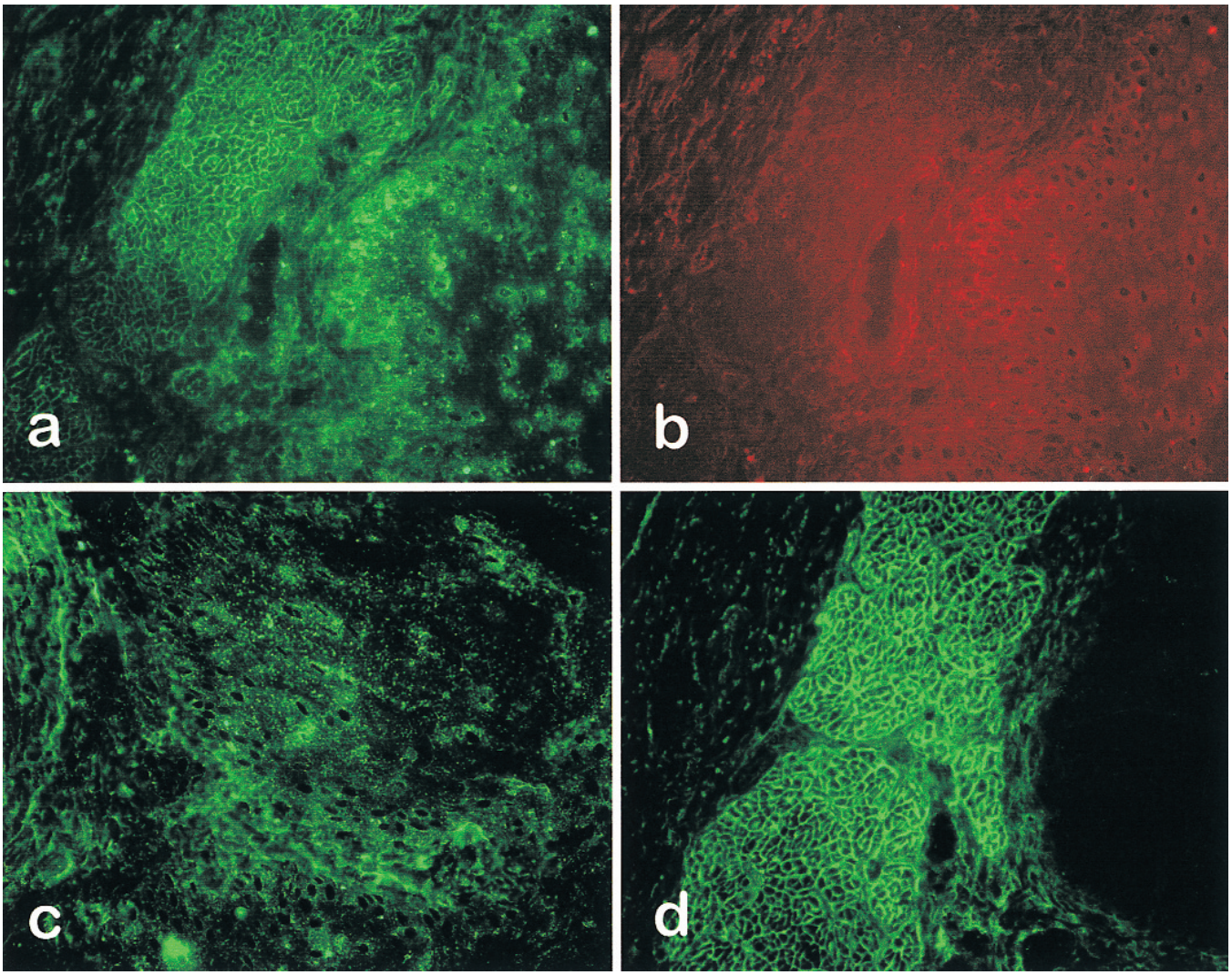

Figure 2. IgA autoantibodies to endomysial antigens (EMA) detected by indirect immunofluorescence on monkey esophagus substrate. The sera of a patient with Sjögren's syndrome with anti-SSA/Ro, SSB/La, and anti-tTG measured by ELISA shows the typical EMA pattern of staining when IgA (a) but not IgG (b) secondary antibodies are used. There is also a strong IgA cytoplasmic speckled staining pattern in all tissue layers (a and c). In comparison, the serum of a CD patient with high titers of anti-tTG shows only the EMA pattern of staining (d) (original magnification ×400).

lence of anti-tTG antibodies among subjects with SS compared to SLE, RA, and healthy controls ($p = 0.04$). In addition, false positive results were surprisingly uncommon (up to 2.6% overall) and 4 of the 7 false positives had anti-tTG levels < 30 units (Table 2). Since some patients were lost to followup and could not be evaluated for CD, the rate of 2.6% could be considered the maximum false positive rate because some of these patients might have unrecognized CD. This point is supported by our observations of the control group where a laboratory technologist was found to have a positive tTG and only on subsequent investigation was found to have unequivocal small bowel biopsy findings of CD.

The identification of tissue transglutaminase (tTG) as a primary autoantigen in CD has introduced an improved serological screening test for this disease[13]. tTG is a calcium-dependent enzyme that cross-links specific collagen types and fibronectin during tissue injury[29]. The potential role of a quantitative IgA anti-tTG ELISA in SS and other autoimmune conditions associated with CD[26,27,30,31] as a replacement for the more time consuming and subjective IgA EMA IIF, or the more invasive small bowel biopsy, has important implications in terms of cost reduction and standardization of results. In the context of systemic rheumatic diseases, such as SS, RA, SLE, and SSc, where GI symptoms and comorbid GI disease can be present, the tTG assay has the potential to provide important clinical information and raise the suspicion of CD. In addition, these patients can have IgA autoantibodies to a variety of intra- and extra-cellular antigens[32-39] including ds DNA, SSA/Ro, cardiolipin, topoisomerase 1 (Scl-70), and α-fodrin, potentially making the IgA EMA IIF assay difficult

to interpret. This is a particular problem when screening sera for EMA at the manufacturer's recommended dilution of 1:5 or even 1:10. We noted that the SS sera had IgA antibodies that reacted with a cytoplasmic antigen in tissue layers represented as speckles of various sizes. Since this finding did not correlate with anti-SSA/Ro or anti-SSB/La status, and because the IgG staining was weak or negative, it is unlikely that there was reactivity to these antigens. Further, since the speckled cytoplasm was not observed in CD sera without SS, it is unlikely that this staining represents reactivity to gliadin. Hence the identity of this antigen targeted by IgA in SS sera is unknown.

Many retrospective studies have compared IgA anti-tTG ELISA to IgA EMA-IIF in CD populations, and high specificities and sensitivities have been reported[14-16,22]. Our study investigated the frequency of anti-tTG in a cohort of unselected SS patients and estimated the positive predictive value to be 83%, negative predictive value 100%, sensitivity 100%, and specificity 97%. The latter 3 values may have been overestimated since our study was not designed to assess the rate of false negative anti-tTG results by investigating asymptomatic patients for biopsy evidence of subclinical disease. Although EMA have been previously described in SS, the relative lack of sensitivity in biopsy-proven CD[8] suggests that a tTG ELISA may have advantages in this clinical setting. The apparent decreased sensitivity in SS-CD may be due to the variety of other autoantibodies that may obscure an otherwise unequivocal EMA test (Figure 2). In this setting, an ELISA that detects antibodies to a specific autoantigen of interest (e.g., tTG) can be a very important diagnostic aid before small bowel biopsy is contemplated. Our previous study of children[22] found a discordance between the conventional IIF EMA and anti-tTG ELISA, findings that were consistent with reports from other centers[14-16]. Interestingly, we found excellent agreement (kappa 0.80) between the EMA and anti-tTG assays in patients with known CD.

The clinical relevance of this study of anti-tTG in SS is akin to patients with type I diabetes mellitus (DM) who have a higher prevalence of CD[22,23,40,41]. It has been suggested that the association between DM and CD is due to immune dysfunction and altered dietary protein tolerance caused by non-specific activation of the immune system[42]. The association of SS with CD may be explained in part by a similar genetic profile, namely the DQ2 heterodimer coded by DQA1*0501 and DQB1*0201 alleles[8,43,44]. Further, it is interesting that both CD and SS have an autoimmune epitheliitis as a common pathological feature[45]. Indeed, it has been shown that as many as 31% of SS patients with normal gut villous morphology have increased levels of IgA anti-gliadin antibodies suggesting that there is subclinical inflammation of the gut in the absence of CD[8]. These studies suggest that, in addition to monitoring for liver, lung, and neoplastic diseases, physicians attending SS patients should also be aware of possible autoimmune bowel involvement.

Several studies have shown that serological markers used to screen for CD may be predictive of latent disease in high-risk patients such as those with Type I DM[46-48]. Such studies report patients with positive serum IgA EMA but normal biopsies who later developed biopsy-confirmed CD. Of interest, 2 asymptomatic patients in our SS group that initially had a positive anti-tTG and EMA, developed persistent symptoms and 2 years later had increased anti-tTG titers and a positive small bowel biopsy. The patient with asymptomatic SS has not had a bowel biopsy but will continue to be followed.

In addition to the problem of subclinical or latent disease as evidenced by normal biopsies, other possible explanations for false positive tests exist. One is the presence of autoantibodies that bind to other autoantigens in tissue substrates or to trace contaminants in purified preparations of tTG. To avoid these problems, assays based on purified human recombinant tTG, which have significantly higher sensitivity and specificity than the conventional EMA assay, have been developed and are now commercially available[49–52]. The performance of these assays in a prospective clinical setting may prove to be superior to current protocols. The second explanation for false positive tTG results is related to observations that anti-tTG is also found in other inflammatory bowel diseases, including Crohn's disease[53]. Of interest, one of the SLE patients in this study with a positive anti-tTG also had Crohn's disease.

Anti-tTG antibodies were detected in 12% of patients with SS, a prevalence 3 times that observed in non-SS controls ($p = 0.05$). The IgA anti-tTG ELISA used in our study appeared to be equivalent to a conventional IgA EMA IIF assay as a screening test in IgA-sufficient SS patients. Although intestinal biopsy remains the gold standard for the diagnosis of CD in patients with suspected malabsorption, the tTG ELISA can be a valuable screening test, particularly in patients with other autoimmune diseases who have multiple autoantibodies that confound conventional serological tests for CD. Lastly, although sera from SS patients have a high frequency of hypergammaglobulinemia, there was only one apparent false positive anti-tTG result that had a low level of reactivity. Patients with apparently false positive tests should be followed with serial antibody testing as they may represent a subpopulation with latent CD who may develop the disease at a later date.

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