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Tissue Transglutaminase-Serology Markers for Coeliac Disease[1)]

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Serology markers of coeliac disease (CD) – antigliadin IgA/IgG antibodies (AGA/AGG) with purified α-gliadin, antiendomysium IgA antibodies (EmA) and anti-tissue transglutaminase (atTG) IgA/IgG antibodies – determined in 1451 serum samples, were analysed with respect to different screening algorithms. Determination of atTG using five ELISA methods was compared taking into account the impact of human recombinant antigen and IgG class of atTG. A subgroup of 119 patients undergoing small intestinal biopsy was used to calculate sensitivity and specificity of CD markers. The highest sensitivity (94%) was obtained for AGG, and the highest specificity (93.5%) was obtained for EmA. All coeliac disease patients were detected using the combination of all four CD markers, resulting in 100% sensitivity. CD and type 1 diabetes mellitus autoantigens were determined in 139 diabetic patients. The atTG IgA mean value (16.7 IU/ml) was higher in the antiglutamate dehydrogenase antibody (GAD)-positive subgroup, where at least one CD marker was positive in 83.6% subjects. In the GAD-negative subgroup atTG IgA was 8.73 IU/ml and at least one CD marker was positive in 57.4% subjects. atTG in IgA and IgG classes could be recommended as valuable serological markers of CD in the differential diagnosis of malabsorption as well as in various screening algorithms. ELISA determination of atTG with human antigen could increase the specificity, especially in patients with other autoimmune diseases. Clin Chem Lab Med 2002; 40(5):485–492

Key words: Coeliac disease; Anti-tissue transglutaminase; Serological markers; Autoimmunity; Diabetes mellitus.

Abbreviations: AGA, antigliadin antibodies of IgA class; AGG, antigliadin antibodies of IgG class; atTG, antitissue transglutaminase antibodies; CD, coeliac disease; ELISA, enzyme-linked immunosorbent assay; EmA, antiendomysium antibodies; GAD, antiglutamate dehydrogenase antibodies; gp-atTG, antitissue transglutaminase antibodies raised with guinea pig antigen; GSE, gluten-sensitive enteropathy; HLA, human leukocyte antigen (major histocompatibility antigen); hc-atTG, antitissue transglutaminase antibodies raised with human cell antigen; hr-atTG, antitissue transglutaminase antibodies raised with human recombinant antigen; IgA, immunoglobulin class A; IgG, immunoglobulin class G; NOD, non obese diabetic; tTG, tissue transglutaminase.

Introduction

Tissue transglutaminase (tTG; EC 2.3.2.13) – the enzyme converting glutamine residues in peptides to glutamate – seems to be the key enzyme in the pathogenesis and diagnostics of coeliac disease (CD). Coeliac disease is a common chronic small-bowel disorder of autoimmune origin occurring in both children and adults, and is one of the most commonly underdiagnosed diseases in general practice with incidence 1:200 (1, 2). This disease is genetically determined, has a strong HLA association (3) with DQ2 (DQA1*0501/DQB1*02), and gliadin peptides derived from wheat gluten were identified as precipitating factors. Numerous studies describe attempts to isolate and identify a specific, toxic peptide sequence (4–6). The highest biological activity was described for α-gliadins, especially for peptide fragments containing -Q-P-Q-. The amino acids sequence 56–75 of A-gliadin deamidated by tissue transglutaminase may produce a neoepitope specific for T cell responses of patients with CD (7).

tTG was identified as the major (8), if not the sole (9), autoantigen in CD (gluten-sensitive enteropathy; GSE) in 1997, and since that time 142 papers have been published reporting the relationship between tTG and CD or GSE (Medline database), 270 papers including abstracts (Web of Science database). CD displays many characteristics of autoimmune disorders and its incidence is nearly ten times higher in patients with such diseases as type 1 diabetes mellitus or autoimmune thyroiditis (10). The manifestation of CD is changing, and better knowledge of GSE has permitted the identification of atypical, asymptomatic, silent and latent forms of CD (11, 12). The strict, lifetime gluten-free diet is the only treatment for the disease and, moreover, strict adherence to the diet may prevent neoplastic and systemic complications associated with the disease (13, 14). The beneficial impact of gluten-free diet was reported to delay or prevent the onset of diabetes in non-obese diabetic (NOD) mice (15).

The final diagnosis of CD is based on a characteristic histological pattern (total or partial villous atrophy, crypt hyperplasia and increased number of intraepithelial lymphoctes) in jejunal biopsy, remission on gluten-free diet and relapse during subsequent gluten chal-

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lenge (16), and three biopsies are required according to the diagnostic criteria established in 1969. The working group of the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) revised these criteria in 1989 leaving the initial biopsy only, clinical response to a gluten-free diet and positive test for antigliadin antibodies. The major challenge is not to confirm the CD but to identify individuals who may have CD, *i.e.* asymptomatic individuals with silent or latent form. A considerable effort is therefore spent on evaluating diagnostic algorithms using available test methods, including immunological and serological detection of different antibodies, function tests, breath tests and other detection markers, with a special emphasis on screening programmes. Serological test methods have gained high priority in screening, in diagnostic work-up and in follow-up of CD. Sera from patients with CD contain IgA and IgG antibodies to gliadin, endomysium, reticulin, tissue transglutaminase, calreticulin and other autoantigens (17–19).

The purpose of this study was to clarify a number of contradictory results concerning the specificity and sensitivity of anti-tissue transglutaminase tests, the validity of antitissue transglutaminase antibodies (atTG) determined in IgG class, the use of human recombinant antigen, and the importance of antigliadin (AGA) antibodies in the screening algorithm. We analyzed selected serum samples from our laboratory databank comprising almost 3000 patient sera.

Materials and Methods

Patients

Serum from 2971 patients was obtained from consecutive samples on which the measurement of CD markers had been requested since May 1992. There were 621 patients with suspected CD or malabsorption syndrome, 478 with type 1 diabetes mellitus, 117 with type 2 diabetes mellitus and 201 with thyroid disease. A subset of 1451 patients used for screening strategy evaluation was characterized by following parameters: mean age 40.2 years: 982 females (66%) with mean age 41.8 years, 510 males (34%) with mean age 37.2 years. All four CD markers have been determined continuously as requested. Patients included in each individual study comparing atTG ELISA methods were selected from the subset mentioned above and were sex- and age-matched. Serum samples were stored in 1 ml aliquots at –20 °C.

Methods

Antigliadin antibodies in IgA (AGA) and IgG (AGG) classes (2971 samples) were determined by ELISA method developed in our laboratory with purified α-gliadin as antigen (20). Dilutions 1:20 and 1:100 were used for IgA antibodies, 1:100 and 1:500 for IgG antibodies, second antibodies SwAHu-IgA and IgG were conjugated with peroxidase (Sevapharm, Prague, Czech Republic), and *o*-phenylendiamine (Sigma, St.Louis, USA) was used as substrate. Results were expressed as index to our intralaboratory CD standard serum measured at 490 nm. Upper limits of normal values for both IgA and IgG antibodies were 30 and were calculated previously as mean +2 SD determined in a control group.

Antiendomysium antibodies (EmA) of IgA class have been determined routinely in our laboratory since April 1998 (1539 samples) using an indirect immunofluorescent method on primate smooth muscle sections (Immco, Buffalo, USA/Dialab, Prague, Czech Republic). All serum samples were diluted 1:20 in buffer, and fluorescein isothiocyanate fluorescence was evaluated using a fluorescent microscope, blindly by two observers unaware of the patient's clinical status and of other test results. The agreement rate was 98.75%.

atTG in IgA class have been routinely determined in our laboratory since January 1999 (1451 samples) by ELISA method (Genesis, Littleport, UK/Dialab, Prague, Czech Republic) with guinea pig antigen. Samples were diluted 1:100. IgG class atTG antibodies were determined by ELISA method with human recombinant tTG antigen (DPC, Los Angeles, USA/Biovendor, Brno, Czech Republic). Samples were diluted 1:100. For comparative study, five commercially available ELISA kits were used (Table 1).

Subjects suspected for CD underwent small intestinal biopsy (274 subjects since May 1992) performed by an experienced gastroenterologist at Gastroenterology Clinic of General Faculty Hospital in Prague, and serology markers were evaluated in our laboratory. Small intestinal biopsies were performed by suction Crosby's capsule with subsequent histopathological and histochemical analysis. All CD markers were determined in 119 cases (50 florid, active cases of CD, seven cases of CD in remission, 38 with normal small bowel mucosa and 24 with isolated lactase deficiency).

Statistical analysis

Bivariate correlations, including Pearson, Kendall's tau-b and Spearman's r coefficients, were calculated to compare atTG

Table 1 Comparison of IgA – atTG ELISA determined with five commercially available kits.

ELISA producer	Antigen source	Calcium activation	Substrate	Measurement (nm)	ICV (%)	Sample dilution	Positive values (IU/ml)	Grey zone (IU/ml)
DPC	Human recombinant	No	TMB	450/620	5.2	1 : 100	> 10	7–10
Genesis	Guinea pig	Yes	TMB	450/620	<12	1 : 100	> 10	No
Immco	Guinea pig	Yes	pNPP	405	9.2	1 : 50	> 25	20–25
Medipan	Guinea pig	Yes	TMB	450/620	n.d.	1 : 50	> 25	No
Orgentec	Human cells	No	TMB	450/620	7.1	1 : 100	> 15	No

TMB: 3,3',5,5'-tetramethylbenzidine, pNPP: 4-nitrophenyl phosphate,
ICV: manufacturer's declared intra-assay coefficient of variation

ELISA methods. Cohen's kappa and common percentage of concordance were calculated for the agreement between atTG and EmA tests. All calculations were performed using statistical package SPSS for Windows version 10 (SPSS Inc., Illinois, USA). Sensitivity, specificity, accuracy, and positive and negative predictive values were calculated for each of serological markers as well as for screening algorithms.

Results

Screening strategy and algorithms

Various screening algorithms were evaluated by analysis of 1451 patient results, where all four CD markers had been determined with special attention to the distribution of 50 cases with active, florid coeliac disease confirmed by histology. Calculation of sensitivity and specificity of CD markers (AGA, AGG, EmA, atTG) was based on a subset of 119 patients who underwent small intestinal biopsy. Patients with a histological pattern of florid CD were considered to be the positive group (seven cases with CD in remission were excluded) and subjects with normal histology or isolated hypolactasia only were considered to be the negative group (Tables 2 and 3). Three algorithms were compared with subsequent determination of CD markers. Accuracy of CD markers in the first step, as well as in all possible variants of the second step are summarized in Table 4.

a) Positive antigliadin antibodies in the IgA class (AGA +) or IgG class (AGG +) as the initial step. Two variants were described with the following EmA determination (Figure 1a) or atTG IgA determination (Figure 1b). Both algorithms with AGA/AGG as the initial step had the highest effectivity identifying 48 of 50 CD (96%) cases, but the number of 839 samples to be tested in the second step was the highest, too. Accu-

Table 2 Sensitivity and specificity of CD markers in the literature and in the present study.

Study	AGA – IgA		AGG – IgG		EmA – IgA		atTG – IgA	
	SN	SP	SN	SP	SN	SP	SN	SP
Stern (22)	73.8–89.3	72.5–89.3	78.2–92.4	66.2–84.8	82.7–95.2	93.9–99.9	86.5–97.2	87.4– 98.2
Chan and Murray (23, 12)	31–100	85–100	46–100	67–100	52–100	89–100	52–100	85–100
Present study*	84.0	71.0	94.0	40.3	68.0	93.5	88.0	64.6

*50 CD patients, 62 patients with normal small bowel mucosa and isolated lactase deficiency;
SN: sensitivity, SP: specificity

Table 3 The frequency of positive values of CD markers in a subgroup of 119 patients with results confirmed by histopathology.

Subject group (number of cases)		AGA – IgA	AGG – IgG	EmA – IgA	atTG – IgA
CDF	(n=50)	42/50	47/50	34/50	44/50
CDR	(n=7)	3/7	5/7	0/7	2/7
HL	(n=24)	5/24	16/24	1/24	5/24
N	(n=38)	13/38	21/38	3/38	17/38

CDF: florid, active CD, CDR: CD in remission, HL: isolated lactase deficiency,
N: normal small bowel mucosa

Table 4 Summary of evaluation of two-step screening algorithms based on 1451 serum samples.

First step CD marker	Effectivity	Accuracy I	Number entering second step	Accuracy II
AGA/AGG	96%	0.598	839	0.821 (EmA) 0.750 (atTG)
EmA	68%	0.821	115	0.821 (AGA/AGG) 0.804 (atTG)
atTG	88%	0.750	323	0.750 (AGA/AGG) 0.804 (EmA)

Effectivity: percentage of CD patients (from 50 bioptically proven) catched by the first marker only; accuracy I: correctly positive/negative (from all biopsy-proven) ranked by the first CD marker only, accuracy II: calculated for two-step process using any of two remaining markers, number entering second step: number of subjects entering the second step of screening (positive by the first step)

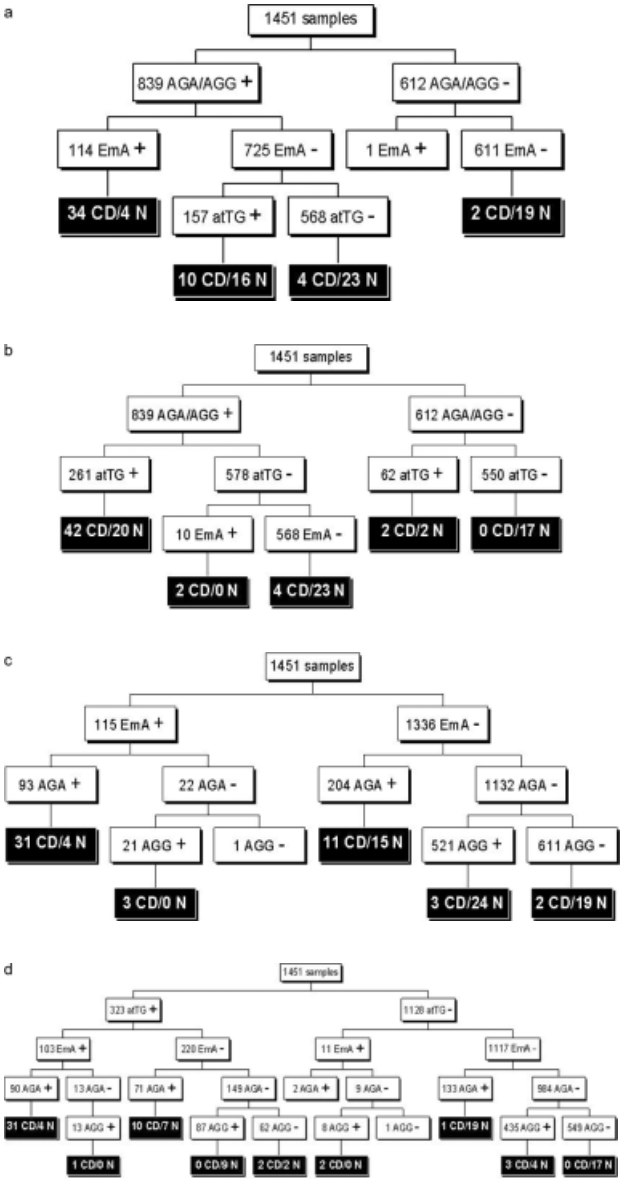

Figure 1 Distribution of 50 cases with biopsy-confirmed CD and 62 cases with normal histology (black-boxes) in the group of 1451 patients with all four CD-markers determined according to different screening algorithms: a) AGA/AGG as the first step followed by EmA as the second, b) AGA/AGG as the first step followed by atTG as the second, c) EmA as the first step with atTG not included, d) atTG as the first step followed by EmA as the second.

racy increased from 0.598 (for AGA/AGG only) to 0.821 for EmA or 0.750 for atTG used in the second step.

b) Positive EmA IgA antibodies as the initial step. Distribution displays lowest effectivity identifying only 34 of 50 CD (68%) cases and also the lowest number of subsequent tests, which would be only 115 (Figure 1c). Accuracy value 0.821 did not increase when AGA/AGG

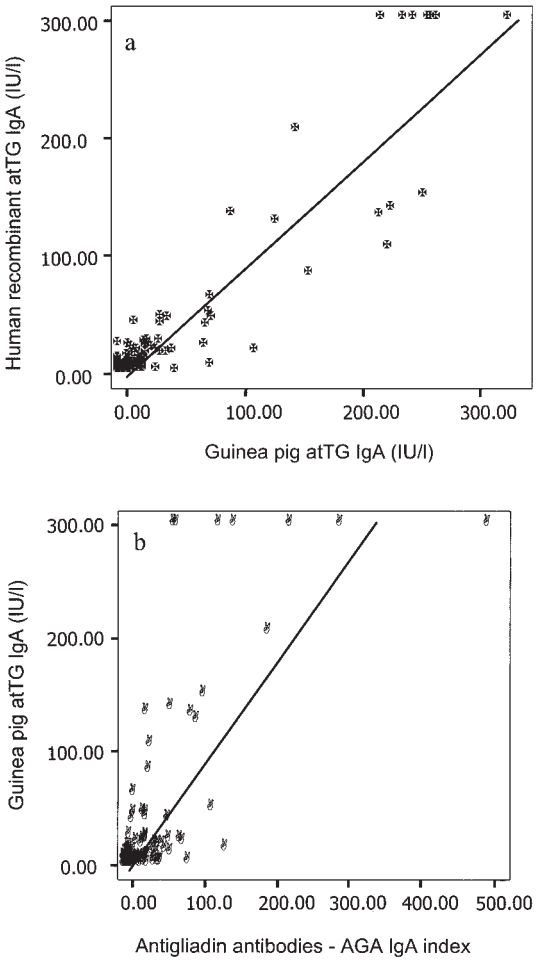

Figure 2 Correlation between a) atTG IgA antibodies determined using human recombinant antigen (hr, DPC-method) and guinea pig antigen (gp, Genesis-metod), Spearman's r=0.781. b) hr-atTG IgA antibodies (DPC-method) and antigliadin IgA antibodies (AGA) determined using purified α-gliadin, Spearman's r=0.706.

Table 5 Comparison of Ig-atTG ELISA determined with four commercially available reagent kits with the method routinely used in our laboratory.

ELISA producer	Antigen source	Correlation coefficient (r)	Concordance (%)	Cohen's κ	Number of samples (n)
DPC	Human recombinant	0.781	85.0	0.61	153
Routine method (Genesis)	Guinea pig	n.d.	84.1	0.40	1451
Immco	Guinea pig	0.509	90.7	0.45	161
Medipan	Guinea pig	0.306	57.5	0.26	40
Orgentec	Human cells	0.343	80.7	0.31	119

The concordance with EmA (as percentage of concordant results) was calculated for all five methods and expressed as also Cohen's κ coefficient of agreement.Correlation with atTG (Genesis) is expressed as Spearman's r coefficient.

were used in the second step and decreased to 0.804 using atTG, as two cases which were EmA-positive were atTG-negative.

c) Positive atTG IgA antibodies determined in the first step before EmA determination (Figure 1d). This algorithm showed 88% effectivity identifying 44 of 50 CD cases, and the number of samples to be tested in the second step was 323. Additional three cases could be identified if atTG IgG class antibodies were determined in subjects with IgA defficiency, increasing effectivity to 94%. Accuracy value 0.750 did not increase when AGA/AGG were used in the second step and increased to 0.804 using atTG in the second step.

Comparison of ELISA kits for atTG IgA determination

Four commercially available kits were compared (Table 5) with routinely used method – GD-70 ELISA (Genesis, Littleport, UK/Dialab, Prague, Czech Republic). a) DPC ELISA assay (MKATGA 1; DPC, Los Angeles, USA/Biovendor, Brno, Czech Republic) with human recombinant tTG antigen was compared using a set of selected 153 serum samples (33 EmA-positive). We found the closest correlation (r=0.781; Figure 2a) among all compared atTG ELISA kits. The concordance with EmA positivity was 85%, and we found also a very close correlation with AGA (r=0.706; Figure 2b). b) Immco ImmunLisa ELISA assay (Immco, Buffalo, USA/Dialab, Prague, Czech Republic) with guinea pig antigen was compared using a set of 161 serum samples from diabetic patients (132 patients with type 1 diabetes mellitus, seven EmA-positive, 29 with type 2 diabetes mellitus). We found a correlation (r=0.509) and concordance with EmA in 90.7%. c) Comparative studies with other two kits were performed on randomly selected serum samples and correlation coefficients for Medizym anti-Trans G (Medipan, Selchow, Germany/Biovendor, Brno, Czech Republic) and ORG 540-A (Orgentec, Mainz, Germany/Dialab, Prague, Czech Republic) were 0.306 and 0.343, and the concordance with EmA was 57.5% and 80.7%, respectively.

Transglutaminase antigen – human or guinea pig?

Methods using human cell (hc) and human recombinant (hr) transglutaminase as antigens were compared with atTG method GD-70 ELISA (Genesis, Littleport, UK/Dialab, Prague, Czech Republic) using guinea pig (gp) antigen, in IgA class only.

a) Impact of human cell antigen usage in atTG antibody determination was tested in a subgroup of 119 patients (CD markers positivity: AGA 24.4%, AGG 53.8%, EmA 10.9%, gp-atTG 37.0%). ELISA assay ORG 540-A (Orgentec, Mainz, Germany/Dialab, Prague, Czech Republic) was used with the following results: positivity for hc-atTG was found in 26 cases (21.8%), correlation with gp-atTG antibodies was 0.343 and concordant positivity/negativity was 74.8%. The concordance between atTG antibodies and EmA antibodies positivity/negativity was increased from 70.6% (gp-atTG) to 80.7% (hc-atTG).

b) Impact of human recombinant antigen usage on atTG antibody determination was tested in a subgroup of 153 patients (CD markers positivity: AGA 25.5%, AGG 83.7%, EmA 21.6%, gp-atTG IgA 40.5%). DPC ELISA assay (MKATGA 1; DPC, Los Angeles, USA/Biovendor, Brno, Czech Republic) was used with the following results: positivity for hr-atTG was found in 46 cases (30.1%), correlation with gp-atTG antibodies was 0.781 and concordant positivity/negativity was 85.6%. The concordance between atTG antibodies and EmA antibodies positivity/negativity was increased from 74.5% (gp-atTG) to 85.0% (hr-atTG). Cohen's κ-coefficient of agreement increased from moderate value 0.43 to substantial one of 0.61.

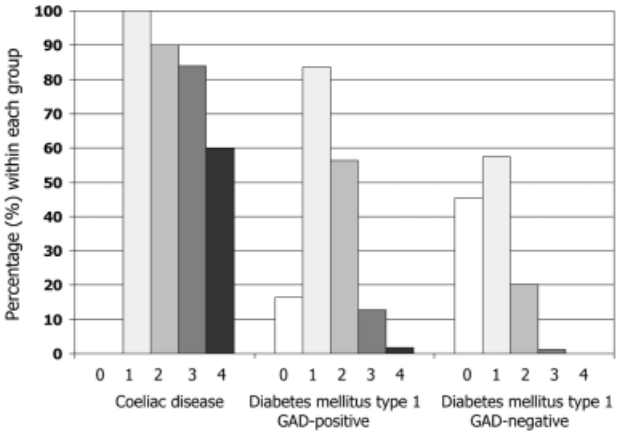

Figure 3 Cummulative percentage of positive CD-markers (zero, at least one, any two, any three or all four) in the three groups of patients: florid CD (50 cases), diabetes mellitus GAD-positive (55 cases) and diabetes mellitus GAD-negative (84 cases).

Diagnostic benefit of atTG IgG measurement

The impact of atTG IgG class antibody determination was tested in the group of 153 patients (described above). DPC ELISA assay (MKATGG 1; DPC, Los Angeles, USA/Biovendor, Brno, Czech Republic) was used with the following results: positivity for hr-atTG IgG was found in 16 cases (10.5%), in which other CD markers were also positive. AGA were found in nine cases (56.3%), AGG in 15 cases (93.8%), EmA in eight cases (50%), and gp-atTG IgA in nine cases (56.3%). Five patients had IgG class antibodies only (AGG and IgG atTG).

Screening for CD in diabetic patients

We compared serum levels of autoantigens for CD and diabetes mellitus in a subgroup of 139 samples. The atTG IgA mean level was 16.57 IU/ml, nearly two times higher in a subgroup of antiglutamate dehydrogenase antibodies (GAD)-positive diabetics (GAD value >32 ng/ml) compared to GAD-negative diabetics (8.73 IU/ml). CD markers determined in the group of 273 diabetic subjects were positive in 55.7% (at least one CD marker), all four CD markers were positive in seven cases (2.6%). Small intestinal biopsy was performed in four patients who agreed to this examina-

tion, three of them were confirmed as florid, but asymptomatic-silent, CD. Figure 3 shows the cumulative positivity of CD markers in 139 type 1 diabetes mellitus patients (55 GAD-positive, 84 GAD-negative) compared to the group of 50 confirmed CD patients. The values represent percentage of subjects with zero, at least one, any two, any three or all four CD markers. Results in GAD-positive subjects were siginificantly higher (16.4%, 83.6%, 56.3%, 12.7%, 1.8%) than in GAD-negative (45.3%, 57.4%, 20.2%, 1.2%, 0%) compared to CD patients (0%, 100%, 90%, 84%, 60%).

Discussion

Screening strategies and diagnostic algorithms for the detection of CD, especially concerning serological markers, are included in research priorities identified at the Ninth International Symposium on Celiac Disease (Baltimore, Maryland, (21)). The European Working Group on Serological Screening for Celiac Disease defined robust noncommercial test protocols for AGA/AGG, EmA and atTG antibodies (22). The specificity and sensitivity of serological markers were reported in numerous studies (Table 2) for individual antibodies, ranging from 31 to 100. Our data confirm results that no marker could be neither 100% specific or 100% sensitive, and that a combination able to detect all 100% coeliac cases (50 in this study) should include four CD markers; AGA, AGG, EmA IgA and atTG IgA class.

AGA and AGG were first ELISA methods available for patient follow-up and screening. The determination of antigliadin antibodies has the highest heterogeneity among antigens used for ELISA. We used well purified fraction of α-gliadin (20) prepared in our laboratory, and we achieved the highest sensitivity (96%) for screening, if AGA- or AGG-positive sera were selected for further examination. We also compared other commercially available ELISA tests for AGA/AGG (24), and the best correlation was found with another assay using purified α-gliadin (Eurospital, Trieste, Italy). Antigliadin antibodies are directed to an environmental, dietetic factor – gliadin, in contrast to all other CD markers, and could be therefore used as an early predictor of gluten ingestion (25). Antigliadin antibodies are recommended as the cheaper strategy for higher risk populations (26).

EmA in IgA class were determined by immunofluorescent method on slices of primate smooth muscle using Immco kit. EmA IgA antibodies are also considered to be nearly 100% sensitive and 100% specific for CD. For screening, the 1:5 dilution is highly recommended (22), the correlation of EmA positivity with other antibodies depends considerably on sample dilution (27, 28), and sensitivity of EmA is dependent on mucosal stage according to Marsh classification (29). Different results were reported if EmA antibodies were determined using primate oesophagus, umbilical vein, rat kidney/stomach or other substrates (11, 22, 29, 30). We used a single 1:20 dilution from the range of dilutions (1:5 to 1:80) proposed in the instruction manual of immunofluorescent reagent kit, because of economical aspects, usefulness for routine screening and preferring specificity to sensitivity in this method. Using the 1:20 titre we achieved the highest specificity (93.5%) for IgA EmA compared to other CD markers. Negative EmA IgA were found in all patients with CD in remission on gluten-free diet, even though two of seven cases had positive atTG and five of seven cases had positive AGA. We cannot recommend the use of EmA IgA antibodies as a first screening test for CD, as CD patients with negative EmA IgA, and not IgA deficiency, would be missed (31). Determination of IgG class EmA antibodies is also discussed (32, 33).

atTG as CD marker was described by Dietrich *et al.* (8) in 1997. Since that time 387 papers have been published reporting on CD markers, 293 of them concerning atTG antibodies. The contradictory results were published on the superiority of atTG compared to EmA determination (34–36). Independently of the antigen used, atTG IgA class ELISA determination can be used as quantitative and observer-independent alternative to the traditional and time-consuming EmA (37). Antitissue transglutaminase of IgA class could be used in successive screening as the first step instead of AGA/AGG, with lower accuracy but also with significantly lower number of subjects to be tested in the second step. The ELISA method with guinea pig antigen seems to be unsuitable because of higher false positivity compared to human recombinant antigen (38). We have used atTG with human antigen in routine diagnostics since October 2001, and a comparable group of patients is being evaluated to verify the advantage of hr-atTG.

Several methods for human recombinant or human cell atTG IgA antibody detection have been described, including ELISA (36, 37), radioimmunoprecipitation assay (39) and dot-blot assay (40). Usage of human cell antigen was shown to be superior to guinea pig antigen, with the specificity and sensitivity comparable to EmA detection by immunofluorescence in patients with CD and dermatitis herpetiformis (35). Our results are comparable, showing better concordance with EmA positivity for both human cell and human recombinant antigens in comparison with guinea pig antigen. The specificity of atTG IgA with human antigens is increased mainly in the subgroup of type 1 diabetes mellitus patients.

Positive impact of determining IgG class atTG was described in both IgA-deficient and IgA-non-deficient CD patients (33, 41). False positivity of atTG in both IgA and IgG classes was described in Down's syndrome and in patients with systemic autoimmune disorders (42). Combined IgA and IgG classes atTG determination was suggested to have 100% sensitivity in CD patients with florid disease (42). The concordant positivity of EmA and IgA and IgG class atTG was described to be 92.5%–100% and 32%–96.7%, respectively (41, 43). In the present study these values are 56.2% and 50.0%, respectively. Determination of IgG atTG could be recommended as additional serology marker, especially in

IgA deficiency, where parallel determination of both IgA and IgG classes increases the efficiency of screening.

High incidence of silent, asymptomatic CD has been reported in various autoimmune diseases leading to the concept of autoimmune polyendocrine syndrome, and there the incidence of CD is increased 10- to 30-fold in comparison to the general population (44, 45). The positivity of CD markers in children and adults with type 1 diabetes mellitus has been described in numerous studies (46, 47), atTG IgA test was a more sensitive parameter than EmA (48, 49), even though atTG antibodies have lower specificity (37). Our results confirm higher sensitivity of atTG and, moreover, an increase in specificity using human recombinant antigen, particularly in the screening of diabetic patients. We recommend, in accordance with Holmes (50) that serology screening for CD should be a part of routine investigation in diabetic patients.

Conclusion

IgA and IgG class atTG could be recommended as valuable serology markers of CD. ELISA determination of atTG using human antigen could increase the specificity, especially in patients with other autoimmune diseases. All patients with type 1 diabetes mellitus should be tested, as 12.7% of patients displayed positivity for at least three CD markers.

Simultaneous determination of all four CD markers could be recommended for focused diagnostics in all cases of malabsorption syndrome and in other high risk subjects. Basic screening could be started with IgA and IgG class antigliadin antibodies with purified α-gliadin, as it is cheaper and sensitive enough.

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