

Diagnostic Accuracy of IgA Anti-Tissue Transglutaminase Antibody Assays in Celiac Disease Patients with Selective IgA Deficiency

D. VILLALTA,^a M. G. ALESSIO,^b M. TAMPOIA,^c E. TONUTTI,^d
I. BRUSCA,^e M. BAGNASCO,^f G. PESCE,^f AND N. BIZZARO^g

^a*Immunologia Clinica e Virologia, DML, A.O. "S. Maria degli Angeli," Pordenone, Italy*

^b*Laboratorio Analisi Chimico-cliniche, Ospedali Riuniti, Bergamo, Italy*

^c*Patologia Clinica, Policlinico Consorziale, Bari, Italy*

^d*Immunopatologia e Allergologia, A.O. "S. Maria della Misericordia," Udine, Italy*

^e*Laboratorio di Patologia Clinica, Ospedale "Buccheri La Ferla," Palermo, Italy*

^f*Laboratorio di Autoimmunità, DIMI, Università di Genova, Genova, Italy*

^g*Laboratorio di Patologia Clinica, Ospedale Civile, Tolmezzo, UD, Italy*

ABSTRACT: Clinical studies have estimated a 10- to 20-fold increased risk for celiac disease (CD) in patients with selective IgA deficiency (SIgAD). For this reason, screening for CD is mandatory in SIgAD patients, but it represents a special challenge since the specific IgA class antibodies against gliadin (AGA), endomysium (EMA), and tissue-transglutaminase (tTG) are not produced in patients with CD. IgG class counterparts of these antibodies may be informative; in particular IgG EMA has been demonstrated to be a valid marker for diagnosing CD in SIgAD cases, but it is not used much in clinical laboratories, because it is cumbersome and involves some technical difficulties. Even if it was widely used in clinical laboratories, the measuring of IgG AGA has shown a less-than-optimum diagnostic accuracy, so that now it tends to be substituted by tests for anti-tTG IgG, for which the few available studies have shown diagnostic performances superior to AGA. Since it is not known whether various available methods for measuring IgG anti-tTG antibodies offer similar diagnostic performances, we have compared the results obtained from nine second-generation commercial methods (D-tek, Phadia, Immco, Orgentec, Radim, Euroimmun, Inova, Aesku, Generic Assays), measuring

Address for correspondence: Danilo Villalta, Immunologia Clinica e Virologia, A.O. "S Maria degli Angeli," Via Montereale 24, 33170 Pordenone, Italy. Voice: +39-0434-399281; fax: +39-0434-399344. danilo.villalta@aopn.fvg.it

IgG anti-tTG antibodies in 20 patients with CD and SIgAD and in 113 controls (9 patients with SIgAD without CD, 54 patients with chronic liver disease, and 50 healthy individuals). Diagnostic sensitivity, calculated by means of ROC plot analysis, ranged between 75% and 95%, and specificity ranged from 94% to 100%. In the same population, the diagnostic sensitivity and specificity of AGA IgG were 40% and 87%, respectively. Even though they perform differently, all IgG anti-tTG methods evaluated are reliable serological assays for the diagnosis of CD in SIgAD patients, with diagnostic accuracy superior to the AGA IgG method. The methods that use a mix of tTG and gliadin peptides as the antigenic preparation have a specificity slightly lower than that of the methods that use only tTG.

KEYWORDS: IgG anti-tissue transglutaminases antibodies; celiac disease; selective IgA deficiency; antigliadin antibodies; antideaminated gliadin peptides antibodies

SELECTIVE IgA DEFICIENCY

Immunoglobulin (IgA) is quantitatively the most important isotype in humans and the second most abundant serum immunoglobulin, playing a key role in host protection of respiratory and intestinal mucosal surfaces.

Selective IgA deficiency (SIgAD) is the most common primitive immunodeficiency, occurring with a prevalence of 1:300–1:800 in the European normal population.^{1–3} It is a heritable condition associated with the ancestral haplotype HLA-A1, Cw7, B8, DR3, DQ2,⁴ characterized by total IgA serum level below 0.05 g/L with IgM and IgG serum levels remaining within normal ranges.

At present, we know that IgA expression in B cells is controlled by cytokines and interactions with T cells, and in particular that the cross-linking of the B cell CD40 exerted by the T cell CD40 ligand, in combination with transforming growth factor- β (TGF- β) or interleukin-10 (IL-10), induces class-switch recombination from IgM to IgA.^{5,6} However, the molecular basis of the SIgAD disorder remains elusive. Recently, Hummelshoj *et al.*⁷ showed that B cells from IgA-deficient subjects have a reduced expression of both activation-induced cytidine deaminase (an ssDNA deaminase essential for class-switch recombination and strictly induced in stimulated B cells undergoing class switching) and of α -germline transcripts upon stimulation with TGF- β , interferon- γ (IFN- γ), and IL-10. They also showed that IgA-deficient B cells are capable of producing IgA, but have a defect in signaling through the TGF- β /IL-10 pathway.

Despite the fact that most IgA-deficient subjects are not ill, IgA deficiency has been associated with many specific disorders,³ such as sinopulmonary infections, gastrointestinal disorders, allergy, malignancies, and autoimmune diseases, including celiac disease (CD).

SELECTIVE IgA DEFICIENCY AND CD

CD is a genetically determined autoimmune disorder related to gluten intolerance and it may be estimated from population-based studies that its frequency is 0.5–1%.^{8–10} Early clinical studies showed a 10- to 20-fold increased risk for CD in SIgAD subjects^{11–13} and these data have recently been confirmed by other studies conducted on very large cohorts of IgA-deficient patients.^{14,15}

CD symptoms at presentation are similar in patients with or without SIgAD, but a higher prevalence of the silent form was observed in CD-associated SIgAD.^{7,11} For this reason, screening for CD is mandatory in patients with SIgAD, but it represents a special challenge since the specific IgA-class autoantibodies against gliadin (AGA), endomysium (EMA), and tissue transglutaminase (tTG) are not produced.

SEROLOGICAL DIAGNOSIS OF CD IN SIgAD PATIENTS

In 1989, Beutner *et al.*¹⁶ documented for the first time the case of a celiac patient with SIgAD who was negative for IgA EMA but positive for EMA, anti-reticulin, and AGA IgG antibodies. Since then, other authors have evaluated the diagnostic performance of various serological markers for CD of the IgG class in patients with CD and SIgAD^{11,14,15,17–23}; TABLE 1 is a schematic representation of the data so obtained. As one can see, the test that seems to have greater diagnostic accuracy is the EMA IgG test, because, except in the case of the data obtained by van Meensel *et al.*,²³ its sensitivity and specificity approach 100%. However, immunofluorescence technique used to measure EMA IgG antibodies is cumbersome and dependent on subjective interpretation. In addition, it is influenced by the type of tissue substrate used. In fact, using monkey esophagus substrates, false-positive results occur because of cross-reactivity with connective tissue fibers. The use of fetal tissues, such as umbilical cord from premature neonates, or of secondary antibody conjugates specific for IgG1 may help, to some extent, to overcome this background staining.²⁰ For the reasons cited above, however, research on EMA IgG, although having a higher diagnostic accuracy when done by experts, has not been used much in clinical laboratories. Of decisively greater use in these labs is the IgG AGA, insofar as enzyme-linked immunosorbent assay (ELISA) methods are more objective and can be automated. Data on the diagnostic performance of IgG AGA, such as those that emerged from the study reported in TABLE I are different, however. Compared to the higher sensitivity found in some studies,^{11,17,19,21–23} Lenhardt *et al.*¹⁵ found them to be of low diagnostic sensitivity. Numerous studies, in addition, have shown such serological markers to be characterized by poor specificity.^{15,17,23}

Since tTG has been identified as the main antigen recognized by EMA,²⁴ ELISA tests have been developed to identify both IgA and IgG anti-tTG antibodies. IgG class assays using recombinant human (rh)-tTG as antigen have

TABLE 1. Antibody profile in patients with CD and SIgAD and in the control population

Authors (reference)	No.	IgG-EMA +	IgG AGA +	IgG anti-tTG+
SIgAD with CD				
Beutner <i>et al.</i> 1989 (16)	1	1	1	ND
Meini <i>et al.</i> 1996 (17)	5	ND	5	ND
Rittmeyer <i>et al.</i> 1996 (18)	2	ND	1	ND
Cataldo <i>et al.</i> 1997 (19)	12	ND	12	ND
Cataldo <i>et al.</i> 1998 (11)	54	ND	51	ND
Korponay-Szabó <i>et al.</i> 1999 (20)	2	2	1	ND
Cataldo <i>et al.</i> 2000 (21)		20	20	20
Kumar <i>et al.</i> 2002 (22)	14	14	14	13
Korponay-Szabó <i>et al.</i> 2003 (14)	78	77	ND	77
Lenhardt <i>et al.</i> 2004 (15)	11	ND	5	11
van Meensel <i>et al.</i> 2004 (23)	5	2	5	2 to 5 *
Controls				
SIgAD without CD				
Meini <i>et al.</i> 1996 (17)	60	ND	11	ND
Cataldo <i>et al.</i> 2000 (21)	10	0	0	2 **
Kumar <i>et al.</i> 2002 (22)	10	0	0	0
Korponay-Szabó <i>et al.</i> 2003 (14)	157	0	ND	0
Lenhardt <i>et al.</i> 2004 (15)	112	ND	22	4 ***
Healthy without SIgAD				
Cataldo <i>et al.</i> 2000 (21)	25	0	0	1 **
van Meensel <i>et al.</i> 2004 (23)	69	ND	17	0 to 39 *

* Results obtained using nine different IgG anti-tTG methods.

** Results obtained with a method using as antigen tTG from guinea pig.

*** Subjects with normal biopsy but HLA DQ2/DQ8 +.

proved to be highly reliable serological tests for the diagnosis of CD in patients with SIgAD.^{14,15,22} In a comparative study of nine methods for measuring class-IgG anti-tTG on five SIgAD patients, however, van Meensel *et al.*²³ have shown how the diverse commercial methods presented significant differences in terms of sensibility and specificity (TABLE 1), where in general the most sensitive methods are also the ones that give the highest number of false positives in the control population. Since, however, the data obtained by van Meensel *et al.*²³ cannot be considered conclusive, insofar as they are done on a limited cohort, recently, we conducted a comparative study of nine methods used for measuring anti-tTG IgG antibodies in a larger cohort of patients with CD and SIgAD and of controls.

COMPARATIVE EVALUATION OF NINE COMMERCIAL ASSAYS FOR IgG ANTI-tTG DETECTION IN SIgAD

Patients and Methods

In our study we included 20 consecutive CD patients with SIgAD, diagnosed according to the revised ESPGAN criteria²⁵ and not on a gluten-free

diet, and 113 controls (9 patients with SIgAD and without CD but affected by other gastrointestinal disorders, 54 patients suffering from chronic liver diseases, and 50 healthy subjects). The research on anti-tTG IgG antibodies was conducted in the sera of all the patients with CD and in all the controls using 9 different commercial methods: 6 using rh-tTG as antigen (D-tek, Mons, Belgium; ReCombi, Phadia Diagnostics, Freiburg, Germany; Immco Diagnostics, Buffalo, NY; Orgentec Diagnostika, Mainz, Germany; Radim, Pomezia, Italy; Euroimmun, Lübeck, Germany); 1 using native human (nh)-tTG (Inova Diagnostics, San Diego, CA, USA); 1 using rh-tTG cross-linked with gliadin-specific peptides (Aesku Diagnostics, Wendelsheim, Germany); and 1 using purified human endomysial antigen and gliadin fragments (Generic Assays, Dahlewitz, Germany). All ELISAs were performed according to the manufacturers' instructions.

Sensitivity and specificity for each kit were calculated both using the manufacturers' cutoff and the optimal decision threshold obtained by receiver operating characteristic (ROC) curves analysis.

All the sera were also tested for AGA IgG by a commercial ELISA test (Radim) and by a new test using a preparation of purified deaminated gliadin peptides (DGP) as antigen (Quanta Lite Gliadin II, Inova Diagnostics, San Diego, CA).

Results and Discussion

Sensitivity and specificity of the nine anti-tTG kits are shown in TABLE 2. At the cutoff levels provided by the manufacturers, diagnostic sensitivity ranged between 75% (1 kit) and 95% (5 kits), and specificity between 88% (1 kit) and 100% (2 kits). At the cutoff levels obtained on the basis of ROC plot analysis the overall diagnostic accuracy increased: the sensitivity ranged between 75% (1 kit) and 95% (7 kits), and the specificity between 94% (1 kit) and 100% (5 kits).

The IgG AGA assay showed lower sensitivity and specificity, while the anti-DGP IgG antibodies provided higher diagnostic accuracy than IgG AGA, but generally lower accuracy than the anti-tTG IgG antibodies.

Our study has shown that most of the commercial methods tested have optimal diagnostic accuracy. Only the D-tek kit had low sensitivity, but it was nevertheless higher than the sensitivity of IgG AGA, while the two methods that use tTG associated with gliadin as antigen source with the intention of increasing diagnostic sensitivity, ended up slightly less specific than the other methods, probably because of the link to antibodies against gliadinic epitopes. The choice of cutoff on the basis of the ROC curve, the higher number of cases, and the selection of patients with CD on a clinical basis, irrespective of their antibody status, might be able to explain the better results we obtained relative to those of van Meensel *et al.*²³ In terms of diagnostic accuracy, almost all of

TABLE 2. Sensitivity (SE) and specificity (SP) of the different IgG anti-tTG assays according to both the cutoffs suggested by the manufacturers and to the threshold values providing the best combination of SE and SP on the basis of ROC curves analysis

Manufacturer	Cutoff	SE% (CI 95%)	SP% (CI 95%)	Cutoff	SE% (CI 95%)	SP% (CI 95%)
IgG anti-tTG						
Aesku	15 U/mL	85 (62 – 97)	95 (90 – 96)	11.5 U/mL	95 (75 – 100)	94 (88 – 97)
D-tek	25 U/mL	75 (51 – 91)	97 (92 – 99)	31.4 U/mL	75 (51 – 91)	98 (90 – 99)
Euroimmun	1 (ratio)	80 (56 – 94)	100 (97 – 100)	0.7 (ratio)	95 (75 – 100)	100 (97 – 100)
Phadia	7 U/mL	95 (75 – 100)	100 (97 – 100)	4.0 U/mL	95 (75 – 100)	100 (97 – 100)
Generic Assays	20 U/mL	95 (75 – 100)	91 (84 – 96)	33.8 U/mL	90 (68 – 98)	95 (75 – 100)
Immco	20 U/mL	95 (75 – 100)	91 (84 – 96)	56.1 U/mL	95 (75 – 100)	100 (97 – 100)
Inova	20 U/mL	75 (51 – 91)	99 (97 – 100)	11.6 U/mL	95 (75 – 100)	99 (97 – 100)
Orgentec	10 U/mL	95 (75 – 100)	94 (88 – 97)	31.5 U/mL	95 (75 – 100)	100 (97 – 100)
Radim	7 U/mL	95 (75 – 100)	88 (80 – 93)	11.0 U/mL	95 (75 – 100)	100 (97 – 100)
IgG AGA (Radim)				15 RU/mL	40 (19 – 68)	87 (76 – 94)
IgG anti-DGP (Inova)				7 U/mL	80 (56 – 94)	98 (90 – 99)

Diagnostic SE and SP of IgG AGA and IgG anti-DGP are also reported.

the methods used demonstrated performances equivalent to those achieved by other authors using EMA IgG determination,^{14,21} confirming, therefore, the higher correlation between IgG anti-tTG and IgG EMA.^{14,21,26} Taken together, our results indicate that the diagnostic accuracy of anti-tTG IgG in SIgAD is comparable to that of anti-tTG IgA in IgA-sufficient patients and that anti-tTG IgG represents the recommended test for screening CD in subjects with SIgAD.

Finally, the higher sensitivity and specificity obtained by tests that use DGP in comparison to tests that use gliadin, would confirm the hypothesis that gliadin antibodies from CD patients exhibit enhanced binding to gliadin that has been deaminated by the enzymatic action of transglutaminase.²⁷

CONCLUSIONS

The second-generation IgG anti-tTG methods are reliable serological marker for the diagnosis of CD in patients with SIgAD. The assays containing both tTG and gliadin peptides in the antigenic preparation seem burdened by a lower specificity. Measuring anti-tTG IgG antibodies with currently available methods, therefore, is to be recommended for screening all subjects with SIgAD for CD.

Because of its low efficiency, testing for AGA IgG should not be routinely used for CD in SIgAD patients; on the other hand, the new assay for AGA detection that uses deaminated gliadin peptides as antigen can be used if there is a high suspicion of CD and IgG anti-tTG is negative.

REFERENCES

1. KOISTINEN, J. 1975. Selective IgA deficiency in blood donors. *Vox Sang.* **29**: 192–202.
2. AMMAN, A.J. 1991. Selective IgA deficiency. In *Basic and Clinical Immunology*, 7th ed. D.P. SITES & A.L. TERR, Eds.: 77–79. Lange. East Norwalk, CT.
3. CUNNINGHAM-RUNDLES, C. 2001. Physiology of IgA and IgA deficiency. *J. Clin. Immunol.* **21**: 303–309.
4. VORECHOVSKY, I., M. CULLEN, M. CARRINGTON, *et al.* 2000. Fine mapping of IGAD1 in IgA deficiency and common variable immunodeficiency: identification and characterization of haplotypes shared by affected members of 101 multiple case families. *J. Immunol.* **164**: 4408–4416.
5. DEFRANCE, T., B. VANBERVLIET, F. BRIERE, *et al.* 1992. Interleukin 10 and transforming growth factor beta cooperate to induce anti-CD40-activated naïve human B cells to secrete immunoglobulin A. *J. Exp. Med.* **175**: 6714–6722.
6. MCINTRE, T.M., M.R. KEHRY, C.M. SNAPPER, *et al.* 1995. Novel *in vitro* model for high-rate IgA class switching. *J. Immunol.* **154**: 3156–3161.

7. HUMMELSHOJ, L., L.P. RYDER, L.K. NIELSEN, *et al.* 2006. Class switch recombination in selective IgA-deficient subjects. *Clin. Exp. Immunol.* **144**: 458–466.
8. CATASSI, G., I.M. RATSCH, E. FABIANI, *et al.* 1994. Coeliac disease in the year 2000: exploring the iceberg. *Lancet* **343**: 200–203.
9. MAKI, M., K. MUSTALAHTI, J. KOKKONEN, *et al.* 2003. Prevalence of celiac disease among children in Finland. *N. Eng. J. Med.* **348**: 2517–2524.
10. VOLTA, U., S. BELENTANI, F.B. BIANCHI, *et al.* 2001. High prevalence of celiac disease in Italian general population. *Dig. Dis. Sci.* **46**: 1500–1505.
11. CATALDO, F., V. MARINO, A. VENTURA, *et al.* 1998. Prevalence and clinical features of selective immunoglobulin A deficiency in coeliac disease: an Italian multicentre study. *Gut* **42**: 362–365.
12. COLLIN, P., M. MAKI, O. KEYRILAINEN, *et al.* 1992. Selective IgA deficiency and celiac disease. *Scand. J. Gastroenterol.* **27**: 367–371.
13. PRINCE, H.R., G.L. NORMAN & W.L. BINDER. 2000. Immunoglobulin A (IgA) deficiency and alternative celiac disease-associated antibodies in sera submitted to a reference laboratory for endomysial IgA testing. *Clin. Diag. Lab. Immunol.* **7**: 192–196.
14. KORPONAY-SZABÓ, I.R., I. DAHLBOM, K. LAURILA, *et al.* 2003. Elevation of IgG antibodies against tissue transglutaminase as a diagnostic tool for celiac disease in selective IgA deficiency. *Gut* **52**: 1567–1571.
15. LENHARDT, A., A. PLEBANI, F. MARCHETTI, *et al.* 2004. Role of human-tissue transglutaminase IgG and anti-gliadin IgG antibodies in the diagnosis of celiac disease in patients with selective immunoglobulin A deficiency. *Dig. Liv. Dis.* **36**: 730–734.
16. BEUTNER, E.H., V. KUMAR, T.P. CHORZELSKI, *et al.* 1989. IgG endomysial antibodies in IgA-deficient patient with celiac disease. *Lancet* **1**: 1261–1262.
17. MEINI, A., N.M. PILLAN, V. VILLANACCI, *et al.* 1996. Prevalence and diagnosis of celiac disease in IgA-deficient children. *Ann. Allergy Asthma Immunol.* **77**: 333–336.
18. RITTMAYER, C. & J.M. RHOADS. 1996. IgA deficiency causes false-negative endomysial antibody results in celiac disease. *J. Pediatr. Gastroenterol. Nutr.* **23**: 504–506.
19. CATALDO, F., G. MARINO, G. BOTTAIO, *et al.* 1997. Celiac disease and selective immunoglobulin A deficiency. *J. Pediatr.* **131**: 306–308.
20. KORPONAY-SZABÓ, I.R., J.B. KOVACS, A. CZINNER, *et al.* 1999. High prevalence of silent celiac disease in preschool children screened with IgA/IgG antiendomysium antibodies. *J. Pediatr. Gastroenterol. Nutr.* **28**: 26–30.
21. CATALDO, F., D. LIO, V. MARINO, *et al.* 2000. IgG1 antiendomysium and IgG antitissue transglutaminase (anti-tTG) antibodies in coeliac patients with selective IgA deficiency. *Gut* **47**: 366–369.
22. KUMAR, V., M. JARZABEK-CHORZELSKA, J. SULEJ, *et al.* 2002. Celiac disease and immunoglobulin A deficiency: how effective are the serological methods of diagnosis? *Clin. Diagn. Lab. Immunol.* **9**: 1295–1300.
23. VAN MEENSEL, B., M. HIELE, I. HOFFMAN, *et al.* 2004. Diagnostic accuracy of ten second-generation (human) tissue transglutaminase antibody assays in celiac disease. *Clin. Chem.* **50**: 2125–2135.
24. DIETERICH, W., T. EHNS, M. BAUER, *et al.* 1997. Identification of tissue transglutaminase as the autoantigen of coeliac disease. *Nat. Med.* **3**: 797–801.
25. WORKING GROUP OF ESPGAN. 1990. Revised criteria for diagnosis of celiac disease. *Arch. Dis. Child.* **65**: 909–911.

26. DAHLBOM, I., M. OLSSON, N.K. FOROOZ, *et al.* 2005. Immunoglobulin G (IgG) anti-tissue transglutaminase antibodies used as markers for IgA-deficient celiac disease patients. *Clin. Diagn. Lab. Immunol.* **12**: 254–258.
27. ALEANZI, M., A.M. DEMONTE, C. ESPER, *et al.* 2001. Celiac disease: antibody recognition against native and selectively deaminated gliadin peptides. *Clin. Chem.* **47**: 2023–2028.