International Journal of Celiac Disease, 2017, Vol. 5, No. 3, 97-100
Available online at http://pubs.sciepub.com/ijcd/5/3/1

DOI:10.12691/ijcd-5-3-1

Anti-tTg-IgA is neither a Solved Problem nor a "closed case" in Celiac Disease Diagnosis

Aaron Lerner[1,2,*], Sandra Neidhöfer[2], Torsten Matthias[2]

[1]B. Rappaport School of Medicine, Technion-Israel Institute of Technology, Haifa, Israel
[2]AESKU.KIPP Institute, Wendelsheim
*Corresponding author: aaronlerner1948@gmail.com

Abstract Anti-tissue transglutaminase (tTg) IgA are considered the most frequently used serological marker for celiac disease diagnosis. Despite its recommended leading position by the 2012 ESPGHAN diagnostic criteria, it exposes multiple false positive and negative titers. In view of the critical opinions expressed lately in the literature against the application of those criteria, the bias in the central place occupied by tTg-IgA in the new ESPGHAN CD Diagnostic Guidelines and the emergence of newer serological marker for celiac disease, it is hoped that the revised guidelines will open up the limited, problematic and single Tg2-IgA antibody for other or additional single or combined serological diagnostic markers.

Keywords: *celiac disease, antibody, diagnosis, tissue transglutaminase, neo-epitope, microbial transglutaminase*

Cite This Article: Aaron Lerner, Sandra Neidhöfer, and Torsten Matthias, "Anti-tTg-IgA is neither a Solved Problem nor a "closed case" in Celiac Disease Diagnosis." *International Journal of Celiac Disease*, vol. 5, no. 3 (2017): 97-100. doi: 10.12691/ijcd-5-3-1.

1. Introduction

Celiac disease (CD) is increasing in incidence and despite powerful serological markers its diagnosis present a great challenge [1,2]. The rise in worldwide gluten consumption, the evolutionary growth in wheat gluten content and toxicity, changing phenotype toward older age, a/hypo symptomatic presentation, multi-extraintestinal organs affected, non-gluten associated environmental factors involved and the sequential changes in the diagnostic criteria, make the suspicion, awareness and diagnosis more complex [1-14]. This is the raison why Villanueva et al, should be congratulated for tackling the topic of IgA and IgG anti-tissue transglutaminase (tTg) antibodies place in CD diagnosis [15]. Based on up-to-date literature, the authors concluded that: 1. it is insufficient to understand the difference of classes and subclasses detected in CD and other autoimmune conditions, 2. Data does not support the use of anti-tTg IgG for diagnosing CD in IgA-sufficient individuals and therefore 3. IgG should not be used in the routine diagnostic process of CD. Personally, I fully support the last two conclusions, but would like to expand on the first one. The present editorial will widen on the diagnostic reliability of the anti-tTg IgA, on the problematic new ESPGHAN diagnostic flow chart based primarily on anti-tTg IgA [16], the newer serological markers in CD diagnostics [17,18,19] and the potential functions of anti-tTg IgA- in CD induction or progression.

Table 1. Outside of normal limits: false positive and negative anti-tTg IgA antibodies (Adapted from reference [20])

	False positive	False negative
1	In face of Marsh 1 degree of intestinal injury	Complete IgA deficiency
2	Autoimmune diseases: IBD, primary biliary cirrhosis, Good pasture's syndrome, Wegener granulomatosis, rheumatoid arthritis, SLE, systemic sclerosis, type 1 diabetes, pemphigus	Refractory CD
3	Non-autoimmune disease: connective tissue diseases, non-autoimmune cirrhosis, linear IgA dermatosis, herpes gestationis, vasculitis	Small intestinal bacterial overgrowth
4	Increased IgM rheumatic factor	Age dependency, especially in the elderly
5	In face of positive anti-endomysial antibody	In face of anti-tTg subepithelial deposits
6	Transient, fluctuating positivity	During gluten-free diet
7	Childhood cerebral palsy	Some non-atrophic CD patients
8	Infectious febrile diseases	Transient, fluctuating negativity
9	End-stage heart failure	Genetic risk

2. False Positive and Negative of Anti-tTg IgA

Anti-tTg IgA- antibodies are quite sensitive and specific non-invasive celiac diagnostic markers. It is the most frequently used to screen for CD and it is recommended by ESPGHAN [16,19]. Several drawbacks still exist since its reflection of the intestinal damage and monitoring of disease activity, is not good enough. Its performance in children is better than in adulthood and elderly. It has multiple limitations and Table 1 summarizes its false positivity and negativity, as reflected in the current literature.

It is worthwhile mentioning that the antibody is directed against the enzyme tTg. The enzyme wide abundance and corporal distribution, its specialized structural conformation, vast substrate specificity, and indispensable cellular functions, explain its involvement in multiple physiological, as well as pathological conditions [21,22]. tTg is involved in genetic, metabolic, senescence, rheumatic, cancerous, nephrogenic, pulmonary, endocrine, hepatic, neurodegenerative and multiple autoimmune diseases [4,5,6,8-14,21,23]. The enzyme pleiotrophism, ubiquitous expression and pivotal biological functionality might explain some of false result of the anti-tTg IgA antibodies directed against it (Table 1).

3. Bias in the Central Place Occupied by anti-tTg IgA in the 2012 ESPGHAN CD Diagnostic Guidelines

According to the new ESPGHAN CD diagnostic criteria, additionally to other fulfilled parameters, if the titer of anti-tTg IgA is above 10 times the upper limit of the normal, the diagnosis of CD can be established without a duodenal biopsy [16]. Since then, several CD specialized groups expressed discomfort, and even criticized those guidelines [24-30].

The main criticisms of the central place occupied by anti-tTg IgA autoantibodies in the 2012 ESPGHAN guidelines, to omit, in certain circumstances, intestinal biopsy in a symptomatic child are:

1. Lack of serological markers standardization and relative definitions of the upper limit of normal cut-off levels [19,20,24,25,26,27].
2. Lack of more extended, multicenter data on the optimal multiplication times of the upper limit of normal cut-off, to be used [26,27].
3. The subjectivity and inter-observer variability of the anti-endomysial antibodies that confirm anti-tTg IgA positivity [31].
4. Insufficient understanding by the clinicians of the performance of their celiac serological tests [30].
5. Lack of adherence to and/or understanding of the guidelines, even by subspecialists [32].
6. Even the gold diagnostic standard of mucosal histology is debatable in several aspects: grading [32], intraepithelial lymphocyte count [33], and pathologist's reproducibility [34]. Notably, the decision of anti-tTg IgA antibody leading position was based on this gold criteria.
7. Geographical and national variability in CD diagnostic tools applicability, due to lack of resources [35].
8. The choice of the anti- tTg IgA as the main serological diagnostic marker for CD was challenged by multiple clinical and laboratory professionals, advocating combined serology [19,26,36-44]. And finally, to set up the stage for the next topic:
9. Lack of comparison of CD additional specific autoantibodies to challenge anti-tTg IgA premiership in the guidelines, for example with the tTg neo-epitope [17,38,40,45,46,47,48,49]. Recent observations show that the tTg neo-epitope outperforms tTg [17,39,49] and also a combination test including IgA and IgG isoforms [18]. Adding an additional autoantibody can detect Marsh 3 intestinal damage among subjects with moderate anti-tTg levels [50].

4. Newer Serological Markers in CD Diagnostics

The CD associated serological markers are continuously expanding. After more than 30 year, since the early 80th when EMA IgA was found [51,52], we witnessed the tTg, DGP IgG or IgA isotypes and combined IgA and IgG isotype emergence, in CD screening and diagnosis [18,19]. Recently, two additional CD associated markers emerged. But this family of markers represents a new concept in CD pathogenesis. tTg and its family member, the microbial Tg (mTg) are well known to deamidate gliadin, but they can also cross-link gliadin, resulting in a new 3-dimensional complex where new immunogenic epitopes are exposed, namely: tTg-neo and mTg-neo-epitope complexes. The CD patients, in face of new immunogenic molecules, mount specific antibodies, namely: anti-tTg neo-epitope and anti-mTg neo-epitope antibodies of IgG and IgA isotypes. The anti-tTg-neo-epitope IgA and IgA+IgG were shown, in multiple studies to be very reliable in CD diagnosis [17,18,19,26,39,40,45,45,47,48,49,53-58]. In fact, anti-tTg neo-epitope was shown most recently to be more reliable than the anti-tTg IgA antibody [49].

mTg is a secreted microbial transglutaminase that is very important for bacterial survival. Its sequence homology to tTg is poor, but at their active site, the homology is much higher, resulting in shared functions, mainly in cross-linking gliadin peptides [17,23,59,60,61,62,63]. Most recently, mTg-neo-epitope was shown to induce specific IgG antibodies in CD children, compared to controls. It was shown, for the first time that mTg-neo-epitope is immunogenic in a CD population [62]. Not less important, in the last ESPGHAN 2017 meeting in Prague, the pathogenicity of the mTg and gluten was shown on CD duodenal biopsies and human originating intestinal cell line. (Sebastian S, Zimmer K-P, Giessen, Germany-personal unpublished data). The authors showed that when incubated with CD intestinal biopsy or with the cell line, mTg may influence the intracellular localization of gliadin in the intestinal mucosa of CD patients. Much more, mTg reaches the lamina propria, indicating an antigenic interaction with cells of the immune system.

5. The Potential Functions of Anti-tTg IgA in CD Induction or Progression

The specific autoantibody against tTg is multifunctional, affecting many of the enzyme activities. Notably, many of them induce the loss of enzyme function, while fewer functions are associated with gain of function of the tTg. It should be stressed that until today, no beneficial protective effects, but only pathogenic ones, were assigned to those CD associated autoantibodies. In summary, the anti-tTg antibodies potentially promote small bowel intestinal or extra-intestinal damage in CD patients. A word of caution: The majority of the anti-tTg autoantibody activities where studied in vitro and ex-vivo, very few though in animals but none in vivo, in human. Its differential role in CD induction or progression is far from being discovered. Unraveling them might open some new therapeutic strategies for CD.

References

[1] Lerner A, Jeremias P, Matthias T. The world incidence of celiac disease is increasing: a review. Internat. J. Of Recent Scient. Res. 2015;7:5491-5496.

[2] Lerner A, Jeremias P, Matthias T. The world incidence and prevalence of autoimmune diseases is increasing: A review. Internat. J. Celiac Dis. 2015;3:151-155.

[3] Lerner A., Reif, S. Nonnutritional environmental factors associated with Celiac disease: The Infectome. In: Infections and Autoimmunity. Eds: Shoenfeld, Y., Agmon-Levine, N., Rose, N.R. 2nd Ed.Elsevier B.V. 2015, Chapter 50, pages 829-837.

[4] Lerner, A., Blank, M. Hypercoagulability in celiac disease-an update. Autoimmun. Rev. 2014;13:1138-41.

[5] Lerner A, Makhoul B, Eliakim R. Neurological manifestations of celiac disease in children and adults. Eur. Neurolog. J. 2012;4: 15-20.

[6] Lerner A, Matthias T. Rheumatoid arthritis-celiac disease relationship: joints get that gut feeling. Autoimm. Rev. 2015;14:1038-47.

[7] Lerner A, Matthias T. Increased knowledge and awareness of celiac disease will benefit the elderly. Internat. J. of Celiac Dis. 2015;3:112-114.

[8] Lerner A, Matthias T. Editorial: Celiac disease: intestinal, heart and skin inter-connections. Internat. J. Celiac Dis.2015: 3:28-30.

[9] Lerner A, Matthias T. Gut-bone cross talks and implications in celiac disease. Internat. J. Celiac Dis, 2016;4:19-23.

[10] Lerner A, Matthias T. The Gut-stomach Axis: Helicobacter pylori and Celiac Disease. Internat. J. Celiac Dis. 2016; 4:77-79.

[11] Lerner A, Matthias T. Extraintestinal manifestations of CD: Common pathways in the gut-remote organs' axes. Internat J Celiac Dis. 2017;5:24-27.

[12] Lerner A, Jeremias P, Matthias T. The gut-thyroid axis: beyond the thyroid: what's happening in the gut. Endocrinol Connections, in press, 2017.

[13] Lerner A, Berthelot L, Jeremias P, Abbad L, Matthias T, Monteiro RC. Gut-kidney axis: gluten, transglutaminase, celiac disease and IgA nephropathy J Clin Cell Immunol, 2017;8: In Press

[14] Eliyah Livshits O, Shauol R, Reifen R, Matthias T. Lerner A. Can Celiac Disease Present Along With Childhood Obesity? Internat J Celiac Dis. 2017;5:19-23.

[15] Villanueva M, Rojas M, Araya M. IgA and IgG Anti-transglutaminase 2 Antibodies in the Diagnosis of Celiac Disease. Internat J Celiac Dis, 2017;5(2):43-47.

[16] Husby S, Koletzko S, Korponay-Szabo IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. Journal of pediatric gastroenterology and nutrition. 2012;54:136-160.

[17] Lerner A, Jeremias P, Neidhöfer S, Matthias T. Comparison of the reliability of 17 celiac disease associated bio-markers to reflect intestinal damage. J of Clin & Cell Immunology. 2017;8: 1.

[18] Lerner A., Neidhöfer S., Matthias T. Serological markers and/or intestinal biopsies in the case-finding of celiac disease. Editorial, Internat. J. Celiac dis. 2015;3:53-55.

[19] Lerner A. Serological Diagnosis of Celiac Disease –Moving Beyond the Tip of the Iceberg. Internat. J. of Celiac Dis. Editorial. 2014;2:64-66.

[20] Lerner A, Jeremias P, Matthias T. Outside of Normal Limits: False Positive/Negative Anti TG2 Autoantibodies. Internat J Celiac Disease, 2015;3:87-90.

[21] Lerner A, Neidhöfer S, Matthias T. Transglutaminase 2 and anti transglutaminase 2 autoantibodies in celiac disease and beyond: Part A: TG2 double-edged sword: gut and extraintestinal involvement. Immunome Research, 2015;11:101-105.

[22] Lerner A, Neidhöfer S, Matthias T. Transglutaminase 2 and anti transglutaminase 2 autoantibodies in celiac disease and beyond. Part B: Anti- Transglutaminase 2 autoantibodies: friends or enemies. Immunome Research, 2015;11:3-7.

[23] Lerner A, Matthias T. Don't forget the exogenous microbial transglutaminases: it is immunogenic and potentially pathogenic. Biophysics, 2016;3:529-535.

[24] Schirru E, Jores RD, Congia M. Prudence is necessary in the application of the new ESPGHAN criteria for celiac disease omitting duodenal biopsy: a case report. Eur J Gastroenterol Hepatol. 2014;26:679-80.

[25] Zevit N, Shamir R. Diagnosis of celiac disease: where are we heading after the ESPGHAN 2012 guidelines? J Pediatr Gastroenterol Nutr. 2014;59 Suppl 1:S13-5.

[26] Barak M., Rozenberg O., Froom P., Grinberg M., Reginashvili D., Henig C., Pacht A., Lerner A. Challenging our serological algorithm for celiac disease (CD) diagnosis by the ESPGHAN guidelines. Clin Chem Lab Med. 2013;51:e257-259.

[27] Vermeersch P, Geboes K, Mariën G, Hoffman I, Hiele M, Bossuyt X. Defining thresholds of antibody levels improves diagnosis of celiac disease. Clin Gastroenterol Hepatol. 2013;11:398-403

[28] Tye-Din JA, Cameron DJ, Daveson AJ, Day AS, Dellsperger P, Hogan C et al. Appropriate clinical use of human leukocyte antigen typing for coeliac disease: an Australasian perspective. Intern Med J. 2015;45:441-50.

[29] Atherton R, Ross A, Jessop F, Williams R, Heuschkel R, Zilbauer M. Coeliac disease in children with type 1 diabetes: are current guidelines proving difficult to implement in practice? J Pediatr Gastroenterol Nutr. 2014;59:600-3.

[30] Gidrewicz D, Potter K, Trevenen CL, Lyon M, Butzner JD. Evaluation of the ESPGHAN Celiac Guidelines in a North American Pediatric Population. Am J Gastroenterol. 2015;110:760-7.

[31] 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.

[32] Marsh MN, Johnson WM, Rostami K. Mucosal histopathology in celiac disease: a rebuttal of Oberhuber's sub-division of Marsh III. Gastroenterol Hepatol Bed Bench. 2015;8:99-109.

[33] Lerner A, Matthias T. Intraepithelial lymphocyte normal cut-off level in celiac disease: The debate continues. Internat J Celiac Dis.2016;4:4-6.

[34] Mubarak A, Nikkels P, Houwen R, Ten Kate F. Reproducibility of the histological diagnosis of celiac disease. Scand J Gastroenterol. 2011;46:1065-73.

[35] Smarrazzo A, Misak Z, Costa S, Mičetić-Turk D, Abu-Zekry M, Kansu A, Abkari A, et al. Diagnosis of celiac disease and applicability of ESPGHAN guidelines in Mediterranean countries: a real life prospective study. BMC Gastroenterol. 2017;17:17.

[36] Sugai E, Moreno ML, Hwang HJ, Cabanne A, Crivelli A, Nachman F, Vázquez H, et al. Celiac disease serology in patients with different pretest probabilities: is biopsy avoidable? World J Gastroenterol. 2010;16:3144-52.

[37] Gujral N1, Freeman HJ, Thomson AB. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. World J Gastroenterol. 2012;18:6036-59.

[38] Bizzaro N1, Tozzoli R, Villalta D, Fabris M, Tonutti E. Cutting-edge issues in celiac disease and in gluten intolerance. Clin Rev Allergy Immunol. 2012;42:279-87.

[39] Rozenberg O, Lerner A, Pacht A, Grinberg M, Reginashvili D, Henig C, Barak M. A novel algorithm for the diagnosis of celiac disease and a comprehensive review of celiac disease diagnostics. Clin Rev Allergy Immunol. 2012;42:331-41.

[40] Rozenberg O, Lerner A, Pacht A, Grinberg M, Reginashvili D, Henig C, Barak M. A new algorithm for the diagnosis of celiac disease. Cell Mol Immunol.2011;8:146-9.

[41] Volta U, Granito A, Parisi C, Fabbri A, Fiorini E, Piscaglia M, Tovoli F, Grasso V et al.Deamidated gliadin peptide antibodies as a routine test for celiac disease: a prospective analysis. Clin Gastroenterol. 2010;44:186-90.

[42] Agardh D. Antibodies against synthetic deamidated gliadin peptides and tissue transglutaminase for the identification of childhood celiac disease. Clin Gastroenterol Hepatol. 2007;5:1276-81.

[43] Niveloni S, Sugai E, Cabanne A, Vazquez H, Argonz J, Smecuol E, Moreno ML, Nachman F, Mazure R, et al. Antibodies against Synthetic Deamidated Gliadin Peptides as Predictors of Celiac Disease: Prospective Assessment in an Adult Population with a High Pretest Probability of Disease. Lin Chem 2007;53:2186-2192.

[44] Holding S, Wilson F, Spradbery D. Clinical evaluation of the BioPlex 2200 Celiac IgA and IgG Kits - A novel multiplex screen incorporating an integral check for IgA deficiency. J Immunol Methods. 2014; 405:29-34.

[45] Tozzoli R., Kodermaz G., Tampoia M., Visentini D., Tonutti E., Bizzaro N. [detection of autoantibodies specific for transglutaminase-gliadin peptides complex: a new way to explore the celiac iceberg.] It J Lab Med. 2010;6;28-35.

[46] Matthias T., Pfeiffer S., Selmi C., Eric Gershwin M. Diagnostic challenges in celiac disease and the role of the tissue transglutaminase-neo-epitope. Clin Rev Allergy Immunol. 2010; 38:298-301.

[47] Remes-Troche JM, Ramírez-Iglesias MT, Rubio-Tapia A., Alonso-Ramos A., Velazquez A., Uscanga LF. Celiac disease could be a frequent disease in Mexico: prevalence of tissue transglutaminase antibody in healthy blood donors. J Clin Gastroenterol. 2006;40:697-700.

[48] Remes-Troche JM, Rios-Vaca A., Ramírez-Iglesias MT, Rubio-Tapia A., Andrade-Zarate V., Rodríguez-Vallejo F., López-Maldonado F., Gomez-Perez FJ, Uscanga LF. High prevalence of celiac disease in Mexican Mestizo adults with type 1 diabetes mellitus. J Clin Gastroenterol.2008; 42:460-5.

[49] Lerner A, Jeremias P, Neidhöfer S, Matthias T. Antibodies against neo-epitope tTg complexed to gliadin are different and more reliable then anti-tTg for the diagnosis of pediatric celiac disease. J Immunol Methods. 2016;429:15-20.

[50] Schirru E, Danjou F, Cicotto L, Rossino R, Macis MD, Lampis R et al. Anti-actin IgA antibodies identify celiac disease patients with a Marsh 3 intestinal damage among subjects with moderate anti-TG2 levels. Biomed Res Int. 2013;2013:630463.

[51] Kumar V, Beutner EH, Lerner A, Jain N, Chorzelski TP. Comparison of disease specificity of antiendomysial and antigliadin antibodies. In: "Immunopathology of the skin", Eds: EH Beutner, TP Chorzelski, V Kumar. John Wiley, New York, pp 483-488, 1987.

[52] Rossi TM, Kumar V, Lerner A, Heitlinger LA, Tucker N, Fisher J. Relationship of endomysial antibodies to jejunal mucosa pathology: specificity towards both symptomatic and asymptomatic celiacs. J Pediatr Gastroenterol Nutr 1988;7:858-863.

[53] Bizzaro N., Tozzoli R., Villalta D., Fabris M., Tonutti E. Cutting-edge issues in celiac disease and in gluten intolerance. Clin Rev Allergy Immunol. 2012; 42:279-87.

[54] Matthias T., Pfeiffer S., Selmi C., Eric Gershwin M. Diagnostic challenges in celiac disease and the role of the tissue transglutaminase-neo-epitope. Clin Rev Allergy Immunol. 2010; 38:298-301.

[55] Matthias T, Neidhöfer S, Pfeiffer S, Prager K, Reuter S, Gershwin ME. Novel trends in celiac disease. Cell Mol Immunol. 2011;8: 121-5.

[56] Porcelli B, Ferretti F, Vindigni C, Terzuoli L. Assessment of a Test for the Screening and Diagnosis of Celiac Disease. J Clin Lab Anal. 2016;30:65-70.

[57] Lytton SD, Antiga E, Pfeiffer S, Matthias T, Szaflarska-Poplawska A, Ulaganathan VK, Placek W, Fabbri P, Hall R, Caproni M. Neo-epitope tissue transglutaminase autoantibodies as a biomarker of the gluten sensitive skin disease--dermatitis herpetiformis. Clin Chim Acta. 2013 ;415:346-9.

[58] Lerner A, Neidhöfer S, Jeremias P, Matthias T. The diversities between the neo-epitope and the IgA- tissue transglutaminase autoantibodies in celiac disease. In: Autoantigens, autoantibodies, autoimmunity. Volume 10th , Eds: K Conrad, Chan EKL, Andrade LEC, Steiner G, Pruijn GJM, Y Shoenfeld. Page 220-226, e-pub, 2015.

[59] Lerner A, Matthias T. Possible association between celiac disease and bacterial transglutaminase in food processing: a hypothesis. Nutr Rev. 2015;73:544-552.

[60] Lerner A, Matthias T. Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease. Autoimmun Rev. 2015;14:479-89.

[61] Lerner A, Matthias T. Microbial transglutaminase is a potential environmental inducer of celiac disease. In: From Autoantibody Research to Standardized Diagnostic Assays in the Management of Human Diseases. Volume 10th, Eds: K Conrad, Chan EKL, Andrade LEC, Steiner G, Pruijn GJM, Y Shoenfeld. 12th symposium on autoantibodies, 2015, Dresden, Germany. Page 227-233, Pabst Science Publishers, Lengerich, Germany, e-pub.

[62] Matthias T, Jeremias P, Neidhöfer S, Lerner A. The industrial food additive microbial transglutaminase, mimics the tissue transglutaminase and is immunogenic in celiac disease patients. Autoimmun Rev, 2016;15:1111-1119.

[63] Lerner A, Matthias T. Are Microbial Enzymes Used Safe in the Processed Food Industries? Food and Bioprocess Technology, 2016;9: 2125-2126.