03

THE NEOEPITOPE TISSUE TRANSGLUTAMINASE PERFORMANCES IN CELIAC DISEASE DIAGNOSIS. PROF. AARON LERNER
NEOEPITOPE EXPERIENCE: A LUSTRUM WORKING WITH NEOEPITOPE ANTIGEN FOR THE DETECTION OF ANTI TRANSGLUTAMINASE ANTIBODIES. DR. SANDRA VERBEKE

PERSPECTIVES IN AUTOIMMUNITY

ISSUE 3. CELIAC DISEASE: ANTI-NEOEPITOPE TISSUE TRANSGLUTAMINASE AUTOANTIBODIES

INTRODUCTION

Celiac disease is an immune-mediated inflammatory disorder in which, in people with a genetic predisposition, the ingestion of gluten (a protein found in wheat, barley and rye) causes an immune reaction that primarily affects the small intestine. Over time, the resulting damage to the lining of the small intestine prevents nutrients being absorbed and can lead to serious complications. Other parts of the body can also be affected.

Celiac disease affects about 1% of the population globally, although this may be an underestimation as many people with the disorder remain undiagnosed [Lebwohl et al. 2018]. The prevalence of celiac disease varies between different parts of the world, probably due to differences in the frequency of HLA-DQ2 and HLA-DQ8 and gluten intake. However, the prevalence is increasing worldwide [Lebwohl et al. 2018].

Celiac disease can develop at any age. It is 1.5 times more common in women than men [Caio et al. 2019]. People with a first-degree relative (parent, sibling, child) with celiac disease are at increased risk. Celiac disease often coexists with other disorders, such as type 1 diabetes, autoimmune thyroid disease, autoimmune liver disease, Down's syndrome, or IgA deficiency [Caio et al. 2019; Al-Bawardy et al. 2017].

In this third issue of Perspectives in Autoimmunity, Prof. Aaron Lerner from Zabludowicz Center for Autoimmune Diseases of Sheba Medical Center, Tel-Hashomer, Israel, and Dr. Sandra Verbeke, head of Immunology section (Autoimmunity and Proteins) from Laboratory of Santa María Clinic (2002 - 2019), Santiago de Chile, describe the importance of the anti-neoepitope tissue transglutaminase (tTG) autoantibodies as a new biomarker for the diagnosis of celiac disease (CD), and its diagnostic performance compared to the classical biomarkers used so far.

In his article, Prof. Lerner introduce the basis of the anti-neoepitope tTG autoantibodies and addresses the diagnostic performance of this new biomarker compared to the classical ones such as: the tTG autoantibodies, the anti-gliadin antibodies, and the anti-deamidated gliadin peptide antibodies. The author describes how the adoption of anti-neoepitope tTG autoantibodies for the diagnostic of celiac disease could improve the diagnostic performance for celiac patients, currently being underdiagnosed or late diagnosed in many cases.

GRIFOLS

In the second article of this issue, Dr. Verbeke explains her real world laboratory experience with more than 4.000 specimens tested for anti-neoepitope tTG autoantibodies. Dr. Verbeke shows the advantages and limitations of this new biomarker and compares it with the conventional technique using recombinant human transglutaminase. Finally, the author highlights the importance of achieving a good resolution of both positive and negative results far away from the "indeterminacy zone" with this new biomarker, and the need for an early diagnosis to prevent complications and future associations with other autoimmune diseases that celiac patients may suffer.

Articles

THE NEOEPITOPE TISSUE TRANSGLUTAMINASE PERFORMANCES IN CELIAC DISEASE DIAGNOSIS

PROF. AARON LERNER
Zabludowicz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Israel.

1. INTRODUCTION

The current educational newsletter named Perspectives in Autoimmunity is dedicated to the neoepitope tissue transglutaminase (tTG) autoantibody performances in celiac disease (CD) diagnosis. But, before plunging into the topic, CD will be summarized in a nutshell, the neoepitope complexes concept and CD serological markers will be presented.

1.1 Celiac disease in a nutshell

Celiac disease is an autoimmune condition presented in genetically predisposed individuals upon intake of gluten-containing prolamins (i.e. wheat, barley, rye and oat) or their ingredients[1]. CD affects around 1-1.5% of Western populations, with a North to South and a West to East gradient[2-4]. Geo-epidemiological co-localization of increased gluten consumption, HLA-DQ2/8 genotypes frequency, accompanied by CD prevalence's surge, reinforce the genetic and environmental interplay in CD development[5]. It is known that quite frequently there is misdiagnosis and the ratio of diagnosed/undiagnosed individuals can mount to 1/7, respectively[6]. Many facts contribute to the under/misdiagnosis of the disease. Its constantly changing epidemiology, clinical presentation, phenotype and incidence[2-4,7]. Its multi-organ affection and distribution, presenting numerous enteric and extraintestinal manifestations[8]. Finally, the worldwide increasing rate of gluten consumption and its detrimental side effects[9,10] and many other reasons summarized recently[3,11], contribute to the delay and to the masking of CD awareness and early prompted diagnosis. On top of that, we are currently witnessing an ongoing pandemic of CD of a large scale. In parallel to the surge in autoimmune incidences, geo-epidemiological screenings witness a gradual rise of CD incidences, spanning Western and Eastern societies over the last decades[2,12-14]. Adding the frequent a/hypo symptomatic presentation, the silent and potential CD occurrence, the atypical clinical phenotypes and the multiple genetic and autoimmune conditions associated with CD, one can understand the necessity and urgency of prompt and early detection of the disease[15]. The concept of a "CD iceberg", with only the "tip" of the patients being diagnosed properly

describes this phenomenon[16]. Increased awareness to the above-mentioned CD facets will increase the diagnostic rate[2-4,6,8,16-19].

A question arises what are the consequences of missed or delayed CD detection? Firstly, undiagnosed CD patients cannot receive timely and beneficial gluten withdrawal therapy[20]. Being undiagnosed and untreated they are at risk of definitive stunted growth, developing secondary autoimmune disorders, infertility, osteoporosis/osteopenia, several malignancies and overall increased morbidity and mortality[7,21-23]. The economic burden of under/missed diagnosis is huge and should be taken in account by the economical and health regulatory authorities[24].

Notably, the under diagnostic rate, the high a/hypo symptomatic presentations, the consequences of delayed detection, and above all, the increased performances of the serological tests, put the CD associated antibody's markers in the front line of CD screening, earlier detection and improved diagnosis[21,25]. The title "Paediatric coeliac disease: early diagnosis for better lifelong health" reinforces the early diagnosis for enhanced health[26].

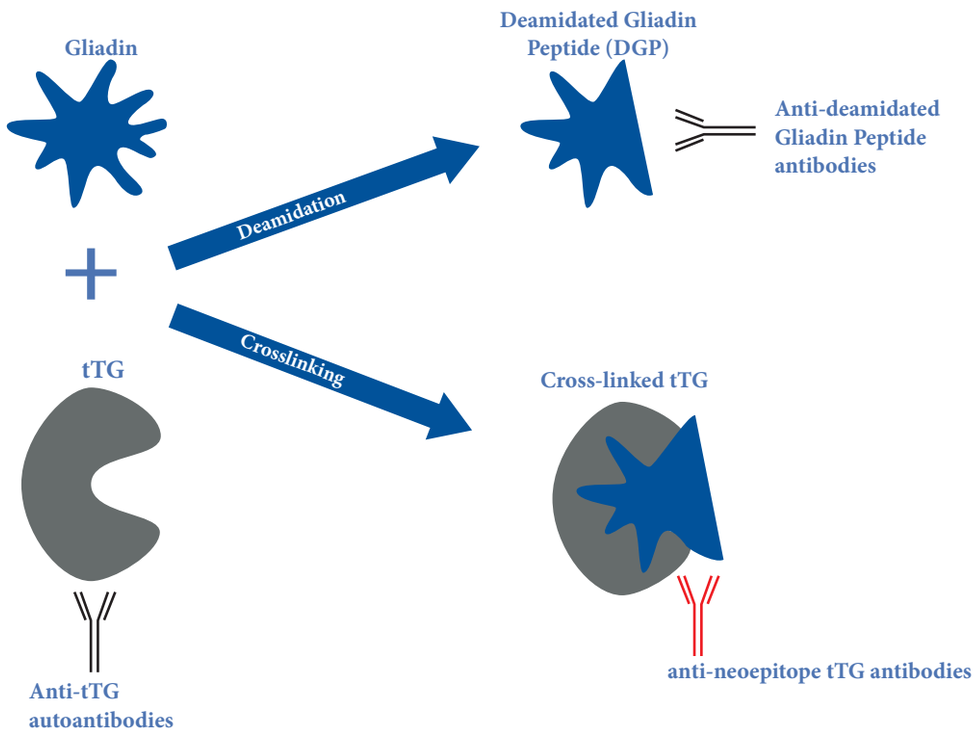
1.2 The repertoire of celiac disease associated serological markers

Multiple autoantibodies were described in CD patients' sera[27], yet only few are considered associated with CD diagnosis[21,25]. Historically, the anti-gliadin antibody, described in the early 80th, was the first one. It is not an autoantibody, since it is directed against the nutritional gliadin and its specificity and sensitivity are below 90% with a very low positive predictive value[25,28,29]. The autoantibody directed against the endomysium, named anti-endomysial antibody took over in 1983, for the next 14 years[25,30,31] till Dieterich W, et al. discovered the anti-tTG, which is directed against the autoantigen of CD[32-34]. The anti-tTG-IgA autoantibody is the most prevalent marker used worldwide and it is recommended by several gastrointestinal associations, including by ESPGHAN[25,35,36]. Despite being a prime marker, the anti-tTG antibody has its limitations, false + and – which were summarized lately[37,38]. Before switching to the newer anti-neoepitope tTG antibodies, one should mention the anti-deamidated gliadin peptide antibody, established in 2011[25,39], hence, criticized lately for its lower diagnostic performances[40].

1.3 The concept of antibodies directed to neoepitope complexes

One of the driving mechanisms of autoimmune disease is posttranslational modification of proteins (PTMP)[41]. The human gut is heavily populated with active enzymes capable of transforming naïve proteins/peptides to immunogenic one, thus losing tolerance to those modified molecules[41,42]. PTMP pathway may contribute to the aberrant modification of enteric luminal or host proteins thus generating an autoimmune cascade, by the host, driving autoimmune genesis. Rheumatoid arthritis and CD are classical examples where the enzymes peptidyl arginine deiminase or tTG induce citrullination or deamidation/transamidation, respectively, in the disease evolvement[43, 44]. The enteric PTMP enzymatic machinery generates a neo complex and exposes new (neo) epitopes that face the local immune systems, resulting in autoantibody production. The CD associated neoepitope tTG is such an example (figure1). In CD, the endogenous tTG heavily active in the sub-epithelial compartment and as most recently shown, derived from shed enterocytes into the gut lumen[45]. The authors hypothesized that gut luminal tTG derived from shed enterocytes is the source of pathogenic tTG in CD. The neoepitope tTG are increasingly used as reflected by the steady

Figure 1: A schematic presentation of transglutaminase involvement in anti tTG and anti-neoepitope tTG production in celiac disease.



Abbreviations: CD- celiac disease; tTG- tissue transglutaminase; mTG- microbial transglutaminase; PTMP- posttranslational modification of protein

rise in the yearly number of publications in the last decade (figure 2). Here is the logical place to introduce the microbial transglutaminase (mTg) which is operating in the human gut lumen and functionally imitates its family member: the tTG. Both are capable to deamidate and transamidate gluten/gliadin peptides[42,46]. In fact, since 2015 the mTg is suggested as a new potential environmental factor in CD induction[41,42,46-51]. More and more data are accumulating for the hypothesis that the CD process is starting in the human intestinal lumen where gluten/gliadin peptides encounter tTG and mTg that initiate the PTMPs gluten/gliadin induced neo molecules. Most probably, the tTG/mTG cross linked gliadin peptides, with their exposed neoepitopes are the first spark to start the CD avalanche.

2. LITERATURE SURVEY ON NEOEPITOPE tTG AUTOANTIBODIES PERFORMANCES IN CELIAC DISEASE

In the last decade the IgA and the combined IgA+IgG neoepitope tTG autoantibodies have gained share in CD diagnosis. It is induced while the endogenous enzyme tTG crosslink gliadin-specific peptides to form a neo-complex, thus, changing the electrical, structural and conformational features to create and expose neoepitopes. These PTMPs are crucial for changing the gliadin from a naive tolerant to auto-immunogenic cross-linked molecule. The antibodies against neoepitopes of the tTG-gliadin complex provide a new screening and diagnostic test in CD. Table 1 describes the main characteristics of the neoepitope tTG autoantibody in CD.

Figure 2: The timeline of the number of neoepitope transglutaminase publications per year. (Adopted from PubMed on 4, July, 2020).

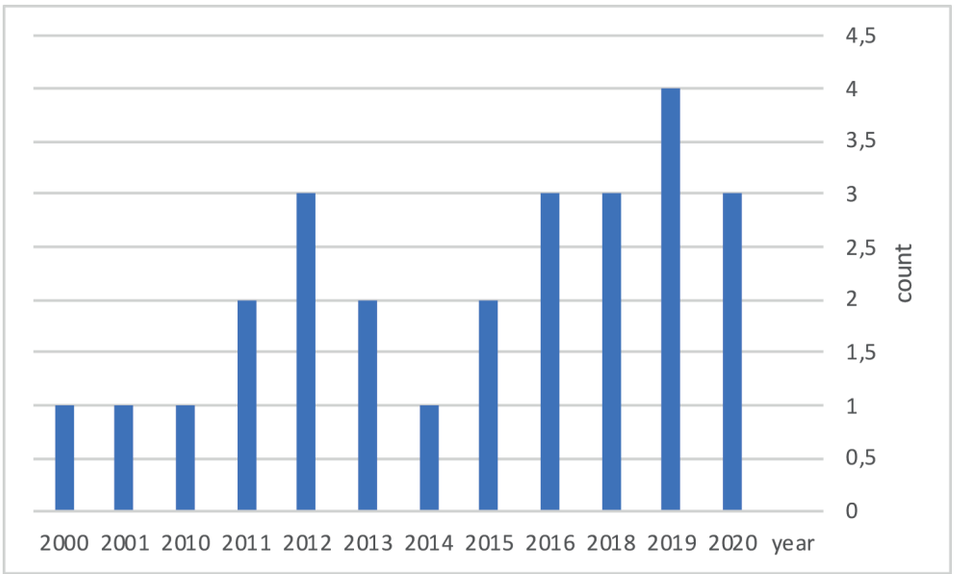


Table 1: The neoepitope tTG autoantibody's performance in CD compared to other CD associated antibodies.

Neoepitope tTG performances	ex vivo/in vitro studies	Values/comments	references
sensitivity	Ex vivo	91-98%	47, 52-56
specificity	Ex vivo	90.4-100%	47, 52-56
Reflection of duodenal pathology	Ex vivo	r2=0.645, p<0.0001 r2=0.649, p<0.0001 r2=0.795, P<0·0001 r2=0.957, p<0.0001	47 55 56 54
Early appearance	Ex vivo	Comparable to anti-tTG appearance	56, 57
Good during infancy	Ex vivo	Below 2 y	56
Predictive ability	Ex vivo	Done in Italy	52, 53
Reflect other gluten dependent conditions	Ex vivo	Dermatitis herpetiformis	58

cut-offs estimated from receiver operating characteristic (ROC) curve	In vitro	0.96-0.99	47, 55, 56
Potential shared epitopes with neoepitope mTg but not with IgA-tTG	In vitro	Competition studies	47, 54
Compared to tTG	ex-vivo	Better performances	47, 54-56
Compared to endomysial autoantibodies	ex-vivo	Better performances	55, 56
Compared to deamidated gliadin peptide antibodies	ex-vivo	Better performances	55, 56, 59
Synthetic neoepitope tTG	ex-vivo	Good performance but never compared to non-synthetic neoepitope tTG	60

Looking at table 1 content, it can be concluded that the neoepitope tTG autoantibodies have a very high sensitivity, specificity and cut-offs estimated from receiver operating characteristic curve. They reflect significantly the duodenal damage, have predictive ability and appear earlier during life cycle and even during infancy. When compared to other CD serological markers the neoepitope tTG outperforms tTG, deamidated gliadin peptide and the anti- endomysial antibodies' performances. It seems that, at least as for today, the neoepitope tTG wins the race in serological diagnosis of CD. The future will disclose if the synthetic neoepitope tTG is good enough to compete with the non-synthetic one. A back to back comparison between the two is highly needed. In fact, several studies have shown the benefits of screening for CD using the neoepitope tTG complex strategy in the general population[53,61] in high-risk subjects[62-66] and with other gluten dependent conditions[58].

3. WHAT ARE RECENT, BACK TO BACK, COMPARISONS OF SEROLOGICAL MARKERS FOR CELIAC DISEASE TELLING US? /WHAT CAN WE LEARN FROM RECENT COMPARISONS?

Several recent studies compared all the available CD antibodies on a well characterized CD/control sera biobank. When reliability of 17 CD associated biomarkers to reflect intestinal damage were explored, the neoepitope tTG stood out as the most reliable one[55]. Intriguingly, in 2020, Agardh D et al. reaffirm the good performances of neoepitope tTG on a multicultural Swedish CD population[56]. Taking into account the performance based on AUC, enteric damage reflection and predictability at an early age, the combined anti-neoepitope tTG IgA+IgG was the most effective diagnostic biomarker for pediatric CD. Recent studies joined others who found the neoepitope tTG to perform well, compared to other serological markers of CD[47,54,62-66]. Based on the necessity and urgency to improve the rate of CD diagnosis and implement a gluten-free diet as soon as possible, it is suggested that anti-neoepitope tTG autoantibodies should be preferably used to detect, monitor dietary restriction compliance and reflect the mucosal pathology in CD. It is suggested that the revised ESPGHAN criteria for pediatric CD diagnosis will include anti-neoepitope tTG antibodies in the next and updated diagnostic flow chart. 1603 W

Abbreviations: CD- celiac disease; tTG- tissue transglutaminase;
mTG- microbial transglutaminase; PTMP- posttranslational modification of protein

"This publication was funded by Grifols and Prof. Aaron Lerner received an honorarium for the preparation of this article."

4. REFERENCES

1. Lerner A. The enigma of oats in nutritional therapy for celiac disease. International Journal of Celiac Disease. 2014;2:110-114.
2. 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.
3. Lerner A, Matthias T. A Silent or Hypo-symptomatic Disease Can Erupt: Acute Presentations of Celiac Disease. International Journal of Celiac Disease. 2017, 5(4), 129-132.
4. Lerner A, Lopez F, Schmiedl A, Matthias T. The Underdiagnosed Enemy: Africa Goes Celiac? International Journal of Celiac Disease. 2019, 7(1), 9-12.
5. Lionetti E, Catassi C. Co-localization of gluten consumption and HLA-DQ2 and -DQ8 genotypes, a clue to the history of celiac disease. Dig Liver Dis. 2014;46:1057-63.
6. Tommasini A, Not T, Kiren V, Baldas V, Santon D, Trevisiol C, et al. Mass screening for celiac disease using antihuman transglutaminase antibody assay. Arch Dis Child 2004; 89:512-515.
7. Lerner A. New Therapeutic Strategies for Celiac Disease. Autoimmun. Rev. 2010;9:144-147.
8. Lerner A, Matthias T, Wusterhausen P. Autoimmunity in celiac disease: Extra-intestinal manifestations. Autoimmun Rev. 2019 Mar;18(3):241-246.
9. 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 Jun;14(6):479-89.
10. Lerner A, Shoenfeld Y, Matthias T. A Review: Gluten ingestion side effects and withdrawal advantages in non-celiac autoimmune diseases. Nutr Rev. 2017 Dec 1;75(12):1046-1058.
11. Lerner A, Matthias T. Gluten free diet- tough alley in torrid time. International Journal of Celiac Disease, 2017, Vol. 5, No. 2, 50-55.

12. Lerner A, Jeremias P, Matthias T. The world incidence and prevalence of autoimmune diseases is Increasing. International Journal of Celiac Disease, 2015, Vol. 3, No. 4, 151-155.
13. Ramakrishna BS. Celiac disease: can we avert the impending epidemic in India? Indian J Med Res. 2011 Jan;133(1):5-8.
14. Wang H, Zhou G, Luo L, J. Bart A. Crusius JBA, Yuan A, Kou J, et al. Serological Screening for Celiac Disease in Adult Chinese Patients with Diarrhea Predominant Irritable Bowel Syndrome. Medicine (Baltimore). 2015 Oct;94(42):e1779.
15. Green PH, Lebwohl B, Greywoode R. Celiac disease. J Allergy Clin Immunol. 2015 May;135(5):1099-106.
16. Catassi C. New Celiac Icebergs Are Spotted, Other Are Slowly Emerging. J Pediatr Gastroenterol Nutr. 2017 Dec;65(6):601-602.
17. Lerner A, Matthias T. Celiac disease clinical, epidemiological, pathophysiological and therapeutical repertoire is expanding. International Journal of Celiac Disease, 2019, Vol. 7, No. 3, 66-68.
18. Lerner A, Matthias T. Increased knowledge and awareness of celiac disease will benefit the elderly. International Journal of Celiac Disease, 2015, Vol. 3, No. 3, 112-114.
19. Eliyah Livshits O, Shauol R, Reifen R, Matthias T, Lerner A. Can celiac disease present along with childhood obesity? International Journal of Celiac Disease Vol. 5, No. 1, 2017, pp 19-23.
20. Lerner A, Matthias T. Gluten free diet- tough ally in torrid time. International Journal of Celiac Disease. 2017, 5(2), 50-55.
21. Lerner A. Serological Diagnosis of Celiac Disease –Moving Beyond the Tip of the Iceberg. International Journal of Celiac Disease, 2014, Vol. 2, No. 2, 64-66.
22. Marafini I, Monteleone G, Stolfi C. Association Between Celiac Disease and Cancer. Int J Mol Sci. 2020 Jun 10;21(11):4155.
23. Stenson WF, Newberry R, Lorenz R, Baldus C, Civitelli R. Increased Prevalence of Celiac Disease and Need for Routine Screening Among Patients with Osteoporosis. Arch Intern Med. 2005 Feb 28;165(4):393-9.
24. Mearns ES, Taylor A, Boulanger T, Craig KJ, Gerber M, Leffler DA, et al. Systematic Literature Review of the Economic Burden of Celiac Disease. Pharmacoeconomics. 2019 Jan;37(1):45-61.
25. Lerner A, Ramesh A, Matthias T. Serological diagnosis of celiac disease: new biomarkers. Gastroenterol Clin North Am. 2019 Jun;48(2):307-317.
26. Kelly D, Mearin ML, Ribes-Koninckx C. Paediatric Coeliac Disease: Earlier Diagnosis for Better Lifelong Health. J Pediatr Gastroenterol Nutr. 2018 Nov;67(5):e106.
27. Shaoul R. Lerner A. Associated authoantibodies in celiac disease. Autoimmun. Autoimmun Rev. 2007 Sep;6(8):559-65.
28. Tucker NT, Barghuthy FS, Prihoda TJ, Kumar V, Lerner A, Lebenthal E. Antigliadin antibodies detected by enzyme-linked immunosorbent assay as a marker of childhood celiac disease. J Pediatr. 1988 Aug;113(2):286-9.
29. 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.
30. Chorzelski TP, Sulej J, Tchorzewska H, Jablonska S, Beutner EH, Kumar V. IgA class endomysium antibodies in dermatitis herpetiformis and coeliac disease. Ann N Y Acad Sci. 1983;420:325-34.

31. Rossi TM, Kumar V, Lerner A, Heitlinger LA, Tucker N, Fisher J. Relationship of endomysial antibodies to jejunal mucosal pathology: specificity towards both symptomatic and asymptomatic celiacs. J Pediatr Gastroenterol Nutr. Nov-Dec 1988;7(6):858-63.
32. Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med. 1997 Jul;3(7):797-801.
33. Dieterich W, Riecken EO, Schuppan D. Immunoassay for detection of IgA antitissue transglutaminase in patients with celiac disease. Methods Mol Med. 2000;41:241-5.
34. Reif S, Lerner A. Tissue transglutaminase--the key player in celiac disease: a review. Autoimmun Rev. 2004 Jan;3(1):40-5.
35. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr. 2012 Jan;54(1):136-60.
36. Husby S, Koletzko S, Korponay-Szabo I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al. European Society Paediatric Gastroenterology, Hepatology and Nutrition Guidelines for Diagnosing Coeliac Disease 2020. J Pediatr Gastroenterol Nutr. 2020 Jan;70(1): 141-156.
37. Lerner A, Jeremias P, Matthias T. Outside of Normal Limits: False Positive/Negative Anti TG2 Autoantibodies. International Journal of Celiac Disease Vol. 3, No. 3, 2015, pp 87-90.
38. Lerner A, Neidhöfer S, Matthias T. Anti-tTg-IgA is neither a solved problem nor a "closed case" in celiac disease diagnosis. International Journal of Celiac Disease. 2017, 5(3), 97-100.
39. Aleanzi M, Demonte AM, Esper C, Garcilazo S, Waggener M. Celiac disease: antibody recognition against native and selectively deamidated gliadin peptides. Clin Chem. 2001 Nov;47(11):2023-8.
40. Lerner A, Haimi M, Matthias T. Deamidated Gliadin Peptide Antibodies in Celiac Disease: A Diagnostic Driver or just along for the Ride. International Journal of Celiac Disease. 2019, 7(2), 42-45
41. Lerner A, Aminov R, Matthias T. Dysbiosis may trigger autoimmune diseases via inappropriate posttranslational modification of host proteins. Front Microbiol. 2016 Feb 5;7:84.
42. Lerner A, Rustam Aminov R, Matthias T. Transglutaminases in Dysbiosis As Potential Environmental Drivers of Autoimmunity. Front Microbiol. 2017; 8: 66.
43. Lerner A, Matthias T. Rheumatoid arthritis-celiac disease relationship: joints get that gut feeling. Autoimmun Rev. 2015 Nov;14(11):1038-47.
44. Lerner A, Neidhöfer S, Matthias T. Beyond the joint: what's happening in the gut. International Journal of Celiac Disease. 2016, 4(4), 127-129.
45. Iversen R, Amundsen SF, Kleppa L, Fleur du Pré M, Stamnaes J, Sollid LM. Evidence that pathogenic transglutaminase 2 in celiac disease derives from enterocytes. Gastroenterology. 2020 Apr 14;S0016-5085(20)30491-1.
46. Lerner A, Matthias T. Possible association between celiac disease and bacterial transglutaminase in food processing: a hypothesis. Nutr Rev. 2015 Aug;73(8):544-52.
47. 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 Dec;15(12):1111-1119.
48. Lerner A, Matthias T. Processed Food Additive Microbial Transglutaminase and Its Cross-Linked Gliadin Complexes Are Potential Public Health Concerns in Celiac Disease. Int J Mol Sci. 2020 Feb 8;21(3):1127.

49. Lerner A, Matthias T. Microbial transglutaminase should be considered as an environmental inducer of celiac disease. World J Clin Cases. 2019 Nov 26;7(22):3912-3914.
50. Lerner A, Matthias T. Microbial Transglutaminase is Beneficial to Food Industries but a Caveat to Public Health. Med One. 2019;4:e190001.
51. Lerner A, Matthias T. Microbial transglutaminase: a new potential player in celiac disease. Clin Immunol. 2019 Feb;199:37-43.
52. 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 Jun;42(3):279-87.
53. 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.
54. 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 Feb;429:15-20.
55. Lerner A, Jeremias P, Neidhöfer S, Matthias T. Comparison of the reliability of 17 celiac disease associated bio-markers to reflect intestinal damage. J Clin Cell Immunol. 2017;8:486.
56. Agardh D, Matthias T, Wusterhausen P, Neidhöfer S, Heller A, Lerner A. Antibodies against neo- epitope of microbial and human transglutaminases' complexes as biomarkers of childhood celiac disease. Clin Exp Immunol. 2020 Mar;199(3):294-302.
57. Matthias T, Pfeiffer S, Selmi C, Gershwin M. Diagnostic challenges in celiac disease and the role of the tissue transglutaminase-neo-epitope. Clin Rev Allergy Immunol. 2010 Apr;38(2-3):298-301.
58. 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 Jan 16;415:346-9.
59. Lerner A, Haimi M, Matthias T. DGP Deamidated Gliadin Peptide Antibodies in Celiac Disease: A Diagnostic Driver or just along for the Ride? International Journal of Celiac Disease. 2019, 7(2), 42-45.
60. Choung RS, Rostamkolaei SK, Ju JM, Marietta EV, Dyke CTV, Rajasekaran JJ, et al. Synthetic Neoepitopes of the Transglutaminase-Deamidated Gliadin Complex as Biomarkers for Diagnosing and Monitoring Celiac Disease. Gastroenterology. 2019 Feb;156(3):582-591.e1.
61. 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 Sep;40(8):697-700.
62. 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 Jun;42(3):331-41.
63. 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 Mar;8(2):146-9.
64. 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 Jul 10;51(11):e257-9.

65. 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. May-Jun 2008;42(5):460-5.
66. Tonutti E, Visentini D, Fabris M et al. Antibodies to the transglutaminase-deamidated gliadin complex: A new serological approach to the diagnosis of celiac disease. Proceedings of the 7th International Congress on autoimmunity, Ljubljana, 5-9 May, 2010.

Articles

NEOEPITOPE EXPERIENCE: A LUSTRUM WORKING WITH NEOEPITOPE ANTIGEN FOR THE DETECTION OF ANTI-TRANSGLUTAMINASE ANTIBODIES

DR. SANDRA VERBEKE PALMA
Head of Immunology section (Autoimmunity and Proteins), Laboratory, Santa María Clinic (2002 - 2019). Santiago de Chile

1. INTRODUCTION

The clinical laboratory delivers analytical results that are used in both medical clinical and public health contexts, so they should reflect the patient's clinical and pathophysiological condition as accurately as possible. When deciding to implement a new diagnostic aid technique, or even to change the existing one for another, this decision should first consider offering examinations that avoid delays in correct diagnosis, unnecessary treatments, additional diagnostic tests or even the lack of a suitable treatment. In other words, said change must offer better results, the most accurate and reliable possible for the examinations in question[1].

These concepts become stronger when we are faced with the need to collaborate in the diagnosis of pathologies whose clinical presentation includes a wide spectrum of signs and symptoms, involving various medical specialities and a broad differential diagnosis. This is the case of celiac disease, whose natural history, both in forms of presentation and in the advancement of knowledge on its pathophysiological mechanisms, has presented to those of us working in immunological laboratories with the permanent challenge of providing new, increasingly sensitive and specific techniques to efficiently detect the marker autoantibodies that collaborate in the investigation and monitoring of these patients[2].

More than a decade ago, three specific situations made me reflect on the need to test and incorporate more specific techniques for the detection of anti-transglutaminase antibodies. One of them was the gradual but constant increase in adults, with mainly extra-intestinal signs and symptoms, referred to the laboratory for differential diagnostic tests for coeliac disease. This undeniably changed the universe of study, since samples often reflect associated clinical conditions, for example due to other concomitant diseases, which could have interfered with the results obtained with the techniques used up to that time. The second consider-

ation was migration of applications from the traditional anti-endomysium/gliadin antibody pair to endomysium/transglutaminase and subsequently transglutaminase/endomysium in a 3:1 request growth ratio in the final pair. This leads us to the third situation, which refers to the changes that the antigens used in the various detection kits were undergoing due to technological advancement, and that we were adopting at all times, starting with the extracts of transglutaminase, guinea pig transglutaminase, purified human transglutaminase and, finally, recombinant human transglutaminase. Although the limitations observed with each of these antigens gradually decreased as they were molecularly purified and perfected, the greater the number of samples we received and the more heterogeneous the universe of patients became, the more the minimal remaining interferences were accentuated[3,4].

2. EXPERIENCE

The foregoing led us to compare our conventional technique using recombinant human transglutaminase with a neoantigen: transglutaminase plus gliadin peptides obtained as a protein complex that is produced under physiological conditions in vivo, called "tTG neocomplex" or transglutaminase/deaminated peptides, also known as neoepitope, and which, in coeliac patients, induces the formation of anti-neoepitope tTG autoantibodies against the different parts of this protein group, being a highly reactive and more immunogenic compound than native antigens. Rozenberg et al. among other authors, agree that this new technique should be implemented as a first step in the diagnostic algorithm, and that obtaining a positive result would imply continuing the study with the recombinant/endomysial human transglutaminase pair. However, this is impractical in our laboratories, owing to both cost and operational availability for the timely delivery of results to the patient and their physician. Taking duodenal biopsy and antiendomysial antibodies as the reference technique, the comparison of both techniques revealed a higher sensitivity and specificity obtained with the new neoepitope technique (S: 100 - E: 92.3%) than with the recombinant human transglutaminase technique (S: 88.3 - E: 78.9%), in line with that published by Porcelli *et al.*, who evaluated several kits on the market. This could be explained, in part, by that stated by Torsten *et al.* and Lerner *et al.*, since this neoantigen could detect a broader group of autoantibodies, increasing the sensitivity of the assay and being useful in those "problem" patients where the serology is negative[5-8].

Routine use techniques should be able to detect early on the unusual situations currently present in the diagnosis of CD, especially in the adult population,[6-10] and achieve good resolution of both positive and negative results, as far away of the cutting zone of the technique, to decrease the "indeterminacy zone" or with uncertain results, avoiding the possibility of obtaining values close to the cut, which greatly disorient the clinician, due to the diversity of gluten-related pathologies, currently known and that make up a set of presumptive diagnostic entities. When reviewing the results obtained during one year, having processed approximately 4,000 samples, of which 94% were clearly negative, only 3.6% were recorded in the uncertainty zone up to the upper cut-off limit value.The analysis of the characteristics of this latter group, patients with values in the indeterminacy zone at the cut-off limit (18 U/ml), added to those that were detected with values between 18 and 20 U/ml, and whose final diagnosis and histology defined them as non-coeliac patients, led us to propose a cut-off value for our population of 20 U/ml. In our experience, this allows: a) an increase in the PPV value from 74.8% (positive cut-off value > 18) to 81.9% (positive cut-off value > 20 U/ml); b) a reduction in the number of patients in the "zone of indeterminacy", incorporat-

ing the idea that with this reduction, in turn, cut-off points more related to the reality of the population being studied locally and geographically could be established, avoiding, on many occasions, an unnecessary period of subsequent observation and the continuity of the diagnostic algorithm in each patient with an indeterminate result[7,8]; and c) a decrease in the number of false positives due to other autoimmune diseases and due to abnormal characteristics of the liver profile[9-11].

It should also be noted that for the laboratory worker it is vitally important to know the cross-reactions, interferences and false positives that a new antigen used as a captor may present, and which may be due to the pathophysiology of concomitant diseases at the time of the study of the markers for differential diagnosis of CD. Our analysis, in agreement with other authors[7,8] showed that certain situations, such as fatty liver, abusive consumption of alcohol, bilirubin, transaminases and/or elevated GGT, thyroid autoantibodies, anti smooth muscle, or in pathologies such as viral hepatitis, cirrhosis, autoimmune liver disease or other gastrointestinal diseases such as irritable bowel syndrome, are factors that seem to interfere with this technique, ultimately obtaining diagnoses other than CD.

Although establishing one's own cut-off value considerably reduces false positives, it is important to suggest interpreting a positive result with caution in the absence of another marker (EMA or DPG) or negative histology and proposing follow-up of the case until its definitive diagnosis.

One final important point to note is that neoepitope detects early those patients with different presentation situation and especially, transgressors of the gluten-free diet, who can present negative or weakly positive serology with conventional techniques. In our experience, a greater number of patients with mild or even involuntary transgressions, or in monitoring improvement or following their diet from the moment of diagnosis, were detected, showing their ability to detect that the duodenal mucosa has not yet normalised its architecture, despite the gluten-free diet, which fully agrees with that stated by Porcelli *et al.*[8] and Rozenberg *et al.*[6] who refer to similar situations in their work. Silvester *et al*. showed that the habitual markers were not good indicators of histological normalisation, and that it could perhaps be inferred that the new neoepitope technique has a better correlation with histological improvement, or better still, with the detection of mucosa even with histological damage, even though the patient is on a gluten-free diet[12]. It is important to remember how relevant rigorous compliance with the gluten-free diet is for the patient, and the importance of having a highly sensitive and specific technique capable of detecting minimal violations and alerting about them, to allow the clinician to search, with the patient, for the possible foods that are causing this transgression[13,14].

When laboratories decide to introduce neoepitope to detect anti-transglutaminase antibodies, although the enzyme immunoassay (ELISA) is indicated in the report template received by the patient as the technique used for their process, it is also highly important to incorporate the use of neoepitope as an antigen in said technique, since, owing to the foregoing, the results will often not be comparable to conventional techniques. This, in addition to avoiding uncomfortable situations, will allow better communication with clinicians, who must finally interpret and make recommendations to their patients.

Considering the importance of early diagnosis to prevent complications and future associations with other autoimmune diseases that a coeliac patient may suffer from not being introduced to a gluten-free diet in a timely fashion, it is essential to adopt the idea of "lowering the line and increasing the tip of the iceberg" as expressed by Lerner *et al.*,[15] giving a new approach to the diagnostic algorithm, and in which *neoepitope* has a preponderant role as a diagnostic tool.

3. CONCLUSIONS

The neoepitope technique could be considered a more accurate tool as the first routine marker, both for diagnosis and for follow-up of coeliac patients, in both the paediatric and adult populations.

We highlight its greater ability to resolve positive and negative results, presenting a "minimum indeterminacy zone" and a low proportion of results close to the cut-off value.

Neoepitope allows the early detection of patients with different presentation situation and transgressors of the gluten-free diet, who can present negative or weakly positive serology with conventional techniques.

We recommend interpreting positive results not consistent with histology with caution, and we suggest following up and/or complementing the complete serological and histological algorithm.

We suggest doing the exercise of one's own cut-off value, wherever possible regional, with a representative universe study of processed samples, in relation to the biopsy and the final diagnosis of the enrolled patients. This could significantly increase the PPV of the technique.

"This publication was funded by Grifols and Dr. Sandra Verbeke received an honorarium for the preparation of this article."

4. REFERENCES

1. Sistema de gestión de la calidad en el Laboratorio (LQMS): Manual. Organización Mundial de la Salud, Clinical and Laboratory Standards Institute y CDC. Biblioteca OMS 2016.
2. Volta U, Villanacci V. Celiac Disease: Diagnostic Criteria in Progress. Cell Mol Immunol. 2011 Mar;8(2):96-102.
3. Caja S, Mäki M, Kaukinen K, Lindfors K. Antibodies in Celiac Disease: Implications Beyond Diagnostics. Cell Mol Immunol. 2011 Mar;8(2):103-9.
4. Fasano A, Catassi C. Clinical practice. Celiac disease. N Engl J Med. 2012 Dec 20;367(25):2419-26.
5. Rozengerg O, Lerner A, Pacht A, Grinberg M, Reginashvili D, Henig C et al. A new algorithm for the diagnosis of celiac disease. Cell Mol Immunol. 2011 Mar;8(2):146-9.
6. Torsten M, Pfeiffer S, Selmi C, Gershwin M. Diagnostic Challenges in Celiac Disease and the Role of the Tissue Transglutaminase-Neo-Epitope. Clin Rev Allergy Immunol. 2010 Apr;38(2-3):298-301.
7. Rozenberg O, Lerner A, Pacht A, Grinberg M, Reginashvili D, Henig C, et al. A novel algorithm for the diagnosis of celiac disease and a comprehensive review of celiac disease

diagnostics. Clin Rev Allergy Immunol. 2012 Jun;42(3):331-41. doi: 10.1007/s12016-010-8250-y.

8. Choung R, Rostamkolaei S, Ju j, Marietta E, Van Dyke C, Rajasekaran J, et al. Synthetic Neoepitopes of the Transglutaminase–Deamidated Gliadin Complex as Biomarkers for Diagnosing and Monitoring Celiac Disease. Gastroenterology. 2019 Feb;156(3):582-591. e1.
9. Porcelli B, Ferretti F, Vindigni C and Terzuoli L. Assessment of a test for the screening and diagnosis of celiac disease. J Clin Lab Anal. 2016 Jan;30(1):65-70.
10. Lerner A, Ramesh A, Matthias T. Serologic Diagnosis of Celiac Disease: New Biomarkers. Gastroenterol Clin N Am. 2019 Jun; 48(2):307-317. doi: 10.101.2019
11. Torrutti E, Bizzaro N. Diagnosis and classification of celiac disease and gluten sensitivity. Autoimmun Rev. Apr-May 2014;13(4-5):472-6.
12. Silvester J, Kurada S, Szwajcer A, Kelly C, Leffler DA and Duerksen D. Test for serum transglutaminase and endomysial antibodies do not detect most patients with celiac disease and persistent villous atrophy on gluten-free diet: a meta-analysis. Gastroenterology. 2017 Sep;153(3):689-701.e1. doi: 10.1053/j.gastro.2017.05.015.
13. Cruchet S, Verbeke S. Dieta libre de gluten y disminución del riesgo de asociación de enfermedades autoinmunes en el paciente celíaco. Rev. chil. nutr. [online]. 2003, vol.30, n.2, pp.102-105.
14. Verbeke S, Cruchet S, Gotteland M, Ríos G, Hunter B, Chávez E, Brunser O, Araya M. Risk markers for insulin-dependent diabetes mellitus. Rev Med Chil. 2004 Aug;132(8):979-84.
15. Lerner A. Serological diagnosis of celiac disease – Moving beyond the tip of the iceberg. International Journal of Celiac Disease, 2014, Vol. 2, No. 2, 64-66.

TRENDING TOPICS

THE NEOEPITOPE TISSUE TRANSGLUTAMINASE PERFORMANCES IN CELIAC DISEASE DIAGNOSIS

ANTIBODIES AGAINST NEO-EPITOPE tTG COMPLEXED TO GLIADIN ARE DIFFERENT AND MORE RELIABLE THEN ANTI-tTG FOR THE DIAGNOSIS OF PEDIATRIC CELIAC DISEASE.

Lerner A, Jeremias P, Neidhöfer S, Matthias T.
J Immunol Methods. 2016 Feb;429:15-20.
https://pubmed.ncbi.nlm.nih.gov/26684936/

SYNTHETIC NEOEPITOPES OF THE TRANSGLUTAMINASE-DEAMIDATED GLIADIN COMPLEX AS BIOMARKERS FOR DIAGNOSING AND MONITORING CELIAC DISEASE.

Choung RS, Rostamkolaei SK, Ju JM, Marietta EV , Van Dyke CT, et al.
Gastroenterology. 2019 Feb;156(3):582-591.e1.
https://pubmed.ncbi.nlm.nih.gov/30342033/

EUROPEAN SOCIETY PAEDIATRIC GASTROENTEROLOGY, HEPATOLOGY AND NUTRITION GUIDELINES FOR DIAGNOSING COELIAC DISEASE 2020.

Husby S, Koletzko S, Korponay-Szabo I, Kurppa K, Mearin ML, Ribes-Koninckx C, et al.
J Pediatr Gastroenterol Nutr. 2020 Jan;70(1): 141-156.
https://pubmed.ncbi.nlm.nih.gov/31568151/

EVIDENCE THAT PATHOGENIC TRANSGLUTAMINASE 2 IN CELIAC DISEASE DERIVES FROM ENTEROCYTES.

Iversen R, Amundsen SF, Kleppa L, Fleur du Pré M, Stamnaes J, Sollid LM.
Gastroenterology. 2020 Apr 14;S0016-5085(20)30491-1.
https://pubmed.ncbi.nlm.nih.gov/32302613/

NEOEPITOPE EXPERIENCE: A LUSTRUM WORKING WITH NEOEPITOPE ANTIGEN FOR THE DETECTION OF ANTI-TRANSGLUTAMINASE ANTIBODIES

A NOVEL ALGORITHM FOR THE DIAGNOSIS OF CELIAC DISEASE AND A COMPREHENSIVE REVIEW OF CELIAC DISEASE DIAGNOSTICS.

Rozenberg O, Lerner A, Pacht A, Grinberg M, Reginashvili D, Henig C, et al.
Clin Rev Allergy Immunol. 2012 Jun;42(3):331-41.
https://pubmed.ncbi.nlm.nih.gov/21279475/

ASSESSMENT OF A TEST FOR THE SCREENING AND DIAGNOSIS OF CELIAC DISEASE.
Porcelli B, Ferretti F, Vindigni C and Terzuoli L.
J Clin Lab Anal. 2016; 30: 65-70.
https://pubmed.ncbi.nlm.nih.gov/25385391/

TEST FOR SERUM TRANSGLUTAMINASE AND ENDOMYSIAL ANTIBODIES DO NOT DETECT MOST PATIENTS WITH CELIAC DISEASE AND PERSISTENT VILLOUS ATROPHY ON GLUTEN-FREE DIET: A META-ANALYSIS.
Silvester J, Kurada S, Szwajcer A, Kelly C, Leffler DA and Duerksen D.
Gastroenterology. 2017 Sep;153(3):689-701.e1.
https://pubmed.ncbi.nlm.nih.gov/28545781/

SEROLOGIC DIAGNOSIS OF CELIAC DISEASE: NEW BIOMARKERS.
Lerner A, Ramesh A, Matthias T.
Gastroenterol Clin N Am. 2019 Jun; 48(2):307-317.
https://pubmed.ncbi.nlm.nih.gov/31046977/

GNL-SDX1-2000004

Edited by: Grifols, S.A. Parc empresarial Can Sant Joan Av. de la Generalitat 152-158 08174 Sant Cugat del Vallès, Barcelona, SPAIN
Contact details and information: medaffairs.diagnostic@grifols.com