Journal of
Clinical & Cellular Immunology
ISSN: 2155-9899

Lerner et al., J Clin Cell Immunol 2017, 8:1
DOI: 10.4172/2155-9899.1000486

Research Article Open Access

Comparison of the Reliability of 17 Celiac Disease Associated Bio-Markers to Reflect Intestinal Damage

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

[1]*Rappaport School of Medicine, Technion-Israel Institute of Technology, Haifa, Israel*
[2]*ESKU.KIPP Institute, Wendelsheim, Germany*

Abstract

In view of the increasing importance of serological biomarkers for screening and diagnosing celiac disease (CD) and the lack of back-to-back comparison of their differential performance to their reliability to reflect the intestinal damage in children with CD, their performances were evaluated.

95 pediatric CD patients (mean age 8.3), 45 nonspecific abdominal pain children (AP) (mean age 7.3), 99 normal children (NC) (mean age 8.5) were tested with following ELISAs (detecting IgA, IgG or both, IgA and IgG (check)): *AESKULISA*® Gliadin (AGA), *AESKULISA*® DGP (DGP), *AESKULISA*® tTg "New Generation" (Neo-epitope tTg complexed to gliadin=tTg-neo), tTg (for in house research purpose only), *AESKULISA*® mTg neo-epitope and mTg (RUO). Anti-endomysial antibodies (EMA) were checked via immunofluorescence test. Results were compared to the degree of intestinal injury, using the revised Marsh criteria. Scatter diagrams and regression analysis comparing the 17 antibodies' activities to the degree of the intestinal damage were performed.

Most of the assays were able to discriminate patients with low and high degree of intestinal damage. Comparing the different correlations of CD associated IgA and IgG antibodies' isotypes, the tTg-neo IgA (r2=0.6165, p<0.0001) and tTg-neo check (r2=0.6492, p<0.0001) stood out, significantly, as the best indicators for intestinal damage in CD. EMA-IgA, tTg, DGP checks and mTg-neo IgG correlated closely to the mucosal injury.

The highest optical densities (medium 2.94 ± 1.2, p<0.0001) were measured in the tTg-neo IgA ELISA of patients with Marsh 3c.

As a conclusion, it is suggested that tTg-neo IgA/IgG antibodies should be used preferably to closely reflect intestinal damage during screening and diagnosing childhood CD. EMA-IgA, tTg and DGP checks and mTg-neo IgG titers followed the tTg-neo check performance. mTg-neo IgG may present a new serological biomarker for CD.

Keywords: Celiac disease; Neo-epitope tissue transglutaminase; Tissue transglutaminase; Microbial transglutaminase; Deamidated gliadin peptide; Antibodies; Serology; Bio-markers; Intestine; Pathology

Introduction

Celiac disease (CD) is a small intestine inflammatory autoimmune disorder in genetically susceptible individuals, triggered by digestion of prolamins contained in wheat, barley, rye and partially in oat. The accepted incidence in Western countries is 1-1.5%. The majority of patients are still under-diagnosed, but increasing awareness and improved serological performances, raise the diagnostic yield. In contrast, in high-risk populations the average risk of CD can reach 5-10% [1]. In the last decades CD frequency is increasing, joining the surge in autoimmune disease incidence and prevalence worldwide [2,3].

CD is a life-long multi-faced condition with an increased risk of complications. Hematological and gastrointestinal malignancies, osteoporosis/penia and other extraintestinal manifestations, decreased height, malnutrition and nutritional deficiencies, fertility impairment, stillbirth, dismaturity, hypercoagulability, psychosocial compromise, impairment of quality of life and-if left untreated-increased mortality and additional autoimmune conditions, to name a few [4]. The epidemiology and phenotype of CD are constantly changing, early diagnosis and subsequent adherence to a gluten-free diet is highly recommended. It has been shown that the classic intestinal clinical picture of malnutrition, chronic diarrhea and nutritional deficiencies are disappearing and extraintestinal presentations are emerging. Nowadays, we are witnessing an epidemiological shift in the disease phenotype toward a more advanced age, and increased prevalence of latent, hyposymptomatic or asymptomatic presentations [5]. All these changes make reliance on symptomatology more remote and therefore diagnosis of the disease more difficult [6]. Recent, major improvements in serological markers performance, with sensitivities and specificities above 90%, are an additional reason why serological screening of CD has achieved prime importance [1].

In the beginning of 2012 the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) released a new set of guidelines for the diagnosis of CD, based on both: the significant progress in the development of specific antibody tests and the understanding of the high prevalence of specific HLA haplotypes [7]. These advances were the base of updated guidelines for CD diagnosis in symptomatic and asymptomatic children, where, in certain circumstances, small bowel biopsy can be omitted. Contrasting, multiple adult guidelines still advocate serological screening followed by obligatory intestinal biopsies for the diagnosis.

Since only the tip of the CD iceberg is above the waterline and a much larger portion remains under water undetected, it can be

***Corresponding author:** Aaron Lerner. AESKU.KIPP Institute. Mikroforum Ring 2, Wendelsheim 55234, Germany, Tel: 49-6734-9622-1010; Fax: 49-6734-9622-2222; E-mail: aaronlerner1948@gmail.com

Received: October 18, 2016; **Accepted:** January 24, 2017; **Published:** January 27, 2017

Citation: Lerner A, Jeremias P, Neidhöfer S, Matthias T (2017) Comparison of the Reliability of 17 Celiac Disease Associated Bio-Markers to Reflect Intestinal Damage. J Clin Cell Immunol 8: 486. doi: 10.4172/2155-9899.1000486

expected that prevalence of the disease will increase continuously. Therefore, presenting symptoms will continue to change towards a-/hyposymptomatic, supporting the need for improved serological screening methods. There are multiple serological tests on the market: IgA anti-endomysial antibody (EMA), IgA and/or IgG tissue transglutaminase (tTg), IgA and/or IgG deamidated gliadin peptide (DGP), whereby IgA-tTg is the most frequently used and ESPGHAN's recommended one. Several combination tests are available too: DGP IgA+G, tTg IgA+G, Gliadin IgA+G (check), CeliCheck IgA, IgG or IgA+IgG (autoantibodies against the cross-linked complex of tTg and gliadin peptides (tTg neo-epitope) and the recently described IgG and/or IgA or IgG+IgA combined mTg neo-epitope and mTg (*AESKULISA*® mTg neo-epitope and mTg, RUO) [1]. Being a new serological biomarker of CD mTg neo-epitope deserves some background information. Microbial transglutaminase (mTg) is capable of cross-linking numerous molecules (including gliadin), thereby creating an mTg-gliadin neo-complex. This post-translational modification of gliadin imitates its family member (human tTg) involved in CD.

For the last 26 years, numerous studies evaluated only one serological marker in relation to intestinal pathology, mainly EMA and later on, tTg [8,9]. Fewer studies checked two antibodies, mainly IgA-tTg and IgA-EMA, the latest being in 2012-13 [10-13]. Only in two Israeli studies, 5 different antibodies' levels were correlated to the mucosal damage [14,15]. Parizade M. et al. found that high antibody concentrations were predictive of villous atrophies for IgA+IgG-tTg, IgA+IgG-DGP and IgA-EMA [14], where EMA and IgA-tTg stood out to be the best. The second multi-marker study evaluated IgA-tTg, IgA+G-DGP, and IgA+G-EMA were the last one had the best correlation [15]. The present study extended the serological repertoire and checked, for the first time, 17 sub-types of the most frequently used antibodies to screen in order to diagnose CD.

In view of the increasing importance of serological biomarkers for screening and diagnosis of CD, their differential performance, and the lack of back to back comparison, we undertook the task to evaluate the reliability of those individual or combined antibodies (ABs) to reflect the intestinal histological injury in CD children.

Material and Methods

Patient populations

Three groups of patients were investigated:

Group 1: 95 pediatric CD patients (CD), mean age 8.3 ± 4.4 years, F/M (1/0.9). The CD group was divided according to the degree of intestinal injury, using Marsh criteria, to 6 groups M0, MI, MII, MIII a/b/c. With M0 representing a normal intestinal biopsy and MIII c total villous atrophy [16]. Those CD sub-groups contained MI=7, MII=13, MIII a=41, MIII b=27, MIII c=7 children, respectively.

Group 2: 45 children with nonspecific abdominal pain (AP), mean age 7.3 ± 5.1 years, F\M 1:0.9, served as a pathological control group (Marsh criteria=M0).

The CD and the AP groups underwent esophago-gastro-duodenoscopy using GIF-xp 20 endoscope (pentax, Tokyo, Japan). At least 5 biopsies were obtained: 4 from the second part of the duodenum and 1 from the antrum, for the diagnosis or exclusion of CD. The biopsies were immediately fixed in buffered formalin and embedded on edge in paraffin. Sections were stained with hematoxylin-eosin and Giemsa, analyzed by the pathologist and graded according to Marsh criteria, as previously described [16].

Celiac disease was diagnosed according to the revised criteria of the European Society for Pediatric Gastroenterology and nutrition, based on specific serology (anti-tissue transglutaminase antibodies, by ELISA) and duodenal biopsies [17]. All participants were on gluten containing diet and had physical examination, laboratory work-up and celiac serology. On the day of endoscopy, 5 ml of peripheral blood was withdrawn, centrifuged at 5000 c/sec for 10 minutes and the serum was kept in -80° Celsius until used in serology assays.

Group 3: 99 normal children, recruited from a normal school, mean age 8.5 ± 4.2 years, F\M 1:0.7, served as a normal control group (NC).

The study was approved by the Carmel Medical Center Helsinki committee and participants or legal guardians signed an informed consent.

Antibody determination by ELISA

The following ELISAs, detecting IgA, IgG separated or combined IgA and IgG (check) were used: *AESKULISA*® Gliadin (AGA), tTg (tTg; RUO (for in house research use only)), *AESKULISA*® DGP (DGP), *AESKULISA*® tTg New Generation (tTg neo-epitope), *AESKULISA*® mTg neo-epitope and mTg (RUO), according to the manufacturer instructions.

Antibody determination by IFA

AESKUSLIDES® EMA (endomysium antibodies) were used for indirect fluorescent IgA and IgG determination of human anti-endomysium antibodies, according to the manufacturer instruction.

The endomysium is the supporting, conective structure that surrounds the smooth fibers, located in the middle third of the esophagus. A representative positive EMA is shown in Figure 1.

Statistics

Statistical analyses were performed using the Software MedCalc® (V 15.6.1). Normally distributed values were expressed as mean ± standard deviation (SD) and were compared by the Student`s t test. Correlations were assessed by the Pearson correlation test. $p<0.05$ was considered as significant.

Scatter diagrams and regression analysis comparing the 17 antibodies' OD activities to the degrees of the intestinal damage were correlated. Antibody results were compared to the degree of intestinal injury, using revised Marsh criteria.

Results

The performance of the different tests for the 99 pediatric CD, were compared to 45 pathological controls with AP and 99 normal control children, with similar age and sex ratio. All antibodies were detected via ELISA, except for EMA which was checked via immunofluorescence.

A general overview of the different IgA and IgG antibodies' U/ml titers is shown in Figure 2. All CD associated mean antibody's levels were above the cut-off levels. IgA isotype (Figure 2A) levels were higher than of IgG isotypes (Figure 2B), except for the DGP-IgA and mTg-neo IgA, here IgG dominated. The tTg neo-epitope IgA test had the highest immunoreactivity ($P<0.0001$) and tTg neo-epitope IgG had a higher immunoreactivity compared to single tTg-IgG. The following Figures 3-6 correlate the various ABs activities to the degree of intestinal damage, as characterized by Marsh criteria.

All assays were able to differentiate between patients of low and

high degree intestinal damage. Comparing the different correlations between CD associated IgA and IgG antibodies' isotypes, EMA-IgA, tTg-neo IgA and tTg-neo check, stood out significantly as the best indicators of CD caused intestinal damage. The highest OD values (mean 2.94 ± 1.2, $p<0.0001$) were achieved using the tTg-neo IgA ELISA with Marsh 3c patients (Figure 3). The correlations, as well as their corresponding statistical significance of each AB isotype, are summarized (in increasing order) in Table 1.

Since EMA testing is performed via immunofluorescence (Figure 1), which is semi quantitative and operator-depended, it is shown separately in Figure 6. The IgA and not the IgG isotype reflects the intestinal pathology best.

For better comparison of diagnostic reliability, Table 1 lists various ABs' activities of 5 combined (checks) and 12 single isotypes associated with CD. Many of the antibodies had high specificity and low sensitivity. Combining the specificity and sensitivity by analyzing the AUC, tTg-neo IgA and EMA-IgA, in the single isotype, and tTg-neo check, in the combined isotype, stood out as best performers. Similarly, tTg-neo check and EMA-IgA reflect best the intestinal damage ($p<0.005$, Table 1). Of note, mTg-neo IgG and not the IgA isotype, had a high AUC (0.95) and reflected significantly the intestinal histology in the CD group.

Discussion

The present study explored 15 ELISA kits encompassing 5 different families of antigens (gliadin, tTg, DGP and tTg neo-epitope and mTg neo-epitope) for specific IgA, IgG, combined IgA+G reactivities and 2 immunofluorescence EMA kits. The performances of all Kits were assessed with the same blood sample, and then compared against the rigid criterion of the corresponding biopsy-demonstrated duodenal histology, further enhancing the uniformity of the study. The analysis showed that antibody levels were well correlated to the duodenal damage degree in CD children, but there was a hierarchy among the evaluated tests. The tTg neo-epitope IgA Kit occupied the first place in the IgA isotype group, DGP-IgG and tTg neo-epitope IgG shared the first place of IgG isotype Tests. The combined IgA+IgG isotypes had low correlations to intestinal histology, except for the tTg-neo check, which showed better performance than tTg-neo IgG or IgA kits . Concerning the differential performance of Kit groups, IgA Kits surpassed IgG in reflecting intestinal damage, except for the mTg-neo IgG, which showed better performance than the mTg-neo IgA kit.

Our own experience [16,18,19] and that of many others [1] favors combination tests to screen for CD. The main single antigen ELISA kit candidates are anti-tTg IgA and anti-DGP IgG, competing with the new CeliCheck combination of IgA and IgG thus omitting screening for IgA deficiency [16,18-21]. However, when reflection of intestinal pathology is concerned, single isotype Test are preferable, mainly the tTg-neo IgA.

The tTg-neo IgA kit uses a neo-epitope formed by complexing tTg and gliadin, the main antigens in CD. The basic idea is that tTg not only able to deamidate gliadin peptides but also to cross-link with a high catalytic rate [22-24]. In the latter case, tTg/gliadin complexes are formed, resulting in the formation of new epitopes (neo-epitopes), evidence has been shown in-vitro. More so, formation of the tTg-DGP complex was suggested to involve epitope spreading from gliadin to tTg [21]. The antibodies against neo-epitopes of the tTg-gliadin complex provide a new screening and diagnostic test in CD. Multiple studies have exhibited diagnostic sensitivities of 95% and specificities of 97% or more, when compared with those of traditional antibody assays [25,26]. The neo-tTg/DGP complex is potentially able to drive the development of newly formed epitopes derived from the cross-linkage between the enzyme and the substrate. It is foreseeable that the autoantibodies generated against the neo-epitope may represent the best means for screening populations, diagnosing high-risk groups and identify silent or latent patients. In fact, several studies have shown the superiority of screening for CD using the neo-tTg/DGPs complex strategy in the general population [26,27] or in high-risk groups' subjects [16,18,19,28-30]. The present results go along this serological diagnostic strategy and add the aspect of intestinal damage reflection.

Several debates and disagreements exist in the literature concerning the cross talks between serological markers and reflection of the intestinal pathology in CD.

On the pathological aspect a concern exists that intestinal biopsy is the gold standard for diagnosis. Recently incorrect biopsy interpretation causing under diagnosis [31], lack of uniformity in the use of Marsh-Oberhuber classification [32] and unrecognized, misleading pathological features that are positively associated with more advanced stages of the disease were described [33]. More so, even the cut-off for the intraepithelial lymphocyte count, a hallmark of CD intestinal pathology, is debatable [34-35]. In the serological domain assays uniformity, lack of standardization, plethora of commercial immune fluorescent and ELISA kits with variable cut-off levels are some

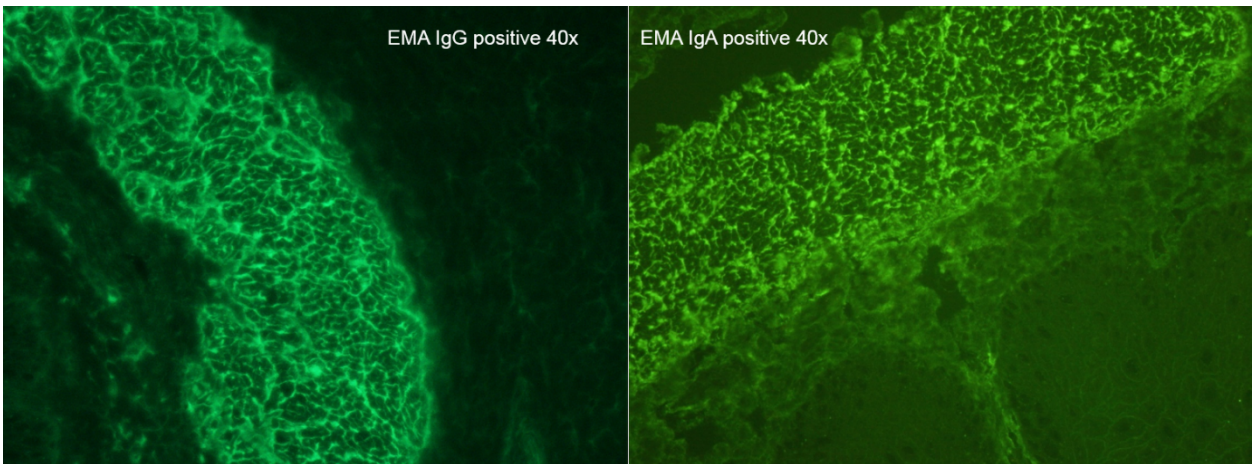

Figure 1: EMA IFA positive results as shown on the upper third esophageal slide. On the left positive-EMA IgG sera and on the right- positive EMA IgA sera, with a total magnification of 40x.

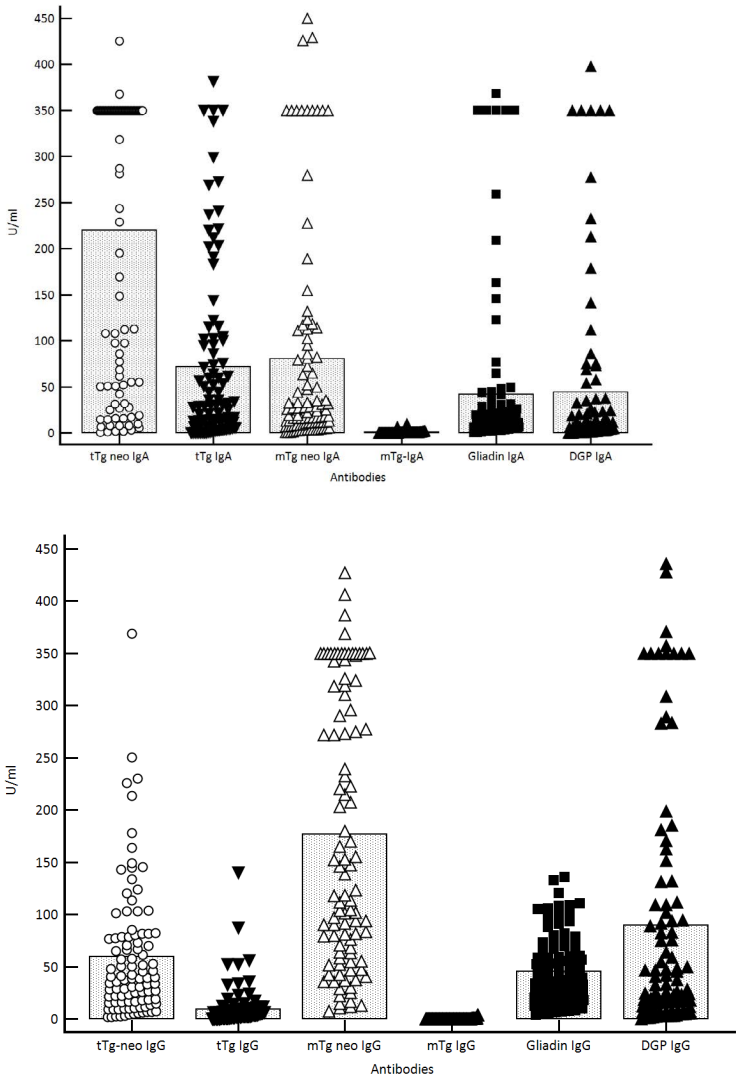

Figure 2: Comparison of A. IgA and B. IgG celiac disease associated antibody activities.

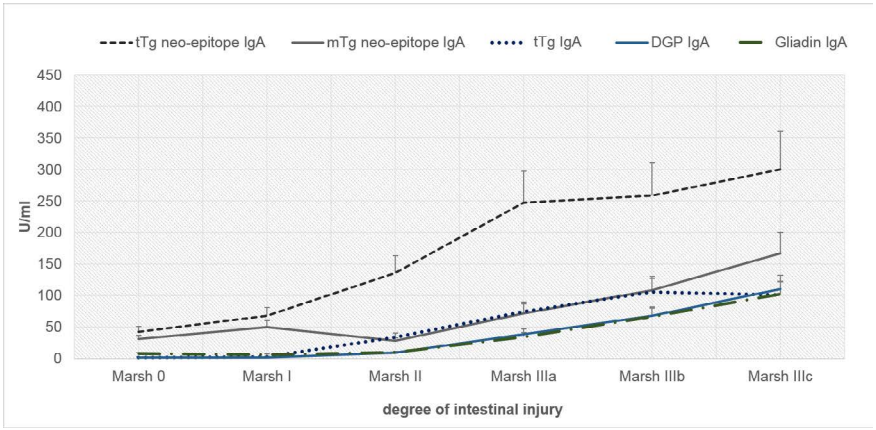

Figure 3: Correlations between IgA antibody activities and intestinal injury in celiac disease.

Assay	sensitivity	specificity	npv	ppv	AUC	corr	P
tTg IgG	13,13	98,99	53,26	92,86	0,56	0,2601	<0,0001
mTg neo IgA	64,65	98,99	73,68	98,46	0,82	0,3018	0,0003
Gliadin IgA	37,37	98,99	61,25	97,37	0,68	0,324	<0,0001
DGP IgA	66,67	98,99	74,81	98,51	0,83	0,359	<0,0001
Glia Check	57,58	98,99	70	98,28	0,78	0,3836	<0,0001
tTg IgA	60,61	98,99	71,53	98,36	0,80	0,4692	<0,0001
DGP IgG	70,71	98,99	77,17	98,59	0,85	0,4922	<0,0001
mTg neo Check	90,91	87,88	90,62	88,24	0,89	0,5127	<0,0001
Gliadin IgG	84,85	98,99	86,73	98,82	0,92	0,5181	<0,0001
tTg neo IgG	77,78	98	81,67	97,47	0,88	0,5334	<0,0001
mTg neo IgG	95,96	93,94	95,88	94,06	0,95	0,5633	<0,0001
DGP Check	82,83	98,99	85,22	98,78	0,91	0,5902	<0,0001
EMA IgG	55,7	99,3	N.D.	N.D:	0,78	0,5996	<0,0001
tTg Check	79,8	98,99	83,05	98,75	0,89	0,6093	<0,0001
tTg neo IgA	88,89	98,99	89,91	98,88	0,94	0,6165	<0,0001
tTg neo check	97,98	98,99	98	98,98	0,98	0,6492	<0,0001
EMA IgA	90,2	94,1	N.D.	N.D.	0,94	0,8094	<0,0001

Table 1: Antibodies diagnostic performances in pediatric celiac disease and their correlations between their activity and the degree of intestinal damage.

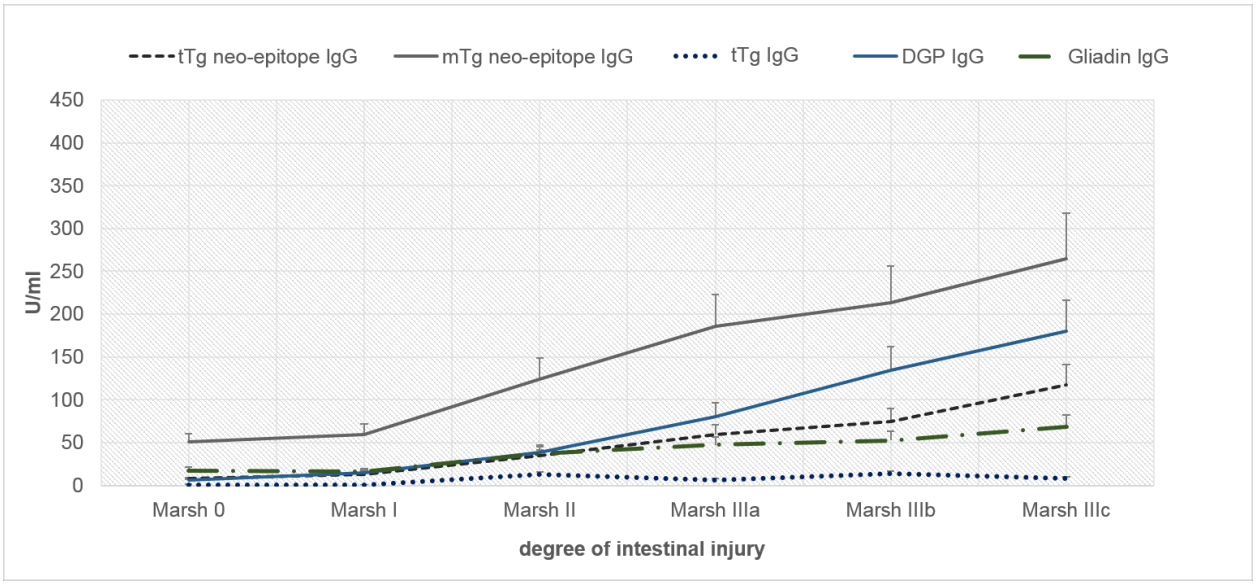

Figure 4: Correlations between IgG antibody titers and intestinal injury in celiac disease.

of the problematic aspects. On top of it, being on gluten containing diet, CD associated antibodies fluctuates or disappears spontaneously in CD children [36]. Positive celiac serology coexists with normal villous morphology [37]. Normalization of serology does not predict normalization of intestinal histology [38]. A recent study from the UK national external quality assessment service center (UK NEQAS) states that not all commercial available IgA-tTg kits are reliable and that the ESPGHAN guidelines are not readily transferable to use in all centers and should not be used in the UK [39]. Interestingly, even in the most recommended autoantibodies for CD diagnosis, multiple false positive and negative exist [40].

Screening some recent reviews and guidelines, comparing reliability of the celiac specific serological markers, one sees the vast variability in their sensitivity and specificities [1,7,41-43].

The present study has several advantages:

1. Much more CD associated antibodies were evaluated in a back-to-back experimental design (12 *vs.* 5).

2. It is the first study to incorporate the neo-epitopes of tTg/mTg in correlation to the intestinal injury.

3. The group of AP is unique in composing pathological controls with normal intestinal biopsy.

4. It is the only study that compared 17 CD associated serological markers, encompassing IgA, IgG and check isotypes.

5. The addition of the IgA+IgG-AGA is important in reference to less recent historical data.

6. All determinations were done in the same laboratory, by the same person, under same conditions for better uniformity and comparability.

7. The antibodies' activities were correlated to the same intestinal Marsh degree of atrophies.

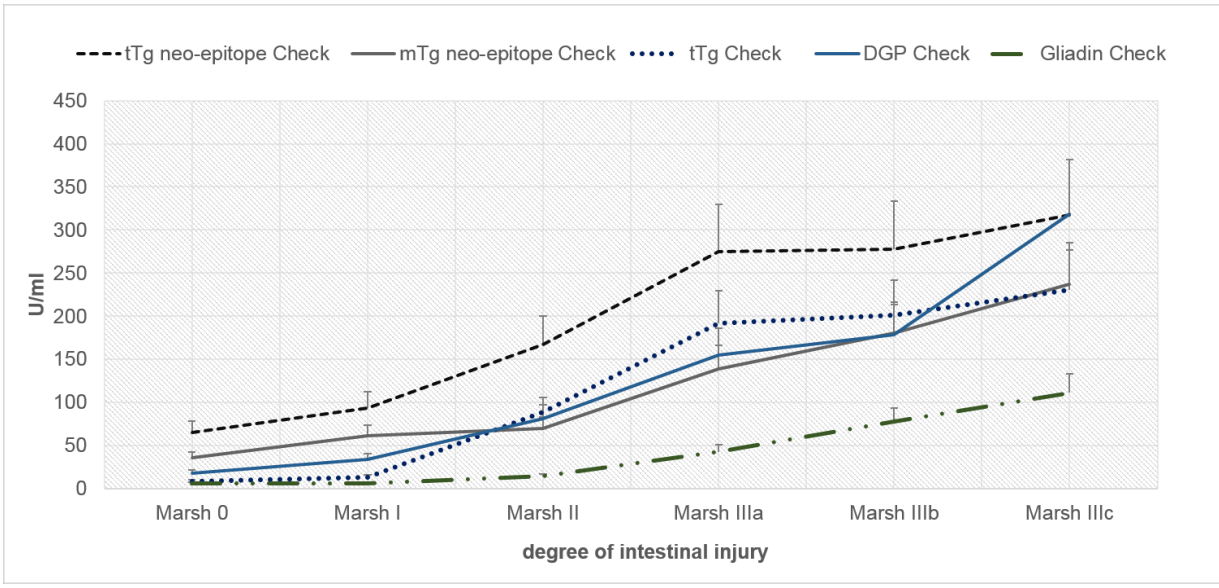

Figure 5: Correlations between IgA and IgG (check) antibody activities and intestinal injury in celiac disease.

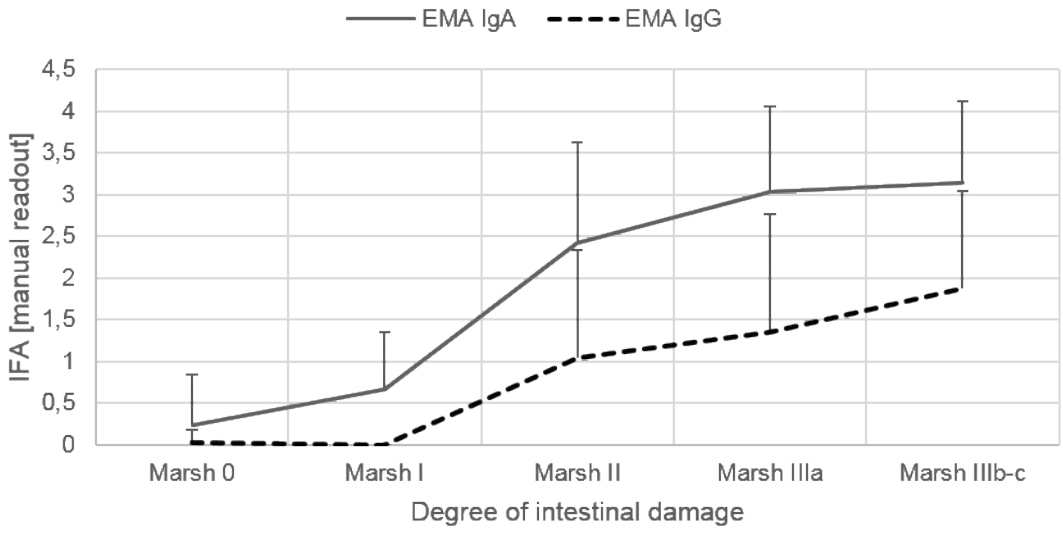

Figure 6: Correlations between EMA-IgA and IgG activities and intestinal injury in celiac disease.

On the other hand, several limitations should be mentioned:

1. A multicenter study would have better represented the global clinical reality. Moreover, a multicenter-approach could have normalized the potential biases encountered in one single center.

2. Comparing extended study populations would have increased the statistical power and the conclusion's reliability.

3. The present study represents only one aspect (intestinal pathology reflection) in the decisional algorithm of the best serological marker for CD screening and diagnosis.

For transparency, some of the data were published separately:

tTg IgA compared to tTg-neo isotypes [24]

mTg-neo isotypes compared to other ABs (but not to DGP and EMA isotypes) [44] Since mTg-neo ABs isotypes are the newest published CD biomarkers of the 17 presently compared, in the following, some facts will be summarized. mTg-neo ABs isotypes are not autoimmune ABs, they are directed against neo-epitopes, formed during the conformational changes happening in the formation mTg/gliadin cross-link complex [22, 44-46]. Since mTg is heavily used by the processed food industries, it is consumed daily [44,47]. mTg neo-epitopes have, most recently, been shown to be immunogenic in active CD patients [44,46]. Presently, it represents a good reflector of CD intestinal atrophy, although, not the best one. CD is an IgA mediated disease, so it is not astonishing that antibodies against tTg and tTg-neo are of IgA isotype. Interestingly, the predominant antibody against mTg-neo is of IgG isotype, reflecting the immune response against an

following advantages over the single tTg-IgA ELISAs:

- Higher sera reactivity, Better reflection of intestinal pathology.
- Higher sensitivity, though comparable specificity, directed against different/new epitopes compared to the tTg antibody.
- It is suitable for IgA deficient patients, since it includes IgG isotype.

In the hierarchy of the 17 bio-markers, the three autoantibodies best reflecting CD intestinal damage, tTg-neo IgA, tTg-neo check and EMA IgA, won the competition. Therefore, it is suggested that tTg neo-epitope antibodies should be preferably used to screen, diagnose and monitor compliance in CD patients. The mTg neo-epitope IgG represent a novel serological biomarker for CD. Its significance, pathogenic role, involvement in other autoimmune diseases or in non-celiac gluten sensitivity, awaits further scientific explorations.

References

1. Lerner A (2014) Serological Diagnosis of Celiac Disease–Moving Beyond the Tip of the Iceberg. Internat J Celiac Dis 2: 64-66.
2. Lerner A, Jeremias P, Matthias T (2015) The world incidence of celiac disease is increasing: a review. Internat J Recent Scient Res 7: 5491-5496.
3. Lerner A, Jeremias P, Matthias T (2015) The world incidence and prevalence of autoimmune diseases is increasing: A review. Internat J Celiac Dis 3: 151-155.
4. Lerner A, Agmon-Levin N, Shapira Y, Gilburd B, Reuter S, et al. (2013) The thrombophilic network of autoantibodies in celiac disease. BMC Med 11: 89.
5. Lerner A (1994) Factors affecting the clinical presentation and time of diagnosis of celiac disease: the Jerusalem and the West Bank-Gaza experience. Isr J Med Sci 30: 294-295.
6. Katz KD Rashtak S, Lahr BD, Melton LJ, Krause PK (2011) Screening for celiac disease in a North American population: sequential serology and gastrointestinal symptoms. Amer J Gastroentrol 106: 1333-1339.
7. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, et al. (2012) European Society for Pediatric Gastroenterology Hepatology and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 54: 136-160.
8. Rossi TM, Kumar V, Lerner A, Heitlinger LA, Tucker N, et al. (1988) Relationship of endomysial antibodies to jejunal mucosal pathology: specificity towards both symptomatic and asymptomatic celiacs. J Pediatr Gastroenterol Nutr 7: 858-863.
9. Beltran L, Koenig M, Egner W, Howard, M, Butt A, et al. (2014) High-titre circulating tissue transglutaminase-2 antibodies predict predict small bowel villous atrophy, but decision cut-off limits must be locally validated. Clin Exp Immunol 176: 190-198.
10. Mubarak A, Wolters VM, Gmelig-Meyling FH, Ten Kate FJ, Houwen RH (2012) Tissue transglutaminase levels above 100 U/mL and celiac disease: a prospective study. World J Gastroenterol 18: 4399-4403.
11. Licata A, Cappello M, Arini A, Florena AM, Randazzo C, et al. (2012) Serology in adults with celiac disease: limited accuracy in patients with mild histological lesions. Intern Emerg Med 7: 337-342.
12. Alessio MG, Tonutti E, Brusca I, Radice A, Licini L et al. (2012) Correlation between IgA tissue transglutaminase antibody ratio and histological finding in celiac disease. J Pediatr Gastroenterol Nutr 55: 44-49.
13. Makovicky P, Rimarova K, Boor A, Makovicky P, Vodicka P, et al. (2013) Correlation between antibodies and histology in celiac disease: incidence of celiac disease is higher than expected in the pediatric population. Mol Med Rep 8: 1079-1083.
14. Parizade M, Bujanover Y, Weiss B, Nachmias V, Shainberg B (2009) Performance of serology assays for diagnosing celiac disease in a clinical setting. Clin Vaccine Immunol 16: 1576-1582.
15. Hojsak I, Mozer-Glassberg, Segal Gilboa N, Weinberger R, Hartman C, et al. (2012) Celiac disease screening assays for children younger than 3 years of age: the performance of three serological tests. Dig Dis Sci 57: 127-132.
16. Rozenberg O, Lerner A, Pacht A Grinberg, M, Reginashvili D, et al. (2011) A new algorithm for the diagnosis of celiac disease. Cellul molec Immunol 8: 146-149.
17. Walker-Smith JA, Guandalini S, Schmitz J, Shmerling DH, Visakorpi JK (1990) Revised criteria for the diagnosis of celiac disease. Report of the working group of European Society of Pediatric Gastroenterology and nutrition. Arch Dis Child 65: 909911.
18. Rozenberg O, Lerner A, Pacht A, Grinberg M, Reginashvili D, et al. (2012) A novel algorithm for the diagnosis of celiac disease and a comprehensive review of celiac disease diagnostics. Clin Rev Allergy Immunol 42: 331-341.
19. Barak M, Rozenberg O, Froom P, Grinberg M, Reginashvili D, et al. (20132) Challenging our serological algorithm for celiac disease (CD) diagnosis by the ESPGHAN guidelines. Clin Chem Lab Med 51: e257-259.
20. Matthias T, Neidhöfer S, Pfeiffer S, Prager K, Reuter S, et al. (2011) Novel trends in celiac disease. Cell Mol Immunol 8: 121-125.
21. Matthias T, Pfeiffer S, Selmi C, Eric Gershwin M (2010) Diagnostic challenges in celiac disease and the role of the tissue transglutaminase-neo-epitope. Clin Rev Allergy Immunol 38: 298-301.
22. Lerner A, Matthias T (2015) Possible association between celiac disease and bacterial transglutaminase in food processing: a hypothesis. Nutr Rev 73: 544-552.
23. Lerner A, Neidhöfer S, Matthias T (2015) Transglutaminase 2 and anti transglutaminase 2 autoantibodies in celiac disease and beyond: Part A: TG2 double-edged sword: gut and extraintestinal involvement. Immunome Res 11: 101-105.
24. Lerner A, Jeremias P, Neidhöfer S, Matthias T (2016) 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 429: 15-20.
25. Bizzaro N, Tozzoli R, Villalta D, Fabris M, Tonutti E (2012) Cutting-edge issues in celiac disease and in gluten intolerance. Clin Rev Allergy Immunol 42: 279-287.
26. Tozzoli R, Kodermaz G, Tampoia M, Visentini D, Tonutti E, et al. (2010) Detection of autoantibodies specific for transglutaminase-gliadin peptides complex: a new way to explore the celiac iceberg. It J Lab Med 6: 28-35.
27. Remes-Troche JM, Ramírez-Iglesias MT, Rubio-Tapia A, Alonso-Ramos A, Velazquez A, et al. (2006) Celiac disease could be a frequent disease in Mexico: prevalence of tissue transglutaminase antibody in healthy blood donors. J Clin Gastroenterol 40: 697-700.
28. Remes-Troche JM, Rios-Vaca A, Ramírez-Iglesias MT, Rubio-Tapia A, Andrade-Zarate V, et al. (2008) High prevalence of celiac disease in Mexican Mestizo adults with type 1 diabetes mellitus. J Clin Gastroenterol 42: 460-465.
29. Tozzoli R, Kodermaz G, Porcelli B (2010) Clinical relevance and diagnostic accuracy of new ELISA method for the detection of autoantibodies to gliadin-transglutaminase complex. Proceedings of the 7th International Congress on autoimmunity, Ljubljana: 5-9.
30. Tonutti E, Visentini D, Fabris M (2010) Antibodies to the transglutaminase-deamidated gliadin complex: a new serological approach to the diagnosis of celiac disease. Proceedings of the 7thInternational Congress on autoimmunity, Ljubljana: 5-9.
31. Arguelles-Grande C, Tennyson CA, Lewis SK, Green PH (2012) Variability in small bowel histopathology reporting between different pathology practice settings: impact on the diagnosis of coeliac disease. J Clin Pathol 65: 242-247.
32. Picarelli A, Borghini R, Donato G, Di Tola M, Boccabella C, et al. (2014) Weaknesses of histological analysis in celiac disease diagnosis: new possible scenarios. Scand J Gastroenterol 49: 1318-1324.
33. Brown IS, Smith J, Rosty C (2012) Gastrointestinal pathology in celiac disease: a case series of 150 consecutive newly diagnosed patients. Am J Clin Pathol 138: 42-49.
34. Siriweera EH, Qi Z, Yong JLC (2015) Validity of Intraepithelial Lymphocyte Count in the Diagnosis of Celiac Disease: A Histopathological Study. Internat. J celiac Dis 3: 156-158
35. Lerner A, Matthias T (2016) Intraepithelial Lymphocyte Normal Cut-off Level in Celiac Disease: The Debate Continues. Internat. J of Celiac Dis 4: 4-6.
36. Simell S, Hoppu S, Hekkala A, Simell T, Ståhlberg MR, et al. (2007) Fate of five celiac disease-associated antibodies during normal diet in genetically at-risk

children observed from birth in a natural history study. Amer J Gastroenterol 102: 2026-2035.

37. Tanpowpong P, Broder-Fingert S, Katz AJ, Camargo CA Jr (2013) Characteristics of children with positive coeliac serology and normal villous morphology: potential coeliac disease. APMIS 121: 266-271.

38. Tursi A, Brandimarte G, Giorgetti GM (2003) Lack of usefulness of anti-transglutaminase antibodies in assessing histologic recovery after gluten-free diet in celiac disease. J Clin Gastroenterol 37: 387-391.

39. Egner W, Shrimpton A, Sargur R, Patel D, Swallow K (2012) ESPGHAN guidance on coeliac disease 2012: multiples of ULN for decision making do not harmonise assay performance across centres. J Pediatr Gastroenterol Nutr 55: 733-735.

40. Lerner A, Jeremias P, Matthias T (2015) Outside of Normal Limits: False Positive/Negative Anti TG2 Autoantibodies. Internat. J Celiac Dis 3: 87-90.

41. Leffler DA, Schuppan D (2010) Update on serologic testing in celiac disease. Am J Gastroenterol 105: 2520-2524.

42. Guandalini S, Assiri A (2014) Celiac disease: a review. JAMA Pediatr 168: 272-278.

43. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA, American College of Gastroenterology (2013) ACG clinical guidelines: diagnosis and management of celiac disease. Am J Gastroenterol 108: 656-676.

44. Matthias T, Jeremias P, Neidhöfer S, Lerner A (2016) The industrial food additive microbial transglutaminase, mimics the tissue transglutaminase and is immunogenic in celiac disease patients. Autoimm Rev 15: 1111-1119.

45. Lerner A, Matthias T (2015) Food Industrial Microbial Transglutaminase in Celiac Disease: Treat or Trick. Internat J Celiac Dis 3: 1-6.

46. Lerner A, Matthias T (2015) 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, ed: K Conrad, Chan EKL, Andrade LEC, Steiner G, Pruijn GJM, Y Shoenfeld. Page 227-23, Pabst Science Publishers, Lengerich, Germany, e-pub.

47. Lerner A, Matthias T (2015) Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease. Autoimm Rev 14: 479-489.