Clinical experience with a new Generation tTG for diagnosis of Celiac disease

Tapasztalatok a cöliákia megbetegedés kimutatására használt új generációs tTG-vel.

Hugo Ribeiro
Aesku Diagnostics

Introduction/Bevezetés

- Incidence/Előfordulás
- Genetics/Genetika
- Prevalence/Elterjedés
- Pathomecanism
- New generation tTG
- Új generációs tTG

Clinical experience/Klinikai tapasztalat

- Routine use/
- Rutin felhasználás
- Research studies
- Kutatási eredmények

- Results and Discussion
- Eredmények, megbeszélés

- Summary/Összegzés

CELIAC DISEASE - (Gluten-sensitive Enteropathy, Sprue)
Cöliákia (Glutén érzékeny enteropathia)

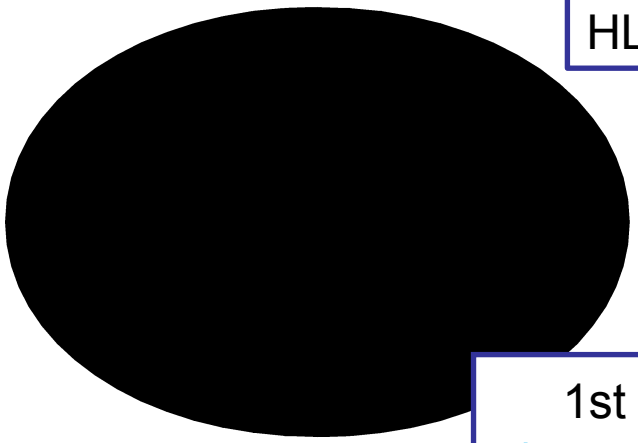
- **Complex autoimmune disease/megbetegedés**
  - Chronic inflammatory disorder of the small intestine/A vékonybél krónikus gyulladásos rendellenessége
- **Wheat Gluten/Búza glutén**
  - Tg2□ Crosslinking or deamidation of gliadine/Tg2 □ keresztkötés, vagy gliadin deamidáció
  - Formation of immunoreactive antigens /Immunreaktív antigének képződése

Autoantibodies against Tg2 and gliadine used for CD screening
Tg2 ellenes autoantitestek és cöliákia szkríningre használt gliadin

Incidence & Genetics /Előfordulás&genetika

30-40

1-3 Months after first wheat containing food supply /1-3 hónappal az első búza tartalmú étel bevitele után



HLA-DQ2 and HLA-DQ8

1st Grade relatives 4-12% /Elsőfokú rokonság esetén:4-12%

PREVALENCE /ELTERJEDÉS

- **Common disorder worlwide (1-2%) /Gyakori rendellenesség világszerte (1-2%)**
  - *Substantially increasing numbers in American and Finnish populations /Jelentékenyen növekvő számok az amerikai és finn lakosságban*

- **Chance to develop CD with genetic Determination /Lehetőség a CD genetikai meghatározásának fejlesztésére**
  - *HLA-DQ8 5-10 %*
  - *HLA-DQ2 25%*

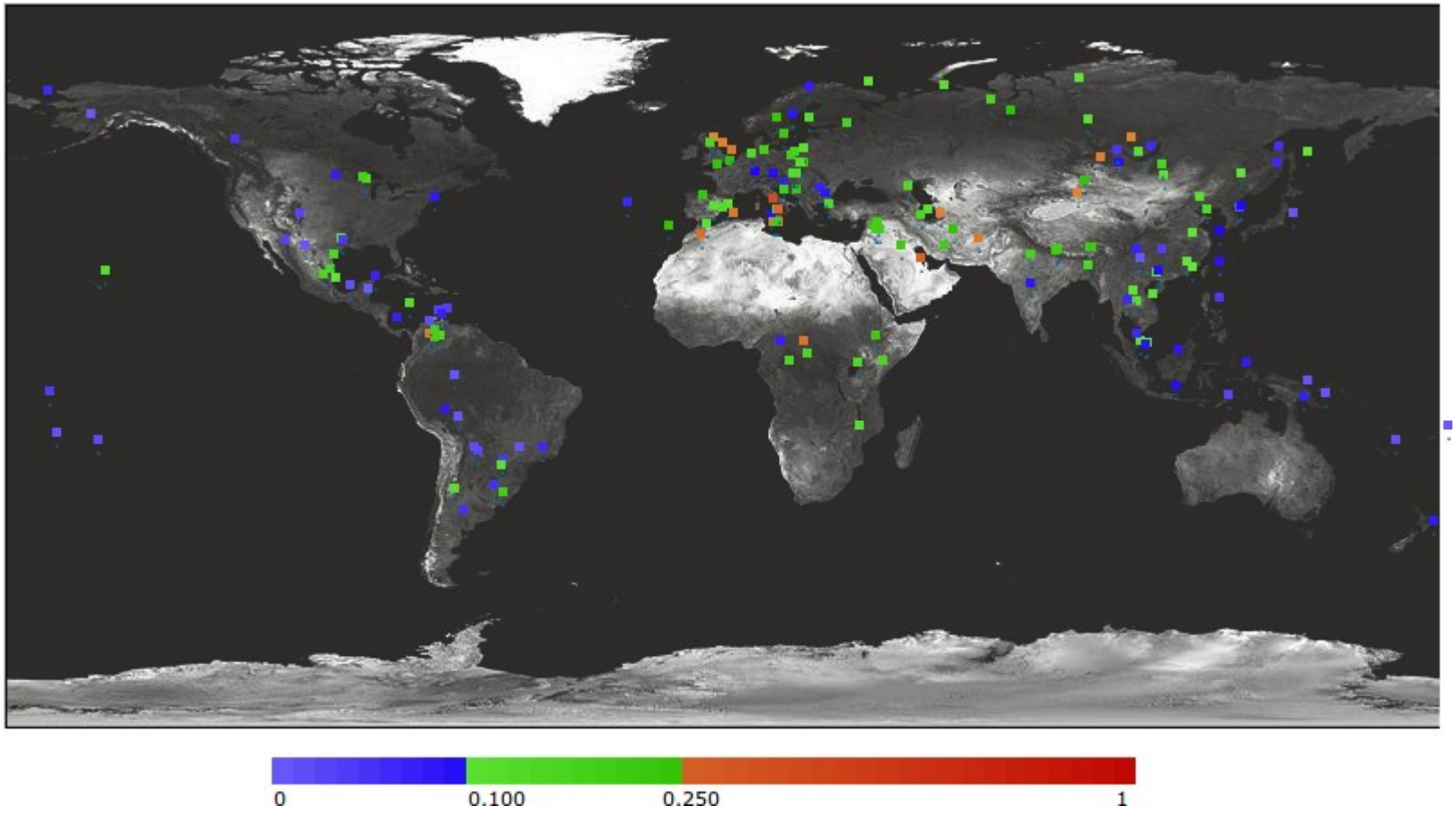
- **Systematic data aquisition /Módszeres adatgyűjtés**
  - *Mainly China and Eastern countries /Főként Kína és keleti országok*

- **``Coeliac disease in China, a field waiting for exploration``**

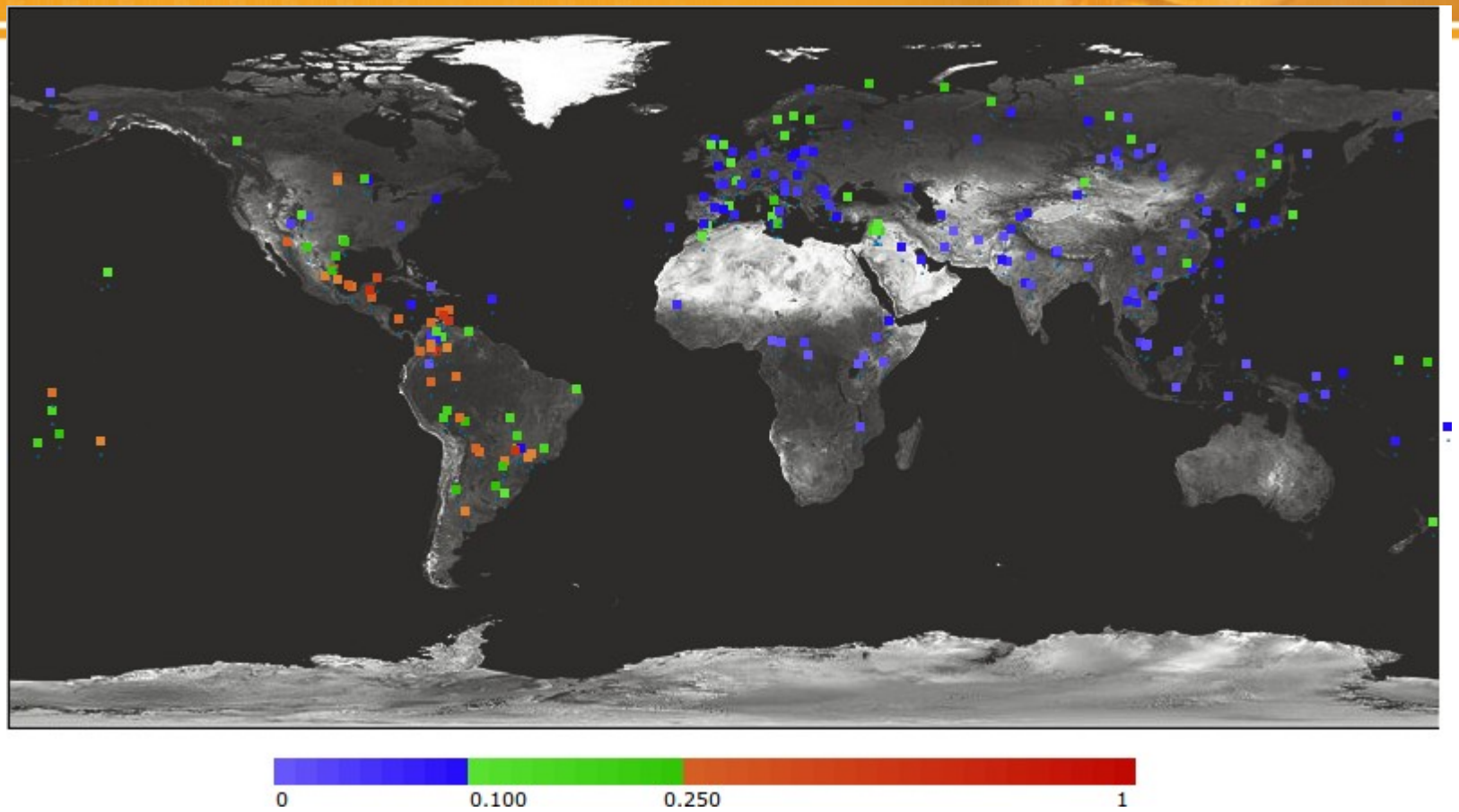
  ”A cöliákia Kínában még egy felderítésre váró terület”

[J. Wu et al. 2010]

HLA-DQ2



HLA-DQ8



"…The average delay in diagnosis was 11 years and the average of doctors consulted prior to confirmation of the CD was 6…"
"…A diagnózis átlagos késedelme 11 év volt és átlagosan 6 orvos konzultált a cöliákia megerősítését megelőzően…"

"…The mean delay to diagnosis from the first symptoms was 9.7 years, …"
"…Az első tünetektől a diagnózisig terjedő átlagos késedelem 9,7 év volt…"

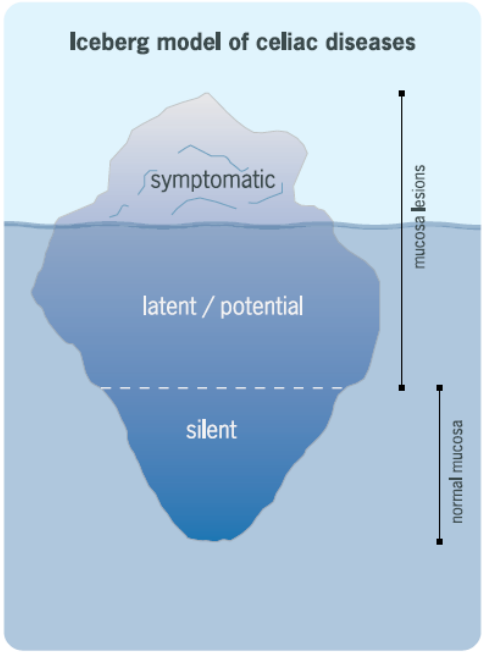
"…Untreated CD results in poor life quality, which improves to the level of the general population if diagnosed and treated..."
„A kezeletlen cöliákia életminőség romlást eredményez, amely diagnosztizálás és kezelés esetén az átlag populáció színvonalára javítható…"

"…Osteoporosis or osteopenia were nearly twice as common among those who took more than five years to be diagnosed…"
„Osteoporosis és osteopenia majd kétszer olyan gyakori volt az 5 évnél később diagnosztizáltak esetében…"

Sources:
Delay to celiac disease diagnosis and its implications for health-related quality of life. Norström F, Lindholm L, Sandström O, Nordyke K, Ivarsson A. Gastroenterol. 2011 Nov 7;11:118.
Quality of life and diagnosis process in adult celiacs from the Valencian Community. Pelegrí Calvo C, Soriano Del Castillo JM, Mañes Vinuesa J. Nutr Hosp. 2012 Aug;27(4):1293-7. doi: 10.3305/nh.2012.27.4.5871.

Celiac disease subtypes with symptoms, histological presentation and serology /Cöliákia altípusok & tünetei, szövettani bemutatás & szerológia

CD Subtype	Symptoms	Histology	Serology
Classic	Diarrhea malabsorption symptoms	Villous atrophy Marsh 3a-c	Positive
Refractory	Classic unresponsive to GFD	Classic unresponsive to GFD	Positive unresponsive to GFD
Latent	Atypical reduced under GFD	Marsh 1-2	Positive
Potential	Inconspicuous	Normal, up to Marsh 1	Positive
Silent	Inconspicuous	Normal	Positive

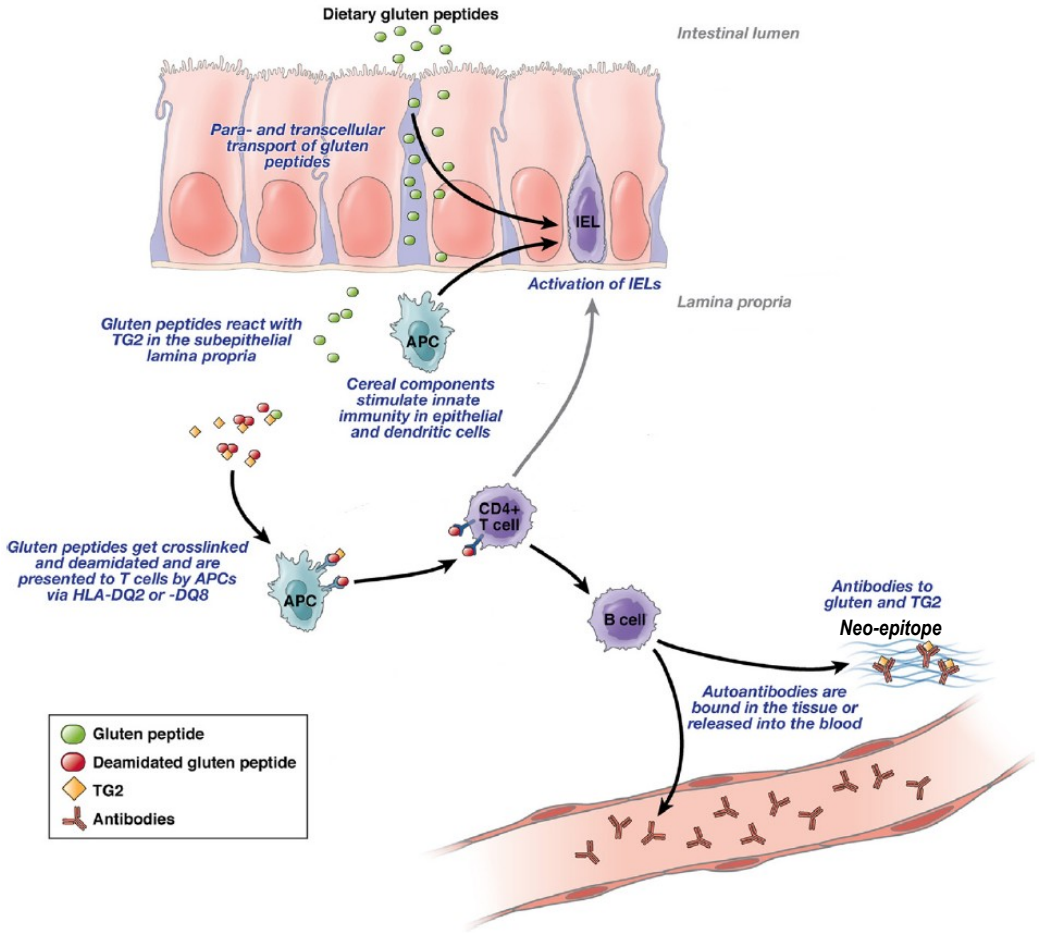


Recent reviews suggest to move away from biopsy dogma being the celiac gold standard back to the final decision of the clinician taking into account all serological, histological, genetical and clinical information*. /Az újabb beszámolók a biopsziától, a cöliákia arany standard dogmájától való eltávolodást szorgalmazzák, abba az irányba, amikor is a klinikus figyelembe vesz minden szerológiai, szövettani, genetikai és klinikai információt.

*Matthias,T. *et al.* (2011) Novel trends in celiac disease. *Cell Mol. Immunol.*

Pathogenesis of celiac disease /Cöliákia kórtörténete

Celiac neo-epitopes – antigen key players / Cöliákia – antigén kulcsszereplők



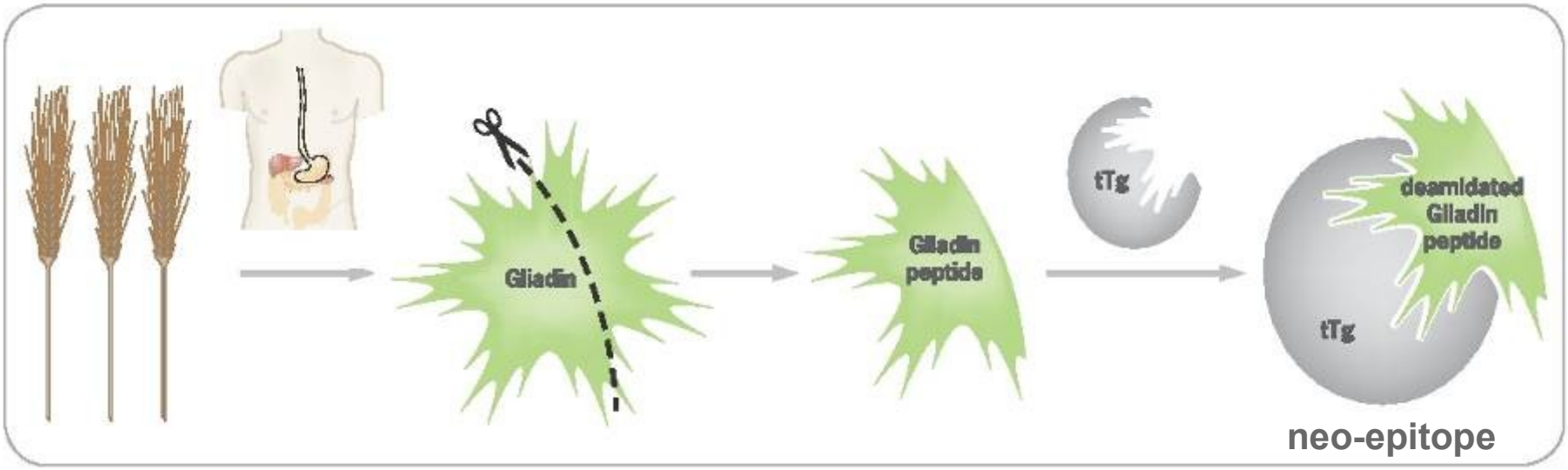
- Gliadin (Dicke; 1950)
- Gliadin peptides (Bronstein;1966)
- Crosslinked gliadin by tTg of RBC (Scabolcs; 1987)/tTg,v.RBC kereszt-kötéses gliadin
- tTg modified gliadin peptides (1997 Molberg/Sollid)/tTg módosított gliadin peptidek
- Tissue transglutaminase (Dieterich /Schuppan 1997)/Szöveti transglutamináz
- Celiac Neo-Epitopes (AESKU 2002) Cöliákia új epitóp (AESKU 2002)

Schuppan,D. *et al.* (2009) Celiac disease: from pathogenesis to novel therapies. *Gastroenterology.* 137, 1912-1933

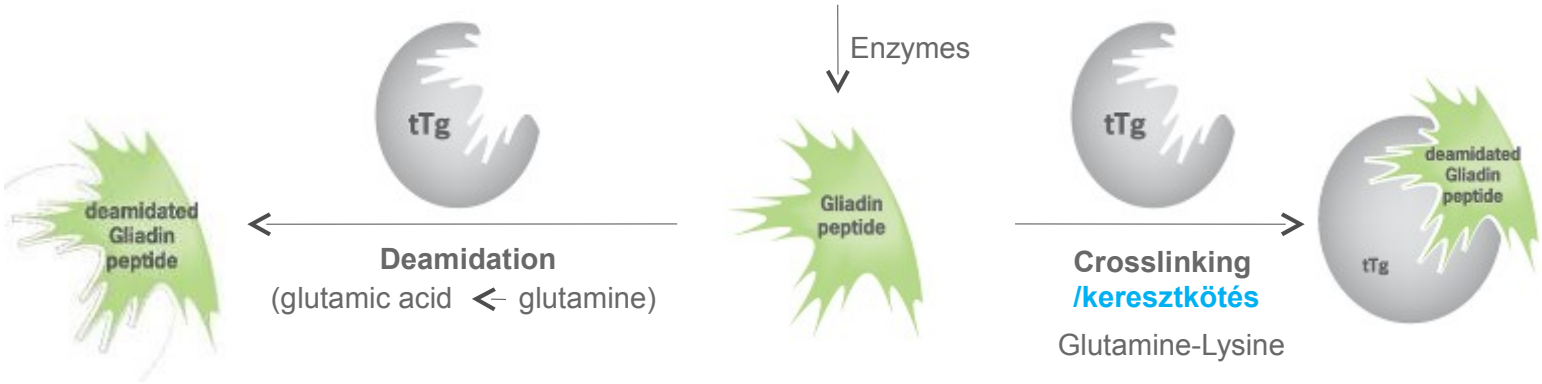
The Neoepitope structure / Az új epitóp szerkezet

The resulting gliadin-tTg complex forms a **neo-epitope** which can actually be the physiologically relevant antigen. Therefore this gliadin-tTg neo-epitope might be the ideal ELISA antigen.

A keletkező gliadin-tTg komplex egy új epitópot hoz létre, mely maga a fiziológiailag lényeges antigén. Ennek köszönhetően ez a gliadin-tTg új epitóp lehet az ideális ELISA antigén.



Scientific evidence / Tudományos bizonyíték



Gliadin peptides
Rich in glutamine
/Glutaminban gazdag

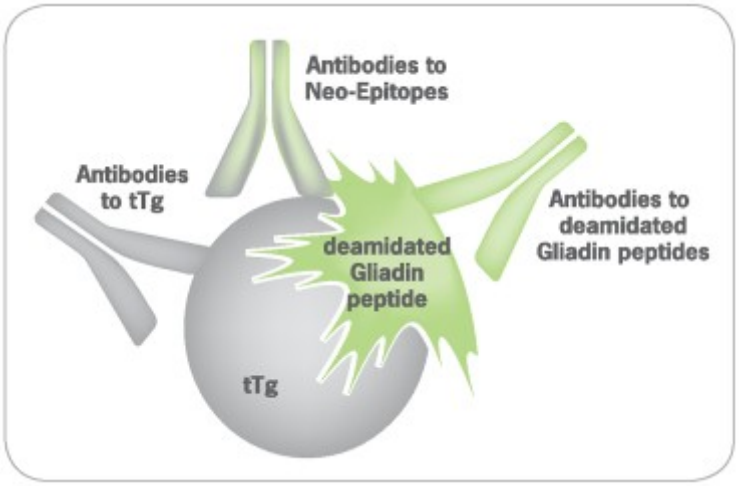
DGPs 25 %

Neo 75 %

Scientific evidence confirming the existence of the Neo-Epitope:
/Tudományos bizonyíték az új epitóp létezésére:

In-vivo:

„Mindent egybevetve, eredményeink azt sugallják, hogy a **tTg és gliadin** mind a normális, mind a beteg duodenális nyálkahártyán közvetlenül egy **szupramolekuláris komplexet alkotnak**, és aktív cöliákia esetén mindkét molekula szintje emelkedett.”



*“Taken together, our results suggest that **tTg and gliadin form supramolecular complexes** directly in normal and diseased duodenal mucosa and that in active CD the levels of both molecules are increased.”* [7]

R. Ciccocioppo et.al. Gliadin and tissue transglutaminase complexes in normal and celiac duodenal mucosa. **Clin.Exp.Immunol.** 134 (2003) 516-524

Scientific evidence /Tudományos bizonyíték az új epitóp létezésére:

/Az úgynevezett új epitóp a szöveti transzglutamináz és a gliagin peptidek komplexe, melyek bizonyítottan *in-vitro* és *in vivo* léteznek.

In-vitro:

> *"Incubation of tTg ... with the known substrate peptide B-αI* ***resulted in complex formation between the peptide and tTg...*** *These covalent complexes might derive from thioester formation between Cys-277 in the active site of tTg and the side chain of Gln-65 in B-αI. Alternatively, tTg itself could act as an acyl acceptor molecule, resulting in isopeptide bond formation."*[5]

„A tTg az ismert B-αI szubsztrát peptiddel történő inkubációja ***a peptid és a t-Tg közötti komplex kialakulását eredményezte.*** *Ezek a kovalens komplexek a tTg aktív területén található Cys-277 és a B-αI Gln-65 oldallánca közötti tioészter keletkezéséből eredhetnek. Ugyanakkor, a tTg önmagában is képes acyl akceptor molekulaként viselkedni, mely izopeptid kötés kialakulását eredményezi.*

Fleckenstein B. et.al. Molecular characterization of covalent complexes between tissue transglutaminase and gliadin peptides. **J.Biol.Chem.** 279 (2004) 17607-16

> "The binding of the epitope peptide to tTg supports the hypothesis of Sollid et.al (1997 Gut 41) that T cell immune response to gliadin would drive antibody responses towards tTg that is cross-linked to gliadin T cell epitopes. The crosslinking occurs, as demonstrated in this paper and recently by others (Fleckenstein JBC 2004) also outside the active site of tTg."
> ***"...we found a ratio between deamidation and transamidation (cross-linking) of 1:4 for tTg..."*** [6]

„Az epitope peptid tTg-hez történő kötődése alátámasztja Sollid és mások (1997 Gut 41) feltevését, hogy a T sejt gliadinra adott immunválasza az antitest választ a T-sejt epitóhoz keresztkötéssel kapcsolódó tTg irányába tereli. A keresztkötés, amint azt jelen tanulmány és újabban mások is (Fleckenstein JBC 2004) bemutatják szintén a tTg aktív oldalán kívül történik."
„… 1:4-es, tTg-re vonatkozó arányosságot találtunk a deamidáció és transamidáció között (keresztkötés)…"

Skovbjerg H. et.al. Deamidation and crosslinking of gliadin peptides by transglutaminases and the relation to celiac disease. **Biochim.Biophys.Acta** 1690 (2004) 220-30

Clinical experience with the New gen tTG
Klinikai tapasztalatok az új generációs tTG-ről

UNDERNEATH THE CD ICEBERG

A cöliákia jéghegye alatt

Europ.J.Gastro.
Hepat.2006

Diagnostic accuracy of coeliac serological tests: a prospective study

Glenn E.M. Reeves[a], Marline L. Squance[a], Anne E. Duggan[a], Rajathurai R. Murugasu[a], Robert J. Wilson[b], Richard C. Wong[b], Robert A. Gibson[a], Richard H. Steele[c], Wendy K. Pollock[d] and the Multicentre Coeliac Study Group[a–h]

Australian multicenter study, starting with 2684 patients of which 254 celiac suspect patients were included in the final study

Ausztráliában több központban végzett tanulmány 2648 beteggel indult, melyből végül 254 cöliákia gyanús beteget vontak be.

The New generation tTG IgG/A appeared as the best screening test for CD (in comparison with 11 companies).

Az új generációs tTG IgG/A teszt bizonyult a legjobb szűrőtesztnek a cőliákia tekintetében (11 céget összehasonlítva).

Europ.J.Gastro. Hepat.2006

Australian multicenter study, 254 celiac patients

Ausztrál multicenter tanulmány, 254 cöliákiás beteg

Reeves et.al. Europ.J.Gastro.Hepat.2006

Kit	Sensitivity %	Specificity %
Aeskulisa Celi-Check (IgG+IgA)	92,31	82,89
Aeskulisa IgA	84,62	86,40
Aeskulisa IgG	84,62	89,04

Kit	Sensitivity %	Specificity %
Celicheck Dual (IgA+IgG)	**92,31**	**82,89**
D-Tek Dual (IgA+IgG)	90,48	80,77
Aeskulisa IgA	**84,62**	**86,40**
Bindazyme IgA	80,77	93,69
Celikey IgA	80,77	93,86
D-Tek IgA	16,67	97,08
Euroimmun IgA	80,77	83,70
Eurospital IgA	92,31	81,14
Fidis IgA	88,46	84,65
Genesis IgA	73,08	94,74
Inova IgA	76,92	87,22
Orgentec IgA	88,46	83,77
Aeskulisa IgG	**84,62**	**89,04**
Bindazyme IgG	65,38	85,59
Celikey IgG	66,67	82,05
D-Tek IgG	16,67	89,78
Euroimmun IgG	76,92	85,09
Eurospital IgG	76,92	85,53
Genesis IgG	69,23	82,89
Inova IgG	42,31	85,96
Orgentec IgG	69,23	81,50

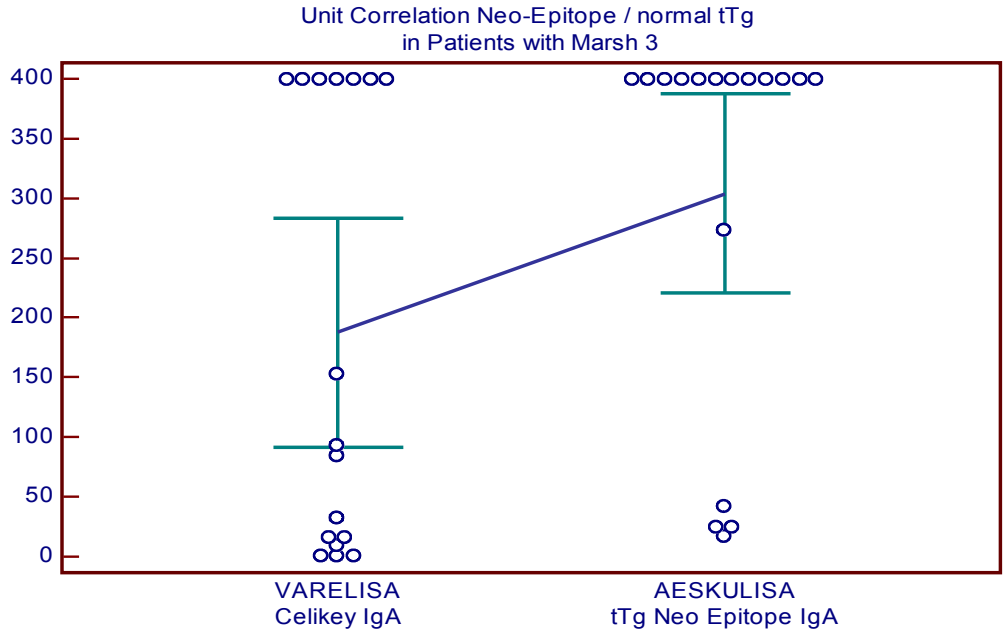
International Autoimmunity congress, 2008

Comparison of tTg New Generation with classical tTg (Celikey)
/Az új generációs tTG összehasonlítása a klasszikus tTG-vel (Celikey)

6th INTERNATIONAL CONGRESS ON AUTOIMMUNITY
PORTO, PORTUGAL
SEPTEMBER 10-14, 2008

TTG NEO-EPITOPES: A COMPLEX OF DEAMIDATED GLIADIN PEPTIDES CROSSLINKED WITH TISSUE TRANSGLUTAMINASE AS AN IDEAL SCREENING TOOL FOR CELIAC DISEASE IN COMPARISON TO TISSUE TRANSGLUTAMINASE

Margarita García Marcos[a]; Beatriz Ortega Carballo[a]; Rocio Lopez Barberá[a]; Mercedes Herranz[a];
Hugo Ribeiro[b]; Sascha Pfeiffer[b]; Torsten Matthias[c]
[a]Centro de Especialidades Vicente Soldevilla Laboratorio, Madrid, SPAIN, [b]AESKU.DIAGNOSTICS, Wendelsheim, GERMANY,
[c]AESKU.KIPP INSTITUTE, Wendelsheim GERMANY

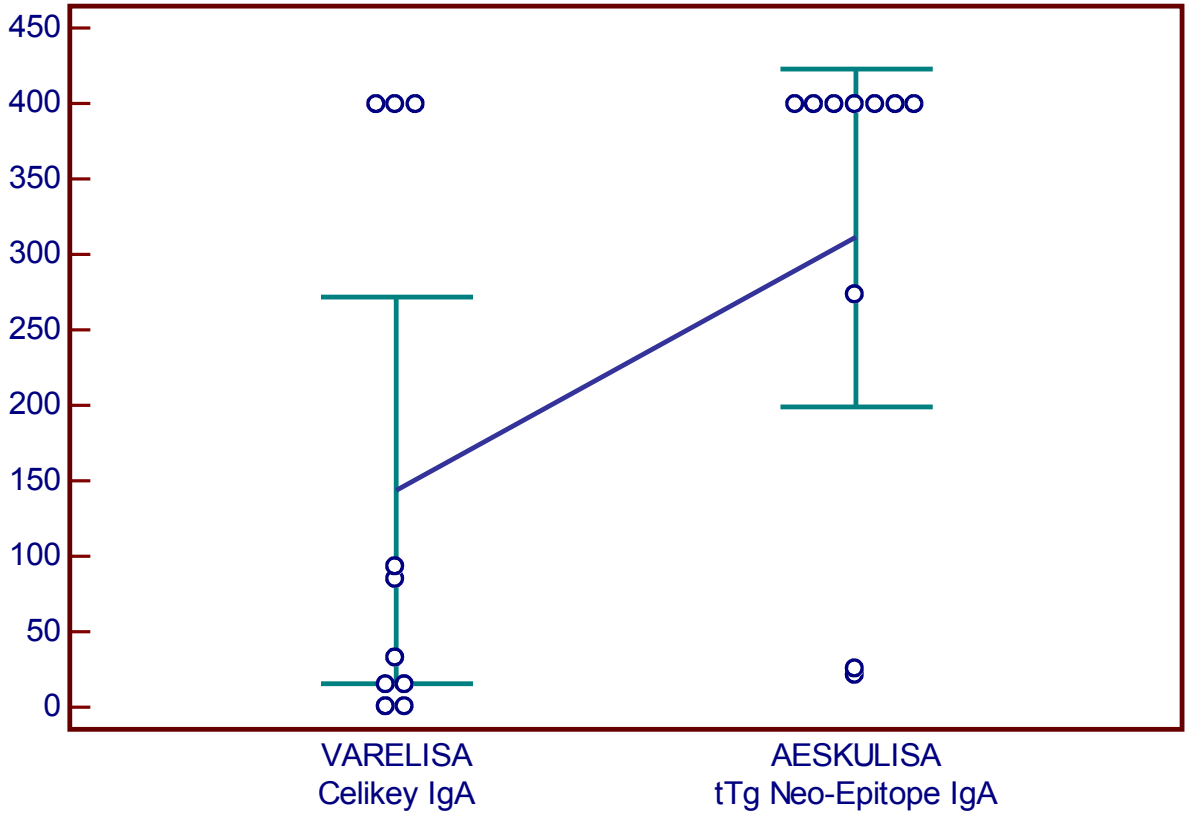


AESKULISA Neo-Epitope IgA	DISEASE POS	NEG	Total
POS	36	12	48
NEG	1	189	190
Total	37	201	238

Sensitivity	97.3%
Specificity	94.0%

New Gen tTG Ab concentration correlates better with the MARSH criteria
/New Gen tTG Ab koncentrácó jobb korrelációt mutat a MARSH kritérium tekintetében

Celiac Pediatric patients (0-8 yrs)
with positive biopsy



n=10 pediatrics (1-8 yrs)
Neoepitope 100% sensitivity
mean value ~300 Units/ml

AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS

International Autoimmunity congress, 2008

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Short summary
Rövidösszegzés

- If you use tTg only based assays, they might be missing patients
- Csak tTg alapú vizsgálatok használata esetén betegeket mulaszthatunk el

- Assay can also be used in pediatric samples
- Gyermek minták esetén is végezhető vizsgálat

- Assay has a better correlation with the MARSH criteria
- A vizsgálat a MARSH klritériummal jobban korrelál

- Better clinical performance than the classic tTg based kit (Phadia), which was one of the first EIA tTg kits on the market
- Az egyik első kereskedelmi forgalomba került klasszikus tTg alapú kitnél (Phadia), jobb klinikai teljesítményt nyújt

AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS

13TH International Coeliac Disease Symposium 2009
April 6 — 8, 2009
Amsterdam, the Netherlands

Relationship between coeliac serology and small bowel biopsy findings in clinical practice.

S.A.McMillan[1], M.B Loughery[2], D.F Hughes[3]

Regional Immunology Service[1] and Department of Pathology[2], Belfast Health and Social Care Trust and Department of Pathology[3], Western Health and Social Care Trust, Northern Ireland.
stan.mcmillan@belfasttrust.hscni.net

A cöliákia szerológia és a vékonybél biopszia találatai közötti kapcsolat a klinikai gyakorlatban.

- Over seven years of data from which 1556 positive CD patients were included
- Több, mint 7 év adatai, melyek közül 1556 pozitív volt cöliákiára

- The AESKULISA tTg New Generation IgA kit was used for screening all patients
- Az AESKULISA tTg új generációs IgA kit minden páciens szűrésére használták

- High positive results for tTg New Generation IgA correlate with severe mucosal damage.
- Az új generációs tTg erősen pozitív eredményei korreláltak a súlyos nyálkahártya károsodással.

- Review of biopsies from patients with high levels of TGA +/- EMA and “normal” biopsy histology showed that a significant number had either Marsh 1, 2 and 3 lesions
- Magas szintű TGA +/- EMA betegektől származó biópszia, valamint normál biopszia szövettani beszámolók azt mutatták, hogy jelentős szám rendelkezett Marsh 1, 2 és 3 sérüléssel.

- A confirmatory biopsy might not be necessary in patients with very high antibody titres
- Nagyon magas antitest titerű betegek esetében megerősítő biopszia elhagyható

Amsterdam poster Apr09 (2).pdf - Adobe Reader

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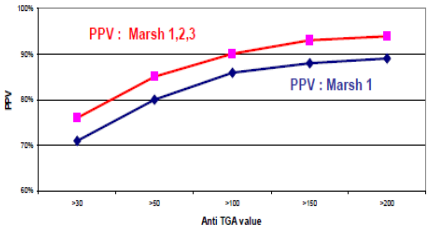
diagnostic criteria.

3. Patients with both high positive TGA (>100 u/ml) and positive endomysial antibody (EMA) showed a PPV of 97% (Marsh 1,2 or 3).
4. There was a slight difference between PPV's obtained from the two pathology reporting centres.
5. Review of biopsies from patients with high levels of TGA +/- EMA and "normal" biopsy histology showed that a significant number had either Marsh 1, 2 and 3 lesions.

Positive predictive value for CD (Marsh 3 and 1,2,3) of anti TGA at different levels



Anti TGA value	>30	>50	>100	>150	>200
PPV Marsh 1,2,3	76	85	92	94	95
PPV Marsh 1	71	80	86	88	89

positive IgA-TGA, (604 also had positive endomysial antibody) from two areas of Northern Ireland.

Corresponding small bowel biopsy results were obtained at the nearest time point after the serology result (within 3 months) from two pathology centres (Belfast and Londonderry). Biopsies were graded as normal, Marsh 1,2 or 3.
Biopsy details were available from 899 of the above patients.

The Positive Predicative Value (PPV) was calculated at different anti TGA values and EMA positivity using as diagnostic criteria either Marsh 3 or Marsh 1,2,or 3 lesions.

Recommendations:

- A confirmatory small bowel biopsy may not be needed in patients with high level CD antibodies (TGA / EMA), thus speeding up time to treatment.
- Patients suspected of CD because of high level CD antibodies but with "normal" biopsies, should have their biopsies reviewed for early histological changes of CD, possibly with the aid of CD3 immunohistochemistry looking for significant numbers of intraepithelial T-cells. As the histological changes of CD can be patchy, repeat biopsy may need to be considered

References:
1. Collin P et al. Anti endomysial and antihuman recombinant tissue transglutaminase antibodies in the diagnosis of coeliac disease: a biopsy-proven European multicentre study. Eur J Gastroenterol Hepatol 2005; 17: 85-91.
2. Hill PG, Holmes GKT. Coeliac disease: a biopsy is not always necessary for diagnosis. Aliment Pharmacol Ther 2008;27:572-7.

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Short summary:
Összegzés

- Our assay is used in the hot spot of celiac disease (Ireland)
- Vizsgálatainkat a cöliákia által leginkább érintett területeken használják (Írország)

- The clinical value of our assay is sustained by a study/reference center who has been using it for more then 5 years with thousands of samples
- Tesztjeink klinikai értékét egy több, mint 5 éve, minták ezreit feldolgozó tudományos/referencia központ támasztja alá

- Our assay is widely used by the experts/opinion leaders in the field
- Tesztjeinket a szakterület szakemberei, szaktekintélyei széles körben használják

- Our assay correlates well with MARSH criteria
- Viszgálataink jól korrelálnak a MARSH kritériumokkal

- Biopsy results should not always go undiscussed and should be reevaluated in case of conflicting results
- Biopszia eredményeket nem szabad konzultáció nélkül hagyni és ellentmondó eredmények esetén újra kell értékelni őket.

- Sometimes a biopsy might even not be necessary /Olykor elkerülhető a biopszia

TISSUE TRANSGLUTAMINASE (TTG) NEO-EPITOPE ELISA: A USEFUL TOOL FOR THE DETECTION OF CELIAC DISEASE IN PEDIATRIC SAMPLES

Troy D. Jaskowski,[1] Matthew R. Donaldson,[2] Harry R. Hill,[1,3]
John J. Zone[2] and Linda S. Book[4]

ARUP LABORATORIES
An enterprise of the University of Utah and its Department of Pathology

[1] ARUP Laboratories, Salt Lake City, Utah - USA; [2] Department of Dermatology, University of Utah Medical Center, Salt Lake City, Utah - USA; [3] Department of Pathology, University of Utah Medical Center, Salt Lake City, Utah - USA; [4] Pediatric Gastroenterology, Univ. of Utah Medical Center , Salt Lake City, Utah - USA

The University of Utah
Department of Pathology

13TH International Coeliac Disease Symposium 2009
April 6—8, 2009
Amsterdam, the Netherlands

	tTg NG IgA	tTg NG IgG	Glia IgA	Glia IgG
Cut-Off*	20	30	20	20
Sensitivity	88.9%	68.5%	57.4%	75.9%
Specificity	95.5%	94.0%	92.5%	80.6%
PPV	88.9%	82.2%	75.6%	61.2%
NPV	95.5%	88.1%	84.4%	89.3%
Agreement	93.6%	86.7%	82.4%	79.3%
AUC	0.969	0.892	0.867	0.862

ROC analysis showing that the best results were achieved if the cut-off values for ***pediatric samples*** are adjusted as follows:

ROC analízis azt mutatja, hogy a legjobb eredmények a ***gyermek minták*** esetén születtek és így alakultak:

- tTg New Generation IgA: >20 is positive
- tTg New Generation IgG: >30 is positive
- Gliadin IgA and IgG: >20 is positive

TISSUE TRANSGLUTAMINASE (TTG) NEO-EPITOPE ELISA: A USEFUL TOOL FOR THE DETECTION OF CELIAC DISEASE IN PEDIATRIC SAMPLES

Troy D. Jaskowski,[1] Matthew R. Donaldson,[2] Harry R. Hill,[1,3]
John J. Zone[2] and Linda S. Book[4]

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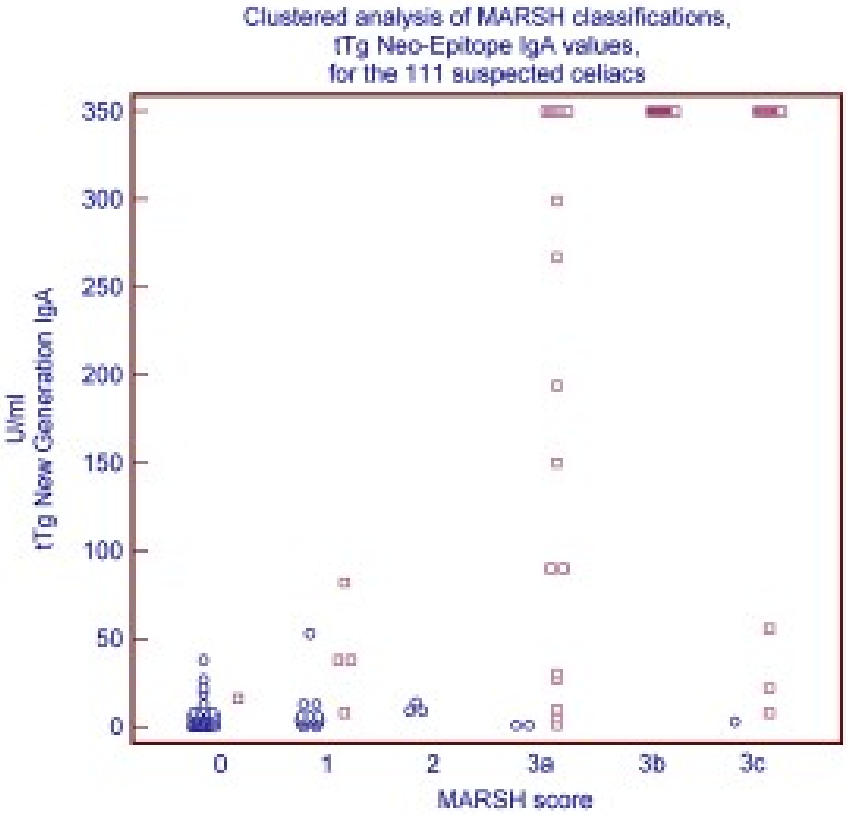
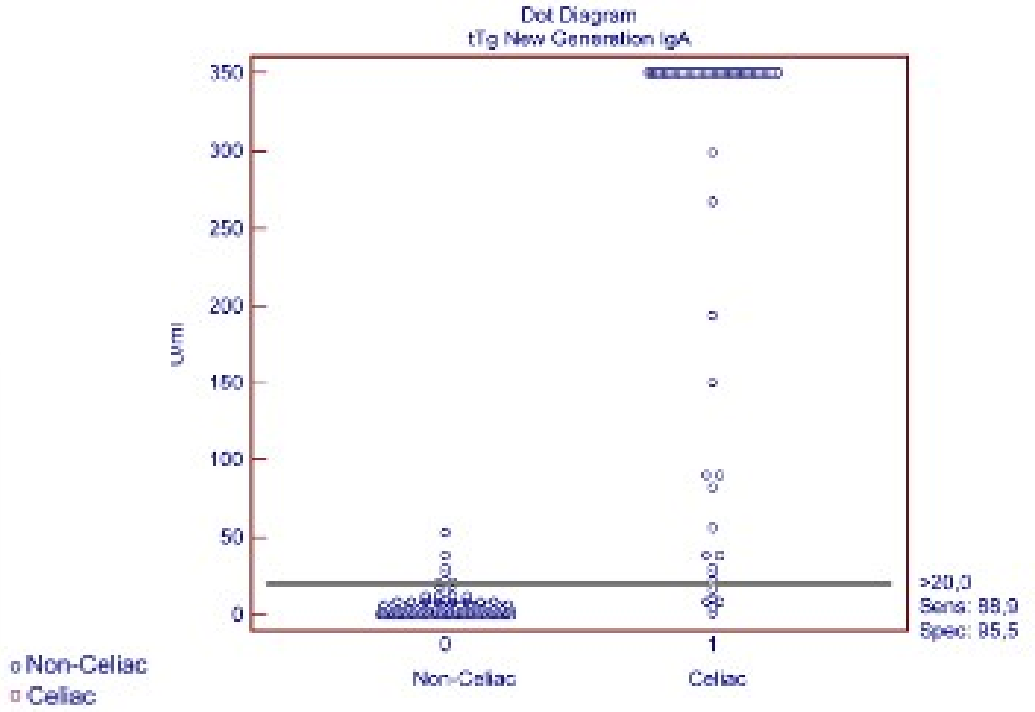




high positive results for tTg New Gen IgA correlate well with severe mucosal damage (MARSH3a,b,c)

/erősen pozitív tTg új. gen. eredmények jól korrelálnak anyálkahártya károsodással (MARSH3a,b,c).

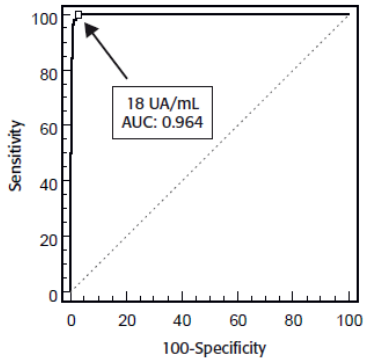
CLINICAL RELEVANCE AND DIAGNOSTIC ACCURACY OF A NEW ELISA METHOD FOR THE DETECTION OF AUTOANTIBODIES TO THE GLIADIN-TRANSGLUTAMINASE COMPLEX

R. Tozzoli[1], G. Kodermaz[1], B. Porcelli[2], L. Terzuoli[2], E. Tonutti[3], M. Tampoia[4], N. Bizzaro[5]
Clinical Pathology Laboratories of [1]Latisana, [3]Udine, [4]Bari, [5]Tolmezzo
and [2]Department of Internal Medicine of Siena, Italy

A gliadin-TG konplex kimutatására szolgáló új ELISA módszer klinikai relevanciája és diagnosztikai pontossága

A total of 895 patients were studied,
Összesen 895 beteg vizsgálata történt meg,
81 affected by untreated/kezeletlen CD,
486 with other autoimmune diseases /egyéb autoimmun megbetegedés
208 with nem/non-autoimmune diseases
120 healthy subjects / egészségesek

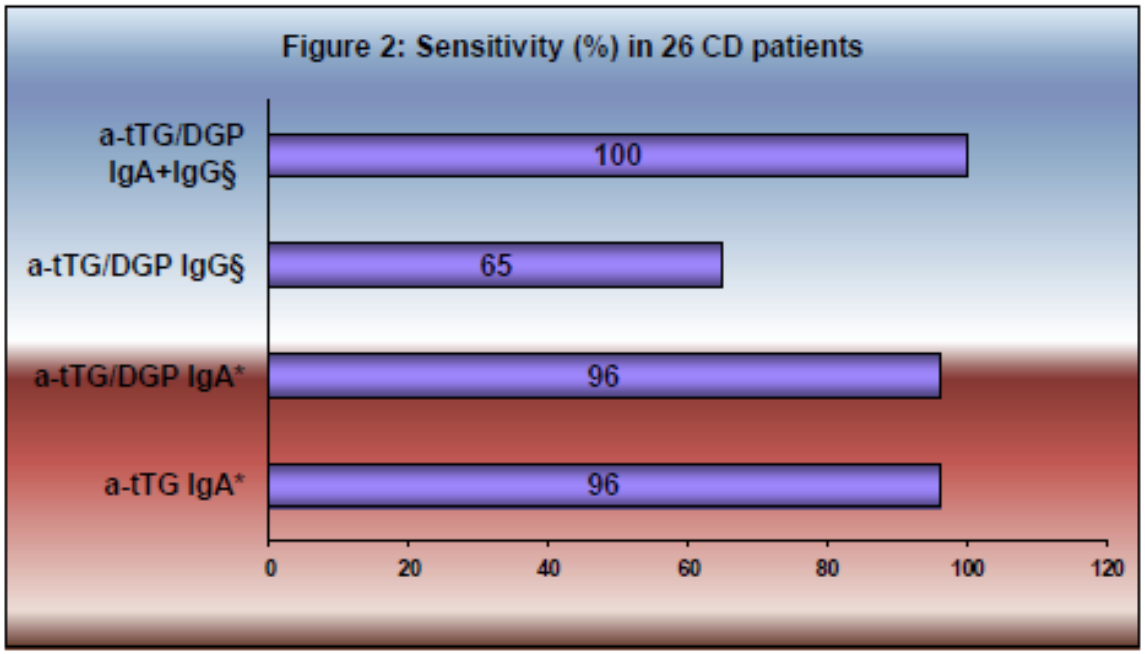
97.5% Sensitivity
98.6% Specificity



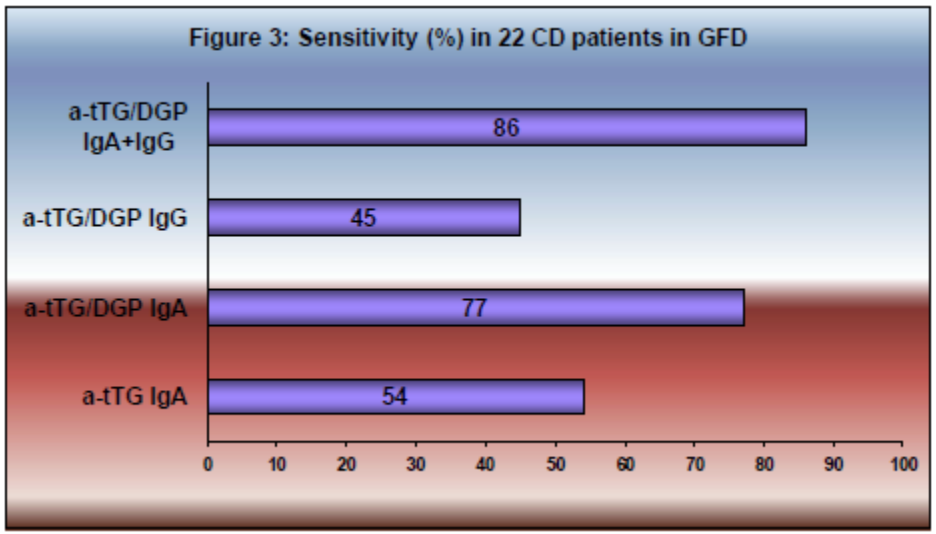
Patients	no.	GPTC (> 18 U/mL)	Celiac disease	Others
Celiac Disease	**81**	**79**	**79/81: 97.5%**	
SLE	80	1	1 (classic)	
RA	50	3	2 (classic)	1
SSc	46	1	1 (silent)	
AIT	166	5	2 (silent)	3
GD	42	2	1 (silent)	1
PBC	67	1		1
BSA	20	1	1 (classic)	
AIH	15	0		
Other autoimmune diseases	**486**	**14**	**8 (4 classic, 4 silent)**	**6**
LC	12	1		1
OID	66	5	3 (classic)	2
ID	130	1		1
Other non autoimmune diseases	**208**	**7**	**3 (classic)**	**4**
Healthy controls	**120**	**0**	**0**	
Total	**814**	**21**	**11/814: 1.4%**	**10**

A new assay for the detection of antibodies to the tissue trasglutaminase-deamidated gliadin peptide neoepitope increases the diagnostic sensitivity for celiac disease.

Elio Tonutti, Daniela Visentini, Martina Fabris, Eleonora Pavan, Nadia Blasone, Patrizia Molinaro, Nicola Bizzaro
Laboratory of Immunopathology and Allergy, Azienda Ospedaliero-Universitaria of Udine, Udine, Italy; Clinical Pathology, General Hospital of Tolmezzo, Udine, Italy



A szöveti transzglutamináz deamidált gliadin peptid új epitóp antitestjeinek kimutatására szolgáló új vizsgálat növeli a cöliákiára vonatkozó diagnosztikai szenzitivitást



Az a-DPG/tTg IgA az a-tTg IgA-hez viszonyítható diagnosztikai teljesítményt nyújt; ugyanakkor a betegek azon csekély részénél akiknél végül cöliákia alakulhat ki, korábban ad pozitív eredményt, mint az a-tTG IgA, vagy a-DPG. Glutén mentes diétát folytató, cöliákiában szenvedő betegek monitorozása esetében az a-tTG/DGP komplex IgA nagyobb szenzitívitást mutat, mint az a-tTG IgA.

Conclusions: The a-DPG/tTG IgA test has diagnostic performance comparable to a-tTG IgA; however, in a small part of patients who may eventually develop CD, it becomes positive earlier than a-tTG IgA or a-DPG. In monitoring CD patients in GFD a-tTG/DGP complex IgA assay demonstrates higher sensitivity than a-tTG IgA assay. Our study confirms the data of Reeves et al. which have compared various methods for measuring autoantibodies related to CD, showing a very high sensitivity of the analytical method for measuring anti-complex antibodies. Detection of a-DGP/tTG complex antibodies might therefore be able to integrate in one single diagnostic assay the information provided by either a-tTG or a-DGP assays and could be used as a reliable test for screening in the general population or in at-risk groups.

Udine Study:/Udinei tanulmány:

Evaluation of the performance (teljesítmény értékelése) of AESKULISA tTg New Generation Test (IgA and IgG)

1006 consecutive patients referred for a-tTG (case finding study) from January to March 2009 / 1006 egymást követő páciens a-tTG vizsgálat (esettanulmány) 2009 januártól márciusig.

Median age (átlag életkor) 38 (1-92 years); F= 705, M= 301

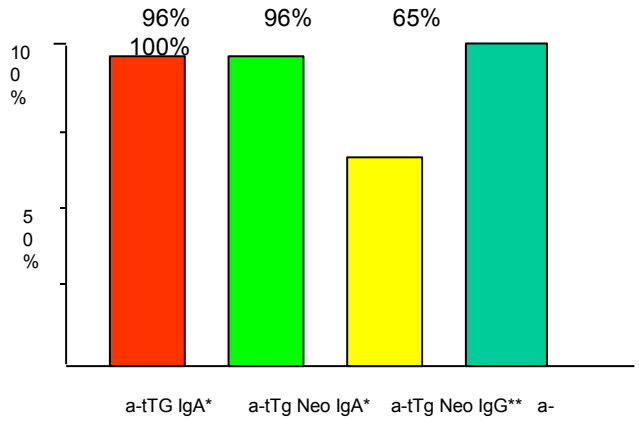
the following assays were performed (az alábbi vizsgálatokat végezték el):

- a-tTG IgA (ELISA Orgentec) and total IgA
- a-tTG IgG (ELISA Orgentec) in IgA deficiency
- INOVA a-DPG IgA and IgG INOVA in patients aged <5 years
- anti-endomysium IgA (EMA) in a-tTG IgA positive sera

and

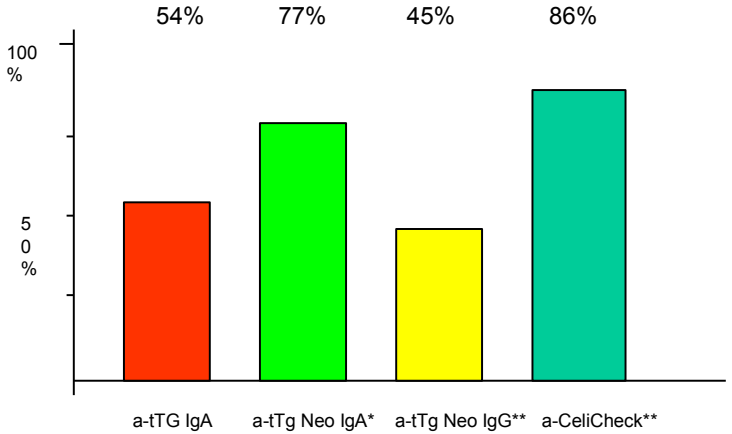
- antibodies to the AESKU tTg New Generation (IgA and IgG)

RESULTS / EREDMÉNYEK

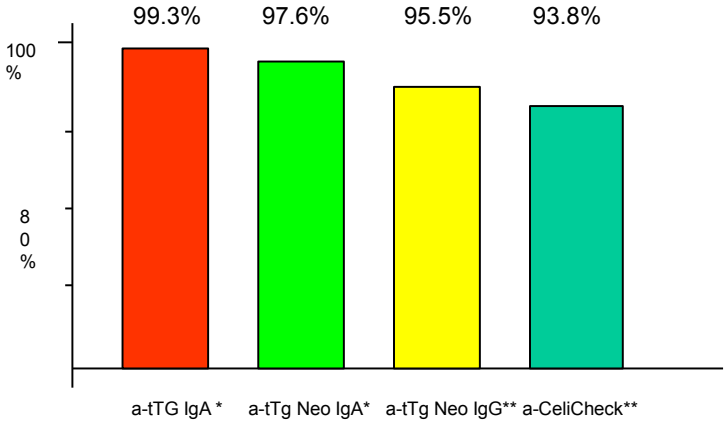


Sensitivity on 26 celiac patients with first diagnosis /első diagnózis

*not included 2 CD patients with IgA deficit (kihagyva 2 IgA deficited cöliákiás beteg) , ** included (beleszámítva) 2 CD patients with IgA deficit



Sensitivity on 22 CD patients in gluten-free-diet (3-38 months) (22 cöliákiás beteg esetén a szenzitívitás glutén mentes diétával: 3-38 hó)



Specificity on 958 patients in whom a diagnosis of CD was excluded (Specificitás 958 betegnél: cöliákia kizárva a diagnózis során)

RESULTS /EREDMÉNYEK

60 patients were **a-tTg New Gen positive and a-tTG negative. How to proceed???**

	tTg Neo IgA +	tTg Neo IgG +	CeliCheck+ IgA/IgG
60	18	36	6

34/60 were revaluated (újra mérve) after 6 months:

- clinical evaluation
- HLA DQ2/DQ8 haplotype
- retesting for a-tTg New Gen, a-tTG, and a-DGP

16 (47%) were HLA DQ2 or DQ8 positive

18 (53%) were HLA DQ2/DQ8 negative (false-positives)

* 18 of 1006 are < 2% of the samples tested and are very close to equivocal zone (nagyon közel a bizonytalan zónához)

6 out of the 16 (16-ból 6) DQ2/DQ8 positive patients were a-tTg Neo-Epitope negative in a second sample tested after 6 months (6 hónap múlva újra mérve egy második mintából).

RESULTS

Of the 16 DQ2/DQ8 positive patients, 2 showed the following results (retested a third time after 1 year /1 év múlva újra mérve harmadszor is)

BE = stipsis
BS = mother and sister (anya és lány testvér cöliákiás) celiacs; no symptoms

		BASAL SAMPLE						SECOND SAMPLE (6 months)				THIRD SAMPLE (12 months)				
ID	AGE/SEX	a-tTG IgA	EMA	a-DGP U/mL		a-TDPGC U/mL		a-tTG IgA	EMA	a-TDPGC U/mL		a-tTG IgA	EMA	a-TDPGC U/mL		HLA
				IgA	IgG	IgA	IgG			IgA	IgG			IgA	IgG	
		c.off = 4		c.off = 20	c.off= 20	c.off= 18	c.off = 18	c.off= 4		c.off= 18	c.off =18	c.off = 4		c.off= 18	c.off =18	
BE	4/F	2	NEG	16	4	28	16	6	POS	49	13	5	POS	55	21	DQ2
BS	8/F	1	NEG	35	9	241	61	2	NEG	80	26	4	POS	70	35	DQ2/DQ7

Conclusion:
tTg Neo Epitope can be positive up to 12+ months before tTG and DGP antibodies can be observed (Neo-Epitope antibodies!)*
Már 12+ hónapon át pozitívitást mutathat a tTg új epitope, mielőtt a tTG és a DGP antitestek észlelhetők lesznek (Új epitóp antitestek!).

*Bizzaro,N. *et al.* (2010) Cutting-Edge Issues in Celiac Disease and in Gluten Intolerance. *Clin. Rev. Allergy Immunol.*

A Novel Algorithm for the Diagnosis of Celiac Disease and a Comprehensive Review of Celiac Disease Diagnostics

Orit Rozenberg · Aaron Lerner · Avi Pacht · Maya Grinberg · Dina Reginashvili · Clara Henig · Mira Barak

"…In the present study, we challenged the performance of this algorithm in order to empower its diagnostic yield in biopsy proven samples of children as well as adults…"

Evaluation of the best screening assay for Celiac Disease in pediatric patients
A gyermek betegminták legjobb cöliákia szűrővizsgálatának értékelése
The study population consisted of 107 sera samples of newly diagnosed children:
/A vizsgált csoport 107 újonnan diagnosztizált gyermek mintából állt:
55 with positive biopsy results / 55 pozitív biopszia eredmény
52 samples with negative biopsy results. / 52 minta negatív biopsziás eredménnyel

The kits that were evaluated (értékelt kittek):

1 AESKU CeliCheck (New Gen)
2 DiaSorin (Liaison) tTG IgA
3 DPC (Immulite) tTG IgA
4 EUROIMMUN GAFX3 DGP IgG
5 IMMCO new G+ IgA
6 IMMCO new G+ IgG
7 IMMCO new tTG IgA
8 IMMCO new tTG IgG
9 IMMCO tTG IgA
10 INOVA DGP screen
11 INOVA tTG-DGP screen
12 ORGENTEC tTG IgA
13 ORGENTEC tTG IgG
14 ORGENTEC tTG screen
15 PHADIA Varelisa - Celikey tTG IgA

RESULTS: /EREDMÉNYEK

80 samples were tested with all the 15 evaluated assays, and another 27 samples were further tested with the 5 best performing assays.
/80 minta mérése történt meg 15 bevizsgált méréssel és 27 minta tovább vizsgáltata az 5 legjobban teljesítő teszttel

The 5 best -performing kits:

1 AESKU CeliCheck
2 DiaSorin (Liaison) tTG IgA
3 INOVA tTG-DGP screen
4 ORGENTEC tTG IgA
5 ORGENTEC tTG screen

Aesku kit showed **higher specificity** with the evaluated samples, thus it was chosen **as the screening test in our algorithm**.
/Az AESKU kit magasabb specificitást mutatott a mért mintákban, így az algoritmus szűrőtesztjeként került kiválasztásra.

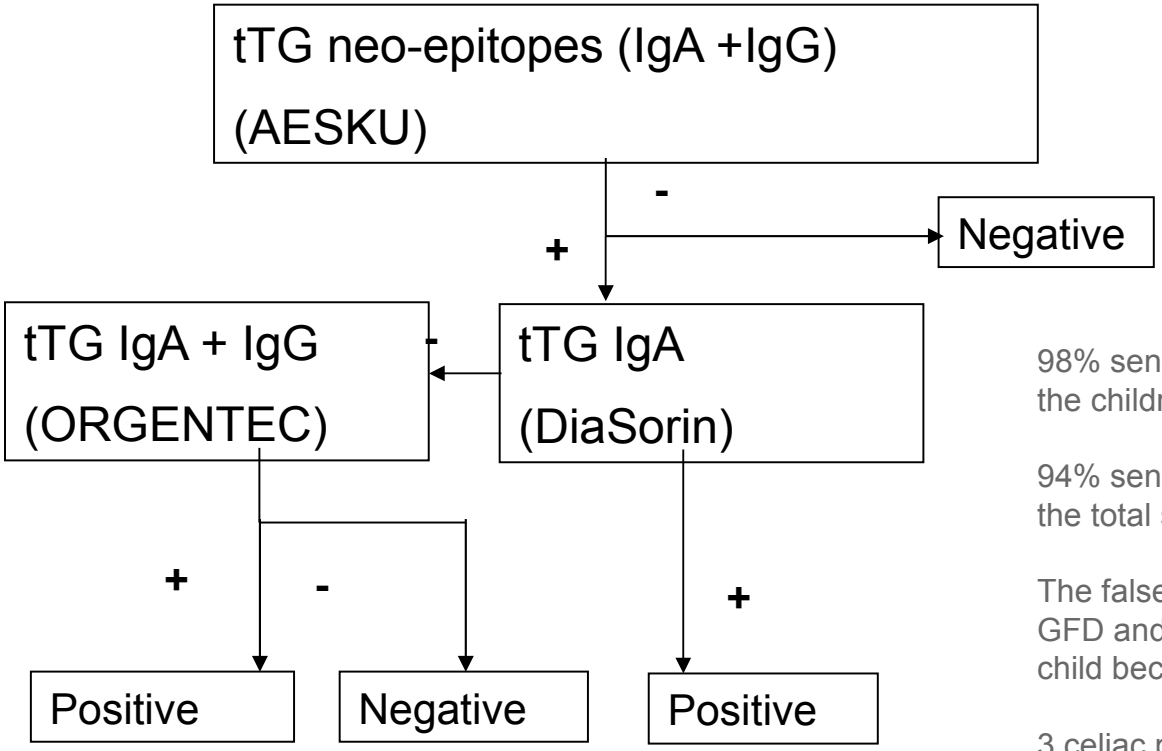
The Liaison and the Orgentec screen kits were chosen as confirmatory (megerősítő tesztként kiválasztva) tests in our algorithm.

Table 3 Calculated sensitivities, specificities and ROC curves

Manufacturer	Manufacturer cut-off or borderline range	Sensitivity	Specificity	AUC	ROC cut-Off	ROC sensitivity	Sensitivity (95% specificity)	ROC specificity	Percent difference between cut-offs (%)
AESKU	16–24 U/ml	1.00	0.95	1.00	*14.2*–23.3	1.00	1.00	0.95	0
PHADIA	5–8 U/ml	0.85	1.00	0.99	2.4	0.97	0.97	0.95	51
INOVA DGP screen	20–30 U	0.95	0.93	0.99	11.9	1.00	0.95	0.93	41
INOVA tTG-DGP screen	20 U	1.00	0.90	1.00	22.9	1.00	1.00	0.95	15
EUROIMMUN GAF-3X	25 RU/mL	0.87	0.95	0.98	4.7	1.00	0.90	0.88	81
DiaSorin (Liaison)	7.2–8.8 AU/mL	0.95	0.98	1.00	4.8	1.00	1.00	0.95	34
IMMCO tTG IgA	20–25 EU/mL	0.87	0.93	0.97	16.3	0.95	0.82	0.90	19
ORGENTEC tTG IgA	10 PGL U/mL	0.97	0.95	0.99	7.4	1.00	0.97	0.93	26
ORGENTEC tTG screen	15 PGL U/mL	0.97	0.95	0.97	*12.0*–15.1	0.97	0.97	0.95	0
DPC (IMMULITE)	4 U/mL	0.97	0.92	0.98	2.7–*5.4*	0.97	0.94	0.95	0
IMMCO new tTG IgA	20–25 EU/mL	0.92	0.90	0.99	*24.9*–27.8	0.92	0.92	0.98	0
IMMCO new tTG IgG	20–25 EU/mL	0.67	0.93	0.93	6.6	0.95	0.62	0.83	67
IMMCO new G+IgA	20–25 EU/mL	0.82	0.80	0.91	1.3	1.00	0.31	0.73	94
IMMCO new G+IgG	20–25 EU/mL	0.95	0.90	0.97	*19.4*–20.2	0.95	0.85	0.90	0
ORGENTEC tTG IgG	10 PGL U/mL	0.79	0.98	0.98	6.2	0.90	0.90	0.98	38

The statistical parameters were calculated with the MedCalc statistical software. In cases where the ROC CO (italicized numbers) was the closest to the recommended manufacturer CO, the next point in the ROC curve was also listed in the table. Manufacturer recommended lowest borderline value was used as cutoff

Celiac Diagnosis–NEW Algorithm



98% sensitivity, 93% specificity and 95% accuracy in the children group

94% sensitivity, 92% specificity and 93% accuracy in the total studied population.

The false negative cases in the adults group due to GFD and the single false negative result in a young child became true positive after six months.

3 celiac patients were monitored before and after diagnosis and found that the algorithm may be suitable for disease monitoring.

Confirmation - new algorithm performance

58 sera samples newly diagnosed children:
27 samples with positive biopsy results
31 samples with negative biopsy results

ELISA	Biopsy: Positive	Biopsy: Negative	
Positive	27	5	
Negative	0	26	58.00

sensitivity	1.00
specificity	0.84

Patient 1: 15 yrs old boy. Type I Diabetes. Positive in all 5 best-performing kits.

5

Patient 2: 18 yrs old boy. G6PD deficiency. Also positive in 2009.

Patient 3: 9 yrs old boy. Also positive in 2008, 2009.

Patient 4: 11 yrs old boy. Also positive in 2008, 2009.

Patient 5: 6 yrs old boy. Positive for anti-streptolysin.

Possible reasons for discrepancy between biopsy and serology /a biopszia és szerológia eltéréseinek lehetséges okai) :

1. Latent celiac disease /Látens cöliákia
2. Changes in atrophy or serology with time /Idővel változik az atrófia, vagy szerológia
3. Regions biopsied in the intestine /A bél biopszia területei
4. Other autoimmune diseases /Más autoimmun megbetegedés
5. Drugs (immunosuppressant, steroids) /Gyógyszerek, immunszupresszáns, szteroidok
6. Gluten-free diet /Glutén mentes diéta
7. Misinterpretation / Sample processing and clerical mistakes /Félreértelmezés/ Minta feldolgozási és adminisztrációs hiba

The new algorithm is more sensitive and more specific

Az új algoritmus érzékenyebb és specifikuasabb

The new algorithm is more cost effective and can economize 36,000€/Yr.

Az új algoritmus költséghatékonyabb (36.000.-€ évi megtakarítás).

Published
January 2012

European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease

Gyermekekben és fiatalokban a cöliákiára utaló jelek, vagy tünetek, és a 10-szeres ULN szintnél magasabb anti-TG2 titer esetén, a bolyos sorvadás (Marsh 3) valószínűsége magas.

In Which Patients Can the Diagnosis of CD Be Made Without Duodenal Biopsies?

In children and adolescents with signs or symptoms suggestive of CD and high anti-TG2 titers with levels >10 times ULN, the likelihood for villous atrophy (Marsh 3) is high. In this situation, the paediatric gastroenterologist may discuss with the parents and patient (as appropriate for age) the option of performing further laboratory testing (EMA, HLA) to make the diagnosis of CD without biopsies. Antibody positivity should be verified by EMA from a blood sample drawn at an occasion separate from the initial test to avoid false-positive serology results owing to mislabeling of blood samples or other technical mistakes. If EMA testing confirms specific CD antibody positivity in this second blood sample, then

ESPGHAN Guidelines for Diagnosis of Coeliac Disease

ULN=upper limit normal range

APPENDIX I
COMPARISON OF HIGH SERUM ANTI-TG2 ANTIBODY LEVELS OBTAINED BY DIFFERENT COMMERCIAL TESTS

TABLE A. Median values obtained in 2009 for the same UKNEQAS positive test samples in 306 European clinical laboratories by the 14 most frequently applied serum anti-TG2 IgA antibody assays

Test kits	Sample I (13.6 U)	Sample II (18 U)	Sample III (30.1 U), high positive	Cutoff	Times ULN for the high sample
Aesku	48	63	135	15	9.0
Binding Site	18	24.1	33.3	4	8.3
BMD Luminex	32.5	27	43	15	
Diasorin	28.6	37.5	57	8	7.1
Euroimmun	171.9	186	200	20	10.0
Eurospital*	70	80.1	95	7	13.6
Generic assays	39.9	44.3	89	20	4.5
Genesis	36.9	48.8	69	7	9.9
Immco	25.9	29.8	48.3	20	2.4

TABLE A. (*Continued*)

Test kits	Sample I (13.6 U)	Sample II (18 U)	Sample III (30.1 U), high positive	Cutoff	Times ULN for the high sample
Inova*	56	69	95.5	20	4.8
Orgentec	25.8	33.2	65.5	10	6.6
Phadia ELIA	35	45	69	7	9.9
Phadia Immuno CAP	34.9	43.5	71	7	10.1

Figure A shows that most tests can distinguish slightly (4 times ULN), moderately (6 times ULN), and highly (10 times ULN) positive anti-TG2 levels

Conclusion

-CD is sub or mis diagnosed and the incidence its much higher than what we believe. Cöliákia alálbecsülése, v. félrediagnosztizálása sokkal gyakoribb, mint gondolnánk

-New serology markers (Neoepitopes) correlate with the activity of the disease:

-/Új szerológiai markerek (új epitópok) korrelálnak a betegség aktívitásával

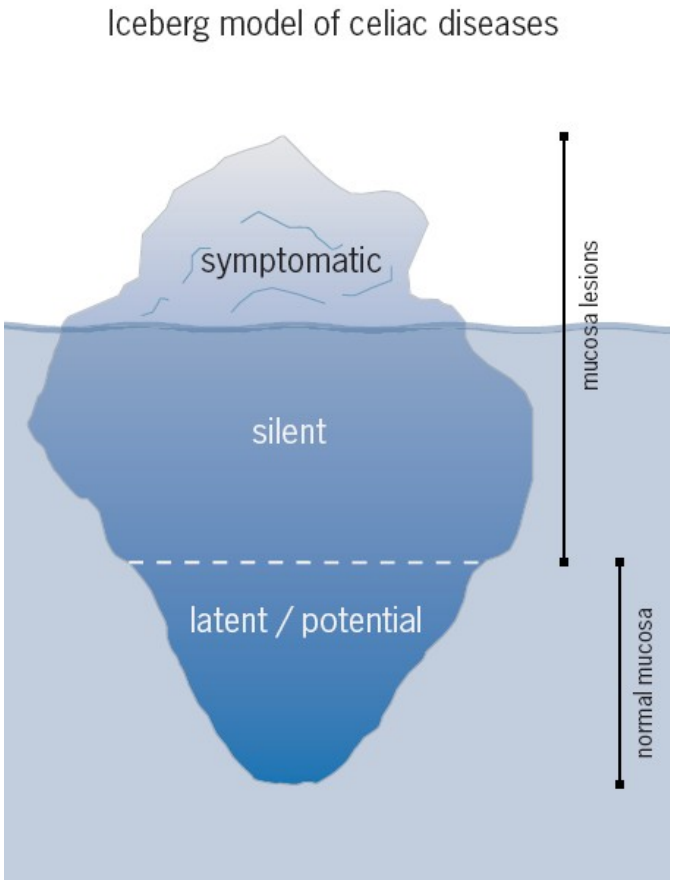
Marsh criteria

- Mucosal damage
- T-Lymphocyte infiltration
- if 10>ULN biopsy might be avoided (biopszia elkerülhető)
- up to 6 month earlier detection of CD (a cöliákia akár 6 hónappal korábbi kimutatása)

More and more studies proof that the serological testing are the fastest and cheapest method to Screen patients for CD (a szerológiai vizsgálat a leggyorsabb és legolcsóbb a cöliákiás betegek szűrésére)

Accordingly with the clinical experience Neoepitopes are an optimal screening tool for diagnosing CD and further knowledge of the pathomechanisms can confirm that.

(optimális szűrő eszköz lehet a cöliákiára, a pathomechanizmus további ismerete megerősítheti)



Thank you for your attention