Laboratory Methods for Diagnosing Celiac Disease

Vijay Kumar, PhD, FACB
IMMCO Diagnostics, Inc.
Buffalo, NY

Prevalence of Celiac Disease

Group	Incidence (%)
With Symptoms	
Adults	1.47
Children	4.00
Associated Symptoms	
Chronic diarrhea	3.85
Abdominal pain	3.20
Constipation	2.63
Anemia	4.17
Short stature	4.00
Fatigue	2.94
Joint pain	3.23
Osteoporosis	2.56

Group	Incidence (%)
Without Symptoms	
Adults	0.95
Children	0.31
Family Members	
First-degree	4.55
Second-degree	2.59
Other Disease Associated	
Type I diabetes	4.35
Autoimmune thyroiditis	2.00
Sjogren's syndrome	2.00
Infertility of unknown etiology	6.25
Down Syndrome	9.09
Asthma	2.63

Arch Intern Med 2003; 163:286-292

Clinical Manifestations of CD

1. **Gastrointestinal Manifestations**

   Early onset — **Age<2 years, Features-diarrhea/steatorrhea, poor weight gain, abdominal distention, muscle wasting, apathy/irritability, hypotonia**

   Late onset — **Age variable (childhood to adulthood). Features—diarrhea (variable/intermittent), nausea/vomiting, abdominal discomfort/pain, bloating, weight loss, constipation**

2. **Musculoskeletal Manifestations**
   - **Short stature**
   - **Osteoporosis**
   - **Dental enamel defects**
   - **Arthritis**
   - **Myopathy**

3. **Mucocutaneous Manifestations**
   - **Dermatitis herpetiformis**
   - **Recurrent aphthous stomatitis**
   - **Vasculitis**

Clinical Manifestations of CD

4. Hematological Manifestations
   - **Anemia—unexplained (iron/folate/B12 deficiency)**
   - **Leucopenia**
   - **Thrombocytopenia**
   - **Vitamin K deficiency**

5. Reproductive System Manifestations
   - **Infertility**
   - **Recurrent abortions**
   - **Delayed Puberty**
   - **Menstrual irregularities**

6. Neurological Manifestations
   - **Epilepsy with cerebral calcifications**
   - **Cerebella ataxia**
   - **Peripheral neuropathy**
   - **Dementia**

7. Autoimmune

8. Miscellaneous Manifestations
   - **Elevated liver enzymes**
   - **Unexplained weight loss**
   - **Lassitude/weakness**
   - **Intestinal lymphoma**

Hill J. et al Pediatr Gastroenterol Nutr. **35**:2002

Available Diagnostic Tools for Celiac Disease

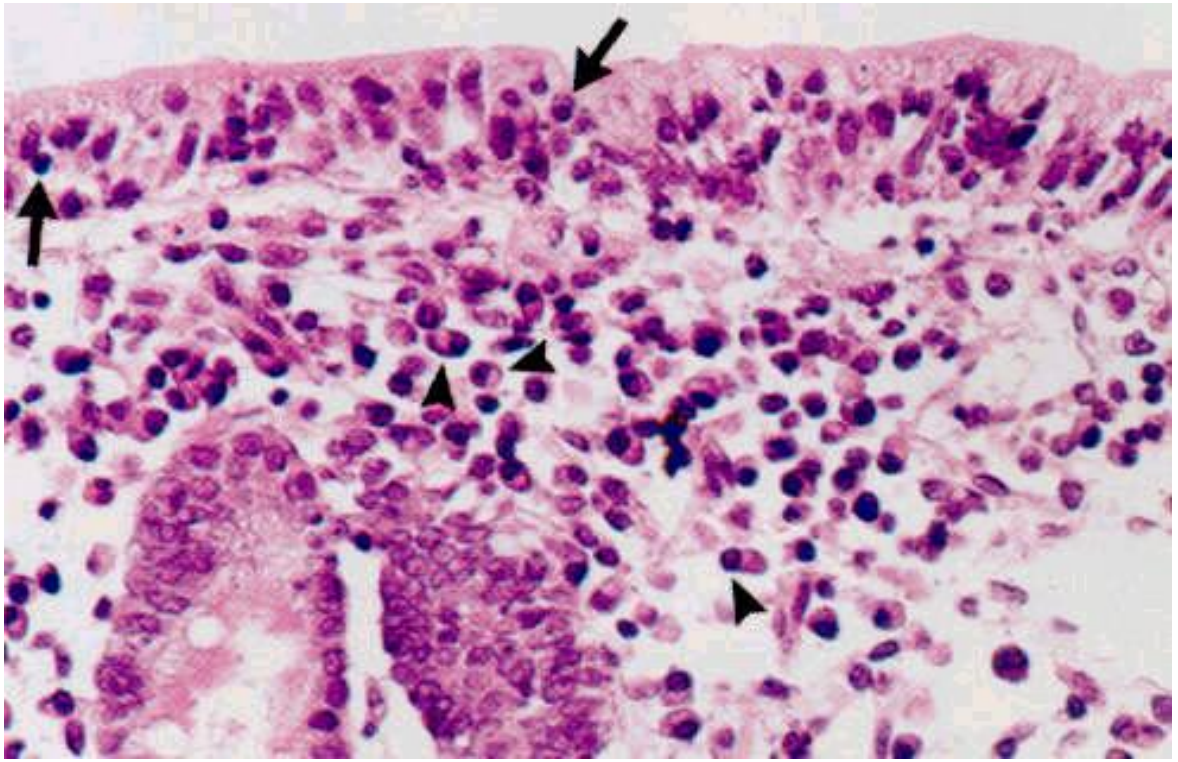
- **Histopathology**
- **Serology**
  - Endomysial antibodies
  - Reticulin antibodies
  - Gliadin, IgG & IgA antibodies.
  - tTG antibodies
  - Deamidated gluten peptide antibodies

Histopathology

Duodenal specimen showing total villous atrophy and crypt hyperplasia.

Histopathology

Marked increase in intraepithelial lymphocytes (arrows) in duodenal surface epithelium. Numerous plasma cells are present in the lamina propria (arrowheads).

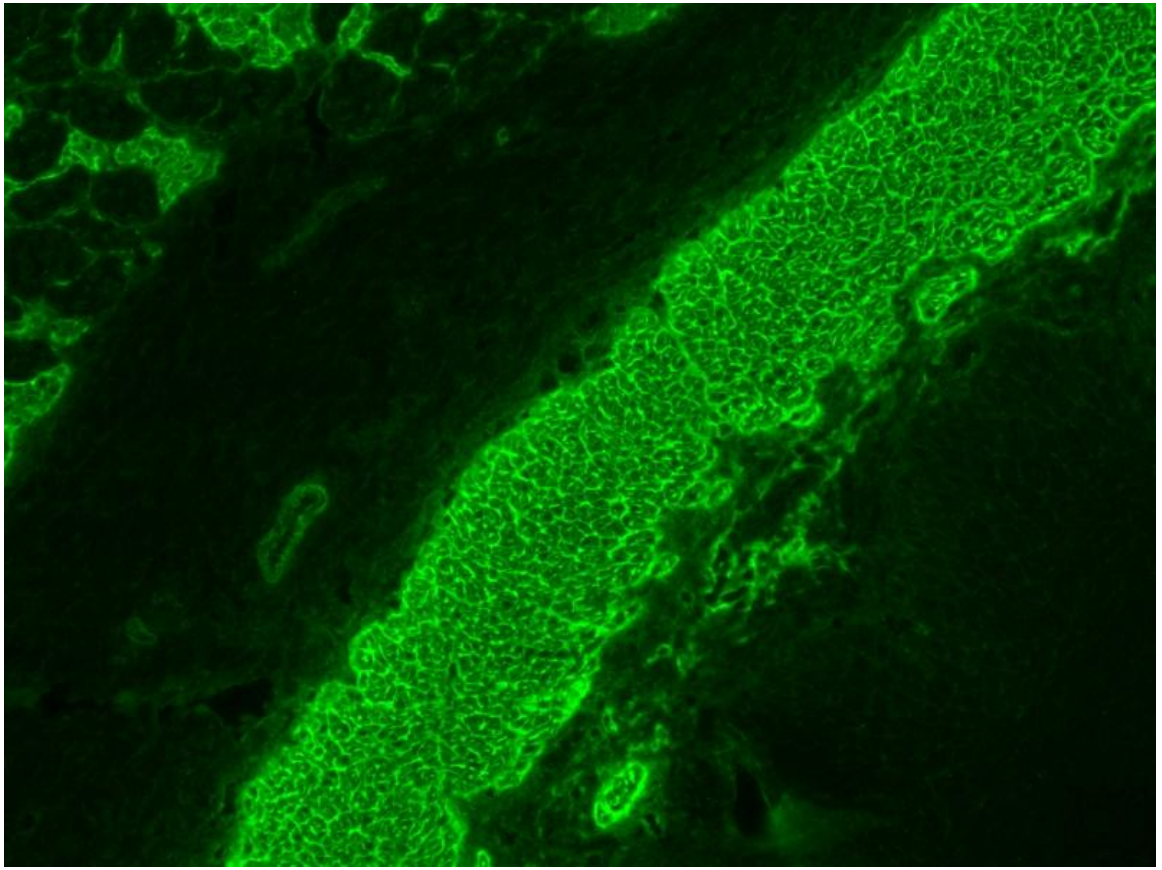


Limitations of Histopathological Diagnosis

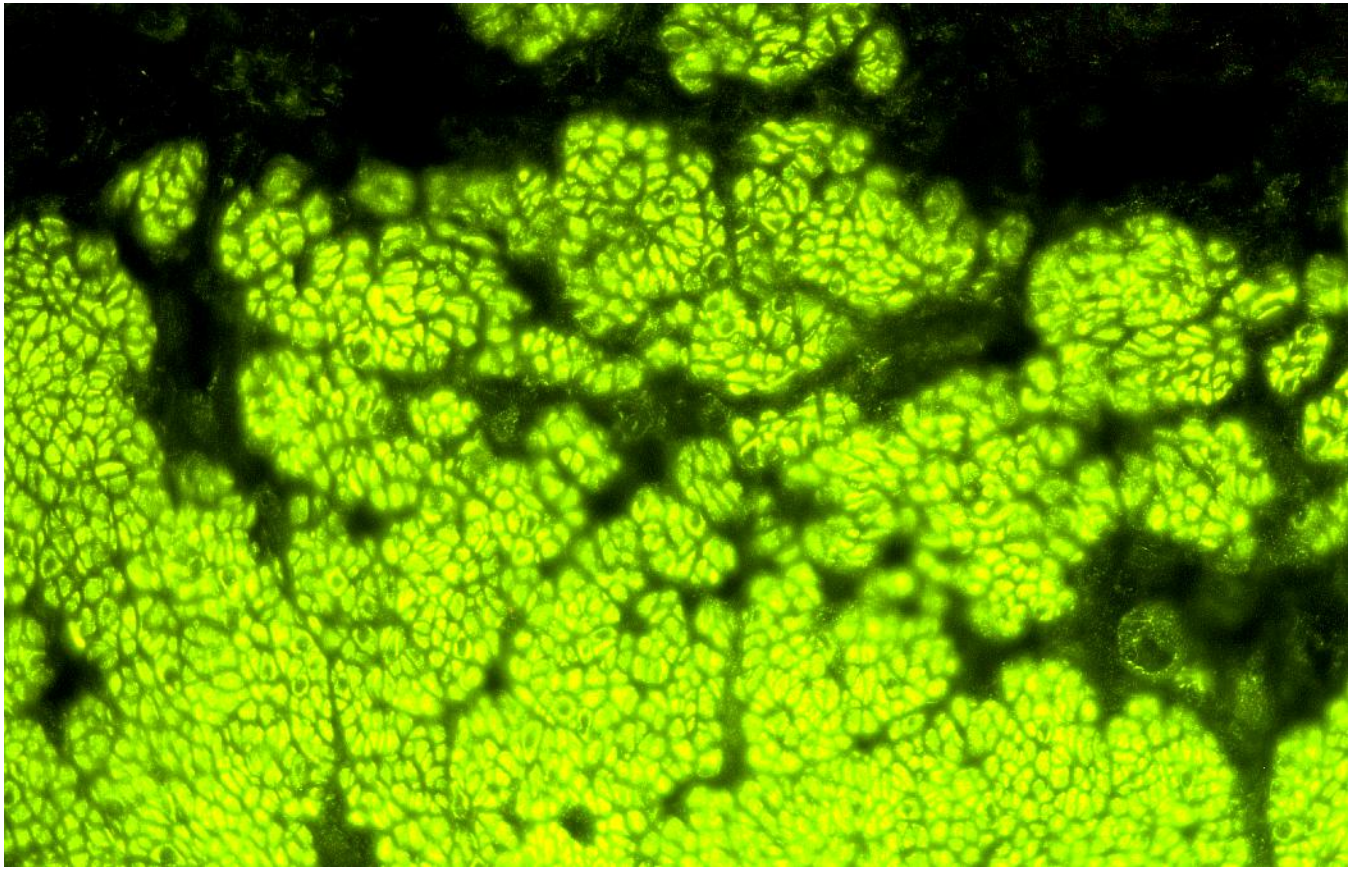
- Discomfort related to intestinal biopsy
- A biopsy with abnormally high density of intraepithelial lymphocyte with a normal villous and crypt architecture may be reported as normal
- Latent Celiac patients may show normal biopsy.
- Other diseases may have morphological changes mimicking CD

Endomysium

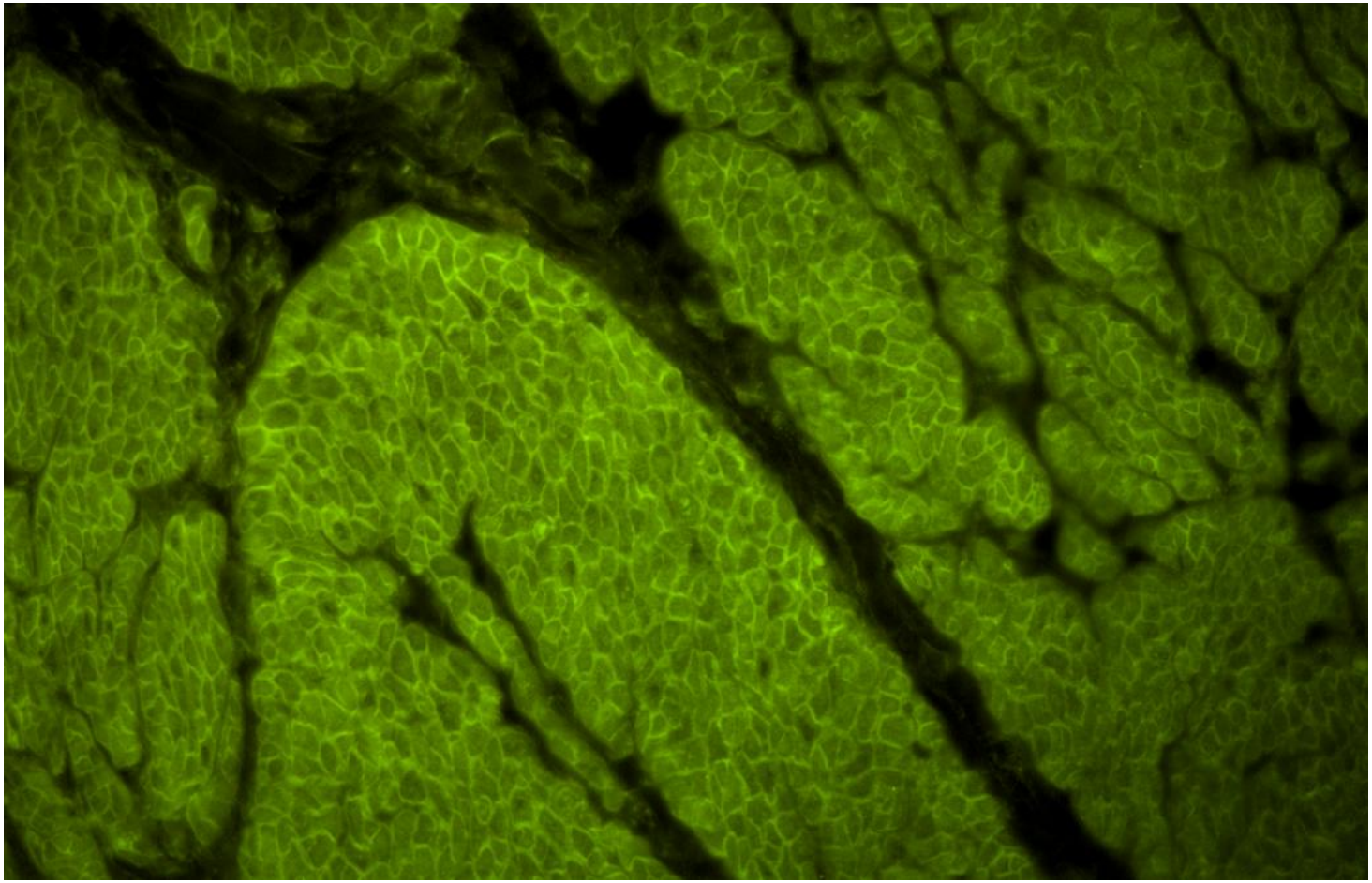
Endomysial Antibodies



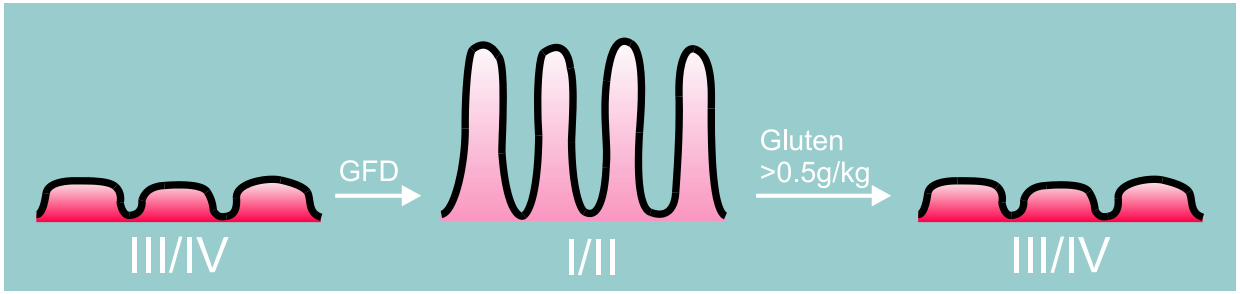
Smooth Muscle Antibodies



Non-Endomysial Reaction



CD Confirmed by ESPGAN Criteria

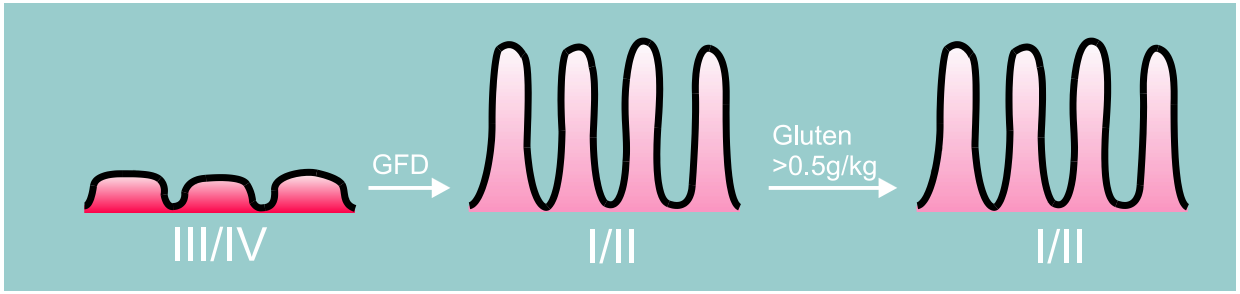


No. cases with above bx. Findings	**133**	**122**	**133**
		11 cases with no biopsy	
Total EMA	**133**	**133**	**130**

*** IgA deficiency in one case of celiac disease**

**** EmA did not reappear in three cases**

CD Ruled Out by ESPGAN Criteria



No. cases with above bx. Findings	**31**	**31**	**31**
EMA	**Negative**	**Negative**	**Negative**

Specificity of EmA: 100%

EMA in Controls

Age Group	Diagnosis	IgG-EmA Positive/Total
Children	Infectious diarrhea	0/210
	Toddler's diarrhea	0/170
	Recurrent diarrhea	0/124*
	Milk protein sensitivity	0/60
	Mucoviscoidosis	0/4
	Ulcerative colitis	0/39
Adults	Healthy adults	0/87
	Duodenal ulcers	0/83
Specificity: 100%		Total: 0/777

* 104 negative challenges controlled with jejunal biopsy studies and IgA-EmA tests; 20 negative challenges controlled with IgA-EmA only.

Reported Effectiveness of EMA in Screening for CD

Study	Sensitivity	Specificity	+ Pred Value	- Pred Value
Rossi et al	100	100	100	100
Kumar et al	100	100	100	100
Karpah et al	84	100	100	79
Fereira et al	100	99	91	100
Vogelsans et al	100	100	100	100
Cataldo et al	94	96		
Lerner et al	97	98	97	98
Valdimarsson et al	74	100	100	96
Del Rosario et al	100	100	100	100
McMillan et al	89	100	100	96
Unsworth DJ	95	94	94	97
Ascher et al	98	100		

EMA Reappearance after Gluten Challenge of 12 Proven CD Cases

Time after challenge (months)	No. positive IgA-EmA/total	
	Normal gut mucosa	Subtotal villous atrophy*
3-5	10/12	
6-8		12/12

* Exceptional cases with subtotal villous atrophy and negative IgA-EMA have been found in larger groups of patients.

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REGULAR ARTICLE

IgA endomysium antibodies – an early predictor for celiac disease in children without villous atrophy

E Grodzinsky (Ewa.Grodzinsky@lio.se)[1], K Fälth-Magnusson[2], L Högberg[3], G Jansson[4], P Laurin[2], L Stenhammar[2,3]

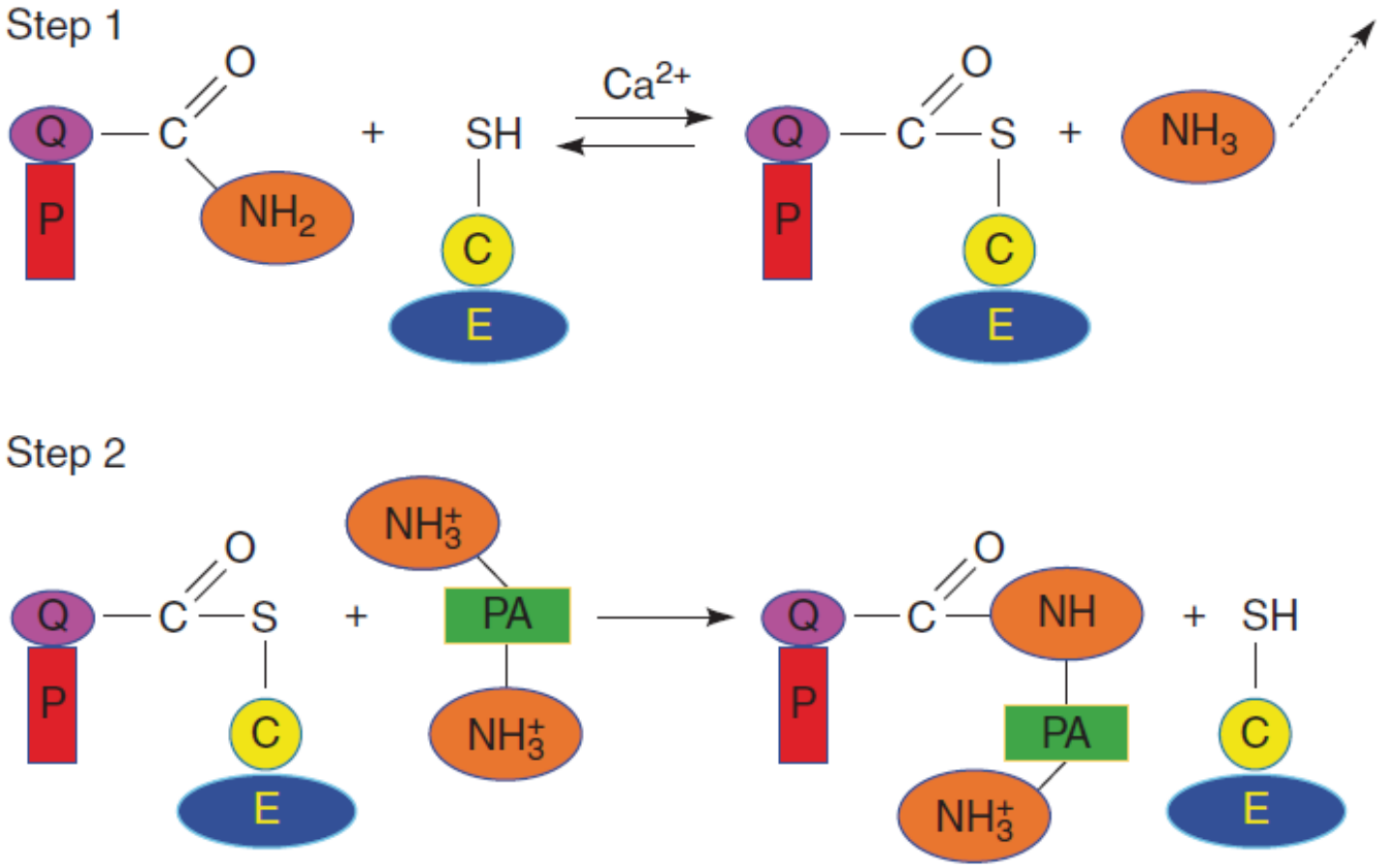
Conclusion: EMA positivity in the absence of small bowel enteropathy could be a very early predictor for later overt CD, and necessitates further follow-up, especially if the child is AGA positive and there is a family history of CD.

Transglutaminase

Table 1. TGase Isoenzymes and their Major Biological Functions where Known

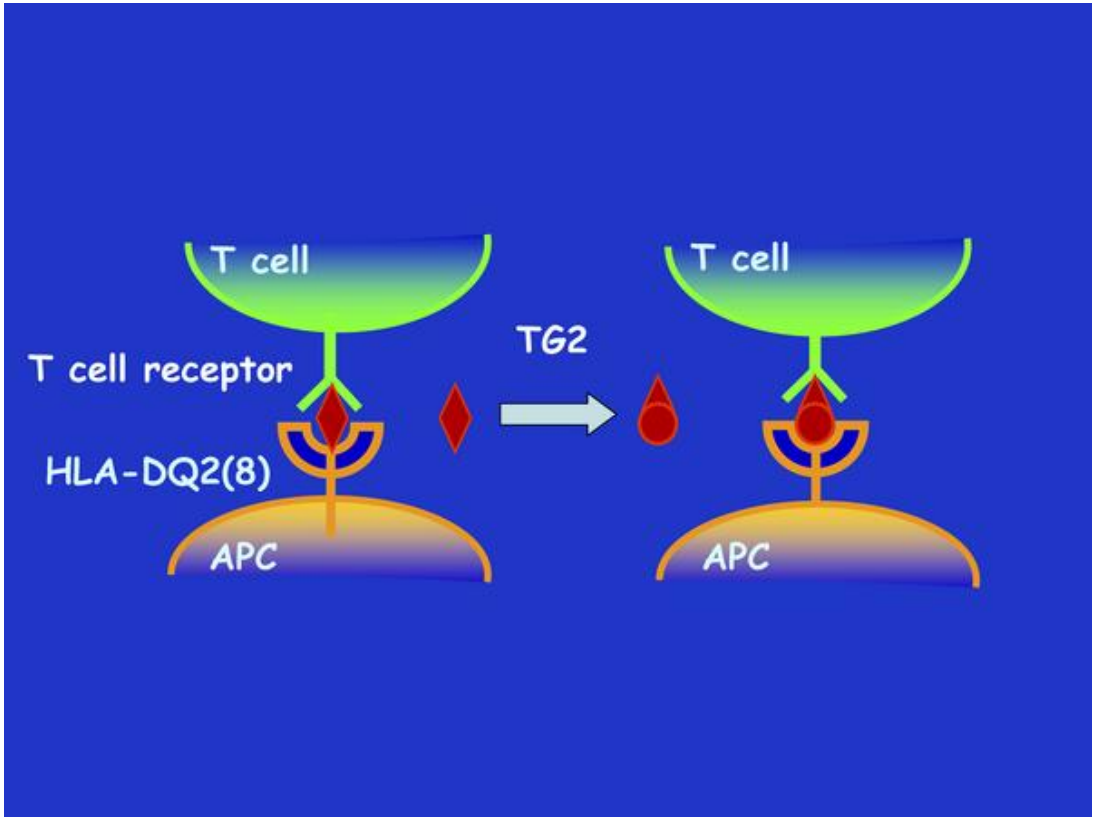
TGase	Biological function	Gene map locus
Factor XIIIa	Blood clotting	6p24-25
TGase 1 (Keratinocyte TGase, kTGase)	Skin differentiation	14q11.2
TGase 2 (Tissue TGase, tTGase, cTGase)	Apoptosis, cell adhesion, signal transduction	20q11-12
TGase 3 (Epidermal TGase, eTGase)	Hair follicle differentiation	20q11-12
TGase 4 (Prostate TGase, pTGase)	Suppression of sperm immunogenicity	3q21-22
TGase 5 (TGase X)	Epidermal differentiation	15q15.2
TGase 6 (TGase Y)	Unknown function	20q11
TGase 7 (TGase Z)	Unknown function	15q15.2

***Current Medicinal Chemistry*, 2006, *13*, 1895-1902**



Annals of Botany **102**: 145–152, 2008

The Role of TG2 in CD



Incidence of Antibodies to Tissue Transglutaminase

Study	Celiac Disease	Control
Sulkanen S et al, Gastroenterology 1998;115:1322-25	129/136 (95%)	13/207 (94%)
Dieterich W et al, Gastroenterology 1998; 115:1317-21	102/106 (95%)	6/114 (95%)
Humbel et al 4th, Dresden Symposium, 1998	85/117 (73%)	6/204 (97%)
Kumar et al, 1999	137/150 (92%)	2/90 (97%)

Association of EMA to tTg Antibodies

EMA Titer	No. Tested	tTg Antibody			
		Negative	Borderline	Positive	Strong Pos.
<10	198	118	39	39	2
10 – 40	35	5	6	13	10
40 – 320	61	0	0	14	47
>640	8	0	0	2	6

Conrad K et al., 4th Dresden Symposium 1998

tTG-IgA Autoantibodies

		EMA		
		Positive	Negative	Total
tTG	Positive	142	5	147
	Negative	1	138	139
	Total	143	143	286

Relative Sensitivity	99%
Relative Specificity	97%
Relative Agreement	98%

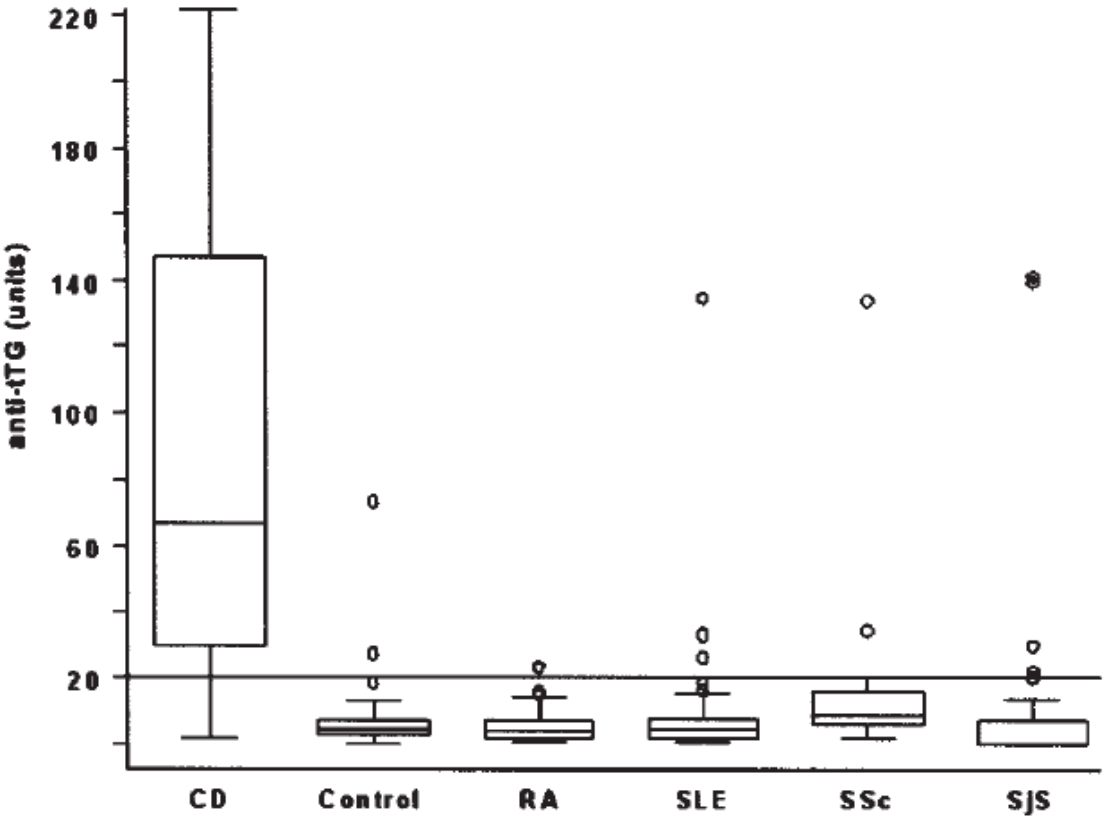
tTG-IgA Autoantibodies

		Disease Group		
		CD	Normal	Total
tTG	Positive	60	0	60
	Negative	0	20	20
	Total	60	20	80

Relative Sensitivity	100%
Relative Specificity	100%
Relative Agreement	100%

Autoantibodies to Tissue Transglutaminase in Sjögren's Syndrome and Related Rheumatic Diseases

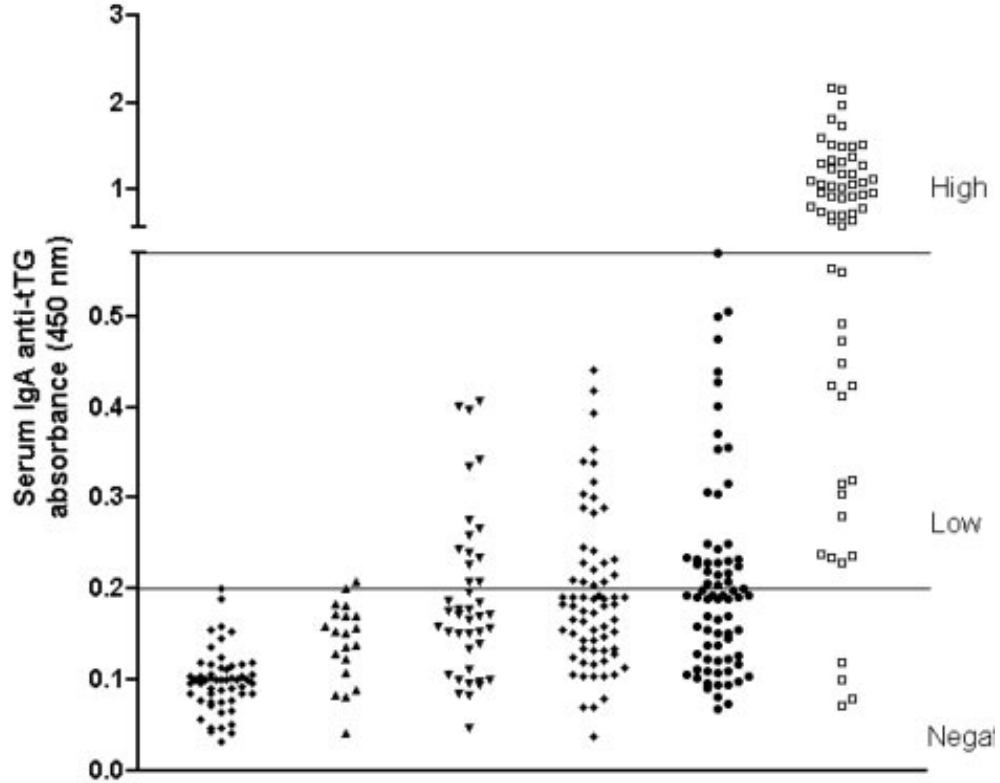
LEEANNE M. LUFT, SUSAN G. BARR, LIAM O. MARTIN, EDWARD K.L. CHAN, and MARVIN J. FRITZLER



Clinical Chemistry 49, No. 12, 2003

Anti-Tissue Transglutaminase Antibodies in Arthritic Patients: A Disease-specific Finding?

*Antonio Picarelli,1**



	Controls (n=54)	OA (n=20)	AS (n=42)	PsA (n=67)	RA (n=74)	CD (n=60)
First quartile	0.078	0.108	0.124	0.132	0.124	0.424
Median	0.1	0.153	0.173	0.182	0.193	0.909
Third quartile	0.114	0.172	0.238	0.229	0.233	1.288

Available online at www.sciencedirect.com
ScienceDirect
Clinica Chimica Acta 376 (2007) 126–135
www.elsevier.com/locate/clinchim

Tissue transglutaminase ELISA positivity in autoimmune disease independent of gluten-sensitive disease

Miklós Sárdy [a,*], Márta Csikós [a], Christof Geisen [b], Klaudia Preisz [a], Zoltán Kornseé [a], Erika Tomsits [c], Ulrich Töx [d], Nicolas Hunzelmann [e], Jörgen Wieslander [f], Sarolta Kárpáti [a], Mats Paulsson [g], Neil Smyth [g]

Results: Many sera from patients with autoimmune disorders gave a positive signal in the human TGc ELISAs. The signal appeared related to minor impurities in the recombinant human TGc used and to raised serum IgA antibody levels rather than to the occurrence of TGc specific antibodies in these patients.
Conclusions: No association of anti-TGc Abs and autoimmune conditions independent of gluten-sensitive disease could be shown. Care should be taken to exclude copurification of chaperones, like heat shock protein 70, where preparing antigens for TGc ELISAs.

Journal of Clinical Laboratory Analysis 20:184–189 (2006)

Low Specificity of Anti-Tissue Transglutaminase Antibodies in Patients With Primary Biliary Cirrhosis

N. Bizzaro,[1]* M. Tampoia,[2] D. Villalta,[3] S. Platzgummer,[4] M. Liguori,[5] R. Tozzoli,[6] and E. Tonutti[7]

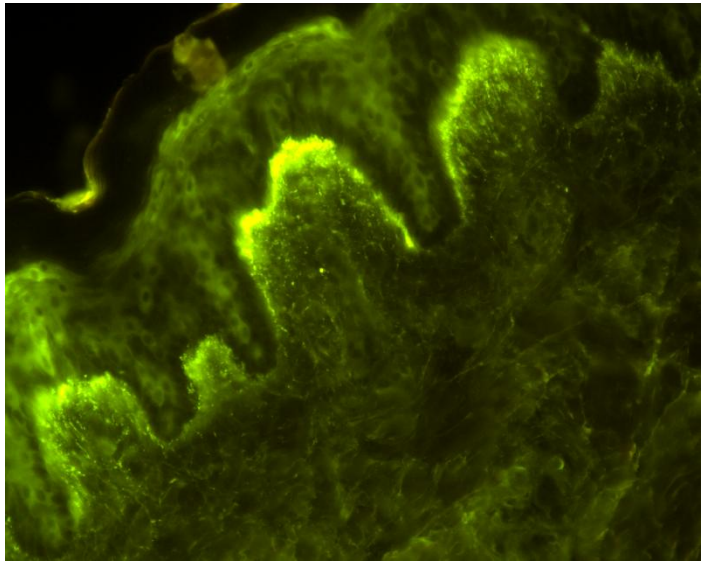
The association between celiac disease (CD) and primary biliary cirrhosis (PBC) is well documented in medical literature; however, a high frequency of false positive results of the anti-transglutaminase (anti-tTG) test has been reported in patients with PBC. To verify if the positive results for anti-tTG autoantibody are false positives due to cross reactivity with mitochondrial antigens, we studied 105 adult patients affected with PBC, positive for anti-mitochondrial M2 antibodies. Anti-tTG IgA antibodies were studied by using six different immunoenzymatic assays that employ the tTG antigen obtained from different sources (human recombinant, placenta, red blood cells, and guinea pig liver). On the whole, 28 out of 105 PBC subjects tested positive for anti-tTG IgA antibodies, but only two were eventually found to be affected by CD; the other 26 were shown to be false positive. The specificity of the various antigenic substrates ranged from 88.5% of the human erythrocytes tTG to 97.1% of the human recombinant tTG. The results of this study showed that a true association between PBC and CD was present in only 2% of the patients and that, in most cases, the false positive results were attributable to the type of substrate utilized in the assay. J. Clin. Lab. Anal. 20:184–189, 2006.

Gliadin

Anti-gliadin Antibodies

70 year old female

Clinical impression: Dermatitis herpetiformis



EMA	Neg
ARA	Neg
tTG	Neg
AGA	Pos

IgA Deficiency

- Defined as the total absence or severe deficiency of IgA
- Occurs one in 400 to 2,000 individuals.
- Incidence varies across racial and ethnic lines
  - 1 in 500 to 1 in 700 in European ancestry
- Serum levels for IgA deficient persons are usually found to be 7 mg/dL or less, while serum IgA in normal adults ranges from 90 to 450 mg/dL.
- IgA deficiency has been found in some patients with
  - Ataxia-telangiectasia
  - Frequent respiratory infections (ear infections, sinusitis, and/or pneumonia)
  - Allergies (asthma and food allergies)
  - Autoimmune disorders

dGP IgA Autoantibodies

		Disease Group		
		CD	Normals	Total
Celiac G+	Positive	94	0	94
	Negative	0	35	35
	Total	94	35	129

Relative Sensitivity	100%
Relative Specificity	100%
Relative Agreement	100%

dGP IgG Autoantibodies

		Disease Group		
		CD	Normals	Total
Celiac G+	Positive	73	0	73
	Negative	0	14	14
	Total	73	14	87

Relative Sensitivity	100%
Relative Specificity	100%
Relative Agreement	100%

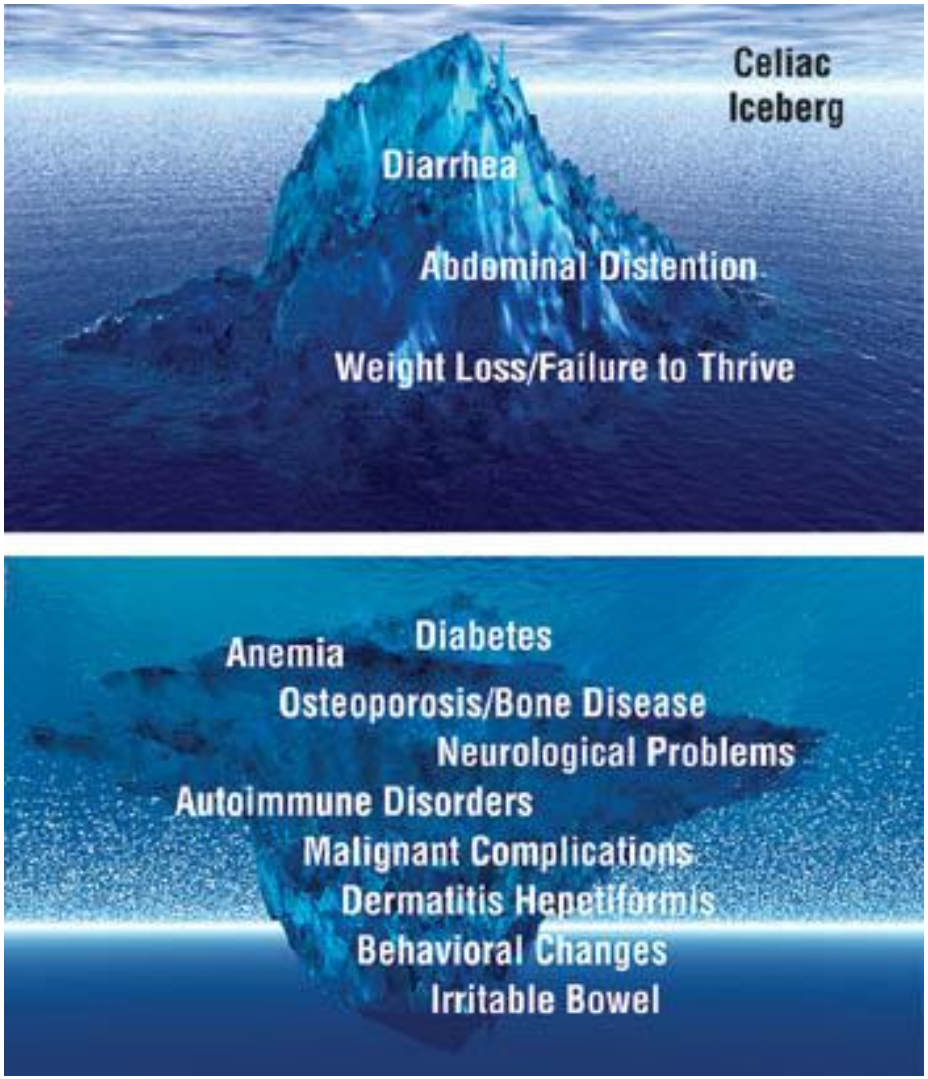
CD group Includes 23 subjects with IgA deficiency (children 14; adults 9)

Role of Serology in CD Diagnosis

- Patients who have villous atrophy in combination with positive serology have CD.
- Symptoms should disappear and serology should normalize on GFD.
- Therefore, gluten challenge may not be necessary.

Wolters VM J Pediatr Gastroenterol Nutr. 2009

Conclusion


Celiac
Iceberg
Diarrhea
Abdominal Distention
Weight Loss/Failure to Thrive
Anemia
Diabetes
Osteoporosis/Bone Disease
Neurological Problems
Autoimmune Disorders
Malignant Complications
Dermatitis Hepetiformis
Behavioral Changes
Irritable Bowel

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Is Intestinal Biopsy Always Needed for Diagnosis of Celiac Disease?

Riccardo Scoglio, MD, Giuseppe Di Pasquale, MD, Giuseppe Pagano, MD, Maria Cristina Lucanto, MD, Giuseppe Magazzù, MD, and Concetta Sferlazzas, MD

Department of Pediatrics, GI Unit, University of Messina, Messina, Italy