

Celiac disease serology in dermatitis herpetiformis. Which is the best option for detecting gluten sensitivity?

Emilia Sugai, Edgardo Smecuol, Sonia Niveloni, Horacio Vázquez, Marcelo Label, Roberto Mazure, Andrea Czech, Zulema Kogan, Eduardo Mauriño, Julio C Bai

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Summary

Background: Dermatitis herpetiformis (DH), a well-established gluten-sensitive skin disorder presenting variable degrees of enteropathy, constitutes a very useful model in order to assess the utility of the celiac disease (CD)-related serology in patients with mild intestinal damage. **Objective:** Our aim was to explore comparatively the performance of a panel of CD-related serologic tests in patients with DH. **Methods:** We assessed a series of 18 consecutive patients with skin biopsy proven DH presenting the overall spectrum of intestinal damage ranging from normal mucosa (n=6) to total villous atrophy (TVA) (n=6) through partial villous atrophy (PVA) (n=6). Sera were obtained from all patients while consuming a gluten containing diet. Serologic tests were antiendomysial, anti-tissue transglutaminase and anti-gliadin antibodies, and newly developed tests detecting both antibody isotypes (IgA and IgG) against deamidated synthetic gliadin-derived peptides (a-GDP). **Results:** Serologic tests had a variable behaviour depending on the degree of enteropathy. While the majority of tests detected patients with TVA, only 50% of those with normal histology had positive assays. Patients with PVA had discordant results. Classical CD-specific tests were positive in only some patients with mild damage while all of them were identified by a single assay detecting both isotypes of a-GDP. **Conclusion:** The detection of a-GDP antibodies was the most reliable tool in order to identify gluten

sensitivity in DH patients presenting a wide range of intestinal damage. Further studies should explore if these findings can be extrapolated to patients with CD having mild enteropathy.

Index (palabras claves): Celiac disease, dermatitis herpetiformis, serology, intestinal permeability, gluten sensitivity.

Resumen

Serología de enfermedad celiaca en dermatitis herpetiforme. ¿Cuál es la mejor opción para detectar sensibilidad al gluten?

Introducción: la dermatitis herpetiformis (DH), una lesión dermatológica consecuencia de sensibilidad al gluten y asociada a grados variables de enteropatía, constituye un modelo muy útil con el objeto de evaluar la eficacia de la serología de la enfermedad celiaca (EC) en pacientes con daño intestinal leve. **Objetivo:** explorar comparativamente la utilidad de una serie de anticuerpos empleados en EC en pacientes con DH. **Métodos:** analizamos una serie de 18 pacientes consecutivos con diagnóstico de DH por biopsia de piel que presentaban el más amplio espectro de daño intestinal variando desde una mucosa normal (n=6) a la atrofia vellosa total (AVT) (n=6) y pasando por atrofia vellosa parcial (AVP) (n=6). Se obtuvo plasma de todos los pacientes mientras consumían gluten. Las pruebas serológicas empleadas fueron anticuerpos antiendomysio, anti-transglutaminasa y atigliadina, y unas pruebas recientemente desarrolladas que detectan anticuerpos IgA e IgG dirigidos contra péptidos sintéticos deamidados derivados de la gliadina (a-GDP). **Resultados:** las diferentes pruebas tuvieron un comportamiento variable dependiendo del grado de lesión intestinal. Mientras que la mayoría de las pruebas detectaron a todos los pacientes con AVT, sólo el 50% de aquellos con histología normal tuvieron resultados positivos. Los pa-

Small Bowel Section, Department of Medicine, Hospital de Gastroenterología "Dr. Carlos Bonorino Udaondo", Buenos Aires, Argentina.

Correspondence: Julio C Bai
Department of Medicine - Hospital de Gastroenterología "Carlos Bonorino Udaondo", Av. Caseros 2061, (1264) Buenos Aires; Argentina
E-mail: jbai@intramed.net
Phone: 54-11-4306-4641 ext. 117
Fax: 54-11-4304-1018

cientos con AVP tuvieron resultados discordantes. Así las pruebas clásicas fueron positivas en sólo algunos pacientes con daño leve, mientras que todos ellos fueron positivos a una prueba para detectar ambos isotipos del a-GDP. Conclusión: la determinación de anticuerpos a-GDP fue la herramienta más confiable con el objeto de identificar serológicamente la sensibilidad al gluten en pacientes con DH que presentan variables grados de daño intestinal. Otros estudios deberían explorar si estos hallazgos podrían ser extrapolados a pacientes con EC con enteropatía de grado leve.

The widespread availability of serologic tests has permitted diagnosis of celiac disease (CD) in a more simple manner than before.¹ Nevertheless, intestinal biopsy is still the mandatory diagnostic gold standard recommended by the wide majority of researchers.² In the last decade, anti-connective autoantibodies have become the serologic tests of choice due to their very high sensitivity and almost absolute specificity.^{3,4} Interestingly, current knowledge has shown that the available serologic armamentarium lacks sensitivity when used in practice settings, in patients with mild enteropathy and in those cases with minor clinical compromise.^{2,5} Dermatitis herpetiformis, a gluten-dependent, papulovesicular, pruritic rash, considered the skin expression of CD, is a very interesting model characterized by a wide spectrum of enteropathy ranging from normal appearing structure to a flat mucosa.⁶ Our aim in this study was to assess the performance of a complete panel of CD-related antibody tests in a series of patients with a well-documented diagnosis of DH in whom, intestinal histology was categorized either as: normal, partial villous atrophy (PVA) or total villous atrophy (TVA). As a novel aspect, the serology armamentarium employed in this study includes newly developed assays to detect antibodies against deamidated synthetic gliadin-derived peptides (a-DGP) that recently have shown impressive statistical values when compared to other well-established tools.⁷

Patients and methods

We prospectively enrolled a series of 20 non-IgA deficient patients with a well-established diagnosis of DH attending the Small Bowel Section of the Gastroenterology Hospital in Buenos Aires. Diagnosis of DH was based on classical histological and immunopathological skin features determined in

punch biopsy specimens of non-involved skin. At the time of diagnosis and before any specific therapeutic intervention, patients underwent small bowel biopsy (three samples from the distal duodenum) through an upper GI endoscopy and serum sampling for serology. At the same time, all patients performed sugar permeability tests after ruling out the intake of nonsteroidal anti-inflammatory agents, acetylsalicylic acid or alcohol consumption in the last two weeks before the study. All patients were informed of the purpose of the study and agreed to participate. Only 18 patients had all serologic tests performed and due to the aims of the study the results presented will only refer to these patients. We compared serologic results in DH patients with those obtained in a series of 391 subjects without histological evidence of CD. The control non CD population was obtained from an study aiming to determine the performance of serology in order to detect CD in subjects with a low pre-test probability (individuals undergoing upper GI endoscopy due to causes primarily non related with CD).

Laboratory determinations

The CD-related serology consisted in the determination of: 1- IgA a-tTG (QUANTA Lite TM, h-tTG IgA, INOVA Diagnostic Inc.; San Diego, CA) by ELISA (cut-off provided by the manufacturer: 20 AU/mL); 2- IgA EmA by immunofluorescence on primate esophagus substrate (INOVA Diagnostics Inc.; San Diego; CA; USA) tested at a 1:5 dilution; 3- a newly developed enzyme-linked immunosorbent assay (ELISA) to detect IgA and IgG a-DGP antibodies (IgA a-DGP and IgG a-DGP) using a kit provided by the manufacturer for research use only (QUANTA Lite Gliadin IgA and IgG II- INOVA Diagnostic Inc.; San Diego, CA), (cut-off provided by the manufacturer: 20 AU/mL); 4- a single kit to assess simultaneously the presence of both antibody isotypes (QUANTALite, Celiac DGP Screen; INOVA Diagnostic Inc.; San Diego, CA). It uses a fully synthetic selectively deamidated peptide that incorporates several B-cell epitopes. The peptide is constructed so that each epitope is presented in a proper conformational shape. The conjugate is a blend of both anti-human IgA and IgG with most of the reactivity biased towards the IgG (approximate IgG vs. IgA ratio: 70% to 30%). 5- IgA and IgG types anti-gliadin antibodies (AGA), (INOVA Diagnostics Inc. San Diego, CA. USA); 5- IgA type anti-actin antibodies (AAA) determined using a modification of a

commercial ELISA assay for IgG type anti-actin antibodies (QUANTA Lite Actin; INOVA Diagnostics Inc. San Diego, CA. USA); 6- Lactulose/mannitol sugar permeability tests were performed in 16 patients reported in this study.⁸

Small bowel histology

At least 3 biopsy samples were obtained from the distal duodenum by duodenoscopy following a standard protocol. Oriented samples were assessed stained with H&E. Morphology and quantitative evaluations (intraepithelial lymphocyte density) were performed by a pathologist unaware of the clinical and laboratory findings of the subjects, and histology was categorized according to the modified Marsh's classification.^{5,6} For the purposes of this study, patients were categorized as: 1- normal histological appearance considered when patients had Marsh's type 0; 2- PVA includes cases with mild enteropathy from Marsh's type I to type IIIb, and 3- TVA in cases with Marsh's IIIc lesions. In the control population, exclusion of CD was based on the presence of a normal histology (Marsh's type 0).

Results

From the histological point of view, six DH patients had a normal histological appearance, six others had PVA and the six remaining cases had TVA. As was reported before, all those patients tested for sugar permeability (n=16) had abnormal tests irrespective of the histological appearance.⁸ Furthermore, IgA concentration was normal in all subjects.

As shown in tables 1 and 2, no single serologic test was able to detect all DH patients. In contrast, while only one patient with normal mucosa was negative for all tests, 17 of 18 (94%) were positive for at least one test. Overall, EmA was positive in 11 of 18 (61%) DH patients, a-tTG antibodies were positive in 13 of 18 (72%) cases, IgA AGA in 8 of 18 cases (44%) and IgG AGA in 9 of 18 (50%). Both isotypes (IgA and IgG) of a-DGP were positive in 14 of 18 (78%) patients. Interestingly, the determination of both antibody isotypes in a single assay had the greater yield detecting 15 cases (87%) confirmed that was the best single serologic option to select patients.

The performance of serology tests was different according to the degree of intestinal histological damage (figure 1). As expected, both AGA isotypes were scantily sensitive in any subgroup of patients. Only half of patients with normal histological ap-

pearance at the duodenal biopsy (but abnormal permeability) were detected by a-tTG, EmA and a-DGP antibodies. In contrast, all patients with TVA were detected by both, a-tTG, EmA and the three variants of a-DGP antibodies determinations. The group of patients with PVA was critical in terms of sensitivity of the different tests. Thus, EmA was positive in only two of six patients, a-tTG in four and both isotypes of a-DGP in five. Interestingly, the blend of IgA and IgG a-DGP detected all six patients with PVA.

According to the histological assessment of duodenal biopsies where a Marsh's type II or more severe mucosal damage was considered as diagnostic of CD, 391 subjects had not evidence of the disorder and, therefore, assumed as the control population. Among them, 17 individuals were positive for IgA a-tTG (specificity: 95.6%), 18 for IgA a-DGP (95.4%), four for IgG a-DGP (99.0%), six for the conjugate of IgA and IgG a-DGP (98.5%), and 37 for the IgA AAA (90.5%).

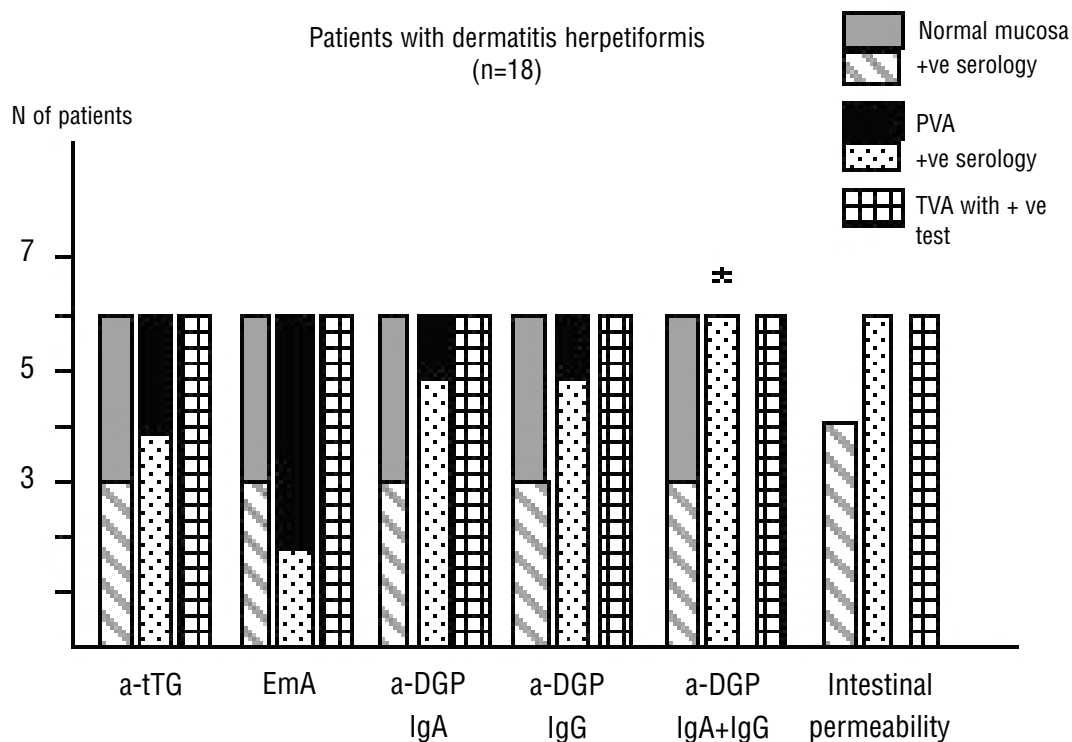
Table 1. Demographic data, histological information and serologic results of DH patients and controls. Histology was characterized as normal (N), partial villous atrophy (PVA) or total villous atrophy (TVA). a-tTG: anti-tissue transglutaminase antibodies; AGA: antigliadin antibodies; EmA: endomysial antibodies; a-DGP: anti-deamidated gluten peptide antibodies. N: number of patients; ND: not done.

	DH patients	Controls
N (female/male)	18 (9/9)	391 (260/131)
Age (range) yr.	38 (14-72)	46 (16-87)
Small bowel histology (N)		
Normal	6	391
PVA	6	-
TVA	6	-
Serology (N of positive cases)		
a-tTG IgA	11	17
AGA IgA	8	ND
AGA IgG	9	ND
EmA IgA	11	ND
a-DGP IgA	14	18
a-DGP IgG	14	4
a-DGP IgA+IgG	15	6

Table 2. Combination analysis of serology tests and histological characteristics of patients with dermatitis herpetiformis. Histology was characterized as normal (N), partial villous atrophy (PVA) or total villous atrophy (TVA). a-tTG: anti-tissue transglutaminase antibodies; AGA: anti-gliadin antibodies; EmA: endomysial antibodies; a-DGP: anti-deamidated gluten peptide antibodies; +: positive results; -: negative results.

a-tTG IgA	AGA IgA	AGA IgG	EmA IgA	DGP-IgA	DGP-IgG	DGP-IgA + IgG	Intestinal Histology	N of patients
+	-	-	+	+	+	+	N	2
+	-	-	-	+	+	+	N	1
-	+	-	-	-	-	-	N	1
-	-	+	+	-	-	-	N	1
-	-	-	-	-	-	-	N	1
+	+	+	+	+	+	+	PVA	1
+	-	+	+	-	+	+	PVA	1
+	+	+	-	+	+	+	PVA	1
-	-	-	-	+	+	+	PVA	2
+	+	-	-	+	-	+	PVA	1
+	+	+	+	+	+	+	TVA	4
+	-	+	+	+	+	+	TVA	1
+	-	-	+	+	+	+	TVA	1

Figure 1. Positive (+ve) results of serology tests according to the degree of severity of histological damage (normal mucosa; partial villous atrophy -PVA- and total villous atrophy -TVA-). a-tTG: anti-tissue transglutaminase antibodies; AGA anti-gliadin antibodies; EmA: endomysial antibodies; a-DGP: anti-deamidated gluten-derived peptide antibodies.



Discussion

The recently questioned performance of the classical CD-related serology for cases with milder histological damage raises the concern of how they can select patients in those clinical settings where many newly diagnosed patients may have minor histological changes. Dermatitis herpetiformis is an ideal gluten-sensitive disorder for assessing the performance of the CD serology in patients exhibiting varied histological damage.^{6,8} We recently had the opportunity to assess a series of DH patients presenting the complete spectrum of histological damage.⁸ Here, we aimed to explore comparatively the performance of a complete set of CD serology tests in the assessment of a series of patients with well-established DH. The different tests used included classical anti-connective autoantibodies (EmA and a-tTG), the AGA's and the newly-developed a-DGP assays detecting antibodies to deamidated synthetic gliadin-derived peptides which have shown a very interesting performance in a previous study.⁷ The a-DGP antibodies set employed included assays for the independent determination of the two main antibody isotypes (IgA and IgG) and an assay detecting both isotypes in a single test.

Our study based on a reduced number of patients diagnosed using the immunopathologic characteristics at skin biopsy as gold standard, has demonstrated that the abnormal intestinal permeability determined by the lactulose/mannitol ratio was the best indicator of the intestinal mucosal damage, even better than the microscopic characterization of mucosal samples.⁸ However, sugar tests lack specificity and the utility of the method in practice seems very limited due to a series of technical factors. So far serology was the best indicator of the gluten dependence of the disorder and can be used as a marker for further diagnostic approach. However, we confirm that the utility of the serologic tests explored, on one hand, was variable depending on the degree of intestinal damage and on the other the type of antibody assayed. As expected, only half of patients exhibiting the pre-infiltrative state of the intestinal mucosal (normal appearance) could be detected by serology. In contrast, all patients with TVA were positive for all tests assessed. Most interestingly, while classical methods such as EmA or a-tTG antibodies

were positive in only some patients with milder forms of enteropathy, the a-DGP detected most cases and the single assay addressing both isotypes of a-DGP was positive in all of them. These findings suggest that the new tests can have potential advantages over to other conventionally used assays. Furthermore, both the IgG a-DGP and the combination of both isotypes of a-DGP assays have shown the greatest specificities compared with other tests, even greater than IgA a-tTG.

Potential biases of our study are the limited number of patients enrolled and the reduced size of each of the three histological-based categorizations. However, if these findings could further be expanded to other CD populations involving a greater number of patients with mild intestinal damage, these results could have a major impact in clinical practice.

In conclusion, according to our findings from this pilot study it seems very likely that a-DGP antibodies could be employed with advantages in comparison with other serology tests in order to detect CD patients with milder intestinal damage who are likely to be missed by other CD-related antibodies.

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