



# ANTIBODIES TO SYNTHETIC GLIADIN-RELATED PEPTIDES FOR DETECTING CELIAC DISEASE IN A POPULATION WITH HIGH PRE-TEST PROBABILITY

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## ABSTRACT

**Introduction:** A novel antibody assays based on synthetic gliadin-related peptides (AGA II) has shown very high diagnostic yield for identification of celiac disease (CD) in selected populations. Aim: to determine the value of these antibodies for detecting CD in individuals with high pre-test probability undergoing intestinal biopsy due to clinical suspicion of small bowel disorder. **Materials:** From October 2004 to September 2005, 122 unselected consecutive patients attending the Malabsorption Clinic of our institution were prospectively enrolled in the study. We excluded patients with prior CD-related serology, intestinal histology or diagnosis of CD or dermatitis herpetiformis. At the time of endoscopy and biopsy, serum samples were obtained for CD-related serology which includes: AGA II IgA, AGA II IgG (QUANTA Lite Gliadin IgA and IgG II- INOVA Diagnostic Inc., San Diego, CA), anti-tissue transglutaminase antibodies (a-tTG) type IgA. Diagnosis of CD and characterization of patients were based on histological criteria (Marsh's type II lesion or greater) and was supported by a-tTG and the response to a gluten-free diet in discordant patients. **Results:** While 57 patients (47%) were finally diagnosed as having CD, 65 had no evidence of the disorder. Compared with controls, CD patients had very significantly higher mean absolute values of both AGA II antibodies ( $p < 0.0000$ ). Sensitivity (Sen), specificity (Spe), diagnostic accuracy (Acc), positive and negative predictive values (PPV and NPV, respectively), and positive and negative likelihood ratios (+LR and -LR, respectively) are reported in the table:

Tests	Sen (%)	Spe (%)	Acc (%)	PPV (%)	NPV (%)	+LR	-LR
AGA II IgA	98.2	92.3	95.1	91.8	98.4	12.7	0.02
AGA II IgG	96.5	100	98.4	100	97.0	96.5	0.03
a-tTG IgA	94.7	96.9	95.9	96.4	95.5	30.5	0.05

Although all assays exhibited outstanding results, the AGA II IgG assay had the best statistical value, even better than the well-recognized a-tTG. **Conclusions:** Our study based on a population of individuals with high pre-test probability has shown that the new gliadin-related peptide antibody assays are observer-independent tests having the highest diagnostic accuracy for detecting CD that should be employed in the setting of high pre-test probability populations.

## BACKGROUND

For more than 30 years, the so-called CD-related serology has been used as valuable marker for diagnosis, screening and non-invasive follow-up of patients. Since the seminal times, the celiac serology was evolving in different ways such as, the identification of newer and more specific antibodies and the improvement of technical approaches in order to minimize failures

During the last 10 to 15 years the serologic diagnosis of CD was dominated by the well-known connective autoantibodies endomysial (EmA) and anti-tissue transglutaminase antibodies (a-tTG). Based on their performance, these autoantibodies are considered the most outstanding non invasive tools for revealing a disorder in all internal medicine

Recently, we have reported a highly encouraging performance for ELISA assays to detect novel antibodies addressed against synthetic gliadin-related peptides (AGA-II)

Studies assessing the diagnostic performance of the available CD serologic tests have shown several potential bias. Among others, the use of pre-selected populations and the inherent interdependence between tests and the diagnostic criteria used to define CD are the most obvious defects affecting results

Ideally, a marker for screening of a disorder must have high predictive values (both positive and negative). The predictive value of a test is determined by the sensitivity and specificity of the test and the prevalence or pre-test probability of the disorder in the population tested

## AIMS

- To determine the prospective value of peptide antibodies for detecting CD in individuals with high pre-test probability undergoing intestinal biopsy due to clinical suspicion of small bowel disorder
- To compare the value of the new tests with other well-established markers of CD using the small bowel histology as a gold standard.

## MATERIALS

### Patients

Between December 2004 and December 2005, we studied a series of serum samples collected prospectively from a group of 141 consecutive adult patients attending the Small Bowel Diseases Clinic of the “Carlos Bonorino Udaondo” Gastroenterology Hospital

- All patients underwent intestinal biopsy irrespective of the clinical suspicion
- We excluded subjects with a previous diagnosis of CD, a former treatment with a gluten-free diet or those having a CD-related serology performed prior to the enrollment

## METHODS

### Diagnosis of CD

The categorization of patients and controls was based on currently accepted histological criteria of CD consisting in the presence of a type II or severer enteropathy (Marsh's modified classification)

The diagnosis of CD was further supported by the serology (a-tTG or EmA) and/or the assessment of the effect of a gluten-free diet

### Celiac disease-related serology

Serum samples obtained at the time of endoscopy were kept frozen until the assay was performed

**Peptide antibodies assays (AGA-II) type IgA and IgG:** ELISA (QUANTA Lite Gliadin IgA and IgG II- Inova Diagnostic Inc.; San Diego, CA).

**Anti-tissue transglutaminase (a-tTG) type IgA:** ELISA (QUANTA Lite TM, h-tTG IgA, INOVA Diagnostic Inc.; San Diego, CA)

**Endomysial antibodies (EmA) type IgA** (INOVA Diagnostics Inc.; San Diego; CA; USA) tested at a 1:5 dilution

**Anti-actin antibodies (AAA) type IgA** (QUANTA Lite Actin; INOVA Diagnostics Inc. San Diego, CA. USA)

**A combination of AGA-II type IgA and IgG in a single assay:** Final mix was 70% of IgG subtype and 30% of IgA

## RESULTS

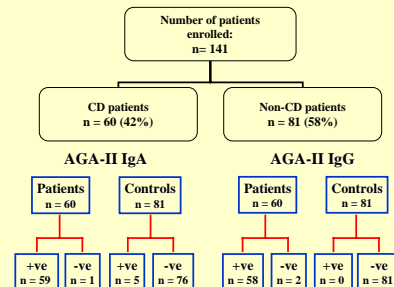


Figure 1. Flowchart of positive (+ve) and negative (-ve) AGA-II tests in CD patients and controls.

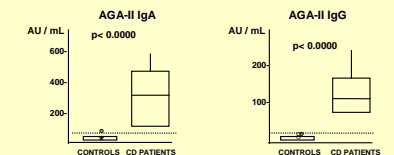


Figure 2. Distribution AGA-II types IgA and IgG absolute values distribution in controls and CD patients at diagnosis

### Celiac disease patients

n	AAA	AGA-II IgA	AGA-II IgG	AGA-II IgA+IgG	a-tTG	Biopsy
50	+	+	+	+	+	+
5	-	+	+	+	+	+
2	-	+	+	+	-	+
1	+	+	+	+	-	+
1	-	-	-	-	+	+
1	+	+	-	+	+	+

Table 1. Distribution of positive and negative serologic tests in CD patients

### Controls

n	AAA	AGA-II IgA	AGA-II IgG	AGA-II IgA+IgG	a-tTG	Biopsy
70	-	-	-	-	-	-
4	+	-	-	-	-	-
4	-	+	-	-	-	-
1	-	+	-	-	+	-
1	-	-	-	+	-	-
1	-	-	-	-	+	-

Table 2. Distribution of positive and negative serologic tests in controls

TESTS	Sen (%)	Spe (%)	Acc (%)	PPV (%)	NPV (%)	+LR	-LR
AAA-IgA	86.7	95.1	91.5	92.8	90.6	17.7	0.14
AGA-II IgA	98.3	93.8	95.7	92.2	98.7	15.8	0.02
AGA-II IgG	96.7	100	98.6	100	97.6	INF	0.03
AGA-II IgA + AGA-II IgG	98.3	98.8	98.6	98.3	98.8	81.9	0.02
a-tTG IgA	95.0	97.5	96.4	96.6	96.3	38.0	0.05
Endoscopic view	100	100	100	100	100	INF	0

Table 3. Statistical performance of serologic tests and endoscopic markers. Sen: Sensitivity; Spe: Specificity; Acc: Accuracy; PPV: Positive predicted value; NPV: Negative predicted value; +LR: Positive likelihood ratio, -LR: Negative Likelihood ratio

## SUMMARY

- Prevalence of CD in this population with a high pre-test probability was 42%.
- Both AGA-II isotypes and their combination in a single assay had the highest sensitivity.
- Specificity was absolute for AGA-II IgG followed by the combination
- The combination of both isotypes of AGA-II in a single assay and the AGA-II IgG had the highest diagnostic accuracy, PPV and NPV.
- 83% of patients had all test positives.
- One CD patient was positive for a-tTG but negative for all others and another patient had two positive tests (a-tTG and AAA).
- Ten non-CD patients had one serologic test positive and one subject had two positive tests.
- The test with more false positive results was AGA-II IgA (n=5) followed by AAA (n=4) and a-tTG (n=2).
- The endoscopic view of the duodenum had 100% sensitivity, specificity, PPV and NPV.

## CONCLUSIONS

- Our prospective study aimed to establish the performance of the CD-related serology in a population of individuals with high pre-test probability based on the intestinal biopsy as a gold standard. The study has shown that the new gliadin-related peptide antibody assays (AGA-II) are observer-independent tests presenting the highest diagnostic accuracy for detecting CD.
- We also established that a combination of both isotypes of AGA-II in a single assay has the highest performance, even better than the classically used a-tTG.
- Based on their diagnostic accuracy, we suggest that these new assays could be employed as the only diagnostic tool in the setting of a population with a high pre-test probability. These tests would allow to avoid intestinal biopsy in the great majority of patients.