

Proposed Serological Panel for Diagnosing Celiac Disease

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Celiac Disease, also called Celiac Sprue, is a gastrointestinal disease that affects the small bowel (Falchuk, 1999). It is triggered by the consumption of the gluten protein which is found in food products that contain wheat, malt, barley, or rye (Mayo, 2008). The gluten protein causes the villi of the small intestine to become inflamed which inhibits the absorption of nutrients causing malnutrition. The inflamed small intestine can also cause auto-antibodies. Other general symptoms can include weight loss, fluid retention, diarrhea, abdominal pain, malodorous flatulence, upset stomach, fatigue, joint pain, muscle cramps, and mouth sores (Mayo, 2008).

Celiac Disease can be asymptomatic or masked by other associated autoimmune disorders such as insulin dependent diabetes and dermatitis herpetiformis (Dahl, 2000). Celiac Disease is one of the most misdiagnosed diseases within the United States. Many people go undiagnosed or are misdiagnosed due to the many manifestations of the disease. Celiac Disease is usually diagnosed by an intestinal biopsy. However, intestinal biopsies are invasive procedures that are both time consuming and costly. Serological testing can help detect disease associated antibodies and is less invasive. Two main serological methods, Enzyme Linked Immuno-Sorbent Assay (ELISA) and Indirect Immuno-Fluorescent Assay (IFA or IIFA), are used in diagnosing the specific antibodies associated with Celiac Disease. Antibodies associated with Celiac Disease include gliadin (AGA), endomysium (EMA), reticulin (ARA), and tissue transglutaminase (tTG). Both IgG gliadin and IgA gliadin subtypes are produced and are collectively called anti-gliadin antibodies (Ryan, 1996). The tissue transglutaminase enzyme was

recently identified as the major auto-antigen in Celiac Disease and as the antigen target recognized by endomysial antibodies. The tissue transglutaminase enzyme is capable of binding and deamidating proteolytically cleaved glutamine-rich gliadin peptides to yield highly immunogenic peptides with glutamate residues which can cause tissue transglutaminase specific B cells to engulf tissue transglutaminase gliadin peptide complexes eventually leading to the formation of auto-antibodies to tissue transglutaminase. It is thought that such acidic peptides make up the pathogenic pool of antigens triggering Celiac Disease (Ankelo 2007). These acidic peptides are called deamidated gliadin peptides (DGP) and can be measured using the ELISA method.

The purpose of this study is to see if it is possible to produce a cost effective test panel that will more quickly and accurately diagnose Celiac Disease through less-invasive techniques. Through review of literature, it was concluded that the IFA method of testing has some noted disadvantages. Many variables may affect the IFA method including the light source, level of ambient light, training and experience of the operator, substrate used, and the initial screening dilution (Murray, 2000). The test for EMA is highly specific for Celiac Disease approaching 100 percent accuracy. However, the EMA assay is more expensive and time consuming than ELISA testing. In addition, the EMA assay is subject to operator interpretation making the results more subjective than those for the tTG test (Green, 2007).

The ELISA method of testing has a much greater advantage in producing accurate results than the IFA method. This method is not subject to interpretation like the IFA. The response is measured on an instrument that calculates the amount of light absorbed by the solution at a particular wavelength and prints out a numerical result. In addition to the patient sample, ELISA plates are processed with three to eight control sera which include a negative control serum and at least two positive control sera

containing one high level of antibody and one low level of antibody. Each control has a minimum and maximum number that must be seen by the instrument in order for it to be a valid test (Ryan, 1996).

In cases of IgA deficiency, either the IgG EMA and/or IgG tTG have excellent sensitivity and specificity. IgG-based tests are markedly less sensitive and specific than the IgA-based tests in patients with normal levels of IgA. Measurement of the serum IgA level is the next appropriate step in individuals who test negatively for IgA EMA or IgA tTG in whom Celiac Disease is still suspected (AGA, 2006).

The sensitivity and specificity for the AGA tests are highly variable. The AGA-IgG test sensitivity and specificity range from 57-100% and 69-87%, while for the AGA-IgA test they range from 54-100% and 79-100% respectively (Hill, 2005). EMA-IgA is both highly sensitive and specific with ranges of 87-95% and 95-100% respectively (Hill, 2005). The EMA method is more costly due to the fact that monkey esophagus or human umbilical cord is needed to perform the test. The tTG-IgA test has a sensitivity of more than 90 percent (range 90-100%). This test also has a specificity of more than 95 percent (range 95-100%) (AGA, 2006).

The ARA-IgG test has a sensitivity range of 65-94% and a specificity range of 93-100% (Hill, 2005). IgA class reticulon antibodies are found only in Celiac Disease and dermatitis herpetiformis (DH), and of the patients with Celiac Disease, only 65% of the patients present with these antibodies. Therefore, this test is slowly falling out of use due to better tests being made available (Ryan, 1996).

The DGP test has a sensitivity of 91% and a specificity of 98% (Hill 2005). According to the Celiac Sprue Association (2009), deaminated gliadin peptide (DGP) antibody tests, developed in 2007, in combination with tTG antibodies, have better accuracy than the native gliadin antibodies. Multiplex immunoassay (MIA) measures multiple antibodies simultaneously providing reduced turnaround time and cost. This test for antibodies is as accurate as ELISA for the presence of Celiac Disease. Rational

combination testing can help identify patients who need intestinal biopsy which is still considered as the “gold standard” for officially diagnosing Celiac Disease.

It is suggested through these results, and the review of literature, that a cost effective Celiac Disease panel should include the tTG test, the DPG test, and both the AGA-IgG and AGA-IgA tests. The combination of these tests’ sensitivity and specificity should accurately detect the Celiac Disease associated antibodies. However, the EMA test could be used as a confirmatory test, although, the cost would increase for the institution and the patient. Further research should be conducted to try to reduce the cost of this method of testing. The ARA test should not be included due to the low incidence of the antibody being present in patients with Celiac Disease which could yield false negatives.

As the afore mentioned serological tests require serum, the patient need only to endure a quick, minimally invasive venipuncture as opposed to a more time consuming, invasive surgical procedure. Also, serological tests have a faster turn around time and are more cost effective. Therefore, for the benefit of the patient and institute, a panel of tests that include: the tTG test, the DPG test, and both the AGA-IgG and AGA-IgA tests should be the initial step in diagnosing Celiac Disease.

References

- Ankelo, M., Kleimola, V., Simell, S., Simell, O., Knip, M., Jokisalo, E., et al. (2007). Antibody responses to deamidated gliadin peptide show high specificity and parallel antibodies to tissue transglutaminase in developing coeliac disease. *Clinical and Experimental Immunology* , 285-293.
- Association, C. S. (2009). Diagnosis of Celiac Disease. *Celiac Sprue Association* , 1-3.
- Dahl, M. R. (2000). Celiac Disease: The Great Mimic. *Celiac Sprue Association* , 1-2.
- Falchuk, M. Z. (1999). The Hows and Whys of Celiac Disease. *Lifeline* , 1-4.
- Green, M. P., & Cellier, M. P. (2007). Celiac Disease. *The New England Journal of Medicine* , 1731-1743.
- Hill, M. I. (2005). What Are the Sensitivity and Specificity of Serological Tests for Celiac Disease? Do Sensitivity and Specificity Vary in Different Populations? *Journal of Gastroenterology* , 25-32.
- Ryan, T. (1996, July 26). *Interpretation of Celiac Disease Blood Test Results*. Retrieved October 14, 2009, from Celiac: <http://www.celiac.com/articles/57/1/Interpretation-of-Celiac-Disease-Blood-Test-Results/Page1.html>
- Staff, M. C. (2008). Celiac Disease. *Mayo Clinic* , 1-13.