Commentary: Advances in the Laboratory Diagnosis of Celiac Disease

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Anti-endomysial autoantibodies are very useful in the diagnosis and monitoring of celiac disease (gluten-sensitive enteropathy) due to their high sensitivity and specificity for that disorder. The recent discovery that the autoantigen responsible for the endomysial pattern is tissue transglutaminase (tTG) has led to the commercial development of automated enzyme immunoassays for quantitation of that autoantibody. These assays are standardized to provide highly accurate and comparable testing between laboratories for anti-tTG autoantibodies. Celiac disease is a common genetic disease in populations of Europe

and the United States. It has a spectrum of expression ranging from silent or mild to severe, with resulting malabsorption that produces multiple-organ system effects due to malnutrition. Many cases miss the diagnosis because the symptoms are not classic or the clinical syndrome is not severe. Because the treatment of celiac disease (avoidance of wheat products) is so effective and inexpensive and because celiac disease is so common in selective populations, a highly reliable test for its detection such as anti-tTG should find wide application in clinical practice. *J. Clin. Lab. Anal.* 15:105–107, 2001. © 2001 Wiley-Liss, Inc.

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Two different groups (1,2) report in this issue on new methods for diagnosis of celiac disease (also called gluten-sensitive enteropathy or celiac sprue) in which the human enzyme designated tissue transglutaminase (tTG) is employed as the specific antigen in an enzyme immunoassay for detection of anti-tTG autoantibodies (3). This development marks the use of still another purified autoantigen in diagnostic tests that have previously relied on indirect immunofluorescent assays with tissue sections to detect clinically relevant autoantibodies according to their microscopic staining patterns.

Tissue transglutaminase has only recently been shown to be the autoantigen corresponding to the anti-endomysial antibodies that are highly sensitive and specific for the diagnosis of celiac disease (4). Tissue transglutaminase has the enzymatic activity of cross-linking proteins through covalent bond formation between residues of glutamine and lysine, entailing deamidation of the glutamine. Such a chemical modification of dietary gliadin (the protein of wheat) may enhance T-lymphocyte reactivity against gliadin in the intestine, thereby triggering or contributing to the immune process leading to celiac disease (5,6). This deamidation of gliadin allows it to be bound more avidly to HLA-DQ2, which may be the immediate mechanism for activation of celiac disease in select individuals of a population (7). Celiac disease is a hereditary disorder in which the genetic susceptibility of an individual interacts with gliadin in the diet and perhaps other

environmental stimuli to produce disease activity ranging from latent through overt (8–10).

Celiac disease is manifested as an inflammatory disorder of the small intestine when an affected individual ingests wheat or other grains (such as rye, barley, and oats) that contain the sensitizing protein gliadin. Celiac disease frequently arises in childhood when babies switch to a solid diet containing cereal derivatives. An affected child develops persistent diarrhea with foul-smelling stool, loses weight, and fails to thrive due to a generalized malabsorption condition. The diagnostic evaluation of that clinical situation can be very wide with infectious agents, immunodeficiencies, and congenital malabsorption syndromes entering the differential diagnosis. Because wheat products are introduced into so many commercially prepared food products (generically listed on labels as “modified food starch”), avoidance of them is difficult to achieve. Thus it may take parents and pediatricians quite a while to consider and diagnose celiac disease.

Many other patients have even less distinct clinical scenarios, although they do experience abdominal symptoms

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(bloating, pain, and gas), anemia and neurologic abnormalities suggesting malnutrition, oral sores, and a skin rash termed dermatitis herpetiformis (characterized by blistering over extensor surfaces), loss of dental enamel, and osteoporosis. These individuals may escape diagnosis until much later in life and suffer malabsorption to some extent throughout that time.

The treatment of celiac disease is simple in principle: avoid wheat and related grains. Patients with celiac disease appear to revert to health and have normal life expectancies when they avoid gluten. (The practicality of avoiding gluten is altogether another question due to the admixture of wheat in commercially processed foods. Fortunately, specialty food stores have a variety of alternative products available that use corn or rice in place of wheat, and which are strictly labeled for content.) If patients remain exposed to gluten, they run a slightly increased risk of developing lymphomas (perhaps linked to the chronic inflammation and immunologic stimulation) in addition to experiencing chronic malnutrition which affects multiple organ systems (11).

The diagnosis of celiac disease can be made through examination of small bowel mucosa biopsy taken while the patient is on a wheat-containing diet and is symptomatic. Positive findings include flattening and atrophy of villi and a diffuse enteritis with intraepithelial lymphocytes and cellular infiltrate of the lamina propria. The proximal small intestine tends to show more distinct changes than does the distal small intestine, probably because the exposure to wheat is greater there. To rule out the possibility that these findings may be due to another cause (such as tropical sprue), the patient is placed on a wheat-free diet and biopsied once more. If the histopathologic abnormalities persist, the disorder is presumed not to be due to gluten sensitivity. If they disappear, the abnormalities are presumed to be due to gluten sensitivity; however, confirmation can be done by placing the patient on wheat again and performing a third biopsy which should demonstrate recurrence of mucosal changes (12).

This very exacting and unpleasant sequence of dietary manipulations and multiple biopsies is almost a parody of itself, and patient compliance cannot be good. Consequently the use of serological tests for celiac disease has great appeal for the patient as well as for the physician who is trying to keep the patient returning for care. Thus a group of different assays have found wide use in diagnosing celiac disease (13). The measurement of serum antibodies against gliadin is a very logical one, although the circulating humoral response may not perfectly reflect immunologic sensitivity at the intestinal mucosa (14). Both IgG and IgA anti-gliadin antibodies are measured in serum, typically with an enzyme-linked immunosorbent assay (ELISA) that depends heavily on the quality of the gliadin antigen that is extracted from wheat. Other conventional tests involve indirect immunofluorescence assays for serum autoantibodies against reticulin (interstitial staining between tubules of mouse kidney) and against en-

domysium. The anti-endomysial autoantibodies recognize a reticulin component in smooth muscle endomysium of primates (typical substrate is monkey esophagus). Endomysial IgA autoantibodies have very high sensitivity and specificity for diagnosing celiac disease and also for detecting dermatitis herpetiformis (15–17). As patients abstain from wheat, the titers of these antibodies fall so they can also be used for monitoring patient progress and for confirming that the patient has successfully eliminated wheat from the diet over the long term (18,19). Serologic testing can also be used in suspected cases of celiac disease to screen for positive patients who should then be directed to small intestine biopsy.

The use of an automated assay with high diagnostic sensitivity and specificity for celiac disease that is based on a standardized autoantigen preparation of tTG has enormous operational advantages for the clinical laboratory. Variability in operator interpretation of staining patterns, quality of substrate preservation, and the sheer availability of scarce materials such as monkey tissues can potentially be minimized or eliminated altogether. Measurements of anti-tTG could also be standardized to a universal calibration, and interlaboratory variation in detecting and quantifying this autoantibody could be minimized.

Why should these technological advances be welcomed? Don't we already have sufficiently expert clinical diagnostic acumen and robust laboratory tests for celiac disease? We do, for the flagrant cases; but many others are unsuspected because they cause less obvious symptoms, probably related to chronic low-grade malabsorption with fatigue, anemia, neurologic dysfunction, bone changes, or skin rash (20). Celiac disease has been called the most common genetic disease in Europe, and it may be vastly under diagnosed both there and in the United States (21–24). The estimated prevalence in high-risk populations ranges from 1 in 150 to 1 in 300 persons.

A disease as common as celiac disease, with simple, effective, and inexpensive treatment deserves to be sought out aggressively. It is not too unreasonable to think that a widely available assay for anti-tTG autoantibodies would be used extensively, particularly for the evaluation of patients who present with typical gastrointestinal complaints, but even more so for persons whose symptoms are much less specific. For example, children who are at the fifth percentile or lower for height and weight may be particularly good candidates for celiac disease screening by a standardized and automated method. Virtually any child with failure to thrive or one who has persistent diarrhea might be screened, as well as individuals who exhibit milder signs of malabsorption such as anemia and neurologic dysfunction or those with dermatitis (15). Patients with osteoporosis are at greater risk for celiac disease, and so persons with premature loss of bone density could be routinely screened as well (24).

Thus the spectrum of patients for whom celiac disease testing might be considered ranges from the very young to adults and even to the elderly. Because the potential benefit of detecting celiac disease is so high and the treatment can be so

effective, mass screening programs using automated testing could complement present protocols of screening neonates for metabolic disorders and also become a staple of wellness testing in adults.

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