Sorting out celiac disease with serologic testing

Valerie Neff Newitt

April 2023—Celiac disease incidence is up and the diagnostic rate is low, and it can be years from onset of symptoms to diagnosis.

“It’s a long diagnostic odyssey, and so in the laboratory business, we’re all in to help,” says Annette Taylor, MS, PhD, associate vice president at Labcorp where she is strategic director of pharmacogenomics and scientific director of molecular genetics. “The whole field is trying to increase the awareness of patients and physicians about celiac disease,” she says, “to decrease the prolonged journey to diagnosis.” One reason cases may be missed, she says, is that many who have celiac disease have extraintestinal symptoms or no symptoms at all.

The prevalence of celiac disease is about one percent globally, though there is variation by geographic location, gender, and age, said Vijayalakshmi Nandakumar, PhD, MS, D(ABCC), medical director of clinical immunology at ARUP Laboratories and assistant professor, University of Utah School of Medicine, in an AACC session last year. “The incidence of diagnosed celiac disease has rapidly increased during the 21st century,” she said, citing a recent meta-analysis (King JA, et al. *Am J Gastroenterol.* 2020;115[4]:507–525). Some of the increase is attributed to the greater ability to diagnose the disease since tTG (transglutaminase) IgA became available in the early 2000s.

But there is evidence that incidence has increased independent of detection. Benjamin Lebwohl, MD, of Columbia University, and Alberto Rubio-Tapia, MD, of Cleveland Clinic, write in a 2021 article that there is “accumulating evidence that, beyond increased awareness, the true incidence of celiac disease has increased, which is affecting the prevalence of celiac disease” (Lebwohl B, et al. *Gastroenterology.* 2021;160[1]:63–75). They say this has been shown by cohort studies that included celiac disease screening, such as the Diabetes Autoimmunity Study in the Young.

Says Dr. Taylor: “The knowledge that celiac is common, globally relevant, and an increasing health problem is important for clinician awareness. And having it on the radar for laboratorians to test when appropriate is important for treatment and the best care of patients.” Only 50 percent of people or fewer with celiac disease know they have it, she says.

To increase access to testing, Labcorp last fall added a celiac disease antibody test to its list of on-demand wellness screening tests. “It empowers the patient and it’s convenient,” Dr. Taylor says, “and it’s another way to help increase the diagnostic rate, since people with a result showing that celiac disease is likely can follow up with their health care professional to get a definitive diagnosis.” Blood is drawn at a Labcorp patient service center, and the test fee is $119.

The screening test begins with tTG IgA, “but at the same time total IgA is tested because a certain percent of people is IgA deficient, and in people with celiac disease, it can be as high as two percent,” Dr. Taylor says. tTG IgA won’t be accurate as an indicator of celiac disease in those patients, so the test begins as a combination and then reflexes to deamidated gliadin peptide (DGP) IgG and tTG IgG in people with low IgA.

“It’s a pretty comprehensive test when all is said and done,” she says. “And we’re funneling everybody into health care” with advice to see a professional.

Diagnosis for celiac disease requires a combination of clinical presentation, serology, and histology. In Dr. Nandakumar’s AACC presentation last July, she focused on serology. And in an article published online March 1 in *Archives of Pathology & Laboratory Medicine*, she and colleagues reported on their study of the Aptiva automated multianalyte system for celiac disease antibody detection as compared with the Quanta Lite manual ELISA method. They concluded that it supports the use of a greater than or equal to 10 times the upper limit of normal anti-tTG IgA biopsy-free approach for serologic diagnosis of celiac disease. “Larger studies are warranted for a widespread adoption of Aptiva’s multianalyte system for CD serology,” they write (Novis CL, et al. *Arch Pathol Lab Med.*

Published online March 1, 2023. doi:10.5858/arpa.2022-0385-OA).

The FDA cleared the Aptiva system and Aptiva celiac disease IgA assay in 2021.

Total IgA and tTG IgA antibody testing is a powerful combination for celiac disease screening, Dr. Nandakumar said in her presentation. Anti-DGP (IgA and IgG) is a newer test but not used for the initial screen because of its low predictive power. It's suited to selected situations, one of which is IgA deficiency, for which IgG can be useful.

"But keep in mind," she said, "that IgA isotope is more specific than IgG for celiac disease diagnosis." Anti-DGP IgG antibody is more sensitive than tTG IgG in IgA-deficient individuals. In low to moderate anti-tTG IgA positive individuals, DGP antibody utility is debatable, she said, "due to its high false-positive rate in patients with type one diabetes and an overall low diagnostic accuracy."

Anti-endomysial (IgA and IgG) antibody testing by immunofluoresence is highly specific for celiac disease but expensive, subject to operator interpretation, and recommended only as a secondary test.

Dr. Nandakumar

HLA DQ2 and DQ8 are the focus of celiac genetic testing, and the power of celiac HLA testing comes from its negative predictive value, she said. "When there is a negative result, you can exclude the diagnosis of celiac disease, but when the result is positive for HLA DQ2 or DQ8, that doesn't confirm a celiac disease diagnosis." HLA testing is not recommended for routine screening but can be used to rule out celiac disease when seronegative enteropathy is present or when the patient has already started a gluten-free diet.

The 2013 guideline of the American College of Gastroenterology recommended serology but required a biopsy for confirmation of the final diagnosis. It called for testing with tTG IgA as the preferred single test, and total IgA when the probability of celiac disease is high, with testing for an IgG serology if total IgA was low. When any antibody is positive, the guideline said, the patient should proceed to biopsy. In children under age two, the guidelines recommended combining tTG IgA with DGP IgA and IgG. "tTG is less sensitive in this age group and it is common for IgA levels to be low, so DGP tests, particularly IgG, are useful," Dr. Nandakumar said.

Discussion related to a biopsy-free diagnosis began in 2012 with the guideline of the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition. The ESPGHAN guideline said that when a patient is symptomatic and the tTG IgA levels are greater than 10 times the upper limit of normal, EMA is positive on a second sample, and HLA is positive, "the patient can be considered for a biopsy-free diagnosis. If the tTG IgA levels are greater than 10 times the upper limit of normal and any one of those tests is negative, the patient should still proceed to biopsy," Dr. Nandakumar said. "And that holds true when your tTG IgA levels are less than 10 times the upper limit of normal."

The criteria for the patient to be symptomatic and HLA positive were removed from the 2020 ESPGHAN guideline update.

In the U.S., the American Gastroenterological Association issued a practice update in 2019, written by the same lead author as the ESPGHAN guidelines, which says that when a tTG IgA level greater than 10 times the upper limit of normal is combined with a positive EMA antibody in a second blood sample, the positive predictive value for celiac disease is virtually 100 percent and is reliable for diagnosing celiac disease.

The American College of Gastroenterology issued a guideline update at the start of this year. It suggests a combination of high-level tTG IgA (greater than 10 times upper limit of normal) with a positive EMA in a second blood sample as reliable tests for diagnosis of celiac disease in children. “In symptomatic adults unwilling or unable to undergo upper GI endoscopy, the same criteria may be considered after the fact, as a diagnosis of likely CD,” the authors write (Rubio-Tapia A, et al. *Am J Gastroenterol.* 2023;118[1]:59–76).

At the AACC conference, Dr. Nandakumar presented the following case scenario and asked which test would be best to confirm the diagnosis: A 53-year-old female has symptoms of diarrhea, occasional bloating, and weight loss. She has a history of breast cancer and is six months post-surgery and chemotherapy. She tests positive for tTG IgA (35 U/mL, negative, <3 U/mL). She doesn’t want a biopsy procedure. The best test to confirm the diagnosis of likely celiac disease: anti-endomysial (EMA) IgA.

Assay methods for tTG IgA consist of chemiluminescent, enzyme-linked, and fluorescence enzyme immunoassays and particle-based multianalyte fluorescence platforms. EIA is used in the high-volume ARUP Laboratories, but Dr. Nandakumar and colleagues recently evaluated the Aptiva technology and the clinical performance of the Aptiva system’s anti-tTG IgA assay.

Sera samples from 703 patients were tested for anti-tTG IgA and IgG and for anti-DGP IgA and IgG antibodies on both Quanta Lite EIA and Aptiva platforms. Of the 703 patients, 127 were classified as celiac disease positive (58) and celiac disease negative (69) based on biopsy results. Dr. Nandakumar and coauthors report in their article published in *Archives of Pathology & Laboratory Medicine* that “anti-tTG IgA detection showed equal clinical sensitivity and specificity of 91% sensitivity and 99% specificity on both platforms.” They add, “Anti-tTG IgG resulted in moderate sensitivity of 69% and 72%, but high specificity of 100% and 94% on Aptiva and Quanta Lite, respectively.”

For anti-DGP IgG, they report sensitivities of 90 percent and 81 percent, and specificities of 94 and 99 percent, on Aptiva and Quanta Lite, respectively. For anti-DGP IgA, they found an 83 percent sensitivity on Quanta Lite and 69 percent on Aptiva, and similar specificities of 97 percent (Quanta Lite) and 98 percent (Aptiva).

They write, “At ≥10 × ULN levels for anti-tTG IgA, Aptiva displayed a sensitivity of 72% and a specificity of 100%, and Quanta Lite showed a sensitivity of 69% and a specificity of 100%.”

Their study, they say, supports the ESPGHAN recommendations for diagnosis of celiac disease in patients with greater than or equal to 10 times the upper limit of normal anti-tTG IgA titers without a biopsy using the Aptiva system. Both Aptiva and Quanta Lite at greater than or equal to 10 times ULN anti-tTG IgA “could be considered for a biopsy-free diagnosis,” they write, when the clinical scenario precludes biopsy, though not in patients with type one diabetes. The patient diagnosed without a biopsy should be monitored “to corroborate the reversal of serology and reconstitution of villi” with the start of a gluten-free diet.

Some patients who do their best to adhere to a gluten-free diet still have symptoms and persistent villous atrophy, Dr. Taylor says. Labcorp in late 2021 introduced a quantitative gluten test for stool samples to monitor patients for diet adherence and accidental gluten consumption and as an aid in assessing refractory celiac disease not related to accidental gluten exposure. “It makes it possible to directly measure the GI disease-triggering source in the patient,” she says.

Areas of future research in celiac disease include a possible relationship between the disease and the gut microbiome—whether changes in the intestinal microbiome play a causal role. “There are papers showing that microbiome signatures differ between people with celiac disease and control participants,” says Dr. Taylor. It raises another question, she says: “Is there a role for probiotics?”

“There are some promising clues, and research is active.”

Dr. Taylor

Research is ongoing into an HLA-DQ-gluten tetramer-based assay to detect gluten-reactive T cells that drive the immune response in people with celiac disease, Dr. Taylor says. The test is designed to measure interleukin-2 release in people on a gluten-free diet after they ingest one bolus of gluten. “People with celiac disease have an increase in IL-2, which distinguishes them from people with non-celiac gluten sensitivity,” she says. The test is not yet available, “but it appears we might be going in that direction.”

The test would be an advance because it could potentially help to diagnose celiac disease in people on a gluten-free diet with no more than a single ingestion of gluten, Dr. Taylor says. Diagnosing celiac disease in such patients is difficult now because it requires a gluten challenge for several weeks, which causes the symptoms to return. “Celiac antibody tests are not reliable as indicators of celiac disease in the context of a gluten-free diet because healing from the diet reduces the antibodies.”

Drs. Lebwohl and Rubio-Tapia in their 2021 article wrote, “If proven accurate and scalable, assays that detect gluten-HLA tetramer complexes might be used in diagnosis to be made in the context of a gluten-free diet without intestinal biopsy.”

For now, Dr. Taylor says, it’s important for all to know celiac disease is underdiagnosed. “Increased awareness will help health care respond to celiac disease and the needs of patients.”

“I see laboratory professionals as educators,” she says.

Valerie Neff Newitt is a writer in Audubon, Pa.