Q&A column

Editor: Frederick L. Kiechle, MD, PhD

Submit your pathology-related question for reply by appropriate medical consultants. CAP TODAY will make every effort to answer all relevant questions. However, those questions that are not of general interest may not receive a reply. For your question to be considered, you must include your name and address; this information will be omitted if your question is published in CAP TODAY.

Q. What is the most specific serologic test for diagnosing IgG4-related disease?

A.February 2023—IgG4-related disease (IgG4-RD) is a fibroinflammatory disease that can affect any organ and was first recognized as a unique clinical entity in 2003. The most affected organs include the pancreas, bile duct, major salivary glands, lacrimal glands, retroperitoneum, and lymphatic ducts. Key features of the disorder include elevated serum IgG4 concentrations, neoplastic-like swelling of the affected organs, as shown on an imaging test, specific histopathological characteristics on immunostaining, as well as a good response to treatment with glucocorticoids.

Diagnosis is largely based on exclusion. Symptoms at presentation may be linked to affected organs and can mimic a host of conditions. In fact, several well-known clinical conditions, such as autoimmune pancreatitis and Hashimoto's thyroiditis, are being reclassified on the IgG4-RD spectrum. It is likely that reclassification will increase as clinical data accumulate. However, this effort is made more difficult because IgG4-RD and distinct autoimmune conditions may coexist in patients.

The clinical workup of IgG4-RD requires ruling out infectious etiology and neoplasm and demonstrating improvement after treatment with glucocorticoids or other immunosuppressive agents. While serological testing for IgG4 is available, results may be elevated or normal in patients with IgG4-RD. There is an emerging role for IgG4 quantification in the phenotypic stratification of patients with IgG4-RD. Recent findings suggest that the degree of IgG4 elevation could correlate with disease burden and multifocal or multiorgan involvement, but additional research is needed to confirm these findings. Relatively nonspecific laboratory findings commonly found in patients with IgG4-RD include total IgG elevation, IgG subclass elevations (aside from IgG4), IgE elevation, eosinophilia on complete blood count, and low-titer antinuclear antibodies with no associated extractable nuclear antigen or double-stranded DNA antibodies detected.

Imaging studies can be misleading, as the lesions typically reveal organ enlargement or pseudotumors. However, findings depend largely on the organs involved and the imaging studies selected in the workup. Not all IgG4-RD lesions are hypermetabolic, so uptake-related imaging studies may not detect lesions.

Because serological testing and imaging provide variable data that are typically inconclusive, biopsy is the best means of confirmation. Common histological features of IgG4-RD include dense lymphoplasmacytic infiltrates (largely represented by IgG4-positive plasma cells), storiform or cartwheel fibrosis, obliterative phlebitis, and eosinophilia in the affected tissues. Acutely affected tissues often demonstrate more infiltrates, while chronically affected tissues may have a "burned out" appearance with more fibrosis.

The first comprehensive diagnostic criteria for IgG4-RD were published in 2012 by Umehara, et al., and include 1) organ dysfunction, 2) IgG4 concentration >135 mg/dL, and 3) the aforementioned biopsy-based findings consistent with IgG4-RD. Involvement of all three criteria made the diagnosis definite; confirmation of Nos. 1 and 3 made the diagnosis probable; and confirmation of Nos. 1 and 2 made the diagnosis possible. However, many inconclusive cases based on these criteria made it clear to the rheumatology community that additional review was necessary. Subsequently, the American College of Rheumatology and European League Against Rheumatism released a 2019 classification of IgG4-RD. It required that at least one of 11 possible organs is involved in a manner consistent with

IgG4-RD; all 32 exclusion criteria are applied to the case; and, after excluding the eliminating factors, eight weighted inclusion criteria that include clinical, serological, radiological, and pathological findings are applied. While this classification system requires thorough testing and clinical workup, it presents the best approach to date and is a template for potential work-up algorithms.

There are no genetic associations that point specifically to IgG4-RD, but studies relating to human leukocyte antigen (HLA) and non-HLA associations are underway. While the pathogenesis of IgG4-RD is incompletely characterized, there is evidence that environmental and occupational exposures can trigger disease flares. This is a first step toward recognizing that the disorder is immune mediated. Furthermore, patients with IgG4-RD who have been treated with anti-CD20 antibody therapy (e.g. rituximab) have demonstrated significant clinical improvement; hence, there is strong B-cell involvement in disease pathogenesis. Further, biopsies from patients with active IgG4-RD express a cytokine profile consistent with regulatory T cell and T helper 2 cell involvement.

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Q. Our new endocrine clinic is monitoring estradiol levels in transgender male patients (female to male) and asked if our standard estradiol immunoassay is appropriate to use in this setting. What do you recommend?

A.Using gender-affirming hormone therapies is increasingly common in transgender and nonbinary populations, and monitoring hormone concentrations may help provide optimal outcomes for these patients.

Testosterone hormone therapy may be used by patients who desire masculinizing effects. Estradiol (E2) concentrations are expected to decrease concurrently with use of testosterone hormone therapy. However, guidelines do not address specific ranges or preferred testing methods for hormone measurements in these patients.[1-3] The ideal concentrations will depend on the desired effects and vary by individual.

Overall, given sensitivity concerns, differences among testing platforms, and the possibility of immunoassay

interference,[4] liquid chromatography-mass spectrometry (LC-MS) methods are preferred when low E2 concentrations are expected. Low E2 concentrations may be expected in individuals desiring E2 concentrations below cisgender male reference intervals, prepubertal patients, and individuals using estrogen-suppressing therapy (e.g. testosterone or aromatase inhibitors). While there are notable differences between methods, if optimal gender-affirming effects are obtained at E2 concentrations above the cisgender male reference interval, immunoassay methods may provide results that are clinically comparable to those of LC-MS[5] and, therefore, may suffice. However, LC-MS methods may be useful if immunoassay results do not align with the clinical situation or expected results.

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2. Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2017;102(11):3869–3903.
3. Coleman E, Radix AE, Bouman WP, et al. Standards of care for the health of transgender and gender diverse people, version 8. *Int J Transgend Health.* 2022;23(suppl 1):S1–S259.
4. Rosner W, Hankinson SE, Sluss PM, Vesper HW, Wierman ME. Challenges to the measurement of estradiol: an Endocrine Society position statement. *J Clin Endocrinol Metab.* 2013;98(4):1376–1387.
5. Greene DN, Schmidt RL, Winston-McPherson G, et al. Reproductive endocrinology reference intervals for transgender men on stable hormone therapy. *J Appl Lab Med.* 2021;6(1):41–50.

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