

Distinguishing Immunoglobulin G4-Related Disease From Its Pancreatobiliary Mimics: Are We There Now?

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Immunoglobulin G subclass 4 (IgG4)-related disease is a fibroinflammatory disorder characterized histopathologically by tumefactive lesions with storiform fibrosis and an IgG4-positive, plasma cell-enriched lymphocytic infiltrate. It was initially described in the late twentieth century as a sclerosing type of autoimmune pancreatitis. In 2003, extrapancreatic manifestations (e.g., biliary) were reported in a series of Japanese patients with IgG4-related (i.e., type 1 autoimmune) pancreatitis, thus giving rise to the current concept that this disorder in fact represents a systemic clinicopathological entity.⁽¹⁾ Indeed, although IgG4-related pancreatitis is regarded as the most frequent manifestation of IgG4-related disease, synchronous and/or metachronous lesions are now known to

occur, with considerable frequency, in a variety of organs.^(1,2)

In many cases of IgG4-related disease (pancreatic or otherwise), increased concentrations of IgG4 can be detected in peripheral blood. Of the four subclasses of the IgG isotype, IgG4 is the least abundant among healthy individuals, was the last to be discovered, and may perhaps be the most immunobiologically enigmatic.^(1,2) Its serum concentrations are highly variable among, yet fluctuate minimally within, healthy individuals, the mechanisms of which are uncertain. Indeed, the biosynthesis of IgG4 is poorly understood, though recent data suggest that interleukins 4, 10, and 12 play an inductive role.⁽¹⁻³⁾ Owing to amino acid residue differences in the constant (ostensibly the structural hinge) region of the IgG4 protein, half-IgG4 molecules can become detached and combine with other half-molecules through a process referred to as “antigen-binding fragment (Fab) arm exchange,” thereby forming asymmetrical, bispecific antibodies.^(1,2) Because of this unique phenomenon, IgG4 has low Fc receptor and complement binding affinity and, intriguingly, can in fact function as an anti-inflammatory mediator. Despite the aforementioned, an increased serum concentration of IgG4 protein is a diagnostic biomarker of IgG4-related disease which provides a readily available, noninvasive clinical clue suggesting this diagnosis. However, approximately 30% of patients with IgG4-related pancreatitis (and/or cholangiopathy) have normal levels of serum IgG4;^(4,5) moreover, elevated IgG4 levels can be seen in up to 30% of patients with primary sclerosing cholangitis (PSC) or pancreatobiliary malignancy, which may also share imaging characteristics with IgG4-related disease.^(6,7) Considering the nature of this differential and the challenges in making an accurate diagnosis, a multimodal evaluation is typically required for suspected IgG4-related disease.

Perhaps the most widely referenced diagnostic criteria in this context are embodied in the acronym “HISORt,”

Abbreviations: BCR, B-cell receptor; IgG, immunoglobulin G; PCR, polymerase chain reaction; qPCR, quantitative PCR; PSC, primary sclerosing cholangitis.

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Potential conflict of interest: Dr. Lindor advises Intercept and Shire.

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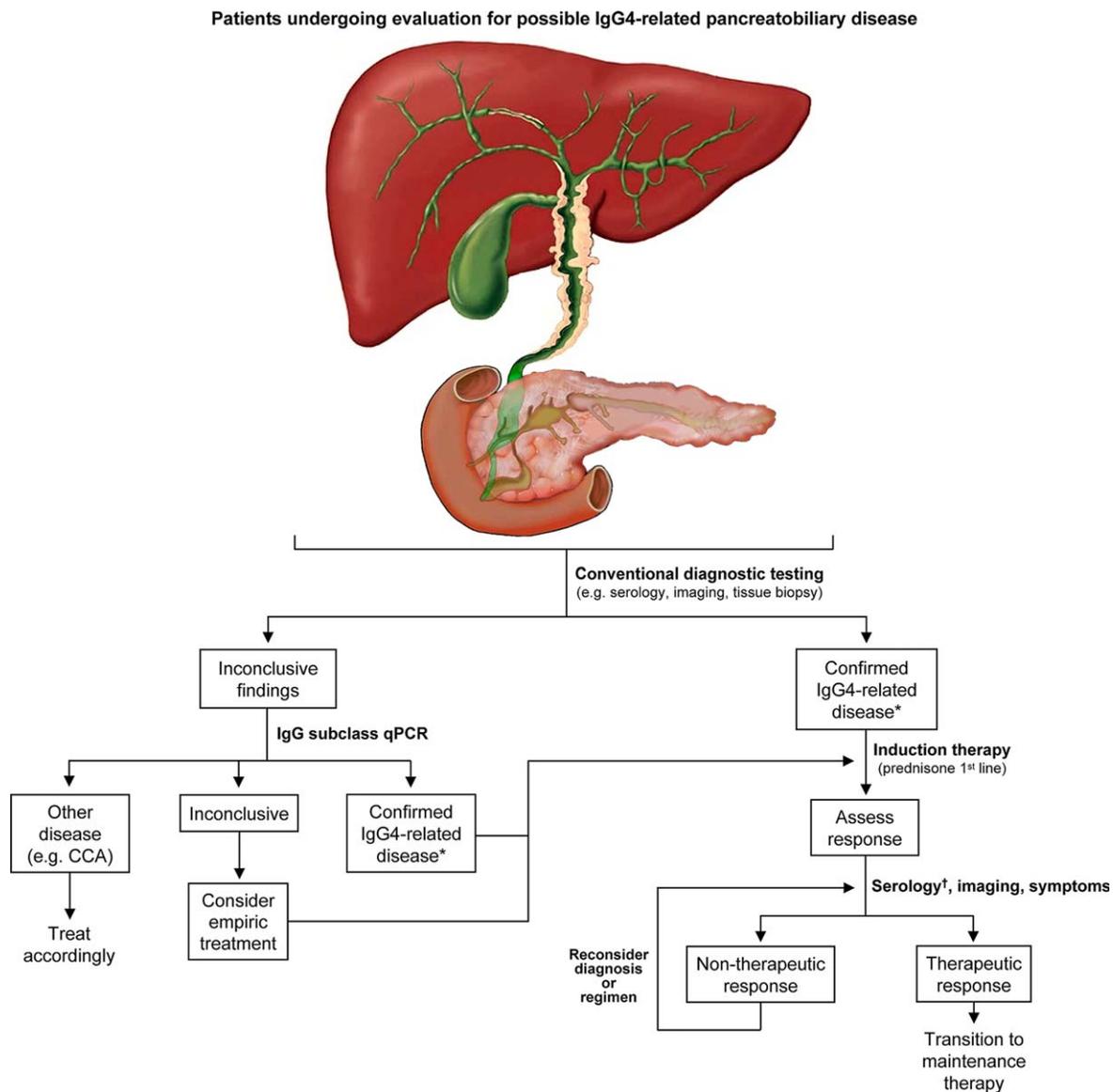


FIG. 1. Proposed algorithm for studies of diagnosis and management of IgG4-related pancreatobiliary disease. *If the clinicodemographic features are compatible with an alternative entity (e.g., PSC with elevated serum IgG4 concentration), the diagnosis of IgG4-related disease may be further scrutinized. †Perform IgG4 qPCR if baseline serum IgG4 concentration is not elevated. Abbreviation: CCA, cholangiocarcinoma.

which encompasses histology, imaging, serology, other organ involvement, and response to therapy.⁽⁸⁾ Despite these and other criteria,⁽⁹⁾ however, confirming a diagnosis of IgG4-related disease and distinguishing it from PSC, pancreatobiliary malignancy, and other mimics remains oftentimes challenging. Moreover, inadequacy of biliary and/or pancreatic specimens, a recognized shortcoming of available endoscopic and other nonoperative tissue acquisition techniques, hampers application of these criteria. Therefore, accurate, noninvasive, widely

applicable, high-throughput tests for diagnosing IgG4-related pancreatobiliary disease are needed.

In this issue of HEPATOLOGY, Doorenspleet et al. provide novel and timely insight regarding a blood-based assay for diagnosis of as well as disease monitoring in IgG4-related pancreatobiliary disease.⁽¹⁰⁾ The investigators aimed to extend their previous work wherein they found expanded dominant IgG4⁺ B-cell receptor (BCR) clones by next-generation sequencing in blood and in major duodenal papillary biopsies of six

patients with IgG4-related cholangiopathy but not in healthy or disease controls. They therefore conducted a larger, prospective case-control study of patients at two European medical centers. Cases consisted of patients who had (1) undergone pancreatobiliary resection for presumed malignancy or core biopsy revealing IgG4-related disease, (2) classical imaging features and elevated serum IgG4 levels, or (3) suspicious pancreatobiliary imaging characteristics together with elevated serum IgG4 levels, other organ involvement, and/or bile duct biopsy with >10 IgG4⁺ B cells/high-power field plus improvement of pancreatobiliary imaging findings and biochemical indices following 4 weeks of corticosteroid treatment. Forty-eight patients with PSC, 27 with pancreatobiliary malignancy, and 10 with chronic pancreatitis due to other (unspecified) causes served as disease controls. Using peripheral venous blood from cases and controls, total RNA was isolated, complementary DNA was synthesized by standard reverse-transcription protocols (with 250 ng RNA input), and linear amplification of the complete immunoglobulin repertoire was performed using optimized polymerase chain reaction (PCR) primers covering all functional heavy chain BCR genes. The resulting amplified nucleic acid samples were magnetic bead-purified, further amplified by PCR, repurified, subjected to next-generation sequencing, and then analyzed bioinformatically. RNA processing and data analyses were performed in a blinded fashion.

The results of the study were remarkable, revealing a striking difference in the distribution of IgG1, IgG2, IgG3, and IgG4-positive clones between IgG4-related disease and controls. Furthermore, the proportion of the BCR repertoire taken up by IgG4⁺ versus IgG4⁻ BCRs was significantly greater in IgG4-related disease compared to controls. This observation led the investigators to hypothesize that IgG4⁺ RNA may comprise an expanded proportion of the total IgG message and thus be a more accurate diagnostic than IgG4 protein. Indeed, quantitative PCR (qPCR) distinguished IgG4-related disease from controls significantly better than serum IgG4 protein. The investigators found a cutoff of 5% of all IgG⁺ RNA molecules being IgG4⁺ to have excellent performance characteristics, with overall sensitivity and specificity of 94.0% and 98.7%, respectively (area under the curve = 0.991). In addition, in a small subset of patients, the investigators found that the IgG4 RNA percentage could also be potentially used to monitor disease activity, noting a decrease in mean IgG4 RNA from 20.3% to 5.8% after 4 weeks and 1.9% after 8 weeks of corticosteroids.

A few study limitations are worth considering. First, there was a paucity of information regarding the histologic features (e.g., degree of hepatic and pancreatic fibrosis) of a sizable proportion of cases as well as disease controls. This is important from an applicability perspective given that the single false-positive qPCR result in the PSC group occurred in a patient with advanced hepatic fibrosis (and based on the available data, only two patients with cirrhosis were studied). Second, patients on immunosuppressive regimens or with severe inflammatory bowel disease (and presumably those postcolectomy), both of which are encountered not infrequently in this patient population, were excluded. Similarly, it is unknown if any patients had renal disease or how the next-generation sequencing or qPCR assay would perform in patients with other autoimmune disorders, e.g., autoimmune hepatitis or thyroid disease, both of which commonly have immunoglobulin profile alterations. Third, it is unclear how the serum ratio of IgG4:total IgG RNA by qPCR compares to the ratio of IgG4:IgG1 protein by nephelometry for diagnosis or for disease monitoring;⁽¹¹⁾ it is possible that the former may be superior, especially in cases with low serum concentrations, but this remains untested. Lastly, further investigation is needed to determine generalizability to non-European cohorts.

The overall findings herein represent advances in the understanding and management of IgG4-related pancreatobiliary disease. The investigators' highly accurate, quantitative assay using a widely available platform (qPCR) to diagnose and monitor this disorder may provide veritable clinical utility. Some of the gaps in the study will need to be addressed to better delineate the strengths, potential weaknesses, and implementation of this biomarker and assay; to this end, we believe that a standardized algorithmic approach which readily lends itself to prospective studies (Fig. 1) may be useful. As this qPCR assay is further refined and validated, it may well be advanced to a first-line modality and potentially help expedite accurate, noninvasive diagnosis of IgG4-related disease.

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