

# Increased IgG4+ Cells in Duodenal Biopsies Are Not Specific for Autoimmune Pancreatitis

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**Key Words:** Serum IgG4 concentration; Autoimmune pancreatitis type I; Ampullary biopsy

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Upon completion of this activity you will be able to:

- cite clinical and radiologic findings that prompt consideration of testing duodenal or ampullary biopsies for immunoglobulin (Ig) G4 plasma cells.
- list disease processes in which increased IgG4 plasma cells may be increased in duodenal or ampullary biopsies.
- outline the immunologic responses that have been shown to cause B-cell class switching leading to IgG4-producing plasma cells.

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## Abstract

*Endoscopic ampullary biopsies showing increased immunoglobulin (Ig) G4+ plasma cells have been reported as an alternative to pancreatic biopsy in diagnosing autoimmune pancreatitis (AIP). This study assessed whether increased IgG4+ cells can be seen outside the context of AIP. Fifty-four cases (45 duodenal or ampullary biopsies, 9 ampullae from pancreatic resections) were selected, and all specimens were immunostained for IgG4 and IgG. Duodenal or ampullary biopsies containing normal duodenal mucosa (n = 6) and increased intraepithelial lymphocytes without villous blunting (n = 7) were negative for IgG4. Increased IgG4+ cells (>10 per high-power field) were found in 7 cases of 18 serologically confirmed celiac disease patients and in 3 of 14 patients with duodenitis or gastric heterotopias. Two of 6 ampullae from patients with pancreatic cancer showed increased IgG4+ cells. In summary, 12 of 51 patients without AIP had duodenal biopsies or ampullae showing increased IgG4+ plasma cells. The finding of increased IgG4+ cells in duodenal biopsies is not specific for AIP without the correct clinical context.*

Immunoglobulin (Ig) G4-related disease, characterized by elevated serum IgG4 concentration and tissue infiltration by IgG4+ plasma cells, can present as a tumefactive process in various organs.<sup>1</sup> This is particularly problematic in the pancreas, where the disease, also called *autoimmune pancreatitis type 1* (AIP), can mimic pancreatic cancer clinically and radiologically.<sup>2,3</sup> In such cases, patients may undergo unnecessary pancreatic resection for a condition that is amenable to steroid therapy.<sup>4</sup> To prevent unwarranted surgery, tissue biopsy is desirable, but pancreatic biopsy is invasive and may not be an option in all hospitals. Ampullary biopsy has been proposed as an alternative because it is easily accessible on endoscopy.<sup>5</sup> Several studies indicate that the presence of increased IgG4+ plasma cells (>10/high-power field [hpf]) noted in ampullary biopsies is characteristic of, and putatively specific for, AIP compared with chronic pancreatitis and pancreatic cancer.<sup>6,7</sup> Studies like these might prompt the endoscopist to request up-front IgG4 immunostaining on duodenal or ampullary biopsy specimens in certain clinical situations, such as recurrent pancreatitis of unknown etiology. However, the significance of increased IgG4 positivity in the context of more common settings such as duodenitis is not well documented in the literature. Moreover, the effect of up-front requests for immunostaining on cost of care cannot be disregarded. With these observations in mind, the present study was designed to assess whether IgG4+ cells can be increased in duodenal and ampullary biopsy specimens outside the already reported contexts of AIP, chronic pancreatitis, and pancreatic cancer.

Materials and Methods

Case Selection

The study was approved by the University of Washington (Seattle) Human Subjects Research Division. The files of University of Washington Medical Center were searched for patients with the following histologic diagnoses: normal duodenum, duodenitis, gastric heterotopia, increased intraepithelial lymphocytes, celiac sprue, AIP, and pancreatic ductal adenocarcinoma **Table 1**. The diagnosis of duodenitis arose from biopsy findings of increased plasma cell infiltration, intraepithelial neutrophils, and gastric surface metaplasia (foveolar metaplasia). *Gastric heterotopia* is defined as the presence of oxyntic glands within the duodenal mucosa. Fifty-four patients were evaluated in this study, 29 male and 25 female patients, ranging in age from 20 to 77 years. We included ampullary or duodenal biopsy specimens from 45 patients and ampullary sections from 9 patients who had undergone Whipple procedures. Among the biopsy specimens, 6 were of normal mucosa, 7 had increased intraepithelial lymphocytes without villous blunting, 18 had blunted villi and increased intraepithelial lymphocytes worrisome for celiac sprue, and 14 had either duodenitis or gastric heterotopia. In all those who underwent biopsy, an average of 4 samples were taken; the average size of each sample was 0.5 cm, for a cumulative mean linear dimension of 2 cm per individual. Three of 9 ampullae from the resections were those of patients with AIP (n = 3); the remainder

(n = 6) were from patients with pancreatic carcinoma. All selected samples were stained immunohistochemically for IgG4 and IgG. IgG immunostaining was performed because of previously published findings that IgG4 to IgG ratios are more accurate in determining an increase in IgG4+ plasma cells than IgG4 counts alone.<sup>2,8</sup>

Immunohistochemical Methods

Immunohistochemistry for IgG4 and IgG was performed on 4-μm-thick formalin-fixed, paraffin-embedded tissue sections. The antibodies, clones, dilutions, pretreatment conditions, and sources are listed in **Table 2**. In brief, sections were dewaxed and then rehydrated. All staining steps were performed on an automated platform (Leica Bond III, Leica Microsystems, Buffalo Grove, IL). After exposure to a dilute solution of hydrogen peroxide in buffer to block endogenous peroxidase activity, sections were subjected to heat-induced epitope retrieval in either EDTA/Tris (sections for IgG4) or citrate (for IgG) buffers for 20 minutes. Slides were then rinsed and incubated with the primary antibody for 15 minutes, washed in buffer, and incubated with peroxidase-labeled polymer. Slides were again washed, and antibody binding was localized using a peroxidase reaction with 3,3'-diaminobenzidine tetrahydrochloride (DAB+, DakoCytomation, Carpinteria, CA) as a chromogen. Slides were washed with water, counterstained, dehydrated, and mounted. Appropriate positive and negative controls were used throughout the procedures.

**Table 1**  
Histologic Features and Summary of IgG4 Immunohistochemical Results in Biopsy and Resection Specimens

| Specimen Type | Histologic Features            | No. of Each Specimen Type | No. of Specimens With >10 IgG4+ Cells/hpf | No. of IgG4+ Cells/hpf in Each Specimen With >10 IgG4+ Cells/hpf | Mean No. of IgG4+ Cells/hpf in Specimens With >10 IgG4+ Cells/hpf |
|---------------|--------------------------------|---------------------------|-------------------------------------------|------------------------------------------------------------------|-------------------------------------------------------------------|
| Biopsy        | Normal                         | 6                         | 0                                         |                                                                  |                                                                   |
|               | Increased IELs                 | 7                         | 0                                         |                                                                  |                                                                   |
|               | Blunted villi + increased IELs | 18                        | 7                                         | 12 (4 cases), 17, 20, 75                                         | 23                                                                |
|               | D or GH                        | 14                        | 3                                         | 11 (GH), 19 (GH), 70 (D)                                         | 33                                                                |
|               | Total                          | 45                        | 10                                        |                                                                  |                                                                   |
| Resection     | AIP                            | 3                         | 3                                         | 44, 70, 93                                                       | 69                                                                |
|               | Pancreatic cancer              | 6                         | 2                                         | 11, 23                                                           | 17                                                                |
|               | Total                          | 9                         | 5                                         |                                                                  |                                                                   |

AIP, autoimmune pancreatitis; D, duodenitis; GH, gastric heterotopia; hpf, high-power field; IEL, intraepithelial lymphocytes; Ig, immunoglobulin.

**Table 2**  
Panel of Antibodies Used in This Study

| Antibody | Clone      | Dilution  | Antigen Retrieval     | Source                          |
|----------|------------|-----------|-----------------------|---------------------------------|
| IgG4     | MRQ-44     | 1:200     | EDTA at pH 8.9-9.1    | Cell Marque, Rocklin, CA        |
| IgG      | Polyclonal | 1:128,000 | Citrate at pH 5.9-6.1 | DakoCytomation, Carpinteria, CA |

Ig, immunoglobulin.

## Quantification of IgG4+ Plasma Cells

The stained slides were evaluated independently by 2 pathologists (M.W. and K.M.C.) who were blinded to the original diagnosis.

## Single Immunohistochemical Stain for IgG4

Plasma cells present in the lamina propria were considered IgG4+ in the presence of discrete cytoplasmic brown staining. Areas with the highest density of IgG4+ plasma cells were identified by scanning at low magnification for each slide. Within these areas, 2 tissue levels of the same hpf (magnification  $\times 400$  [0.2 mm<sup>2</sup>]) were evaluated, and an average number of IgG4+ plasma cells per hpf was determined. Based on other studies, a count of less than 10 was considered negative, and 10 or more was considered positive.<sup>6,7</sup>

## Results

### Normal Biopsy Specimens Are Negative for IgG4 in Contrast to AIP

All normal biopsy specimens, with their usual lamina propria plasmacytic population, showed 0 to 1 (mean 0 positive cells) IgG4 plasma cells per hpf. Specimens with increased intraepithelial lymphocytes but no villous blunting also had 0 or rare IgG4+ plasma cells (mean, 1 positive cell) per hpf. The ampullae in the 3 patients with AIP had numerous IgG4+ plasma cells (mean, 69 positive cells) per hpf, a finding in keeping with earlier published studies.

### Increased IgG4+ Cells Are Also Found on Biopsy in Patients With Cancer, Celiac Sprue, and Duodenitis

Two of 6 patients with pancreatic carcinoma also had more than 10 IgG4+ plasma cells per hpf in their ampullae (mean, 17 positive cells); no histologic features of AIP were present in these cases. Moreover, 7 of 18 patients whose biopsy specimens showed blunted villi and increased intraepithelial lymphocytes (indicative of celiac sprue) had 10 or more IgG4+ cells in at least 1 hpf (mean, 23 positive cells in biopsy specimens with increased IgG4) ■Image 1■. Higher numbers of positive cells were found in biopsy specimens with comparatively more severe villous blunting. Three of the 14 cases of duodenitis or gastric heterotopia also showed more than 10 IgG4+ cells (mean, 33 positive cells in biopsy specimens with increased IgG4) ■Image 2■.

### Clinicoradiologic Correlation of Non-AIP Patients Whose Biopsy Specimens Showed Increased IgG4

Imaging had previously been performed in 6 of the 10 patients with increased IgG4 positivity in their biopsy

specimens who did not have AIP or carcinoma; all of these patients had normal pancreata. Celiac serologic studies (anti-tissue transglutaminase, antiendomysial, anti gliadin antibodies) were performed in 6 of the 10 patients and were positive in all. Three patients particularly stood out in having significantly dense IgG4 positivity ■Table 3■. One patient had recurrent pancreatitis and ulcerative colitis, another had primary sclerosing cholangitis, and the third had celiac sprue.

## IgG Staining Is Difficult to Interpret

IgG immunostaining was performed on all biopsy specimens to calculate IgG4-IgG ratios. However, heavy background staining of debris, vessels, and epithelial tissue precluded reliable assessment of these stains ■Image 3■.

## Discussion

Our study shows that positive staining for plasma cells expressing IgG4 on ampullary and duodenal biopsies is not a useful indicator of AIP when stain selection is not guided by the clinical and radiologic context. In addition, a cutoff value higher than 10 IgG4+ cells per hpf is not specific for AIP. The ampullae in patients with AIP had numerous IgG4+ plasma cells, which is in agreement with findings reported in previous studies.<sup>6,7</sup> However, more than 10 IgG4+ plasma cells—using cutoff values for positivity as defined in studies focused on AIP—were found not only in patients with AIP but also in patients with pancreatic cancer, duodenitis, gastric heterotopia, and villous blunting. In fact, of the 51 cases not related to AIP, 24% had more than 10 IgG4+ plasma cells (Table 1). We found that 7 of 18 patients whose biopsy specimens showed blunted villi and increased intraepithelial lymphocytes had 10 or more IgG4+ plasma cells in at least 1 hpf. In contrast to previously published studies, the ampullae in 2 of 6 patients with pancreatic cancer also had more than 10 IgG4+ plasma cells, and no features of concomitant AIP were present in their pancreata.

To our knowledge, this is the first report showing that IgG4+ plasma cells can be increased in duodenal biopsy specimens from patients without AIP. When we focused on the 10 cases that had pathology other than AIP and pancreatic cancer, we found that 6 of these 10 patients, who had positive serologic findings for celiac sprue, had increased IgG4+ plasma cells without radiologic evidence of pancreatic abnormalities. Three of these 10 patients had biopsy specimens that showed innumerable IgG4+ plasma cells in the absence of pancreatic abnormalities on imaging. Of these 3 patients, one had a history of recurrent pancreatitis and ulcerative colitis, another had a history of primary sclerosing cholangitis and ulcerative colitis, and the third had a history of celiac sprue (Table 3). The clinical history of the patient with recurrent

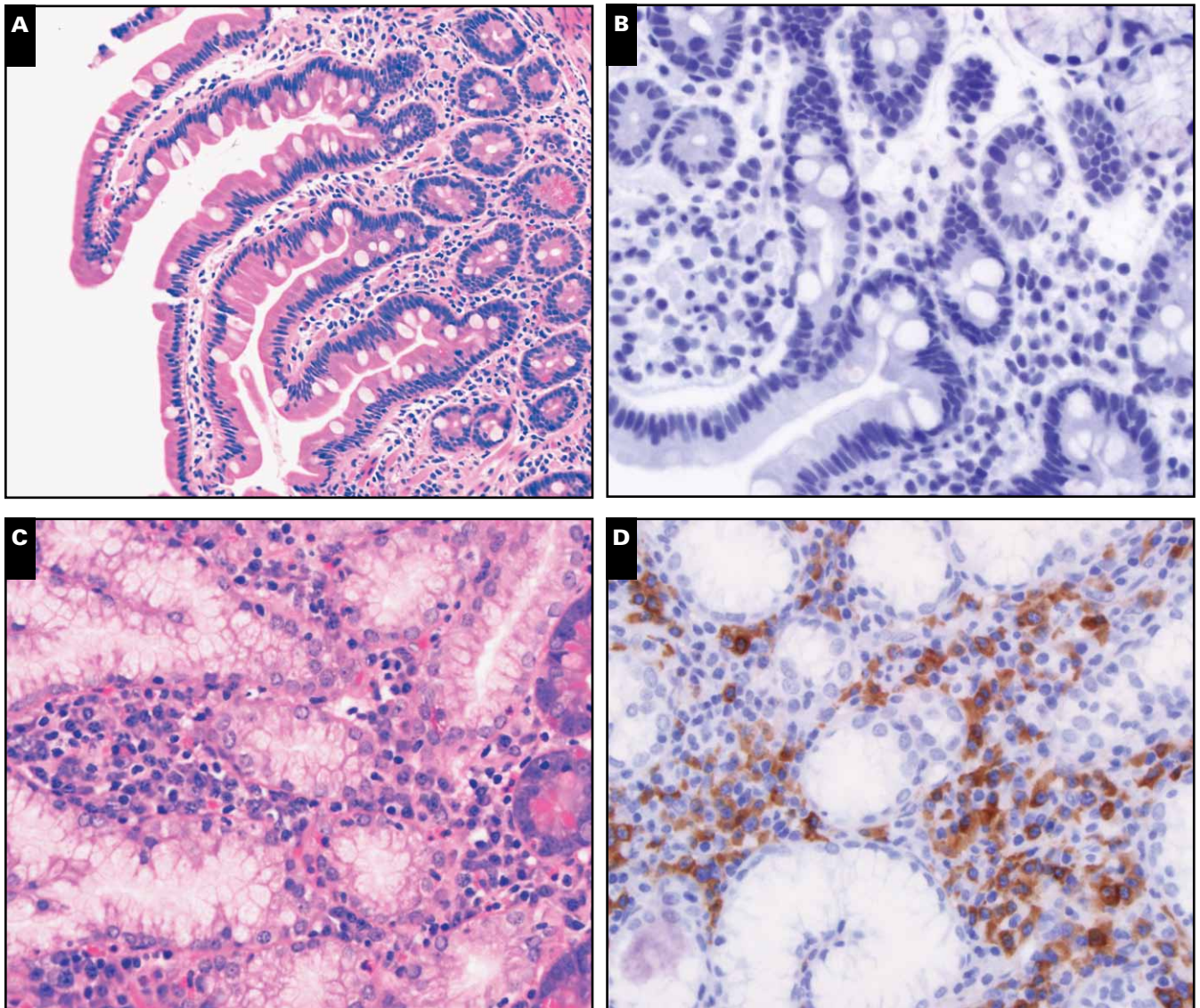


**Image 1** Immunoglobulin (Ig) G4 immunohistochemical staining in normal biopsy specimens vs cases of autoimmune pancreatitis (AIP). **A**, Normal biopsy specimens with their usual lamina propria plasmacytic population (H&E, ×200). **B**, Normal biopsy specimens with IgG4 immunostaining (×400) are negative for IgG4. **C**, Ampullary portion of resections with AIP (H&E, ×40); note that the ampullae of affected pancreata do not show phlebitis or storiform fibrosis characteristic of IgG4 sclerosing disease, rendering it difficult to suspect ampullary involvement on biopsy. **D**, Ampullary portion of resections with AIP (×400) had numerous IgG4+ cells as reported in previous studies.

pancreatitis and ulcerative colitis could be predicted from earlier reports in the literature showing that increased IgG4+ plasma cells may be seen in the gastrointestinal tract in the setting of idiopathic inflammatory bowel disease, predominantly in the colon but also in the duodenum.<sup>9</sup>

Given the clinical histories and histologic findings of the patients whose biopsy specimens contained innumerable IgG4+ plasma cells, it is quite possible that the IgG4+ plasma cells represent an appropriate response to an underlying inflammatory condition. It has been shown that production of IgG4-producing plasma cells is influenced by T helper 2 (Th2) cytokines that mediate allergic reactions and IgE production.

In this milieu of Th2 cytokines, the addition of the regulatory cytokine interleukin 10, produced by both Th2 cells and T regulatory cells, is thought to induce class-switching of B cells to IgG4-producing plasma cells.<sup>10</sup>

T regulatory pathways regulate the immune response to help ensure self-tolerance. In IgG4-related sclerosing disease, in contrast to classic autoimmune conditions in which T regulatory pathways are impaired, these seemingly protective pathways are activated.<sup>2,11,12</sup> In addition, transforming growth factor  $\beta$ , a cytokine that induces fibrosis, tends to be overexpressed in IgG4-related sclerosing disease.<sup>13</sup> It is not clear whether the IgG4+ plasma cells are direct mediators

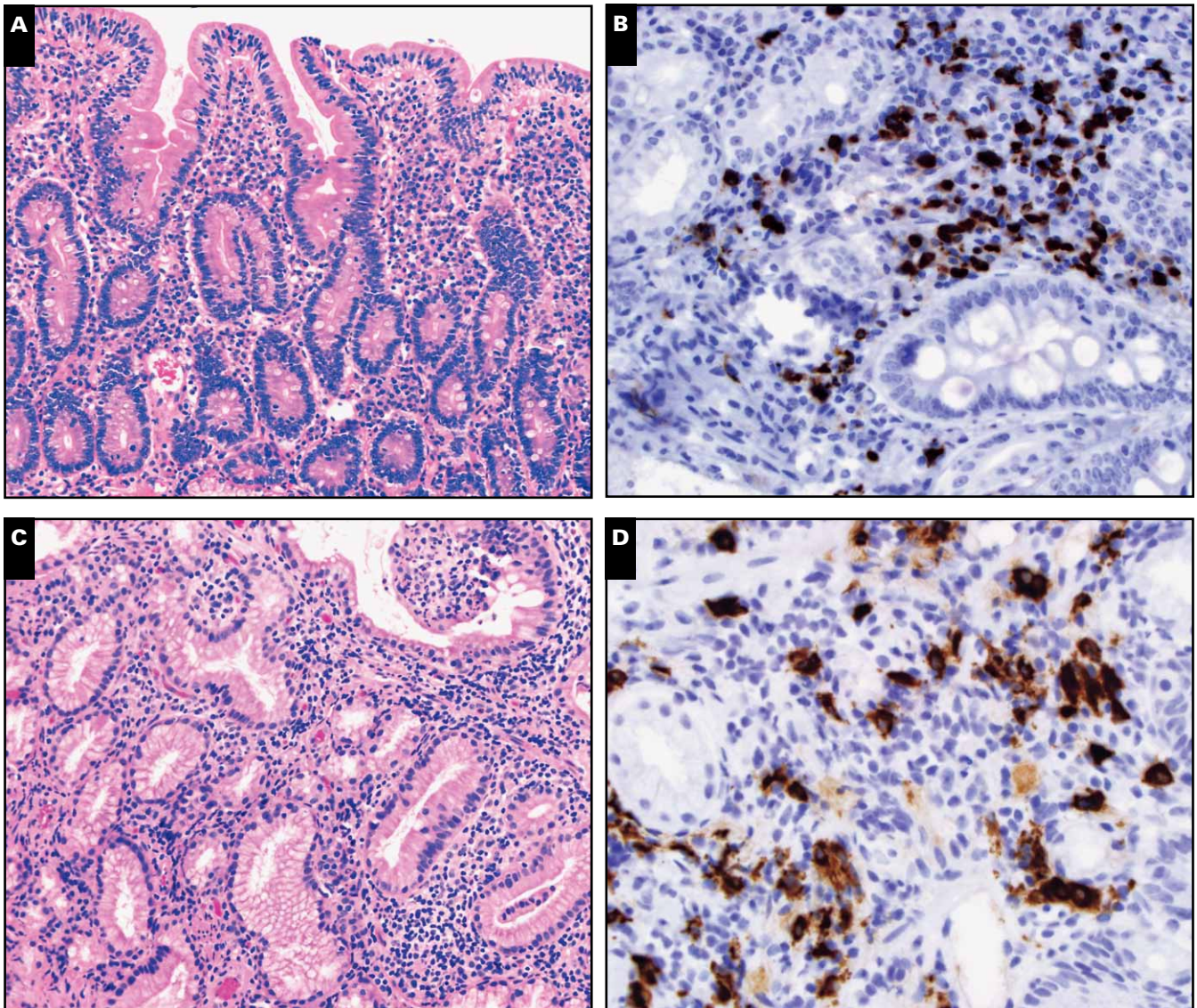


**Image 2** Immunoglobulin (Ig) G4 immunohistochemical staining in biopsy specimens that show blunted villi and increased intraepithelial lymphocytes and in biopsy specimens that show duodenitis. **A**, Biopsy specimen shows features of celiac sprue such as villous blunting and intraepithelial lymphocytosis (H&E,  $\times 200$ ). **B**, The biopsy specimen has features of celiac sprue, ( $\times 400$ ) showing dense IgG4+ plasma cells. **C**, Biopsy specimen shows active inflammation (H&E,  $\times 200$ ). **D**, The biopsy specimen shows duodenitis ( $\times 400$ ) with dense IgG4+ plasma cells.

**Table 3**  
Clinical Data for Biopsies Showing Dense Numbers of IgG4+ Cells

| Age/Sex | History                                           | Imaging                                                                                       | Endoscopy                                                                                     | Serum IgG4              | Other Serology                                                      | Histology                        |
|---------|---------------------------------------------------|-----------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------|---------------------------------------------------------------------|----------------------------------|
| 36/F    | Recurrent pancreatitis, UC                        | Normal abdominal MRI                                                                          | Normal esophagus, stomach, duodenum, and major papilla                                        | 18.4 mg/dL <sup>a</sup> |                                                                     | Gastric heterotopia              |
| 51/M    | PSC, UC                                           | Diffuse intrahepatic changes of sclerosing cholangitis on ERCP; normal pancreas by ultrasound | Diffuse clean-based ulcers in antrum and duodenum; portal gastropathy; esophageal varices     | Not performed           |                                                                     | Villous blunting, increased IELs |
| 20/F    | Epigastric and abdominal pain, worse after eating | Normal pancreas by ultrasound                                                                 | Unremarkable esophagus and stomach; edematous mucosa in the duodenal bulb and second duodenum | Not performed           | Positive antiendomysial IgA and IgG, anti-TTG, and anti-gliadin IgG | Villous blunting, increased IELs |

ERCP, endoscopic retrograde cholangiopancreatography; IEL, intraepithelial lymphocytes; Ig, immunoglobulin; MRI, magnetic resonance imaging; PSC, primary sclerosing cholangitis; TTG, tissue transglutaminase; UC, ulcerative colitis.

<sup>a</sup> Reference range for serum IgG4: 2.4 to 121.0 mg/dL (0.02-1.21 g/L).

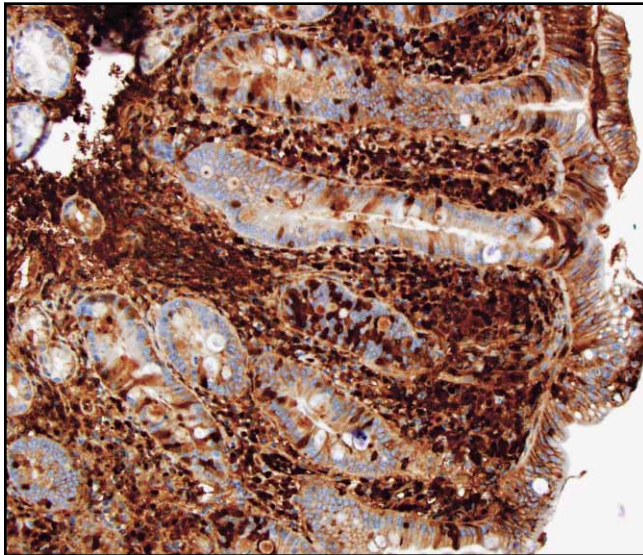


**Image 3** Immunohistochemistry for immunoglobulin G shows heavy background staining, precluding accurate determination of positive plasma cells (H&E, ×200).

of the disease process or whether they are present as a protective response to an inflammatory insult. In any case, the presence of IgG4+ plasma cells in our patient samples is potentially secondary to an immune mechanism related to various inflammatory conditions. Given the systemic nature of IgG4-related disease, many researchers have sought to evaluate the presence of IgG4+ plasma cells in other inflammatory conditions of the gastrointestinal tract, including pernicious anemia<sup>14</sup> and inflammatory bowel disease.<sup>9</sup>

Selected published studies emphasize the importance of the IgG4-IgG ratio when assessing for the presence of IgG4+ plasma cells, particularly when the number of plasma cells is decreased, such as in long-standing fibrotic lesions or after steroid therapy.<sup>2,8</sup> Unfortunately, assessment of the IgG4-IgG ratio in our patients' biopsy specimens was precluded by heavy background staining of epithelium, vessels, and stroma (a problem that pervades our clinical endoscopic biopsy material as well). In addition, when using step sections to evaluate for IgG, the plasma cell areas of interest were not necessarily representative of what was stained on the IgG4-stained slides. As such, we did not find immunohistochemistry for IgG to be a practicable or useful tool for comparing IgG4+ plasma cells to overall IgG+ plasma cells. The use of IgG4 stains alone may nonetheless be an appropriate means of study. In a recent study by Dhall et al,<sup>15</sup> sensitivity of IgG4 stains for AIP in ampullae from pancreatic resection specimens was 84% when a cutoff value of more than 50 IgG4+ plasma cells per hpf (×40 [0.2 mm<sup>2</sup>]) was used. This study suggests that a higher cutoff for IgG4+ plasma cells could improve the sensitivity of ampullary or duodenal biopsies for AIP while avoiding the

potential problems associated with using anti-IgG antibodies. Given the possibility for the presence of increased IgG4+ plasma cells in inflammatory processes other than AIP, our study also suggests that a higher cutoff for IgG4+ plasma cells could improve the sensitivity, as well as specificity, of this test for AIP.

The results of our study illustrate several reasons why IgG4 immunostaining and the reliance on the cutoff value of more than 10 IgG4+ cells per hpf, the current recommended cutoff in the literature, are not specific for AIP and why IgG4 immunohistochemistry should not be used regularly to screen for AIP, particularly when the history is limited. We found increased IgG4 in duodenal biopsy specimens from patients with duodenitis, gastric heterotopia, villous blunting, celiac sprue, and idiopathic inflammatory bowel disease. A few patients with no evidence of pancreatic abnormalities had dense infiltrates of IgG4+ plasma cells with substantially more than 10 positive cells per hpf. In addition, ampullary biopsies in patients with AIP did not show any other helpful histologic features associated with AIP (or IgG4-related sclerosing disorders in general), such as fibrosis or phlebitis; this limited our ability to determine whether the biopsy specimen was from a patient with AIP.<sup>13</sup> In contrast to current reports in the clinical literature, we conclude that the presence of increased IgG4+ plasma cells greater than 10 positive plasma cells per hpf in duodenal biopsies is not specific for AIP. They should perhaps only be interpreted in patients with a clinical suspicion of AIP or a radiologic impression of pancreatic abnormality. The potential value of serologic IgG4 studies should also be considered.

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