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Tissue IgG4-positive plasma cells in inflammatory bowel disease: a study of 88 treatment-naive biopsies of inflammatory bowel disease

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The distinction of Crohn's disease from ulcerative colitis is based on clinical, endoscopic, radiological, and histological findings, a paradigm that remains unchanged despite the advent of new understanding of the immunological and genetic basis of inflammatory bowel disease. There is a strong correlation between inflammatory bowel disease, predominantly ulcerative colitis, and autoimmune pancreatitis. We hypothesized that colonic biopsies from patients with inflammatory bowel disease would demonstrate increased numbers of IgG4-positive plasma cells and that this elevation might be restricted to ulcerative colitis. We examined a cohort of 78 cases of inflammatory bowel disease: 50 ulcerative colitis and 38 Crohn's disease. We identified treatment-naive biopsies. Additionally, four cases of inflammatory bowel disease associated with autoimmune pancreatitis and 15 cases of lymphocytic/collagenous colitis were also identified. Immunohistochemical stains for IgG4 were performed. Biopsies from patients with ulcerative colitis showed significantly higher numbers of IgG4-bearing plasma cells than those with Crohn's disease (mean IgG4 counts per high-power field (hpf) 9.8 *vs* 2.8, $P=0.001$). Samples from 19 (38%) ulcerative colitis patients had IgG4 counts >10/hpf, compared with only two (5%) patients with Crohn's disease; the sensitivity and specificity of a cutoff at 10 IgG4-positive plasma cells per hpf was 38 and 95%, respectively. Among individuals <18 years, there were no statistically differences in the IgG4 counts between the two subforms of inflammatory bowel disease. Among adult patients, a cutoff of 5 IgG4+ plasma cells distinguished ulcerative colitis from Crohn's disease with a sensitivity of 53% and specificity of 83%. In comparison to inflammatory bowel disease, patients with lymphocytic/collagenous colitis showed significantly lower numbers of IgG4-positive plasma cells ($P=0.0001$). Ulcerative colitis with pancolitis showed higher numbers of IgG4-bearing plasma cells (mean IgG4 12.8 *vs* 5.8 per hpf; $P=0.09$). An immunohistochemical stain for IgG4 may aid in making the distinction between ulcerative colitis and Crohn's disease (with exclusion of the pediatric cases), albeit with a relatively low sensitivity. This study also provides additional support to the hypothesis that a subset of ulcerative colitis cases is associated with a Th2 response.
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A diagnosis of inflammatory bowel disease with its two subsets, Crohn's disease (Crohn's disease) and ulcerative colitis (ulcerative colitis), is generally based on clinical, endoscopic, radiological, and histological grounds. This paradigm of classifying inflammatory bowel disease remains unchanged despite the advent of new technologies for the examination of genetic sequences. Patients with ulcerative colitis typically present with uniform, continuous inflammation that extends proximally from the rectum but remains limited to the colon. In contrast, Crohn's disease typically demonstrates inflamed mucosal areas alternating with non-inflamed mucosal surfaces ('skip

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lesions'). One important histological criterion for distinguishing ulcerative colitis from Crohn's disease is the presence of granulomas. However, granulomas are detected in only 40–60% of Crohn's disease cases in resection specimens and much less frequently in biopsy samples (15–36%).[1] Transmural inflammation, a feature of Crohn's disease rather than ulcerative colitis, requires operative specimens, because colonic biopsies obtained at endoscopy generally do not extend beyond the lamina propria. In inflammatory bowel disease patients whose manifestations are confined to the colon and/or are not characterized by typical endoscopic or histological findings, the distinction between the two disease subsets is often challenging. Moreover, up to 15% of patients with inflammatory bowel disease are considered to have 'indeterminate colitis', a term reserved for cases that cannot be categorized as either Crohn's disease or ulcerative colitis.[2,3]

Genome-wide association studies have provided novel insights into the genetic underpinnings of inflammatory bowel disease, but little progress has been achieved thus far in the area of tissue-based biomarkers.[4] The finding of granulomas on mucosal biopsies represents the only decisive histological criterion for distinguishing between Crohn's disease and ulcerative colitis. Nevertheless, strong consensus exists that there are important differences between the mucosal inflammatory repertoires of the two major inflammatory bowel disease subsets. Studies of inflammatory bowel disease in mice and humans implicate dysregulation of intestinal T-cell subsets in the pathogenesis of these diseases. In Crohn's disease, there is increased production of the Th1 cytokines interferon-γ and TNF-α within the intestinal mucosa.[5–7] In ulcerative colitis, a Th2 cytokine orientation exists in addition to increased IL-17.

Our interest in examining the significance of tissue IgG4-positive plasma cells arose from the now widely accepted association of inflammatory bowel disease—predominantly ulcerative colitis—with autoimmune pancreatitis.[8] Autoimmune pancreatitis is strongly associated with elevated serum concentrations of IgG4 as well as the infiltration of pancreatic tissue with IgG4-positive plasma cells. IgG4 is normally the least abundant subclass of IgG, and increased serum and tissue IgG4 concentrations are often associated with an exaggerated Th2 response.[9] We hypothesized that colonic biopsies from patients with inflammatory bowel disease would demonstrate increased numbers of IgG4-positive plasma cells and that this elevation would be restricted to ulcerative colitis.

Materials and methods

We used our electronic pathology database to identify consecutive inflammatory bowel disease patients who underwent either an endoscopic or surgical biopsy at the Massachusetts General Hospital in the year 2000. Using this list, we sought to identify patients' initial pretreatment biopsy, thereby identifying biopsies performed as early as 1986. We excluded cases for which no pretreatment biopsy was available. Two additional cohorts were included in this study: consecutive patients with biopsy-proven lymphocytic/collagenous colitis and four cases of inflammatory bowel disease that arose in patients with autoimmune pancreatitis. These biopsies were included in a microarray (2-mm-sized core). A biopsy fragment with the highest amount of chronic inflammation was selected for inclusion in the tissue microarray.

Demographic information collected included age and sex. The majority of these patients were followed up at the inflammatory bowel disease center at this institution and the electronic medical records were examined for features that could distinguish ulcerative colitis from Crohn's disease. The distinction between ulcerative colitis and Crohn's disease was based on well-established clinical guidelines.[10] The presence of granulomas or significant ileitis greater than that accepted as backwash ileitis on the index biopsy or any subsequent biopsy indicated Crohn's disease.

The pretreatment colonic biopsies were examined for histopathological indicators of inflammatory bowel disease severity. The active inflammation was graded as mild (cryptitis), moderate (crypt abscesses), and severe (erosions or ulcerations). The most severe grade in any biopsy was recorded for cases from which multiple biopsies were available. However, this analysis was limited to the initial pretreatment biopsy.

In addition, we identified 21 consecutive cases with inflammatory bowel disease from the year 2009. Similar to the first cohort, on each of these cases we retrieved the biopsy obtained at the time of initial presentation of the disease. Thus, only cases in which a naive (pre-therapy) biopsy could be identified were included in the study.

Whole sections from biopsies of patients with inflammatory bowel disease from the '2009' cohort were evaluated using immunohistochemistry for IgG4, as were the tissue microarray sections that included inflammatory bowel disease cases from the '2000' cohort as well as cases with microscopic colitis and autoimmune pancreatitis-associated colitis. Immunohistochemistry for IgG4 was performed as described previously.[7] Briefly, immunohistochemical studies using antibodies to IgG4 (Zymed, 1:200 dilution) were performed. Antigen retrieval was performed using protease digestion, and antigen detection was conducted using the UltraView diaminobenzidine chromogen (Ventana Medical Systems, AZ). One high-power field (HPF area $= 0.2375\,mm^2$) with the highest number of IgG4-positive cells was recorded.

Statistical Analysis

Statistics were calculated using the SPSS version 20.0 (SPSS, Chicago, IL, USA). Differences between

the groups were evaluated using the chi-square test or the Fisher exact test for qualitative variables and the Student's *t*-test for quantitative variables. *P*-values <0.05 were considered significant.

Results

Inflammatory Bowel Disease Cohort

Demographic data. This cohort included 78 cases of inflammatory bowel disease: 50 cases of ulcerative colitis (28 males and 22 females) and 38 with Crohn's disease (21 males and 17 females). The mean age of the ulcerative colitis cohort was 42.3 years (range 8–76 years) and that of the Crohn's disease cohort was 30 years (range 11–81 years).

Sites of involvement and categorization of disease severity in inflammatory bowel disease. Thirty of the Crohn's disease patients showed involvement of the ileum and colon, while the other eight patients did not show involvement of the ileum. The disease in the ulcerative colitis cohort was confined to the colon.

The severity of disease was evaluated on histological sections. Twelve patients showed mild active colitis, 25 patients showed moderately active colitis, ie, crypt abscesses, and the remaining 13 patients showed foci of erosions/ulcerations (severely active colitis). In the Crohn's disease cohort, 19 patients showed moderately active colitis, while 8 patients each showed mildly and severely active colitis. There was no difference in the level of activity in the two cohorts ($P=0.06$).

Tissue IgG4 immunohistochemistry. Biopsies from patients with ulcerative colitis showed significantly higher numbers of IgG4-bearing plasma cells than those with Crohn's disease (mean IgG4 counts 9.8 *vs* 2.8, $P=0.001$; Figure 1 and Figure 2; Table 1). Samples from 19 (38%) of the 50 ulcerative colitis patients had IgG4 counts >10/hpf, compared with only two (5%) of the 38 patients with Crohn's disease. The sensitivity and specificity of a cutoff at 10 IgG4-positive plasma cells per HPF was 38 and 95%, respectively.

If the analysis was restricted to children (<18 years), there were no statistically significant differences in the IgG4 counts between the two subforms of inflammatory bowel disease. Thus, when children were excluded from the analysis, a cutoff point of >5 IgG4-positive plasma cells distinguished ulcerative colitis from Crohn's disease with the sensitivity of 53% and the specificity of 83%.

Correlation between IgG4 counts and measures of disease severity in ulcerative colitis. The IgG4 counts correlated with the histological activity of disease at presentation. Cases of ulcerative colitis with erosions/ulceration showed significantly higher numbers of IgG4-bearing plasma cells than patients who lacked these findings (mean IgG4 17.6 *vs* 6.2, $P=0.034$).

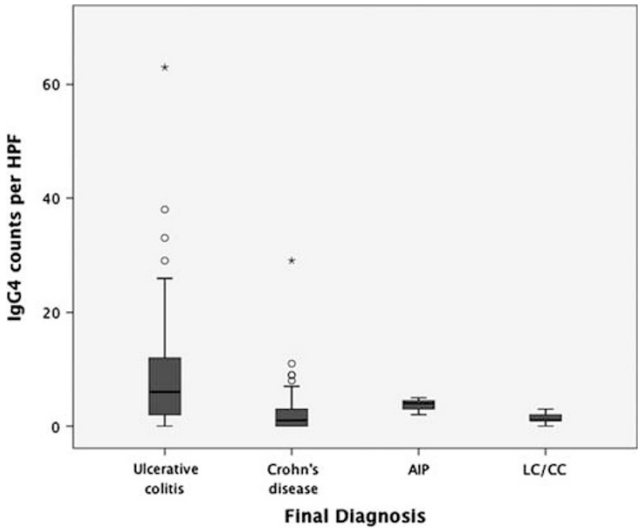

Figure 1 Final diagnosis. AIP, autoimmune pancreatitis; CC, collagenous colitis; LC, lymphocytic colitis.

Within the ulcerative colitis cohort, elevated numbers of IgG4 plasma cells correlated with the extent of disease. Cases of ulcerative colitis with pancolitis showed higher numbers of IgG4-bearing plasma cells (mean IgG4 12.8 *vs* 5.8), although this difference did not achieve statistical significance ($P=0.09$). With patients with Crohn's disease, at a cutoff point of 5 IgG4-positive cell per HPF, there was no correlation between the IgG4 counts and level of activity ($P=0.85$).

Autoimmune Pancreatitis-Associated Inflammatory Bowel Disease Cases

All four patients had ulcerative colitis, and pancolitis was identified in all the four individuals. A total colectomy was performed in three cases. Mean IgG4 counts in the autoimmune pancreatitis group was 3.67 (s.d. 1.5), lower than the ulcerative colitis cohort ($P=0.38$). However, it should be noted that we could not identify a pretreatment biopsy in this cohort, and hence these results may reflect the use of immunosuppressive therapy.

Comparison of Inflammatory Bowel Disease with Lymphocytic/Collagenous Colitis

We evaluated six patients with lymphocytic colitis and nine cases of collagenous colitis. Biopsies from these patients showed significantly lower numbers of IgG4-bearing plasma cells compared with the patients with inflammatory bowel disease. The mean IgG4 counts of the inflammatory bowel disease cases was 6.8 (s.d. 10.2), while that of lymphocytic/collagenous colitis cases was 1.47 (s.d. 0.9) ($P=0.0001$). Ulcerative colitis showed significantly higher numbers of IgG4-positive plasma cells

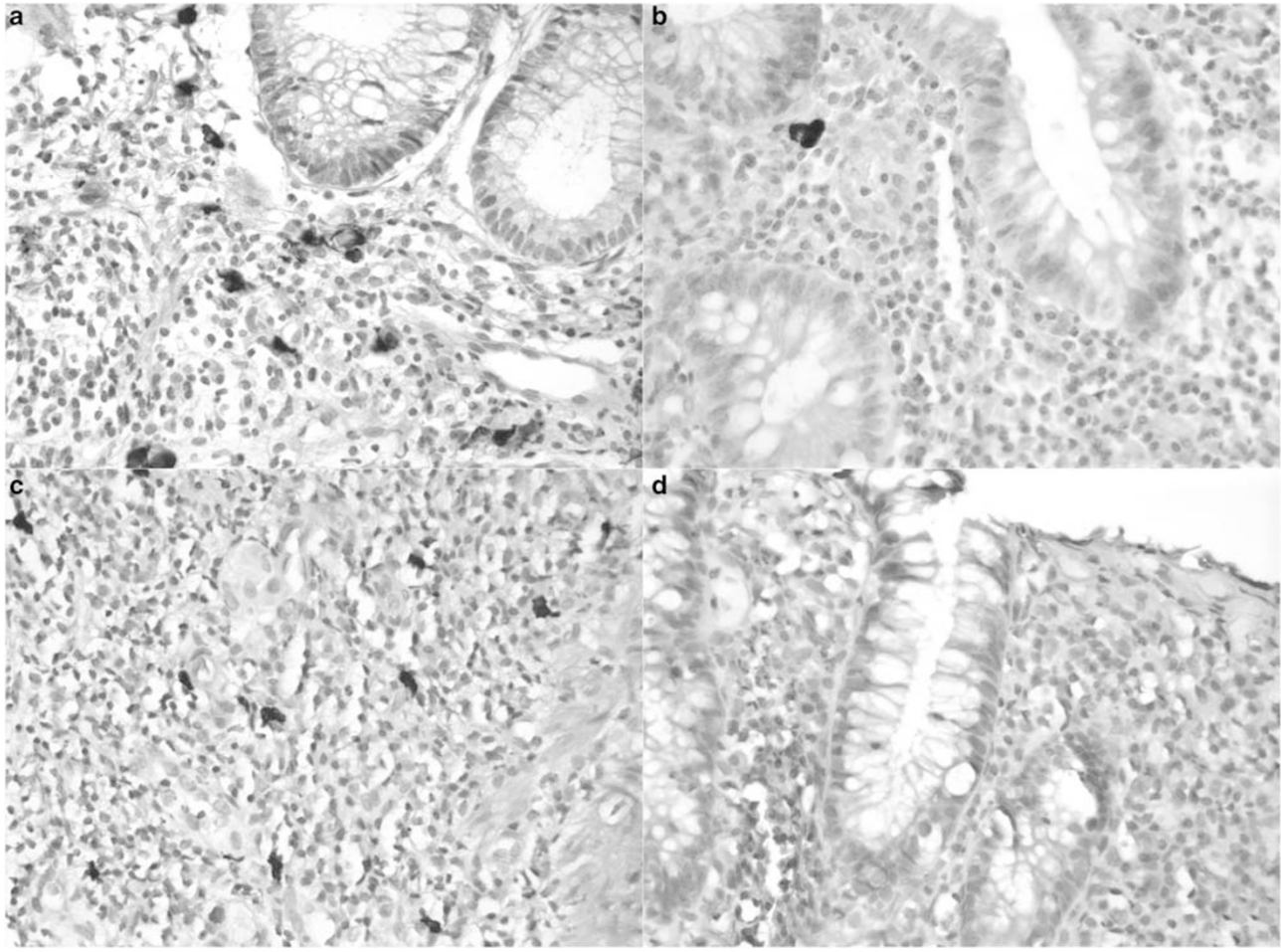

Figure 2 Immunohistochemical stains for IgG4 in ulcerative colitis (**a**), Crohn's disease (**b**), autoimmune pancreatitis-related ulcerative colitis (**c**), and collagenous colitis (**d**).

Table 1 IgG4 counts in inflammatory bowel disease

	Ulcerative colitis (n = *50*)	*Crohn's disease* (n = *38*)	P *value*
All case mean IgG4 counts (s.d.)	9.77 (11.9)	2.79 (5.3)	0.001
IgG4 counts >10 per high-power field	19 (38%)	2 (5.3%)	0.0001
Children (≤18 years) *N* = 15	9.43	4.8	0.355
Adults (≥18 years)	10.0	2.3	0.001
IBD cases on TMA (s.d.)	9.7 (12.2)	3.36 (6)	0.02
IBD cases—whole sections (s.d.)	10 (11.1)	1 (1.4)	0.03

than microscopic colitis ($P = 0.0001$), while there was no statistically significant difference between Crohn's disease and microscopic colitis ($P = 0.25$).

Discussion

This study explores a neglected area of inflammatory bowel disease investigation: the use of tissue-based biomarkers to distinguish ulcerative colitis from Crohn's disease. We demonstrate here that cases of ulcerative colitis show significantly higher numbers of IgG4-positive plasma cells than Crohn's disease. Based on a cutoff point of 10 IgG4-positive plasma cells per HPF, the sensitivity in this study is 38% and the specificity 95%. If the pediatric cases were excluded, the sensitivity of a cutoff of 5 IgG4 + plasma cells rose to 53% (specificity 83%). Nonetheless, these numbers cannot justify the routine use of a tissue IgG4 stain to distinguish ulcerative colitis from Crohn's disease. However, in select cases the presence of >10 IgG4-positive plasma cells per HPF could further buttress a presumptive diagnosis of ulcerative colitis.

Interestingly, these statistically significant differences in IgG4 levels were not seen in children, although the number of cases evaluated was small. It is certainly possible that a larger cohort may reveal differences, and this requires further study. Alternatively, this difference may reflect the underlying biological differences between inflammatory bowel disease in children and adults. Indeed, there are clinical, serological, and histological differences between inflammatory bowel disease in adults and children.[11] Children with ulcerative colitis tend to have more extensive disease than adults. Although

serological assays are potentially valuable to the diagnosis of inflammatory bowel disease in older children and adults, this is not true in young children.[12] Histologically, the changes of chronicity may take several years to evolve, unlike adults where the crypt architectural changes and basal plasmacytosis develop within weeks of the onset of the disease.[13]

Remarkably, patients with lymphocytic and collagenous colitis showed lesser numbers of IgG4-positive plasma cells: none of the cases we evaluated showed levels of >5 IgG4-positive plasma cells per HPF. Generally, distinguishing inflammatory bowel disease from lymphocytic/collagenous colitis is straightforward; however, some cases of collagenous colitis may show mild architectural distortion accompanied by active colitis, thus mimicking ulcerative colitis. In such instances, a tissue IgG4 stain may provide an additional parameter to refute a diagnosis of 'microscopic colitis'. Interestingly, like Crohn's disease, microscopic colitis is characteristically a Th1-type T-cell response.[14]

Although there are several approaches to document the severity of disease in ulcerative colitis, the retrospective nature of the study limited our options in this regard. Nonetheless, patients of ulcerative colitis with erosions or ulceration showed higher IgG4 counts than those who lacked these histological features. The second measure of severity we choose to examine was the presence of pancolitis, either at presentation or at follow-up. Although patients with pancolitis did show higher numbers of IgG4-positive plasma cells, this difference did not reach statistical significance ($P=0.09$).

We were careful to include only biopsies from treatment-naive patients. It is widely recognized that IgG4 levels are exquisitely sensitive to immunosuppressive therapy. Following the institution of immunosuppressive therapy, a swift decline in serum IgG4 concentration is observed.[9,15] After treatment with glucocorticoids, for example, patients with IgG4-related disease show depletion of IgG4-bearing plasma cells.[8,9] When using a tissue IgG4 stain to make the distinction between ulcerative colitis and Crohn's disease, it is thus paramount to identify a pretreatment biopsy.

Another facet of this study that we would like to emphasize is the fact that although ulcerative colitis is associated with elevated levels of IgG4-positive plasma cells, this disease is by no means a IgG4-related disease. The diagnosis of IgG4-related disease requires both a characteristic histopathological profile and elevated numbers of IgG4-positive plasma cells.[15] Ulcerative colitis lacks both storiform fibrosis and phlebitis and hence does not fit the profile of IgG4-related disease.

These quantitative differences between the two forms of inflammatory bowel disease are based on sound biological principles. Conventionally, two immune phenotypes are recognized: Th 1 response characterized by secretion of interleukin-2, tumor necrosis factor-α, and interferon γ, responses associated with delayed-type hypersensitivity reactions; Th2 responses are characterized by the secretion of IL-4, IL-5, and IL-10, a profile conventionally associated with allergy. Traditionally, Crohn's disease (as well as lymphocytic and collagenous colitis) has been associated with a predominantly Th1 response; conversely, ulcerative colitis has been linked with a Th2 response.[7,16] Several of the key cytokines associated with the Th2 response are also critical for IgG4 class switching.

This, however, is a relatively simplistic model of inflammatory bowel disease: genome-wide association studies and subsequent investigations have uncovered a role for the Th17 response in inflammatory bowel disease.[4] It is now widely recognized that Th17 cytokine response has a significant role in the pathogenesis of both ulcerative colitis and Crohn's colitis. Nonetheless, this 'proof of concept' study could provide impetus to exploit the immunological differences between ulcerative colitis and Crohn's disease and develop tissue-based biomarkers that distinguish the two diseases. The lack of IgG4-positive plasma cells in a substantial minority of cases of ulcerative colitis may reflect the immunological non-homogeneity of this disease.

There are only a handful of studies that have explored the differences in the interleukin profile of patients with ulcerative colitis and Crohn's disease. Interestingly, in virtually all of the studies no attempt was made to distinguish between treatment-naive patients and those exposed to immunosuppressive therapy. This may partially account for the often contradictory results from these studies.[17,18] In one study, interleukin-4 was detectable in ulcerative colitis patients in 78% of patients, while it was undetectable in patients with Crohn's disease.[18] In other study, the number of lamina propria interferon-gamma-positive cells was significantly increased as compared with controls, while a similar increase was not seen in the ulcerative colitis cohort.[17] A more contemporary study examined Th17-related cytokine transcripts in inflammatory bowel disease.[6] Although these transcripts were elevated in both the forms of inflammatory bowel disease, they were more abundant in ulcerative colitis.[6]

We acknowledge several shortcomings of the study. The first of these is the retrospective nature of the study. Furthermore, we did not have access to serum IgG4 levels. We were also limited in our ability to correlate the severity of the disease with IgG4 counts. However, previous work has shown a correlation between the severity of inflammatory bowel disease-related pouchitis and elevated numbers of IgG4-bearing plasma cells.[19] And finally, although every effort was made to identify a pretreatment biopsy, many of these individuals were diagnosed in the 1990s: it is thus difficult to entirely exclude the possibility that some of these patients were exposed to immunosuppressive

therapy before the 'index' biopsy. There is clearly a need to perform a prospective study that would evaluate the diagnostic and prognostic value of a tissue IgG4 stain. In this exploratory study, we elected not to evaluate cases of 'indeterminate colitis', and this represents another fertile area for investigation.

In conclusion, we show that an immunohistochemical stain for IgG4 may aid in making the distinction between ulcerative colitis and Crohn's disease, albeit with a relatively low sensitivity. This data also lend support to the hypothesis that ulcerative colitis is associated with a Th2-type T-cell response. We anticipate that this study would encourage further investigations that evaluate the use of tissue-based biomarkers as predictive and prognostic markers in inflammatory bowel disease.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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