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Diagnostic Potential of Zymogen Granule Glycoprotein 2 Antibodies as Serologic Biomarkers in Chinese Patients With Crohn Disease

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Abstract: The need for reliable biomarkers for distinguishing Crohn disease (CD) from ulcerative colitis (UC) is increasing. This study aimed at evaluating the diagnostic potential of anti-GP2 antibodies as a biomarker in Chinese patients with CD. In addition, a variety of autoantibodies, including anti-saccharomyces cerevisiae antibodies (ASCA), perinuclear anti-neutrophil cytoplasmic antibodies (PANCA), anti-intestinal goblet cell autoantibodies (GAB), and anti-pancreatic autoantibodies (PAB), were evaluated.

A total of 91 subjects were prospectively enrolled in this study, including 35 patients with CD, 35 patients with UC, 13 patients with non-IBD gastrointestinal diseases as disease controls (non-IBD DC), and 8 healthy controls (HC). The diagnosis of IBD was determined based on the Lennard-Jones criteria, and the clinical phenotypes of the IBD patients were determined based on the Montreal Classification.

Anti-GP2 IgG antibodies were significantly elevated in patients with CD, compared with patients with UC ($P = 0.0038$), HC ($P = 0.0055$), and non-IBD DC ($P = 0.0063$). The prevalence of anti-GP2 IgG, anti-GP2 IgA and anti-GP2 IgA, or IgG antibodies in patients with CD was 40.0%, 37.1%, and 54.3%, respectively, which were higher than those in non-IBD DC (anti-GP2 IgG, 15.4%; anti-GP2 IgA, 7.7%; and anti-GP2 IgA or IgG, 23.1%) and those in patients with UC (anti-GP2 IgG, 11.4%; anti-GP2 IgA, 2.9%; and anti-GP2 IgA or IgG, 14.3%). For distinguishing CD from UC, the sensitivity, specificity, positive predictive value (PPV) and positive likelihood ratios (LR+) were 40%, 88.6%, 77.8%, and 3.51 for anti-GP2 IgG, 37.1%, 97.1%, 92.9%, and 13.0 for anti-GP2 IgA, and 54.3%, 85.3%, 79.2%, and 3.69 for anti-GP2 IgA or IgG. For CD diagnosis, the combination of anti-GP2 antibodies with ASCA IgA increased the sensitivity to 68.6% with moderate loss of specificity to 74.3%. Spearman's rank of order revealed a significantly positive correlation of anti-GP2 IgG with ileocolonic location of disease (L3) ($P = 0.043$) and a negative correlation of anti-GP2 IgA with biologic therapy ($P = 0.012$).

Our findings suggest that anti-GP2 antibodies could serve as a biomarker for distinguishing patients with CD from patients with UC, and the combination of anti-GP2 antibodies with ASCA IgA may improve the predictive power.

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Abbreviations: ANCA = anti-neutrophil cytoplasmic antibodies, ASCA = anti-saccharomyces cerevisiae antibody, CD = Crohn disease, ELISA = enzyme-linked immunosorbent assay, GAB = anti-intestinal goblet cell autoantibody, GI = gastrointestinal, GP2 = glycoprotein 2, IBD = inflammatory bowel disease, IIF = indirect immunofluorescence, LR− = negative likelihood ratios, LR+ = positive likelihood ratios, M cell = microfold or membranous cell, PAB = pancreatic autoantibody, pANCA = perinuclear anti-neutrophil cytoplasmic antibodiey, PP = Peyer's patches, PPV = positive predictive value, UC = ulcerative colitis.

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of chronic relapsing intestinal inflammation of unknown etiology and heterogeneous clinical symptoms and course.[1] A combination of genetic, environmental, and immunological mechanisms has been proposed to cause and/or contribute to IBD.[1–3] Crohn disease (CD) and ulcerative colitis (UC) are the 2 major clinical phenotypes of IBD.[1,2] Both CD and UC present a series of symptoms and signs, including intestinal and extra-intestinal involvements.[1–3] However, CD and UC display substantial difference in terms of lesion location in the gastrointestinal (GI) tract. Specifically, CD can affect any part of the GI with the lesion formation in the entire bowel wall, whereas UC only affects large intestine with the lesion formation restricted to the epithelial lining of the gut.[1–3] The different characteristics between CD and UC result in different clinical managements and therapies, especially when it comes to surgical interventions.[1–3] In addition, diagnostic dilemma can also come from other disorders affecting the GI, which may present similar symptoms to those seen in IBD patients.[1–3] Therefore, accurate diagnosis is essential for proper clinical interventions.

A number of serological biomarkers have been identified for distinguishing IBD from non-IBD and for distinguishing CD from UC. Anti-saccharomyces cerevisiae antibodies (ASCA) and perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) have been widely used as routine tests for patients with clinical suspicion of IBD.[4,5] However, the sensitivity of

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ASCA in CD patients is far from satisfactory.[4] Recent data showed that the sensitivity of ASCA was 46.3% in Chinese patients with CD.[4] Interestingly, 2 studies suggested the prevalence of ASCA was much lower in Chinese patients with CD in terms of either ASCA IgA[6] or ASCA IgG,[7] challenging the role of ASCA in the diagnosis of different subtypes of IBD. Taken together, these studies indicate a strong need for additional biomarkers to improve the diagnostic sensitivity and accuracy.

Pancreatic autoantibodies (PAB) have been recognized as CD-specific biomarkers.[8–10] It has been reported that PAB can be detected in approximately 30% of patients with CD but less than 5% of patients with UC or non-IBD and health controls.[9–10] However, detection of PAB exclusively depends on indirect immunofluorescence (IIF) on pancreatic tissues. Thus, the clinical utility of PAB has been hampered due to its unidentified antigenic targets. Excitingly, zymogen granule glycoprotein 2 (GP2) was recently described as the major autoantigen of CD-specific PAB. GP2 is a highly glycosylated protein, accounting for around 40% of the zymogen granule membrane proteins of pancreatic acinar cells.[11] Importantly, overexpression of GP-2 has been identified in the intestinal tissue in patients with CD, but not in patients with other immune-mediated enteropathies, such as UC, suggesting a direct involvement of GP2 in the inflammatory process in CD.[11] In addition, GP-2 was found on the surface of microfold (M) cells of the intestinal Peyer's patches (PP),[12] which have been considered the original location of CD inflammation.[13]

Recent studies indicate that anti-GP2 IgA and/or IgG were present in 21–45% of patients with CD and in 2–19% of patients with UC.[11,14–18] Most importantly, 8–24% of ASCA-negative CD patients were positive for anti-GP2 IgA and/or IgG,[16–17] suggesting that anti-GP2 IgA and/or IgG could be a promising biomarker for distinguishing CD from UC, and the combination of ASCA with anti-GP2 IgA and/or IgG could synergistically strengthen the overall performance. Of note, the prevalence and diagnostic performances can be affected by a variety of factors, and ethnic/geographic background is an important one among these factors. Thus, it is of paramount importance to assess the diagnostic potential of anti-GP2 IgA and/or IgG as a biomarker in Chinese patients with CD. In this study, we determined the sensitivity, specificity, and positive and negative predictive values (PPV and NPV) of a variety of autoantibodies, including anti-GP2 antibodies, ASCA, anti-PAB, pANCA, and anti-intestinal goblet cell autoantibodies (GAB), and investigated their individual diagnostic values as well as combinational diagnostic values in distinguishing CD from UC (Figure 1).

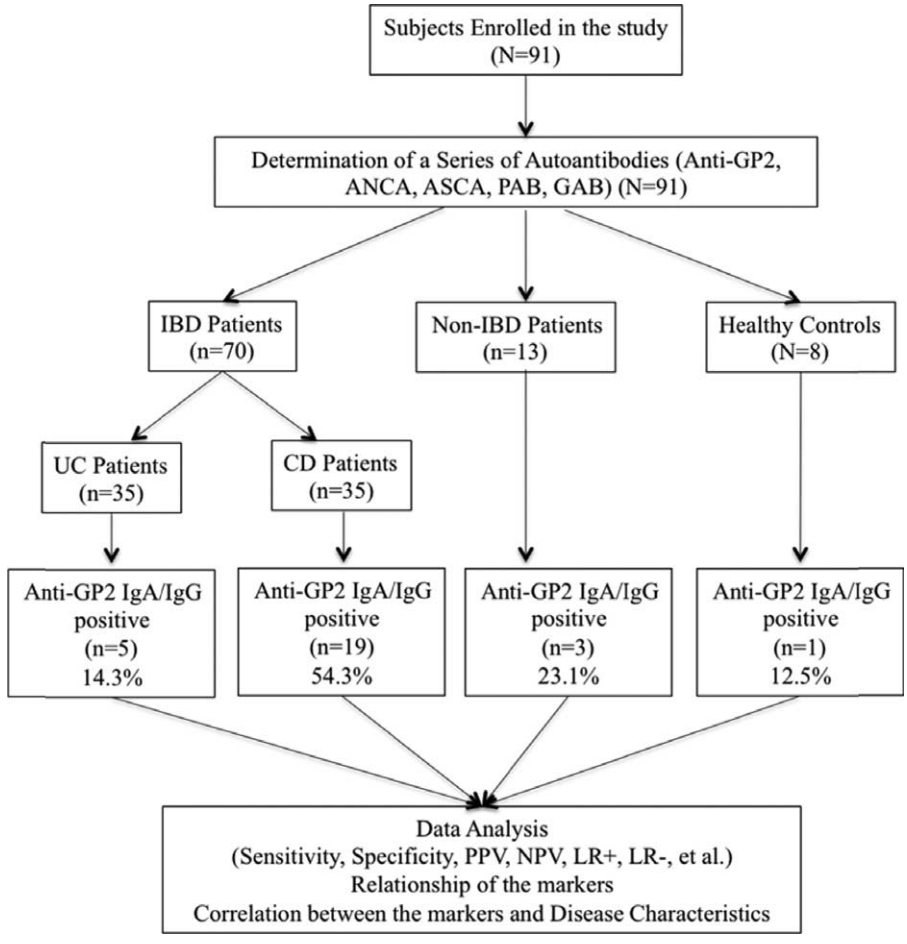

FIGURE 1. Evaluation of multiple autoantibodies, including anti-GP2, ANCA, ASCA, PAB, GAB antibodies in the diagnosis of Chinese patients with CD.

METHODS

Subjects and Specimen Collections

A total of 91 subjects were prospectively enrolled in this study, including 35 patients with CD, 35 patients with UC, 13 patients with non-IBD gastrointestinal diseases as disease controls (non-IBD DC), and 8 healthy controls (HC). The non-IBD DC included patients with intestinal Behcet disease (n = 5), intestinal tuberculosis (n = 6), ischemic colitis (n = 2), and infectious colitis (n = 2). HC included subjects without any signs of infection or inflammation or other significant illnesses. All patients were diagnosed and managed at the Department of Gastroenterology, Peking Union Medical College Hospital (PUMCH). The diagnosis of IBD was determined based on the Lennard-Jones criteria.[19] Specifically, subjects were diagnosed with CD or UC based on a combination of standard criteria that included clinical symptoms, physical examination, colonoscopy, imaging (bariums studies and CT enterography), and histopathology. Enteric infections, intestinal tuberculosis, ischemia, nonsteroidal anti-inflammatory drug-induced ulceration, and radiation colitis were excluded. Clinical phenotypes of the IBD patients were determined based on the Montreal Classification.[20] Specifically, CD is described by A, L, and B classifications. A represents age at diagnosis (A1, below 17 yr; A2, between 17 and 40 yr; A3, above 40 yr), L represents the location of disease (L1, ileal; L2, colonic; L3, ileocolonic; L4, upper disease), and B represents disease behavior (B1, nonstricturing, nonpenetrating; B2, stricturing; B3, penetrating; P, perianal disease modifier). UC is described by E classifications (E1, proctitis, lesions limited to the rectum; E2, left-sided colitis, lesions below the splenic flexure; E3, pancolitis, lesions exceeded the splenic flexure). The activity of UC was defined by the Simple Clinical Colitis Activity Index (SCCAI) as mild (3–5 scores), moderate (6–11 scores), and severe (above 12 scores). The demographics and clinical characteristics of the CD and UC patients are shown in Table 1. Study protocols were reviewed and approved by the Ethical Committee of PUMCH and informed consents were obtained from all participants. All sera were stored at −20°C until analysis.

TABLE 1. Demographics of Patients With Inflammatory Bowel Disease

	CD (n = 35)	UC (n = 35)
Female, n (%)	10 (28.6)	19 (54.3)
Median age at study (max, min)	17 (69, 13)	38 (75, 18)
Median duration (max, min)	3 (39, 0.1)	5(38, 0.2)
Age at diagnosis, n (%)		
Below 17 years (A1)	12 (34.3)	2 (5.7)
Between 17 and 40 years (A2)	16 (45.7)	21 (60.0)
Above 40 years (A3)	7 (20.0)	12 (34.3)
CD location, n (%)		
Ileal (L1)	5 (14.3)	NA
Colonic (L2)	7 (20.0)	NA
Ileocolonic(L3)	23 (65.7)	NA
Upper disease, modifier (L4)	3 (8.6)	NA
CD behavior, n (%)		
Nonstricturing, nonpenetrating (B1)	10 (28.6)	NA
Stricturing (B2)	20 (57.1)	NA
Penetrating (B3)	12 (34.3)	NA
Perianal disease (p)	10 (28.6)	NA
UC extent, n (%)		
Proctitis (E1)	NA	1 (2.9)
Left-sided colitis (E2)	NA	18 (51.4)
Pancolitis (E3)	NA	16 (45.7)
UC severity, n (%)		
Mild	NA	13 (37.1)
Moderate	NA	14 (40)
Severe	NA	8 (22.9)
Extraintestinal manifestations		
Musculoskeletal	2 (5.7)	7 (20)
Dermatologic	9 (25.7)	1 (2.9)
Ocular	1 (2.9)	0 (0)
Outcome, n (%)		
Biologic therapy	8 (22.9)	0 (0)
Immunosuppressive therapy	8 (22.9)	2 (5.7)
Surgery	15 (42.9)	5 (14.3)

CD = Crohn disease; NA = not applicable; UC = ulcerative colitis.

Serum Antibodies Determination

Serum anti-GP2 autoantibodies (IgG and IgA) were determined by ELISA (Generic Assays, Dahlewitz/Berlin, Germany), according to the manufacturer's instructions. The cutoff value for positivity was set to 20 U/mL for both anti-GP2 IgG and anti-GP2 IgA antibodies, as recommended by the manufacturer. Serum anti-saccharomyces cerevisiae antibodies (ASCA) (IgG and IgA) were determined by ELISA (Euroimmune, Luebeck, Germany). Values above 20 U/mL were considered positive according to the manufacturer's instructions. Serum anti-neutrophil cytoplasmic autoantibodies (ANCA) (IgG and IgA), anti-intestinal goblet cell autoantibodies (GAB), and pancreatic autoantibodies (PAB) were tested by indirect immunofluorescent assay (IFA) (Euroimmune, Luebeck, Germany), in accordance with the manufacturer's instructions. IFA testings were performed starting with an initial dilution of 1/10. Serial dilutions of 1/20, 1/40, 1/80, and 1/160 were further performed for all positive samples. Two experienced technologists interpreted the results.

Statistical Analysis

SPSS 20.0 statistical software package (SPSS Inc, Chicago, IL) and Prism 5.02 (GraphPad Software, San Diego, CA) were utilized for statistical analyses. For comparison of continuous variables, the independent t-test or Mann–Whitney *U* test was performed. For comparison of categorical variables, the χ^2 test or Fisher exact test was employed. The association of anti-GP2, ASCA, or anti-PAB antibodies with disease characteristics was assessed by Spearman's rank of order. For all statistic analyses, *P* values of less than 0.05 were considered statistically significant.

RESULTS

Levels of Anti-GP2 IgG Antibodies Were Significantly Elevated in Patients With CD

As shown in Figure 2A, anti-GP2 IgG antibodies were significantly elevated in patients with CD, compared with patients with UC ($P = 0.0038$), HC ($P = 0.0055$), and non-IBD DC ($P = 0.0063$). However, no significant difference was observed in anti-GP2 IgA antibodies in patients with CD, compared with patients with UC ($P = 0.0704$), HC ($P = 0.0834$), and non-IBD DC ($P = 0.0616$) (Figure 2B),

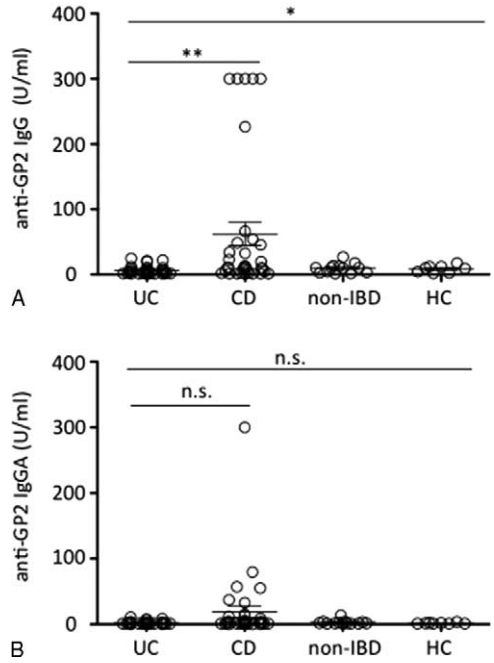

FIGURE 2. Anti-GP2 IgG (A) and anti-GP2 IgA (B) antibodies levels in patients with UC, patients with CD, non-IBD patients, and healthy controls (HC). n.s. = nonsignificant, $^{*}P < 0.05$; $^{**}P < 0.005$.

although a trend of increased levels of anti-GP2 IgA was observed in patients with CD (Figure 2B).

Prevalence of Multiple Autoantibodies in Patients With CD and UC

The prevalence of a series of IBD-relevant autoantibodies was evaluated among all the subjects, and the results are summarized in Table 2. Overall, the prevalence of anti-GP2 IgG, anti-GP2 IgA, and anti-GP2 IgA or IgG antibodies in patients with CD was 40.0%, 37.1%, and 54.3%, respectively, which were higher than those in non-IBD DC (anti-GP2 IgG, 15.4%; anti-GP2 IgA, 7.7%; and anti-GP2 IgA or IgG, 23.1%) and those in patients with UC (anti-GP2 IgG, 11.4%; anti-GP2 IgA, 2.9%; and anti-GP2 IgA or IgG, 14.3%) (Table 2). Anti-PAB antibodies were detected in patients with CD (IgG, 8.6%; IgA, 5.7%, and IgA or IgG 8.6%) and patients with UC (IgG, 8.6%; IgA, 2.9%, and IgA or IgG, 8.6%), but not in non-IBD DC and HC. ASCA IgA antibodies were present in 25.7% of CD patients, 5.7% of UC patients, 23.1% of non-IBD DC, and 12.5% of HC, respectively. The prevalence of ASCA IgA or IgG antibodies was 25.7%, 11.4%, 30.8%, and 12.5% in CD patients, UC patients, non-IBD DC, and HC, respectively. For the ANCA IgA or IgG antibodies, the prevalence was similar among CD patients, UC patients, and non-IBD DC, ranging from 34.3% to 40.0%. GAB IgA or IgG antibodies were detected in 37.1% of CD patients, 37.1% of UC patients, 15.4% of non-IBD DC, but not in HC. To further assess the potential role of these autoantibodies in distinguishing patients with CD from patients with UC, *P* values were calculated between CD patients and UC patients. A significantly higher prevalence of anti-GP2 IgA or IgG (19/35, 54.3%) was detected in patients with CD, compared with patients with UC (5/35, 14.3%) ($P = 0.0009$). Importantly, the prevalence of both anti-GP2 IgG and anti-GP2 IgA was significantly higher in patients with CD than that in patients with UC (anti-GP2 IgG, $P = 0.013$; anti-GP2 IgA, $P = 0.0006$). In addition, the prevalence of ASCA IgA was significantly higher in patients with CD (9/35, 25.7%), compared with patients with UC (2/35, 5.7%) ($P = 0.045$). No significant difference was found in other autoantibodies, either in IgG subtype or in IgA subtype (Table 2).

Predictive Power of Serologic Markers for Distinguishing Patients With CD From Patients With UC

Assay performance characteristics for the detection of anti-GP2 antibodies (IgA and/or IgG) were compared to corresponding ASCA and PAB values, and the results are

TABLE 2. Prevalence of Autoantibodies in Patients With Inflammatory Bowel Disease and Controls

	CD (n = 35)	UC (n = 35)	Non-IBD (n = 13)	HC (n = 8)	*P* Value*
Anti-GP2 IgG, n (%)	14 (40.0)	4 (11.4)	2 (15.4)	1 (12.5)	0.013
Anti-GP2 IgA, n (%)	13 (37.1)	1 (2.9)	1 (7.7)	0 (0)	0.0006
Anti-GP2 IgA or IgG, n(%)	19 (54.3)	5 (14.3)	3 (23.1)	1(12.5)	0.0009
PAB IgG, n (%)	3 (8.6)	3 (8.6)	0 (0)	0 (0)	1.000
PAB IgA, n (%)	2 (5.7)	1 (2.9)	0 (0)	0 (0)	1.000
PAB IgA or IgG, n(%)	3 (8.6)	3 (8.6)	0 (0)	0 (0)	1.000
ASCA IgG, n (%)	3 (8.6)	3 (8.6)	1 (7.7)	0 (0)	1.000
ASCA IgA, n (%)	9 (25.7)	2 (5.7)	3 (23.1)	1 (12.5)	0.045
ASCA IgA or IgG, n(%)	9 (25.7)	4 (11.4)	4 (30.8)	1 (12.5)	0.218
ANCA IgG, n (%)	12 (34.3)	10 (28.6)	5 (38.5)	0 (0)	0.807
ANCA IgA, n (%)	9 (25.7)	8 (22.9)	4 (30.8)	0 (0)	1.000
ANCA IgA or IgG, n(%)	14 (40.0)	12 (34.3)	5 (38.5)	0 (0)	0.805
GAB IgG, n (%)	13 (37.1)	13 (37.1)	2 (15.4)	0 (0)	1.000
GAB IgA, n (%)	1 (2.9)	2 (5.7)	0 (0)	0 (0)	1.000
GAB IgA or IgG, n (%)	13 (37.1)	13 (37.1)	2 (15.4)	0 (0)	1.000

ANCA = anti-neutrophil cytoplasmic antibodies; ASCA = anti-saccharomyces cerevisiae antibodies; CD = Crohn disease; GAB = anti-intestinal goblet cell autoantibodies; GP2 = glycoprotein 2; HC = health control; IBD = inflammatory bowel disease; PAB = pancreatic autoantibody; UC = ulcerative colitis.

* The difference of the autoantibodies prevalence in patients with CD and inpatients with UC.

TABLE 3. Predictive Power of Serologic Markers for Differentiation Among Patients With Crohn Disease and Ulcerative Colitis

	Sensitivity	Specificity	PPV	NPV	LR+	LR−
CD vs. UC						
Anti-GP2 IgG	40%	88.6%	77.8%	59.6%	3.51	0.68
Anti-GP2 IgA	37.1%	97.1%	92.9%	60.7%	13.0	0.65
Anti-GP2 IgA or IgG	54.3%	85.3%	79.2%	64.4%	3.69	0.54
PAB IgG	8.6%	91.4%	50%	50%	1.00	1.00
PAB IgA	5.7%	97.1%	66.7%	50.8%	1.96	0.97
PAB IgA or IgG	8.6%	91.4%	50%	50%	1.00	1.00
ASCA IgG	8.6%	91.4%	50%	50%	1.00	1.00
ASCA IgA	25.7%	94.3%	81.8%	55.9%	4.51	0.79
ASCA IgA or IgG	25.7%	88.6%	69.2%	54.4%	2.25	0.84
UC vs. CD						
ANCA IgG	22.2%	73.7%	45.5%	48.5%	0.84	1.06
ANCA IgA	22.9%	74.3%	47.1%	49.1%	0.89	1.04
ANCA IgA or IgG	34.3%	60%	46.2%	47.7%	0.86	1.10
GAB IgG	37.1%	62.7%	50%	50%	0.99	1.00
GAB IgA	5.7%	97.1%	66.7%	50.8%	1.96	0.97
GAB IgA or IgG	37.1%	62.7%	50%	50%	0.99	1.00

ANCA = anti-neutrophil cytoplasmic antibodies; ASCA = anti-saccharomyces cerevisiae antibodies; CD = Crohn disease; GAB = anti-intestinal goblet cell autoantibodies; GP2 = glycoprotein 2; LR− = negative likelihood ratio; LR+ = positive likelihood ratio; NPV = negative predictive value; PAB = pancreatic autoantibody; PPV = positive predictive value; UC = ulcerative colitis.

summarized in Table 3. For distinguishing CD from UC, anti-GP2 IgA or IgG demonstrated the highest sensitivity (54.3%), followed by anti-GP2 IgG (40.0%), anti-GP2 IgA (37.1%), and ASCA IgA or IgG (25.7%) and ASCA IgA (25.7%). The sensitivities of PAB IgA, IgG, IgA or IgG, and ASCA IgG were less than 10% (Table 3). The specificities of all of these markers were similar, ranging from 85.3% to 97.1%. Anti-GP2 IgA showed the highest positive predictive value (PPV) (92.9%) and positive likelihood ratios (LR+) (13.0), followed by ASCA IgA (PPV: 81.8%, LR+: 4.51), and anti-GP2 IgA or IgG (PPV: 72.9%, LR+: 3.69) (Table 3). For distinguishing UC from CD, GAB IgG and GAB IgA or IgG showed the highest sensitivity (37.1%), followed by ANCA IgA or IgG (34.3%), ANCA IgA (22.9%), and ANCA IgG (22.2%) (Table 3).

As both anti-GP2 antibodies and ASCA IgA demonstrated a good performance in distinguishing CD from UC, we evaluated the predictive power of combination of anti-GP2 antibodies and ASCA IgA in distinguishing CD from UC. The double positive of anti-GP2 IgA and ASCA IgA, or the triple positive of anti-GP2 IgA, anti-GP2 IgG, and ASCA IgA strikingly raised the specificity and PPV to 100%, but decreased the sensitivity to 8.3% (Table 4). In contrast, either anti-GP2 IgA positive, or anti-GP2 IgG positive, or ASCA IgA positive increased the sensitivity from 54.3% (the sensitivity of anti-GP2 IgA or IgG) to 68.6%, with moderate loss of specificity from 85.3% (the specificity of anti-GP2 IgA or IgG) to 74.3% (Table 4).

Relationships Between Serological Markers (Anti-GP2, ASCA, and Anti-PAB Antibodies) in the CD Cohort and UC Cohort

As more than one of the described autoantibodies (anti-GP2, ASCA, anti-PAB) was found in several patients, we illustrate the distribution of these antibodies in CD patients by Venn diagram (Figure 3A). Of note, 31.4% of patients with CD were negative for all of the 3 antibodies, and the remaining 68.6% of patients with CD reacted to at least 1 marker. Only 2.9% of patients with CD were reactive to all of the markers. Importantly, 42.8% of ASCA negative CD patients were positive for anti-GP2 IgA and/or IgG antibodies, whereas only 14.3% of anti-GP2 negative CD patients were positive for ASCA IgA and/or IgG antibodies. The distribution of autoantibodies (ANCA and anti-GAB antibodies) is illustrated by Venn

TABLE 4. Combined Analysis of Anti-GP2 and ASCA for Differentiation Among Patients With Crohn Disease and Ulcerative Colitis

	Sensitivity	Specificity	PPV	NPV	LR+	LR−
Anti-GP2 IgA or IgG or ASCA IgA	68.6%	74.3%	72.7%	70.3%	2.67	0.42
Anti-GP2 IgA and IgG and ASCA IgA	8.6%	100%	100%	52.2%	NA	0.91
Anti-GP2 IgA or ASCA IgA	54.3%	85.7%	79.2%	65.2%	3.8	0.53
Anti-GP2 IgA and ASCA IgA	8.6%	100%	100%	52.2%	NA	0.91

ASCA = anti-saccharomyces cerevisiae antibodies; GP2 = glycoprotein 2; LR− = negative likelihood ratio; LR+ = positive likelihood ratio; NA = not applicable; NPV = negative predictive value; PPV = positive predictive value.

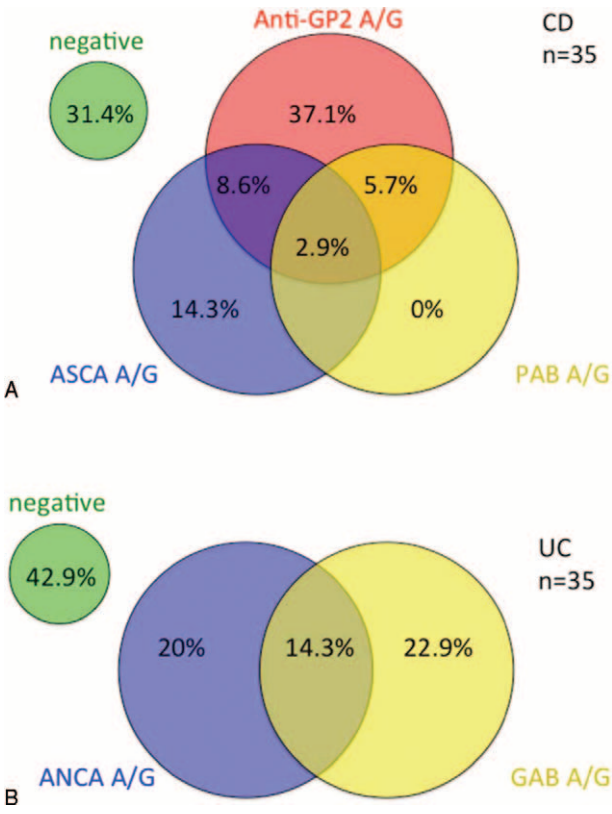

FIGURE 3. Venn diagram describing the relationships between serological markers (Anti-GP-2, ASCA, and PAB) in the CD cohort (n = 35) by presence vs. absence (A), Venn diagram describing the relationships between serological markers (ANCA and GAB) in the UC cohort (n = 35) by presence vs. absence (B). The percentage of positive patients for each marker, or any combination of 2 markers, is shown.

diagram in Figure 3B. Overall, 42.9% of patients were negative for ANCA and anti-GAB antibodies. Approximately 57% of patients with UC were positive for at least 1 marker, and 14.3% of the patients with UC were reactive to both of the markers (Figure 3B).

Association of Anti-GP2, ASCA, or Anti-PAB Antibodies With Disease Characteristics of Patients With CD

The association of anti-GP2, ASCA, or anti-PAB antibodies with disease characteristics was evaluated in patients with CD. Spearman's rank of order revealed a significantly positive correlation of anti-GP2 IgG with ileocolonic location of disease (L3) ($P = 0.043$) and a negative correlation of anti-GP2 IgA with biologic therapy ($P = 0.012$) (Table 5). ASCA IgG ($P = 0.011$) and ASCA IgA and IgG ($P = 0.011$) were positively correlated with patients with an age less than 17 years at diagnosis. In addition, PAB IgG ($P = 0.035$) and PAB IgA or IgG ($P = 0.035$) were positively correlated with colonic location of disease (L2), and PAB IgA ($P = 0.003$), PAB IgG ($P = 0.035$), PAB IgA or IgG ($P = 0.035$), and PAB IgA and IgG ($P = 0.003$) were positively correlated with nonstricturing, nonpenetrating disease (B1).

DISCUSSION

In this study, we evaluated the prevalence and diagnostic potential of anti-GP2 antibodies in Chinese patients with IBD. The major findings include the following: first, the levels of anti-GP2 IgG antibodies were significantly elevated in patients with CD; second, the prevalence of anti-GP2 (either IgA or IgG subtype) antibodies was significantly higher in patients with CD than that in patients with UC; third, the double positive of anti-GP2 IgA and ASCA IgA, or the triple positive of anti-GP2 IgA, anti-GP2 IgG and ASCA IgA had a strong indication of CD with a specificity and PPV of 100%; fourth, anti-GP2 IgG was positively correlated with ileocolonic location of disease (L3) and anti-GP2 IgA was negatively correlated with biologic therapy. Our findings suggest that anti-GP2 antibodies could serve as a biomarker for distinguishing patients with CD from patients with UC.

Previous studies from several groups in Europe showed that anti-GP2 antibodies were present in 25–30% of patients with CD and in 5–12% of patients with UC.[14,21,22] In this study, we found that the prevalence of anti-GP2 antibodies was 54.3% in Chinese patients with CD, which is higher than that reported in previous studies.[14,21,22] However, the prevalence of anti-GP2 antibodies in UC patients in our study is similar to that reported in other studies.[14,21,22] As different genetic, immunologic, and environmental factors contribute to the pathogenesis of CD, the higher prevalence of anti-GP2 antibodies in Chinese patients with CD could be due to the combination of these factors. More importantly, the increased sensitivity of anti-GP2 antibodies did not sacrifice the specificity, PPV and LR+ values. Therefore, our data suggest that anti-GP2 antibodies could be a promising biomarker for distinguishing CD from UC.

ASCA has been recognized as the most widely used biomarker for CD.[23] Interestingly, in our study, 42.8% of ASCA-negative CD patients were identified positive for anti-GP2 antibodies (IgA and/or IgG), whereas only 14.3% of anti-GP2-negative CD patients were positive for ASCA (IgA and/or IgG), indicating that anti-GP2 antibodies might be more sensitive in identifying patients with CD. More importantly, the combination of ASCA and anti-GP2 antibodies could synergistically strengthen the power of distinguishing CD from UC. In addition, we noticed that 31.4% of patients with CD were negative for all of the 3 antibodies (ASCA, anti-PAB, and anti-GP2 antibodies), indicating other biomarkers, such as anti-CUZD1 or anti-MZGP2 antibodies,[24,25] might be helpful in CD diagnosis. Further studies on evaluation of these biomarkers are needed.

GP2 are the specific receptors in the exocrine pancreas as well as on M cells of intestinal Peyer's patches (PP).[12,26] Interestingly, M-cell-associated GP2 is suggested to be involved in the interaction between the immune system and intestinal microbiota.[27] Indeed, it has been reported that GP2 can function as an endogenous immunomodulator by modulating both innate and adaptive immune responses.[26–28] Anti-GP2 antibodies are the autoantibodies targeting GP2, and it has been proposed that anti-GP2 antibodies are generated during ileal inflammation, and the inflamed ileal environment contributes to the release of GP2 by M cells and the continual exposure of GP2 to the immune system.[22] Consistent with this assumption, Pavlidis et al reported that CD patients with ileal (L1) or extensive disease (L3) presented higher prevalence of anti-GP2 IgG.[16] We also found anti-GP2 IgG was positively correlated with ileocolonic location of disease (L3). Interestingly, no significant difference on the correlation of anti-GP2 IgG with

TABLE 5. Association of Anti-GP2, ASCA, or PAB Autoantibodies With Disease Characteristics of Patients With Crohn Disease

		anti-GP2 n (*P* Value)				ASCA n (*P* value)				PAB n (*P* value)			
	N	IgA	IgG	IgA or IgG	IgA and IgG	IgA	IgG	IgA or IgG	IgA and IgG	IgA	IgG	IgA or IgG	IgA and IgG
Age at diagnosis													
Below 17 years (A1)	12	5 (0.700)	6 (0.398)	7 (0.738)	4 (0.300)	5 (0.126)	3 (0.011)	5 (0.126)	3 (0.011)	0 (0.307)	1 (0.972)	1 (0.972)	0 (0.307)
Between 17 and 40 years (A2)	16	5 (0.522)	5 (0.347)	8 (0.652)	2 (0.191)	3 (0.402)	0 (0.102)	3 (0.402)	0 (0.102)	1 (0.904)	1 (0.664)	1 (0.664)	1 (0.904)
Above 40 years (A3)	7	3 (0.736)	3 (0.868)	4 (0.870)	2 (0.698)	1 (0.454)	0 (0.380)	1 (0.454)	0 (0.380)	1 (0.288)	1 (0.560)	1 (0.560)	1 (0.288)
Location													
Ileal (L1)	5	2 (0.891)	1 (0.339)	2 (0.503)	1 (0.874)	3 (0.061)	1 (0.339)	3 (0.061)	1 (0.339)	0 (0.566)	0 (0.474)	0 (0.474)	0 (0.566)
Colonic (L2)	7	3 (0.736)	1 (0.128)	3 (0.512)	1 (0.560)	2 (0.852)	0 (0.380)	2 (0.852)	0 (0.380)	1 (0.288)	2 (0.035)	2 (0.035)	1 (0.288)
Ileocolonic (L3)	23	8 (0.700)	12 (0.043)	14 (0.293)	6 (0.543)	4 (0.126)	2 (0.972)	4 (0.126)	2 (0.972)	1 (0.642)	1 (0.229)	1 (0.229)	1 (0.642)
Upper disease, modifier (L4)	3	0 (0.173)	0 (0.147)	0 (0.050)	0 (0.339)	1 (0.761)	0 (0.592)	1 (0.761)	0 (0.592)	0 (0.667)	0 (0.592)	0 (0.592)	0 (0.667)
Behavior													
Nonstricturing, nonpenetrating (B1)	10	3 (0.736)	3 (0.868)	4 (0.870)	2 (0.698)	0 (0.086)	0 (0.380)	0 (0.086)	0 (0.380)	2 (0.003)	2 (0.035)	2 (0.035)	2 (0.003)
Stricturing (B2)	20	7 (0.770)	9 (0.500)	12 (0.448)	4 (0.654)	7 (0.155)	2 (0.737)	7 (0.155)	2 (0.737)	0 (0.098)	1 (0.398)	1 (0.398)	0 (0.098)
Penetrating (B3)	12	5 (0.700)	7 (0.116)	8 (0.302)	4 (0.300)	2 (0.391)	1 (0.972)	2 (0.391)	1 (0.972)	0 (0.307)	0 (0.202)	0 (0.202)	0 (0.307)
Perianal disease (p)	10	4 (0.831)	4 (1.000)	6 (0.679)	2 (0.806)	2 (0.637)	1 (0.854)	2 (0.637)	1(0.854)	0 (0.372)	1 (0.854)	1 (0.854)	0 (0.372)
Extraintestinal manifestations													
Musculoskeletal	2	0 (0.276)	1 (0.774)	1 (0.904)	0 (0.443)	0 (0.406)	0 (0.667)	0 (0.406)	0 (0.667)	0 (0.729)	0 (0.667)	0 (0.667)	0 (0.729)
Dermatologic	9	2 (0.433)	1 (0.074)	2 (0.061)	1 (0.442)	3 (0.400)	0 (0.339)	3 (0.400)	0 (0.339)	0 (0.443)	1 (0.663)	1 (0.663)	0 (0.443)
Ocular	1	0 (0.173)	0 (0.147)	0 (0.050)	0 (0.339)	0 (0.300)	0 (0.592)	0 (0.300)	0 (0.592)	0 (0.667)	0 (0.592)	0 (0.592)	0 (0.667)
Outcome													
Biologic therapy	8	0 (0.012)	4 (0.525)	4 (0.789)	0 (0.084)	3 (0.400)	2 (0.061)	3 (0.400)	2 (0.061)	0 (0.443)	0 (0.339)	0 (0.339)	0 (0.124)
Immunosuppressive therapy	8	2 (0.433)	3 (0.874)	5 (0.608)	0 (0.084)	2 (0.960)	1 (0.663)	2 (0.960)	1 (0.663)	0 (0.443)	0 (0.339)	0 (0.339)	0 (0.443)
Surgery	15	7 (0.327)	5 (0.500)	8 (0.925)	4 (0.654)	2 (0.155)	0 (0.124)	2 (0.155)	0 (0.124)	0 (0.219)	0 (0.124)	0 (0.124)	0 (0.443)

ASCA = anti-saccharomyces cerevisiae antibody; GP2 = glycoprotein 2; PAB = pancreatic autoantibody.

disease location was observed in Czech Republic CD patients.[29] Bogdanos et al revealed the association between anti-GP2 IgG with structuring behavior (B2) and perianal disease in CD patients.[17] However, no significant difference of anti-GP2 IgG with structuring behavior (B2) and perianal disease was observed in our study with Chinese CD patients.

Several limitations in this study need to be pointed out. First, the sample size of our study was small, which may introduce analysis bias. Further studies with large cohorts are needed. Second, the subjects in our study were from a single institution, and these subjects were homogenous Han Chinese ethnic group. A multicenter study with various ethnic groups is needed to evaluate the generalizability of our results.

In summary, our data suggest that anti-GP2 antibodies could serve as a biomarker for distinguishing patients with CD from patients with UC, and the combination of anti-GP2 antibodies with ASCA IgA may improve the predictive power.

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REFERENCES

1. Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med.* 2009;361:2066–2078.
2. Fakhoury M, Negrulj R, Mooranian A, et al. Inflammatory bowel disease: clinical aspects and treatments. *J Inflamm Res.* 2014;7:113–120.
3. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet.* 2007;369:1627–1640.
4. Zhou F, Xia B, Wang F, et al. The prevalence and diagnostic value of perinuclear antineutrophil cytoplasmic antibodies and anti-Saccharomyces cerevisiae antibodies in patients with inflammatory bowel disease in mainland China. *Clin Chim Acta.* 2010;411:1461–1465.
5. Lewis JD. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. *Gastroenterology.* 2011;140:1817–1826.
6. Lawrance IC, Murray K, Hall A, et al. A prospective comparative study of ASCA and pANCA in Chinese and Caucasian IBD patients. *Am J Gastroenterol.* 2004;99:2186–2194.
7. Prideaux L, Kamm MA, De Cruz P, et al. Inflammatory bowel disease serology in Asia and the West. *World J Gastroenterol.* 2013;19:6207–6213.
8. Joossens S, Vermeire S, Van Steen K, et al. Pancreatic autoantibodies in inflammatory bowel disease. *Inflamm Bowel Dis.* 2004;10:771–777.
9. Klebl FH, Bataille F, Huy C, et al. Association of antibodies to exocrine pancreas with subtypes of Crohn's disease. *Eur J Gastroenterol Hepatol.* 2005;17:73–77.
10. Seibold F, Weber P, Jenss H, et al. Antibodies to a trypsin sensitive pancreatic antigen in chronic inflammatory bowel disease: specific markers for a subgroup of patients with Crohn's disease. *Gut.* 1991;32:1192–1197.
11. Roggenbuck D, Hausdorf G, Martinez-Gamboa L, et al. Identification of GP2, the major zymogen granule membrane glycoprotein, as the autoantigen of pancreatic antibodies in Crohn's disease. *Gut.* 2009;58:1620–1628.
12. Hase K, Kawano K, Nochi T, et al. Uptake through glycoprotein 2 of FimH(+) bacteria by M cells initiates mucosal immune response. *Nature.* 2009;462:226–230.
13. Gullberg E, Söderholm JD. Peyer's patches and M cells as potential sites of the inflammatory onset in Crohn's disease. *Ann N Y Acad Sci.* 2006;1072:218–232.
14. Roggenbuck D, Reinhold D, Wex T, et al. Autoantibodies to GP2, the major zymogen granule membrane glycoprotein, are new markers in Crohn's disease. *Clin Chim Acta.* 2011;412:718–724.
15. Op De Beéck K, Vermeire S, Rutgeerts P, et al. Antibodies to GP2, the major zymogen granule membrane glycoprotein, in inflammatory bowel diseases. *Gut.* 2012;61:162–164.
16. Pavlidis P, Romanidou O, Roggenbuck D, et al. Ileal inflammation may trigger the development of GP2-specific pancreatic autoantibodies in patients with Crohn's disease. *Clin Dev Immunol.* 2012;2012:640835.
17. Bogdanos DP, Roggenbuck D, Reinhold D, et al. Pancreatic-specific autoantibodies to glycoprotein 2 mirror disease location and behaviour in younger patients with Crohn's disease. *BMC Gastroenterol.* 2012;12:102.
18. Pavlidis P, Forbes A, Bogdanos DP. Antibodies to glycoprotein 2 (GP2) in patients with inflammatory bowel diseases from UK. *Clin Chim Acta.* 2011;412:1163–1164.
19. Lennard-Jones JE. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl.* 1989;170:2–6.
20. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol.* 2005;19:5A–36A.
21. Roggenbuck D, Reinhold D, Werner L, et al. Glycoprotein 2 antibodies in Crohn's disease. *Adv Clin Chem.* 2013;60:187–208.
22. Bogdanos DP, Rigopoulou EI, Smyk DS, et al. Diagnostic value, clinical utility and pathogenic significance of reactivity to the molecular targets of Crohn's disease specific-pancreatic autoantibodies. *Autoimmun Rev.* 2011;11:143–148.
23. Reese GE, Constantinides VA, Simillis C, et al. Diagnostic precision of anti-Saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol.* 2006;101:2410–2422.
24. Komorowski L, Teegen B, Probst C, et al. Autoantibodies against exocrine pancreas in Crohn's disease are directed against two antigens: the glycoproteins CUZD1 and GP2. *J Crohns Colitis.* 2013;7:780–790.
25. Pavlidis P, Shums Z, Koutsoumpas AL, et al. Diagnostic and clinical significance of Crohn's disease-specific anti-MZGP2 pancreatic antibodies by a novel ELISA. *Clin Chim Acta.* 2015;441:176–181.
26. Roggenbuck D, Reinhold D, Schierack P, et al. Crohn's disease specific pancreatic antibodies: clinical and pathophysiological challenges. *Clin Chem Lab Med.* 2014;52:483–494.
27. Schierack P, Rödiger S, Kolenda R, et al. Species-specific and pathotype-specific binding of bacteria to zymogen granule membrane glycoprotein 2 (GP2). *Gut.* 2015;64:517–519.
28. Werner L, Paclik D, Fritz C, et al. Identification of pancreatic glycoprotein 2 as an endogenous immunomodulator of innate and adaptive immune responses. *J Immunol.* 2012;189:2774–2783.
29. Kohoutova D, Drahosova M, Moravkova P, et al. Anti-outer membrane protein C and anti-glycoprotein 2 antibodies in inflammatory bowel disease and their association with complicated forms of Crohn's disease. *BMC Gastroenterol.* 2014;14:190.