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Original Article

Prospective value of serologic antibodies in Chinese patients with inflammation bowel disease

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Abstract: Serological antibodies have been widely applied for diagnostics and potential clinical phenotypes of inflammatory bowel disease (IBD). The aim of this prospective study was to assess the clinical value of multiple antibodies, including anti-neutrophil cytoplasmic antibody (ANCA), anti-Saccharomyces cerevisiae antibody (ASCA), Escherichia coli outer membrane porin C antibody (anti-OmpC), anti-glycoprotein 2 antibody (GP2), and goblet cell antibody (GAB), by means of ELISA testing, in Chinese patients with inflammation bowel disease. The study investigated 130 Crohn's disease (CD) and 120 ulcerative colitis (UC) patients, compared with 80 healthy subjects. IBD phenotype was classified according to the Montreal classification. A combination of ASCA IgA and IgG had a sensitivity of 58.5% in CD. ANCA showed a sensitivity of 46.7% for UC. Prevalence of anti-OmpC (IgA) and GP2 (IgG) were significantly higher in CD patients than UC patients. Positivity of GAB (IgG) was higher in UC than in CD. Hence, a combination of ASCA/anti-OmpC IgA/GP2 (IgG) showed the best specificity in distinguishing CD from UC. In CD, ASCA IgG, anti-OmpC IgA and GP2 (IgG) were identified to be greater in complicated CD patients (B2/B3) than in simple phenotype (B1) patients. Furthermore, positivity of ANCA was associated with disease activity of UC. GAB (IgG) positivity implied extensive location in UC. Even with a relatively low prevalence, this study suggests certain serological antibodies as biomarkers for disease phenotypes and behavior in IBD.

Keywords: Inflammatory bowel disease, diagnostic antibodies, ANCA, ASCA, Anti-OmpC, Anti-glycoprotein 2 antibody, Goblet cell antibody

Introduction

Inflammatory bowel diseases (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), are chronic and relapsing gastrointestinal disorders with an unclear etiology. Incidence and prevalence in Asia have rapidly increased over the past ten years [1]. The current estimated average incidence of IBD, UC, and CD in mainland China is 1.80, 1.33, and 0.46/1,000,000 persons, respectively [2].

Current pathogenetic theories have suggested that IBD results from a dysregulated immune response to bacterial microorganisms in genetic sensibility individuals [3]. These interactions maybe play a vital role in phenotypes, progression, and complications of IBD [4]. A wide range of antibodies against self-antigens and microbial have been reported in IBD. However, their clinical potential in diagnosing and predicting disease behavior, as well as treatment outcomes, of IBD remains unconfirmed.

The most extensive relevant antibodies are ASCA (anti-Saccharomyces cerevisiae antibody) and ANCA (anti-neutrophil cytoplasmic antibody), first discovered in 1990. However, a large panel of serum antibodies have increasingly been identified to illustrate whether the novel biomarkers are superior or whether they augment value to conventional biomarkers [5].

Current emerging novel markers include Escherichia coli outer membrane porin C antibody (anti-OmpC), exocrine pancreatic antibody (PAB), intestinal goblet cells antibody (GAB), anti-glycan antibody, bacterial flagellin CBir1 antibody (anti-CBir1), and pseudomonas fluorescens-associated sequence I2 antibody (anti-I2) [6].

Anti-OmpC antibody is aimed at proteins derived from a bacterial outer membrane of *Escherichia coli* [7]. Baseline positivity for anti-OmpC antibody in IBD has been described in a few studies and considered as a biomarker for risk for surgery [8].

Pancreatic autoantibodies (PAB) have served as specific biomarkers for CD patients [9]. GP2 is a highly glycosylated protein. Excitingly, anti-glycoprotein 2 antibody (GP2) has been recently recognized as the major target of CD-specific PAB [10]. GP-2 is expressed on the epithelium of intestinal Peyer's patches. It, therefore, might play a vital role in CD immunity [11].

Autoantibody against goblet cell (GAB) has been described in patients with IBD and other autoimmune diseases [12, 13]. However, the significance of GAB in IBD patients remains unclear.

Overall, most studies concerning serological antibodies have been performed abroad. Studies have identified that incidence and prevalence of serum antibodies differ modestly between regions or ethnic groups. Research of serological biomarkers concerning the clinical value of IBD in China has been carried out to a certain extent. However, there are no identical sufficiency relevant reports. Thus, consistent research is necessary to draw guidance that could be widely applied to all regions of China.

The present study aimed to investigate the clinical potential of the abovementioned serological antibodies (ANCA, ASCA, anti-OmpC, GP2, and GAB) in a large cohort of Chinese patients with IBD, examining their value for diagnosis of IBD, disease phenotypes, and long-term disease course.

Materials and methods

Patients

This prospective study was conducted among 250 IBD patients (130 CD patients and 120 UC patients) treated in the Department of Gastroenterology, the First Affiliated Hospital of Soochow University and the North District of the Affiliated Suzhou Hospital of Nanjing Medical University (Jiangsu, China), between May 2017 and May 2018. Age-and gender-matched healthy individuals (a total of 80), from the Physical Examination Department of the North District of the Affiliated Suzhou Hospital of Nanjing Medical University, served as the control group. Diagnosis of IBD was established by clinical manifestations, radiological findings, and endoscopic and histological criteria.

Sex, age, smoking history, and duration were collected by experienced clinicians, reviewing and completing medical questionnaires. IBD phenotype (duration, location, behavior, severity) was classified according to the Montreal classification. IBD patients that should be conformed to a confirmed diagnosis for more than 1 year were recorded.

A total of 2 mL venous whole blood obtained, prospectively, for ANCA, ASCA, anti-OmpC, GP2, and GAB. All samples were separated at a speed of 1,000 × g for 15 minutes, within 2 hours, and the upper serum was stored at -80°C before use.

Written informed consent was obtained from all IBD patients and controls, respectively. The present study protocol was approved by the Ethics Committees of the First Hospital Affiliated to Soochow University.

Serologic antibody detection

Samples were tested at the Digestive and Nutrition Center (Suzhou, China). Serological antibody detection was investigated using a standardized enzyme-linked immunosorbent assay, testing the five antibodies (ELISA kit from Heng Yun Technology Co. Ltd. Shanghai, China). The panel detected the five antibodies: ANCA, ASCA (IgA and IgG), anti-OmpC (IgA), GP2 (IgG), and GAB (IgG). Values were assessed as ELISA units (EU/mL). Serum antibodies were defined as positive if they exceed the following values: ANCA>20 U/mL, ASCA (IgA>20 U/mL and IgG>20 U/mL), anti-OmpC (IgA>16 U/mL), GP2 (IgG>20 U/mL), and GAB (IgG>15 U/mL). Positive reference ranges were defined according to R. S. Choung et al. [14]. Testing was investigated by researchers blinded to all patient samples.

Statistical analysis

Statistical analysis wa s performed using SPSS 19.0 (SPSS Inc, IBM, USA) for windows and GraphPad Prism 7.04 (GraphPad Software, San Diego, CA). Continuous variables are expressed as mean ± standard deviation. Positive predictive values (PPV) and negative predictive values

Table 1. Clinical characteristics of patients and controls

Characteristic	CD (n=130)	UC (n=120)	HC (n=80)
Male/female	82/48	64/56	46/34
Mean age (years)	40.8±12.8	45.4±13.7	42.9±14.9
Range (years)	17-70	22-75	20-70
Current smoker n (%)	23.1	13.3	42.5
Disease duration (years)	5.1±3.1	5.1±3.4	
Disease location: CD, n (%)			
Terminal ileum, L1	60 (46.2)		
Colon, L2	30 (23.1)		
Ileocolon, L3	40 (30.8)		
Disease activity: CDAI, n (%)			
Mild	38 (29.2)		
Moderate	56 (43.1)		
Severe	36 (27.7)		
Disease behavior: CD, n (%)			
Non-stricture and penetrating, B1	70 (53.8)		
Stricture, B2	44 (33.8)		
Penetrating, B3	16 (12.3)		
Disease location: UC, n (%)			
Rectum, E1		40 (33.3)	
Left side, E2		56 (46.7)	
Extensive, E3		24 (20.0)	
Disease severity: n (%)			
Mild		38 (31.7)	
Moderate		54 (45.0)	
Severe		28 (23.3)	

HC: Healthy control, CD: Crohn's disease, UC: ulcerative colitis.

(NPV), as well as sensitivity and specificity of ANCA, ASCA (IgA and IgG), anti-OmpC (IgA), GP2 (IgG), and GAB (IgG), were calculated to distinguish the different groups. Association between serum antibody positivity and study groups was determined by Chi^2 test or Fisher's exact test. For comparison of serum antibodies, Mann-Whitney U-test and independent t-tests were performed. Association between serum antibodies titers and disease phenotypes was assessed by Spearman's correlation assay. Univariate and multivariate logistic regression were used to identify risk factors. Comparisons were adjusted by sex and age. *P* values <0.05 indicate statistical significance.

Results

Characteristics of the patients

Baseline characteristics of the IBD patients and controls are summarized in **Table 1**. A total of 330 subjects were studied. There were 130 patients with CD (82 males [63.1%], mean age 40.8 years), 120 patients with UC (64 males [53.3%], mean age 45.4 years), and 80 healthy controls (46 males [57.5%], mean age 42.9 years). The disease duration at the study point was (5.1± 3.1) years for CD and (5.1± 3.4) years for UC, respectively. Of the cohort, 46.2% of the CD patients were terminal ileum (L1), 23.1% were colon (L2), and 30.8% were ileocolon (L3). According to the Montreal classification, 60 out of the 130 patients [46.2%] had a complicated disease behavior (B2 or B3). Around 33.3% of the UC patients had rectum location (E1), 46.7% had left side location (E2), and 20.0% had extensive colitis (E3). CD activity was classified as mild, moderate, or severe, according to the CD Activity Index (CDAI). The severity of UC patients was measured as mild, moderate, and severe.

Presence of serological antibodies

Serum antibody patterns for the five antibodies are shown in **Table 2**. ANCA was positive in 46.7% of UC patients, compared with 29.2% of CD patients (P=0.04) and only 5.0% of the healthy control group (P<0.001). Presence of ASCA-IgA and IgG in CD (32.3%, 46.2%) was markedly higher than that observed in UC (13.3%, 25.0%) (P=0.01, P=0.01), higher than that in healthy control groups (2.5%, 2.5%) (P<0.001). In addition, prevalence of anti-OmpC IgA was significantly higher in CD than in UC groups (44.6% VS 26.7%, P=0.04). Of patients with CD, 46.2% and 10.8% were positive for GP2-IgG and GAB-IgG, respectively. In UC patients, rates were 15.0% and 25.0%. Obviously, prevalence of GAB-IgG was higher in UC than in CD groups (P=0.04). Moreover, 10 of 65 (15.4%) of the CD patients were negative for all antibodies and 1 of 65 (1.5%) were positive for all antibodies. Prevalence of all negative antibodies in

Table 2. Prevalence of six serological antibodies in IBD and controls

Antibody	CD (n=130)	UC (n=120)	HC (n=80)
ANCA (%)	38 (29.2)	56 (46.7)	4 (5.0)
ASCA IgA (%)	42 (32.3)	16 (13.3)	2 (2.5)
ASCA IgG (%)	60 (46.2)	30 (25.0)	2 (2.5)
Anti-OmpC IgA (%)	58 (44.6)	32 (26.7)	4 (5.0)
GP2 (IgG) (%)	60 (46.2)	18 (15.0)	4 (5.0)
GAB (IgG) (%)	14 (10.8)	30 (25.0)	2 (2.5)

HC: Healthy control, CD: Crohn's disease, UC: ulcerative colitis, ASCA: anti-Saccharomyces cerevisiae antibody, ANCA: antineutrophil cytoplasmic antibody, anti-OmpC: outer membrane porin C antibody of Escherichia coli, GP2: glycoprotein 2, GAB: goblet cell antibody.

Table 3. Predictive power of serum antibodies for CD and UC patients

Antibody	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CD vs HC				
ANCA	29.3	95.0	90.5	45.2
ASCA IgA	32.3	97.5	95.5	47.0
ASCA IgG	46.2	97.5	96.7	57.7
Anti-OmpC IgA	44.6	95.0	93.5	51.4
GP2 (IgG)	46.2	95.0	93.8	52.8
GAB (IgG)	10.8	97.5	87.5	40.2
UC vs HC				
ANCA	46.7	95.0	93.3	54.5
ASCA IgA	13.3	97.5	88.9	42.9
ASCA IgG	25.0	97.5	93.7	46.4
Anti-OmpC IgA	26.7	95.0	88.9	46.3
GP2 (IgG)	15.0	95.0	81.8	42.7
GAB (IgG)	25.0	97.5	93.8	46.4

HC: Healthy control, CD: Crohn's disease, UC: ulcerative colitis, ASCA: anti-Saccharomyces cerevisiae antibody, ANCA: antineutrophil cytoplasmic antibody, anti-OmpC: outer membrane porin C antibody of Escherichia coli, GP2: glycoprotein 2, GAB: goblet cell antibody. PPV, positive predictive value; NPV, negative predictive value.

UC patients was 13 of 60 (21.7%), while 0 of 60 (0%) were positive for all antibodies.

Diagnostic power of a single antibody or combined antibodies for CD and UC

Sensitivity, specificity, PPV, and NPV for the five antibodies are listed in **Table 3**. When detected alone, sensitivity of ASCA-IgA and ASCA-IgG in CD patients was 32.3% and 46.2%, while specificity was 97.5% and 97.5%. PPV was 95.5% and 96.7%, respectively. Sensitivity of anti-OmpC and GP2 IgG in CD patients was 44.6% and 46.2%. Specificity was 95.0% and 95.0%, respectively. In UC patients, sensitivity, specificity, PPV, and NPV of ANCA were 46.7%, 95.0%, 93.3%, and 54.5%, respectively. Sensitivity of anti-OmpC and GAB IgG was 26.7% and 25.0%, respectively, while specificity was 95.0% and 97.5%.

The diagnostic value of the combined four CD-associated serological antibodies is shown in **Table 4**. ASCA-IgG sensitivity for CD was 46.2%. When combined with ASCA-IgA, sensitivity increased to 58.5%. The specificity value of ASCA-IgG/IgA was 95.0%. When ASCA was combined with anti-OmpC IgA, the sensitivity of ASCA and anti-OmpC IgA in CD patients increased to 73.8%, while the specificity value slightly decreased to 90.0%. The combination of ASCA and GP2 IgG had a similar sensitivity and specificity in CD patients (70.8%, 90.0%). When the combined serological antibodies panel (ASCA-IgG/ASCA-IgA/Anti-OmpC-IgA/GP2-IgG) was tested, overall sensitivity and were specificity 80.0% and 85.0% in CD. Hence, it was concluded that the diagnostic value of combined ASCA-IgG/ASCA-IgA/Anti-OmpC-IgA/GP2-IgG was the highest.

When combining two mainly expressed serological antibodies in UC (ANCA/GAB-IgG), the sensitivity increased to 56.7%. Specificity was 92.5%

Diagnostic power of serological antibodies in distinguishing CD from UC

From the abovementioned dates, this study identified that ASCA, anti-OmpC IgA, and GP2-IgG were unique antibodies for CD. ANCA and GAB-IgG were unique antibodies for UC. Single or combined serological antibodies were tested for distinguishing CD from UC (**Tables 5**, **6**). ASCA IgG, anti-OmpC IgA, and GP2 IgG had similar sensibility (44.6% VS 46.2% VS 44.6%, P>0.99) and the specificity was 65.0%, 73.7%, 85.0%, respectively. Combination of ASCA, anti-OmpC IgA, and GP2 IgG had the highest sensibility value of 80%, with a specificity of 46.7% in differentiating between CD and UC.

The sensitivity of ANCA in distinguishing UC and CD was 46.7%, while the specificity was 70.8%. When combining ANCA with GAB IgG, the sensi-

Table 4. Combined analysis of serological antibodies for CD or UC

Antibody	Comparison	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ASCA IgA/ASCA IgG	CD vs HC	58.5	95.0	95.0	63.3
ASCA/Anti-OmpC IgA	CD vs HC	73.8	90.0	92.3	67.9
ASCA/GP2 (IgG)	CD vs HC	70.8	90.0	92.0	65.5
ASCA/Anti-OmpC IgA/GP2 (IgG)	CD vs HC	80.0	85.0	89.7	72.3
ANCA/GAB (IgG)	UC vs HC	56.7	92.5	91.9	58.3

HC: Healthy control, CD: Crohn's disease, UC: ulcerative colitis, ASCA: anti-Saccharomyces cerevisiae antibody, ANCA: antineutrophil cytoplasmic antibody, anti-OmpC: outer membrane porin C antibody of Escherichia coli, GP2: glycoprotein 2, GAB: goblet cell antibody. PPV, positive predictive value; NPV, negative predictive value.

Table 5. Prospective potential of serum antibodies in distinguishing CD patients from UC patients

Antibody	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ANCA	46.7	70.8	59.6	59.0
ASCA IgA	23.3	86.7	72.4	54.2
ASCA IgG	46.2	65.0	66.7	56.3
Anti-OmpC IgA	44.6	73.7	64.4	55.0
GP2 (IgG)	46.2	85.0	76.9	59.3
GAB (IgG)	25.0	89.2	68.2	56.3

CD: Crohn's disease, UC: ulcerative colitis, ASCA: anti-Saccharomyces cerevisiae antibody, ANCA: antineutrophil cytoplasmic antibody, anti-OmpC: outer membrane porin C antibody of Escherichia coli, GP2: glycoprotein 2, GAB: goblet cell antibody. PPV, positive predictive value; NPV, negative predictive value.

tivity in differential diagnosis of UC and CD increased to 56.7%, while the specificity decreased to 64.6%.

Important values of OmpC IgA and GP2 IgG were observed in that ASCA negativity of CD may be positive for OmpC IgA and GP2 IgG. The dates showed that the two identified between ASCA negativity of CD and UC. However, anti-OmpC and GP2 IgG cannot differentiate between ANCA-positivity of CD and UC.

The number of serological positivity of antibodies in the five-antibody panel between CD and UC (**Figure 1**) was calculated. Results indicate that the prevalence of patients that had ≥3 serological positive antibodies in CD was higher than in UC. Results also suggest that CD patients were more inclined to have ≥3 serological antibodies.

Association between serum antibodies and disease phenotype

In CD patients, ASCA IgG positivity was commonly found in terminal ileum (L1), compared with colon (L2) and ileocolon (L3) (63.3% vs 26.7% or 40.0%, respectively, P=0.048) (**Figure 2**). Moreover, the higher occurrence of ASCA IgG was identified in complicated CD patients (stricturing or penetrating, B2/B3), compared with simple phenotype (non-stricturing and penetrating, B1) (70.0% vs 25.7%, P<0.001) (**Figure 3**). When adjusted for sex, age, and smoking, bivariate logistic analysis identified that ASCA IgG positivity was independently correlated with increased risk of complicated behavior (OR 6.74, 95% CI: 2.27-20.14, P=0.001).

However, in the positive titers of ASCA IgG aspect, there were no significant differences among disease location, activity, and behavior. Similarly, ASCA IgA positivity did not show significant differences in location or disease behavior of CD.

In CD with anti-OmpC IgA positivity, there were no significant differences among disease location and activity. In addition, no association was found between the titer of anti-OmpC IgA and disease phenotype. However, incidence of anti-OmpC IgA positivity was significantly higher in B2/B3 phenotype, compared with B1 (60.0% vs 31.4%, respectively, P=0.02) (**Figure 3**). After adjusting for clinical factors, logistic analysis showed that anti-OmpC IgA was also correlated with increased risk of complicated behavior (OR 3.27, 95% CI: 1.18-9.09, P=0.02).

The presence of GP2 IgG positivity was significantly higher in severe activity than in mild and moderate activity in patients with CD (72.2% vs 36.8%, 35.7%, respectively, P=0.03) (**Figure 4**). Seropositivity of anti-GP2 IgG in CD patients with B2/B3 phenotype was higher than in B1 behavior (66.7% vs 28.6%, respectively, P<0.001) (**Figure 3**). Additionally, logistic regression analysis was used to identify independent risks (OR 5.0, 95% CI: 1.74-14.37, P< 0.001).

Table 6. Combined analysis of serological antibodies in distinguishing CD from UC

Antibody	Comparison	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ASCA IgA/ASCA IgG	CD vs UC	58.5	66.7	65.5	59.7
ASCA/Anti-OmpC IgA/GP2 (IgG)	CD vs UC	80.0	46.7	50.0	68.3
ANCA/GAB (IgG)	UC vs CD	56.7	64.6	59.6	61.8

CD: Crohn's disease, UC: ulcerative colitis, ASCA: anti-Saccharomyces cerevisiae antibody, ANCA: antineutrophil cytoplasmic antibody, anti-OmpC: outer membrane porin C antibody of Escherichia coli, GP2: glycoprotein 2, GAB: goblet cell antibody. PPV, positive predictive value; NPV, negative predictive value.

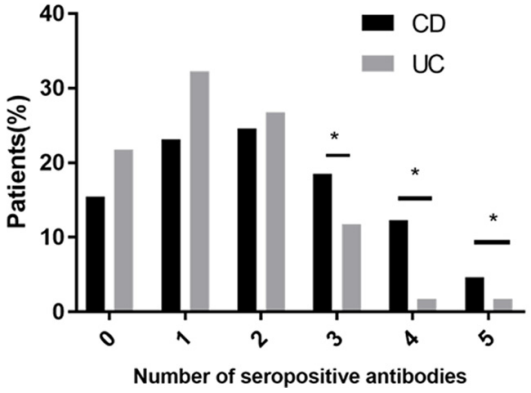

Figure 1. The number of serological positivity of antibodies, CD vs UC. *P<0.05. CD: Crohn's disease, UC: ulcerative colitis.

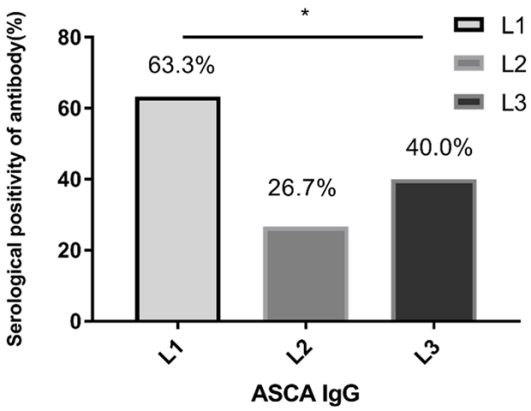

Figure 2. Serological positivity of ASCA IgG in different locations of CD. *P<0.05. ASCA: anti-Saccharomyces cerevisiae antibody. L1: terminal ileum, L2: colon, L3: ileocolon.

GAB IgG showed no association with disease location, activity, and behavior.

In UC patients, the positivity presence of ANCA was obviously higher in severe patients than in mild and moderate patients (78.6% vs 36.8%, 37.0%, P=0.02). The latter two were compared, with no differences indicated (**Figure 4**). The titers of ANCA positivity did not indicate any difference in disease activity.

The GAB IgG positive presence in UC patients was significantly higher than that in CD patients (25.0% VS 10.8%, P=0.04). Moreover, its positivity was obviously shown more in extensive locations than in rectum and left side lesions (66.7% vs 10.0%, 10.7%, P=0.001) (**Figure 5**). There were no differences between rectum and left side lesions.

ASCA IgA, anti-OmpC IgA, and GP2 IgG showed no association with disease location, activity, and behavior in UC. Serum antibodies showed no association with disease duration.

Combined positive serological antibodies were calculated to evaluate CD phenotypes (**Figure 6**). When the number of positive antibodies was 0 or 1, prevalence of simple phenotype in CD behavior was significantly higher than the complicated phenotype. When two antibodies were positive, there were no obvious differences between the simple and complicated phenotype. When there were 3 or more positive serological antibodies, it was more likely to be the complicated phenotype of CD behavior.

Discussion

The current study conducted an extensive serological antibodies test, including classic (ANCA, ASCA IgA, ASCA IgG) and newly discovered antibodies (Anti-OmpC IgA, GP2 IgG, GAB IgG), aiming to identify the clinical power in Chinese IBD patients.

ASCA has been widely considered as the most useful serological antibody for CD, including IgA and IgG [15]. ANCA was identified as an important antibody for UC in 1990. Previous researchers have identified that the serum positivity of ASCA was approximately 39-70% in CD patients and 10-15% in UC patients, respectively [16]. In the present study, the serological positivity of ASCA IgA and IgG was 32.3% and 45.0%, respectively, higher than that in UC and control groups. Similarly, the seroprevalence of ANCA

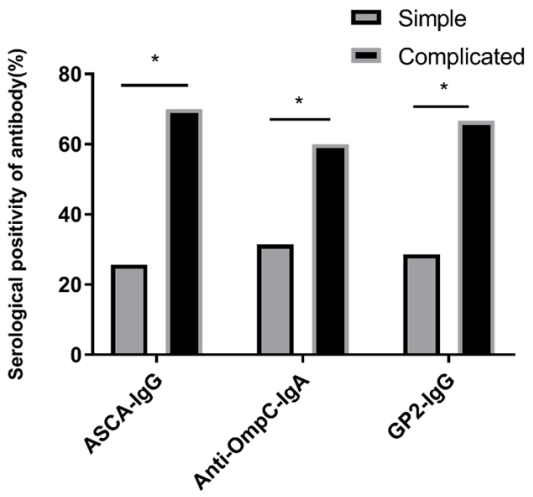

Figure 3. Serological positivity of antibodies in CD behaviors. *P<0.05. ASCA: anti-Saccharomyces cerevisiae antibody. anti-OmpC: outer membrane porin C antibody of Escherichia coli, GP2: glycoprotein 2.

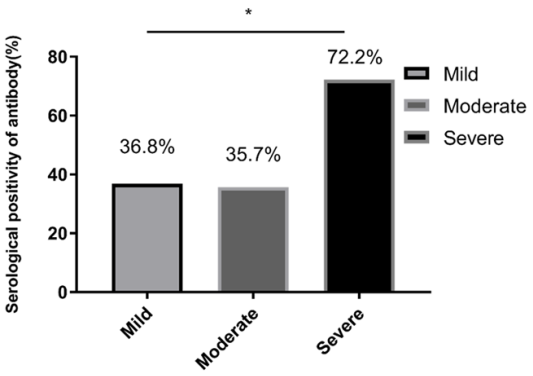

Figure 4. Serological positivity of GP2 IgG in severe activity of CD. *P<0.05. GP2: glycoprotein 2.

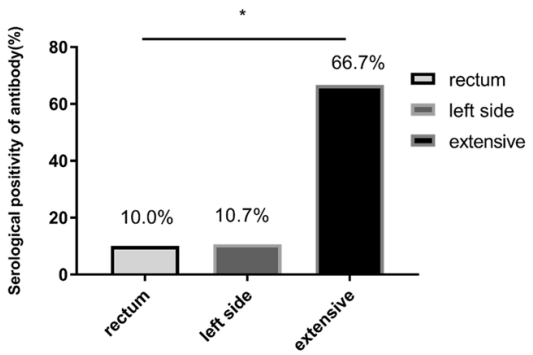

Figure 5. Serological positivity of GAB IgG in UC location. *P<0.05. GAB: goblet cell antibody.

in UC was 46.7%, significantly higher than that in CD patients. These results were consistent with studies performed abroad [15] and in China [17].

Anti-OmpC, GP2, and GAB are newly discovered serological biomarkers. According to previous studies, the seropositivity of anti-OmpC ranged from 20-55% in CD patients, while the prevalence was 5-11% in UC patients [18]. Later studies identified that anti-OmpC IgA had a more common expression in CD [19]. Hence, anti-OmpC IgA was chosen for detection. Interestingly, this study identified that the seropositivity of OmpC IgA was 44.6% in CD and 26.7% in UC, similar to ASCA IgG. Kotlowski et al. may have explained these results by combining the specific adherent *Escherichia coli* with ileal mucosa in CD patients [20].

Anti-GP2 is a specific receptor present in the intestinal Peyer's patches, considered a CD inflammation hotbed [21]. The relationship between anti-GP2 IgA and IgG has been mentioned in CD [19]. The current study identified the value of seropositivity of anti-GP2 IgG among CD, UC, and controls. There was a slight increase in UC patients, compared with healthy controls, in accord with previous data [22]. Higher occurrence of anti-GP2 IgG in CD patients was observed, approximately 46.2%. Moreover, sensitivity and specificity of anti-GP2 IgG were better than anti-OmpC IgA, which suggests that the diagnosis value of anti-GP2 IgG might be superior to anti-OmpC IgA.

The prevalence of goblet cell antibody in IBD (0-30%) has great variation [23]. In the present study, seropositivity of GAB was significantly increased in UC, compared to CD and controls (UC, 25.0%; CD, 10.8%; control: 2.5%). Furthermore, GAB was correlated with the location of UC.

Combined testing has more differential power in identifying IBD subtypes. The ASCA/Anti-OmpC IgA/GP2 IgG profile was the best combination in distinguishing CD from UC (sensitivity 80.0%, specificity 46.7%).

Previous results have shown that the number of serological positivity of antibodies is the better diagnostic value for IBD [24]. The current study revealed that serological positive antibodies ≥3 were more inclined to be diagnosed as CD rather than UC.

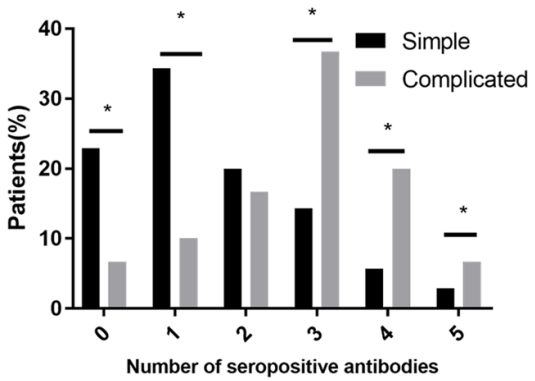

Figure 6. The number of serological positivity of antibodies in CD behaviors. *P<0.05. CD: Crohn's disease, UC: ulcerative colitis.

In this study, anti-OmpC IgA and anti-GP2 IgG helped to distinguish from ASCA-negative CD and ASCA-negative UC, partly in accordance with a previous study [25]. Neither anti-OmpC IgA nor anti-GP2 IgG was able to distinguish ANCA-positive CD from UC. Moreover, GAB was not able to distinguish ANCA-negative UC, in accordance with a western study.

ASCA-IgG has an association with CD location. Its positivity has been commonly found in terminal ileum. Moreover, higher prevalence of ASCA IgG has been identified in complicated CD patients (stricture or penetrating). However, ASCA IgA positivity did not show significant differences in location or disease behavior of CD. This point may be partly different from previous studies [26]. The ASCA titers did not show any differences in disease locations or disease severity.

The previous study identified that anti-OmpC was significantly high in ileocolonic location of CD [19]. Present findings, however, were not consistent with this result. Serum positivity of anti-OmpC IgA was associated with complicated forms of CD. In addition, no association was found between the titer of anti-OmpC IgA and disease phenotype.

Roggenbuck D et al. demonstrated that anti-GP2 is interrelated with CD [27]. An association between anti-GP2 IgG with complicated forms of CD (B2/B3) has been revealed by Bogdanos et al. [28]. The current study is in accord with this result: seropositivity of anti-GP2 IgG in CD patients with B2/B3 phenotype was higher than in B1 behavior. Moreover, the presence of GP2 IgG positivity was significantly higher in moderate and severe activity than in mild activity in patients with CD. This has not been found before. Considering GP2 receptors localized in small bowels, it was assumed that the seropositivity of anti-GP2 might be associated with ileal CD [28]. However, no association was found between GP2 IgG and disease location.

Logistic regression also revealed that ASCA IgG positivity may be the most independently increased risk for CD behavior. Above all, ASCA, anti-OmpC IgA, and Anti-GP2 seemed to be independent factors for disease phenotype.

Brita Ardesjo et al. identified that goblet cell immunoreactivity of IBD sera is associated with regions of gastrointestinal tract [12]. The current study confirmed that GAB IgG positivity is obviously higher in extensive locations than in rectum and left side lesions. However, the number of GAB IgG positivity in UC might not be large enough to demonstrate an affirmative conclusion.

Combined positive serological antibodies indicated that, when the number of positive antibodies were 0 or 1, CD patients were inclined to be simple phenotype in disease behavior. When there were 3 or more positive serological antibodies, it was more likely to be complicated phenotype of CD behavior. This result has not been found previously. Moreover, there was no relationship found between the titer of antibody and IBD phenotype in this study.

Certain limitations in the current study should be noted. First, the number of subjects was not large enough, possibly leading to data deviation. A more diverse population is necessary to make conclusions more applicable. Second, the current research was a retrospective rather than a prospective study. The relationship between serological antibodies and the predictive value of antibody treatment was not traced. Thus, further research is necessary.

In conclusion, ANCA, ASCA, anti-OmpC IgA, GP2 IgG, and GAB IgG are serviceable biomarkers for IBD. Combined with disease phenotype, to some extent, these markers may guide clinical decisions. More understanding concerning serum antibodies will provide novel sight into the pathophysiology of IBD.

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Disclosure of conflict of interest

None.

Abbreviations

CD, Crohn's disease; UC, ulcerative colitis; ASCA, anti-Saccharomyces cerevisiae antibody; ANCA, antineutrophil cytoplasmic antibody; anti-OmpC, outer membrane porin C antibody of Escherichia coli; GP2, glycoprotein 2; GAB, goblet cell antibody; PPV, positive predictive value; NPV, negative predictive value.

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References

[1] Kaplan GG. The global burden of IBD: from 2015 to 2025. Nat Rev Gastroenterol Hepatol 2015; 12: 720-727.

[2] Cui G and Yuan A. A systematic review of epidemiology and risk factors associated with chinese inflammatory bowel disease. Front Med (Lausanne) 2018; 5: 183.

[3] Xavier RJ and Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007; 448: 427-434.

[4] De Souza HS and Fiocchi C. Immunopathogenesis of IBD: current state of the art. Nat Rev Gastroenterol Hepatol 2016; 13: 13-27.

[5] Papp M and Lakatos PL. Serological studies in inflammatory bowel disease: how important are they? Curr Opin Gastroenterol 2014; 30: 359-364.

[6] Kovacs M, Muller KE, Papp M, Lakatos PL, Csondes M and Veres G. New serological markers in pediatric patients with inflammatory bowel disease. World J Gastroenterol 2014; 20: 4873-4882.

[7] Liu YF, Yan JJ, Lei HY, Teng CH, Wang MC, Tseng CC and Wu JJ. Loss of outer membrane protein C in escherichia coli contributes to both antibiotic resistance and escaping antibody-dependent bactericidal activity. Infect Immun 2012; 80: 1815-1822.

[8] Hamilton AL, Kamm MA, De Cruz P, Wright EK, Selvaraj F, Princen F, Gorelik A, Liew D, Lawrance IC, Andrews JM, Bampton PA, Sparrow MP, Florin TH, Gibson PR, Debinski H, Gearry RB, Macrae FA, Leong RW, Kronborg I, Radford-Smith G, Selby W, Bell SJ, Brown SJ and Connell WR. Serologic antibodies in relation to outcome in postoperative Crohn's disease. J Gastroenterol Hepatol 2017; 32: 1195-1203.

[9] Michaels MA, Jendrek ST, Korf T, Nitzsche T, Teegen B, Komorowski L, Derer S, Schroder T, Baer F, Lehnert H, Buning J, Fellerman K and Sina C. Pancreatic autoantibodies against CUZD1 and GP2 are associated with distinct clinical phenotypes of Crohn's disease. Inflamm Bowel Dis 2015; 21: 2864-2872.

[10] Zhang S, Wu Z, Luo J, Ding X, Hu C, Li P, Deng C, Zhang F, Qian J and Li Y. Diagnostic potential of zymogen granule glycoprotein 2 antibodies as serologic biomarkers in chinese patients with crohn disease. Medicine (Baltimore) 2015; 94: e1654.

[11] Gullberg E and Soderholm 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.

[12] Ardesjo B, Portela-Gomes GM, Rorsman F, Gerdin E, Loof L, Grimelius L, Kampe O and Ekwall O. Immunoreactivity against goblet cells in patients with inflammatory bowel disease. Inflamm Bowel Dis 2008; 14: 652-661.

[13] Hori K, Fukuda Y, Tomita T, Kosaka T, Tamura K, Nishigami T, Kubota A and Shimoyama T. Intestinal goblet cell autoantibody associated enteropathy. J Clin Pathol 2003; 56: 629-630.

[14] Choung RS, Princen F, Stockfisch TP, Torres J, Maue AC, Porter CK, Leon F, De Vroey B, Singh S, Riddle MS, Murray JA and Colombel JF. Serologic microbial associated markers can predict Crohn's disease behaviour years before disease diagnosis. Aliment Pharmacol Ther 2016; 43: 1300-1310.

[15] Reese GE, Constantinides VA, Simillis C, Darzi AW, Orchard TR, Fazio VW and Tekkis PP. Diagnostic precision of anti-saccharomyces cerevisiae antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. Am J Gastroenterol 2006; 101: 2410-2422.

[16] Peyrin-Biroulet L, Standaert-Vitse A, Branche J and Chamaillard M. IBD serological panels: facts and perspectives. Inflamm Bowel Dis 2007; 13: 1561-1566.

[17] Zhou F, Xia B, Wang F, Shrestha UK, Chen M, Wang H, Shi X, Chen Z and Li J. 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.
[18] Zholudev A, Zurakowski D, Young W, Leichtner A and Bousvaros A. Serologic testing with ANCA, ASCA, and anti-OmpC in children and young adults with Crohn's disease and ulcerative colitis: diagnostic value and correlation with disease phenotype. Am J Gastroenterol 2004; 99: 2235-2241.
[19] Kohoutova D, Drahosova M, Moravkova P, Rejchrt S and Bures J. 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.
[20] Kotlowski R, Bernstein CN, Sepehri S and Krause DO. High prevalence of escherichia coli belonging to the B2+D phylogenetic group in inflammatory bowel disease. Gut 2007; 56: 669-675.
[21] Roggenbuck D, Reinhold D, Schierack P, Bogdanos DP, Conrad K and Laass MW. Crohn's disease specific pancreatic antibodies: clinical and pathophysiological challenges. Clin Chem Lab Med 2014; 52: 483-494.
[22] Bogdanos DP, Roggenbuck D, Reinhold D, Wex T, Pavlidis P, von Arnim U, Malfertheiner P, Forbes A, Conrad K and Laass MW. Pancreatic-specific autoantibodies to glycoprotein 2 mirror disease location and behaviour in younger patients with Crohn's disease. BMC Gastroenterol 2012; 12: 102.
[23] Folwaczny C, Noehl N, Tschop K, Endres SP, Heldwein W, Loeschke K and Fricke H. Goblet cell autoantibodies in patients with inflammatory bowel disease and their first-degree relatives. Gastroenterology 1997; 113: 101-106.
[24] Prideaux L, De Cruz P, Ng SC and Kamm MA. Serological antibodies in inflammatory bowel disease: a systematic review. Inflamm Bowel Dis 2012; 18: 1340-1355.
[25] Joossens S, Reinisch W, Vermeire S, Sendid B, Poulain D, Peeters M, Geboes K, Bossuyt X, Vandewalle P, Oberhuber G, Vogelsang H, Rutgeerts P and Colombel JF. The value of serologic markers in indeterminate colitis: a prospective follow-up study. Gastroenterology 2002; 122: 1242-1247.
[26] Wang ZZ, Shi K and Peng J. Serologic testing of a panel of five antibodies in inflammatory bowel diseases: diagnostic value and correlation with disease phenotype. Biomed Rep 2017; 6: 401-410.
[27] Roggenbuck D, Hausdorf G, Martinez-Gamboa L, Reinhold D, Buttner T, Jungblut PR, Porstmann T, Laass MW, Henker J, Buning C, Feist E and Conrad K. Identification of GP2, the major zymogen granule membrane glycoprotein, as the autoantigen of pancreatic antibodies in Crohn's disease. Gut 2009; 58: 1620-1628.
[28] Pavlidis P, Romanidou O, Roggenbuck D, Mytilinaiou MG, Al-Sulttan F, Liaskos C, Smyk DS, Koutsoumpas AL, Rigopoulou EI, Conrad K, Forbes A and Bogdanos DP. Ileal inflammation may trigger the development of GP2-specific pancreatic autoantibodies in patients with Crohn's disease. Clin Dev Immunol 2012; 2012: 640835.