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Association of Anti-glycan Antibodies and Inflammatory Bowel Disease Course

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Abstract

Background and Aims: The usefulness of anti-glycan antibodies alone or combined with anti-*Saccharomyces cerevisiae* [ASCA] or perinuclear antineutrophil cytoplasmic [pANCA] antibodies for diagnosis of inflammatory bowel disease [IBD], differentiation between Crohn's disease [CD] and ulcerative colitis [UC], disease stratification including IBD phenotype, and also for determination of the course of the disease, remain unclear.

Methods: A large panel of serological anti-glycan carbohydrate antibodies, including anti-mannobioside IgG antibodies [AMCA], anti-chitobioside IgA [ACCA], anti-laminaribioside IgG antibodies [ALCA], anti-laminarin [anti-L] and anti-chitine [anti-C] were measured in the serum from a cohort of 195 patients with IBD] [107 CD and 88 UC]. The respective accuracy of isolated or combined markers for diagnosis, disease differentiation, stratification disease phenotype, and severity of the disease course, defined by a wide panel of criteria obtained from the past medical history, was assessed.

Results: The positivity of at least one anti-glycan antibody was detected in a significant higher proportion of CD and UC compared with healthy controls [$p < 0.0001$ and $p < 0.0007$, respectively]. Whereas ASCA and ANCA antibody status had the highest efficacy to be associated with CD in comparison with UC (area under receiver operating characteristic curve [AUROC] = 0.70 for each], the adjunction of anti-laminarin antibody substantially improved the differentiation between CD and UC [AUROC = 0.77]. Titres of ACCA [> 51 U/ml] and anti-laminarin [> 31 U/ml] were significantly linked with a higher association with steroid dependency (odds ratio [OR] =2.0 [1.0–4.0], $p = 0.03$ and OR = 2.4 [1.1–5.2], $p = 0.02$, respectively]. We further defined the respective performance of anti-glycan antibodies to discriminate between patients with severe or not severe CD and UC course and determined the associated optimal cut-off values: severe CD course was significantly more likely in case of AMCA > 77 U/ml [OR = 4.3; $p = 0.002$], ASCA > 63 U/ml [OR = 3.5; $p < 0.009$] and at a lesser degree ACCA > 50 U/ml [OR = 2.8; $p < 0.02$] and severe UC course was significantly associated with AMCA > 52 U/ml [OR = 3.4; $p = 0.04$] and ACCA > 25 U/ml [OR = 3.0; $p < 0.04$].

Conclusions: Anti-glycan antibodies are valuable serological markers, especially AMCA antibodies that may help clinicians to promptly classify patients into high risk for severe disease.

Keywords: Serological antibodies; anti-glycans; Crohn's disease; ulcerative colitis; IBD behaviour

Abbreviations

[CD]	Crohn's disease
[UC]	Ulcerative colitis
[IBD]	Inflammatory bowel disease
[TNFα]	perinuclear antineutrophil cytoplasmic antibody Tumor necrosis factor
[anti-TNF]	Anti-Tumor Necrosis Factor
PPV	Positive Predictive Value
NPV	Negative Predictive Value
Ss	Sensibility
Sp	Specificity.

1. Background

Inflammatory bowel diseases [IBD], including Crohn's disease [CD] and ulcerative colitis [UC], are chronic intestinal disorders characterised by alternating relapse and remission periods. Pathogenesis is multifactorial and involves abnormal host-immune-gut microbial interactions, as evidenced by the presence of serum specific antibodies directed against resident luminal bacterial components.[1] Various seroreactivity to specific microbial antigens such as anti-*Saccharomyces cerevisiae* [ASCA] and perinuclear antineutrophil cytoplasmic antibody [pANCA] serve as surrogate markers that can be seen in a subgroup of patients with IBD. Knowledge of other CD-specific antibodies directed against specific microbial antigens has expanded, including anti-*Pseudomonas*-associated sequence I2, outer-membrane-porin C, and anti-bacterial cBir1 flagellin; and all of them have been previously extensively investigated for their respective ability to help clinicians in diagnosis, disease stratification, or disease behaviour in IBD. Reactivity to microbial antigens such as oligomannan ASCA, OmpC and CBir1 were found to be associated with an aggressive CD behaviour.[2,3,4,5] More recently, novel specific seroreactivity to anti-glycan carbohydrate antibodies, including anti-mannobioside carbohydrate IgG antibodies [AMCA], anti-chitobioside carbohydrate IgA [ACCA], and anti-laminaribioside carbohydrate IgG antibodies [ALCA] have been added to the armamentarium of available serological markers that can be detected. They have been also studied for their ability to assess diagnosis of CD[6] and to differentiate CD from UC, and recent evidences have also reported that high levels of anti-glycan antibodies could be associated with complicated disease behaviour and a higher probability of disease-related surgery in CD.[3,7,8,9] It has been shown that the presence of anti-glycans was linked with a high likelihood to develop a complicated IBD behaviour leading to increased risk of IBD-related surgery.[9]

There is a growing interest to determine whether the determination of an immune response directed against glycan-epitopes alone or in combination could be associated with an aggressive disease behaviour or stability, and whether the detection and the measurement of anti-glycan antibody status in the serum of patients with IBD would represent a surrogate marker in determining the clinical course or severity of IBD. Emerging disease-modifying therapies, including anti-tumour necrosis factor [anti-TNF] are capable to change the natural course of IBD if they are used as early enough, as necessary. Therefore early identifying patients at high risk of severe or disabling disease would be of interest in using preemptive therapy. In the present study, we focused on the anti-glycan carbohydrate antibodies by analysing their performance alone or in combination with ASCA or pANCA antibodies for diagnosis of IBD, disease stratification, and disease phenotype, an also whether anti-glycan antibody status may be qualitatively and quantitatively associated with the severity of the course of the disease.

2. Methods

2.1. Patients and study characteristics

All the consecutive in- and outpatients with IBD [107 with CD and 88 with UC] referred to the Department of Gastroenterology of Saint-Etienne's University Hospital were prospectively enrolled over a 12-month period between September 2009 and October 2010. The diagnosis of IBD was assessed on common endoscopic and histological evidences of the disease, according to the Lennard-Jones criteria.[10] Only patients with a confirmed diagnosis of IBD for more than 1 year were enrolled and patients with indeterminate colitis were excluded. The healthy control group [n = 45] were age- and gender-matched to IBD group consecutive healthy volunteers selected from blood donors. All gave their written informed consent for participation in the study, which was previously approved by the local Ethics Committee of the University of Saint-Etienne.

A complete medical chart review of the past history and physical examinations were systematically recorded at inclusion and disease activity was assessed using the Crohn's Disease Activity Index [CDAI] for CD and the Lichtiger severity index for UC. The following additional clinical data were collected at baseline: date of birth, gender, disease duration, age at onset, disease location and behaviour according to the Montreal classification,[11] concomitant perianal disease, extra-intestinal manifestation, smoking history, and previous history of IBD-related surgery. Previous drug-response history (e.g. need for steroid and/or immunosuppressant, steroid dependency and/or resistance, as previously defined by the European Crohn's and Colitis Organisation [ECCO]) was also recorded by reviewing the medical charts.

For the purpose of the study, the presence of at least one of the following composite severity criteria in the IBD-related past medical history was noted: patients with uncontrolled active disease requiring adjunction of biotherapy with anti-TNF after failure with conventional immunosuppressants; two or more IBD-related previous surgeries or bowel resection longer than 70 cm; concomitant active perianal disease with complex fistulas; or spread of bowel disease [defined regarding a length of small bowel greater than 50 cm with diffuse jujenal injury], for patients with CD, and: pancolitis associated with increased C-reactive protein levels and steroid treatment at UC diagnosis; early use of immunosuppressant during the first year of the disease due to steroid-refractory response; use of anti-TNF after failure with immunosuppressant; acute severe UC; or colectomy, for UC patients. These conditions were associated with serological markers.

2.2. Serological marker analysis

All patients and healthy controls provided a venous blood sample [≈ 10 ml]. Serum was rapidly transported to the laboratory,

separated from blood by centrifugation and kept frozen at -80°C until use. All sera were analysed by enzyme-linked immunosorbent assay [ELISA] for a large panel of serological markers including ASCA, ANCA, ACCA, ALCA, AMCA, anti-chitin and anti-laminarin antibodies. Clinical data analyses and serological assessments were performed in a blinded manner without knowledge of the patients' diagnosis and medical history. Commercially available kits were used to detect ANCA IgG [Bioadvance, Philadelphia, PA], ASCA IgA and IgG, ACCA IgA, ALCA IgG, AMCA IgG, anti-chitin IgA and anti-laminarin IgA [Glycominds, Israel] levels in sera. Briefly, serum samples or standard control samples were diluted and incubated on microtitre plates coated with specific antigens. The plates were washed to remove the unbound antibodies. Then biotin-labeled antibodies were added to the wells and the plates were incubated with frequent shaking. The wells were then washed by washing with buffer three times and then 100 µl of streptavidin enzyme conjugate was added to each well. After incubation, the plates were washed and 100 µl of the substrate tetramethylbenzidine [TMB] was added. After short incubation in the dark, the stop solution was added to block colour development after the recommended times and the optical densities [OD] at 450 nm were measured by an ELISA-microplate reader [AP22, Bioadvance]. Results are presented as arbitrary units, which were calculated based on the sample and calibrator optical density. Intra- and inter-assay precision errors were below 10%.

2.3. Statistical analysis

Qualitative data were reported as numbers and percentages. Quantitative data were reported as mean ± standard deviation, median value with first quartile [Q1] and third quartile [Q3], minimum and maximum. Univariate and multivariate analyses were generated to identify potential independent antibodies for CD and UC. These analyses were adjusted for age and sex. Only variables with a prevalence of at least 3% and associated with the risk of pathology according to univariate analyses with a *p*-value of less than 0.15 were included in the multivariate model. The univariate analysis and multivariate models were logistic. A *p*-value of 0.05 or less was used to retain antibody as independent factor of pathology. Data were processed and analysed by SAS-WINDOWS™ software [version 9.2].

3. Results

3.1. Characteristics of the patients

A total of 107 consecutive patients with CD (57 females [53%], median age 41.3 years [20–101]) and 88 consecutive patients with UC (33 females [37%], median age 49.3 years [19–88]) were enrolled during the study period. The median duration of disease at study inclusion was 9.4 years [1–44] for CD, and 10.4 years, [1–34] for UC. More than half of CD patients enrolled had a colonic [L2] or ileocolonic disease location [L3] and 43 out of 107 patients [40%] a complicated behaviour [B2-stricturing or B3-penetrating], according to the Montreal classification. Around 46% of UC patients had a pancolitis [E3] at inclusion. Among the whole population, 26 patients [13%] were treated with anti-TNF antibodies and more than two-thirds of patients were steroid-dependent and/or steroid-refractory in their medical history. Of 107 CD patients, 68 [63%] and 44of 88 UC patients [50 %] fulfilled the criteria for severe disease described below. Demographic and baseline disease characteristics of patients and healthy controls are listed in Table 1.

3.2. Distinguishing IBD patients from controls and UC patients from CD patients

The respective abilities of the large panel of anti-glycan antibodies tested, including ALCA, ACCA, and AMCA, anti-laminarin [anti-L] and anti-chitin [anti-C] antibodies to distinguish IBD patients from healthy controls were analysed. Using predetermined manufacturers' thresholds, all the antibodies investigated, except for anti-C antibodies, were found significantly positive more often in CD [40.6% vs 2.3% for AMCA or 29.2% vs 4.5% for ALCA] when compared with those detected in healthy controls [Supplementary Table 2, available as Supplementary data at *ECCO-JCC* online: as examples, ACCA median of 52.9 vs 37.9, AMCA median of 78.4 vs 39.9 respectively]. In UC, positive anti-glycan antibodies ALCA and AMCA were detected in a significantly higher number of patients [Supplementary Table 2: 19.3% and 29.5% vs 4.5% and 2.3%, respectively] compared with those in healthy controls [$p < 0.04$ and $p < 0.006$, respectively]. In contrast, positivity against ACCA, an anti-L or anti-C antibody was not found statistically different between UC and healthy controls. Using a multivariate analysis, a significantly and independently higher number of CD and UC patients where found positive for at least one anti-glycan antibody compared with healthy controls [$p < 0.0001$ and $p < 0.0007$ for CD and UC, respectively Supplementary Table 2]. Again by a multivariate analysis, the positivity of AMCA seroreactivity [29.5%] was also found significantly associated with UC [$p < 0.005$; Supplementary Table 3, available as Supplementary data at *ECCO-JCC* online].

Among the wide panel of antibodies tested in univariate analysis using manufacturers' thresholds, the best antibodies capable of distinguishing CD from UC patients were ANCA, ASCA, ACCA, and anti-laminarin antibodies. Figure 1 summarizss the median titres of individual antibody measured in CD and in UC patients. Then we performed a multivariate analysis taking into account all significant anti-glycan antibodies adjusted for age and sex, given that these parameters were statistically different between UC and CD [$p = 0.0032$ and $p = 0.0286$, respectively]. We found that only ANCA, ASCA, and anti-laminarin antibodies were able to differentiate CD from UC. For example, ASCA antibodies [IgA/IgG] were positive in 18.2% of UC and 47.2% of CD cases [Supplementary Table 3]. The results of univariate and, in cases of significance, multivariate analysis for all the antibodies are summarised in Supplementary Table 3. In a head-to-head comparison, ASCA and ANCA antibodies had the highest validity to discriminate CD from UC, with an area under the receiver operating characteristic [AUROC] curve of 0.70 for both and, at a lesser degree, for anti-laminarin antibody [AUC = 0.68]. The combination of the three antibodies [ASCA, ANCA, and anti-laminarin] substantially improved the diagnostic value of these three markers with an AUC of 0.77.

3.3. Relationships between anti-glycan antibodies and phenotype of IBD and therapy

We further tested the relationship between the positivity of specific seroreactivity and disease location in a univariate analysis. Only anti-glycan ALCA antibodies were statistically associated with an increased risk of colonic [L2] or ileocolonic [L3] CD, according to the Montreal classification [OR = 2.77 [1.08–7.08], $p = 0.0334$]. In UC, we failed to detect any association between anti-glycan seroreactivity and disease location. We further investigated the relationships between antibodies and IBD behaviour and we failed to detect any significant difference in the levels of expression of anti-glycan antibodies with regard to disease phenotype. According to disease duration, we were also not able to show a significant difference in

Table 1. Demographic and main baseline characteristics of patients and controls

	UC	CD	Controls
Number	88	107	45
Sex ratio[M/F]	55/33	50/57	22/23
Current smoker [*n*, %]	3.4	42.1	
Median age at inclusion, years, [IQ]	49.3 [19–88]	41.3 [20–101]	
Median age at diagnosis years, [IQ]	40.0 [13–79]	32.9 [9–84]	
Median disease duration at inclusion years, [IQ]	10.4 [1–34]	9.4 [1–44]	
Disease location			
Ileal [L1]		14 [19 %]	
Colonic [L2]		12 [15 %]	
Ileocolonic [L3]		52 [66 %]	
Rectum [E1]	15 [18.1 %]		
Distal colitis [E2]	30 [36.1 %]		
Extensive colitis [E3]	38 [45.8 %]		
Disease behaviour			
Non-stricturing and non-penetrating [B1]		61 [58.7 %]	
Stricturing [B2]		28 [26.9 %]	
Penetrating [B3]		15 [14.4 %]	
Perianal disease		22 [21.2 %]	
First-line medications			
Anti-TNF therapy	0 [0 %]	1 [0.9 %]	
Steroids	39 [44.3 %]	73 [68.2 %]	
Immunosuppressants	1 [1.1 %]	8 [7.5 %]	
5-ASA	48 [54.5 %]	25 [23.4 %]	
Concomitant medications			
Anti-TNF therapy	7 [8 %]	19 [17.8 %]	
Steroids	54 [61.4 %]	86 [80.4 %]	
Immunosuppressants	21 [23.9 %]	50 [46.7 %]	
Steroid dependent	60 [68.2 %]	74 [69.2 %]	
Steroid refractory	30 [34.1 %]	18 [16.8 %]	
IBD-related surgery	12 [13.6 %]	41 [38.3 %]	

IQ, interquartile range; anti-TNF, anti tumour necrosis factor; 5-ASA, 5-aminosalicylic acid; IBD, inflammatory bowel disease.

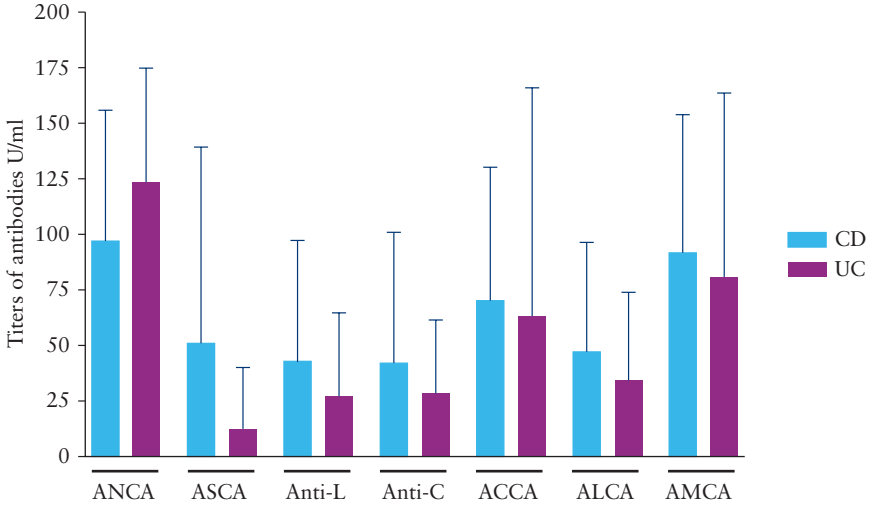

Figure 1. Serum levels of the panel of anti-glycan antibodies in CD and UC patients.Histogram represents the mean ± SD of serum titre [U/ml] for each antibody in CD patients [white bar] and in UC patients [black bar]. CD, Crohn's disease; UC, ulcerative colitis.

term of positivity of antibodies between the patients in whom the disease had evolved for more than 10 years [*n* = 76] or for less than 10 years [*n* = 119]. Moreover, we compare the presence of each antibody in early IBD [less than 2 years' duration; *n* = 28] and very long duration of IBD [more than 10 years; *n* = 76]. No significant difference between these two groups was reported

Considering IBD-related therapy, we have investigated the relationship between the presence of specific anti-antibodies detected

in the cohort and the need for IBD-related surgery. Among all the panel of tested antibodies, only AMCA antibodies tended to be associated with higher risk of CD-related surgery with an odds of 2.1 [0.8–5.1] but the level of significance was not reached [p = 0.10]. We analysed the potential association between the panel of anti-glycan antibodies and the presence of steroid-dependency. Among the 195 IBD patients, 134 experienced a steroid-dependency [74 CD and 60 UC], and only the presence of ACCA was significantly associated with a higher prevalence of steroid dependency [OR = 2.9 [1.2–6.7], p = 0.01] using predetermined manufacturers' thresholds. Given that the thresholds of positivity of antibodies were not specifically defined according to the criterion of steroid-dependency, a ROC curve was further constructed to determine the optimal cut-off points for each anti-glycan antibody. So titres of ACCA antibody higherer than 51.7 U/ml and higher than 31.8 U/ml for anti-laminarin antibody were significantly associated with a higher risk of steroid-dependency [OR = 2.0 [1.0–4.0], p = 0.030 and OR = 2.4 [1.1–5.2], p = 0.023, respectively, for ACCA and anti-laminarin]. The combination of the positivity for ACCA and anti-laminarin antibodies had an AUC of 0.65 and is associated with whether patients have experienced a steroid-dependency in their past medical history. In addition, there was no association between any antibodies and loss of response under anti-TNF treatment [data not shown].

3.4. Association between anti-glycan antibodies and the severity during the disease course

We further investigated the relationships between seroreactivity directed against glycan and the severity of IBD. First, we analysed individually in a univariate analysis all serological markers with each clinical associated parameter for severe IBD [disease behaviour, first medications, concomitant medications, perianal disease, IBD-related surgery, pancolitis, and steroid dependence/refractory response; data not shown]. In this first-step analysis, only anti-laminarin antibodies were significantly associated with perianal disease (p = 0.048, 0.26 [0.07; 0.96]). For other anti-glycans, we observed only trends in the association with clinical parameters such as surgery or treatments due to the lack of power in those groups. So we pooled all clinical parameters related to severe illness to continue our analysis. According to the severe disease criteria defined below, 68 CD patients [63 %] and 44 UC patients [50 %] were reported to have had a disease considered as 'severe'. Using manufacturers' cut-offs, we found that a significant number of patients with severe CD had positive ASCA and anti-glycan AMCA antibodies [n = 32 and n = 35, respectively] when compared with the number of patients who had none of the predefined criteria of severity in their past medical history disease [n = 8 and n = 8, p = 0.011 and p = 0.003, respectively]. In CD, ASCA and AMCA antibodies had the best validity for association with a non-severe course, stable from severe disease, with an AUC of 0.63 for ASCA and 0.65 for AMCA antibodies. If we combined both antibodies, we obtained a better diagnostic precision with an AUC of 0.71. In UC, we failed to detect any significant difference between the positivity of all antibodies tested and disease severity. The associations between individual antibody according to manufacturers' cut-off point and disease severity are summarised in Table 2 for CD and Table 3 for UC. Figure 2 represents the titres of each antibody measured in CD and UC patients according to disease severity.

We finally constructed several ROC curves to define optimal cut-off values to discriminate with the best accuracy a severe from a non-severe disease course using anti-glycan antibodies. For CD, we found that ASCA, ACCA, and AMCA antibodies were the best serological markers to diagnose severe CD with an AUC of 0.68 for ASCA, 0.66 for ACCA, and 0.68 for AMCA antibodies [Table 4]. The thresholds defined with ROC curves and the performances obtained for each antibody tested in CD are summarised in Table 4. A titre of antibody higher than the threshold of 63 U/ml for ASCA, 50.7 U/ml for

Table 2. Statistical analysis of all individual antibodies according to the severity of CD [manufacturers' cut-off].

	Severe CD n=68	Non severe CD n=39	Univariate analysis	Multivariate analysis
ANCA	19 [28.4 %]	6 [15.4 %]	p=0.1346	-
ASCA	32 [47.8 %]	8 [20.5 %]	3.54 [1.42;8.83] p=0.0066	3.41 [1.32;8.82] p=0.0114
Anti-laminarin	22 [32.8 %]	12 [30.8 %]	1.10 [0.47;2.57] p=0.8260	-
Anti-chitin	22 [32.8 %]	6 [15.4 %]	2.69 [0.98;7.37] p=0.0545	1.11 [0.31;3.96] p=0.8733
ACCA	27 [40.3 %]	7 [17.9 %]	3.09 [1.19;8.00] p=0.0204	2.30 [0.82;6.48] p=0.1142
ALCA	21 [31.3 %]	10 [25.6 %]	1.32 [0.55;3.21] p=0.5343	-
AMCA	35 [52.2 %]	8 [20.5 %]	4.24 [1.7;10.56] p=0.0019	4.10 [1.6;10.50] p=0.0033

CD, Crohn's disease; ANCA, mannobioside IgG antibodies; AMCA, anti-chitobioside IgA antibodies; ACCA, anti-laminaribioside IgG antibodies; ASCA, anti-*Saccharomyces cerevisiae* antibodies; ALCA, anti-laminaribioside IgG antibodies.

Table 3. Statistical analysis of all the antibodies according to the severity of ulcerative colitis [UC] course [manufacturers' cut-off].

	Non severe UC n=44	Severe UC n=44	Univariate analysis	Multivariate analysis
ANCA	23 [52.3 %]	19 [43.2 %]	p=0.3939	-
ASCA	3 [6.8 %]	2 [4.5 %]	-	-
Anti-laminarin	4 [9.1 %]	7 [15.9 %]	-	-
Anti-chitin	8 [18.2 %]	5 [11.4 %]	-	-
ACCA	10 [22.7 %]	5 [11.4 %]	2.29 [0.71;7.37] p=0.1635	-
ALCA	12 [27.3 %]	5 [11.4 %]	2.92 [0.93;9.17] p=0.0658	-
AMCA	17 [38.6 %]	9 [20.5 %]	2.45 [0.95;6.34] p=0.0650	-

UC, ulcerative colitis; ANCA, mannobioside IgG antibodies; AMCA, anti-chitobioside IgA antibodies; ACCA, anti-laminaribioside IgG antibodies; ASCA, anti-*Saccharomyces cerevisiae* antibodies; ALCA, anti-laminaribioside IgG antibodies.

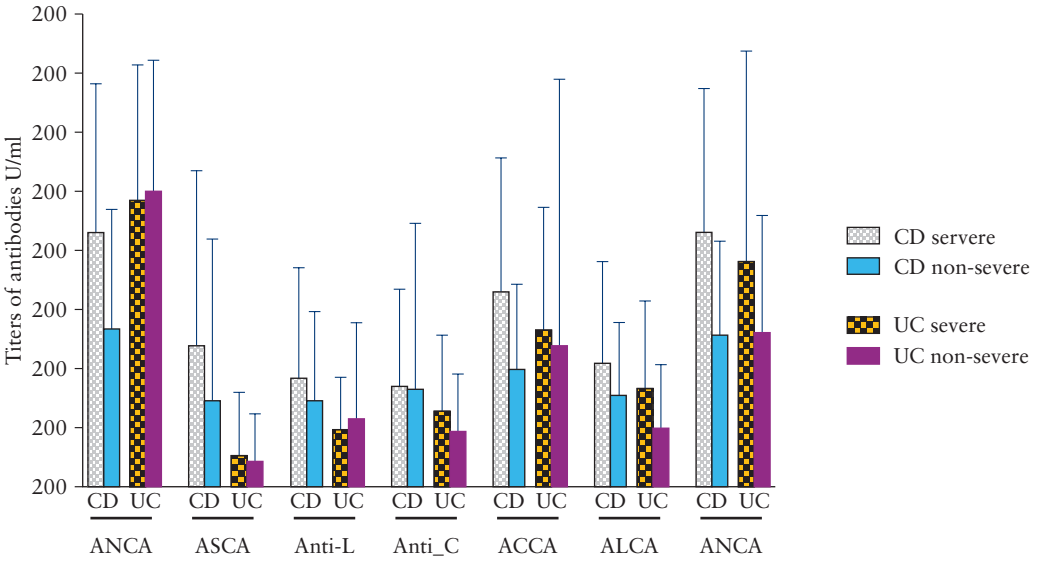

Figure 2. Serum levels of the panel of anti-glycan antibodies in CD and UC patients according to the disease stability status. CD, Crohn's disease; UC, ulcerative colitis. Histogram represents the mean ± SD of serum titre [U/ml] for each antibody in CD patients who experienced a severe [grey bar] or a stable disease [white bar] and in UC patients who experienced a severe [small black bar] or a stable disease [black bar].

Table 4. Optimal thresholds according to ROC curves and subsequent accuracies for each antibody tested according to the severity of the disease course in CD.

Antibody	Best threshold U/ml	Sensitivity %	Specificity %	AUC
ASCA	> 63	65.7	71.8	0.687
Anti-laminarin	> 16.1	88.1	43.6	
Anti-chitin	> 35.7	41.8	79.5	
ACCA	> 50.7	67.2	69.2	0.669
ALCA	> 19.4	83.6	43.6	
AMCA	> 77.4	64.2	74.4	0.680

ROC, receiver operating characteristic, CD, Crohn's disease; AUC, area under the curve; AMCA, anti-chitobioside IgA antibodies; ACCA, anti-laminaribioside IgG antibodies; ASCA, anti-*Saccharomyces cerevisiae* antibodies; ALCA, anti-laminaribioside IgG antibodies.

ACCA and 77.4 U/ml for AMCA antibodies, was significantly associated with severe disease (OR = 3.5 [1.37–9.2], $p = 0.0009$; OR = 2.8 [1.12–7.39], $p = 0.027$; and OR = 4.3 [1.69–11.03], $p = 0.0022$, respectively, for ASCA, ACCA, and AMCA antibodies). The combination of thethree3 antibodies [ASCA, ACCA, and AMCA] improved the diagnosis value associated with severe CD course with an AUC of 0.79. Regarding UC, a similar analysis was performed and we could identify that values of ACCA or AMCA antibodies higher than a threshold of 25.5 U/ml and 52 U/ml, respectively, were significantly associated with a severe UC course (OR = 3.0 [1.0–9.1], $p = 0.045$ and OR = 3.4 [1.0–6.9], $p = 0.045$, respectively, for ACCA and AMCA antibodies). With the new thresholds, the AUC was 0.62 for ACCA and 0.63 for AMCA antibodies. The combination of the two antibodies [ACCA and AMCA] slightly improved the diagnostic value of these two markers, with an AUC of 0.67.

4. Discussion

The present cross-sectional study clearly highlights the value of novel anti-glycan antibodies in the setting of disease diagnosis, differentiation between CD and UC, disease behaviour and severity based on the presence of various criteria in their past medical history in patients with IBD. The determination of ALCA, AMCA, and anti-laminarin antibody status may be useful to distinguish between CD and healthy controls, and the presence of ALCA and AMCA antibodies were found more often positive in UC when compared with non-IBD controls. The qualitative seroreactivity to AMCA was independently associated with UC, and multivariate analysis revealed that the presence of at least one anti-glycan antibody found positive was significantly associated with IBD. Interestingly, the presence of ALCA antibodies in CD was associated with 2-fold increase in odds of having a colonic or an ileocolonic location.

Our findings confirm those from previous studies reporting the value of anti-glycan for the diagnosis of IBD and for disease differentiation and behaviour and extends the relationships between seroreactivity for anti-glycans and the course of IBD.[9,12,13,14] Few studies are available regarding the relationship between anti-glycan antibodies and the severity of IBD past history. Interestingly, in our cohort, a combination of positive ASCA, AMCA, and ACCA antibodies was preferentially present in patients who had experienced a severe CD course. The ability of the measurement of these three autoantibodies allowed discrimination between patients who met the criteria of severe disease course and those who did not, with good accuracy [AUC = 0.79]. In addition, in UC the presence of both ACCA and AMCA antibodies was associated with a 3-fold increase of severe disease. The need for surgery has been associated with the presence of reactivity to oligomannan ASCA, OmpC, and CBir1. In a multicentere study in a paediatric cohort of CD, reactivity to ASCA, anti-I2, anti-OmpC, and anti-CBir1 was measured early in the course of the disease with more than half of the patients within 3 months of diagnosis. The risk of developing fibrostenosing and internally penetrating CD, two conditions favouring the need for surgery, increased in parallel with seroreactivity to increasing number of antigens.[15] High ASCA levels have also been related with complicated disease behaviour and the requirement of small bowel surgery.[16] Based on the seroreactivity status, if our findings are confirmed in a longitudinal prospective trial in which the antibody status would be determined before the occurrence of disease complications, the development of an aggressive IBD behaviour may be anticipated in case of reactivity to microbial antigens,

consequently helping the clinicians to better manage such patients at risk of developing disabling disease. Severe and disabling disease could be associated with an enhanced intestinal permeability consequently favouring seroreactivity to microbial antigens.

Our study has several limitations: the cohort of IBD patients comes from a tertiary referral single –centre, leading to a selection bias; the relative small sample size of the population may have limited the identification of some associations with disease behaviour and severity; the qualitative and quantitative seroreactivity is based on a unique serum sample and assumed that anti-glycan antibodies remain stable over time. A recent longitudinal study from a large IBD cohort has clearly demonstrated that the serological anti-glycan antibodies status remains widely stable over time.[13] Moreover, and regarding the use of anti-glycans for UC diagnosis and association with severity, the low number of UC patients in this study limits the conclusions about the utility of these markers and must be confirmed in a larger number of patients. We also acknowledge that the criteria defining a severe disease course are composite, including heterogeneous parameters, and may be the subject of discussion. However, each of these criteria has been individually recognised as a prognostic factor indicating a more severe history. We focused in the present study on the value of anti-glycan antibodies and therefore the determination of other anti-microbial reactivity, including anti-OmpC, anti-I2, anti-CBir1, was not available even though the impact of a combination of all serological markers, particularly on disease course, would be of paramount interest in patients with IBD. In addition, it was shown that associations of genetic markers, including variants of certain susceptibility genes such as *NOD2*, and seroreactivity to microbial antigens, are linked since a defect in innate immunity can lead to an enhanced adaptive immune response. NOD2 genotype and seroreactivity were both involved in determining the probability of developing fibrostenosing CD.[17] Moreover, the observational design of our study allows reporting only of an association and not a prediction or causality.

Despite a large panel of confounders in our logistic regression analyses, anti-glycan antibodies remained independently associated with severe disease, as well in CD as in UC. The determination of anti-glycan antibody markers may help clinicians to classify patients into a population at high risk for developing a disabling disease course.

In conclusion, a combination of seroreactivities to different cell surface glycans may represent a non-invasive and valuable tool to identify certain IBD patient subgroups that have experienced a severe disease. Longitudinal prospective studies are lacking to confirm these findings and to assess whether the early detection of anti-glycans may predict the course of the disease and impact on the therapeutic management of the patients.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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References

1. Prideaux L, De Cruz P, Ng SC, *et al.* Serological antibodies in inflammatory bowel disease: a systematic review. *Inflamm Bowel Dis* 2012;**18**:1340–55.
2. Arnott ID, Landers CJ, Nimmo EJ, *et al.* Sero-reactivity to microbial components in Crohn's disease is associated with disease severity and progression, but not NOD2/CARD15 genotype. *Am J Gastroenterol* 2004;**99**:2376–84.
3. Ferrante M, Henckaerts L, Joossens M, *et al.* New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut* 2007;**56**:1394–403.
4. Mow WS, Vasiliauskas EA, Lin YC, *et al.* Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. *Gastroenterology* 2004;**126**:414–24.
5. Targan SR, Landers CJ, Yang H, *et al.* Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology* 2005;**128**:2020–8.
6. Dotan I, Fishman S, Dgani Y, *et al.* Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. *Gastroenterology* 2006;**131**:366–78.
7. Papp M, Altorjay I, Dotan N, *et al.* New serological markers for inflammatory bowel disease are associated with earlier age at onset, complicated disease behavior, risk for surgery, and NOD2/CARD15 genotype in a Hungarian IBD cohort. *Am J Gastroenterol* 2008;**103**:665–81.
8. Papp M, Altorjay I, Norman GL, *et al.* Seroreactivity to microbial components in Crohn's disease is associated with ileal involvement, noninflammatory disease behavior and NOD2/CARD15 genotype, but not with risk for surgery in a Hungarian cohort of IBD patients. *Inflamm Bowel Dis* 2007;**13**:984–92.
9. Rieder F, Schleder S, Wolf A, *et al.* Serum anti-glycan antibodies predict complicated Crohn's disease behavior: a cohort study. *Inflamm Bowel Dis* 2010;**16**:1367–75.
10. Elliott PR, Lennard-Jones JE, Hathway N. Simple index of Crohn's disease activity. *Lancet* 1980;**1**:876.
11. Satsangi J, Silverberg MS, Vermeire S, *et al.* The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut* 2006;**55**:749–53.
12. Rieder F, Hahn P, Finsterhoelzl L, *et al.* Clinical utility of anti-glycan antibodies in pediatric Crohn's disease in comparison with an adult cohort. *Inflamm Bowel Dis* 2012;**18**:1221–31.
13. Rieder F, Lopez R, Franke A, *et al.* Characterization of changes in serum anti-glycan antibodies in Crohn's disease a longitudinal analysis. *PLoS One* 2011;**6**:18172.
14. Rieder F, Schleder S, Wolf A, *et al.* Association of the novel serologic anti-glycan antibodies anti-laminarin and anti-chitin with complicated Crohn's disease behavior. *Inflamm Bowel Dis* 2010;**16**:263–74.
15. Dubinsky MC, Lin YC, Dutridge D, *et al.* Serum immune responses predict rapid disease progression among children with Crohn's disease: immune responses predict disease progression. *Am J Gastroenterol* 2006;**101**:360–7.
16. Vasiliauskas EA, Plevy SE, Landers CJ, *et al.* Perinuclear antineutrophil cytoplasmic antibodies in patients with Crohn's disease define a clinical subgroup. *Gastroenterology* 1996;**110**:1810–9.
17. Ippoliti AF, Devlin S, Ynag H, *et al.* The relationship between abnormal innate and adaptive immune function and fibrostenosis in Crohn's disease patients. *Gastroenterology* 2006;**130**[Suppl 2]:A24.