

Pancreatic autoantibodies and autoantibodies against goblet cells in pediatric patients with inflammatory bowel disease (IBD).

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Abstract

Background: Significance of pancreatic autoantibodies determined by using exocrine pancreas (PAB) and recombinant pancreas antigens (rPAB), as well as importance of autoantibodies against goblet cells (GAB) are not known in pediatric patients with inflammatory bowel disease (IBD). Our aim was to determine the complex analysis of PAB, rPAB, GAB, antibodies against *Saccharomyces cerevisiae* (ASCA), and perinuclear components of neutrophils (pANCA) in pediatric IBD patients. Moreover, association with NOD2/CARD15 and disease phenotype was determined.

Methods: 152 pediatric patients (median age 13.9 years) with IBD [103 patients with Crohn's disease (CD) and 49 patients with ulcerative colitis (UC)] and 104 controls were included. Serum autoantibodies were determined by indirect immunofluorescence assay. NOD2/CARD15 variants were tested by polymerase chain reaction/restriction fragment length polymorphism.

Results: The presence of PAB and rPAB was significantly higher in CD (34% and 35.9%) and in UC (20.4% and 24.5%) compared to pediatric control cohort (0% and 0%, $p<0.0001$). In addition, GAB positivity was significantly increased in patients with UC in comparison to CD and controls, respectively (UC, 12.2%, CD, 1.9%, controls, 1.9%, $p=0.02$). Specificity of PAB and rPAB was 100%, however, sensitivity was low. The combination of PAB and/or ASCA/pANCA improved the sensitivity of serological markers in CD (87.4%) and in UC (79.6%); specificities were 89.3% and 93.2%, respectively. Pancreatic autoantibodies (PAB, rPAB) and GAB were not related to clinical presentation, medical therapy or need for surgery in CD or in UC.

Conclusions: Pancreatic autoantibodies and GAB were specific for IBD but the sensitivity was limited as well as there was lack of correlation with clinical phenotype. Combinations of these antibodies have shown increased sensitivity, therefore, it may be recommended in the diagnostic procedure of IBD.

Keywords: PAB, rPAB, GAB, inflammatory bowel disease, pediatric

Introduction

Inflammatory bowel diseases (IBDs), Crohn's disease (CD) and ulcerative colitis (UC) are chronic relapsing and remitting disorders of the gastrointestinal tract. The pathogenesis of IBD is complex and multifactorial. Current evidence suggests that IBD results from an aberrant immune response and loss of tolerance to the normal intestinal flora, leading to

chronic inflammation of the gut in a genetically susceptible host (1). This hypothesis is supported by the occurrence of antibodies directed to microbial antigens and by the identification of NOD2/CARD15, as susceptibility genes to CD (2). Besides genetic predisposition and environmental factors, autoimmune mechanisms are suggested to play a vital part in the pathogenesis of IBD. The search for the underlying trigger of the abnormal intestinal inflammation characteristics of IBD has led to the discovery of antibodies present specifically in the blood of patients with CD and/or UC. Several autoantibodies have been described in IBD.

Relatively low prevalence of exocrine pancreas antibodies (PAB) was detected in adult patients with CD (27-39%) using indirect immunofluorescence (IIF) (3,4). However, increased prevalence of PAB has been found in un-affected first-degree relatives of IBD patients, suggesting a genetic origin of these antibodies (5). The determination of autoantibodies against exocrine pancreas by IIF using human cells transfected with the recently identified proteoglycans CUZD1 and GP2 as recombinant target antigens (recombinant pancreas antigen 1 and 2: rPAg1 and rPAg2) represents a new dimension in the serological diagnosis of IBD (6,7). The studies conducted in adult patients provided conflicting data regarding association between PAB and CD phenotype (4,8,9).

Autoantibodies against intestinal goblet cells (GAB) have previously been described in UC with a prevalence of 28-30% and in 20% of first-degree relatives to IBD patients (3,10). Recent studies, however, suggested a much lower prevalence in both diseases (4,11). Moreover, PAB, rPAB and GAB have not been examined in a large cohort of pediatric IBD, and data on the specificity and sensitivity are contradictory in adult IBD.

The two most intensively studied conservative antibodies are autoantibodies to neutrophils [perinuclear anti-neutrophil cytoplasmic antibodies (pANCA)], primarily associated with UC and anti-Saccharomyces cerevisiae antibodies (ASCA), primarily

associated with CD (2,12). In pediatric IBD the sensitivity/specificity of pANCA in UC ranged between 57-83%, and 65% to 97% respectively, whereas in CD, ASCA showed a sensitivity/specificity in the range of 44% to 76%, and 88% to 95%, respectively (13,14). ASCA positivity or high titers are associated with a complicated disease behavior (penetrating or stenosing disease) and could be useful markers for predicting need for surgery also in pediatric patients (15-17). PANCA is noted for its association with „UC-like” CD phenotype (18,19).

The clinical importance of PAB and GAB is understudied in pediatric IBD patients. The aim of our study was to determine the prevalence of PAB, rPAB, GAB, ASCA, and pANCA in patients with pediatric-onset IBD. In addition, we assessed the association between antibody profile and NOD2/CARD15 status, clinical presentation, response to treatment and extraintestinal manifestations.

Patients and methods

Patients

Our study included 103 consecutive patients with pediatric-onset CD [male/ female (m/f) ratio: 63/40, median age: 13.9 years (range: 5.3-19.6 years)], 49 patients with pediatric-onset UC [m/f ratio: 22/27, median age: 12.5 years (range: 6-19.7 years)], and 104 age-and sex-matched controls. The diagnosis of IBD was based on clinical, radiologic, endoscopic and histological criteria. Disease activity was evaluated according to the Pediatric Crohn's Disease Activity Index (PCDAI) in children with CD and according to the Pediatric Ulcerative Colitis Index (PUCAI) for the UC group (21,22). Activity index >30 is defined as a moderate-severe disease, the index between 11-30 indicates a mild disease, and the index ≤10 refers to an inactive disease.

Age, age at onset, presence of extraintestinal manifestation (EIM); arthritis, ocular manifestations, skin lesions, and hepatic manifestations, frequency of flare-ups (frequent flare up: >1/year), therapeutic effectiveness (e.g.: need for steroid and/or immunosuppressive therapy) steroid resistance need for surgery (resection), the presence of familial IBD were collected by the clinical investigator reviewing the medical charts and completing a questionnaire. The disease phenotype (age at onset, duration, location, and behavior) was determined according to the Montreal Classification. In this study, complicated disease behavior in CD patients was defined as stricturing or penetrating behavior during follow-up. Only patients with a confirmed diagnosis for more than 1 year were enrolled.

Blood samples were obtained prospectively for PAB, rPAB, GAB, ASCA, and pANCA. At the time of taking blood samples, patients had a clinical assessment, including calculation of clinical disease activity scores. Sera for serological markers determination were coded to maintain blinding. After serum separation, blood samples were stored at -80 °C until further analysis.

The study protocol was approved by the Ethical and Science Committee of the Semmelweis University. Each parent of the children was informed about the nature of the study and signed the informed consent form.

Antibody assays for PAB, rPAB, GAB, ASCA, and ANCA

Presence of PAB, rPAB, GAB, ASCA and ANCA was determined in an commercially available IIF assay (EUROIMMUN AG, Luebeck, Germany) according to the manufacturers' instructions. For IIF coated cover glasses with several biological substrates were cut into millimeter-sized fragments (BIOCHIPS, EUROIMMUN AG) and used side by side in the same reaction field, giving the opportunity to investigate one serum on several tissues or prepared cells: PAB: monkey pancreas, recombinant pancreas antigen 1 (rPAg1) CUZD1 and

recombinant pancreas antigen 2 (rPAg2) GP2: human transfected cells, non-transfected cells (control), GAB: human intestinal goblet cell culture, ASCA: *Saccharomyces cerevisiae* fungal smear, ANCA: ethanol (EOH)-fixed and formalin (HCHO)-fixed human granulocytes. Sera were incubated at a 1:10 dilution (ASCA IgG: 1:1000, ASCA IgA: 1:100) in phosphate-buffered saline (PBS)/ Tween for 30 min. After washing with the buffer, slides were then incubated with fluorescein-labelled goat antihuman IgG or IgA antibodies for another 30 minutes. After further washing evaluation and classification of the patterns was performed under ultraviolet light (UV) using fluorescence microscope (EUROIMMUN LED, EUROStar Bluelight, EUROIMMUN AG, Luebeck, Germany). Sera that showed specific fluorescence patterns were classified as follows:

1) Two relevant patterns against pancreas acinus cells can be found: a reticulo-glanular (type 1) and a drop-like fluorescence (type 2). Antibodies against rPAg1 (CUZD1) resulted in a reticulo-granular, while antibodies against rPAg2 (GP2) resulted in a drop-like pattern. 2) GAB positivity can be detected as a cloudy fluorescence with indistinct boundary on intestinal goblet cells. 3) If ASCA were present, the smeared globular yeasts on the reaction fields fluoresced clearly in their entirety. 4) Using ethanol-fixed granulocytes two basic ANCA patterns were detectable: a granular fluorescence which is distributed evenly over the entire cytoplasm, leaving the cell nuclei free (cytoplasmic type, cANCA) or a fluorescence around the cell nuclei (perinuclear type, pANCA). Interference by antinuclear antibodies, which may mimic the pANCA pattern, was excluded by evaluation on HEp-2 cells, liver tissue of primates and formalin-fixed granulocytes. All positive sera were titrated to end-point.

Detection of NOD2/CARD15 mutations

Genomic DNA was isolated from whole blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany). The three NOD2/CARD15 variants, Arg702Trp, Gly908Arg,

Leu1007fs, were typed using polymerase chain reaction/restriction fragment length polymorphism as previously described (22). NOD2/CARD15 variants were detected by denaturing high-performance liquid chromatography (dHPLC, Wave DNA Fragment Analysis System, Transgenomic, UK). Sequence variation, observed in the dHPLC profile, was sequenced on both strands to confirm the alteration. Sequencing reactions were performed with the ABI BigDye Terminator Cycle Sequencing Kit v1.1 (Applied Biosystems, Foster City, CA) and samples were sequenced on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Genotyping was carried out at the National Haematology and Immunology Institute, Budapest, Hungary.

Statistical Analysis

Statistical analysis was carried out using Graph Pad Prism 5 (GraphPad, San Diego CA, USA). The presence of ASCA, pANCA, PAB, rPAB, and GAB were compared between the IBD and control cohorts, as well as within subgroups of IBD patients using χ^2 -test and Fisher's exact test. The differences of age between in subgroups of patients were assessed by Mann-Whitney-test. Logistic regression analysis was also performed to assess the complex associations between clinical phenotype and the serology profile. A p value of <0.05 was considered as significant.

Results

Characteristics of patients with IBD

The clinical phenotype of CD and UC patients is shown in **Table 1**. Median age at diagnosis was 13.9 years. Fifty-one percent of CD patients and 50% of UC patients had active disease, whereas 49% of CD patients and 50% of UC patients were in remission at the time of taking blood samples. Forty pediatric CD patients (38.8%) developed penetrating and /or stricturing

disease after a median follow-up of 18 months and 9.7% underwent surgery. App. 80% of patients were treated with steroids at some point during their disease and the majority (72.1%) was treated with azathioprine. Twenty percent of CD patients received infliximab, 40 % had remission or fistula closure 60% had partial remission or partial fistula closure.

Diagnostic accuracy of PAB, rPAB, and GAB and association with disease phenotype in CD and UC

Sensitivity and specificity data for PAB, rPAB and GAB are given in **Table 2**.

The presence of PAB and rPAB (IgA or IgG) antibodies was significantly higher in CD (34% and 35.9%) and UC (20.4% and 24.5%) compared to controls (0% and 0%, $p<0.0001$). The combination of PAB and/or ASCA/pANCA improved sensitivity of serological markers in both CD (87.4%) and UC (79.6%). Specificities were 89.3% and 93.2%, respectively. The positive predictive value (PPV) was 89.1% in CD for the combination of the markers and negative predictive value (NPV) was 87.6% (in UC, PPV, 93.2%, NPV, 82.2%). The accuracy of serologic antibodies is shown in **Table 2**. A strong association was observed between the reticulo-granular pancreatic IIF pattern and the reactivity of CUZD1 transfected cells (CD, 26 cases, UC, 8 cases) as well as between the droplet pattern and GP2 reactivity (CD, 24 cases, UC, 3 cases). 1 sera showed positive CUZD1 and 3 sera positive GP2 reactions (CD, 1, UC, 2), but no signal with tissue.

GAB positivity was significantly higher in patients with UC compared to CD and controls, respectively (UC, 12.2%, CD, 1.9%, controls: 1.9%, $p=0.02$).

The presence of PAB, rPAB, and GAB was not associated to clinical presentation, medical therapy, need for surgery or extraintestinal manifestations in either CD or in UC. The PAB and rPAB positivity was numerically higher in patients with colonic (28.6% and 27%) and

ileocolonic (60% and 59.4%) CD than in ileal disease (11.4% and 13.5%) [Table 3], however the difference was not statistically significant.

Diagnostic accuracy of ASCA and pANCA and association with disease phenotype in CD and in UC

Of the 103 CD patients studied 72.8% were ASCA positive (either IgA or IgG). Specifically, 63.1% were positive for ASCA IgA, 66.9% for ASCA IgG, and 60.2% for both ASCA IgA and IgG. The presence of ASCA (either IgA or IgG) was significantly higher in CD (72.8%) compared to UC (26.5%), and control (4.8%) patients.

ASCA positivity was associated with complicated disease behavior ($p=0.0003$) [stenosing ($p=0.02$) and/or penetrating disease behavior ($p=0.0003$)] and perianal complications ($p=0.01$) in CD. Association with location in patients with CD was not found [Table 4]. The frequency of stenosing and penetrating disease, and ileocolonic involvement increased with increasing number of immune responses, whereas inflammatory behavior (B1) showed inverse correlation with the number of ASCA [Table 5] There was no significant difference with regard to the need for surgery between ASCA positive and ASCA negative patients (need for surgery: 12% vs. 3.6%). ASCA was not associated with medical therapy and extraintestinal manifestations.

ASCA positivity was found in 13 UC patients (26.5%), however 7 of these patients had progressive sclerosing cholangitis (PSC).

PANCA were detected in 38 (77.5%) of 49 UC and in 34 (33%) of 103 CD patients. The presence of pANCA was not associated with clinical presentation, medical therapy, need for surgery or extraintestinal manifestations neither in CD nor in UC.

NOD2/CARD15 genotype, serum antibodies and phenotype in CD

NOD2/CARD15 genotypes were known for 43 CD patients. Mutations of NOD2/CARD15 were detected in 13 (30.2%) CD patients. There was no association of ASCA, PAB, rPAB and pANCA antibody status to NOD2/CARD 15 genotype. Three NOD2 carriers were PAB and ASCA negative, and two NOD2 carriers were PAB, ASCA and pANCA negative. NOD2 mutations were not related to age at onset, disease location and disease behavior. Nevertheless, NOD2 variants were significantly related to steroid refractory disease and administration of infliximab.

Discussion

This is the first report to assess the diagnostic value of PAB, rPAB and GAB as well as relevant phenotype-serotype associations in large cohort of patients with pediatric-onset IBD. Furthermore, we investigated the accuracy in combination with ASCA and pANCA, and NOD2/CARD15 mutations. In the present study PAB positivity in CD (34%) was similar to previous reports from in adult patients. The prevalence of rPAB was numerically higher than PAB. Prevalence of 27-39% of PAB has been found in adult CD patients, compared with only 0-5 % in UC patients (3,9,23). Stöcker et al. reported that PAB could be determined only in the serum of patients with CD (3). However, other studies found a much higher prevalence of PAB in UC (4,8,24) in accordance with our results (20.4%). Even though the specificity of PAB for CD and UC is high, their sensitivity is low. However, sensitivity can be significantly increased with combinations of different antibodies (see below). Recent studies have demonstrated that GP2 is expressed on the apical surface of intestinal membranous cells of the follicle-associated epithelium, and is essential for host-microbial interaction and the initiation of bacteria-specific mucosal immune responses (24,25).

There are conflicting results of association between PAB and CD phenotype in adult cohorts. In a Belgian study PAB was negatively associated with stricturing disease behavior

in CD (5). In contrast, in other European studies, an increased prevalence of PAB was observed in patients with stricturing or penetrating phenotypes (4,9,26). Lakatos et al. reported association between PAB positivity and perianal disease and extraintestinal manifestations (4). In a French study PAB correlated with early onset of disease in CD (23). In the present study pancreatic autoantibodies were not related to clinical presentation, medical therapy, need for surgery or extraintestinal manifestations in CD or in UC.

Autoantibodies against different colonic antigens have been found in patients with UC; for example GAB. In previous studies GAB have been detected in adult UC patients, the prevalence was 28-30% (3,10). In contrast, other studies suggested a much lower prevalence in both diseases (4,11). Probably, these differences are due to methodological differences, such as in ELISA antigen substrates and in evaluation of fluorescence patterns. GAB produces mucin that has multiple functions: it serves as a lubricant, provides nonspecific protection against unwanted microbial agents, and hosts the normal bacterial flora. Through complicated and strictly regulated glycosylation, mucins act as a decoy in binding a range of different microbes and thereby maintaining a normal intestinal flora. The significance of these antibodies, however has not been established and its remains unclear.

In agreement with the results published by Lakatos et al. and Lawrence et al. in our study the prevalence of GAB in our UC group was lower (12.2%) than previously reported (3,10). One explanation for the lower prevalence of GAB in our study may be the younger age of the patients. In concordance with earlier data (4) we could not demonstrate any association between the presence of GAB and clinical presentation, medical therapy, need for surgery or extraintestinal manifestations in UC patients. Consequently, the clinical utility of GAB for diagnostic purposes in pediatric IBD is limited.

In the present study the prevalence of ASCA in CD (72.8%) was similar to previous pediatric reports (44%-76%) (13-15,27). In concordance with published data we observed association between ASCA positivity and stenosing and penetrating behavior (16,23,28,29). ASCA positivity in our patients with UC was higher compared with earlier reports, perhaps due to the relatively higher proportion of patients with PSC. Of note, in an Italian study ASCA reactivity was detected in 44% of PSC patients (30). In the present study, the presence of ASCA in UC patients without PSC was found similar to other studies (31,32).

The prevalence of pANCA in our UC and CD patients (UC:77.5%, CD:33%) was comparable to earlier reports (57-83%) (13,14,32). Although pANCA has been established as an UC specific marker, approximately 25% of all CD patients also express pANCA (18). Several studies have suggested that pANCA expression is significantly higher in colonic CD (17,19,33), but others have been unable to confirm this finding (34). In the present study we did not observe any correlation between pANCA positivity and colonic CD.

In addition, we evaluated the diagnostic accuracy of combining markers. In concordance with previous data (11) in the present study the specificity of PAB was 100%, however, sensitivity was low (CD, 34%, UC, 20.4%). The sensitivity increased for combinations (e.g. PAB, ASCA and pANCA for CD (87.4%) as well as PAB and pANCA for UC (79.6%)). In combinations the specificity for CD was 89.3% and for UC was 94.2%.

Associations between NOD2 variants and complicated disease course, earlier time to surgery in children with CD were reported (35,36). In the present study we could not demonstrate any association of NOD2/CARD15 genotype, serum antibodies and phenotype in CD. However, in accordance with the results of Roesler et al. (37) we found an association between NOD2 variants and need for more intensive therapy (steroid refractory disease and infliximab use) in our CD cohort.

In conclusion, pancreatic autoantibodies (PAB, rPAB) and GAB were specific for IBD but the sensitivity was limited as well as they were not associated to clinical phenotype. Combinations of these antibodies with conventional serology markers are associated with increased sensitivity, therefore, the use of combinations may be recommended in the diagnostic work-up of selected IBD cases.

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Table 1. Clinical data of IBD patients

	CD n=103	UC n=49
Male/female	63/40 (61/39%)	22/27 (45/55%)
Age (y)	13.9 (range:5.3-19.6)	12.5 (range:6-19.7)
Age at presentation (y)	10.8 (range:2-18)	10.2 (range:1.7-18)
Duration (y)	1.5 (range:0.5-11)	2.3 (range:0.5-13.4)
Location		
	Ileum (L1): 17/103 (16.5%)	Proctitis (E1): 4/49 (7.1%)
	Colon (L2): 30/103 (29.1%)	Left sided (E2):15/49 (35.8%)
	Ileocolon (L3): 56/103 (54.4%)	Extensive (E3): 33/49 (57.1%)
	Upper GI (+L4): 24/103 (23.3%)	
Behavior		
	Inflammatory (B1): 63/103 (61.2%)	
	Stricturing (B2): 18/103 (17.5%)	
	Penetrating (B3): 27/103 (26.2%)	
Perianal disease	21/103 (20.4%)	
Frequent relapse	43/103 (41.7%)	22/49 (48.9%)
Surgery	10/103 (9.7%)	1/49 (2%)
NOD2/CARD15 carrier (n=43)	13/43 (30.2%)	

Table 2. Diagnostic value of serological markers in children with IBD

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	P value
rCD vs. controls n=103	ASCA	72.8	95.2	93.8	77.8	<0.0001
	PAB	34.0	100	100	60.2	<0.0001
	rPAB	35.9	100	100	60.9	<0.0001
	pANCA	33.0	94.2	85.1	58.4	<0.0001
	GAB	1.9	98.1	50.0	50.0	1.0000
	PAB and/or ASCA	79.6	95.2	94.3	82.3	<0.0001
	rPAB and/or ASCA	79.6	95.2	94.3	82.3	<0.0001
	PAB and/or ASCA and/or pANCA	87.4	89.3	89.1	87.6	<0.0001
	rPAB and/or ASCA and/or pANCA	87.4	89.3	89.1	87.6	<0.0001
	PAB and ASCA (both)	29.1	100	100	58.5	<0.0001
	rPAB and ASCA (both)	31.1	100	100	59.2	<0.0001
	PAB and /or ASCA/ pANCA-	53.4	95.2	91.8	67.1	<0.0001
	rPAB and /or ASCA/ pANCA-	53.4	95.2	91.8	67.1	<0.0001
	PAB+/ASCA+/pANCA-	21.4	100	100	56.0	<0.0001
	rPAB+/ASCA+/pANCA-	23.3	100	100	56.6	<0.0001
	ASCA+/pANCA-	51.5	95.2	91.5	66.2	<0.0001
UC vs.	pANCA	77.5	94.2	93.0	80.9	<0.0001

controls n=49	GAB	12.2	98.1	86.5	52.8	0.0196
	PAB	20.4	100	100	55.6	<0.0001
	rPAB	24.5	100	100	57.0	<0.0001
	ASCA	26.5	95.2	84.7	56.4	0.0014
	ASCA*	16.3	95.2	77.3	53.2	0.4419
	PAB and/or pANCA	79.6	94.2	93.2	82.2	<0.0001
	rPAB and/or pANCA	79.6	94.2	93.2	82.2	<0.0001
	PAB and/or pANCA and/or GAB	79.6	94.2	93.2	82.2	<0.0001
	rPAB and/or pANCA and/or GAB	79.6	94.2	93.2	82.2	<0.0001
	GAB+/pANCA+	12.2	98.1	86.5	52.8	0.0136
	PAB+/pANCA+	18.4	100	100	55.1	<0.0001
	rPAB+/pANCA+	22.4	100	100	56.3	<0.0001
	GAB+/PAB+/pANCA+	4.1	100	100	51.0	0.1011

*Diagnostic value of ASCA antibodies in UC patients without PSC

Table 3. PAB and rPAB positivity (%) in patients with CD (n=103) in association with disease location and behaviour

	PAB+ (%) n=35	PAB- (%) n=68	rPAB+ (%) n=37	rPAB- (%) n=66
Location				
Ileum (L1)	4 (11.4%)	13 (19.1%)	5 (13.5%)	12 (18.2%)
Colon (L2)	10 (28.6%)	20 (29.4%)	10 (27%)	20 (30.3%)
Ileocolon (L3)	21 (60%)	35 (51.5%)	22 (59.4%)	34 (51.5%)
Upper GI (+L4)	8 (22.9%)	16 (23.5%)	9 (24.3%)	15 (22.7%)
Behavior				
Inflammatory (B1)	22 (62.9%)	41 (60.3%)	23 (62.1%)	40 (60.6%)
Stenosing (B2)	7 (20%)	11 (16.2%)	7 (18.9%)	11 (16.6%)
Penetrating (B3)	6 (17.1%)	21 (31%)	7 (18.9%)	206 (30.3%)

No statistically significant differences were noted.

Table 4. ASCA positivity (%) in patients with CD (n=103) in association with disease location, behavior and need for surgery

	ASCA+ n=75	ASCA- n=28	P value	OR	95% CI
Location					
L1 (n=17)	12 (16%)	5 (17.9%)	0.77 ^a	0.87	0.27-2.7
L2 (n=30)	19 (25.3%)	11 (39.3%)	0.22 ^b	1.91	0.76-4.78
L3 (n=56)	44 (58.6%)	12 (42.8%)	0.18 ^c	1.89	0.78-4.55
L4 (n=24)	20 (26.6%)	4 (14.3%)	0.29 ^d	0.29	0.67-7.07
Behavior					
B1 (n=63)	38 (50.6%)	25 (89.3%)	0.0003 ^e	0.12	0.03-0.44
B2 (n=18)	16 (21.3%)	2 (7.1%)	0.02 ^f	5.2	1.11-24.91
B3 (n=27)	26 (34.6%)	1 (3.6%)	0.0003 ^g	17.1	2.17-134.3
Perianal disease	20 (26.6%)	1 (3.6%)	0.01	9.8	1.25-77.1
Surgery	9 (12%)	1 (3.6%)	0.27	3.7	0.45-31.47

^aL1 vs.L2+L3, ^bL2 vs.L1+L3, ^cL3 vs.L1+L2, ^dL4 vs.non L4, ^eB1 vs.B2 and/or B3, ^fB2 vs. B1,

^gB3 vs. B1

Table 5. Disease characteristics in CD patients in relation to the number of responses to ASCA

	Serological Marker (ASCA)			Statistical analysis		
	Positivity					
Clinical Phenotype (n=103)	0 (n=28)	1 (n=13)	2 (n=62)	P value (0 vs.2)	OR (0 vs.2)	95% CI (0 vs.2)
Ileum (L1)	5 (17.2%)	2 (15.4%)	10 (16.1%)	1.0	1.13	0.34-3.68
Colon (L2)	11 (37.9%)	2 (15.4%)	17 (27.4%)	0.33	1.71	0.66-4.39
Ileocolon (L3)	12 (41.3%)	9 (69.2%)	35 (56.4%)	0.02	0.35	0.15-0.83
Inflammatory (B1)	25 (89.3%)	9 (69.2%)	29 (46.8%)	0.0001	9.4	2.59-34.7
Stenosing (B2)	2 (7.1%)	3 (23%)	13 (21%)	0.03	0.17	0.03-0.86
Penetrating (B3)	1 (3.6%)	3 (23.1%)	23 (37.1%)	0.0002	0.05	0.006-0.4
Perianal complications	1 (3.6%)	4 (30.8%)	16 (25.8%)	0.01	0.10	0.01-0.84
Surgery	1 (3.6%)	1 (7.7%)	8 (12.9%)	0.26	0.25	0.03-2.1