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Significance of anti-*Saccharomyces cerevisiae* IgA and IgG antibodies in inflammatory bowel disease (ulcerative colitis and Crohn's disease) of Iraqi patients

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Abstract

The present investigation aimed to evaluate the diagnostic and discriminately potentials of anti-*Saccharomyces cerevisiae* (ASC) antibodies of both classes (IgA; aASC and IgG; gASC) in two groups of inflammatory bowel disease (IBD) patients; ulcerative colitis (UC) and Crohn's disease (CD). Seventy-nine Iraqi IBD (54 UC and 25 CD) patients were enrolled in the study, in addition to 10 apparently healthy controls. The patients attended the Gastrointestinal Tract Unit at Al-kadhamyiah Teaching Hospital in Baghdad for diagnosis and treatment during the period March - August 2012. The frequency distribution of positive cases for aASC antibodies showed a significant ($P \leq 0.001$) variation between IBD patients (UC and CD) and controls, and such variation was related to an increased frequency of positive cases in UC (24.1%) and CD (76.0%) compared to controls (0.0%). Ulcerative colitis versus controls revealed no significant difference, while CD patients versus controls showed a highly significant difference ($P = 4.6 \times 10^{-6}$), as well as, CD versus UC patients ($P = 3.3 \times 10^{-5}$). As the case in aASC antibodies, the frequency distribution of positive cases for gASC antibodies showed a significant variation between IBD patients (UC and CD) and controls ($P \leq 0.001$), and such variation was related to an increased frequency of positive cases in total CD (32.0%) patients compared to total UC patients (3.7%). Such variation was more pronounced in female CD patients compared to female UC patients (60.0 vs. 10.0%; $P = 0.014$).

Keywords: Anti-*Saccharomyces cerevisiae* (ASC), Ulcerative colitis, Crohn's disease.

Introduction

Inflammatory bowel disease (IBD) is a group of intestinal inflammatory diseases that can be subdivided into ulcerative colitis (UC) and Crohn's disease (CD) on the basis of clinical manifestations. The former is a transmural chronic inflammation potentially affects any gastrointestinal tract from mouth to anus, while the latter is a non-transmural disease affecting the colon with a caudo-cranial extension without patchiness or skip lesions (Podolsky, 2002). In CD, all layers of the gut may be involved and normal healthy gut may be found between sections of diseased bowel. In contrast, UC causes inflammation and ulcers in top layer lining the large intestine (Danese and Fiocchi, 2006).

When the gut is inflamed, there is a breakdown in intestinal barrier function, abnormal secretion, changes in the patterns of motility and visceral sensation, which contribute to symptoms generation (Shaheen and Annette 2010). These two diseases are often considered together because of multiple similarities; including gastrointestinal inflammation, waxing and waning severity, and unknown etiology, and symptoms which include diarrhea, abdominal pain, rectal bleeding, fever, nausea, weight loss, lethargy and loss of appetite, but clinical and pathological heterogeneity are also found (Actis *et al*., 2011).

The diagnosis of IBD and differentiation between UC and CD is currently based on a combination of clinical, laboratory, radiological, endoscopic and histopathologic criteria; however, in about 15% of colitis patients a definitive diagnosis cannot be made, and a disease category termed indeterminate colitis (IC) has been introduced (Stange *et al*., 2006). Accordingly, antibodies directed against microbial antigens (anti-glycan-antibodies) have been recently suggested as important diagnostic serum markers. They are a panel of antibodies consisting of anti-*Saccharomyces cerevisiae* (ASC), anti-mannobioside carbohydrate (AMC), anti-laminaribioside carbohydrate (ALC), anti-chitobioside carbohydrate (ACC), anti-laminarin carbohydrate (Anti-L) and anti-

chitin carbohydrate antibody (Anti-C) antibodies, which have been reported in independent studies to show a high discriminatory capacity between UC and CD (Ferrante *et al*., 2007; Rieder *et al*., 2010). In this context, ASC antibodies have been specifically associated with CD, with 40-60% sensitivity and 80-90% specificity (Gary, 2012e). Accordingly, the present study was planed with the aims to evaluate the discriminately potential of ASC antibodies of both classes (IgA and IgM) between UC and CD in a sample of Iraqi patients.

Materials and Methods

Patients: The study was approved by the Medical Ethics Committee of the Ministry of Health in Iraq, in which 79 Iraqi patients with IBD were investigated. The patients attended the Gastrointestinal Tract Unit at Al-kadhamyiah Teaching Hospital in Baghdad for diagnosis and treatment during the period March - August 2012. The disease was clinically diagnosed by the consultant medical staff at the hospital, which was based on a clinical evaluation using colonoscopy and a histopathological examination of a biopsy. According to the diagnosis of consultants, the patients were clinically distributed into two clinical groups: UC, which included 54 patients (34 males and 20 females), and their age mean ± S.E. was 38.9 ± 1.7 years; and CD, which included 25 cases (15 males and 10 females), and their age mean ± S.E. was 37.8 ± 2.2 years. In addition to patients, 10 apparently healthy controls of blood donors (5 males and 5 females) matched patients for age (34.4 ± 3.7 years) and ethnicity (Iraqi Arabs) were also enrolled in the study.

Assessment of ASC antibodies: Five milliliters of venous blood was collected from each participating subject. The blood sample was immediately transferred to a plain tube and left to clot at room temperature (20-25°C) for 15 minutes. Then, it was centrifuged at 1000 rpm for 10 minutes to separate serum, which was distributed into aliquots and stored frozen at -20°C until assayed for ASC IgA and IgG antibodies. The assessment was carried out by using QUANTA Lite ASCA (*S. cerevisiae*) IgA and IgG ELISA kits (INOVA, USA). The assay was based on the principles that partially purified and disrupted *S. cerevisiae* antigen was bound to the wells of a polystyrene microwell plate under conditions that preserve the antigen in its native state. Controls (negative, low positive and high positive) and diluted (1:101) patient's sera were added to separate wells, allowing any ASC IgA or IgG antibodies present to bind to the immobilized antigen. Unbound sample was washed, and an enzyme labeled anti-human IgA or IgG conjugate was added to each well. A second incubation allowed the enzyme labeled anti-human IgA or IgG to bind to any sample antibodies, which were attached to the microwells. After washing of unbound enzyme labeled anti-human IgA or IgG, the remaining enzyme activity was measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The absorbance (OD) of each well was read at a wavelength of 450nm within one hour of stopping the reaction.

The reactivity of each sample was calculated by dividing the OD of sample by the OD of the ASC IgA or IgG ELISA Low Positive. The result was multiplied by the number of units assigned to the ASC IgA or IgG ELISA Low Positive (25 units). The sample then was classified as negative, equivocal or positive for IgA or IgG anti-ASC according to the following scheme: Negative: 0.0 - 20.0 units; Equivocal: 20.1 - 24.9 units and Positive: ≥25 units.

Statistical analysis: Significant differences between percentage frequencies of positive and negative cases for anti-glycan (aASC and gASC) antibodies of both IBD patients (UC and CD) and controls were assessed by Pearson Chi-square test. In addition, relative risk (RR) and etiological fraction (EF) were also estimated to define the association between both types of antibodies and IBD. These estimations were made by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package (including the programs and their manuals) is available at http://www.brixtonhealth.com.

To test the usefulness of aASC and gASC antibodies as diagnostic antibodies, sensitivity and specificity, as well as, positive predicative value and negative predicative value were estimated for both classes of anti-glycan antibodies (aASC and gASC) in UC and CD, as shown in table 1. The significance of such association was assessed by two-tailed Fisher's exact probability (Akobeng, 2006).

Table (1): Defining sensitivity, specificity and predictive values.

Test	Patients	Controls	Total Tests
Positive	a	b	Positive (a+b)
Negative	c	d	Negative (c+d)
Total Number	Patients (a+c)	Controls (b+d)	a+b+c+d

Sensitivity: a/(a+c); Specificity: d/(b+d); Positive predictive value: a/(a+b); Negative predictive value: d/(c+d).

Results and Discussion

Anti-*Saccharomyces cerevisiae* IgA Antibody: The frequency distribution of positive cases for aASC antibodies showed a significant variation between IBD patients (UC and CD) and controls ($P \leq 0.001$), and such variation was related to the increased frequency of positive cases in UC (24.1%) and CD (76.0%) patients (Table 3-2). When IBD patients (UC or CD) were distributed by positivity of their sera to aASC antibodies and gender, there was no significant difference between male and female patients of each group of IBD patients, but a much higher frequency of aASCA antibodies was observed in male and female patients of CD (73.3 and 80.0%, respectively) as compared with the corresponding UC patients (26.5 and 20.0%, respectively), such differences were significant ($P = 0.006$ and 0.005, respectively), and associated with RR values of 7.46 and 16.00, respectively, and EF values of 0.64 and 0.75, respectively (Tables 3 and 4).

Anti-*Saccharomyces cerevisiae* IgG Antibody: As in the case of aASC antibodies, the frequency distribution of positive cases for gASC antibodies showed a significant variation between IBD patients (UC and CD) and controls ($P \leq 0.001$), and such variation was related to the increased frequency of positive cases in total CD (32.0%) patients in comparison with total UC patients (3.7%). The RR, EF and P values of such difference were 12.24, 0.29 and 0.002, respectively (Tables 5 and 7). Such variation was more pronounced in female CD patients in comparison with female UC patients (60.0 *vs.* 10.0%; RR = 13.50; EF = 0.56; P = 0.014) (Tables 6 and 7).

Table (2): Total inflammatory bowel disease patients (ulcerative colitis and Crohn's disease) and controls distributed by positivity of their sera to anti-*Saccharomyces cerevisiae* IgA antibody.

Inflammatory Bowel Disease Patients	Number	Anti-*Saccharomyces cerevisiae* IgA Antibodies			
		Positive		Negative	
		No.	%	No.	%
Ulcerative colitis	54	13	24.1	41	75.9
Crohn's disease	25	19	76.0	6	24.0
Controls	10	0	0.0	10	100.0

Pearson Chi-square = 26.334; D.F. = 2; $P \leq 0.001$ (Significant)

Table (3): Inflammatory bowel disease patients (ulcerative colitis and Crohn's disease) and controls distributed by positivity of their sera to anti-*Saccharomyces cerevisiae* IgA antibody and by gender.

Inflammatory Bowel Disease Patients	Total Number (M:F)	Anti-*Saccharomyces cerevisiae* IgA Antibodies							
		Males				Females			
		Positive		Negative		Positive		Negative	
		No.	%	No.	%	No.	%	No.	%
Ulcerative colitis	54 (34:20)	9	26.5	25	73.5	4	20.0	16	80.0
Crohn's disease	25 (15:10)	11	73.3	4	26.7	8	80.0	2	20.0
Controls	10 (5:5)	0	0.0	5	100.0	0	0.0	5	100.0

Table (4): Statistical evaluation of anti-*Saccharomyces cerevisiae* IgA antibody in inflammatory bowel disease patients (ulcerative colitis and Crohn's disease) and controls.

Type of Comparison	Statistical Evaluation		
	RR	EF	P
Ulcerative colitis *vs.* Controls	-	-	N.S.
Crohn's disease *vs.* Controls	-	0.76	4.6×10^{-6}
Crohn's disease *vs.* Ulcerative colitis	9.99	0.68	3.3×10^{-5}
Male Ulcerative colitis *vs.* Male Controls	-	-	N.S.
Male Crohn's disease *vs.* Male Controls	-	-	0.016
Female Ulcerative colitis *vs.* Female Controls	-	-	N.S.
Female Crohn's disease *vs.* Female Controls	-	-	0.014
Male Crohn's disease *vs.* Male Ulcerative colitis	7.46	0.64	0.006
Female Crohn's disease *vs.* Female Ulcerative colitis	16.00	0.75	0.005
Crohn's disease (Males *vs.* Females)	-	-	N.S.
Ulcerative colitis disease (Males *vs.* Females)	-	-	N.S.

RR: Relative risk; EF: Etiological fraction; P: Fisher's exact probability (two-tailed); NS: Not significant ($P > 0.05$).

Table (5): Total inflammatory bowel disease patients (ulcerative colitis and Crohn's disease) and controls distributed by positivity of their sera to anti-*Saccharomyces cerevisiae* IgG antibody.

Inflammatory Bowel Disease Patients	Number	Anti-*Saccharomyces cerevisiae* IgG Antibodies			
		Positive		Negative	
		No.	%	No.	%
Ulcerative colitis	54	2	3.7	52	96.3
Crohn's disease	25	8	32.0	17	68.0
Controls	10	0	0.0	10	100.0

Pearson Chi-square = 15.415; D.F. = 2; P ≤ 0.001 (Significant)

Table (6): Inflammatory bowel disease patients (ulcerative colitis and Crohn's disease) and controls distributed by positivity of their sera to anti-*Saccharomyces cerevisiae* IgG antibody and by gender.

Inflammatory Bowel Disease Patients	Total Number (M:F)	Anti-*Saccharomyces cerevisiae* IgG Antibodies							
		Males				Females			
		Positive		Negative		Positive		Negative	
		No.	%	No.	%	No.	%	No.	%
Ulcerative colitis	54 (34:20)	0	0.0	34	100.0	2	10.0	18	90.0
Crohn's disease	25 (15:10)	2	13.3	13	86.7	6	60.0	4	40.0
Controls	10 (5:5)	0	0.0	5	100.0	0	0.0	5	100.0

Table 7: Statistical evaluation of anti-*Saccharomyces cerevisiae* IgG antibody in inflammatory bowel disease patients (ulcerative colitis and Crohn's disease) and controls.

Type of Comparison	Statistical Evaluation		
	RR	EF	P
Ulcerative colitis *vs*. Controls	-	-	N.S.
Crohn's disease *vs*. Controls	-	-	N.S.
Crohn's disease *vs*. Ulcerative colitis	12.24	0.29	0.002
Male Ulcerative colitis *vs*. Male Controls	-	-	N.S.
Male Crohn's disease *vs*. Male Controls	-	-	N.S.
Female Ulcerative colitis *vs*. Female Controls	-	-	N.S.
Female Crohn's disease *vs*. Female Controls	-	-	N.S.
Male Crohn's disease *vs*. Male Ulcerative colitis	-	-	N.S.
Female Crohn's disease *vs*. Female Ulcerative colitis	13.5	0.56	0.014
Crohn's disease (Males *vs*. Females)	-	-	N.S.
Ulcerative colitis disease (Males *vs*. Females)	-	-	0.04

RR: Relative risk; EF: Etiological fraction; P: Fisher's exact probability (two-tailed); NS: Not significant (P > 0.05).

Sensitivity and Specificity of Anti-glycan Antibodies: The sensitivity (SN) and specificity (SP), as well as, positive predicative value (PPV) and negative predicative value (NPP) were estimated for both anti-glycan antibodies (aASC and gASC) in UC and CD. In UC, they were 20.1, 100.0, 100.0 and 19.6%, respectively for aASC antibodies, while in CD; they were 76.0, 100.0, 100.0 and 62.5%, respectively, but the difference was significant in CD only ($P = 4.6 \times 10^{-6}$). For gASC antibodies, the corresponding figures in UC (3.7, 100.0, 100.0 and 16.1%, respectively) or CD (32.0, 100.0, 100.0 and 37.0, respectively) showed no significant variations (Table 8).

Table (8): Estimation of sensitivity and specificity, positive predicative value and negative predicative value for anti-*Saccharomyces cerevisiae* IgA and antibodies in ulcerative colitis and Crohn's disease patients.

Parameter	Percentage			
	Ulcerative Colitis		Crohn's Disease	
	aASCA	gASCA	aASCA	gASCA
Sensitivity	20.1	3.7	76.0	32.0
Specificity	100.0	100.0	100.0	100.0
Positive predictive value	100.0	100.0	100.0	100.0
Negative predictive value	19.6	16.1	62.5	37.0
Probability	N.S.	N.S.	4.6×10^{-6}	N.S.

aASCA: Anti-*Saccharomyces cerevisiae* IgA antibody.
gASCA: Anti-*Saccharomyces cerevisiae* IgG antibody.
N.S.: Not significant ($P > 0.05$).

The presented results suggest that anti-glycan antibodies were of a significant value in discriminating between UC and CD, and aASC antibodies were more useful than gASC antibodies in such discrimination, especially in CD patients. This is the first observation in Iraqi IBD patients (for the best knowledge of investigator), but other world IBD patients have also shown a similar presentation, in which CD patients have always been characterized by and associated with aASC antibodies. In this regard, there has been a growing interest in the use of anti-glycan antibodies as markers for differentiation and stratification of CD, and most investigations suggest a potential use of these markers for discrimination between CD and UC, as well as prediction of complicated CD behavior and CD-related surgery (Dotan *et al*., 2006; Ferrante *et al*., 2007; Simondi *et al*., 2008; Rieder *et al*., 2010), and one of these antibodies is ASC, which has been found to be more frequently observed in CD patients (50 - 80%) compared to patients with UC (2 - 14%) (Papp *et al*., 2008). The present study share such manner of ASC distribution in UC and CD patients, in which aASC antibody status was positive in 24.1% of UC patients and 76.0% of CD patients, and the corresponding percentages for gASC were 3.7 and 32.0%. However, the question is how these antibodies were generated against *S. cerevisiae*, and why they are more frequent in CD than UC.

Generation of ASC antibodies is poorly understood, but studies have shown that their titers do not correlate with disease activity, as observed in a classical autoimmune disease, and they are regarded to be stable over long periods of time (Mow *et al*., 2004). In agreement with such theme, it has been reported that patients who have had their last flare up of CD more than 20 years ago and currently (at the time of investigation) displayed normal findings in gastroscopy, colonoscopy, and histology, but still had high titers of ASC (Rieder *et al*., 2010). Thus, these antibodies seem to represent stable serological markers, and such stability may highlight their causal relationship with CD, especially if we consider that ASC antibodies have been found to be not related to mucosal disintegrity, and they are independent of disease activity. Therefore, the suggestion was that elevated serum levels of ASC antibodies did not primarily seem to result from a defect in the gut barrier, and food antigens may have their effect (Rieder *et al*., 2011.

Food antigens have long been implicated in the etiology of IBD. Higher serum antibody titers to *S. cerevisiae* (baker's yeast) in patients with CD than in UC or healthy controls evoked much interest (Papp *et al*., 2008). A serologic twin study of antibodies (IgA, IgG and IgM) to yeast cell mannan, a whole yeast (*S. cerevisiae*) preparation, betalactoglobulin, gliadin and ovalbumin was performed, and two important observations were made. In the first, individuals with UC were indistinguishable from healthy twins and controls, while in the second, twins who had developed CD displayed higher antibody titers towards yeast cell mannan in particular, but also to whole yeast (*S. cerevisiae*). These observations suggested that mannan, or some antigens rich in mannose and cross reacting with mannan, may play an etiological role in CD, but not in UC. This study demonstrates that cell wall mannan was the main antigen that triggers production of ASC antibodies (Halme *et al*., 2006). Furthermore, ASC antibodies have been reported in sera of CD patients who had young age at onset (Biank *et al*., 2007). Accordingly, several studies suggested that ASC antibodies reflects the load of occurrence of CD in families due to their earlier exposure to *S. cerevisiae* in their life, but whether ASC antibodies are a familial trait due to a genetic factor or to increased exposure to an environmental factor is still not well-understood (Gruber *et al*., 2012). However, twin studies may help to make some understanding. In one of these studies, 98

twin pairs were investigated and ASC antibodies were found in 57% of twins with CD, 12% of twins with UC, 17% of healthy twin siblings to twins with CD and 14% of healthy twin siblings to twins with UC. Mean ASCA antibody titers in these four groups were 15.8, 4.2, 5.4, and 4.3 U/ml, respectively. There was no increased occurrence of ASC antibodies in healthy twin siblings in discordant monozygotic twin pairs with CD compared with dizygotic twins (Halfvarson *et al*., 2005). These findings contradict the hypothesis that ASC antibodies may be a genetic susceptibility marker for CD and the results rather suggest that ASC antibodies in healthy family members is a marker of shared environment (Rogler, 2011). In concordant monozygotic twin pairs with CD a high degree of similarity in the level of ASC antibody titers was observed within each pair. This suggests that ASC is associated with CD and a marker of response to an environmental antigen (i.e. *S. cerevisiae*), but the level of response might be determined by specific gene (Cooney and Jewell, 2009).

Other studies have explained the association between IBD (more specifically CD) and ASC antibodies in the ground of dysregulation of mucosal immunity, and as IBD patients have abnormal responses to food antigens, it has been suggested that the generated immune response (production of ASC antibodies) is considered to be secondary to inflammation and damage to the integrity of the intestinal wall; causing increased exposure of the immune system to antigenic contents of the bowel lumen, including *S. cerevisiae* antigens in baker's yeast (Oshitani *et al*., 2003). However, the highly specific association of ASC with CD has been hard to explain because there is no correlation between ASC and intestinal permeability (Reumaux *et al*., 2003). Thus the physiological importance of ASC remains unclear. The presence of IgG ASC-reactive antigens in the granulomas of bowel resections and on infiltrating lymphocytes and neutrophils in inflamed tissue from CD patients, are consistent with antigen specificity (or cross-reactivity) and suggest that *S. cerevisiae* itself may have some primary pathogenic role in CD (Severance *et al*., 2013). Therefore, it is hypothesized that a defect in immunological tolerance to *S. cerevisiae* may indicate a global loss of tolerance to fungal antigens, which consequence in production of ASC antibodies, but it remains unclear whether ASCA antibodies are pathogenic per se or only a specific phenomenon accompanying a subset of CD patients. To answer this question it would be important to identify the antigen recognized by ASCA antibodies within the small bowel, which is not well-established (Comito *et al*., 2014).

In conclusion, ASC antibodies can be considered as important diagnostic and discriminatory parameters in UC and CD, and IgA ASC antibodies are better than IgG ASC antibodies in defining CD.

References

Actis, G.C., Rosina, F., Mackay, I.R., 2011. Inflammatory bowel disease: beyond the boundaries of the bowel. Expert Rev. Gastroenterol. Hepatol., 5:401-410.

Akobeng, A.K., 2006. Infliximab for induction and maintenance therapy for ulcerative colitis. J. Pediatr. Gastroenterol. Nutr., 42: 589-590.

Biank, V., Broeckel, U. and Kugathasan, S., 2007. Pediatric inflammatory bowel disease: clinical and molecular genetics. Inflamm. Bowel. Dis., 13: 1430-1438.

Comito, D., Cascio, A. and Romano, C., 2014. Microbiota biodiversity in inflammatory bowel disease. Ital J. Pediatr., 40: 32 (in print).

Cooney, R. and Jewell, D., 2009. The genetic basis of inflammatory bowel disease. Dig Dis., 27: 428-442.

Danese, S., Fiocchi, C., 2006. Etiopathogenesis of inflammatory bowel diseases. World J. Gastroenterol., 12: 4807-4812.

Dotan, I., Fishman, S., Dgani, Y., Schwartz, M., Karban, A., Lerner, A., Weishauss, O., Spector, L., Shtevi, A., Altstock, R.T., Dotan, N. and Halpern, Z., 2006. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. Gastroenterol., 131: 366-378.

Ferrante, M., Henckaerts, L., Joossens, M., Pierik, M., Joossens, S., Dotan, N., Norman, G.L., Altstock, R.T., Van Steen, K., Rutgeerts, P., Van Assche, G. and Vermeire, S., 2007. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. Gut., 56: 1394-1403.

Ferrante, M., Henckaerts, L., Joossens, M., Pierik, M., Joossens, S., Pierik, M., Joossens, S., Dotan, N., Norman, G.L., Altstock, R.T., Van Steen, K., Rutgeerts, P., Van Assche, G. and Vermeire, S., 2007. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. Gut., 56: 1394-1403.

Gary, R.L., 2012. Inflammatory bowel disease. Goldman's Cecil Medicine , 1 : 913-921.

Gruber, L., Lichti, P., Rath, E. and Haller, D., 2012. Nutrigenomics and nutrigenetics in

inflammatory bowel diseases. J. Clin. Gastroenterol., 46: 735-747.

Halfvarson, J., Standaert-Vitse, A., Järnerot, G., Sendid, B., Jouault, T., Bodin, L., Duhamel, A., Colombel, J.F., Tysk, C. and Poulain, D., 2005. AntiSaccharomyces cerevisiae antibodies in twins with inflammatory bowel disese. Gut, 54: 1237-1243.

Halme, L., Paavola-Sakki, P., Turunen, U., Lappalainen, M., Farkkila, M. and Kontula, K., 2006. Family and twin studies in inflammatory bowel disease. World J Gastroenterol., 12: 3668-3672.

Mow, W.S., Vasiliauskas, E.A., Lin, Y.C., Fleshner, P.R., Papadakis, K.A., Taylor, K.D., Landers, C.J., AbreuMartin, M.T., Rotter, J.I., Yang, H. and Targan, S.R. (2004). Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. Gastroenterol., 126: 414-424.

Oshitani, N., Hato, F., Suzuki, K., Sawa, Y., Matsumoto, T., Maeda, K., Higuchi, K., Kitagawa, S. and Arakawa, T. 2003. Cross-reactivity of yeast antigens in human colon and peripheral leukocytes. J. Pathol., 199: 361-367.

Papp, M., Altorjay, I., Dotan, N., Palatka, K., Foldi, I., Tumpek, J., Sipka, S., Udvardy, M., Dinya, T., Lakatos, L., Kovacs, A., Molnar, T., Tulassay, Z., Miheller, P., Norman, G.L., Szamosi, T., Papp, J., Hungarian IBD Study Group and Lakatos, L., 2008. 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., 103: 665-681.

Podolsky, D.K., 2002. Inflammatory bowel disease. N. Engl. J. Med. 347: 417-29.

Reumaux, D., Sendid, B., Poulain, D., Duthilleul, P., Dewit, O. and Colombel, J.F., 2003. Serological markers in inflammatory bowel diseases. Best Pract Res. Clin. Gastroenterol., 17: 19-35.

Rieder, F., Lopez, R., Franke, A., et al. (2011). Characterization of changes in serum anti-glycan antibodies in Crohn's disease - a longitudinal analysis. PLoS One, 6: e18172.

Rieder, F., Schleder, S., Wolf, A., Dirmeier, A., Strauch, U., Obermeier, F., Lopez, R., Spector, L., Fire, E., Yarden, J., Rogler, G., Dotan, N. and Klebl, F., 2010. Serum antiglycan antibodies predict complicated Crohn's disease behavior: a cohort study. Inflamm. Bowel. Dis., 16: 1367-1375.

Rogler, G., 2011. Interaction between susceptibility and environment: examples from the digestive tract. Dig Dis., 29:136-143.

Severance, E.G., Gressitt, K.L., Yang, S., Stallings, C.R., Origoni, A.E., Vaughan, C., Khushalani, S., Alaedini, A., Dickerson, F.B. and Yolken, R.H., 2013. Seroreactive marker for inflammatory bowel disease and associations with antibodies to dietary proteins in bipolar disorder. Bipolar Disord., 6: 10.1111/bdi.12159.

Shaheen, E.L. and Annette, K., 2010. Neuroinflammation in inflammatory bowel disease. Journal of Neuroinflammation . 7: 37.

Stange, E.F., Travis, S.P., Vermeire, S., Beglinger, C., Kupcinkas, L., Geboes, K., Barakauskiene, A., Villanacci, V., Von Herbay, A., Warren, B.F., Gasche, C., Tilg, H., Schreiber, S. W., Schölm, J. and Reinisch, W. 2006. European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. Gut, 55: 1-15.