**ORIGINAL ARTICLE**

**Title:** Diagnostic and clinical significance of Crohn's disease-specific anti-MZGP2 pancreatic antibodies by a novel ELISA

**Short Title**: New anti-MZGP2 ELISA in Crohn's

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**List of Abbreviations:** ANCA, anti-neutrophil cytoplasmic antibodies; ASCA, anti-*Saccharomyces cerevisiae* antibody; CrD, Crohn's disease; GP2, glycoprotein 2; IBD, inflammatory bowel disease; IFA, immunofluorescence assay; MZGP2, major zymogen granule membrane glycoprotein 2; PAB, pancreatic autoantibody; UC, ulcerative colitis

**ABSTRACT (words: 335)**

The recent identification of the pancreas major zymogen granule membrane glycoprotein 2 (MZGP2) as the major autoantigen of pancreatic autoantibody (PAB) has led to the appreciation of anti-MZGP2 antibodies as specific markers of Crohn's disease (CrD). We have recently developed new, robust, highly sensitive and specific IgA and IgG anti-MZGP2 antibody ELISAs and assessed their clinical relevance in the largest inflammatory bowel disease (IBD) cohort tested to date for anti-MZGP2 antibodies. In contrast to currently available anti-MZGP2 ELISAs, the new QUANTA Lite® MZGP2 ELISA (INOVA Diagnostics, San Diego, CA) utilizes the eukaryotically-expressed specific isoform 4 of human GP2 UniProtKB: P55259. A total of 832 sera were studied including 617 consecutive IBD patients (323 CrD and 294 UC) under regular follow-up in a tertiary centre, 112 patients with various diseases, and 103 healthy blood donors. The new ELISA's calculated AUC was 0.5968, 95% CI (0.5552, 0.6383) for IgA anti-MZGP2 [CD *vs* non-CD (UC plus controls)] and 0.6236, 95% CI (0.5813, 0.6659) for IgG anti-MZGP2. The sensitivity of IgA anti-MZGP2 for CrD in the IBD population was 15% and the specificity was 98% (95, 99), while the sensitivity and specificity of IgG anti-MZGP2 was 27% and 97%. IgA and IgG anti-MZGP2 combined testing led to a sensitivity of 31% and specificity of 96%. Positivity for either ASCA (IgA or IgG) or anti-MZGP2 (IgA or IgG) showed a sensitivity of 75% (70, 80) and specificity of 84% (79, 89). Of clinical relevance, IgA anti- MZGP2 antibodies were more prevalent in patients with early disease onset (Montreal classification A1, p=0.011), while patients with localised colonic disease were less likely to be IgG anti-MZGP2 positive. Anti-MZGP2 positive patients more frequently had extensive disease with ileal involvement and stricture formation. Patients with longer disease duration were more likely to have IgG anti-MZGP2 or IgA ASCA antibodies. In conclusion, the new IgA and IgG anti-MZGP2 antibody ELISAs allow accurate autoantibody determination, and can be used as a tool to study the clinical significance and utility of these autoantibodies in patients with IBD.

## INTRODUCTION

The exact mechanisms responsible for the induction of Crohn's disease (CrD) as well as ulcerative colitis (UC), the other form of inflammatory bowel disease (IBD), remain poorly understood [1-4]. Both diseases are characterised by antibody seropositivity against distinct antigens, which complement the endoscopic and histological examinations used for the prompt diagnosis of patients with suspected IBD [5, 6].

The most widely used antibody marker for CrD is anti-*Saccharomyces cerevisiae* antibody (ASCA), while UC is characterized by the presence of anti-neutrophil cytoplasmic antibodies (ANCA) showing an atypical perinuclear (pANCA) pattern by indirect immunofluorescence assay (IFA) [5, 6]. While most other antibody markers failed to meet demanding clinical needs, pancreatic autoantibody (PAB) has emerged as potentially diagnostically and clinically meaningful marker [7]. Antigen-specific PABs against exocrine pancreas are present in 20-30% of patients with CrD, but in less than 2-9% of patients with UC, and in very few patients with non-IBD related pathology [8, 9]. The recent identification of the major zymogen glycoprotein 2 (MZGP2) as the primary autoantigen of PAB [10], has prompted the development of ELISAs or IFA techniques to allow the proper detection of anti-MZGP2 PABs in routine practice [11, 12]. Rodent pancreatic tissue or GP2- over-expressed cell-lines have been used as substrates to test for GP2-specific PABs by IFA, but because IFA procedures are labor-intensive, time-consuming, and require experienced operators, laboratories prefer to use ELISA-based assays [7, 10-19]. ELISA testing for anti-MZGP2 antibodies has recently become available [11], but the assay is not FDA-approved for *in vitro* diagnostic use in the USA. In addition, the performance characteristics of these test systems have only been compared to those obtained by *in-house* assays used for research protocols in a small number of European Institutions [7, 18].

The aim of the present study was to develop new robust and highly sensitive and specific anti-MZGP2 antibody ELISAs for commercial use. The new ELISAs for the accurate

determination of IgA or IgG anti-MZGP2 have been developed and tested using sera from 617 patients with IBD, including 323 patients with CrD and 294 patients with UC, as well as 215 pathological and normal controls. The newly developed ELISAs not only allowed better assessment of the diagnostic significance of anti-MZGP2 antibodies, but also gave us the opportunity to investigate the clinical significance of these autoantibodies and their relevance to distinct clinical phenotypes.

## PATIENTS AND METHODS

### Patients

Six hundred seventeen consecutive patients with a diagnosis of IBD (CrD 323, female/male 176/147, age 40±14.3, disease duration 14 years IQR[7,22]; UC 294, female/male 141/153, age 48.7±15.7, disease duration 14 years IQR[6,25]), under regular follow up in a tertiary centre (University College London Hospitals, United Kingdom) were included in this study.

The IBD patient characteristics are presented in Table 1.The IBD diagnosis was based on current standard clinical, radiological, endoscopic, and histological criteria (Lennard-Jones criteria [20]). Demographics and disease information including age at study, age at diagnosis, disease duration, location/extent and behaviour were extracted from a prospectively updated IBD database. The disease phenotypes were determined according to the Montreal classification[21] .

Additionally, 112 patients with various diseases were studied as pathological controls, including serum samples from patients with the following diagnoses: celiac disease (n=20); chronic pancreatitis (n=19); diabetes mellitus (n=20); primary sclerosing cholangitis (n-21); primary biliary cirrhosis (n=10); autoimmune hepatitis/PSC overlap syndrome (n=6); chronic hepatitis B (n=8); and chronic hepatitis C (n=8}. Finally, 103 randomly selected blood donors (age17-60, sex female/male 64/39) were also studied as normal controls. Investigators

performing tests were blinded to the patients' exact diagnoses. The study was conducted in accordance with the Helsinki declaration and approved by the local ethics committees. Written informed consent was obtained from each individual. All sera had been stored at −20°C before analysis.

**IgA and IgG ASCA testing by ELISA**

Determination of IgA and IgG ASCA antibodies were determined by an FDA-cleared ELISA (QUANTA LIte® ASCA IgG and ASCA IgA, INOVA Diagnostics) following the manufacturer's protocol. A cut off for positivity was set at 25 U (arbitrary units) as recommended by the manufacturer.

**IgG and IgA anti-MZGP2 antibody testing by ELISA**

MZGP2 IgG and IgA antibodies were detected by novel ELISAs (INOVA Diagnostics, Research Use Only) utilizing human recombinant MZGP2, isoform 4 antigen UniProtKB: P55259. Briefly, 100 µLs of pre-diluted control and diluted patient sera (1:100) were added to separate wells of MZGP2 antigen-coated polystyrene microwells and incubated for 30 min at room temperature. Unbound sample was then washed away and peroxidase-conjugated goat anti-human IgG antibody or anti-human IgA antibody was added to each well. After another incubation and washing steps, the remaining enzyme activity was measured by adding tetramethylbenzidine chromogenic substrate for 30 minutes. Stop solution ($H_2SO_4$) was added to terminate the reaction and absorbance read at 450/620 nm. Results, expressed in arbitrary units (U), were calculated in reference to a kit-provided calibrator. Results ≥ 25 U were interpreted as positive

**ANCA testing by IFA**

ANCA for IgG antibodies were evaluated by indirect immunofluorescence using commercially available human neutrophil slides (Nova Lite™, INOVA Diagnostics). Briefly, samples were diluted at 1:20 and tested in accordance with the manufacturer's instructions on ethanol- and formalin-fixed human neutrophil substrate slides. Results were reported as P-ANCA if a perinuclear pattern was observed on ethanol and granular cytoplasmic on formalin slides, C-ANCA if both ethanol and formalin slides resulted in a cytoplasmic pattern, and "atypical" ANCA if the pattern was perinuclear on ethanol and negative on the formalin fixed slide.

**Pancreatic Antibodies (PAB) by IFA**

Pancreatic antibodies (PAB) were detected by IFA on monkey pancreas tissue (NOVA Lite™, INOVA Diagnostics) using sera at a 1:20 dilution and primate-absorbed goat anti-human FITC conjugate.

**Statistical Methods**

Variables were tested for normality with the Kolmogorov-Smirnov test. Age is presented as mean and standard deviation (SD). Non-parametric continuous variables including ASCA and anti-MZGP2 titres are given as median and interquartile range (IQR). The report of the atypical ANCA is qualitative (positive or negative) based on immunofluorescence review by one of the authors (DPB). The cut off for anti-MZGP2 IgA and IgG assays was calculated by plotting a receiver operator characteristic (ROC) curve by using the test results of the patients with CrD versus controls (UC, healthy, other pathological controls). The area under the curve (AUC) values are followed by a 95% confidence interval (CI). The diagnostic value of ASCA, anti-MZGP2 and atypical ANCA for the IBD population was assessed by cross tabulation and calculation of sensitivity, specificity, positive and negative predictive values,

all presented as percentages followed by 95% CI. The clinical significance of the different antibodies was studied with chi-square tests for every clinical variable (2x2 tables) and the results are presented as odds ratios with 95% CI and p values. Associations between variables found on univariate analysis to have statistically significant ($p<0.05$) high prevalence in patients testing positive for individual autoantibodies or autoantibody combinations were further tested by loglinear regression. Comparisons in titre medians between different diseases or disease subgroups were performed using the non-parametric Mann Whitney or Kruskal Wallis tests. Comparisons of parametric variables (ie age) were performed using the upaired t-test. Cross tabulation and loglinear analysis was performed using SPSS (SPSS Inc., Chicago, Illinois, USA) software. Prizm software (by GraphPad Software Inc., La Jolla, California, USA) was used for ROC curve plotting, antibody titre comparisons and figures.

## RESULTS

### Diagnostic accuracy of serological markers

Venn diagrams depicting numbers of CrD or UC cases showing individual reactivities are shown in Figure 1. Scatter plots of anti-MZGP2 antibody reactivities (IgG or IgA) in patients with CrD, UC, pathological, and normal controls are shown in Figure 2. The ROC curves for anti-MZGP2 IgA and IgG assays (CrD *vs* controls) are also presented as inserts in Figure 2. The calculated AUC was 0.5982, 95% CI (0.5567, 0.6398) for IgA anti-MZGP2 [CD *vs* non-CD (UC and controls)] and 0.6240, 95% CI (0.5817, 0.6662) for IgG anti-MZGP2. The sensitivity, specificity and likelihood ratio for different cut offs of anti-MZGP2 are presented in Table 2 (for CrD *vs* non-CrD cohorts, including UC, pathological and normal controls) and in Table 3 (for CrD *vs* UC), respectively.

The sensitivity of IgA anti-MZGP2 for CrD in the IBD population was 15% (11, 19) and the specificity was 98% (95, 99), while the sensitivity of IgG anti-MZGP2 for CD was 27% (22,

32) and the specificity was 97% (94-98) using the manufacturer's cut-off set at 25 U. In comparison, the sensitivity of IgA and IgG ASCA for CrD was 47% (41, 52) and 66% (61, 71), respectively, while the specificity was 95% (92, 97) and 90% (86, 93), respectively for CrD *vs* UC. The combination of positive IgA and IgG ASCA testing increased the sensitivity to 71% (66, 76) but reduced the specificity to 87% (83,91). Positivity for either ASCA (IgA or IgG) or anti-MZGP2 (IgA or IgG) showed a sensitivity of 75% (70, 80) and specificity of 84% (79, 89).

The presence of any one of the autoantibodies (ASCA IgA, ASCA IgG, MZGP2 IgA, MZGP2 IgG) yielded the highest sensitivity at 75% (70, 80), but reduced specificity to 84% (79, 89). In contrast, while only 7% sensitive, the presence of all four autoantibodies (ASCA IgA, ASCA IgG, MZGP2 IgA, MZGP2 IgG) in 23 individuals (Table 4 and Figure 1) was 100% specific for CrD, being negative in all 294 patients with ulcerative colitis. Dual positivity for MZGP2 IgA and IgG was slightly less specific at 99% (11% sensitivity), followed by dual positivity for ASCA IgA and IgG at 98% specificity and 42% sensitivity and single IgA MZGP2 positivity with a specificity of 98% and a sensitivity of 15%.

The sensitivity of IgG atypical (x)ANCA testing for UC in the IBD population was 36% (31, 42) and the specificity was 91% (87,94). Sensitivity, specificity, negative, and positive predictive values are presented for all autoantibodies and their combinations in Table 4.

As expected, ASCA and anti-MZGP2 antibody titres were higher in CrD compared to UC; the difference of the median titres for all antibody reactivities between CrD and UC was statistically significant (Mann Whitney, anti-MZGP2 IgA $p= 0.0045$, anti-MZGP2 IgG $p<0.0001$, ASCA IgA $p<0.0001$ and ASCA IgG $p<0.0001$). Figure 3 shows ASCA levels in IgA or IgG anti-MZGP2 antibody positive and negative patients with CrD.

**Clinical Significance of Antibody**

Table 5 presents the associations between the autoantibodies and different disease characteristics. IgA anti-MZGP2 antibodies were more prevalent in patients with early disease onset (A1< 16 years, OR: 2.3 [1.2, 4.4], p=0.011). Patients positive for IgG ASCA were younger when compared to negative CrDs (mean age 22.92 +/- 0.62 vs. 28.02 +/- 1.44, upaired t test, p=0.0002) and were less likely to have late disease onset (A3) (p=0.003 for IgG and p=0.026 for IgA). Patients with A3 disease onset had lower titres for IgA or IgG ASCA (median IgA ASCA titre for A1: 23.6, A2: 23.4, A3: 9, p=0.002, median IgG ASCA titre for A1:42, A2: 45.5, A3: 16.8, p= 0.001). Also, IgA anti-MZGP2 titres were higher in younger patients (median IgA anti-MZGP2 titre for A1: 7.4, A2: 4.3, A3: 3.8, p=0.04).

Patients positive for IgG anti-MZGP2 or (IgA or IgG) ASCA were more likely to have extensive CrD with ileal involvement (OR: 2.3, 1.7, 1.9, respectively). The presence of both (IgA or IgG) anti-MZGP2 and (IgA or IgG) ASCA increased the OR for extensive disease (L3) to 2.8 (1.5, 5.2). Patients with localised colonic disease were less likely to be positive for these antibodies; IgA ASCA, IgG ASCA and IgG anti-MZGP2 titres were also lower in these patients and higher in patients with extensive disease (median IgA ASCA titre for L1: 24.05, L2: 12.2, L3: 26.6, L4: 15.1, p=0.02; median IgG ASCA titre for L1: 46.95, L2: 16.8, L3: 49.1, L4: 54.5, p=0.001; median IgG anti-MZGP2 titres for L1: 4.55, L2: 3.7, L3: 7.4 0.156, L4: 3.7, p=0.046, (Figure 4).

Stricturing disease (B2) was more likely in patients tested positive for IgG ASCA (OR:2.3 [1.3,4]), while the presence of both (IgA or IgG) anti-MZGP2 and (IgA or IgG) ASCA increased the OR for B2 to 3.1 (1.5, 6.3). Antibody titres were also higher in B2 in comparison to B1, B3 (median ASCA IgA titre for B1: 17.3, B2: 29.1, B3: 25.35, p=0.007, median IgG ASCA titre for B1: 35.2, B2: 53.7, B3: 45.2, p=0.033). IgA ASCA, IgG ASCA or (IgA, IgG) anti-MZGP2 were less prevalent in patients with inflammatory behaviour (B1).

Patients with longer disease duration were more likely to have IgG anti-MZGP2 (difference in medians: 2 years), or IgA ASCA antibodies (difference in medians: 5.5 years).

Atypical ANCA were not associated with sex, age of onset, disease duration, disease extent or the requirement for colectomy and stoma formation in UC patients. UC patients positive for atypical ANCA though, were older on disease onset (mean age: 32.35 vs. 25.67, p=0.02) and had longer disease duration (median duration 16.5 vs. 12 , p= 0.03) when compared to positive patients with CrD.

Of the 13 ulcerative colitis patients positive for MZGP2 IgG and/or IgA, 2 were also positive for both ASCA IgG , ASCA IgA, and both IgG and IgA pancreatic antibodies by IFA. Four other patients showed moderate to strong PAB (IgG and/or IgA) by IFA. Of the 5 normal donors found positive for MZGP2 IgG, 1 was ASCA IgG and IgA positive with1-2+ IgA PAB, and 3 others showed 1-2+ IgA PAB and nonspecific IgG PAB. The one very strong positive chronic pancreatic patient had no clinical features identified which distinguished them from the other chronic pancreatitis patients.

**DISCUSSION**

In the present study, we report development of two robust, highly-specific ELISAs for the detection of IgA and IgG anti-MZGP2 PABs. Using these ELISAs, we have detected anti-MZGP2 antibodies in 31% of patients with CrD and 4% of UC patients. Among the reactive CrD patients, 27% and 15% showed IgG or IgA anti-MZGP2 reactivity, while reactivity to both isotypes was concurrently present in 11% of the CrD patients, but in just 1% of patients with UC.

Cumulatively, the new ELISAs demonstrate enhanced sensitivity and superior specificity for CrD within IBD compared to commercial anti-MZGP2 ELISAs currently available (GA). A recent study using GA's ELISA tested 3 cohorts – two from Germany and one from our centre – and reported an overall (IgA or IgG) anti-MZGP2 sensitivity and specificity of 30.2% and 91%, respectively [13]. Additional testing on a Belgium cohort using the GA ELISA has showed anti-MZGP2 antibodies in 21% of CrD patients and 9% of UC patients [14].

Interestingly, other studies reported GA ELISA anti-MZGP2 antibody reactivity in up to 22% of patients with UC [10-12, 15-19]. The low specificity reported for this assay raises significant concerns for the diagnostic utility of this test and its incorporation into routine testing of individuals assessed for IBD. The lower sensitivity and specificity of the GA ELISA in the Belgian cohorts has been attributed to the difference in the geographic origin of the patients and/or the selection criteria for involvement in the studies [22]. Methodological issues were not raised, as both studies have used the GA ELISA kit and were performed by exceedingly qualified research laboratories [11, 22].

The majority of studies have agreed upon the fact that most IgA anti-MZGP2 antibody positive CrD patients have concurrent IgG antibodies against the same antigen [10, 11, 13-16]. In the present study, we also noted that IgA anti-MZGP2 antibody positivity marginally increases the over-all sensitivity of the test, as only 4.3% (14/323, Figure 1) of the IgG anti-MZGP2 seropositive cases had only IgA anti-MZGP2 antibodies. This finding points towards the limited diagnostic utility of the IgA antibodies, but does not exclude their clinical relevance, as there are several clinical correlates associated with the presence of IgA anti-MZGP2 only (see Table 3).

At a first glance, ASCA (IgA or IgG) remain the most sensitive antibody tests for CrD with a sensitivity of 71% compared to 31% for anti-MZGP2 (IgA or IgG) [6]. However, ASCA testing shows the lowest specificity for CrD in IBD (87%), while that of anti-MZGP2 is much higher (96%). This difference in the overall specificity is clinically significant. The sensitivity of anti-MZGP2 for CrD in IBD could be even higher if the cut off was set to less stringent specificity. For example if a 94% specificity was targeted, the cut off could be lowered to 15 U for both IgA and IgG anti-MZGP2 and the resulting sensitivity of anti-MZGP2 for CrD could reach as much as 24% for IgA and 35% for IgG anti-MZGP2 (Table 2). Studies directly investigating the performance of the two assays in well-defined IBD serum samples, pre-characterised as ‘equivocal’, low, or moderate level seropositive sera from IBD patients and controls are needed to clarify this topic. The relatively low specificity of ASCA for CrD in IBD is a well-

described feature and is one of the greatest limitations of this test [6]. Nevertheless, simultaneous detection of anti-MZGP2 and ASCA (of any isotype) is practically absent in patients with UC. The assays can be used to help "rule out" UC. In other words, if a patient with a suspicion of IBD is seropositive for both ASCA and anti-MZGP2, they are unlikely to have UC (PPV for CD: 100%). Also, 12 out of 87 (14%) CrD ASCA negative patients had anti-MZGP2 antibodies, which could suggest that 14% of clinically suspicious individuals (who were reliant on ASCA positivity for a firm diagnosis of CrD), may have gone unnoticed if anti-MZGP2 antibody testing was not ordered. The detection of the MZGP2 antibodies can alert the clinician to the potential presence of CrD and suggest additional evaluation of the patient.

There is no doubt that the development of these new assays will promote more research on the diagnostic and clinical value of MZGP2 antibodies in IBD [7, 18]. Using our new assays, together with the wealth of detailed clinical data available on the study cohort patients, we have found several associations between patients with IgA or IgG anti-MZGP2 antibody reactivities and clinical parameters. Some of these associations have been previously described and are confirmed with our new assay, while others are newly described [10, 11, 14-19]. Using earlier ELISAs, we have found that anti-MZGP2 antibody reactivity is a characteristic feature of patients with ileocolonic location [13, 16]. We have been able to replicate this finding using the INOVA anti-MZGP2 ELISAs. We also noted in the past that younger age (A1) at diagnosis connotes the characteristic feature of a higher prevalence of anti-MZGP2 antibodies within this group compared to patients diagnosed at older age (A2 and A3) [13]. This finding has also been confirmed by the new ELISA testing. Finally, using the GA ELISA, anti-MZGP2 antibodies have been able to identify patients with stricturing disease. Intriguingly, stricturing disease (B2) was more likely (OD: 3.1) in patients testing positive for the presence of ASCA or anti-MZGP2 by the new ELISA, further underlined the notion that simultaneous testing of these autoantibodies may be of help to treating physicians.

Our study has revealed previously unnoticed associations. For example, patients positive for IgG anti-MZGP2 were more likely to have extensive CrD with ileal involvement, which was also the case for IgA or IgG ASCA (OR: 2.3, 1.7, 1.9, respectively). As this ELISA uses a new form of the MZGP2 protein (Isoform 4) as well as a new assay configuration, the clinical associations reported here must be validated externally. Anti-MZGP2 antibodies have recently been determined by a commercial IFA using GP2-overexpressing cell lines (EUROIMUNN)[12], however the performance relative to the new ELISAs is unknown. In conclusion, our new ELISA permits the accurate detection of GP2 PAB-specific autoantibodies with high efficiency. Though firm conclusions regarding the clinical utility of anti-MZGP2 antibodies cannot be reached at this time, we are confident that the present data mirror results that could be obtained by independent investigators. Significantly, our study enrolled the largest number of CrD and UC patients investigated thus far in a single centre and supports the strength of the study and its conclusions. Some of the findings published in the past have included cumulative data merged from cohorts of various centres, and this may explain inconsistencies amongst publications [7, 18]. We suggest that testing of large series of uniformly collected and selected serum samples from independent cohorts, as was done in the present study, will provide insight into the diagnostic and clinical value of these autoantibodies, in combination or in isolation, for the more effective assessment and management of patients.

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**Legends to the Figures**

**Figure 1.** Venn diagrams of individual IgA or IgG anti-*Saccharomyces cerevisiae* antibody (ASCA) and anti-major zymogen granule membrane glycoprotein 2 (MZGP2) serum antibody reactivity of patients with Crohn's disease (CD) and ulcerative colitis (UC)

**Figure 2**. Anti-MZGP2 antibody reactivity(IgG, left and IgA right) in 323 patients with Crohn's disease (CrD), 294 with ulcerative colitis (UC) patients and in pathological controls including patients with coeliac disease (n=20); chronic pancreatitis (n=19); diabetes mellitus, DM (n=20); primary biliary cirrhosis, PBC (n=10); primary sclerosing cholangitis, PSC (n-21); autoimmune hepatitis(AIH)/PSC overlap syndrome (n=6); chronic viral hepatitis (viral hep) (chronic viral hepatitis B (n=8); and chronic viral hepatiis C (n=8); Normal controls consisted of 103 randomly selected blood donors.

**Figure 3.** ASCA antibody reactivity in anti-MZGP2 antibody positive (n=98) and anti-MZGP2 antibody negative (n=225) patients with Crohn's disease

**Figure 4.** Scatter plot analysis of individual antibody reactivities seen in 323 patients with Crohn's disease stratified in accordance to disease location (L1-L4, Montreal classification). A scatter plot for IgA anti-MZGP2 was not included because comparison of titres did not show statistically significant differences.

**Table 1**. Major demographic and clinical characteristics of the 323 Crohn's disease (CrD) and 294 ulcerative colitis (UC) patients enrolled in the study.

| | **CrD (n=323)** | **UC (n=294)** |
|---|---|---|
| | | |
| **Sex (m/f)** | 147/176, (46% / 54%) | 153/141, (52%/ 48%) |
| **Age** | 40 (±14.3 SD) | 48.7 (±15.7SD) |
| | | |
| **Age at diagnosis (years)** | 24.7 (±11.7SD) | 31.98(±13.9SD) |
| **Disease Duration (years)** | 14 (IQR 7.22) | 14 (IQR 6,25) |
| | | |
| **Age** | A1: 76 (24%) | |
| | A2: 214 (66%) | |
| | A3: 33 (10%) | |
| | | |
| **Location* (L)/ Extent* (E)** | L1: 38 (12%) | E1: 28 (9%) |
| | L2: 53(16%) | E2: 82 (28%) |
| | L3: 227 (70%) | E3: 184 (63%) |
| | L4: 5 (2%) | |
| | | |
| **Behavior *(B)** | B1: 168 (52%) | |
| | B2: 83 (26%) | |
| | B3: 72 (22%) | |
| | Perianal: 72 (22%) | |
| | | |

According to Montreal classification A (Age); A1, below 17 years; A2 between 17 and 40 years; A3, above 40 years; L (Location), L1, ileal; L2, colonic; L3, ileocolonic, and L4, upper disease modifier; B1, non-stricturing; B2, structuring; B3, penetrating behavior

**Table 2.** Diagnostic accuracy of anti-MZGP2 antibodies (IgA, IgG) for different cut offs in Crohn's disease v*ersus* pathological (including ulcerative colitis) and normal controls. Results are presented as sensitivity (% and 95% confidence intervals values), specificity, (% and 95% confidence intervals values) and likelihood ratios.

| IgA anti-MZGP2 cut off | Sensitivity (%) | 95% CI | Specificity (%) | 95%CI | Likehood Ratio |
|---|---|---|---|---|---|
| > 10 | 31.27 | 26.25% to 36.63% | 88.61 | 85.52% to 91.23% | 2.74 |
| > 15 | 23.84 | 19.30% to 28.87% | 93.71 | 91.24% to 95.66% | 3.79 |
| > 20 | 17.96 | 13.93% to 22.59% | 95.87 | 93.76% to 97.43% | 4.35 |
| > 25 | 14.55 | 10.89% to 18.88% | 97.45 | 95.67% to 98.63% | 5.70 |
| > 30 | 12.07 | 8.729% to 16.13% | 98.62 | 97.19% to 99.45% | 8.78 |
| > 40 | 10.22 | 7.138% to 14.05% | 99.02 | 97.72% to 99.68% | 10.40 |

| IgG anti-MZGP2 cut off | Sensitivity (%) | 95% CI | Specificity (%) | 95%CI | Likehood Ratio |
|---|---|---|---|---|---|
| > 10 | 39.94 | 34.56% to 45.51% | 89.98 | 87.04% to 92.45% | 3.99 |
| > 15 | 34.67 | 29.49% to 40.14% | 93.71 | 91.24% to 95.66% | 5.52 |
| > 20 | 30.34 | 25.37% to 35.67% | 95.28 | 93.07% to 96.96% | 6.43 |
| > 25 | 26.63 | 21.88% to 31.80% | 96.66 | 94.71% to 98.04% | 7.97 |
| > 30 | 25.08 | 20.44% to 30.17% | 97.64 | 95.92% to 98.78% | 10.64 |
| > 40 | 22.29 | 17.87% to 27.23% | 98.62 | 97.19% to 99.45% | 16.21 |

**Table 3**. Diagnostic accuracy of anti-MZGP2 antibodies (IgA, IgG) for different cut offs in Crohn's disease v*ersus* ulcerative colitis. Results are presented as sensitivity (% and 95% confidence intervals values), specificity, (% and 95% confidence intervals values) and likelihood ratios.

| IgA anti-MZGP2 cut off | Sensitivity (%) | 95% CI | Specificity (%) | 95%CI | Likehood Ratio |
|---|---|---|---|---|---|
| **> 11** | 28.48 | 23.62% to 33.74% | 89.80 | 85.75% to 93.01% | 2.79 |
| **> 15** | 23.84 | 19.30% to 28.87% | 94.56 | 91.31% to 96.86% | 4.38 |
| **> 20** | 17.96 | 13.93% to 22.59% | 96.26 | 93.40% to 98.12% | 4.80 |
| **> 25** | 14.55 | 10.89% to 18.88% | 97.62 | 95.16% to 99.04% | 6.11 |
| **> 30** | 12.07 | 8.729% to 16.13% | 97.96 | 95.61% to 99.25% | 5.92 |
| **> 40** | 10.22 | 7.138% to 14.05% | 98.30 | 96.08% to 99.45% | 6.01 |
| **> 50** | 8.359 | 5.581% to 11.93% | 98.98 | 97.05% to 99.79% | 8.19 |

| IgG anti-MZGP2 cut off | Sensitivity (%) | 95% CI | Specificity (%) | 95%CI | Likehood Ratio |
|---|---|---|---|---|---|
| **> 11** | 39.32 | 33.96% to 44.88% | 89.46 | 85.37% to 92.72% | 3.73 |
| **> 15** | 34.67 | 29.49% to 40.14% | 93.54 | 90.09% to 96.06% | 5.37 |
| **> 20** | 30.34 | 25.37% to 35.67% | 94.90 | 91.72% to 97.12% | 5.95 |
| **> 25** | 26.63 | 21.88% to 31.80% | 96.60 | 93.83% to 98.36% | 7.83 |
| **> 29** | 25.08 | 20.44% to 30.17% | 96.94 | 94.27% to 98.59% | 8.19 |
| **> 40** | 22.29 | 17.87% to 27.23% | 98.30 | 96.08% to 99.45% | 13.11 |
| **> 50** | 19.20 | 15.05% to 23.92% | 98.98 | 97.05% to 99.79% | 18.81 |

**Table 4**. Summary of sensitivity (% and confidence intervals values), specificity, negative predictive values (NPV) and positive predictive values (PPV) of individual antibody reactivities in 323 Crohn's disease (CrD) and 294 ulcerative colitis (UC) patients.

| | CrD (n=323) (positive n) | UC (n=294) (positive n) | Sens% | Sens CI% | Spec % | Spec CI% | PPV% | NPV% |
|---|---|---|---|---|---|---|---|---|
| IgA anti-MZGP2 pos | 48 | 7 | 15 | 11, 19 | 98 | 95, 99 | 87 | 51 |
| IgG anti-MZGP2 pos | 87 | 10 | 27 | 22, 32 | 97 | 94, 98 | 90 | 55 |
| IgA and/or IgG anti-MZGP2 pos | 99 | 13 | 31 | 25, 36 | 96 | 93, 98 | 88 | 56 |
| IgA and IgG anti-MZGP2 pos | 36 | 4 | 11 | 8, 15 | 99 | 97, 99 | 90 | 50 |
| IgA ASCA pos | 151 | 14 | 47 | 41, 52 | 95 | 92, 97 | 92 | 62 |
| IgG ASCA pos | 213 | 29 | 66 | 61, 71 | 90 | 86, 93 | 88 | 71 |
| IgA and/or IgG ASCA pos | 230 | 37 | 71 | 66, 76 | 87 | 83, 91 | 86 | 73 |
| IgA and IgG ASCA pos | 134 | 6 | 42 | 36, 47 | 98 | 96, 99 | 96 | 60 |
| (IgA or IgG) ASCA and/or (IgA or IgG) anti-MZGP2 pos | 242 | 47 | 75 | 70, 80 | 84 | 79, 88 | 84 | 75 |
| IgA and IgG ASCA pos and IgA and/or IgG anti-MZGP2 pos | 171 | 0 | 53 | 47,59 | 100 | 98, 100 | 100 | 66 |
| IgA and IgG ASCA pos and IgA and IgG anti-MZGP2 pos | 23 | 0 | 7 | 5, 11 | 100 | 99, 100 | 100 | 50 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| X-ANCA (values for UC) | 30 | 106 | 36 | 31, 42 | 91 | 87, 94 | 78 | 61 |
| IgA or IgG ASCA pos and ANCA neg | 214 (66%) | 22 (8%) | 94 | 86,98 | 80 | 64,91 | 90 | 89 |
| IgA or IgG MZGP2 pos and ANCA neg | 87 | 10 | 27 | 22, 32 | 97 | 94, 98 | 90 | 55 |

ANCA. anti-neutrophil cytoplasmic antibodies; ASCA. anti-*Saccharomyces cerevisiae* antibody

**Table 5**. Clinical relevance of antibody reactivities in patients with Crohn's disease; Rows corresponding to L1, L4, B3 and A2 were omitted because statistically significant differences for a given parameter were not obtained; Positive associations are indicated in **bold** and negative associations in *italic*; A (Age), L (Location), and B (Behavior) according to Montreal classification; L1 ileal; L2 colonic; L3 ileocolonic, and L4 upper disease modifier; B1, non-stricturing/non penetrating; B2, stricturing; B3, penetrating behavior

| P<br>OR<br>95%CI | **IgA**<br>**Anti-MZGP2** | **IgG**<br>**Anti- MZGP2** | **IgA or IgG**<br>**Anti-MZGP2** | **IgA**<br>**ASCA** | **IgG**<br>**ASCA** | **IgA or IgG**<br>**ASCA** | **(IgA or IgG) ASCA**<br>**or**<br>**(IgA or IgG) Anti-MZGP2** | **(IgA or IgG) ASCA**<br>**&**<br>**(IgA or IgG) Anti-MZGP2** | **x-ANCA** |
|---|---|---|---|---|---|---|---|---|---|
| **L2** | | *0.002* | *0.007* | *0.0008* | *<0.0001* | *<0.0001* | *<0.0001* | *0.002* | |
| **L3** | | **0.007**<br>**2.3 (1.2, 4.2)** | **0.013**<br>**2 (1.2, 3.6)** | **0.03**<br>**1.7 (1.1, 2.8)** | **0.003**<br>**1.9 (1.2, 3.2)** | **0.012**<br>**1.9 (1.2, 3.2)** | | **0.001**<br>**2.8 (1.5, 5.2)** | |
| **B1** | | | | *0.033* | *0.021* | *0.002* | *0.005* | | **0.038**<br>**2.3 (1.1, 5.3)** |
| **B2** | | | | | **0.006**<br>**2.3 (1.3, 4)** | **0.005**<br>**2.4 (1.3, 4.6)** | **0.001**<br>**3.1 (1.5, 6.3)** | | |
| **A1** | **0.011**<br>**2.3 (1.2, 4.4)** | | | | | | | **0.043**<br>**1.8 (1.0, 3.1)** | |
| **A3** | | | | | *0.003* | *0.026* | *0.015* | | |
| **Duration** | | **0.018** | **0.026** | **<0.0001** | | **0.019** | **0.008** | **0.034** | |