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SEROLOGIC ANTIBODIES IN RELATION TO OUTCOME  
IN POST-OPERATIVE CROHN'S DISEASE

Short title: Serologic Antibodies in Post-operative Crohn's Disease

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**ABSTRACT**

**Background:** Disease recurs frequently after Crohn's disease resection. The role of serological antimicrobial antibodies in predicting recurrence or as a marker of recurrence has not been well defined.

**Methods:** 169 patients (523 samples) were prospectively studied, with testing peri-operatively, and 6, 12 and 18 months post-operatively. Colonoscopy was performed at 18 months post-operatively. Serologic antibody presence (pANCA, ASCA IgA/IgG, anti-OmpC, anti-CBir1, anti-A4-Fla2, anti-Fla-X) and titre were tested. Quartile sum score (range 6-24), logistic regression analysis, and correlation with phenotype, smoking status and endoscopic outcome were assessed.

**Results:** Patients with  $\geq 2$  previous resections were more likely to be anti-OmpC positive (94% vs. 55%  $\geq 1$  v  $< 2$ ,  $P = 0.001$ ). Recurrence at 18 months was associated with anti-Fla-X positivity at baseline (49% v 29%; positive v negative,  $P = 0.033$ ) and 12 months (52% v 31%,  $P = 0.04$ ). Patients positive ( $n=28$ ) for all four antibacterial antibodies (anti-CBir1, anti-OmpC, anti-A4-Fla2 and anti-Fla-X) at baseline were more likely to experience recurrence at 18 months than patients negative ( $n=32$ ) for all four antibodies (82% v 18%,  $P = 0.034$ ; OR 6.4, 95% CI 1.16-34.9). The baseline quartile sum score for all six antimicrobial antibodies was higher in patients with severe recurrence (Rutgeert's i3-i4) at 18 months, adjusted for clinical risk factors (OR 1.16, 95% CI 1.01-1.34,  $P = 0.039$ ). Smoking affected antibody status.

**Conclusions:** Anti-Fla-X and presence of all anti-bacterial antibodies identifies patients at higher risk of early post-operative Crohn's disease recurrence. Serologic screening pre-operatively may help identify patients at increased risk of recurrence.

**Keywords:** Crohn's Disease, Serology, Antibodies, Post-Operative, Smoking

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## INTRODUCTION

Within a year of Crohn's disease resection recurrence occurs in up to 90% of patients.<sup>1, 2</sup> Further surgery is required in a majority of patients within 10 years.<sup>3</sup> We have recently demonstrated in a prospective study that smoking, penetrating disease and previous surgery are associated with an increased risk of earlier disease recurrence.<sup>4</sup> Biomarkers to identify patients at risk of recurrence would be valuable in focusing preventive therapy.

Circulating antibodies in inflammatory bowel disease (IBD) are directed against auto-antigens or enteric microbial antigens. Serological antibodies have been studied in relation to need for surgery<sup>5-9</sup> and development of more complex disease.<sup>9-15</sup> Choung *et al* have recently shown that complex Crohn's Disease patients often have serologic antibodies present pre-diagnosis and higher titres when compared to patients with uncomplicated disease.<sup>16</sup> However, there are limited data on whether such antibodies may predict the development of, or are associated with, recurrent disease after surgery.<sup>17, 18</sup> Previous studies have demonstrated that anti-*Saccharomyces cerevisiae* antibodies (ASCA) alone are not sufficient to predict recurrence.<sup>18-20</sup> A small prospective study found that ASCA IgG and IgA did not predict the need for further surgery.<sup>17</sup> The development of novel serological IBD-related antibodies provides an opportunity to investigate this further.<sup>21, 22</sup>

Microbial antigens known to elicit an antibody response in IBD include oligomannan, cell wall porin proteins and flagellin subunits. Antibodies to mannan cell wall proteins derived from baker's yeast, *Saccharomyces cerevisiae* (ASCA IgG or IgA), are highly prevalent in Crohn's disease.<sup>23, 24</sup> Omp-C is a bacterial outer membrane protein derived from *E. coli*.<sup>14</sup> The antigens CBir1, A4-Fla2 and Fla-X are flagellin subunit proteins linked to *Clostridium* cluster XIVa.<sup>25, 26</sup>

In a randomized controlled trial examining the optimal strategy for preventing recurrence of Crohn's disease after intestinal resection (Postoperative Crohn's Endoscopic Recurrence (POCER) study)<sup>4</sup> patients were stratified according to clinical risk factors, treated to prevent recurrence, and monitored for recurrence using ileo-colonoscopy and faecal calprotectin measurement.<sup>4, 27</sup> We now aim to assess the relationship between the presence and magnitude of serologic antibodies and the post-operative course in this cohort of patients.

## MATERIALS AND METHODS

The POCER Study was a prospective, randomized, multi-centre trial in 174 patients undergoing resection of all macroscopic luminal Crohn's disease.<sup>4</sup> Patients were stratified as high risk if they had one or more of previous resection, smoking or perforating disease, or low risk for those with no risk factors. Patients were randomized (2:1 ratio) to a colonoscopy at 6 months (active care) or to best standard drug therapy. All patients underwent a colonoscopy at 18 months.

Patient phenotype was classified at baseline according to the Montreal Classification<sup>28</sup>, disease activity assessed using the Crohn's Disease Activity Index (CDAI) and all medications recorded. Smoking history was assessed as current, past or never smoker at baseline. All patients received metronidazole (400mg BD) for three months; high risk patients additionally received a thiopurine (azathioprine 2mg/kg or 6-mercaptopurine 1.5mg/kg daily) or adalimumab (160mg initially, 80mg at 2 weeks, and then 40mg fortnightly thereafter) if thiopurine intolerant.

Endoscopic assessment was undertaken using the Rutgeerts Score<sup>29</sup>, with recurrence defined as a score  $\geq 2$ . Patients in the active care arm with endoscopic recurrence at 6 months received intensified drug therapy: low risk patients stepped up to a thiopurine, and patients on a thiopurine commenced combination therapy with the addition of adalimumab 40mg fortnightly. Patients on adalimumab therapy intensified dosing to 40mg weekly.

Five hundred and twenty-three serum samples for antibody testing were taken from 160 patients at baseline (peri-operatively, prior to or within 4 weeks of surgery), 142 patients at 6 months, 111 patients at 12 months and 110 patients at 18 months post-operatively. Overall 169 of the 174 patients provided one or more serum samples. Baseline characteristics of the cohort are shown in table 1.

#### **Antibody Testing**

Antibody testing was performed using a commercial enzyme-linked immunosorbent assay (ELISA; IBD-SGI panel, Prometheus Laboratories, San Diego, CA). The panel measures the following antibodies: ANCA titre and perinuclear staining pattern (pANCA), anti-*Saccharomyces cerevisiae* antibodies (ASCA IgA and IgG), anti-CBir1 IgG, anti-OmpC IgA, anti-A4-Fla2 IgG and anti-Fla-X IgG. Results were expressed as ELISA units (EU/ml), with positivity assessed according to the reference ranges defined in Choung *et al.*<sup>16</sup> Testing was performed blinded to patient data. Assessment for atypical perinuclear-ANCA staining (pANCA) was performed on ANCA titre positive samples, by immunofluorescence on DNase treated and untreated alcohol fixed neutrophil slides, as previously described.<sup>13, 30</sup>

#### **Statistical Analysis**

Data were analysed using STATA Version 12 (StataCorp, Texas, USA). Associations between antibody positivity and patient characteristics were assessed using Chi<sup>2</sup> or Fisher's exact test, while the association between antibody levels and patient characteristics were assessed using the Kruskal-Wallis test. Correlations between continuous variables were assessed using Spearman's Rank Correlation. Associations between binary outcomes for endoscopic recurrence, serologic markers, and phenotype were determined using logistic regression.

The cumulative magnitude of antibody responses for all six tested antimicrobial antibodies (ASCA IgA and IgG, anti-CBir1 IgG, anti-OmpC IgA, anti-A4-Fla2 IgG and anti-Fla-X IgG) were investigated using the quartile sum score method (range 6-24) as previously described.<sup>31</sup>

ANCA titre and pANCA fluorescence (positive or negative) were excluded from the quartile sum score analysis, but pANCA was used as an adjusting factor in subsequent analyses.

The mean quartile sum score and the sum of the number of positive markers was compared between groups using the two-sample t-test, and included in a logistic regression model.

Comparisons were corrected for gender, age, disease location, smoking and pANCA status, in relation to endoscopic recurrence.

Positive predictive values (PPV) and negative predictive values (NPV) were determined for single antibodies, combinations, quartile sum score and number of positive markers, for prediction of endoscopic recurrence. Receiver Operator Characteristic (ROC) curves were plotted (sensitivity v 1-specificity) and the area under the curve calculated (AUROC).

### ***Ethical Considerations***

The POCER Study, including the collection and analysis of serological samples, was approved by the Human Research Ethics Committee of St Vincent's Hospital, Melbourne (HREC-A 077/09), and is registered with ClinicalTrials.gov (NCT00989560). All patients provided written informed consent.

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## RESULTS

### Presence of Serological Antibodies

Of the 160 patients with baseline testing, 24 of 160 (15%) were positive for pANCA, 158 of 160 (99%) for ASCA IgA, 75 of 160 (47%) for ASCA IgG and 74 of 160 (46%) for both ASCA IgA and IgG.

With regard to anti-bacterial antibodies 95 of 160 (59%) of patients were positive for anti-OmpC, 51 of 160 (32%) for anti-CBir1, 67 of 160 (42%) for anti-A4-Fla2 and 89 of 160 (56%) for anti-Fla-X.

32 of 160 (20%) of patients were negative for all bacterial antibodies, and 28 of 160 (17.5%) were positive for all four. Overlapping antibody positivity to the bacterial antigens is shown in figure 1. The largest group of patients (22.5%) were positive for only for anti-OmpC.

Ninety-three percent of patients had ileal or ileo-colonic disease (Table 1). Disease phenotype according to the Montreal Classification<sup>28</sup> was associated as follows: pANCA positively associated with inflammatory (B1) disease (OR 6.1 (95% CI 1.8-20.3), P = 0.003) and negatively associated with penetrating (B3) disease (OR 0.4 (95% CI 0.14 -0.90), P = 0.029). Anti-OmpC was negatively associated with inflammatory (B1) disease (OR 0.27 (95% CI 0.08-0.93), P = 0.038).

### Change in antibodies over time

There was no significant change in mean quartile sum score (all antibodies combined for each patient) between baseline and 18 months ( $\Delta$  -0.32, P = 0.265). However the mean number of

positive markers changed significantly over time ( $\Delta$ -0.33,  $P = 0.006$ ). The mean titre for each antibody changed significantly from baseline to 18 months for all antibodies (figure 2), although the magnitude of change was small, between 3.24EU/ml for ASCA IgG to -10.8EU/ml for anti-Fla-X.

The median values for the change in each individual time period (0-6 months, 6-12 months, 12-18 months) were assessed for variation in titre, to exclude the surgical resection as a cause of titre variation over time. There was significant variation in ANCA ( $P = 0.015$ ), anti-OmpC ( $P = <0.001$ ) and anti-Fla-X ( $P = <0.001$ ) titres between each time-period. No marker was consistently stable across all time periods, although the magnitude of the change for all antibodies was small.

#### **Relationship Between Serological Antibodies, Surgery, Risk Factors for Recurrence and Presence of Disease Recurrence**

##### ***Surgery***

One hundred and eleven of the 160 (69%) patients had had no previous surgery. The 17 patients with two or more previous resections were significantly more likely to be anti-OmpC positive at baseline than the 143 patients with less than 2 previous resections (94% vs. 55%,  $P = 0.001$ ). Anti-A4-Fla2 was negatively associated with previous surgery (OR 0.43 (95% CI 0.2-0.9),  $P = 0.025$ ).

With regard to the indication for current surgery, patients positive for anti-OmpC at baseline were more likely to undergo surgery for failure of medical therapy than those having surgery

for another indication (OR 2.20, 95%CI 1.0-4.7;  $P = 0.044$ ). Patients positive for anti-A4-Fla2 were more likely to have surgery for obstruction (OR 2.1, 95%CI 1.0-4.3;  $P = 0.05$ ).

### ***Prediction of Post-Operative Endoscopic Recurrence***

ANCA titre and atypical perinuclear staining (pANCA) at baseline were not associated with endoscopic recurrence at 18 months, with no difference between patients positive at baseline for ANCA titre or pANCA compared with patients who were negative: ANCA titre 45% v 39%,  $P = 0.600$ , pANCA staining 35% vs 41%,  $P = 0.632$ .

Endoscopic recurrence at 18 months did not differ between patients positive at baseline for ASCA IgA and IgG compared with patients who were negative: ASCA IgA 40% v 50%,  $P = 1.00$ , ASCA IgG 44% vs 37%,  $P = 0.464$ .

Patients with recurrence at 18 months were more likely than those without recurrence to be positive for anti-Fla-X when measured at all time points, with this being significantly more likely at baseline (49% v 29%,  $P = 0.05$ ) and 12 months (52% v 31%,  $P = 0.04$ ), but not significantly so at 6 (48% v 33%,  $P = 0.121$ ) or 18 months (51% v 34%,  $P = 0.115$ ).

Patients positive for all four antibacterial antibodies (anti-CBir1, anti-OmpC, anti-A4-Fla2 and anti-Fla X) at baseline were more likely to have endoscopic recurrence at 18 months than patients who were negative for all antibodies (82% v 18%,  $P = 0.034$ ; OR 6.4, 95% CI 1.16-34.9).

When patients negative for all antibacterial antibodies were compared to patients positive for

one or more antibacterial antibodies, patients who were negative were less likely to have disease recurrence at 18 months (11% v 47%,  $P = 0.004$ ).

There were no significant differences in the mean quartile sum score at baseline and the presence or absence of endoscopic recurrence at 6 or 18 months (table 2; figure 3, panel A). The mean number of positive markers was associated with 18 month endoscopic outcome when measured at baseline [(recurrence v remission; 3.8 v 3.18,  $P = 0.038$ ) (table 2; figure 3, panel B)]. When tested at 18 months, the number of positive antibodies was higher in patients with endoscopic recurrence compared with those in remission although this was not significant (recurrence v remission: 3.24 v 2.75,  $P = 0.07$ ).

The relationship between known clinical risk factors for recurrence and serological antibodies was examined. In the high risk patients, the mean baseline quartile sum score and number of positive markers was higher in those with endoscopic recurrence at 18 months (QSS: 16.4 v 14.6,  $P = 0.045$ , n positive: 3.86 v 3.13,  $P = 0.032$ ). In the low risk patients, neither the quartile sum score nor the mean number of positive markers was associated with disease recurrence at 18 months.

To evaluate the relationship between clinical risk factors, antibody profile and disease recurrence at 18 months the odds ratios for the quartile sum score and number of positive markers were calculated using step-wise logistic regression (Table 3).

Quartile sum score at baseline was significant when adjusted for smoking (OR 1.12, 95% CI 1.0-1.2,  $P = 0.03$ ). When adjusted for the clinical risk factors (smoking, perforating disease and previous surgery) and pANCA the odds ratio was similar (OR 1.13, 95% CI 1.0 -1.3,  $P = 0.02$ ).

The number of positive antibodies (range 0-6) measured at baseline was associated with disease recurrence at 18 months, after adjustment for smoking status (OR 1.36, 95% CI 1.1-1.8, P = 0.018).

The total baseline quartile sum score (but not number of positive markers) was significantly greater in patients with severe recurrence (Rutgeert's i3-i4) than in patients without severe recurrence at 18 months, when adjusted for clinical risk factors (OR 1.16, 95% CI 1.01-1.34, P = 0.039) and additionally adjusted for pANCA status (OR 1.17, 95% CI 1.01-1.36, P = 0.034).

### ***Sensitivity and Specificity***

For the prediction of recurrence at 6 and 18 months the total baseline quartile sum score AUROC was 0.50 and 0.60 respectively, and the number of positive markers AUROC was 0.51 and 0.59 respectively. The AUROC curves for the individual markers at baseline are shown in supplementary figure 1.

### **Other Assessments of Clinical and Disease Recurrence**

#### **Crohn's Disease Activity Index (CDAI) and Faecal Calprotectin**

No antibody titres correlated significantly with subsequent CDAI measurements or faecal calprotectin at any timepoint.

#### **Smoking**

Patients who had never smoked were more likely than current or past smokers to be ASCA IgG positive (56% v 40%  $P = 0.05$ ) and anti-Fla-X positive (67% v 47%,  $P = 0.01$ ) at baseline.

Individual antibody levels stratified based on smoking status are compared in Figure 4.

The relationship between smoking and antibody presence or titre was assessed by comparing baseline antibody results between current ( $n=52$ ) versus never ( $n=77$ ) and past ( $n=43$ ) smokers combined (mean quartile sum score: 14.4 v 15.3 ( $P = 0.184$ ); mean number of positive markers 3.2 v 3.4 ( $P = 0.432$ )).

Comparing current and past smokers with patients who had never smoked revealed a significantly lower quartile sum score [(14.1 v 16.2 ( $P = 0.0015$ )) (Figure 3, panel C)], and lower number of positive markers [3.0 v 3.7 ( $P = 0.006$ )] (Figure 3, panel D)].

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## DISCUSSION

The accuracy of serologic antibodies for identifying patients at increased risk of Crohn's disease post-operative disease recurrence, and identifying patients with recurrence, have not been prospectively addressed in a large cohort. Anti-Fla-X had the greatest value in relation to predicting subsequent recurrence. Recurrence at 18 months was associated with anti-Fla-X positivity at all time points although significantly so only at baseline and at 12 months post-operatively.

Baseline positivity for all four bacterial antibodies (anti-CBir1, anti-OmpC, anti-A4-Fla2 and anti-Fla-Y) were also more likely to have early recurrence than those negative for all antibodies. Seventeen percent of patients were positive for all four antibodies, potentially identifying a group of patients who have a higher risk of recurrence separate to the clinical risk factors. Adjustment for clinical risk factors improved the predictive ability of antibody testing.

Two antibodies, anti-A4-Fla2 and anti-OmpC, related to previous surgery and may be regarded as markers for risk of re-operation. Anti-A4-Fla2 was negatively associated with the risk of surgery, and anti-OmpC positively associated with two or more previous operations. Neither of these two antibodies was predictive for, or associated with recurrent disease. The A4-Fla2 and Fla-X flagellins have been mapped to the family *Lachnospiraceae*, an anaerobic non-pathogenic bacterial family within the Firmicutes phylum.<sup>6,25</sup> OmpC is an *E. coli* outer membrane protein that represents an immune response to the *Enterobacteriaceae* family.<sup>32</sup>

In relation to increased risk of severe recurrence ( $\geq$  Rutgeerts i3) the total baseline quartile sum score (but not number of positive markers) was significantly greater in patients with, than patients without, severe recurrence at 18 months.

The presence and magnitude of many serological antibodies varies little according to disease activity<sup>31</sup>, treatment<sup>33,34</sup> and surgery<sup>17</sup>. ASCA IgA and IgG levels do not vary in relation to surgery or endoscopic recurrence.<sup>17</sup> Our results demonstrate that mean titres of individual antibodies vary significantly (Figure 2) but the magnitude of change is small and unlikely to be pathophysiologically relevant. Measurement of changes in titre of these antibodies over time is therefore not clinically useful.

We have demonstrated lower humoral immune activation in current and past smokers across multiple antibodies. This has been shown previously in periodontal disease<sup>35</sup>, systemic lupus erythematosus<sup>36</sup>, healthy subjects<sup>37,38</sup>, and in a cohort of 113 Crohn's Disease patients using only a single antibody (ASCA).<sup>39</sup> That past smokers cluster with current smokers in the presence and magnitude of these antibodies suggests that immune down-regulation may not resolve on cessation of smoking; this remains to be tested with stricter smoking criteria. The lower titre of antibodies in past or current smokers compared to "never" smokers indicates that the increased risk of recurrence associated with smoking is mediated through a separate mechanism to that reflected in antibody production. When interpreting antibody levels serological testing should be interpreted in the light of smoking status.

The pathophysiology of antibody development to luminal antigens is currently unclear. Antibody levels do not decline after surgery, suggesting that an immune change, such as one

related to the genetic background or gut microbiome, is more important than disease burden. Mutations in microbial pattern recognition receptor genes (NOD2) and autophagy genes (ATG16L) have been linked with the development of ASCA, and IGRM mutations with antibody responses to CBir1 flagellins.<sup>40</sup> Anti-OmpC shows high concordance (Intraclass correlation coefficient of 0.80) between monozygotic twin pairs discordant for CD implying a possible genetic predisposition for antibody development.<sup>41</sup> Because the CBir1 and Fla-X antigens have 84% amino acid sequence overlap<sup>26</sup>, and A4-Fla2 and Fla-X also overlap significantly<sup>25</sup>, a susceptible genetic background may influence the immune reactivity to other closely related flagellin proteins.

The strengths of this study include strict phenotyping and endoscopic assessment rather than outcomes based on clinical recurrence or imaging, and defined time points. This study used established reference ranges to define antibody positivity or negativity.

Our patient population had a high ASCA positivity rate. This may relate to the sensitivity of the IgA and IgG assays used, or may reflect the high proportion of patients with complex disease.<sup>5,</sup>

<sup>16, 17, 42</sup>

In summary, serologic antibodies may add value in the prediction of post-operative disease recurrence. Smoking status should be considered in relation to serologic testing. Their role in providing pathophysiological insights remains to be explored. Patients who demonstrate positivity for multiple serologic antibodies remain at risk of a more complex disease course, and a greater need for surgical intervention.<sup>6-8, 12, 15, 42</sup> These serologic markers may identify

patients who would benefit from more aggressive monitoring and therapy of their disease generally, and following intestinal resection.

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## TABLES

Table 1 Patient demographics at baseline

| <i>Serology Cohort</i> | All |
|------------------------|-----|
|------------------------|-----|

| <i>(at least one measurement) n = 169</i>              |                | n          | %  |
|--|----------------|------------|----|
| n (Male)   |                | 77         | 46 |
| Age > 40 years   |                | 74         | 44 |
| <b>Age, median (years):</b>                            |                | 36 (26-46) |    |
| Inter quartile range (IQR)                             |                |            |    |
| <b>Age at Diagnosis (years):</b>                       |                |            |    |
| < 16 Years   |                | 19         | 11 |
| 17 - 40 Years  |                | 129        | 76 |
| > 40 Years   |                | 21         | 12 |
| <b>Duration of Crohn's disease (years):</b>            |                |            |    |
| median (IQR)   |                | 9 (4-16)   |    |
| ≥ 10 years   |                | 66         | 39 |
| <b>Disease Location at Surgery:</b>                    |                |            |    |
| Ileum only (L1)  |                | 92         | 54 |
| Colon only (L2)  |                | 11         | 7  |
| Ileum and colon (L3)                                   |                | 66         | 39 |
| <b>Disease Phenotype at Surgery:</b>                   |                |            |    |
| B1 (Inflammatory)                                      |                | 16         | 9  |
| B2 (Stricture)   |                | 60         | 36 |
| B3 (Penetrating)                                       |                | 93         | 55 |
| <b>Indication for surgery:</b>                         |                |            |    |
| Failure of drug therapy                                |                | 37         | 22 |
| Obstruction  |                | 48         | 28 |
| Perforation  |                | 84         | 50 |
| <b>Number of prior surgical resections:</b>            |                |            |    |
|  | 0              | 120        | 71 |
|  | 1              | 32         | 19 |
|  | 2              | 9          | 6  |
|  | 3 or more      | 8          | 5  |
| <b>Smoking Status:</b>                                 |                |            |    |
| Active Smoker  |                | 52         | 31 |
| Past Smoker  |                | 41         | 24 |
| Never Smoker   |                | 76         | 45 |
| <b>Immediate Post-Operative Baseline Drug Therapy:</b> |                |            |    |
| Metronidazole alone                                    |                | 27         | 16 |
| Thiopurine   |                | 99         | 59 |
| Adalimumab   |                | 43         | 25 |
| <b>Baseline CDAI</b>                                   | <i>n = 148</i> |            |    |
| CDAI >150  |                | 110        | 74 |
| CDAI >200  |                | 88         | 59 |

**Table 2.** Mean quartile sum score and mean number of positive markers measured at baseline, 6 and 18 months for the prediction of endoscopic outcome (recurrence or remission) at 18 months.

|   | Mean Values      |                   |
|---|------------------|-------------------|
| <b>Baseline Serology for 18 month Endoscopic Outcomes</b> | <b>Remission</b> | <b>Recurrence</b> |
| Mean quartile sum score                                   | 14.86            | 16.31             |
| P Value   | 0.067            |                   |
| Mean n positive markers                                   | 3.18             | 3.82              |
| P Value   | <b>0.038</b>     |                   |
| <b>6 Month Serology for 18 month Endoscopic Outcomes</b>  | <b>Remission</b> | <b>Recurrence</b> |
| Mean quartile sum score                                   | 14.98            | 16.41             |
| P Value   | 0.057            |                   |
| Mean n positive markers                                   | 3.03             | 3.45              |
| P Value   | 0.115            |                   |
| <b>18 month Serology for 18 month Endoscopic Outcomes</b> | <b>Remission</b> | <b>Recurrence</b> |
| Mean quartile sum score                                   | 14.41            | 15.69             |
| P Value   | 0.094            |                   |
| Mean n positive markers                                   | 2.75             | 3.24              |
| P Value   | 0.072            |                   |

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**Table 3.** Adjusted odds ratios for baseline testing in relation to endoscopic outcome at 18 months, on step-wise logistic regression.

|  | TOTAL Quartile Sum Score<br>(range 6-24) |         |             | TOTAL Number of Positive Antibodies<br>(range 0-6) |           |              |
|--|--|---------|-------------|--|-----------|--------------|
|  | Adjusted<br>OR                           | 95% CI  | P Value     | Adjusted<br>OR                                     | 95% CI    | P Value      |
| <i>Adjusted for Smoking only</i>   |  |         |             |  |           |              |
| Baseline   | 1.12                                     | 1   1.2 | <b>0.03</b> | 1.36   | 1.1   1.8 | <b>0.018</b> |
| <i>Adjusted for all clinical risk factors (smoking, previous surgery, perforating disease)</i> |  |         |             |  |           |              |
| Baseline   | 1.13                                     | 1   1.3 | <b>0.02</b> | 1.40   | 1.1   1.8 | <b>0.013</b> |
| <i>Adjusted for all clinical risk factors and pANCA status</i>                                 |  |         |             |  |           |              |
| Baseline   | 1.13                                     | 1   1.3 | <b>0.02</b> | 1.40   | 1   1.8   | <b>0.013</b> |

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## FIGURE LEGENDS

### Figure 1.

Overlapping bacterial antibody positivity at baseline

### Figure 2.

Change in mean antibody titres from baseline to 18 months (EU/ml) for each antibody tested.

P values denote statistically significant variation in mean values (one sample t-test), with the change (delta) in EU/ml given below. All boxes denote median and IQR.

### Figure 3.

**Panel A.** Baseline Quartile Sum Score (quartiles: ASCA IgA and IgG, anti-OmpC, anti-CBir1, anti-A4-Fla2, anti-Fla-X) by 18 month endoscopic outcomes.

**B.** Baseline number of positive markers by 18 month endoscopic outcomes.

**C.** Baseline Quartile Sum Score by smoking status.

**D.** Baseline number of positive markers by smoking status.

All boxes denote median and IQR.

### Figure 4.

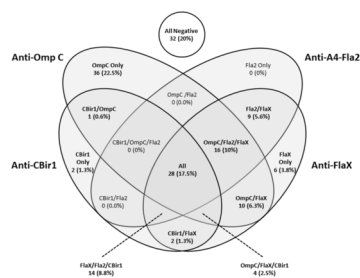
Baseline values for individual antibodies, stratified by smoking status. Red line represents reference range cut off. P values are for current vs. past smokers, and current/past smokers combined vs. never smokers. All boxes denote median and IQR.

**Supplementary Figure 1.**

AUROC curves for baseline serological prediction of endoscopic recurrence at 18 months post operatively for each antimicrobial marker.

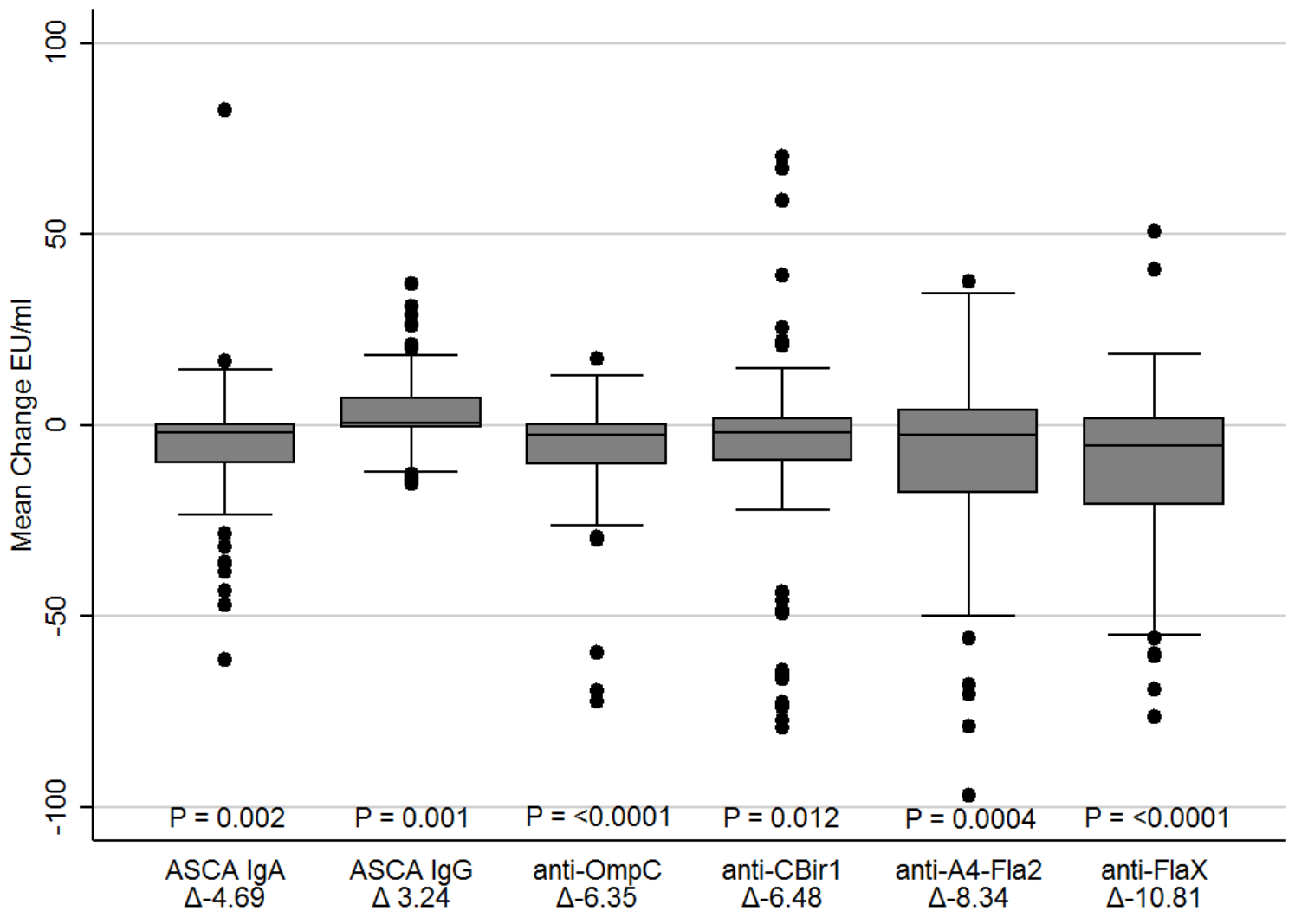
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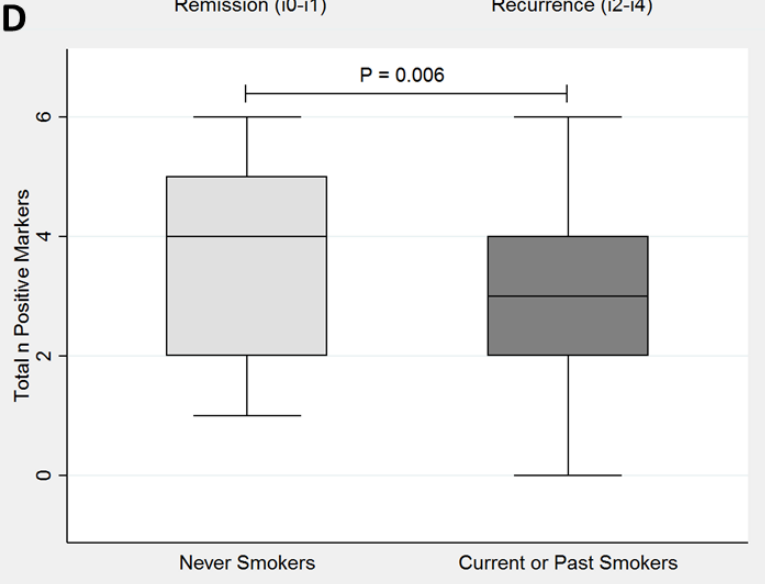
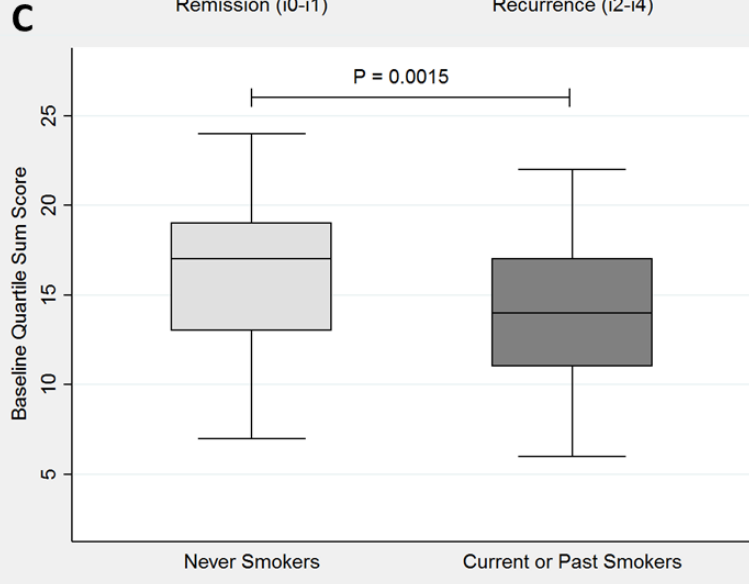
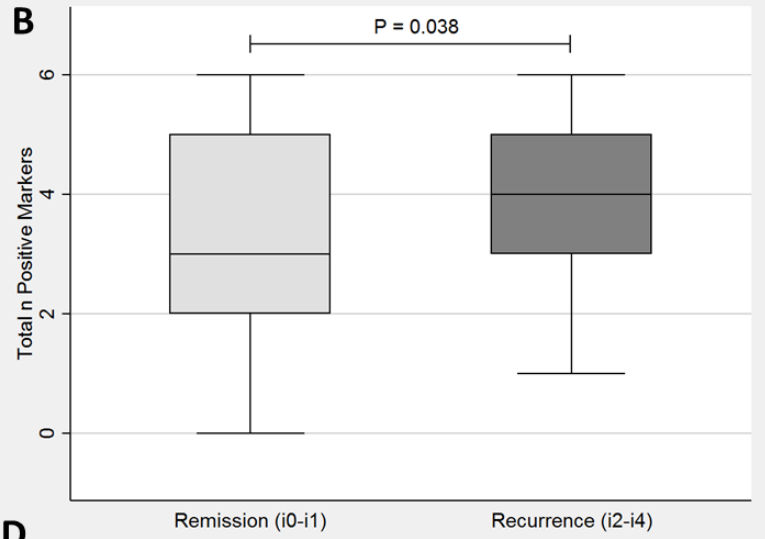
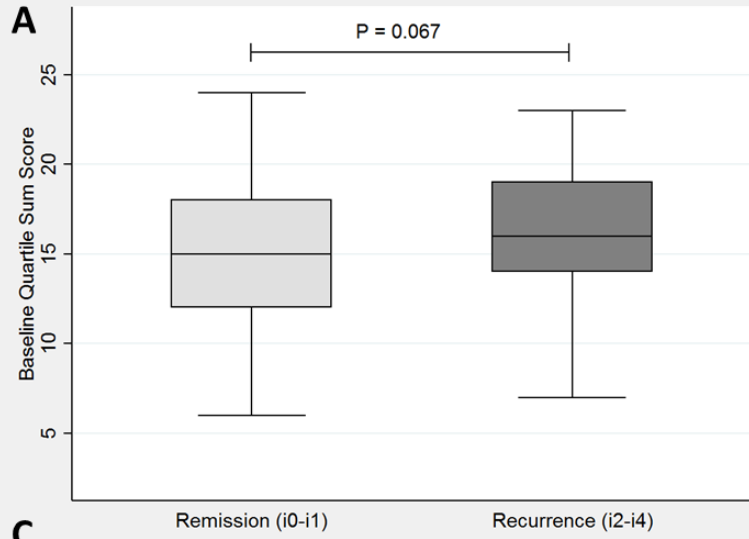
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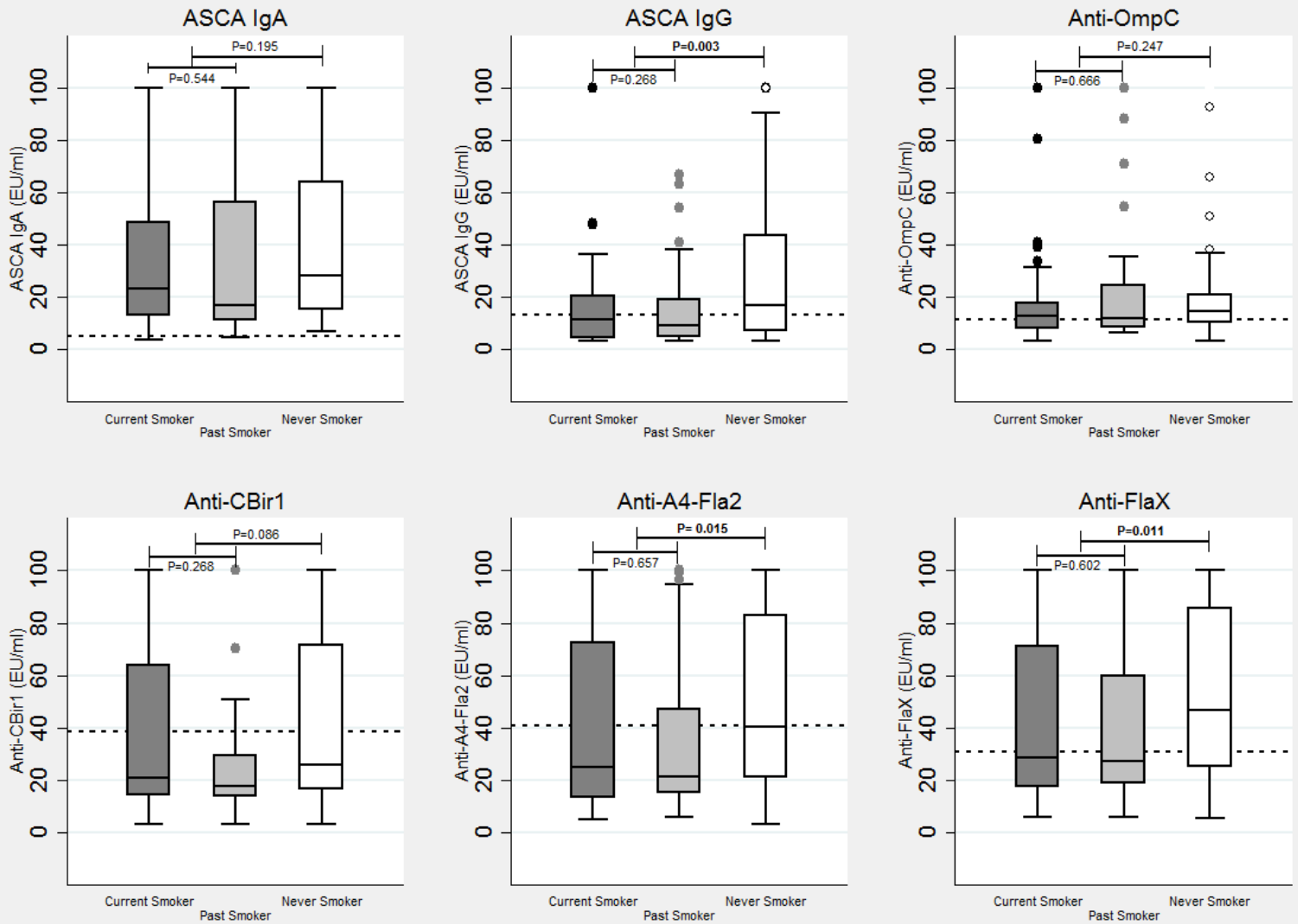
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