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# Prediction of Crohn's disease relapse with fecal calprotectin in infliximab responders: a prospective study

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**Prediction of Crohn’s disease relapse with fecal calprotectin in infliximab responders: a prospective study**

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**Abstract**

**Background:** Fecal calprotectin (FC) is a reliable tool for predicting Crohn’s disease (CD) relapse in patients with sustained remission. Prediction of relapse with FC has been less studied in patients with severe CD treated with anti-TNF.

**Aim:** to identify an association between FC concentration and CD clinical relapse in patients achieving remission with infliximab (IFX).

**Methods:** From February 2007 to October 2008, consecutive patients with refractory luminal CD were prospectively included when they received three IFX infusions (5 mg/kg at weeks 0-2-6) followed by maintenance with an immunomodulator alone. FC and CRP were measured at entry and at week 14 (w14).

**Results:** Sixty-five patients (43W; median age: 30.4 years) were included and 50 (77%) were in clinical remission off steroids at w14; 23/50 (46%) experienced CD **clinical** relapse during the first year of follow up. Median FC level at w14 was similar in patients with and without CD clinical relapse (200 and 150 µg/g, respectively). When considering two suggested FC cut-offs to predict CD relapse, sensitivities and specificities were 61% and 48% for 130 µg/g respectively, and 43% and 57% for 250 µg/g. Neither FC nor CRP at baseline and at w14 could predict relapse even when CD location subgroup analysis was considered.

**Conclusion:** In patients responding to an IFX induction regimen, FC measurement at w14 cannot predict CD **clinical** relapse at one year.

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## Introduction

Inflammatory bowel diseases are chronic disorders characterized by alternating periods of remission and relapse. In current practice, they are generally viewed as unpredictable diseases. Therefore, the putative risk of an unanticipated relapse is considered as one of the main fear by patients <sup>1</sup>.

Surrogate markers of mucosal inflammation could be helpful to predict the clinical course of inflammatory bowel diseases. Prospective studies in patients in clinical remission have been conducted in order to foresee relapse with biomarkers. Since approximately one third of patients in clinical remission has an elevated serum C-reactive protein (CRP), serum inflammatory parameters have been evaluated. A prospective study has shown that Crohn's disease patients in remission have an eightfold risk of short-term relapse when CRP was higher than 20 mg/L and/or erythrocyte sedimentation rate (ESR) above 15 mm <sup>2</sup>.

Fecal biomarkers which are considered as witnesses of infra-clinical disease activity may be also reliable tools for predicting relapse of inflammatory bowel disease, with a best accuracy than CRP level as suggested by comparative studies <sup>3,4</sup>. Calprotectin represents up to 50% of the cytosolic proteins in granulocytes. Its fecal measurement is considered as an accurate measure of neutrophil migration into the gastro-intestinal tract <sup>5,6</sup>. To date, six prospective studies conducted in adults with Crohn's disease in clinical remission have demonstrated the predictive value of fecal calprotectin for clinical relapse with 43-90% sensitivity and 43-88% specificity <sup>3, 4, 7-10</sup>. Thresholds suggested for predicting Crohn's disease relapse varied between 130 and 400 µg/g. However, as most of the patients recruited in these series received maintenance with mesalamine, little is known about fecal calprotectin meaning in more severe Crohn's disease patients treated with biologics.

The significance of fecal calprotectin value in patients with active Crohn's disease has been also prospectively assessed. Thus, it was better correlated to endoscopic activity than to

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clinical symptoms <sup>11, 12</sup>. As mucosal healing is now considered as an important therapeutic goal in Crohn’s disease and is achievable with biologics <sup>13-15</sup>, fecal calprotectin could also predict early clinical and mucosal responses, as suggested in a pilot study <sup>16</sup>. Therefore, the aim of the present study was to identify an association between fecal calprotectin and clinical relapse in patients with active Crohn’s disease responders to infliximab.

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## Patients & Methods

### *Selection of patients and study schedule*

This was a prospective two-centre study conducted in the Gastroenterology departments of Haut-Lévêque and Saint-André Hospitals from the Bordeaux University Hospitals from February 2007 to October 2008.

Eligible Crohn's disease patients were consecutively recruited when they met the following criteria: luminal Crohn's disease diagnosed at least for three months on usual criteria including mucosal ulcerations on ileo-colonoscopy, insufficient response to steroids (resistance defined as active disease despite prednisolone of up to 40 mg/day over a period of 2 weeks; dependence defined as inability to reduce steroids below 10 mg/day or budesonide 3mg/day within 3 months or relapse within 3 months of stopping steroids; intolerance defined as a steroid-side effect leading to discontinuation of the drug) requiring an infliximab induction regimen (5 mg/kg at weeks 0-2-6) associated with maintenance by an immunosuppressant alone (azathioprine/mercaptopurine was given to patients naive for immunosuppressants and methotrexate in case of previous azathioprine/mercaptopurine intolerance or failure)<sup>17, 18</sup>. Immunosuppressant was started at a stable dose within the four weeks before the first infliximab infusion which defined baseline. Subjects with ulcerative colitis, indeterminate colitis, contra-indication for infliximab or immunosuppressant, surgical resection needed, pure ano-perineal fistulising disease or pregnancy were excluded.

All patients received treatment according to clinical need. Drugs used were those normally employed in Crohn's disease, according to licensed or published doses and frequency (infliximab: 5 mg/kg at weeks 0-2-6; azathioprine: 2-3 mg/kg/day; parenteral methotrexate: 25 mg/week). Patients received treatment only after full and informed consent.

Data were collected at baseline and at week 14 and the following characteristics were recorded: date of birth, gender, disease duration, age at diagnosis, disease location and



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behavior according to the Montreal classification<sup>19</sup>, perianal disease, extraintestinal manifestation, smoking history, previous history of intestinal surgery, past treatment with immunosuppressants and biologics, type of response to steroids (resistance, dependence or intolerance as defined previously), clinical (CDAI) disease activity at baseline and at week 14.

### ***Laboratory parameters***

Laboratory tests included CRP (normal value < 5 mg/L) and calprotectin assay (normal value < 50 µg/g). Biomarkers were measured at inclusion and at week 14 in patients with remission off steroids by one experienced biologist (S.M.) blinded to the clinical status and the medications taken by the patients.

Fecal calprotectin was assayed by a sandwich enzyme-linked immunosorbent assay (ELISA) (Calprotectin ELISA®, Bühlmann, Switzerland) using a monoclonal capture antibody highly specific to the calprotectin. Patients provided stool samples within the last 24-hours before the visits which were rapidly addressed to the laboratory and stored at +4°C. Stool samples (50 to 100 mg) were homogenized with the extraction buffer for 30 min on a multi tube vortexer. Supernatants were collected after centrifugation for 5 min at 3000 x g and, diluted 1:50 with incubation buffer. Calibrators, controls and diluted samples - 100 µL each - were added in duplicate into subsequent wells and incubated for 30 min at room temperature on a plate rotator. After 50 min of incubation at room temperature and washing, 100 µL of a horseradish peroxidase-conjugated antibody was added to detect calprotectin bound to the monoclonal antibody coated onto the plate. After 30 min of incubation, plates were washed and 100 µL of tetramethylbenzidine (TMB substrate) was added. Optical density values were read at 405 nm.

### ***Outcomes and objectives***

The primary objective of the study was to determine whether fecal calprotectin at week 14 in patients with remission off steroids achieved with an infliximab induction could predict Crohn's disease relapse until week 52. For this purpose, according to data from two

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prospective cohorts of Crohn's disease patients in clinical remission, two different fecal calprotectin cut-off values predicting relapse -  $130 \mu\text{g/g}$  <sup>7</sup> and  $250 \mu\text{g/g}$  <sup>3</sup> - were studied.

Secondary objectives were: i) to identify predictive factors of clinical remission at week 14 in the whole population; ii) to identify a correlation between fecal calprotectin and CRP levels at baseline and at week 14; iii) to identify factors associated with clinical relapse until week 52 in patients with remission off steroids at week 14.

Clinical remission at week 14 was defined as a CDAI < 150. Patients with clinical remission off steroids were prospectively followed at least 38 additional weeks - until week 52 - with planned visits every three months including physical examination and routine laboratory analyses.

During the follow up period, Crohn's disease **clinical** relapse was defined as increasing symptoms (CDAI > 250 within two weeks or CDAI > 150 with an at least 70 points of increase as compared to CDAI at week 14), or the need for an additional steroid or infliximab course, or the need for a surgical resection.

### **Statistics**

Continuous variables are presented as median and range; categorical variables are presented as percentages. Continuous data were analysed using Mann-Whitney's test. Categorical data were analysed using the Pearson's chi-squared test, or Fisher's exact test if any cell number was <5, for frequencies. Considering fecal calprotectin at week 14, relapse-free survival rates in relation to observation time were estimated using Kaplan-Meier life-table method.

Kendall's coefficient of correlation (tau-b) and their probabilities (p) evaluated the relationship between fecal calprotectin and CRP levels. Analysis of predictive factors of clinical relapse within one year in primary responders to infliximab was performed. Univariate and multivariate analyses were performed to assess impact of clinical, disease, treatment, and biological variables. Continuous variable were dichotomised according to the

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median. Variables analysed were age at inclusion, gender, disease duration, active tobacco smoking, Crohn’s disease location and behaviour, extra-intestinal manifestations, previous intestinal resection, indication for infliximab (luminal or fistulising disease), type of immunosuppressant associated, CRP level (mg/L) and fecal calprotectin (µg/g) at week 14. Difference between relapse and non-relapse groups was assessed by  $\chi^2$  or Fisher’s exact tests when appropriate. A logistic regression model was created using significantly associated variables ( $p<0.10$ ), and the odds ratios (OR) for the variables that remained significant ( $p<0.05$ ) in the model determined.

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## Results

During the study period (from February 2007 to October 2008), 65 patients (median age: 30.4 [range: 15-69] years; 66% female) were prospectively included and received an infliximab induction regimen associated with maintenance by an immunosuppressant alone. The flow chart of patients is given in Figure 1. At entry, 58% of patients had an active luminal Crohn's disease (CDAI > 150), with CRP > 10 mg/L in 57% cases and fecal calprotectin > 250 µg/g in 85%. Baseline characteristics of the whole population are presented in Table 1.

Remission without steroids at week 14 was not achieved in 15 (23%) patients. Among the 50 patients in remission after the infliximab induction regimen, 23 (46%) experienced clinical relapse until week 52 (including 21 with treatment modification - 19 restarted infliximab, 1 had an azathioprine optimization and 1 had surgery; 15/21 with CRP > 10 mg/L at time of relapse - and two patients lost during follow-up). The probability to be in remission was 76% and 52% at week 26 and 52, respectively. Baseline characteristics of patients with relapse (group R) and without relapse (group NR) from week 14 to week 52, which are presented in Table 2, were not different.

### *Biomarkers in infliximab primary responders*

CRP and fecal calprotectin were collected at baseline and at week 14, with a median time of 45 [38-53] days since inclusion (four fecal dosages missing, all in group NR). In the 50 patients in remission off steroids, median CRP and fecal calprotectin values at week 14 were 0 [0-32] mg/L and 200 [10-4400] µg/g in group R, and 0 [0-16] mg/L and 150 [5-7800] µg/g in group NR (NS for CRP and calprotectin).

Considering cut-off values for predicting Crohn's disease clinical relapse, fecal calprotectin at week 14 was > 130 µg/g in 61% in group R and 52% in NR (p=0.323), and > 250 µg/g in 43% and 43%, respectively (p=0.890). Kaplan-Meier curves of survival off relapse according these two cut-offs are shown in Figure 2. Considering the 130 µg/g fecal calprotectin

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threshold, sensitivity and specificity for predicting relapse were 61% and 48%. With the 250  $\mu\text{g/g}$  threshold, they were 43% and 57%, respectively. Using other fecal calprotectin cut-offs, the highest negative predictive value of fecal calprotectin for predicting clinical relapse was 58% with the 100  $\mu\text{g/g}$  cut-off (Table in supporting information). Using the 5 mg/L threshold, CRP sensitivity and specificity were 35% and 78%.

Even when the analysis was restricted to patients with pure colonic Crohn’s disease without ileal involvement (L2 according to the Montreal classification), fecal calprotectin could not predict relapse until week 52 ( $p=0.375$  vs. other locations). Lastly, the median drop of fecal calprotectin from baseline to week 14 was 340  $\mu\text{g/g}$  and was similar in groups R and NR.

*Secondary objectives*

In univariate analysis, no factor associated with clinical remission off steroids at week 14 could be identified.

At baseline in the whole population, no correlation was identified between fecal calprotectin and CDAI ( $r=0.193$ ;  $p=0.200$ ) or CRP ( $r=-0.86$ ;  $p=0.658$ ) at baseline (Figure in supporting information). Considering the 46 assessable patients in clinical remission at week 14, there was a good correlation between the two biomarkers at week 14 ( $r=0.46$ ;  $p=0.001$ ) (Figure in supporting information). However, the combination of both biomarkers at week 14 could not predict relapse until week 52, whatever the cut-off used for CRP (0 or 5 mg/L) and fecal calprotectin (50, 80, 130 or 250  $\mu\text{g/g}$ ).

No factor associated with clinical relapse until week 52 was identified in the univariate analysis conducted in the subgroup of 50 patients in clinical remission at week 14. Relapse was independent of the type of immunosuppressant given as maintenance ( $p=0.232$ ).

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## Discussion

According to studies recruiting patients with sustained remission, most of them being treated by mesalamine, fecal calprotectin is considered as a reliable tool for predicting Crohn's disease relapse<sup>3, 4, 7, 8, 10</sup>. However, fecal calprotectin prognostic value probably differs in patients with more severe disease and may vary with treatments. Thus, calprotectin may behave differently in patients who are on no therapy or receiving mesalamine, which efficacy is limited in Crohn's disease. In series including patients with active disease or just achieving remission with more efficient drugs such as steroids or anti-TNF, fecal calprotectin seems to be a poor predictor of the disease course<sup>20</sup>, as suggested by the results from the present series. For the time being, stool biomarkers, mostly calprotectin, have been validated as discriminating tests between inflammatory bowel disease and irritable bowel syndrome, with the 50 µg/g cut-off value<sup>21</sup>. Considering patients with inflammatory bowel disease, fecal biomarkers are closely correlated to the endoscopic inflammation and provide an easy and accessible method to assess mucosal activity<sup>11, 22, 23</sup>. This has been better observed in ulcerative colitis than in colonic Crohn's disease, and was not conclusive in case of pure ileal involvement<sup>7</sup>. However, fecal calprotectin is now considered and used as a surrogate marker of endoscopic activity in current Crohn's disease controlled trials. Another putative development of stool tests is the prediction of disease course. Although large variations of sensitivity and specificity have been reported and different cut-offs proposed, calprotectin may be helpful to identify patients with the higher risk of relapse, mainly when combined with other surrogate biomarkers such as CRP, as shown recently in a prospective multicentre study<sup>24</sup>. However, the present study could not confirm that biomarkers are good predictors of Crohn's disease relapse.

Nevertheless, our study has some limitations. First, despite a sample size comparable to studies demonstrating the good prognostic value of fecal calprotectin in Crohn's disease, a

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type-2 error cannot be rule out. Second, no data on endoscopic activity, which is better correlated to calprotectin than CDAI <sup>11, 12</sup>, have been collected. However, in patients responding to an infliximab induction regimen, an endoscopic evaluation is not usually performed in current practice and calprotectin may be useful to assess the mucosal response. Furthermore, disease relapse that was the primary endpoint in the present study was solely clinical-based and may be disputable. Instead, the clinical remission rates observed at week 14 and at week 52, i.e. 77% and 41% respectively, appear comparable with results from the azathioprine-naive subgroup of patients receiving three infliximab infusions in the GETAID study <sup>17</sup>. Moreover, as relapser and non-relapser groups have approximatively the same size, it offers the opportunity to identify factors associated with relapse. Lastly, the bridging strategy used in the present study, i.e. an infliximab induction regimen associated with maintenance by a single immunosuppressant started concomitantly, was considered as an alternative to scheduled infliximab when it was designed. However, as observed in two prospective studies, the benefits of this approach are decreasing with time <sup>25, 26</sup> and maintenance with regular infliximab is recommended <sup>18</sup>. Unfortunately, nor fecal calprotectin neither CRP at week 14 could be helpful in predicting relapse. Despite a significant decrease of these biomarkers from baseline to week 14, reaching values comparable to patients with more sustained remission, a remnant infra-clinical inflammation due to absence of complete mucosal healing of severe Crohn's disease lesions in patients just achieving remission with infliximab may explain this feature. Our negative results may be also explained by the high intraindividual variability of fecal calprotectin. In a recent Norwegian study, calprotectin levels were measured in stool samples taken two consecutive days from 63 Crohn's disease patients with mild to moderate activity. A low correlation between the two measurements was observed ( $\kappa = 0.0355$ ) and the largest differences occurred in patients with the highest calprotectin values <sup>27</sup>. Therefore, a single

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calprotectin value should be cautiously interpreted and may be repeated, as other measurements commonly used in gastroenterology such as fecal weight or fat in the stools.

In conclusion, the predictive values for relapse of fecal calprotectin and CRP had not been confirmed in Crohn's disease patients achieving clinical remission with an infliximab induction. Therefore, biomarkers should be cautiously analysed in Crohn's disease patients, taking into account not only the inflamed bowel location but also disease activity and treatments given. Larger prospective studies are now mandatory to define usefulness and predictive value of biomarkers, mainly calprotectin and CRP, in Crohn's disease according to medications, duration and depth of remission.



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**Figure legends**

Figure 1: Flowchart of patients (responders were defined as patients in clinical remission off steroids)

Figure 2: Time to Crohn’s disease relapse in the 50 infliximab primary responders according to fecal calprotectin value at week 14 according cut-off values of 130 µg/g (2A) and 250 µg/g (2B) (Kaplan-Meier curves).

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Table and Figure in supplementary file:

Supplementary Table: Sensitivity (Se), specificity (Sp), positive predictive and negative predictive values (PPV and NPV) for various fecal calprotectin (FC) cut-offs for predicting Crohn's disease clinical relapse

Supplementary Figure: Correlation between fecal calprotectin and CRP levels at baseline in the whole population (A) and in the 46 patients in clinical remission off steroids at week 14 (B).

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Table 1: Main baseline characteristics among the 65 included patients with luminal refractory Crohn’s disease

	Whole population
n	65
Median age, years [range]	30 [15-69]
Female gender, n (%)	43 (66)
Median disease duration, years [range]	4 [0-31]
Age at diagnosis, n (%)	
A1	4 (6)
A2	61 (94)
A3	0
Disease location, n (%)	
L1	15 (23)
L2	21 (32)
L3	29 (45)
L4	0
Disease behaviour, n (%)	
B1	46 (71)
B2	14 (22)
B3	5 (8)
Perianal disease, n (%)	30 (46)
Current smokers, n (%)	23 (35)
Previous intestinal resection, n (%)	10 (15)
Immunosuppressant given in maintenance, n (%)	
Azathioprine	37 (57)
Methotrexate	28 (43)
Median CDAI [range]	205 [36-700]
Median CRP level (mg/L) [range]	19 [0-154]
Median fecal calprotectin (µg/g) [range]	845 [17-8500]

Age at diagnosis, Crohn’s disease location and behaviour according to the Montreal classification<sup>19</sup>.

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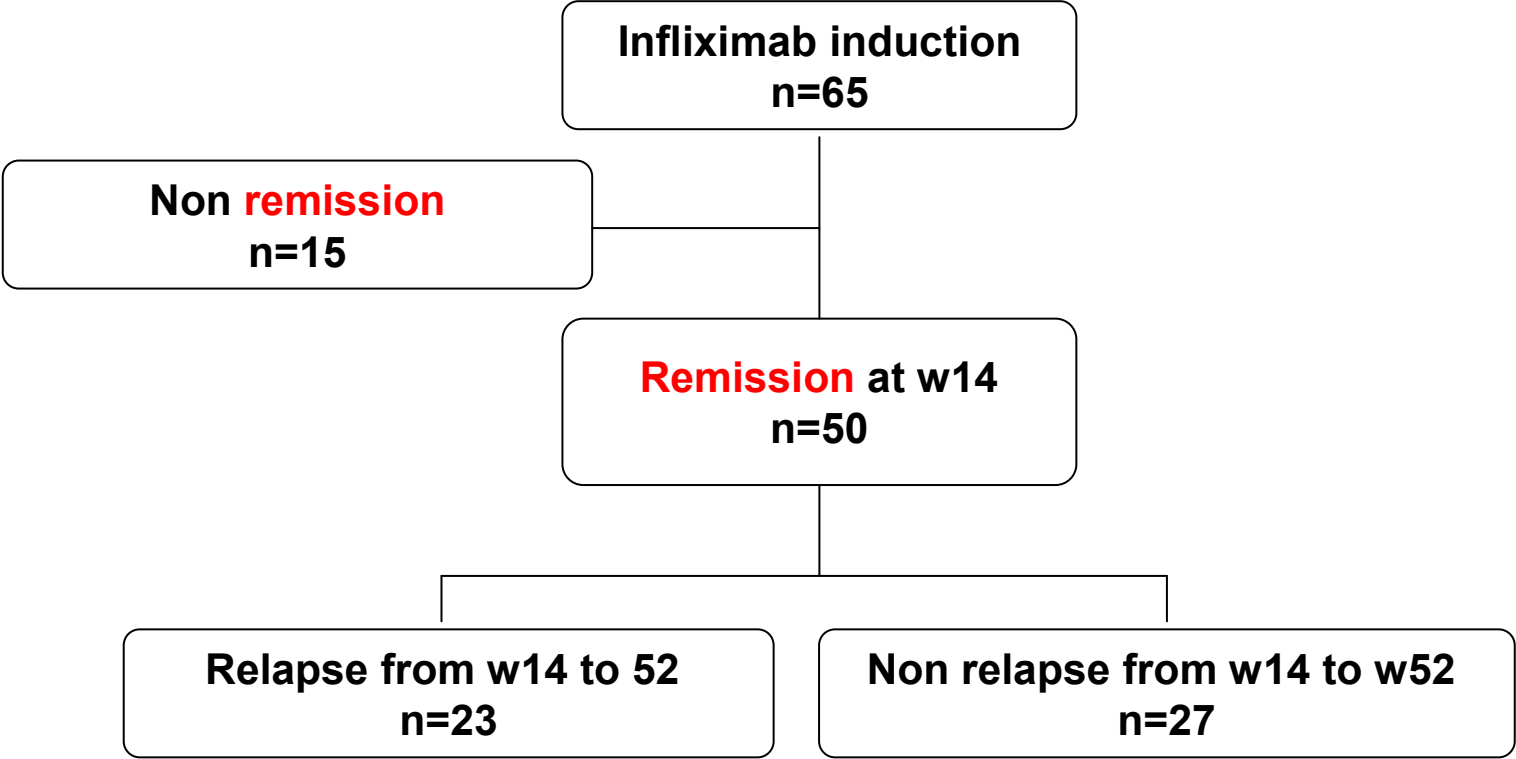
Table 2: Baseline characteristics among the 50 patients in clinical remission at week 14 who experienced (n=23) or not (n=27) relapse until week 52

	Relapsers	Non-relapsers	p-value
n	23	27	
Median age, years [range]	27 [17-65]	31 [15-68]	0.271
Female gender, n (%)	13 (57)	21 (78)	0.192
Median disease duration, years [range]	2 [0-17]	4 [0-31]	0.350
Age at diagnosis, n (%)			1.000
A1	2 (9)	2 (7)	
A2	21 (91)	25 (93)	
A3	0	0	
Disease location, n (%)			0.959
L1	5 (22)	5 (19)	
L2	8 (35)	10 (37)	
L3	10 (43)	12 (44)	
L4	0	0	
Disease behaviour, n (%)			0.155
B1	20 (87)	17 (63)	
B2	2 (9)	7 (26)	
B3	1 (4)	3 (11)	
Perianal disease, n (%)	10 (43)	11 (39)	0.643
Current smokers, n (%)	8 (35)	11 (41)	0.665
Previous intestinal resection, n (%)	3 (13)	5 (19)	0.711
Immunosuppressant given in maintenance, n (%)			0.179
Azathioprine	11 (48)	18 (67)	
Methotrexate	12 (52)	9 (33)	
Median CDAI [range]	170 [17-700]	200 [50-450]	0.961
Median CRP level (mg/L) [range]	19 [0-127]	15 [0-154]	0.930
Median fecal calprotectin (µg/g) [range]	700 [17-5400]	1200 [54-8300]	0.313

Age at diagnosis, Crohn's disease location and behaviour according to the Montreal classification<sup>19</sup>.



Figure 1



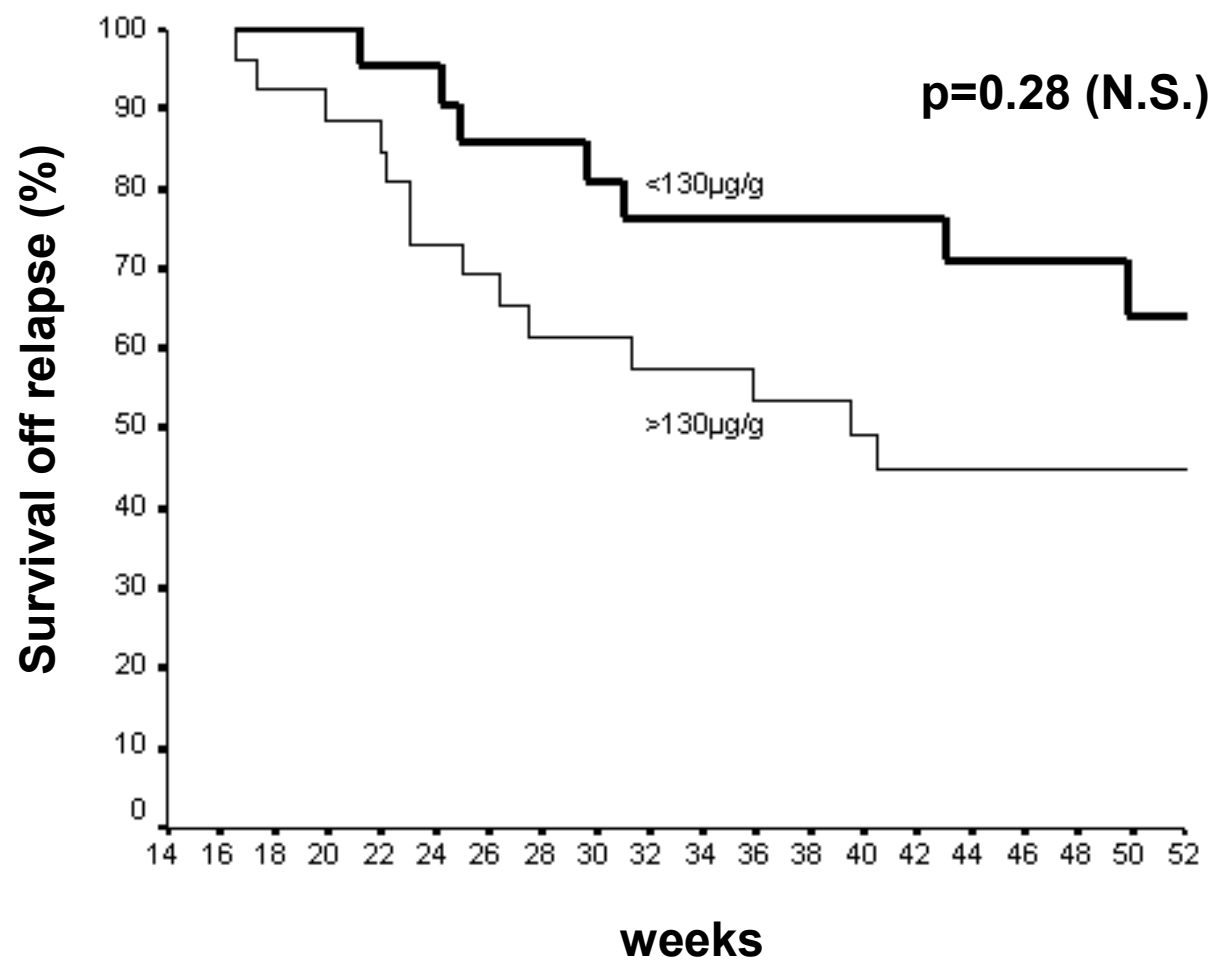
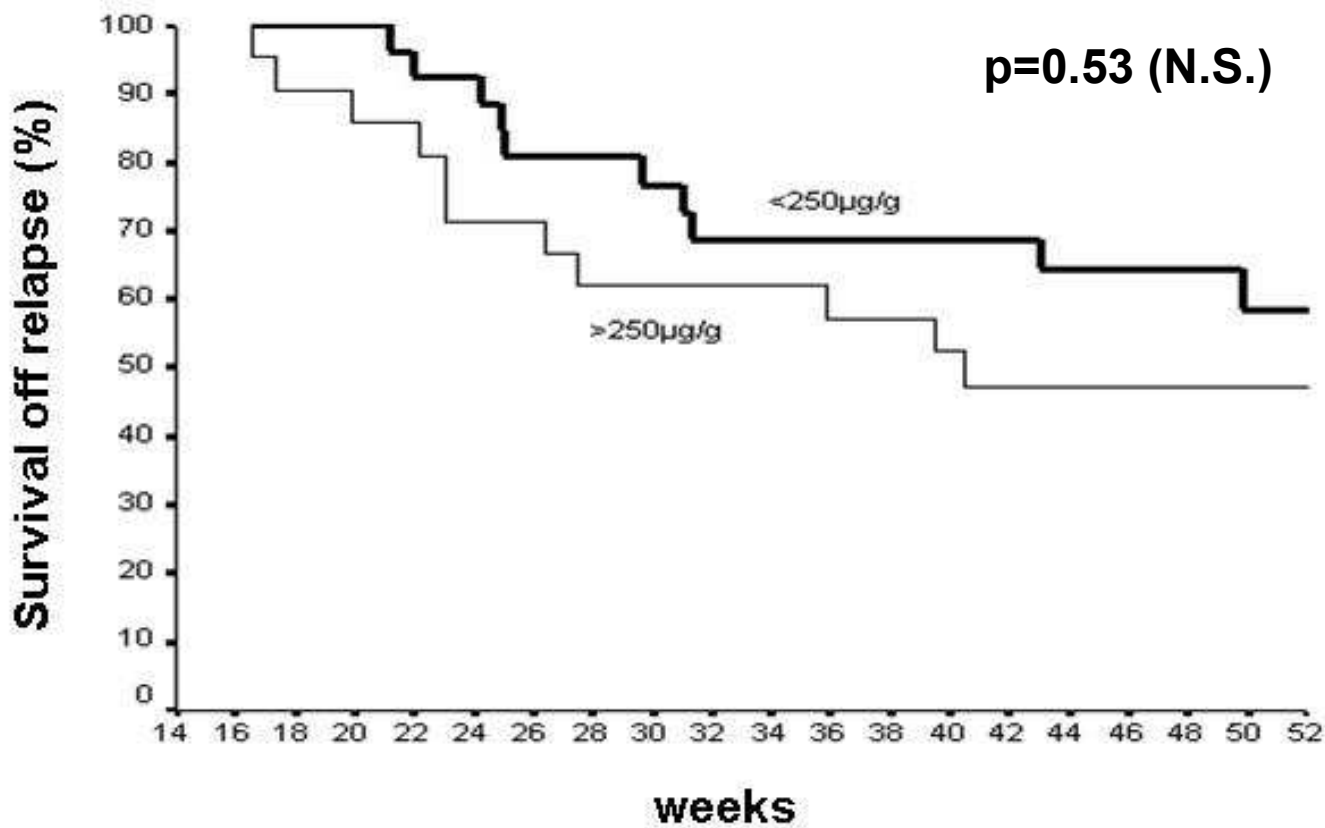
**Figure 2A**

Figure 2B



**Sensitivity (Se), specificity (Sp), positive predictive and negative predictive values for various fecal calprotectin (FC) cut-offs in order to predict Crohn's disease clinical relapse**

FC (µg/g)	Relapse		Se	Sp	PPV	NPV
	no	yes				
< 20	4	3	87%	17%	51%	57%
≥ 20	19	20				
≤ 20	4	5	78%	17%	49%	44%
> 20	19	18				
< 30	5	5	78%	22%	50%	50%
≥ 30	18	18				
≤ 30	6	5	78%	26%	51%	55%
> 30	17	18				
≤ 50	8	7	70%	35%	52%	53%
> 50	15	16				
≤ 75	10	7	70%	43%	55%	59%
> 75	13	16				
< 100	11	8	65%	48%	56%	58%
> 100	12	15				

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# Supporting information

Scatter plot showing the correlation between CRP baseline (mg/L) and Calprotectin baseline (µg/g). The y-axis ranges from 0 to 140 mg/L, and the x-axis ranges from 0 to 10000 µg/g. A negative correlation is indicated by  $r = -0.086$ . Data points are represented by black squares, and a solid regression line is shown.

Figure 3B

