

Calprotectin

NON INVASIVE TOOL FOR DIAGNOSIS AND FOLLOW-UP IN
INFLAMMATORY BOWEL DISEASE PATIENTS

Table of content

- Introduction

- Utility

 - Diagnostic process

 - Symptomatic IBD patient

 - Asymptomatic IBD patient

 - Post-operative IBD patient

- Conclusions

Introduction

1. Diagnosis of Inflammatory Bowel Disease (IBD)

Crohn's Disease (CD) and Ulcerative Colitis (UC)

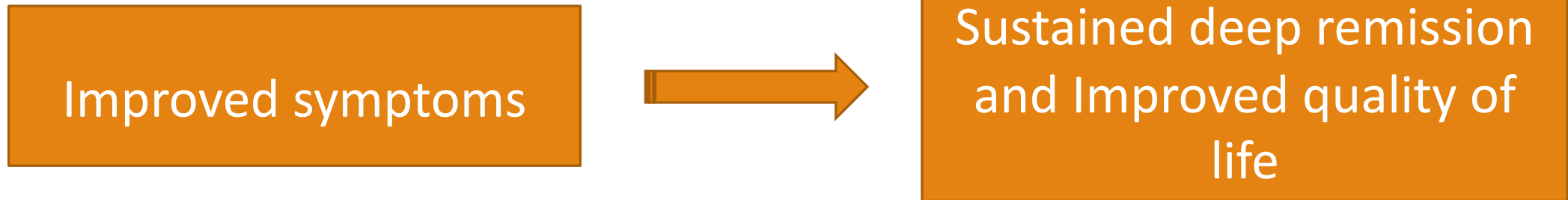
Combination of suggestive clinical, biological, radiological, endoscopic and histological data

AND

Rejecting other diseases with similar clinical manifestations

Introduction

2. IBD : shift in treatment goals



Establishing and maintain mucosal healing

- Higher clinical rates of response (CD, UC)
- Lower relapse rates (CD, UC)
- Lower hospitalization rates (CD, UC)
- Lower risk of colorectal cancer (UC, CD?-lack of data)
- Reduced need for surgery (CD, UC)

Introduction

3. Golden standard for diagnosis and follow-up

Endoscopy (and histology on biopsy specimens)

- Expensive
- Invasive
- The need for repeated examination

➡ **Need for biological markers**

simple, rapid, sensitive, specific, inexpensive and non-invasive

Introduction

4. Available biological markers

Systemic markers

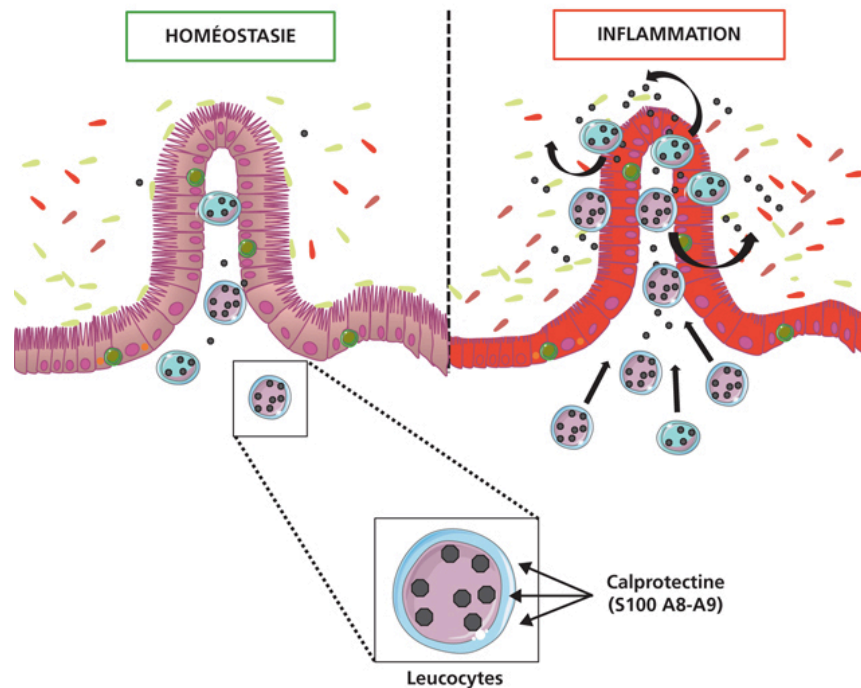
- **CRP** – C-reactive protein (negative in 25% CD patients – often normal in UC patients)
- ESR – erythrocyte sedimentation rate
- ANCA – anti-neutrophil cytoplasmic antibodies
- ASCA – anti-Saccharomyces cerevisiae antibodies

Stool markers

- **Calprotectin**
- Lactoferrin

Introduction

5. Calprotectin



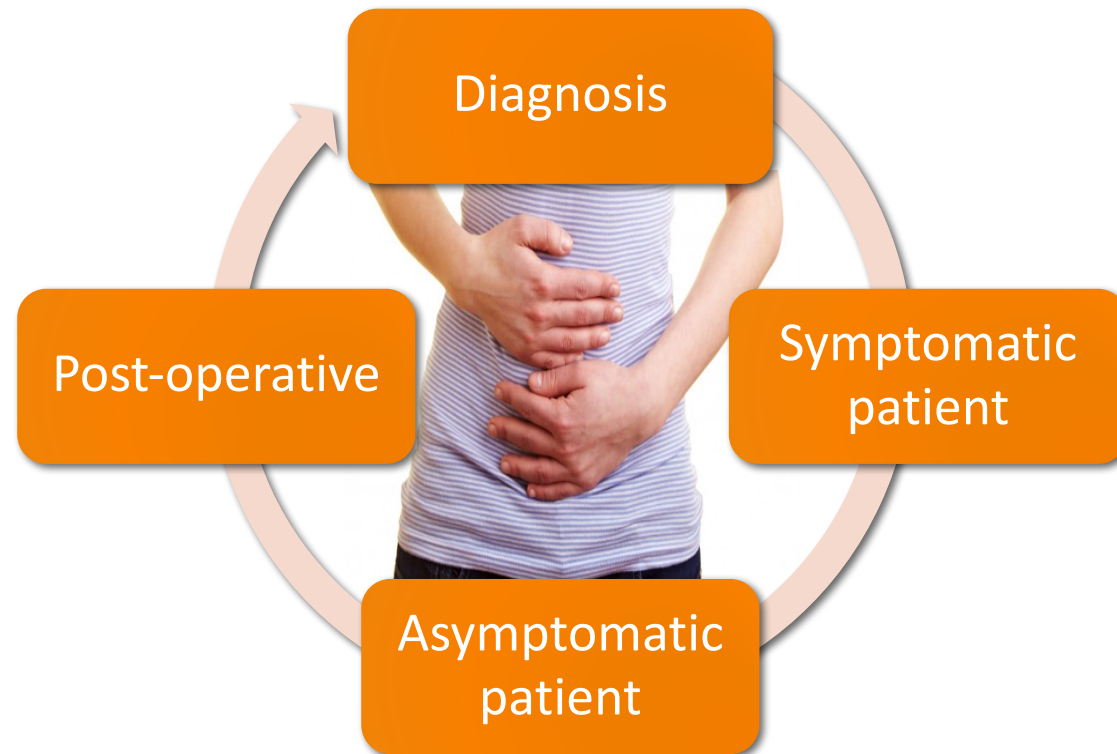
- Calprotectin (S100 A8-A9)
- 36 kDa dimer calcium binding proteins S100A8 and S100A9
- PMN – Macrophages – Monocytes – Epithelial Cells – Keratinocytes
- Secretion : PMN activation – Stress – Death cell – Vascular adhesion of Monocytes
- Immunomodulatory role
- Resistant to enzymatic degradation
- Easily measurable in faeces

Introduction

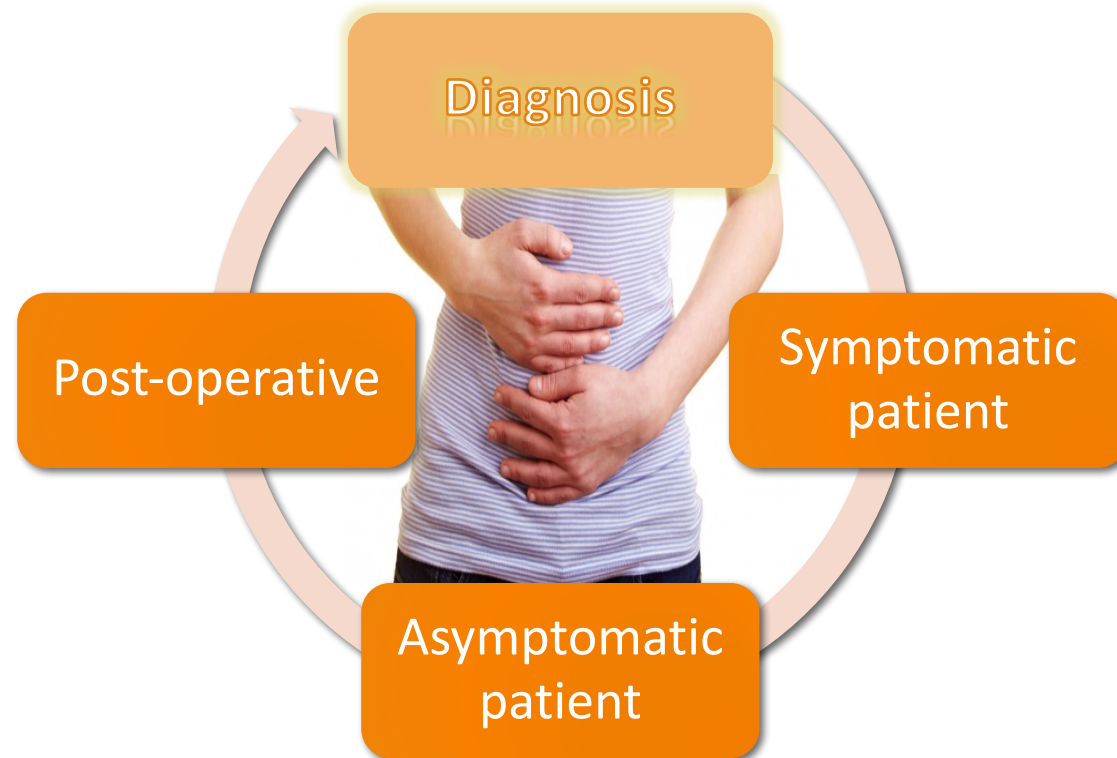
6. Faecal Calprotectin (FC) - disadvantages

- Currently no reimbursement (30 to 35 €)
- No (inter)national guidelines
- Increase after use of NSAIDs (false positive), PPI
- Several diseases other than IBD can increase calprotectin levels, especially
 - Colorectal neoplasia and adenomas
 - Gastrointestinal infections
 - Microscopic colitis
 - Coeliac disease, protein losing enteropathy, etc.

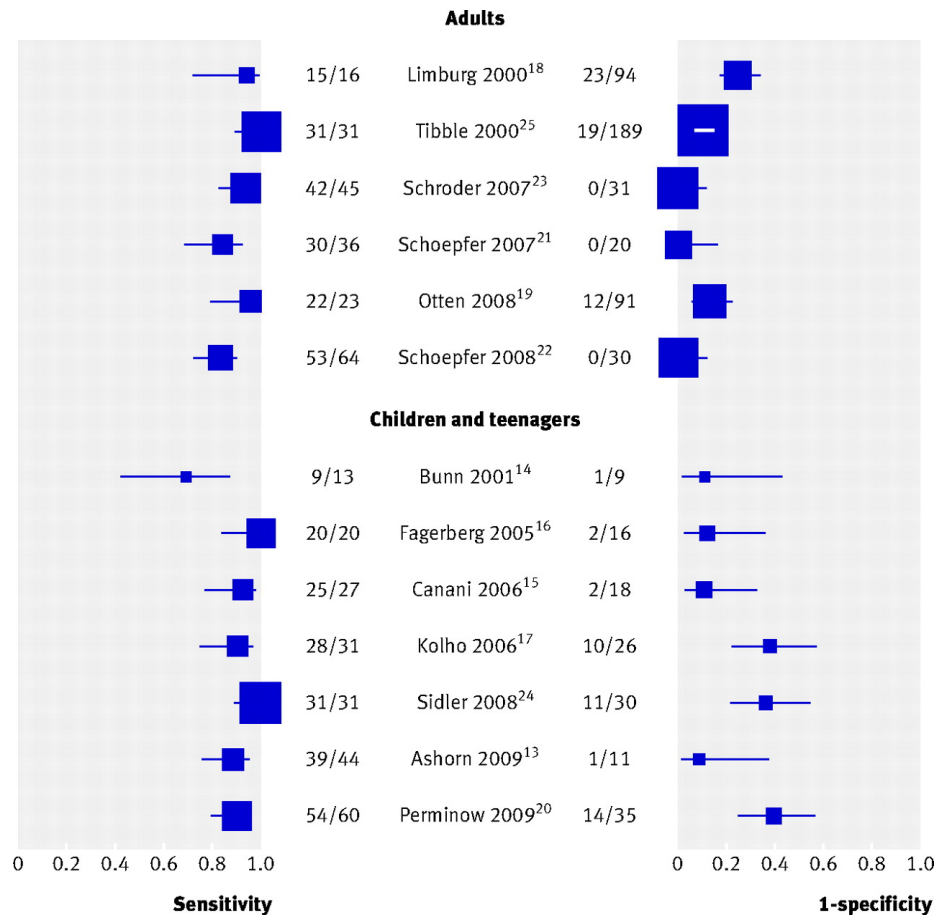
Utility



Utility



Diagnostic process (1)



- Met-analysis
 - 13 studies
 - 6 in adults (n=670)
 - 7 in children (n=371)

- Adults
sensitivity: 0.93; Specificity: 0.96

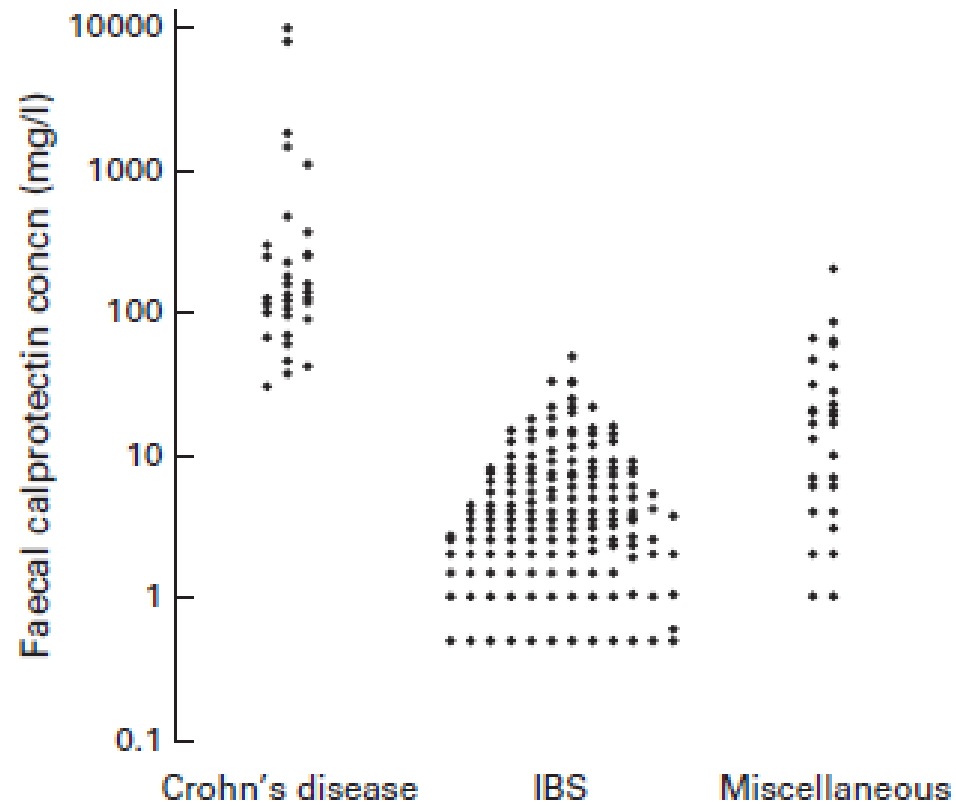
- Children/teenagers
sensitivity: 0.92; Specificity: 0.76

	Adults	Children
Sensitivity	=	
Specificity	>	

- 67% reduction in the number of adults requiring endoscopy
- Delayed diagnosis in 6% of adults because of a false negative test result
- Most of the studies used cut-off of 50-100 µg/g

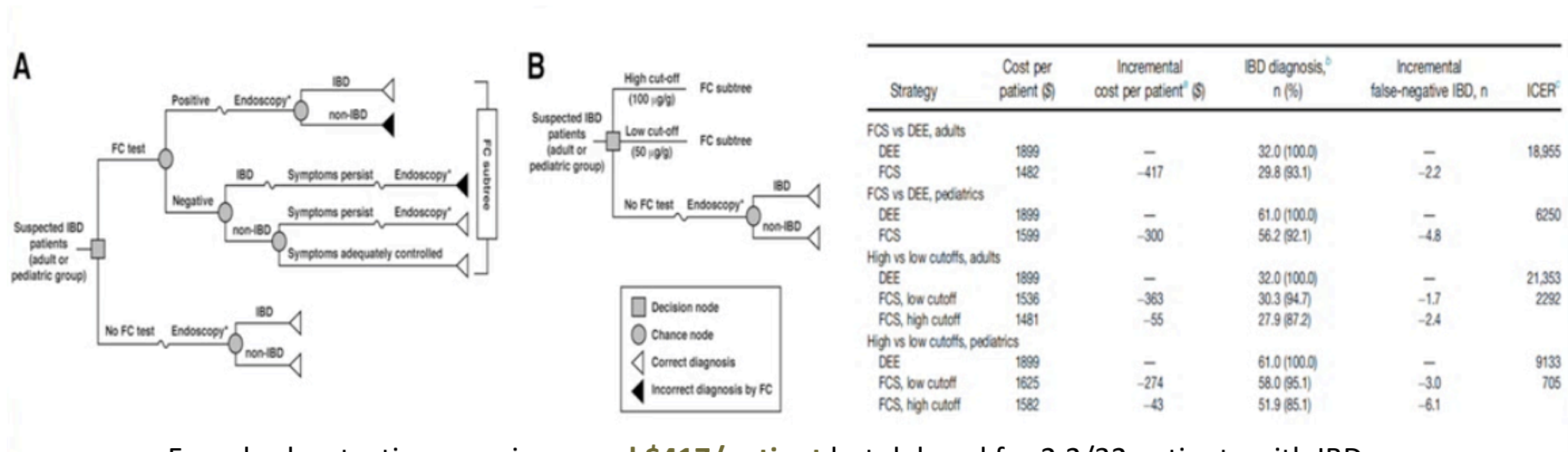
Diagnostic process (2)

- Better accuracy at a cut-off level of 100 microg/g



Cost effectiveness of calprotectin for the diagnosis of IBD

Faecal calprotectin screening versus direct endoscopic evaluation for IBD



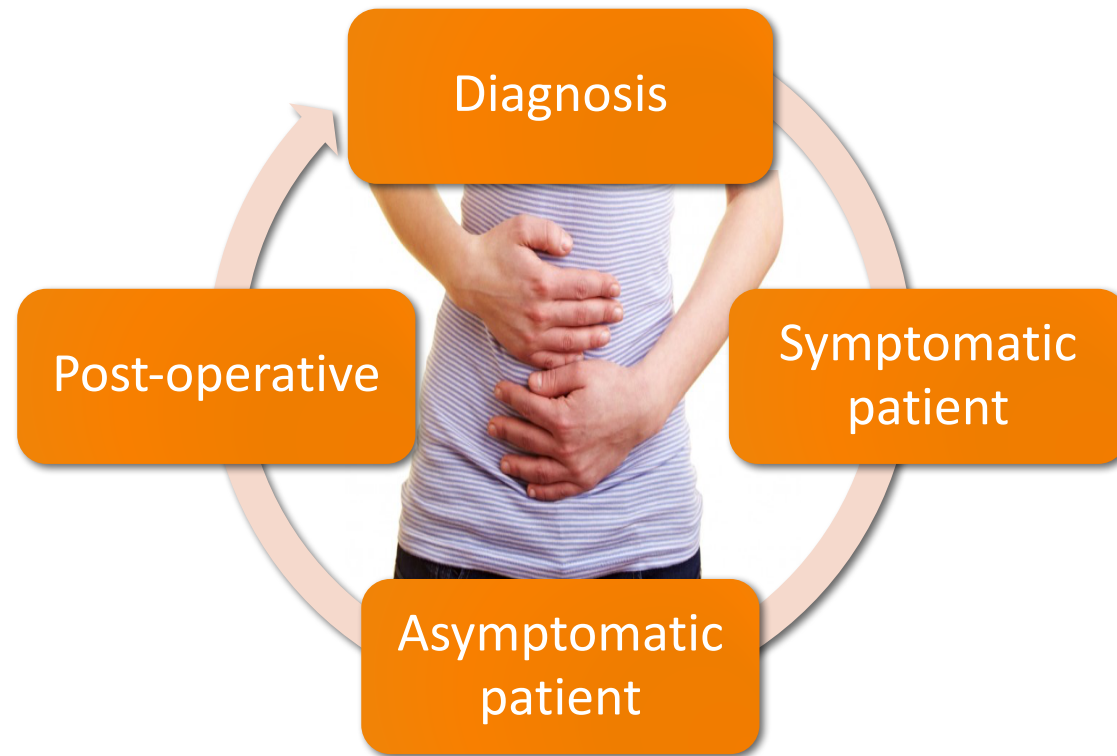
Faecal calprotectin screening **saved \$417/patient** but delayed for 2.2/32 patients with IBD

The cut-off level of 50 mg/g cost an additional \$55
for adults compared to FC **cut-off level of 100 mg/g**

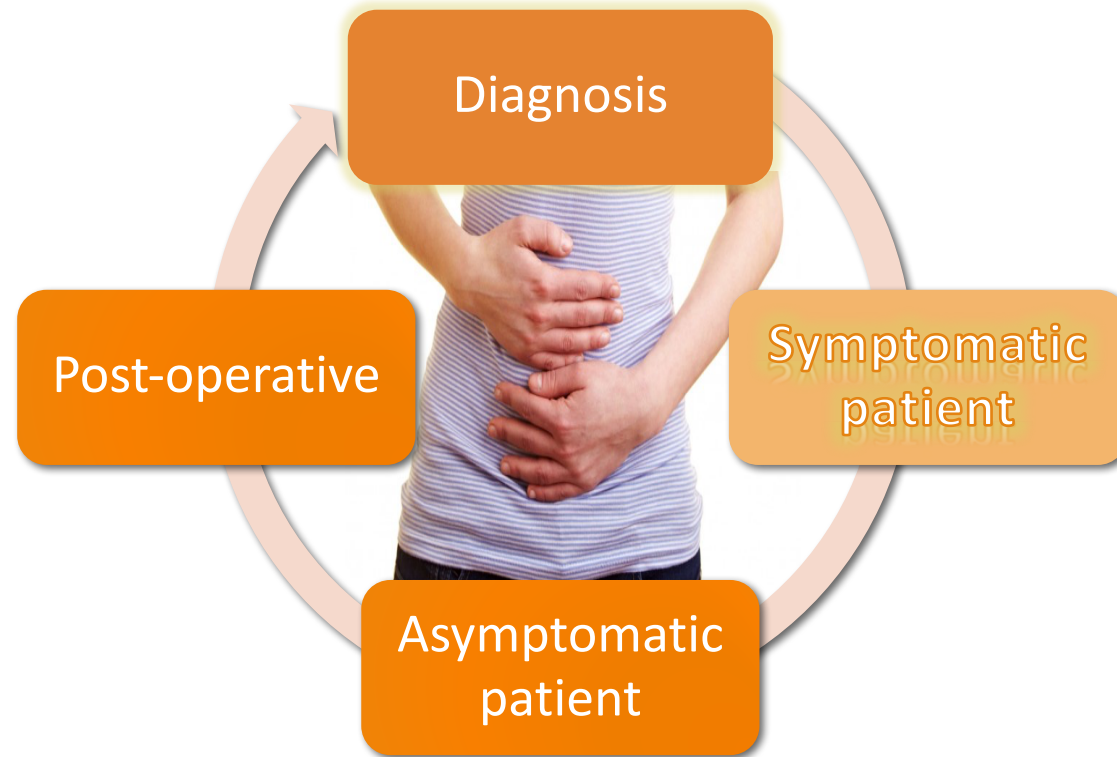
Diagnostic process - summary

- An increased FC level identifies patients who are **most likely to have inflammatory bowel disease** and justifies urgency for endoscopy
- Use of faecal calprotectin as screening test **reduces the number of endoscopies** with negative results in suspected inflammatory bowel disease
- The test **delays diagnosis** in a small and acceptable proportion of patients
- In some conditions **endoscopy is inevitable**

Utility



Utility



Preliminary remarks in IBD

- Careful **evaluation of disease characteristics** at baseline is essential to evaluate disease extend, severity and complications
- **Biomarkers** values must be established **at baseline** for future comparison

Symptomatic IBD patients

- Confirm IBD relaps ->
- Predicting response to treatment
- Predicting endoscopic healing



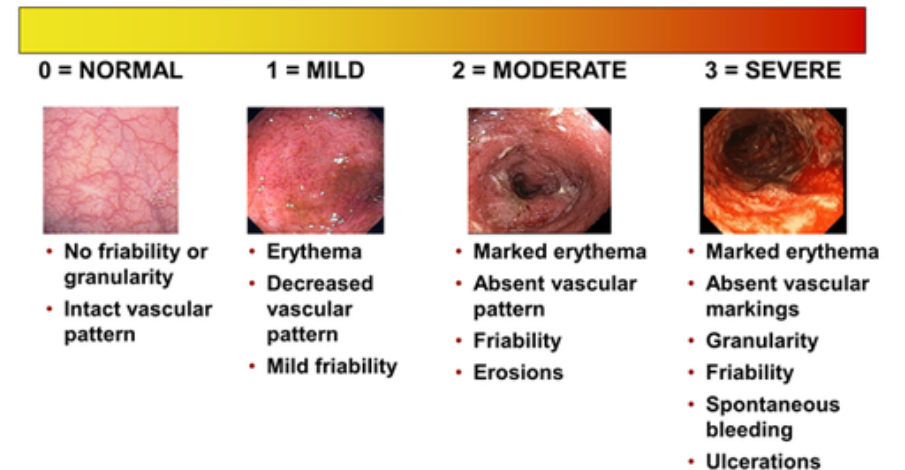
Correlation with endoscopy

Deep remission

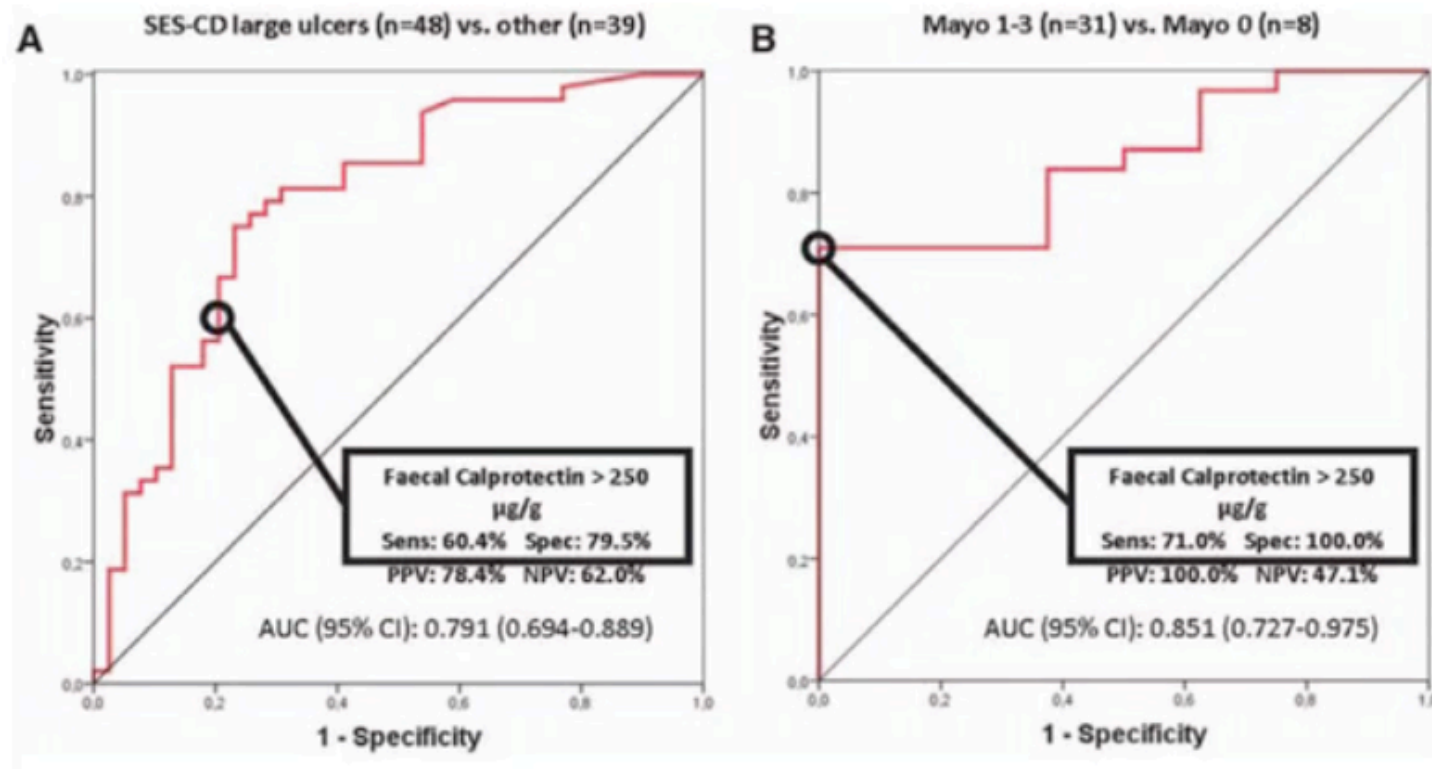
Faecal calprotectin and endoscopic scores

Tableau 2 Simplified Endoscopic Score for Crohn's Disease (SES-CD) [4]						
	Iléon	Côlon droit	Transverse	Côlon gauche	Rectum	Total
Taille des ulcérations (0-3)						
Surface ulcérée (0-3)						
Surface lésée (0-3)						
Sténose (0-3)						
Cotation						
Variable	0	1	2	3		
Taille des ulcérations	Aucune	U aphtoïdes (0,1-0,5 cm)	U larges (0,5-2 cm)	U très larges (> 2 cm)		
Surface ulcérée	Aucune	< 10 %	10-30 %	> 30 %		
Surface lésée	Aucune	< 50 %	50-75 %	> 75 %		
Présence de sténose	Aucune	Unique et franchissable	Multiples et franchissable	Infranchissable		
U : ulcérations.						

Endoscopic Severity of Disease



Faecal calprotectin and endoscopic scores



Faecal calprotectin and endoscopic scores

UC

TABLE 4. Correlation of the Endoscopic Activity Index Subgroups with the Clinical Activity Index, Fecal Calprotectin, CRP, and Blood Leukocytes

Endoscopic Activity Index	Inactive (0–3)	Mild (4–6)	Moderate (7–9)	High (10–12)
No. of patients	34	26	26	48
Clinical Activity Index	3.6±3.2 (0–13)	9.8±4 (4–17)	11.8±4.8 (4–23)	16.3±4.7 (7–27)
P-Value		<0.001	0.119	0.002
Calprotectin µg/g	42±38 (9–141)	210±121 (15–592)	392±246 (39–1044)	730±291 (266–1436)
P-Value		<0.001	0.002	<0.001
CRP mg/L	6±8 (2–44)	10±9 (3–35)	21±19 (3–74)	23±18 (3–69)
P-Value		NS	0.0102	NS
Leukocytes G/L				11.3±3 (5.1–15.6)
P-Value				

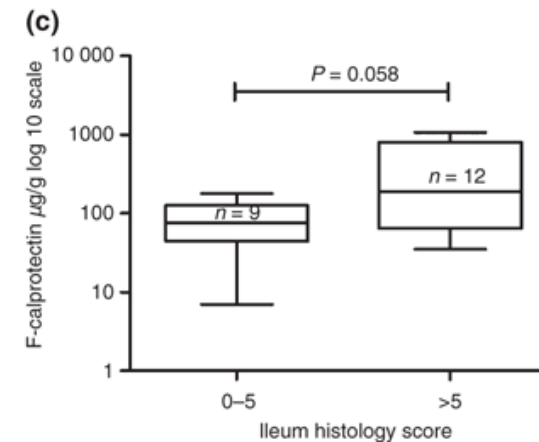
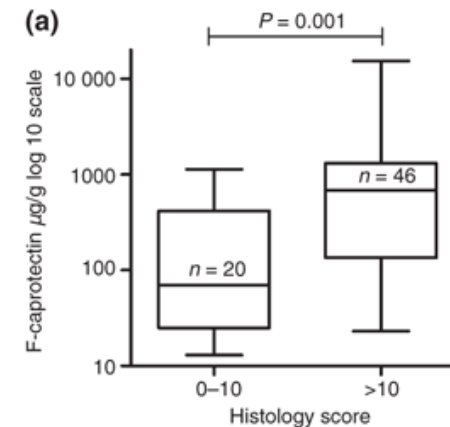
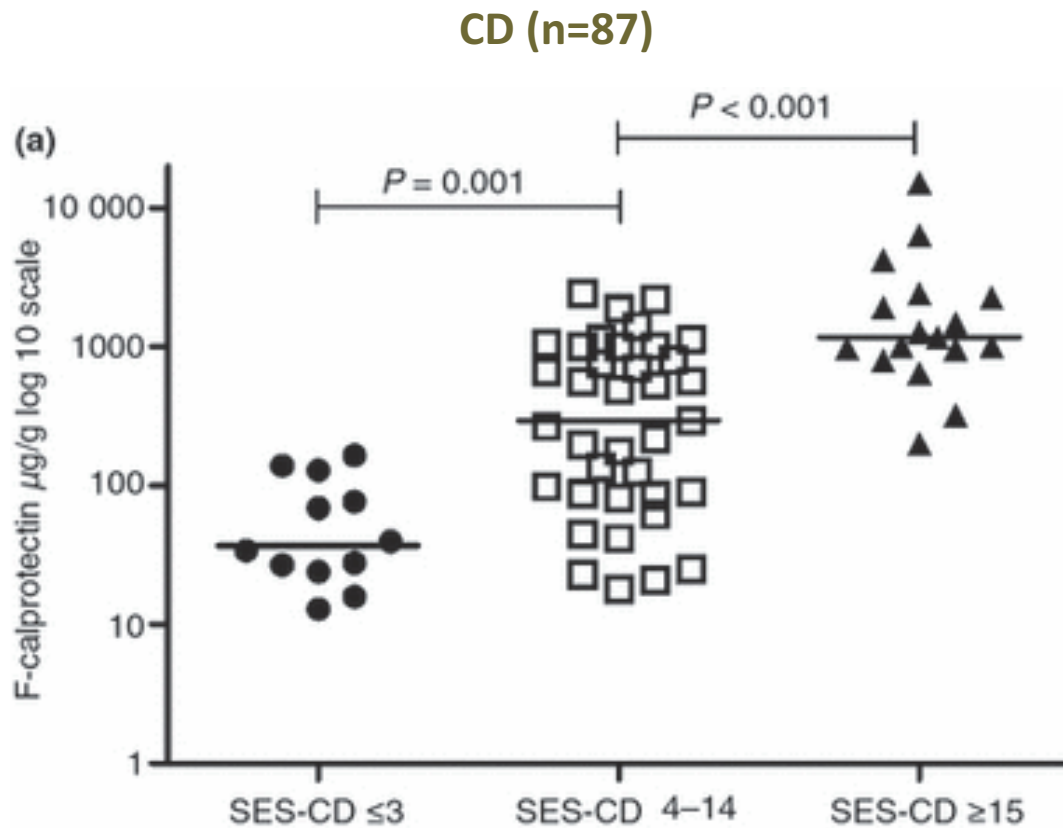
Correlation: FC > clinical activity score

CD

Table 3. Correlation of Endoscopic Activity Subgroups with the Clinical Activity Index, Fecal Calprotectin, CRP, and blood leukocytes

Endoscopic activity	Inactive (0–3)	Mild (4–10)	Moderate (11–19)	High (≥20)
No. of patients	26	40	27	47
CDAI	79±86 (13–281)	85±70 (14–297)	116±47 (44–323)	218±75 (86–417)
P value		0.739	0.201	<0.001
Calprotectin (µg/g)	104±138 (10–725)	231±244 (12–1009)	395±256 (68–912)	718±320 (93–1327)
P value		<0.001	0.008	<0.001
CRP (mg/l)	12±19 (3–94)	8±10 (3–53)	23±31 (3–172)	40±28 (5–121)
P value		0.349	0.013	0.019
Leukocytes (g/l)	7.7±3.1 (4–17.9)	7.6±2.8 (3.7–13.6)	8.8±3.1 (1.4–15.8)	11.1±3.5 (2.9–18.6)
P value		0.903	0.117	0.004

Faecal calprotectin and endoscopic scores/histology (CD)



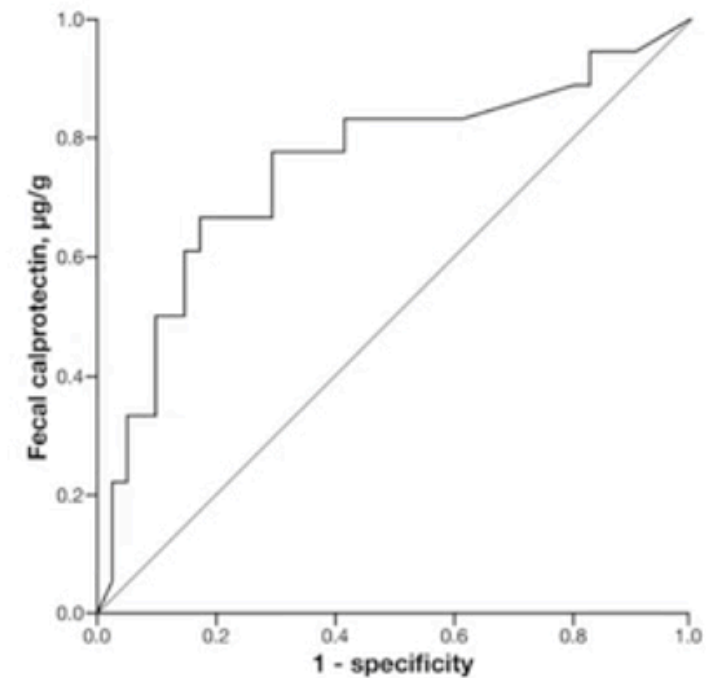
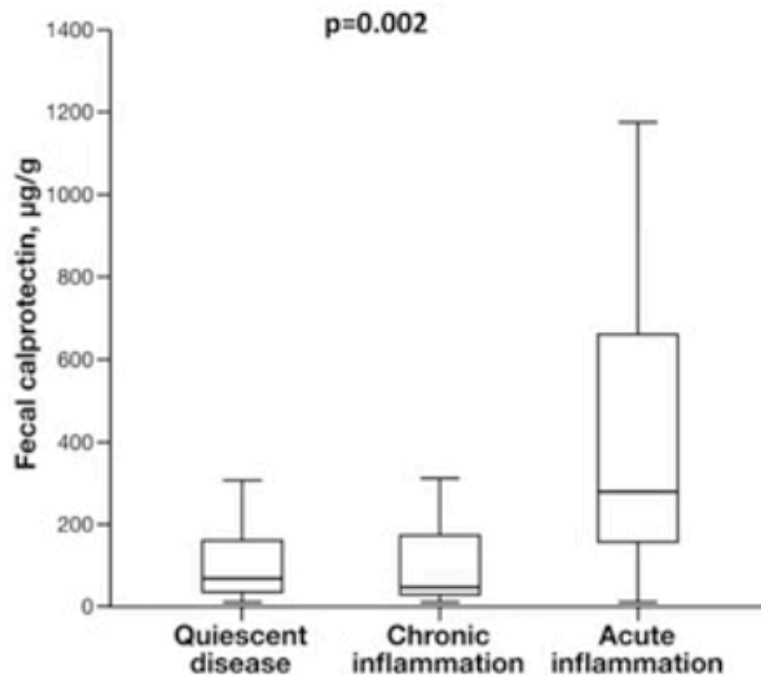
Histological criteria

- Epithelial damage (0-2)
- Extensive pathology
- Architectural changes
- Mononuclear cells in LP
- PMN in LP
- PMN in the epithelium
- Cryptitis
- Erosion or ulcers

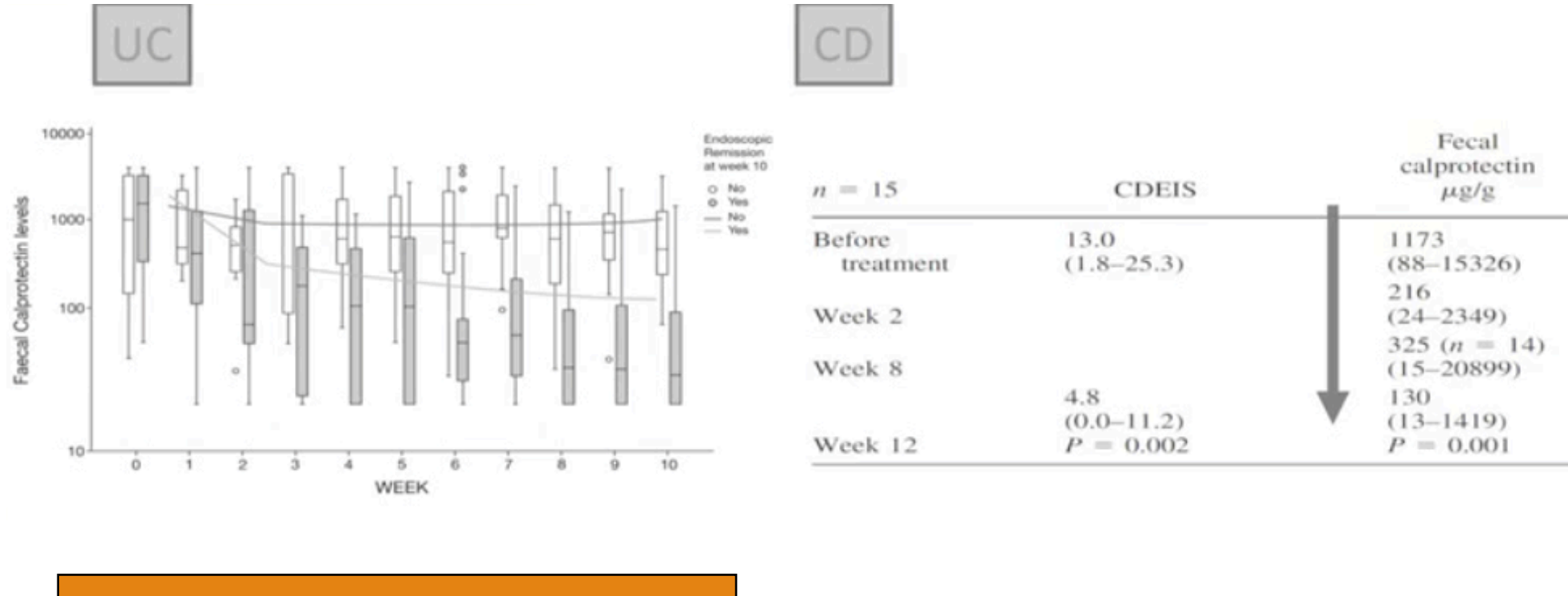
Faecal calprotectin and histology (UC)

Predictive factor of active histologic inflammation:

Faecal calprotectin and MAYO 1 vs 0



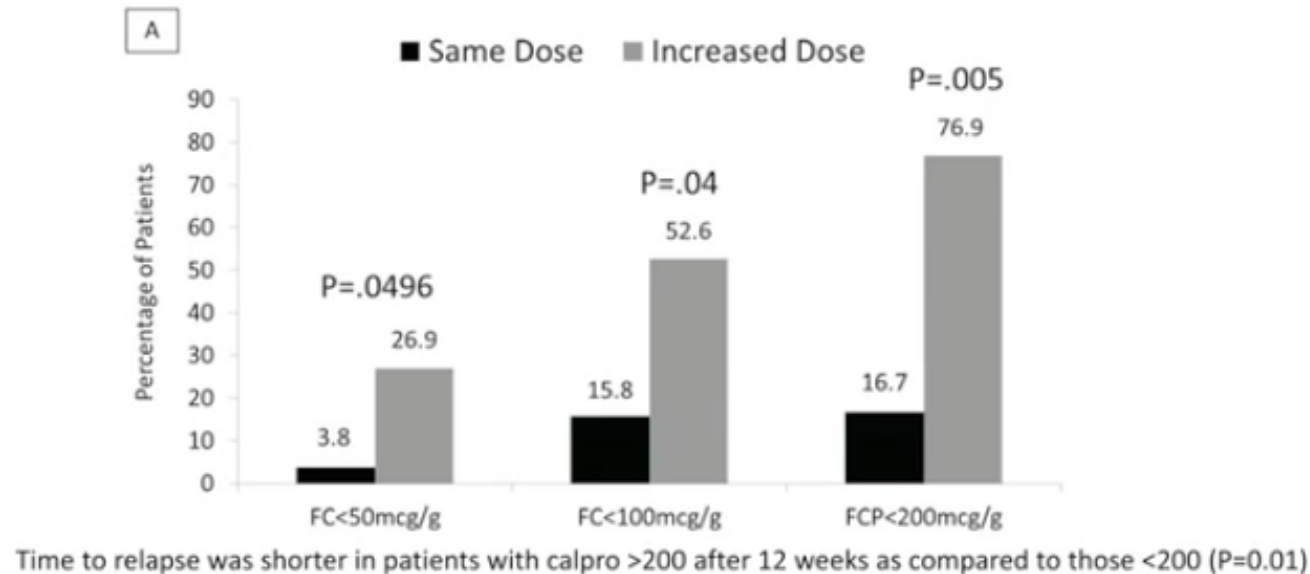
Response to anti-TNF



***FC $\leq 50 \mu\text{g/g}$ or decrease $\geq 80\%$ (W2)
predicts endoscopic remission at W10***

Predicting Response to ASA and clinical relapse in asymptomatic UC patients

Fecal calprotectin decrease after 5ASA dosage increase in patients in remission of both UC with persisting elevation of calpro



Using FC in Acute severe ulcerative colitis

No colectomy vs colectomy

No colectomy (<i>n</i> =39)	Colectomy (<i>n</i> =25)	<i>P</i> value
887 (478.0–1,472.0)	1,200.0 (677.0–1,900.0)	0.04

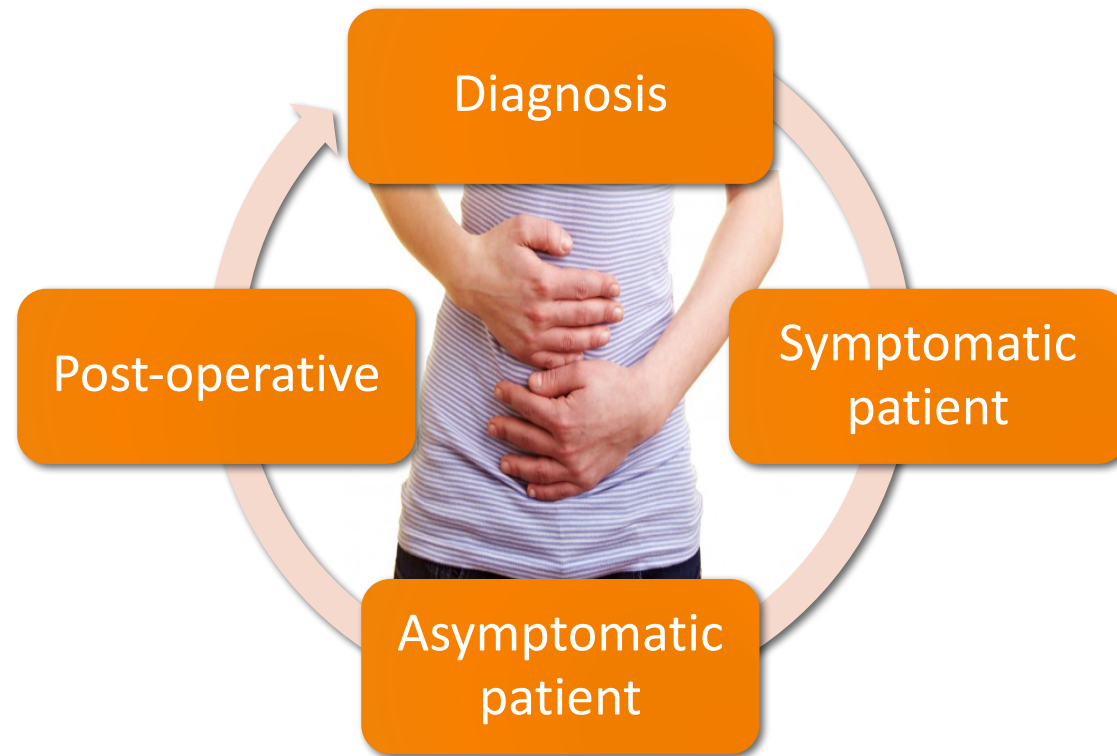
Response to treatment

Corticosteroid responders (<i>n</i> =28)	Corticosteroid nonresponders (<i>n</i> =36)	<i>P</i> value
863.5 (431.8–1,493.0)	1100.0 (663.5–1,817.5)	0.08
Infliximab responders (<i>n</i> =10)	Infliximab nonresponders (<i>n</i> =7)	<i>P</i> value
920.5 (603.8–1,483.8)	1,795.0 (1,208.0–2,170.0)	0.06

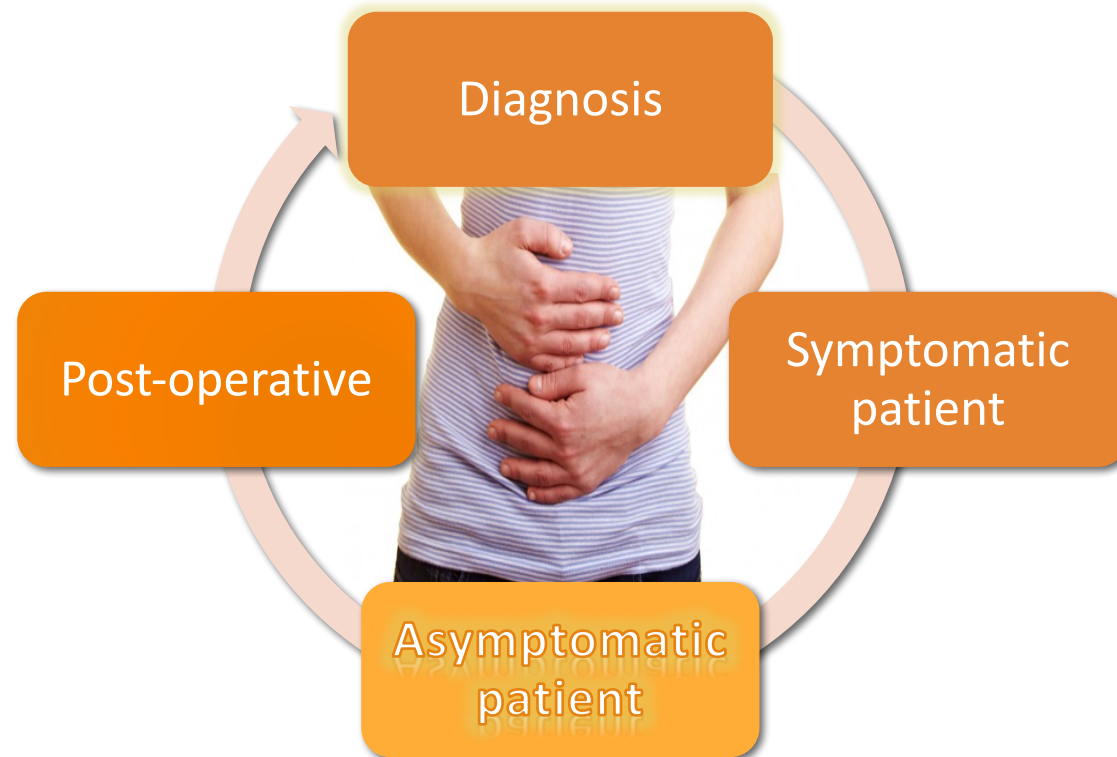
• *FC levels are markedly elevated in ASUC*

• *FC levels are significantly higher in patients who failed conventional medical therapy and require emergency colectomy*

Utility



Utility



Asymptomatic patients

➔ assess the activity of the disease

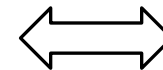
- Predicting clinical relapse
- Predicting endoscopic relapse
(preceding clinical relapse)
- Predicting clinical relapse after top-down treatment

Predicting clinical relapse in quiescent IBD patient

Table 5. Studies Associating Increased Concentrations of Fecal Calprotectin With Relapse in Patients With Exacerbations in Ulcerative Colitis

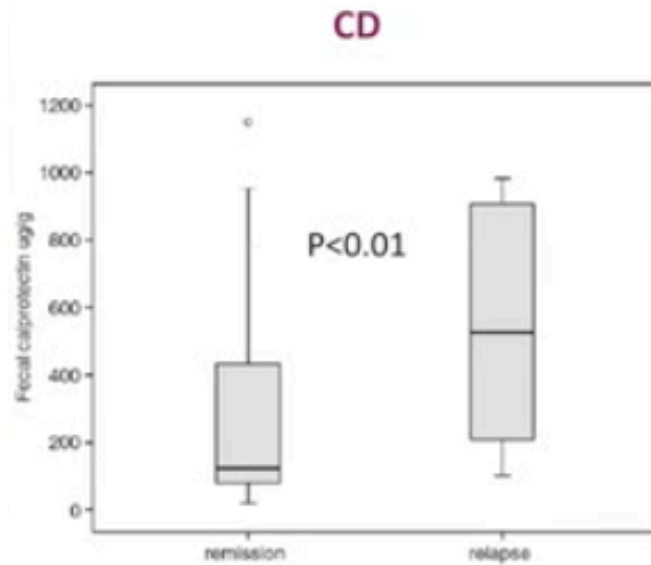
Author	Patient population	Duration of remission at entry	Calprotectin concentration to define elevated level	Relapse rate with low calprotectin, %	Relapse rate with high calprotectin, %
Gisbert et al ⁸¹	UC	>6 mo	>150 µg/g	9	31
Tibble et al ⁸⁰	UC	1–4 mo	>50 µg/g	10 ^a	85 ^a
Tibble et al ⁸⁰	CD	1–4 mo	>50 µg/g	15 ^a	85 ^a
Costa et al ⁷⁹	UC	1–12 mo	>150 µg/g	10	81
Costa et al ⁷⁹	CD	1–12 mo	>150 µg/g	57	87
D’Inca et al ⁸²	UC	3–36 mo	>130 µg/g	30	79
Sipponen et al ⁸³	UC + CD	>3 mo (51% >12 mo)	>100 µg/g	25	39
Walkiewicz et al ⁸⁴	CD	Not stated	>400 µg/g	11	56

^aEstimated from Kaplan-Meier curves

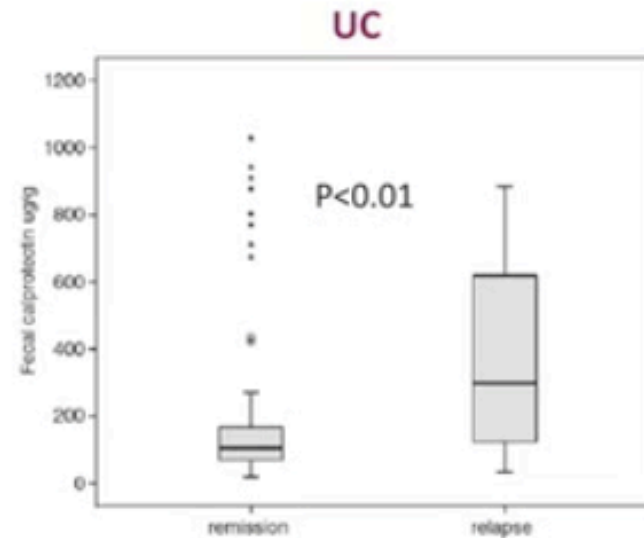


Predicting clinical relapse in asymptomatic UC and CD patients

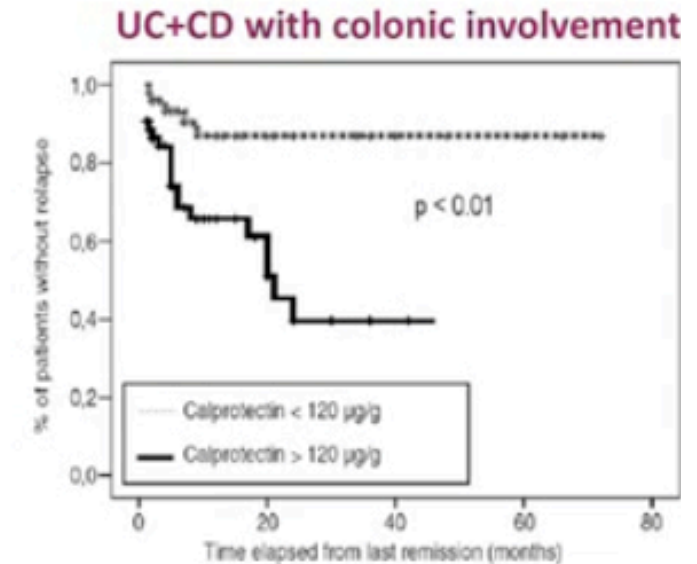
Prospective study during 1 year of UC and CD patients in clinical remission



FC > 200 $\mu\text{g/g}$: RR relapse X4

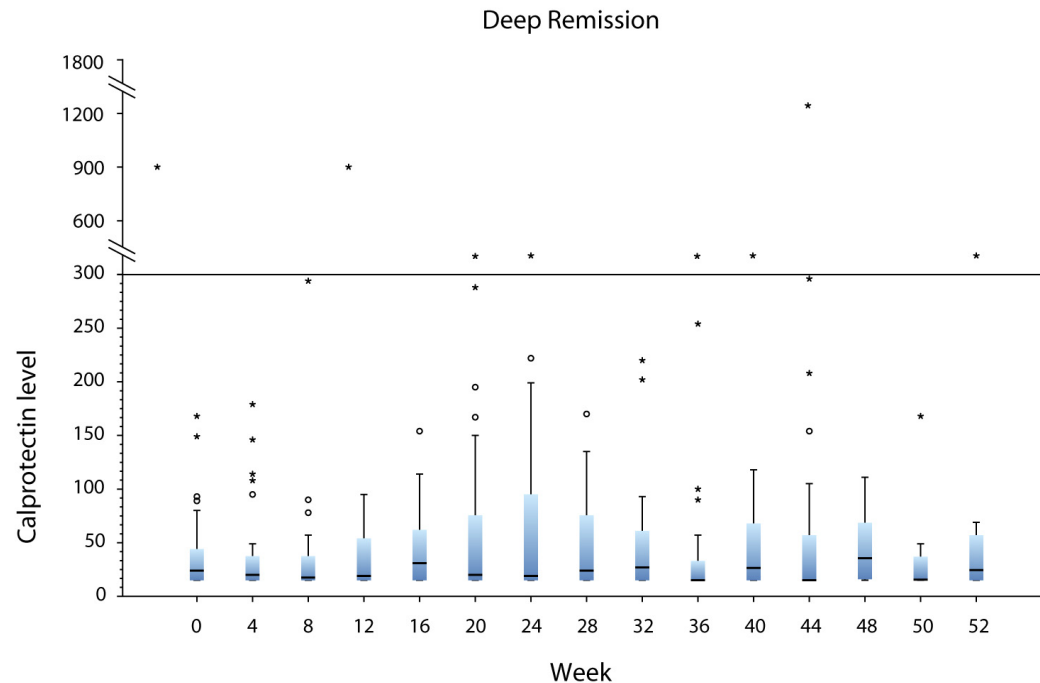


FC > 120 $\mu\text{g/g}$: RR relapse X6

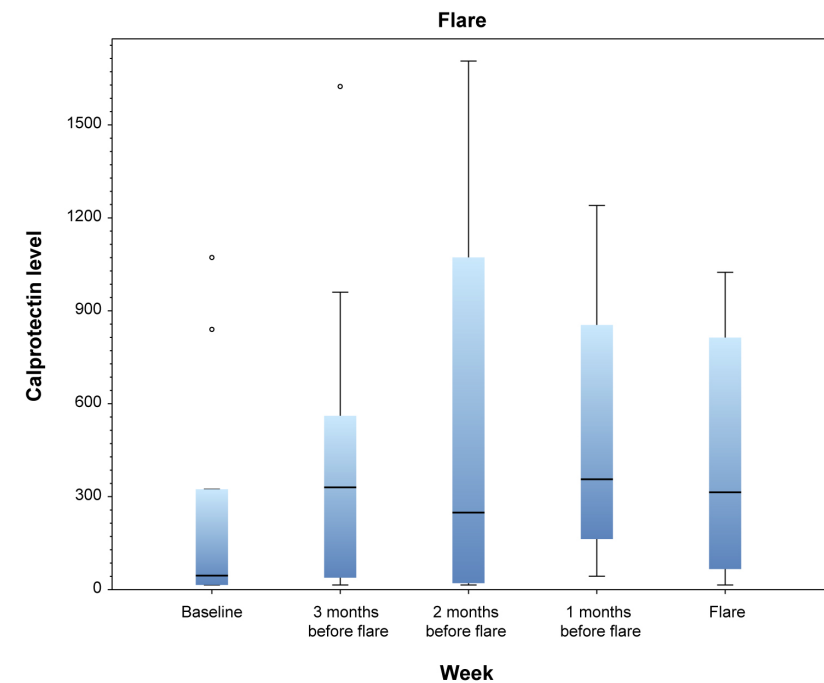


Predictive value similar in colonic CD and UC
Cut-off of 120 $\mu\text{g/g}$:
Sensitivity=80% and Specificity=60%

Predicting clinical relapse in quiescent UC patients on IFX

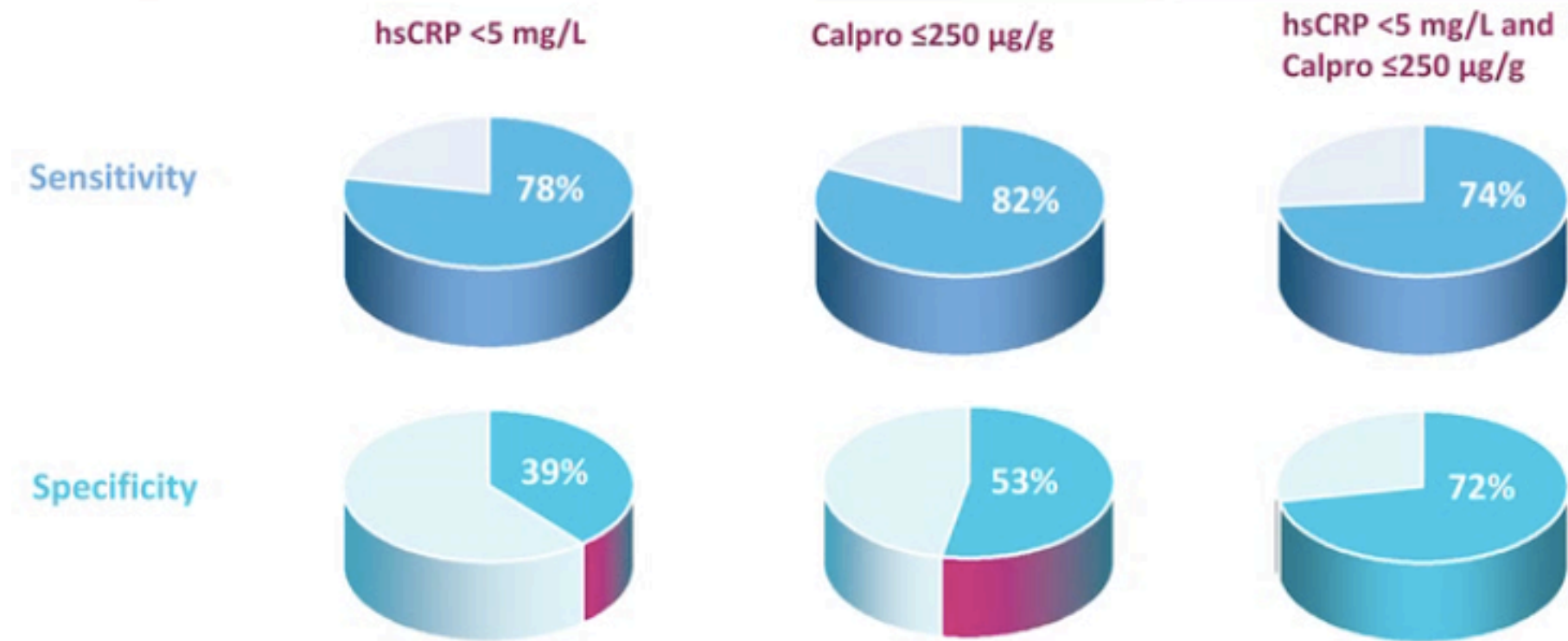


Calprotectin levels $< 50 \mu\text{g/g}$
predicts deep remission (Ss 83%, Sp 83%)



2 consecutive calprotectin
measurements of $> 300 \mu\text{g/g}$
predict flare (Ss 62%, Sp 100%)

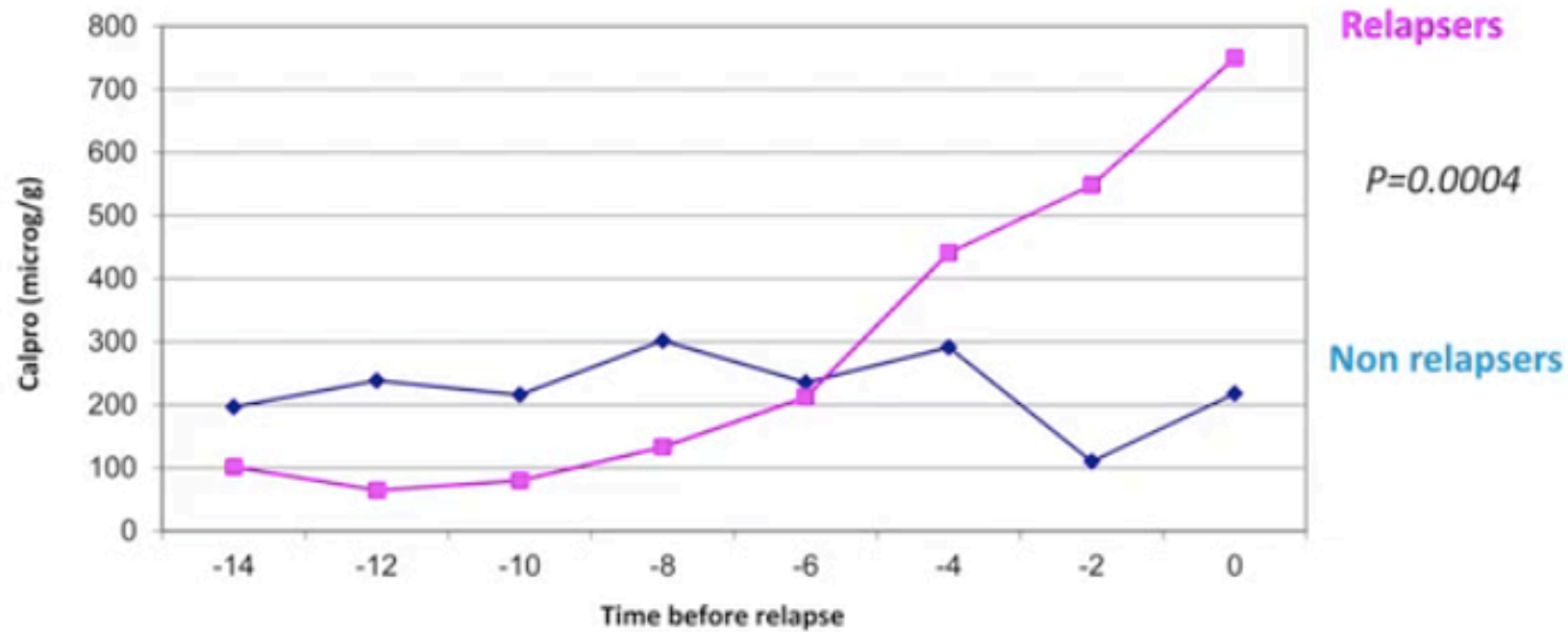
Predicting endoscopic relapse in asymptomatic CD patients



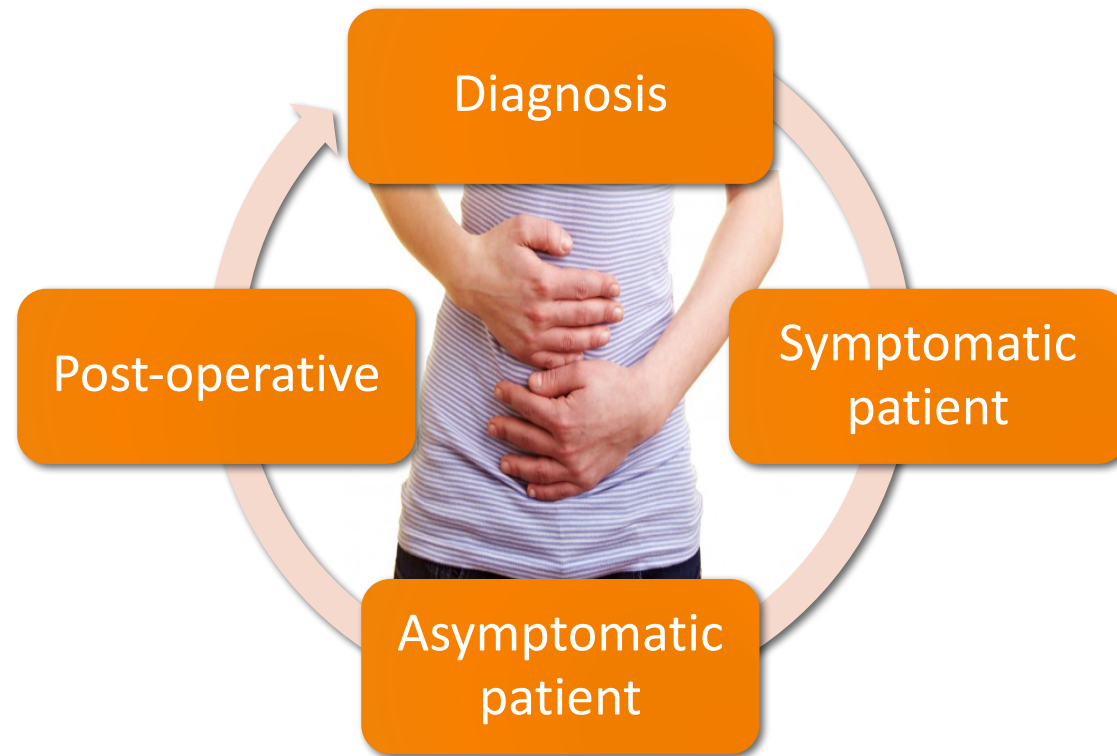
* Mucosal healing defined as CDEIS ≤3

Predicting clinical relapse after top-down strategy

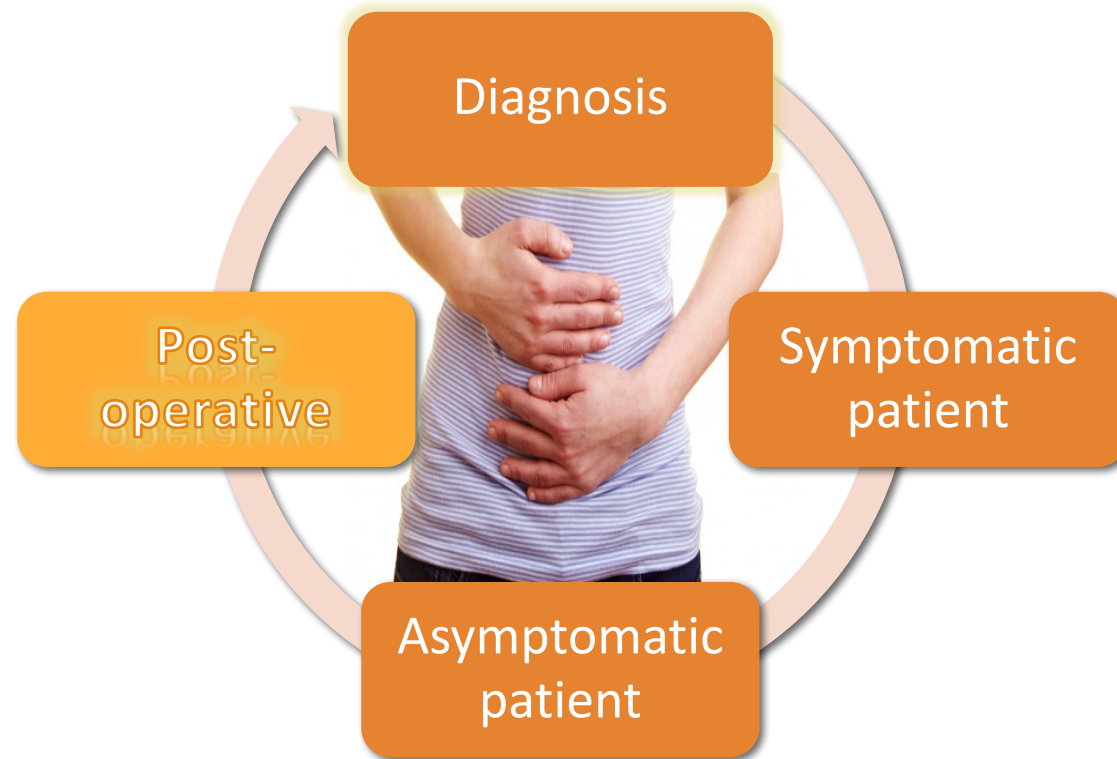
Clinical relapse in clinically quiescent CD patients after IFX interruption



Utility



Utility



Post-operative relapse

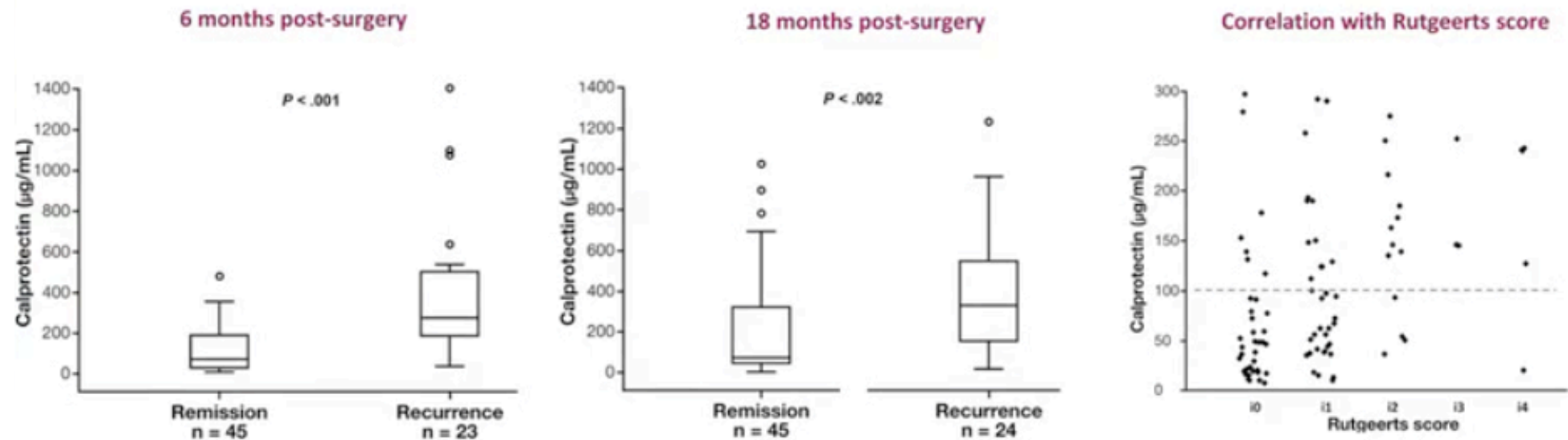
- **50-70%** of CD patients require surgery during their disease course
- Post operative recurrence POR :
 - **15-40%** at 10 years
 - **50-70%** at 20 years
- POR predictors:
 - Risk factors (patient, disease)
 - Endoscopic score 6 months after surgery (**Rutgeerts score**)

Clinical remission 3 years after surgery and Rutgeerts score

Rutgeerts Score		
i ₀	No lesions in the distal ileum	95%
i ₁	Less than 5 aphthous lesions in the distal ileum	85%
i ₂	> 5 aphthous lesions with normal mucosa between the lesions, or skip area of large lesions or lesions confined to ileocolonic anastomosis	
i ₃	Diffuse aphthous ileitis with diffusely inflamed mucosa	60%
i ₄	Large ulcers with diffuse mucosal inflammation or nodules or stenosis in the neoterminal ileum	5%

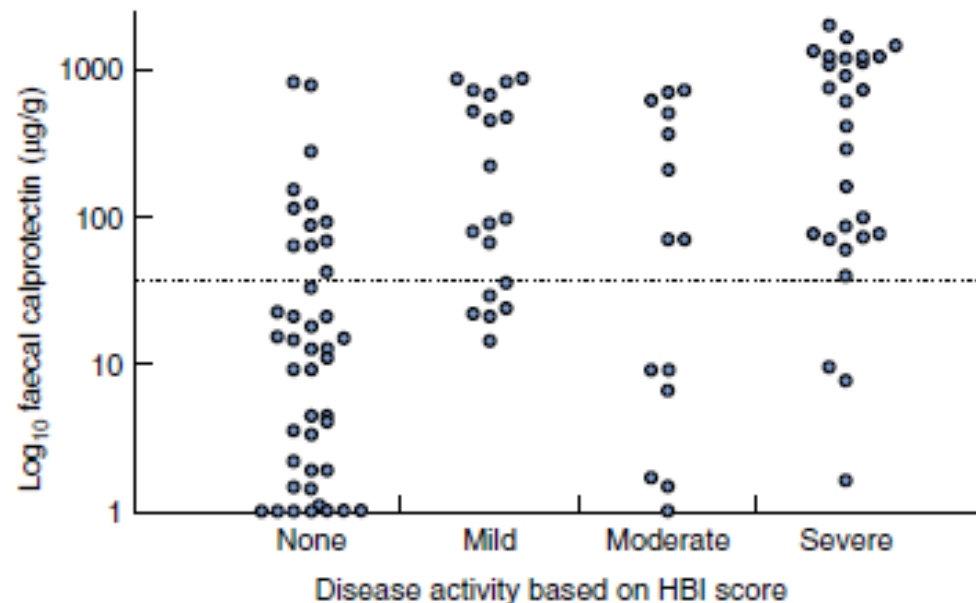
Predicting post-operative relapse

Median pre-operative FC = 1347 $\mu\text{g/g}$



FC > 100 $\mu\text{g/g}$ => 89% sensitivity and 58% specificity, NPV=91%

Post-op correlation between faecal calprotectin and Harvey Bradschaw Index



Clinical activity (Harvey Bradshaw Index score)	Faecal calprotectin levels (µg/g)
None (≤ 3)	70.2(27.1)
Mild (4)	333.7(78.9)
Moderate (5)	242.4(79.2)
Severe (≥ 6)	661.1(119.1)

Values are mean(s.e.).

Significant correlation between FC and HBI ($r=0.53$, $p<0.001$)

Very low levels in clinically inactive disease

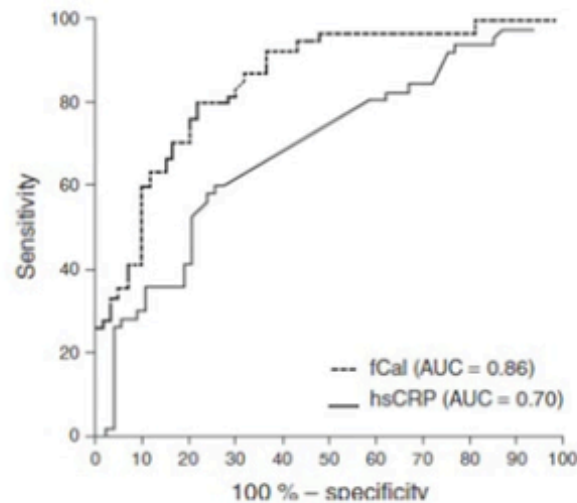
Very high levels in severely clinically active disease

FC values in mild to moderate group are far more heterogeneous

Predicting post-operative relapse

Better performance for fCal compared to hsCRP

Association of fCal ≤ 100 $\mu\text{g/g}$ and hsCRP ≤ 1 mg/l did not increase the performance of fCal concentration alone



fCal in $\mu\text{g/g}$ cutoff	Sen (%)	Spe (%)	PPV (%)	NPV (%)	OA
50	98	33	60	94	66
100	95	54	69	93	77
150	77	82	81	78	79
250	52	91	85	65	71
hsCRP in mg/l Cutoff	Sen (%)	Spe (%)	PPV (%)	NPV (%)	OA
1	87	24	52	58	53
2	72	65	56	64	59
5	43	86	69	59	62
fCal cutoff 100 $\mu\text{g/g}$ and hsCRP cutoff 1 mg/l	—	—	52	59	54



Optimal Cut-Off

Optimal cut-off for FC in different situations	
Situations	Optimal cut-off
IBD or IBS	50-100 µg/g
IDB activity	250 µg/g
Relapse	250 µg/g
POR	100 µg/g
Response to treatment	100-150 µg/g
Mucosal healing	250 µg/g ?
Relaps in top-down strategy	250 µg/g ?

Testing for faecal Calprotectin at home



- Correlation coefficient with ELISA = 0.6285
- Intra-assay coefficient of variation = 4.42%
- Interassay coefficient of variation = 12.49%
- Sensitivity = 82%
- Specificity = 85%
- Positive predictive value = 47%
- Negative predictive value = 97%
- Optimal cut-off = 150 µg/g

Conclusion

- Faecal calprotectin predicts patients with **high risk of IBD** and allows a better selection of patients who require endoscopy. This strategy is **cost saving**.
- Faecal calprotectine is highly correlated with endoscopic and histological scores
 - Accurate marker of deep remission
 - Identifies patients with endoscopic recurrence and high risk of clinical recurrence
- Although faecal calprotectin is correlated with **post-operative endoscopic recurrence** colonoscopy is still mandatory 6 months after surgery
- The **home testing faecal calprotectin** is in development
 - Reliable
 - **New way** of monitoring by eHealth
- No Guidelines.