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Investigating the role of faecal calprotectin in luminal gastroenterology

Daniel R Gaya MB ChB (Hons), FRCP (Glasg), MSc (Edin)

Submitted in fulfilment of the requirements for the degree of MD by published work
Institute of Infection, Immunity and Inflammation
College of Medical, Veterinary and Life Sciences,
University of Glasgow

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Contents

| | |
|--|-------|
| 1. List of publications | 4 |
| 2. List of tables and figures | 5 |
| 3. List of abbreviations | 6 |
| 4. Summary | 7-8 |
| 5. Introduction | 9-10 |
| 6. Chapter 1 – FC in the assessment of Crohn’s disease activity | 11-14 |
| 7. Chapter 2 – Intra-individual variability of FC in CD patients | 15-18 |
| 8. Chapter 3 – Predictive value of FC in CD patients in remission | 19-25 |
| 9. Chapter 4 –Investigatory yield mildly elevated FC in new GI referrals | 26-28 |
| 10. Chapter 5 – Assessment of FC compared to serum calprotectin in GI patients | 29-32 |
| 11. Discussion | 33-44 |
| 12. Conclusion | 45 |
| 13. References | 46-55 |
| 14. Appendices | 56 |
| 15. Published papers | 57 |

List of publications (original papers)

McCann RK, Smith K, **Gaya DR** A prospective single centre evaluation of a serum calprotectin assay in unselected GI patients. *Clinical biochemistry* 2017 (in press)

Seenan JP, Thomson F, Rankin K, Smith K, **Gaya DR**. Are we exposing patients with a mildly elevated faecal calprotectin to unnecessary investigations? *Frontline Gastroenterology* 2015;6:156-160

A prospective evaluation of the predictive value of faecal calprotectin in quiescent Crohn's disease. Naismith GD, Smith LA, Barry SJE, Munro JI, Laird S, Karen K, Morris AJ, Winter JW, **Gaya DR**. *Journal of Crohns and Colitis* 2014;8:1022-1029

Naismith GD, Smith LA, Barry SJ, Munro JI, Laird S, Rankin K, Morris AJ, Winter JW, **Gaya DR**. A prospective single-centre evaluation of the intra-individual variability of faecal calprotectin in quiescent Cohn's disease. *Alimentary Pharmacology & Therapeutics* 2013;37:613-21

Gaya DR, Lyon TDB, Duncan A, Neilly JB, Han S, Howell J, Liddell C, Stanley AJ, Morris AJ, MacKenzie JF. Faecal calprotectin in the assessment of Crohn's disease activity. *QJM* 2005;98:435-441

List of tables

Chapter 2

| | |
|---|----|
| 2.3 Baseline characteristics of patients | 16 |
| 2.3 Percentage of patients classified as normal or 'case' based on FC | 17 |
| 2.3 Reliability of FC in defining a case | 18 |

Chapter 3

| | |
|------------------------------------|----|
| 2.3 Subject demographics | 21 |
| 2.3 Cox proportional hazards model | 24 |

Chapter 4

| | |
|---|----|
| 4.3 Patient demographics | 27 |
| 4.3 Investigations performed and diagnostic yield | 27 |

Chapter 5

| | |
|---|----|
| 5.3 Correlations between FC, SC and CRP | 32 |
|---|----|

List of figures

Chapter 1

| | |
|--|----|
| 1.3 Boxplot of FC concentrations | 12 |
| 1.3 Scatterplots of FC plotted against CDAI and WCS scores | 13 |
| 1.3 ROC curve of FC in predicting positive from negative WCS | 14 |

Chapter 2

| | |
|--|----|
| 2.3 Histograms of FC values in the three samples | 16 |
| 2.3 Scatterplots of three serial FC values | 17 |

Chapter 3

| | |
|--|----|
| 3.3 ROC curve of FC in relapse prediction at 12 months | 22 |
| 3.3 Scatterplot of FC values in relapser and non-relapsers | 23 |
| 3.3 Kaplan-Meier event curve of time to relapse | 24 |

Chapter 4

| | |
|----------------------------|----|
| 4.3 Pie chart of diagnoses | 28 |
|----------------------------|----|

Chapter 5

| | |
|--|-------|
| 5.3 Scatterplots of SC/FC, SC/CRP and FC/CRP | 30-32 |
|--|-------|

List of abbreviations

AOC – area under the curve
CD – Crohn’s disease
CDAI – Crohn’s disease activity index
CRP – C-reactive protein
CT – computerised tomography
CT – computerised tomography
DAMP – disease associated molecular pattern protein
ELISA – enzyme linked immunoassay
ESR – erythrocyte sedimentation rate
FC – faecal calprotectin
GI – gastrointestinal
GRI – Glasgow Royal Infirmary
IBD – inflammatory bowel disease
IBS – irritable bowel syndrome
ICC – intraclass correlation
ICC – intraclass correlation coefficient
IQR – inter-quartile range
NICE – National Institute of Clinical Excellence
NPV – negative predictive value
NSAID – non-steroidal anti-inflammatory drug
PPI – proton pump inhibitor
PPV – positive predictive value
SC – serum calprotectin
REC – research ethics committee
ROC – receiver operating characteristic
UC – ulcerative colitis
WCS – radiolabelled white cell scanning

Summary

Providing a sensitive, non-invasive, cheap marker of disease activity inflammatory bowel disease (IBD) comprises an area of ongoing interest and unmet clinical need. In previous years, options included only serum CRP (poorly sensitive and specific), colonoscopy (invasive, costly, perforation risk, inability to view proximal small bowel), CT (costly, ionising radiation risk) and radiolabelled white cell scanning (costly, poor sensitivity). My thesis describes a series of trials performed to establish the role of faecal calprotectin to define current disease activity in IBD patients.

Prompted by studies demonstrating the potential role for a novel faecal marker of clinical utility in the context of NSAID enteropathy, we chose to investigate the role of this biomarker, faecal calprotectin (FC), in Crohn's disease. To facilitate this I used existing cohorts and then generated new cohorts in which to address fundamental and clinically relevant questions of importance. We compared FC to radiolabelled white cell scanning in our first study which initiated and established a mutually beneficial collaboration between luminal gastroenterology and clinical biochemistry. Thereafter we recruited a rigorously phenotyped cohort of Crohn's disease patients in remission to answer two separate research questions. First, was there a significant intra-individual variability of FC and secondly, would FC sampling in remission predict future relapse over the ensuing 12 months? Thereafter, in a new cohort, we investigated whether we were over-investigating new GP referrals to the GI clinic with only mildly elevated FC values. Finally, and most recently, we sought to investigate whether or not there was any correlation between serum calprotectin and FC in a new unselected GI cohort of patients, thereby potentially obviating the need for our patients to collect stool samples.

Our data demonstrated that FC correlated well with radiolabelled white cell scanning in assessment of Crohn's disease activity, thereby potentially avoiding this costly test as part of disease monitoring. In addition, we defined an acceptable intra-individual variability of FC in Crohn's disease to support the clinical utility of one off testing using FC. Our prospective dataset revealed that an FC in remission can indeed stratify Crohn's disease patients to estimate future relapse risk thereby allowing us to

personalise medical therapies with more aggressive therapeutics employed in those with Crohn's disease in remission but with residual high FC. The work we undertook in our primary care dataset revealed an extremely low yield of investigating mildly elevated FC and thus we developed a new shared protocol with our GP colleagues in which serial FC testing is recommended rather than referral to secondary care for such patients. Lastly, our most recent work demonstrated that there was no significant correlation between serum and FC in an unselected GI cohort meaning the search for a GI-specific serum biomarker of inflammation goes on – this is in accord with a variety of other chronic inflammatory diseases in which circulating biomarkers have proven challenging to find and especially to validate.

This body of work has been presented nationally and internationally at meetings, and has been published in discipline relevant, peer reviewed medical journals. Moreover, it has supported the adoption of FC into everyday NHS GI practice. We were the first UK hospital to establish an NHS service for this biomarker in 2007 when we performed around 50 assays per month. Currently, the test is in widespread use and the Glasgow Royal Infirmary biochemistry lab now analyses 1400 samples per month. This has become an established non-invasive, cheap, sensitive marker of IBD activity in clinical practice, often avoiding the need for colonoscopy for the purposes of disease activity monitoring. This biomarker is also being used to gauge the success or failure of medical therapies in IBD and is a useful tool to differentiate irritable bowel syndrome from IBD. The clinical utility of the test has allowed GPs to triage referrals and often avoid referrals completely and has engaged patients in the self-monitoring of their IBD.

Introduction

The clinical need for a sensitive, cheap, non-invasive measure of inflammatory burden in the GI tract is significant. Faecal biomarkers represent an attractive option in view of the shortcomings of the other available options in luminal gastroenterology including CRP (poorly sensitive and specific), radiolabelled white cell scanning (costly, poor sensitivity), CT scanning (costly, poorly sensitive, ionising radiation dose) and colonoscopy (invasive, costly, perforation risk, inability to visualise the proximal small bowel).

Of the faecal biomarkers of inflammation available, FC has been the most studied. Calprotectin, first described in 1980 (Fagerhol MK 1980), is a protein found in the cytosol of neutrophils and macrophages composed of two subunits S100A8 and S100A9. It can be detected in plasma, urine, cerebrospinal fluid, faeces, saliva, synovial fluid and colonic biopsies (Johns B 1997). It is stable in faeces for up to seven days at room temperature and has a homogenous distribution in faeces (Roseth AG 1992), properties which lend it to testing spot faecal samples. There has been recent emphasis of the involvement of the innate immune system in the pathogenesis of IBD (Kono H 2008). Calprotectin is classed as a damage associated molecular pattern protein (DAMP) having antimicrobial protective properties. DAMPs are released by the innate immune system from damaged or activated cells, initiating and perpetuating the immune response. The extracellular release of calprotectin during times of cell stress/damage makes it an accurate marker of intestinal inflammation.

Over the last 15 years, I have led a clinical research programme looking at FC in luminal gastroenterology. In 2002, I became interested in this faecal biomarker due to its potential utility in our IBD cohort at GRI. We undertook a cross sectional cooperative study of FC, radiolabelled white cell scanning and the CDAI in CD patients attending our out patients clinic (paper). Thereafter we analysed intra-individual variability of FC in quiescent CD (paper). Utilising the same cohort of patients, we prospectively followed these patients to ascertain the predictive value of FC in CD patients in remission (paper). Owing to concerns that we were over-investigating

patients with mildly elevated FC referred to the GI clinic at GRI, we specifically sought to address this question (paper). Most recently, we investigated whether there was any correlation between FC and serum calprotectin in an unselected cohort of GI patients (paper).

Chapter 1 – FC in the assessment of Crohn’s disease activity (Gaya DR et al Q J Med 2005;98:435-441)

1.1 Introduction

The clinical assessment of CD activity is challenging and correlates poorly with endoscopic findings. However endoscopy is invasive, unpleasant for patients, carries significant morbidity and is expensive. Hence a faecal biomarker is an attractive alternative option. We compared radio-labelled white cell scanning (WCS), a validated clinical scoring system (CDAI, see appendix I) and FC in the assessment of CD activity.

1.2 Methods

Patients were recruited from the GI out-patient clinic at GRI with symptoms suggestive of a CD relapse. After signed informed consent, a CDAI was calculated for each patient. Subsequently a WCS and stool sample collection were done on the same day. Exclusions included being on oral prednisolone, a PPI, NSAID or having a positive stool culture or purely perianal CD.

Samples for FC were collected and stored within 6 hours at -20° C and analysed by a commercial ELISA method (Calprest, Calprotech Ltd) as per the manufacturer’s instructions. WCS was performed in the nuclear medicine department of GRI using an established protocol. Interpretation of the WCSs was undertaken by three experienced reporters who were each blinded to the CDAI and FC results.

Statistical analyses were undertaken using Minitab version 13 (Minitab Inc) and the study was granted full ethical opinion by the GRI REC (01GA005).

1.3 Results

A box plot of the FC concentrations from the 35 entrants is shown in figure 1 with ranges from 5 – 7623 µg/ml and 85% having a raised FC. FC correlated significantly with mean total WCS score ($r=0.73$, $p<0.001$) (figure 2), ‘extent’ WCS score ($r=0.71$, $p<0.001$) (figure 3), ‘severity’ WCS score ($r=0.64$, $p<0.001$) (figure 4) and ‘combined extent and severity’ WCS score ($r=0.71$, $p<0.001$) (figure 5). No correlation was seen

between CDAI and FC ($r=0.33$, $p=0.06$) (figure 6) or mean total WCS score ($r=0.21$, $p=0.24$) (figure 7).

Using ROC analysis, at a value of $100\mu\text{g/g}$, FC had a sensitivity of 80%, specificity of 67%, PPV of 87%, NPV of 60% in detecting CD patients with and without inflammation on a WCS. The accuracy of FC in correctly classifying a CD patient, as measured by the AOC, was 87% (figure 8).

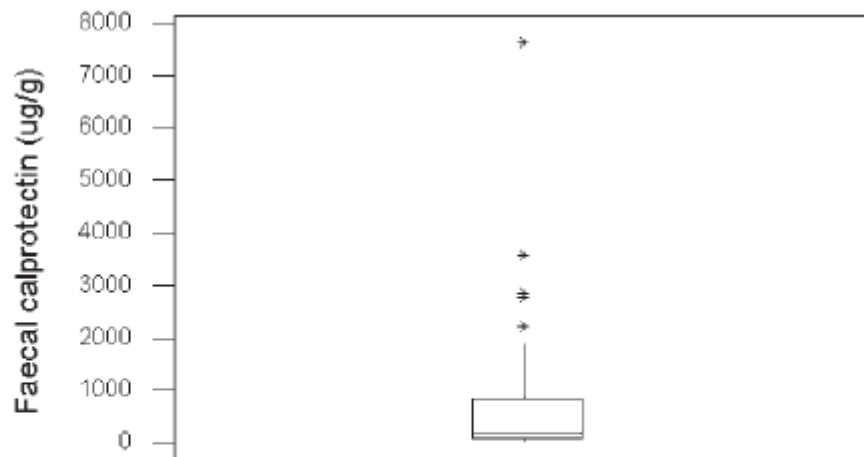


Figure 1. Boxplot of faecal calprotectin concentrations (median $195\mu\text{g/g}$, 25th centile $77\mu\text{g/g}$, 75th centile $850\mu\text{g/g}$) (normal $<50\mu\text{g/g}$).

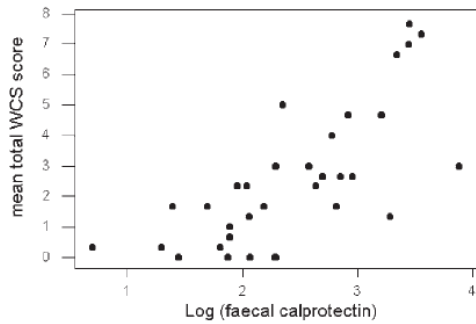


Figure 2. Log of faecal calprotectin plotted against mean total WCS score ($r=0.73$, $p<0.001$).

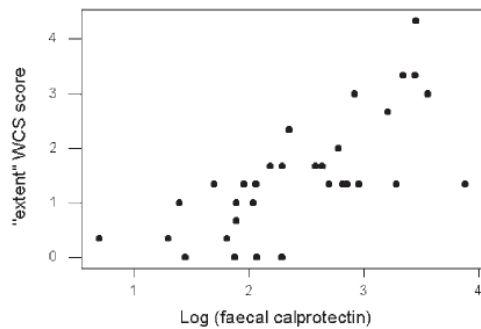


Figure 3. Log of faecal calprotectin plotted against 'extent' WCS score ($r=0.71$, $p<0.001$).

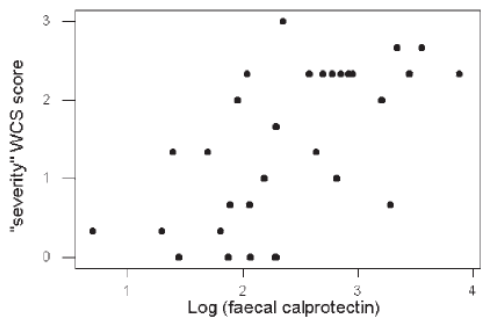


Figure 4. Log of faecal calprotectin plotted against 'severity' WCS score ($r=0.64$, $p<0.001$).

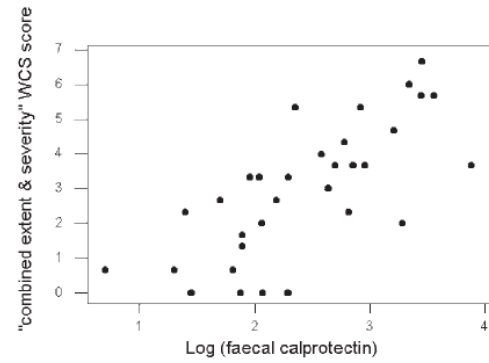


Figure 5. Log faecal calprotectin plotted against 'combined extent & severity' WCS score ($r=0.71$, $p<0.001$).

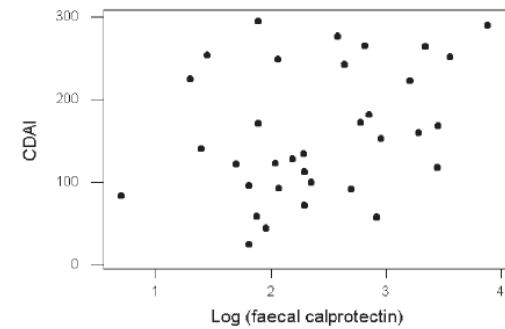


Figure 6. Log faecal calprotectin plotted against CDAI ($r=0.33$, $p=0.06$).

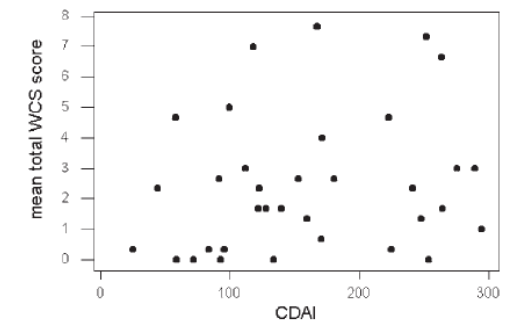


Figure 7. CDAI plotted against mean total WCS score ($r=0.21$, $p=0.24$).

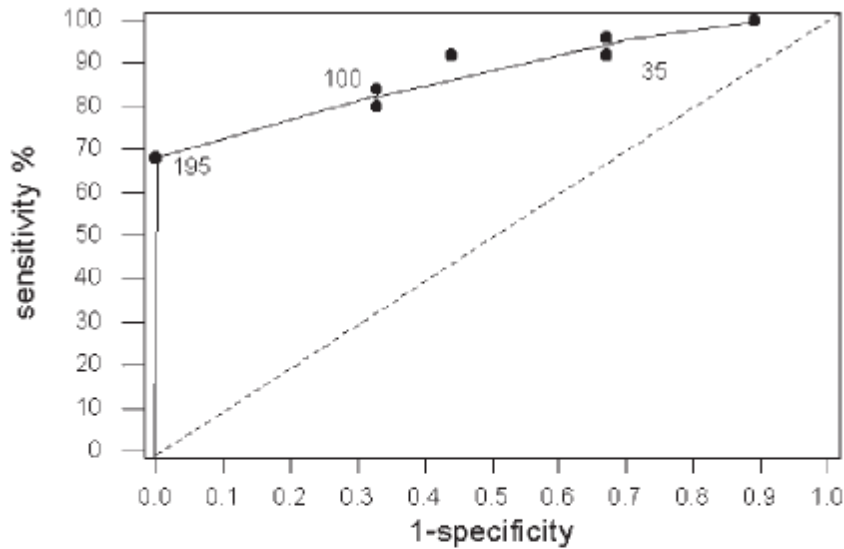


Figure 8. Receiver operating characteristic (ROC) curve of the ability of faecal calprotectin to discriminate between those Crohn's disease patients with and without a positive WCS. The diagonal line from the origin indicates no discriminatory value whatsoever.

1.4 Conclusion

Our data revealed that FC correlated well with WCS in the assessment of CD activity. ROC analysis illustrated that a one off FC gave an accurate reflection of the extent and/or severity of the inflammation. Furthermore, CDAI did not correlate with either FC or WCS and is thus a poor surrogate for inflammatory burden in CD patients.

Chapter 2 – Intra-individual variability of FC in CD patients (Naismith GD et al Aliment Pharmacol Ther 2013;37:613-21)

2.1 Introduction

Whilst FC has been adopted into routine GI clinical practice, there are concerns regarding the variability of FC within the same patient. Thus clinical decision making based on a one off FC may be flawed. We thus investigated the reliability and reproducibility of FC values in CD patients in remission.

2.2 Methods

Between August 2010 and November 2011, CD patients attending the OPC at GRI were recruited with a CDAI<150. Written informed consent was obtained. Candidates were asked to collect stool samples for FC over three consecutive days. The study was approved by the West of Scotland REC (10/S0704/1).

Stool samples were analysed according to the manufacturer's instructions (Buhlmann) using the Roche faecal extraction kit.

The consistency of FC was analysed using the intraclass correlation (ICC) of the log transformed FC values. The reliability was analysed using the kappa statistic. A sample size of 95 patients would give sufficient power to test these parameters adequately

2.3 Results

Of the 143 patients recruited, 98 submitted three stool samples suitable for analysis. The baseline characteristics of these patients are shown in table 1. Despite normal CDAIs, the entrants FC values were wide ranging albeit most had relatively low levels (figure 1). The ICC was 0.84 (95% CI:0.79-0.89) which reveals good overall consistency between the three samples for each study participant (figure 2). The reliability in detecting a 'case' of active CD is revealed by the kappa value of 0.648 (0.511-0.769), 0.603 (0.477-0.720) and 0.732 (0.588-0.853) for FC cut offs of >50µg/g, >100µg/g and >350µg/g respectively (table 2). In the wider population, using an FC cut off of >350µg/g would be highly reliable (table 3).

Table 1 | Baseline characteristics of the 98 patients that took part in the study

| | |
|----------------------------|---------------|
| Age (mean \pm s.d.) | 47 \pm 16 |
| Gender (% male) | 34% |
| Smoker (%) | 19% |
| Stoma (%) | 17% |
| CDAI (mean \pm s.d.) | 50 \pm 39 |
| CD location (%) | |
| Ileal | 15% |
| Ileocolonic | 36% |
| Colonic | 47% |
| CD phenotype (%) | |
| Inflammatory | 57% |
| Stricturing | 30% |
| Fistulizing | 12% |
| Perianal (%) | 15% |
| 5 ASA (%)* | 43% |
| Corticosteroid (%)* | 4% |
| Thiopurine (%)* | 38% |
| Methotrexate (%)* | 3% |
| Anti TNF (%)* | 14% |
| CRP (mg/L) Mean \pm s.d. | 5.7 \pm 7.6 |

* Medications refer to current use.

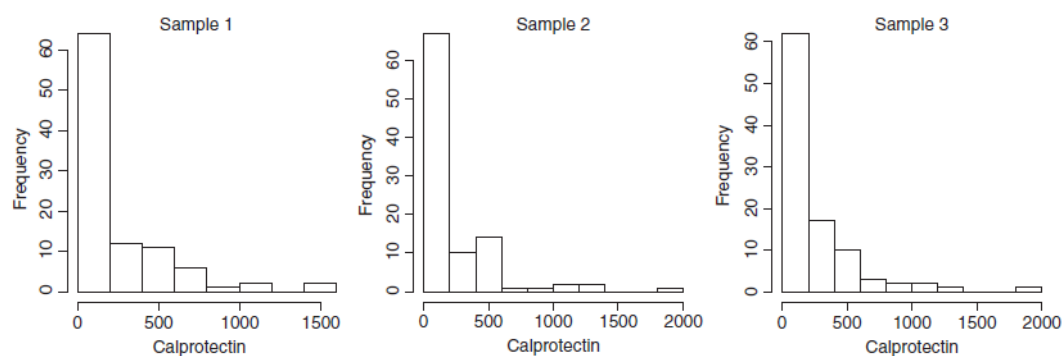


Figure 1 | Histograms of the FC values ($\mu\text{g/g}$) in the three samples.

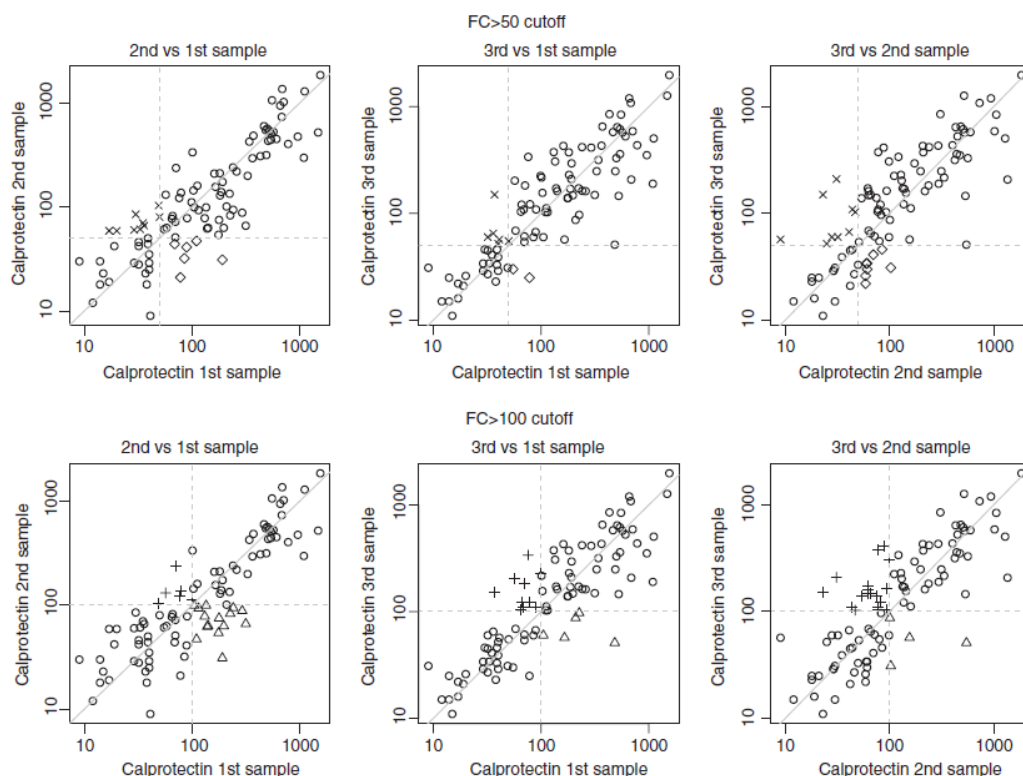


Figure 2 | Scatterplots of the first, second and third sample values, on the log to base 10 (log10) scale, with the >50 $\mu\text{g/g}$ (top row) and >100 $\mu\text{g/g}$ (bottom row) cut offs marked. The axis values on the log10 plots show the raw FC values that each point corresponds to. The differently shaped points represent patients classified differently as case or normal by different samples.

Table 2 | Percentage of the 98 patients classified as case or normal in each sample according to the definition of a case being FC >50, >100 or >350 $\mu\text{g/g}$

Case if FC > 50 $\mu\text{g/g}$ (total agreement = 79%)

| 3rd sample = Normal (N = 24) 1st sample | 2nd sample | | 3rd sample = Case (N = 74) 1st sample | 2nd sample | |
|--|------------|------|--|------------|------------|
| | Normal | Case | | Normal | Case |
| Normal | 15% | 7% | Normal | 4% | 2% |
| Case | 1% | 1% | Case | 5% | 64% |

Case if FC >100 $\mu\text{g/g}$ (total agreement = 71%)

| 3rd sample = Normal (N = 39) 1st sample | 2nd sample | | 3rd sample = Case (N = 59) 1st sample | 2nd sample | |
|--|------------|------|--|------------|------------|
| | Normal | Case | | Normal | Case |
| Normal | 34% | 1% | Normal | 5% | 5% |
| Case | 2% | 3% | Case | 13% | 37% |

Case if FC >350 $\mu\text{g/g}$ (total agreement = 86%)

| 3rd sample = Normal (N = 75) 1st sample | 2nd sample | | 3rd sample = Case (N = 23) 1st sample | 2nd sample | |
|--|------------|------|--|------------|------------|
| | Normal | Case | | Normal | Case |
| Normal | 69% | 1% | Normal | 5% | 0% |
| Case | 2% | 4% | Case | 2% | 16% |

The percentages agreeing across all three samples are in bold, while all nonbold percentages are mismatches. Any percentage discrepancies are due to rounding.

| Table 3 Reliability of defining a 'case' using calprotectin >50, >100 or >350 µg/g | | |
|--|---------------------|--|
| Case definition | Kappa (95% CI) | Level of agreement between samples (agreement based on 95% CI) |
| Calprotectin >50 | 0.648 (0.511–0.769) | Substantial (moderate to substantial) |
| Calprotectin >100 | 0.603 (0.477–0.720) | Moderate (moderate to substantial) |
| Calprotectin >350 | 0.732 (0.588–0.853) | Substantial (moderate to almost perfect) |

2.4 Conclusion

Day to day variability of FC in CD patients in remission is low. The consistency and reliability of the test is good, meaning a one off value can be utilised in this patient cohort for clinical decision making.

Chapter 3 – Predictive value of FC in CD patients in remission (Naismith GD et al J Crohns Colitis 2014;8:1022-1029)

3.1 Introduction

Crohn's disease is characterised by relapses and remissions. Being able to predict which patients are at greater risk of future relapse is of major importance. We assessed whether an FC level in clinical remission was of clinical utility in predicting relapse within 12 months.

3.2 Methods

Of the 98 CD patients in remission recruited to our previous study (chapter 2), 97 were followed prospectively to 12 months. The primary end point was relapse within 12 months whilst a secondary end point was relapse at any point during follow up. Patients were asked to contact our IBD team with any symptoms which could represent a flare of disease. Flare was defined as an unplanned escalation of therapy, progression of disease phenotype by Montreal classification or hospitalisation for emergency medical/surgical treatment of CD. Only patients who were lost to follow up before 12 months were excluded from analysis.

Ethical approval and FC analysis was identical to our previous study (chapter 2).

The Mann-Whitney or t-test were used to test for significant differences in continuous variables (incl FC) between 'relapsers' and 'non-relapsers', while Fisher's exact test was used for categorical variables. The sensitivity and specificity of FC to predict relapse was calculated and the resulting ROC curve was plotted. The resulting AOC was calculated to reveal the predictive power of FC regarding future relapse and the optimal FC cutoff (best sensitivity/specificity, PPV, NPV) was used to calculate Kaplan –Meir cumulative event curves for time to relapse. A Cox proportional hazards model was fitted to assess the impact of an FC value above or below the optimal cutoff on time to relapse at any point during the study.

3.3 Results

Of the 97 recruited patients, 92 were followed to the primary end point or to 12 months (baseline characteristics, table 1). 10 patients (12%) relapsed within 12 months and these patients had higher baseline median FC than those that did not (414µg/g; IQR 259-590 versus 96µg/g; IQR 39-237; p=0.005). ROC curve analysis revealed an

optimal FC cutoff of 240 μ g/g with a sensitivity of 80%, specificity of 74.4% NPV of 96.8% and PPV of 27.6% (figure 1). The AUC for FC to predict CD relapse at 12 months was 77.4% (figure 1). Only two patients with an FC <240 μ g/g relapsed by 12 months whilst only a minority of those with an FC >240 μ g/g did relapse highlighting the low PPV (figure 2). We plotted a Kaplan-Meier cumulative event curve to reveal time to relapse with clear separation between the curves in those with FC > and <240 μ g/g (figure 3). The hazard ratio of relapse with an FC >240 μ g/g was 12.18 (95% CI 2.55-58.2; p=0.002). Table 2 shows the Cox proportional hazard model of FC on time to relapse and shows that patients with an FC>240 is 12.18 times more likely to relapse within 12 months than one who does not. Also this table reveals there was no impact on patient demographics on time to relapse. Table 2

Table 1 Subject demographics, by whether or not they relapsed by 12 months (N = 92). CRP measurements taken within 1 month of the FC sample only were included.

| | | Non-relapsers | Relapsers | p-Value |
|--------------------------------|---------------------------------------|-------------------|---------------------|---------|
| Faecal calprotectin (µg/g) | N _{obs} (N _{miss}) | 82 (0) | 10 (0) | 0.005 |
| | Mean (SD) | 215.0 (279.4) | 529.6 (448.8) | |
| | Median (IQR) | 95.5 (39.2,237.2) | 414.0 (258.8,590.2) | |
| | [Range] | [9.0,1550.0] | [38.0,1480.0] | |
| Age (years) | N _{obs} (N _{miss}) | 82 (0) | 10 (0) | 0.207 |
| | Mean (SD) | 47.9 (16.0) | 41.0 (15.4) | |
| | Median (IQR) | 46.0 (35.2,60.8) | 44.5 (28.2,47.8) | |
| | [Range] | [18.0,83.0] | [18.0,66.0] | |
| Gender | Female | 53 (89.8%) | 6 (10.2%) | 0.742 |
| | Male | 29 (87.9%) | 4 (12.1%) | |
| Surgery | No | 38 (92.7%) | 3 (7.3%) | 0.503 |
| | Yes | 44 (86.3%) | 7 (13.7%) | |
| Stoma ^a | No | 69 (88.5%) | 9 (11.5%) | 1.000 |
| | Yes | 13 (92.9%) | 1 (7.1%) | |
| Smoker | No | 68 (89.5%) | 8 (10.5%) | 0.684 |
| | Yes | 14 (87.5%) | 2 (12.5%) | |
| Age at diagnosis (years) | <16 | 6 (75.0%) | 2 (25.0%) | 0.092 |
| | 17–40 | 58 (87.9%) | 8 (12.1%) | |
| | > 40 | 18 (100.0%) | 0 (0.0%) | |
| Duration of disease (years) | N _{obs} (N _{miss}) | 82 (0) | 10 (0) | 0.367 |
| | Mean (SD) | 10.7 (9.7) | 14.1 (10.8) | |
| | Median (IQR) | 7.5 (3.0,15.8) | 13.0 (4.0,23.2) | |
| | [Range] | [1.0,38.0] | [1.0,30.0] | |
| Location | Ileal | 15 (93.8%) | 1 (6.2%) | 0.493 |
| | Colonic | 29 (93.5%) | 2 (6.5%) | |
| | Ileal colonic | 38 (84.4%) | 7 (15.6%) | |
| | Isolated upper disease | 0 (NaN%) | 0 (NaN%) | |
| Behaviour | Non stricturing non penetrating | 53 (94.6%) | 3 (5.4%) | 0.077 |
| | Stricturing | 23 (79.3%) | 6 (20.7%) | |
| | Penetrating | 6 (85.7%) | 1 (14.3%) | |
| Peri-anal | No | 71 (88.8%) | 9 (11.2%) | 1.000 |
| | Yes | 11 (91.7%) | 1 (8.3%) | |
| C-reactive protein | N _{obs} (N _{miss}) | 40 (42) | 5 (5) | 0.539 |
| | Mean (SD) | 8.62 (15.78) | 2.14 (1.57) | |
| | Median (IQR) | 2.10 (0.98,7.78) | 2.00 (1.10,3.00) | |
| | [Range] | [0.20,77.00] | [0.30,4.30] | |
| <i>Medications at baseline</i> | | | | |
| 5-Amino-salicylic acid | No | 45 (88.2%) | 6 (11.8%) | 1.000 |
| | Yes | 37 (90.2%) | 4 (9.8%) | |
| Thiopurine | No | 54 (93.1%) | 4 (6.9%) | 0.164 |
| | Yes | 28 (82.4%) | 6 (17.6%) | |
| Anti-tumour necrosis factor | No | 71 (88.8%) | 9 (11.2%) | 1.000 |
| | Yes | 11 (91.7%) | 1 (8.3%) | |
| Steroid ^b | No | 81 (90.0%) | 9 (10.0%) | 0.207 |
| | Yes | 1 (50.0%) | 1 (50.0%) | |
| Methotrexate | No | 79 (88.8%) | 10 (11.2%) | 1.000 |
| | Yes | 3 (100.0%) | 0 (0.0%) | |
| No medications ^c | No | 57 (86.4%) | 9 (13.6%) | 0.272 |
| | Yes | 25 (96.2%) | 1 (3.8%) | |

^a One patient underwent elective stoma closure during study period.

^b Long term low dose maintenance steroid only.

^c One quiescent patient later commenced adalimumab for arthritis only.

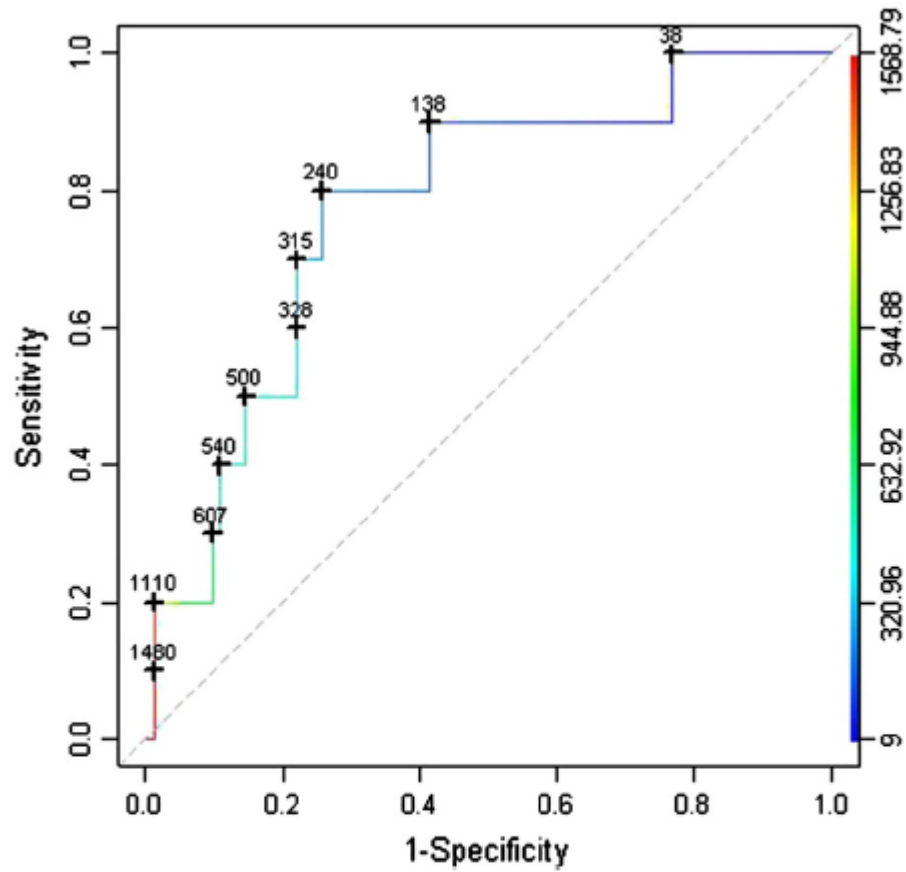


Figure 1 Receiver operating characteristic (ROC) curve of the sensitivity and specificity of FC predicting relapse at 12 months for the 92 patients followed up for at least that length of time, based on various cutoffs of FC.

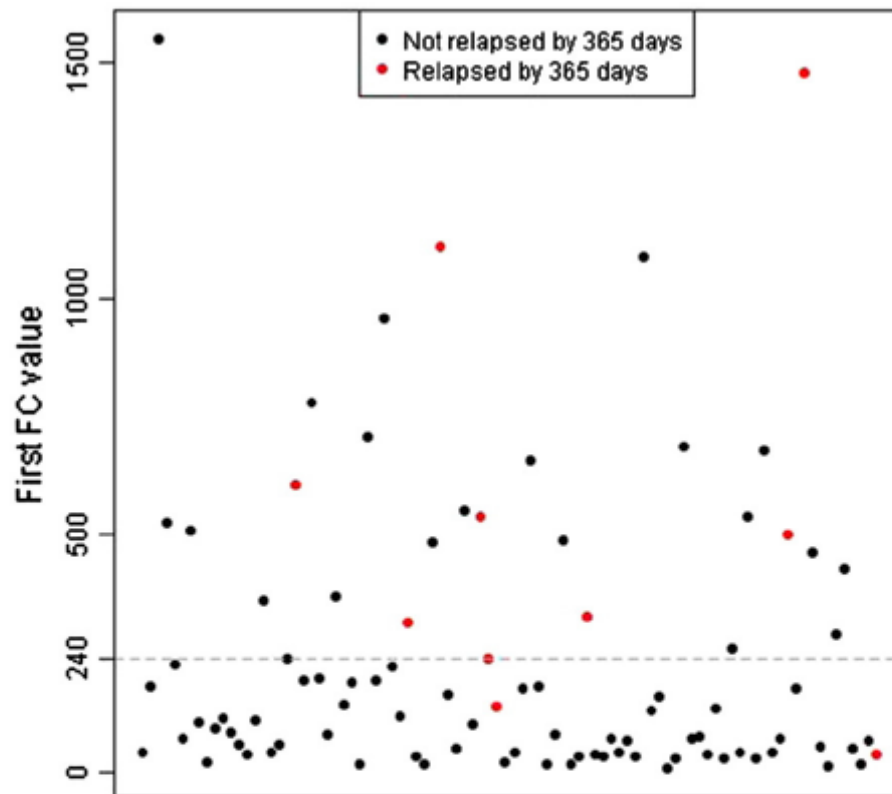


Figure 2 Scatterplot of the FC values of the 92 patients, with those who relapsed by 12 months marked in red, and those who did not marked in black. The optimal cutoff of 240 $\mu\text{g/g}$ marked as a dashed line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

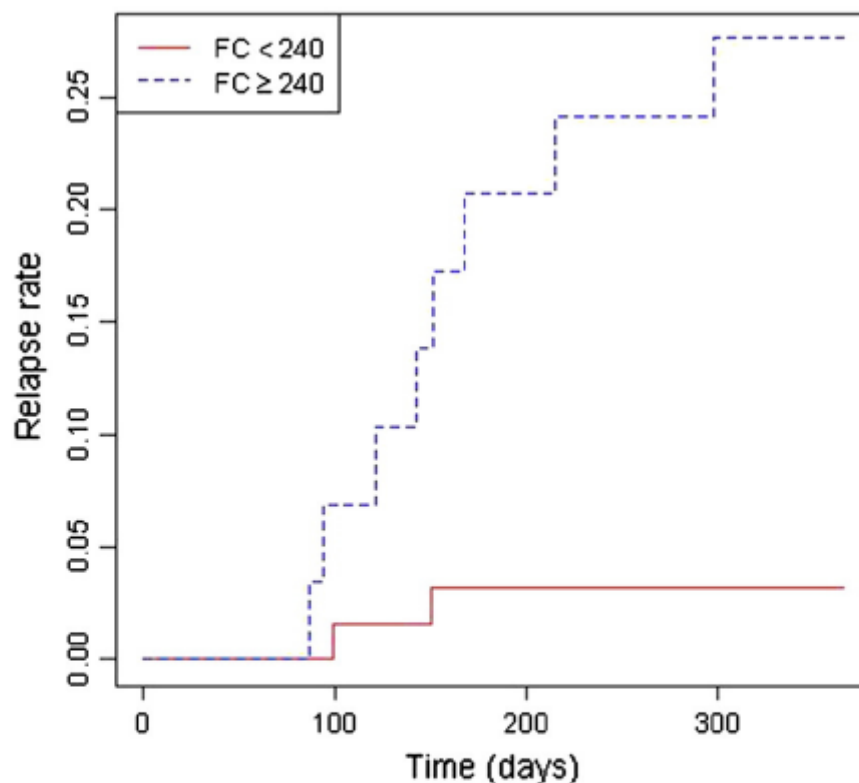


Figure 3 Kaplan–Meier (K–M) cumulative event curves of time to relapse in days for the 92 patients, stratified by whether their FC was below or above 240 $\mu\text{g/g}$.

Table 2 Cox proportional hazards model showing the relationship between time to relapse and high FC, adjusted for demographics (n = 92).

| | Estimate (95% CI) | p-Value |
|--------------------------------|--------------------|---------|
| Age (years) | 0.98 (0.94, 1.02) | 0.250 |
| Gender (male vs female) | 1.25 (0.34, 4.51) | 0.737 |
| Surgery | 3.18 (0.77, 13.25) | 0.111 |
| Stoma | 0.52 (0.06, 4.42) | 0.551 |
| Faecal calprotectin ≥ 240 | 12.18 (2.55, 58.2) | 0.002 |

3.4 Conclusion

Our dataset reveals that adults with quiescent CD with a FC <240 are unlikely to relapse within 12 months and we believe this should become a therapeutic target when assessing patients with CD at the clinic.

Chapter 4 –Investigatory yield mildly elevated FC in new GI referrals (Seenan JP et al Frontline Gastroenterology 2015;6:156-60)

4.1 Introduction

Patients are often referred from primary to secondary care for investigation of lower GI symptoms in the context of a mildly elevated FC. The manufacturers' guidance of a normal FC may be too low for clinical practice meaning patients could be exposed to unnecessary investigations in this setting. We hypothesised that many such patients had IBS rather than IBD. We thus investigated the diagnostic yield of endoscopic, histological and radiological investigation in patients aged <50 presenting with new lower (non-'alarm') GI symptoms and a mildly elevated FC (100-200 μ g/g).

4.2 Methods

All patients with an FC 100-200 μ g/g were identified from our GRI biochemistry database between 2009-2011. Patients aged 16-50 attending the GI OPC with new lower GI symptoms were identified from the EPR. Subjects were excluded if they were taking NSAIDs, had IBD, positive stool culture, 'alarm' symptoms (weight loss, rectal bleeding) or anaemia. Further investigations were at the discretion of the managing consultant gastroenterologist. The latter information was recorded from the EPR.

4.3 Results

In all, 161 patients (103 females) were identified who met the inclusion criteria. Baseline demographics and presenting symptoms are shown in table 1. The mean age was 37.3 years with a mean FC of 147 μ g/g. The mean duration of follow up was 172.4 weeks. The main presenting complaint was diarrhoea in 98 (60.9%) and abdominal pain in 63 (39.1%). A total of 398 endoscopic, radiological and histological investigations were undertaken in 141 patients with an average of 2.8 investigations per patient (table 2). A total of 131 colonoscopies were undertaken with abnormalities detected in only 24 (18.3%). In patients with a macroscopically normal upper GI endoscopy and colonoscopy, the diagnostic yield of further investigations was only 7.3%.

The NPV of an FC 100-200 μ g/g in this cohort was 86.7% for any pathology and 97.5% for significant luminal pathology (IBD, advanced adenoma or colorectal cancer). IBD was the final diagnosis in only 4 patients (2.5%) and IBS in 49.7%. 74% of the patients

were deemed not to require long term secondary care GI follow up. All diagnoses made are revealed in figure 1.

Table 1: Patient Demographics

| | | |
|---------------------------------|----------------|------------|
| Mean Age | 37.3 years | |
| Sex | Male | 58 (36%) |
| | Female | 103 (64%) |
| Primary Symptom | Abdominal Pain | 63 (39.1%) |
| | Diarrhoea | 98 (60.9%) |
| Secondary Symptom | Abdominal Pain | 46 (28.6%) |
| | Diarrhoea | 30 (18.6%) |
| | Constipation | 3 (1.9%) |
| Mean Faecal Calprotectin | 147 µg/g | |

Table 2: Investigations Performed and Diagnostic Yield

| Investigation | Number | Yield |
|---|---------------|--------------|
| All | 398 | 13.3% |
| Colonoscopy | 131 | 18.3% |
| Excluding Colonoscopy | 267 | 10.9% |
| TI/Colonic Biopsies | 119 | 7.6% |
| Barium Meal and Follow-through | 16 | 6.3% |
| CT | 19 | 5.3% |
| MRI Small Bowel | 19 | 16.7% |
| Capsule Endoscopy | 12 | 8.3% |
| Upper Gastrointestinal Endoscopy | 47 | 27.7% |
| Distal Duodenal Biopsies | 36 | 2.8% |

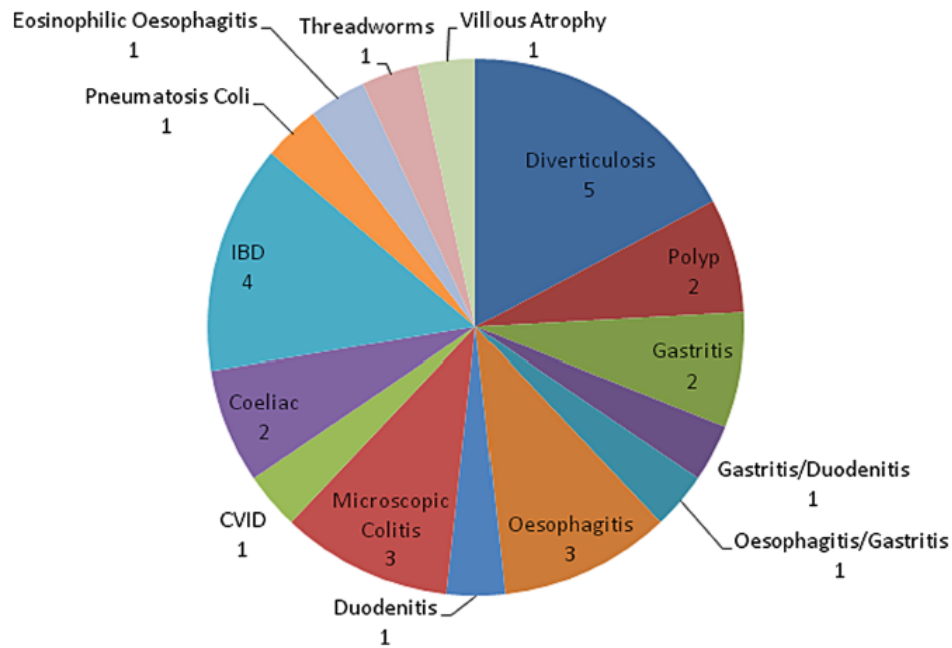


Figure 1: Pie chart of diagnoses

4.4 Conclusion

In adults <50 years of age with new non-‘alarm’ GI symptoms, The NPV of an FC of 100-200 μ g/g is high in excluding significant pathology. These patients often have a functional diagnosis and the yield of investigations is extremely low. We suggest that the manufacturers’ cut-off of <50 μ g/g is too low for use in clinical practice and that repeat FC testing in this cohort, rather than invasive GI investigations, would be a more pragmatic approach.

Chapter 5 – Assessment of FC compared to serum calprotectin in GI patients

(McCann RK et al Clinical Biochem 2017 in press)

5.1 Introduction

5.2 Methods

Between July and October 2015, in and outpatients within the adult GI service at GRI who submitted a stool sample for FC analysis were prospectively identified. Those patients who also had a serum sample obtained within 24h of stool sample collection were identified and the sample stored at -80C for batch analysis of CRP and serum calprotectin. The project was approved by the West of Scotland REC (14/WS/1035).

Both serum and FC were analysed using a Buhlmann quantitative ELISA as per the manufacturer's instructions. CRP was measured on Architect 8000 (Abbott).

Statistical analysis was performed using MedCalc software (version 15.4).

5.3 Results

109 patients were recruited. 68 (62%) were female. The mean age was 51 years (range 18-93). The indications for testing FC were assessment of IBD activity (69, 63%) and assessment of chronic diarrhoeal symptoms (40, 37%).

Mean SC in this group of patients was 6.67 $\mu\text{g/mL}$ (range: 1.06 – 24.00 $\mu\text{g/mL}$). This assay is linear up to 24.00 $\mu\text{g/mL}$ and for the purpose of the statistical analysis, any results greater than 24.00 $\mu\text{g/mL}$ were arbitrarily assigned a value of 24.00 $\mu\text{g/mL}$. Mean FC was 362.72 $\mu\text{g/g}$ (range: 30 - 1800 $\mu\text{g/g}$). This assay is linear between 30 and 1800 $\mu\text{g/g}$ and any results less than 30 or greater than 1800 were arbitrarily assigned values of 30 and 1800 $\mu\text{g/g}$, respectively, for statistical purposes. Mean CRP was 15.3 mg/L (range: 1.0 – 126 mg/L) and any CRP result less than 1.0 mg/L was arbitrarily assigned a value of 1.0 mg/L for statistical analysis.

The log transformed correlation datasets are illustrated on figures 1, 2 and 3 for SC/FC, SC/CRP and FC/CRP respectively. Correlations were expressed as intraclass correlation coefficients (ICC) and were calculated following logarithm transformation of the data (Table 1). The closer the ICC is to 1 the better the agreement.

Excluding data outside the linear range of the three assays created a patient cohort of 73 patients. When the statistical analysis was repeated with this smaller cohort it had no

significant effect on the overall statistical outcome thus indicating that assigning arbitrary values did not create a bias in the dataset.

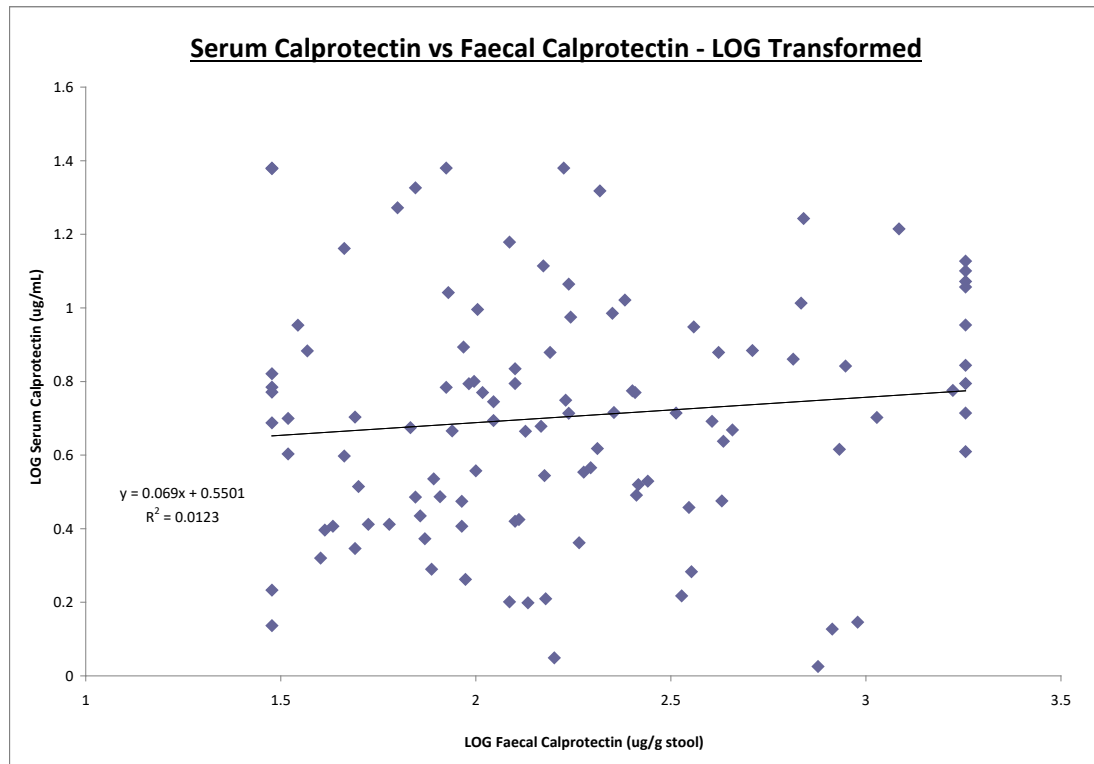


Figure 1: Serum Calprotectin vs Faecal Calprotectin

All data was log transformed. Serum calprotectin results (y-axis) were plotted against faecal calprotectin results (x-axis). Trendline, equation and R^2 value are depicted on the graph.

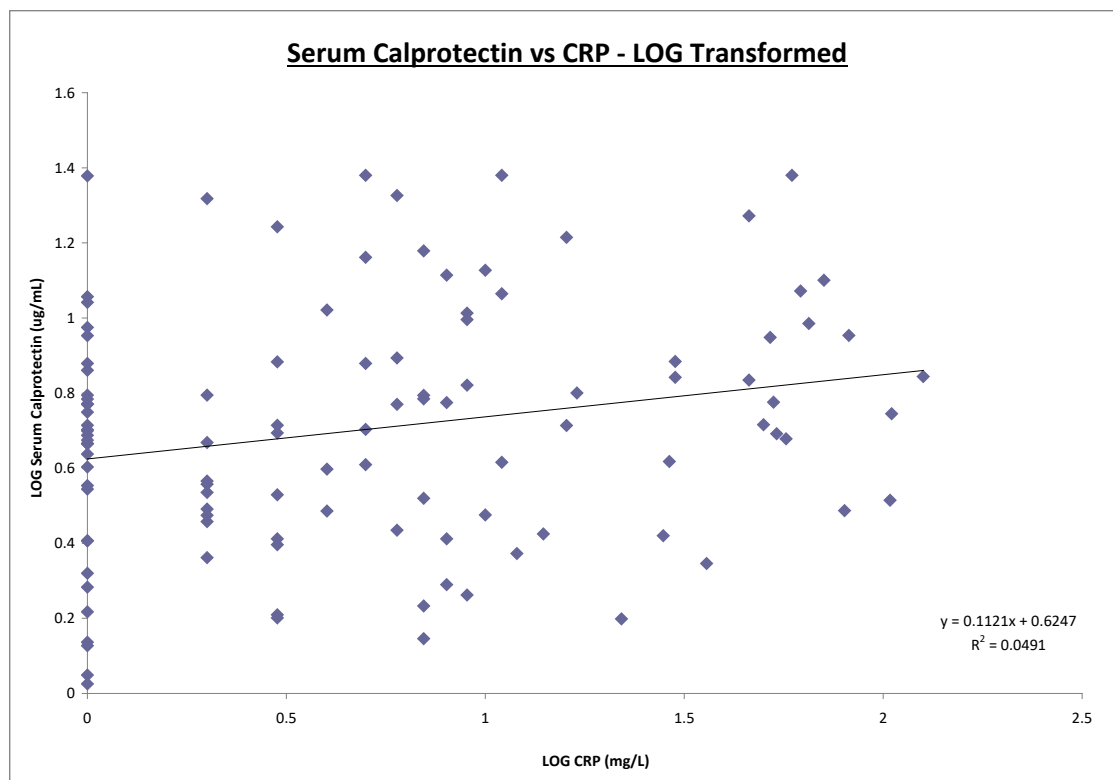


Figure 2: Serum Calprotectin vs CRP

All data was log transformed. Serum calprotectin results (y-axis) were plotted against CRP results (x-axis). Trendline, equation and R^2 value are depicted on the graph.

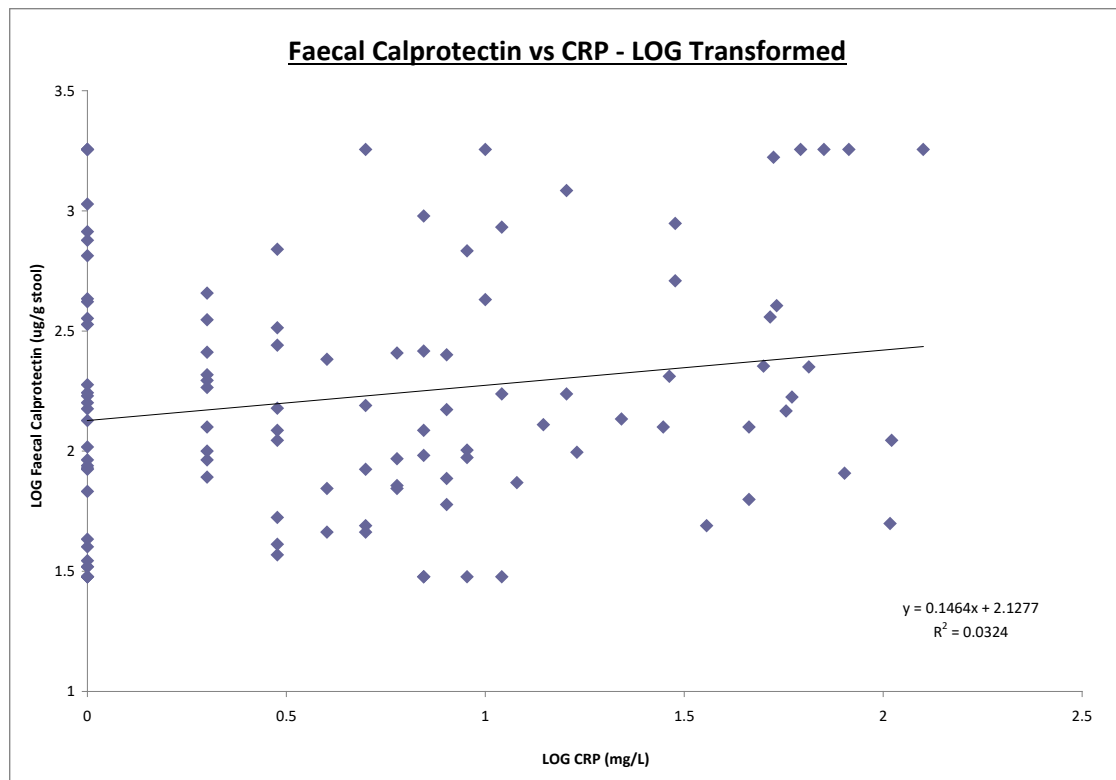


Figure 3: Faecal Calprotectin vs CRP

All data was log transformed. Faecal calprotectin results (y-axis) were plotted against CRP results (x-axis). Trendline, equation and R^2 value are depicted on the graph.

| | Dataset with 109 patients | |
|----------------------|---------------------------|--------------|
| | ICC | 95% CI |
| SC compared with FC | 0.10 | -0.09 – 0.28 |
| SC compared with CRP | 0.18 | -0.01 – 0.35 |
| FC compared with CRP | 0.18 | -0.01 – 0.35 |

Table 1: Correlations between serum calprotectin, faecal calprotectin and CRP

5.4 Conclusion

As a serum marker of intestinal inflammation, serum calprotectin is unlikely to be of clinical utility and the search for a serum marker with a similar profile to FC continues.

10. Discussion

10.1 METHODOLOGICAL ASPECTS OF FC TESTING

After the publication of the first method of FC analysis by ELISA in 1992 (Roseth AG 1992), numerous kits from several competing companies have appeared for the commercial analysis of FC. There is a lack of standardisation of the assay leading to significant inter-assay variability (Whitehead SJ 2013) and the ‘normal’ range is debated and unlikely to be transferrable between the different assays (Lin JF 2014). Indeed recent guidance from NICE highlights that further research was needed before and recommendation could be made about a specific cut off could be made (NICE guidance 2013).

More recently, point of care testing has been developed to facilitate self management of IBD at home (Coorevits L 2013, Lobaton T 2013). Also smart phone technology has been utilised allowing digital analysis and measuring of the FC result which can be sent directly to the secondary care team (Vinding KK 2016).

In terms of intra-individual variability of FC analysis, we have shown good agreement between three samples over consecutive days in CD patients (chapter 2). Recently it has been shown that there is variability in FC depending on the time of day of FC sampling in UC patients and thus testing the first stool of the day has been recommended (Lasson A 2015). Also the authors noted that storage of FC samples for more than 3 days at room temperature is not advisable due to a decline of FC level in samples stored to 7 days.

10.2 FAECAL CALPROTECTIN IN THE DIAGNOSIS OF IBD/IBS

The diagnosis of IBD has historically been based on a combination of clinical history and examination, blood parameters, radiology and endoscopy. The addition of a faecal biomarker able to reduce the need for invasive endoscopic procedures or exposure to radiation is advantageous.

In a study of 110 patients attending for colonoscopy for the investigation of chronic diarrhoea showing that increased faecal calprotectin levels were significantly ($P=0.0001$) associated with the presence of colorectal inflammation (CD, UC, microscopic colitis or diverticulitis)(Limburg PJ 2000). Within the colonic inflammation subgroup, calprotectin concentrations were highest amongst subjects with IBD. The negative predictive value of faecal calprotectin in this dataset was 93%. A large metaanalysis of studies supports the use of FC as a screening tool for organic disease and in determining the need for further investigations (van Rheen PF 2010). In this regard NICE recommended its use in primary and secondary care in adults with recent onset lower gastrointestinal symptoms, where cancer is not suspected (considering factors such as age), and for whom specialist assessment is being considered (NICE guidance 2013).

IBD and irritable bowel syndromes (IBS) can present in a similar clinical fashion with symptoms such as diarrhoea and abdominal pain. Routine colonoscopy in these patients is costly, invasive and has associated morbidity and mortality. Serum markers of inflammation such as C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in isolation are not sufficiently sensitive or specific for the diagnosis of inflammatory bowel disease (Tibble J 2000) The use of faecal calprotectin to distinguish between IBD and IBS has been analysed in several studies. In 2000 Tibble presented results of a prospective study of 220 consecutive patients in whom the principal differential diagnosis was that of either IBS or CD. They excluded patients with UC on sigmoidoscopy and biopsy. A diagnosis of CD was made from a combination of radiological, endoscopic and histological investigations. A diagnosis of irritable bowel syndrome was made on basis of normal investigations and a compatible history fulfilling the ROME criteria. All patients subsequently diagnosed with CD had significantly higher faecal calprotectin concentrations than those with IBS. The investigators found that using a cut-off point of 30 mg/L faecal calprotectin had a 100% sensitivity and 97% specificity in discriminating between active Crohn's disease and IBS.

Schoepfer (Schoepfer AM 2008) looked at the accuracy of faecal biomarkers alone and in combination with the IBD antibodies, antineutrophil cytoplasmic antibody (ANCA) and anti-*Saccharomyces Cerevisiae* manna antibody (ASCA), in discriminating IBD

from IBS. They found that the overall accuracy of faecal calprotectin for discriminating between IBD and IBS was 89% (sensitivity 83%, specificity 100%). There was only a marginal increase in overall accuracy when faecal calprotectin was combined with IBD antibodies to 91%.

Faecal calprotectin has been studied as a tool to predict abnormal small-bowel radiology (Dolwani S 2004). The study looked at 73 consecutive patients attending for small bowel follow through whose presenting symptoms were consistent with a possible diagnosis of IBD. The control group consisted of 25 patients with IBS, 25 normal volunteers and 25 patients with active CD. A faecal calprotectin level above 60µg/g predicted all abnormal barium follow through results. The negative predictive value of a single calprotectin result below 60 µg/g of stool was 100% compared with 91% each for erythrocyte sedimentation rate cut off of 10 mm and C-reactive protein of 6 mg/L. Somewhat in contrast to this Sipponen (Sipponen T 2012) found that faecal calprotectin had a low utility for predicting the presence of small bowel CD on wireless capsule endoscopy, sensitivity was low at 59% with a moderate specificity of 71% using a cut-off of 50µg/g.

A meta-analysis analysed 30 prospective studies comparing the diagnostic precision of faecal calprotectin against a histological diagnosis (von Roon AC 2007). Summary receiver operating characteristic curve analysis showed a sensitivity of 0.95 (95% CI 0.93-0.97), specificity of 0.91 (95% CI 0.86-0.91), and an area under the curve (AUC) of 0.95 for the diagnosis of IBD. The diagnostic precision of faecal calprotectin for IBD was higher in children than adults with better accuracy at a cut-off level of 100 µg/g versus 50 µg/g. This meta-analysis also showed that faecal calprotectin was superior to CRP, ESR, ASCA, pANCA and anti-Escherichia coli outer membrane porin C antibody in diagnosis of IBD.

Thus it can be stated that a normal faecal calprotectin result, in the absence of 'red flag' symptoms and in the context of positive Rome criteria, is associated with a high likelihood of subsequent non-organic diagnosis and further endoscopic or radiological evaluation may be avoided in such patients. A meta-analysis published in 2010 to assess whether the use of faecal calprotectin reduces the number of unnecessary endoscopic procedures in the investigation of suspected IBD showed that screening

with faecal calprotectin would result in a 67% reduction in the number of adults requiring endoscopy. The downside of this screening strategy is delayed diagnosis in 6% of adults because of a false negative test result (van Rheenan PF 2010). Our own data suggests that a mildly elevated FC in patients <40 is most likely to yield a diagnosis of IBS and invasive GI investigations could be avoided (chapter 4).

Previous studies suggest that the trend in FC can be a useful indicator of the likelihood of significant pathology. Demir et al (Demir OM 2013) examined patients referred from primary to secondary care with GI symptoms in whom a FC had been checked. 2663 patients were included. They looked in more detail at patients with a 'minimally elevated' FC (50-150 μ g/g) and those with higher levels (150-3000 μ g/g) who underwent repeat FC testing after an interval of 6-8 weeks. In the higher FC cohort, there were 13 new cases of IBD with a mean increase in FC from 933 to 1666 μ g/g. In 66 patients with a 'minimally elevated' FC, none developed IBD during the 2 years of follow-up and the mean FC fell from 88 to 65 μ g/g. Similarly in the study by Zayyat et al (Zayyat R 2011), in 90% (9/10) of the patients who were ultimately diagnosed with IBD a repeat FC had increased.

Furthermore, a small study by Mohammed et al (Mohammed N 2012) demonstrated that after initial negative radiological or endoscopic GI investigations, longer term follow-up of patients with elevated FC <225 μ g/g failed to identify significant pathology. 67 patients were followed for 3 years with no patients found to have IBD during subsequent review. Recent work by D'Haens et al (D'Haens G 2012) in IBD patients supports the observation that FC levels of this magnitude are not associated with significant mucosal inflammation. In their study which examined 126 patients with IBD, a FC <250 μ g/g was associated with mucosal healing, predicting endoscopic remission (CDEIS \leq 3) with 94.1% sensitivity.

Faecal calprotectin appears to better reflect disease activity in UC rather than CD (Costa F 2005) but faecal calprotectin has not been found to be useful in distinguishing UC from CD. Quail *et al* (Quail MA 2009) looked at faecal calprotectin concentrations in Scottish children with a diagnosis of IBD; there was no statistical difference in

calprotectin concentrations between CD and non-Crohn's patients (UC or IBD type unspecified – IBDU).

10.3 FAECAL CALPROTECTIN IN ASSESSMENT OF DISEASE ACTIVITY AND RESPONSE TO TREATMENT

In IBD, the presence of active gut inflammation is associated with migration of leucocytes, including neutrophils, to the gut mucosa (Vermeire S 2006). As a result the faecal stream contains increased levels of these inflammatory proteins including calprotectin. Faecal calprotectin has been shown to differentiate quiescent from active disease in both patients with CD and UC (Sipponen T 2008, Langhurst J 2005, Xiang JY 2008). Correlation of faecal calprotectin tends to be higher with endoscopic activity than clinical activity indices (Schoepfer AM 2010) and indeed some studies have demonstrated no significant correlation between faecal calprotectin and clinical indices (Gaya DR 2005, Jones J 2008). In general faecal calprotectin correlates better with colonic CD rather than ileal disease (Sipponen T 2008) and an inflammatory rather than a structuring/penetrating phenotype (Sipponen T 2008). Sipponen *et al* showed that in active disease (Crohn's disease endoscopic index of severity CDEIS ≥ 3), faecal calprotectin concentrations were significantly higher in colonic than in ileal CD. Also, in limited ileal disease faecal calprotectin failed to correlate with endoscopic activity.

In UC Ricanek *et al* (Ricanek P 2011) showed that the median faecal calprotectin concentration was higher in patients with extensive and left sided disease distribution compared with proctitis (740 μ g/g, 2106 μ g/g, 86 μ g/g respectively; $p=0.007$ and $p=0.009$). There was no significant difference in faecal calprotectin concentration between extensive and left sided disease distribution.

There have been several studies looking at the use of faecal calprotectin to predict or monitor response to treatment. In a study (Wagner M 2008) looking at 11 patients with relapsing IBD (11 CD and 27 UC) faecal calprotectin was analysed at inclusion and after 8 wk of treatment (end of study). Treatment was individualised medical therapy. A normalised faecal calprotectin concentration at 8 weeks predicted a complete response in 100% patients. There was a significant decline in faecal calprotectin levels ($P<0.001$) in patients with UC responding to treatment defined as normalisation of clinical and

endoscopic scores. Within the small subgroup of patients with CD although 81% of patients achieved a complete clinical response defined clinically as a Harvey Bradshaw Index (HBI) ≤ 5 there was no significant decline in calprotectin levels. This study was limited by small numbers and also the lack of endoscopic evidence of disease activity or remission in CD patients, it is possible that these patients had ongoing subclinical inflammation. In fact it has been shown that in patients with steroid induced clinical remission faecal calprotectin levels can remain elevated (Sipponen T 2010). This finding is in keeping with earlier studies showing incomplete mucosal healing in patients treated with corticosteroids (Modigliani R 1990). Sipponen *et al* (Sipponen T 2010) were able to show a significant decrease in faecal calprotectin ($P=0.005$) in patients with CD who responded both clinically and endoscopically (using CDEIS) to an individualised escalation of treatment. There was no significant change in faecal calprotectin concentration in patients without endoscopic response.

Faecal calprotectin may be able to predict colectomy in patients with acute severe UC. Ho (Ho GT 2009) showed that in patients with acute severe UC requiring inpatient treatment with intravenous corticosteroids faecal calprotectin was significantly higher in patients who failed to respond to medical therapy and required colectomy than those who did not ($P=0.04$). The area under the curve was 0.65 ($P=0.04$) for faecal calprotectin to predict colectomy with a maximum likelihood ratio of 9.23 at a cut-off of 1922.5 $\mu\text{g/g}$ (specificity of 97.4%). Overall in the study faecal calprotectin concentrations were high with 86% of patients having levels of $>500 \mu\text{g/g}$ (median 1020 $\mu\text{g/g}$).

Faecal calprotectin can be used to monitor response to biological therapy. Palmon (Palmon R 2006) showed that faecal calprotectin concentration decreases significantly at week 2 after an infliximab (IFX) infusion in 17 patients with CD on maintenance IFX therapy. Calprotectin levels were noted to rise back to baseline values by week 4 again despite a low median HBI. There was no endoscopic assessment of disease activity in this study. The rise in faecal calprotectin at week 4 may once again indicate a subclinical recurrence of mucosal inflammation. Sipponen (Sipponen T 2008) assessed the role of faecal calprotectin in monitoring clinical, using the Crohn's disease activity index (CDAI) and endoscopic (CDEIS) response to anti-TNF- α therapy (infliximab or adalimumab) in 15 patients with CD. Following 12 wk of treatment faecal calprotectin

levels declined significantly from baseline level ($P=0.001$) and changes in faecal calprotectin correlated to endoscopic appearances as scored using CDEIS (Spearman's rank correlation = 0.561, $P=0.03$) suggesting that faecal calprotectin is a useful non-invasive marker of mucosal response to anti-TNF- α treatment.

10.4 PREDICTING MUCOSAL HEALING

Historically clinical practice has considered interpretation of symptoms and the use of scoring systems such as the CDAI, HBI and the Rachmilewitz ulcerative colitis activity index (CAI) to determine treatment success in IBD. These indices however tend to reflect patient well-being and quality of life rather than the degree of mucosal inflammation (Gaya DR 2005). In both CD and UC there is evidence that mucosal healing is associated with sustained remission and reduced need for surgery (Frosliel KF 2007, Baert F 2010) and following ileal resection the endoscopic appearance of the neoterminal ileum mucosa at 1 year post surgery has been shown to predict symptomatic relapse (Rutgeerts P 1999). Thus mucosal healing is evolving into the new goal of IBD treatment.

Roseth (Roseth AG 2004) demonstrated that normalisation of faecal calprotectin concentration corresponds to endoscopic mucosal healing. 17 patients with CD and 28 with UC clinically in remission who had faecal calprotectin concentrations of <50 mg/L underwent endoscopic assessment of their lower GI tract and macroscopic mucosal appearances were assessed. Biopsies were also taken to assess histological inflammation. All but one of these patients with faecal calprotectin <50 mg/L had inactive mucosal disease on colonoscopy.

Several subsequent studies have on to show that concentration of faecal calprotectin correlates with both histological and endoscopic disease activity in IBD (Langhorst J 2008, Sipponen T 2008, Jones J, Roseth AG 1997). Furthermore, several of the studies included in table 1 show that correlation of faecal calprotectin with endoscopic appearances is stronger than correlation with clinical indices (D'Haens G 2012, Sipponen T 2008, Jones J 2008).

However, Denis (Denis MA 2007) failed to find a significant correlation between CDEIS and faecal calprotectin concentration. This was a small study of 28 patients with CD who had CDAI>150 but a normal serum CRP. This lack of correlation may reflect the population studied as more than half of the patients had isolated ileal disease and overall disease activity was low (median CDEIS 3.4).

Interestingly one study in paediatric patients with IBD showed that calprotectin concentration correlated more closely with histological inflammation rather than endoscopic findings suggesting that faecal calprotectin may be more sensitive than macroscopic endoscopic appearances in evaluating disease activity status (Bunn SK 2001).

10.5 USE OF FAECAL CALPROTECTIN TO PREDICT RELAPSE

Being able to identify patients at high risk of relapse, and those with sub-clinical intestinal inflammation, may allow adjustment of their treatment strategy thus preventing clinical relapse. A non-invasive method of identifying this would reduce cost and risk of morbidity and mortality to patients. As sensitivity and specificity of serum markers of inflammation correlate poorly with intestinal inflammation their ability to predict disease relapse is poor (Tibble JA 2000, Langhorst J 2008). Several studies have looked at the use of faecal calprotectin to predict relapse in patients with IBD and have demonstrated significant differences in faecal calprotectin concentration in relapsers compared with non-relapsers (Naismith G 2012, Laharie D 2011, Kallel L 2010, Gisbert JP 2009). A recent study of patients with endoscopic remission, showed that FC was a better predictor of future relapse than microscopic inflammation in biopsies (Mooiweer E 2015). Serial measurement of FC in IBD patients in clinical and endoscopic remission has been shown to rise before clinical relapse (Molander P 2015). This study evaluated patients stopping anti-TNF- α therapy with an initial FC <100ug/g, with monthly calprotectin for six months, and then bimonthly. Calprotectin rose a median of 94 (13-317) days before clinical symptoms. This implies that early reintroduction of treatment could be made before symptoms recur, on the basis of rising calprotectin, but there is still inadequate data to support such use of FC. Interestingly, faecal calprotectin appears less useful for predicting relapse in patients with ileal CD compared with patients with UC or colonic / ileocolonic CD (D’Inca R 2008, Garcia-

Sanchez V 2010).

The major outlier in the literature on this topic is the study by Laharie (Laharie D 2011) which looked at the use of faecal calprotectin to predict relapse specifically in 65 patients with CD treated with infliximab induction regimen and then maintained on immunomodulator alone. There was no significant difference in faecal calprotectin concentration between those patients who relapsed by 14 weeks post induction and those who did not even when the analysis was restricted to patients with pure colonic disease. No endoscopic evaluation was performed after infliximab induction so although the study did show a median drop in faecal calprotectin concentration of 340µg/g following induction there may have been ongoing subclinical inflammation. Another limitation of this study is that disease relapse was defined on clinical grounds alone.

Mao (Mao R 2012) performed a meta-analysis of the predictive capacity of faecal calprotectin in IBD relapse. Analysing 6 studies they found a pooled sensitivity of 78% and specificity of 73%. Capacity to predict relapse was comparable between CD and UC. Due to the small number of patients the predictive value of faecal calprotectin in ileal only CD patients was not assessed.

Overall, elevated FC in patients in remission is currently used as a screening test to prompt restaging of CD by colonoscopy and/or MRI scanning by clinicians. As it is an insensitive test, being raised in many inflammatory bowel pathologies, therapeutic intervention on the basis of a raised FC alone in CD cannot be recommended.

10.6 DETECTION OF POST-OPERATIVE CD RELAPSE

More than 80% of CD patients require surgery within 10 years of diagnosis and by 3-5 years after surgery around a third of patients will have had a clinical relapse (Bernell O 2007). The role of faecal calprotectin in predicting post-surgical recurrence of CD has been assessed. One study assessed 39 patients with CD undergoing bowel resection (Orlando A 2006). The majority of patients (67%) had ileocolonic disease. Measurements of faecal calprotectin, CDAI and ultrasound examination were performed at 3 months post surgery. Endoscopy was performed at 1 year regardless of

patient symptoms and was considered the ‘gold standard’ for recurrence of disease. All patients were clinically in remission at 3 months. Using a cut-off level of 200mg/L, in predicting endoscopic post-surgical recurrence, faecal calprotectin has a sensitivity of 63% and specificity of 75% leading the authors to suggest that patients with elevated faecal calprotectin levels at 3 months post surgery should be assessed endoscopically for early recurrence.

Lamb (Lamb CA 2009) prospectively followed 13 patients for 12 months post ileocaecal resection for symptomatic CD. Faecal calprotectin was seen to normalise after uncomplicated surgery at 2 months and remained within normal limits in 8 patients who remained clinically in remission. In the remaining 5 patients there was an increase in faecal calprotectin concentration after initial normalisation associated with disease recurrence or post operative intra-abdominal collections. The authors’ conclusion from this small number of patients was that patients with low levels of faecal calprotectin after resection who had symptoms were unlikely to have mucosal inflammation. It should be pointed out though that there was no scheduled endoscopic evaluation of the gut mucosa during the 12 month follow up period. Such datasets have recently been replicated in independent cohorts (Wright EK 2015).

In the POCER trial (De Cruz P 2015), a prospective study to determine optimal medical management of post-operative Crohn’s, a faecal calprotectin >100mcg/g indicated endoscopic recurrence with a sensitivity 0.89 and NPV 91%, potentially allowing avoidance of colonoscopy in 41% of patients.

10.7 USE OF FAECAL CALPROTECTIN TO AID DECISIONS OF WITHDRAWAL OF TREATMENT IN IBD

When and in which patients it is appropriate to withdraw anti TNF alpha therapy is hotly debated and driven by costs and concerns about long term safety. Faecal calprotectin levels may assist in this decision making. Louis *et al* (Louis E 2012) published results of a prospective study (STORI) of CD patients who had been in steroid free remission on infliximab and thiopurine or methotrexate for at least six months. Relapse after infliximab withdrawal was associated with various risk factors including a faecal calprotectin concentration of $\geq 300\mu\text{g/g}$. Other risk factors for relapse

identified on multivariate analysis included male sex, absence of surgical resection, leucocyte counts $>6 \times 10^9/\text{L}$, haemoglobin $\leq 145 \text{ g/L}$ and hsCRP $\geq 5.0 \text{ mg/L}$. Patients with no more than 2 of the above risk factors had a reduced risk of relapse within 1 year (15% compared with 43.9% overall). A low faecal calprotectin can thus be used to aid decision-making about stopping therapy, although once again there is little data on relevant calprotectin thresholds, which most likely vary between patients and assays.

10.8 LIMITATIONS OF FC IN LUMINAL GASTROENTEROLOGY

A number of different commercial calprotectin assays are available and some concerns have been raised about inter-assay variability in the literature, which is due, in part, to a lack of assay standardisation (Lahere D 2014). The derivation of a nationally agreed normal range and an “intermediate” level is thus an area of concern due to this between-assay variability (van Room AC 2007, Henderson P 2014, Dhaliwal A 2014, Kristensen V 2015). Indeed the NICE committee highlighted the different thresholds for interpreting faecal calprotectin results and concluded that there was a need to undertake further research before a recommendation on a particular cut-off could be nationally agreed (NICE guidance 2013).

There is insufficient evidence to rely on faecal calprotectin to diagnose colorectal cancer or polyps. It should not therefore be used to evaluate older patients (with a cut-off varying between 40 and 50 years, best determined by local audit data) where colonoscopy is mandated as part of established care pathways to exclude colorectal neoplasia (van Room AC 2007).

When used in primary care to identify patients with altered bowel habit who require colonoscopy for possible IBD, it is clear that use of the upper limit of the ‘normal range’ for assays, (usually 50ug/g stool) as the threshold for referring for colonoscopy, will result in large numbers of unnecessary procedures. Several authors, including NICE, have proposed the role of an intermediate range of faecal calprotectin for values that are above the reference range, but not clinically significant in assessing possible IBD (Seenan JP 2015). This is perhaps justified by the need to balance sensitivity and specificity. An indeterminate range could include values from 50, to as much as

200ug/g stool (Seenan JP 2015). The setting of a level above which colonoscopy is needed should be determined locally based on audit data, quality assurance processes and in conjunction with clinical assessment. A higher threshold will result in fewer investigations, but more risk of missing IBD. A lower threshold will result in fewer missed diagnoses (increase sensitivity), but lead to more unnecessary investigations (reduce specificity). It has been suggested that specificity could be improved by repeat testing for patients with results in the indeterminate range, assuming that patients with IBD will have persistently raised or rising calprotectin (Zayyat R 2011). There is currently inadequate data to recommend re-testing of patients in this way. The NICE review emphasised the need for research to identify optimal cut-off values and the investigation of repeat testing strategies in people with intermediate levels.

Conclusion

The data presented in this thesis has revealed that FC correlates well with WCS in CD patients and has an acceptable intra-individual variability in CD allowing one off samples to be of clinical utility in this setting. In addition we showed the prognostic value of FC in CD in remission and the potential for over investigation of new patients referred to the GI clinic with mildly elevated FC. Lastly our most recent dataset reveals no correlation between FC and serum calprotectin.

Over the last 15 years, FC has evolved from a little known faecal biomarker to becoming established in routine luminal gastroenterology practice. It is a sensitive, non-invasive marker of intestinal inflammation which has found clinical utility in the fields of aiding a diagnosis of IBS, suggesting a diagnosis of IBD, monitoring disease activity and response to treatment in IBD and assessing for post-operative recurrence in CD. Without any significant challengers on the horizon, I suspect the future clinical utility of FC in luminal gastroenterology is secure.

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