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Original Article

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Beyond Histological Remission: Intramucosal Calprotectin as a Potential Predictor of Outcomes in Ulcerative Colitis

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Abstract

Background and Aims: Histological remission and low faecal calprotectin are positive prognostic factors in ulcerative colitis [UC]. Intramucosal calprotectin [iMC], which can be readily determined by immunohistochemistry, has not so far been evaluated as a predictor of outcome in UC. We aimed to investigate the relationship between iMC and clinical, endoscopic, and histological measures of remission in UC, and the independent prognostic value of iMC.
Methods: Ambulant patients with UC were recruited for a study comparing clinical activity indices. Sigmoidoscopy and biopsy were performed at the index visit. Clinical, endoscopic, and histological activity were scored and iMC semi-quantitatively measured using immunohistochemistry for the S100A8/9 heterodimer on colonic biopsies, scored as the mean number of positive cells in five high-power fields [HPF]. At the end of follow-up [6 years], data on steroid use, hospitalisation, and colectomy ['adverse outcomes'] were collected.
Results: iMC was determined in 83 patients and 20 controls, and correlated with clinical, endoscopic, and histological activity [r = 0.51, 0.65, 0.53, $p > 0.001$, respectively]. iMC was lowest (median 2.4, interquartile range [IQR]: 5.2–5, $p < 0.001$) in patients with concordance between clinical, endoscopic, and histological remission. Median iMC > 5/HPF was associated with adverse outcome (hazard ratio [HR] 3.36, confidence interval [CI] 1.58, 7.15, $p < 0.001$). Only 53%, 33%, and 25% of patients in histological remission with iMC > 5 cells/HPF avoided an adverse outcome after 1, 3, and 6 years, respectively.
Conclusions: iMC was lowest in patients with concordant clinical, endoscopic, and histological remission. Median iMC > 5/HPF was associated with adverse outcomes despite histological remission. Therefore iMC is a potentially useful independent marker of activity.

Key Words: Mucosal calprotectin; predictors of outcome; remission

1. Introduction

The definition of remission in patients with ulcerative colitis [UC] has in the past 5 years moved from solely clinical criteria to more objective endpoints, such as mucosal healing.[1] Therefore histological healing has been proposed as a critical indicator of remission in UC, with the implication that novel therapies in inflammatory bowel

disease [IBD] should aim for this endpoint.[2–6] The rationale for aspiring to histological remission includes evidence demonstrating an association with lower colectomy rates, reduced risk of hospitalisation, and reduced risk of colorectal cancer.[5–10] Histological remission has, at its simplest, been defined as an absence of crypt neutrophil infiltration,[5,11] although the absence of visible neutrophil infiltration does not necessarily equate to the absence of inflammatory activity.

Calprotectin, variously known as L1 protein, myeloid related protein 8/14, calgranulin, cystic fibrosis antigen, and myeloid/histiocyte antigen,[12] is a major product of neutrophils and can comprise approximately 50% of the total cytosolic protein content. Although the function of calprotectin remains unknown, it has been classified as an alarmin, which is an endogenous molecule released in response to tissue damage. It is released by neutrophils and monocytes in the circulation and those that have migrated into tissues. It is also produced by some tissue macrophages and tissue eosinophils.[13] Calprotectin may also be an endogenous ligand for toll-like receptor 4 [TLR 4] and have antimicrobial properties, as well as promoting endothelial activation and transcription of pro-inflammatory cytokines. Despite the potential production of calprotectin by cells other than neutrophils, immunohistochemical staining has been shown to correlate with neutrophil infiltration.[14–18] Therefore immunohistochemical staining for intramucosal calprotectin [iMC] could augment histological assessment of disease activity in UC.

Assaying faecal calprotectin levels, presumed to reflect infiltration by neutrophils in inflamed intestinal tissue, is widely used in clinical trials and in clinical practice, partly because lower faecal calprotectin levels are associated with reduced risk of relapse.[19-23] However, the correlation of faecal calprotectin with endoscopic findings varies greatly and is related to the clinical context,[24–28] and levels also vary widely between individuals.[29,30]

The potential role of iMC as an adjunct to measuring faecal calprotectin has so far not been evaluated, although there is potential for lower inter- and intra-individual variation with an assay that quantifies the biomarker at source in the colonic mucosa. The potential role of iMC for defining remission or predicting outcome has not been explored. Therefore, in this study we aimed to examine how iMC varies in a cohort of patients with UC and a spectrum of disease activity, and to explore a potential role for predicting disease outcomes and defining remission.

2. Materials and Methods

2.1. Study design and patient population

In 2007, at a single university teaching hospital clinic with a large IBD practice, ambulant patients with varying states of UC activity were recruited for a study comparing disease activity indices.[31] All had flexible sigmoidoscopy and mucosal biopsies performed by a single operator at the index visit, according to a defined protocol. Mucosal biopsies were taken from the rectum and/or sigmoid colon, or areas of visible inflammation. Clinical, endoscopic, and histological activities were scored by the Simple Clinical Colitis Activity Index [SCCAI],[32] Baron endoscopy score,[33] and Truelove and Richards' histopathology index,[34] respectively. An experienced IBD specialist gastroenterologist who reviewed patients at the initial visit calculated the SCCAI. Another experienced IBD specialist, blinded to patient identity and clinical score, independently evaluated flexible sigmoidoscopy videos without knowledge of clinical information, to determine the Baron score.

An experienced gastrointestinal histopathologist who was blinded to clinical and endoscopic scores performed histological scoring of biopsies. The clinical, endoscopic, and histological indices were selected because, unlike composite indices such as the Mayo Clinic index, they separate clinical, endoscopic, and histological components. Additionally, the scores could be divided into four strata: quiescent; mild; moderate; and severe. Furthermore, it represented established clinical practice at our centre at that time.

Information on clinical outcomes was collected in 2014 by review of clinical record and data on steroid use, hospitalisation, and colectomy.[5] Of the original cohort of patients, only those with colonic biopsies judged sufficient for immunohistochemistry were included in this study. Control specimens for iMC were taken from histologically normal colonic mucosa included in surgical resection specimens of distal colorectal tumours.

Ethical approval for recording, collating, evaluating, and subsequently analysing clinical, endoscopic, and histological data was obtained from the activity indices' study [LREC 536407Q1605/58ORH],[31] Oxford IBD Cohort study [Research Ethics Committee reference 09/HI204730] and Oxford Gastrointestinal Illness Cohort and Biobank study [Research Ethics Committee reference 09/H606/5].

2.2. Definitions

Remission: SCCAI ≤ 2 was used to define clinical remission.[35] The SCCAI ranges from 0–19, with a score of 3–5 being consistent with mild activity, 6–9 with moderate activity, and 10–19 with severe activity.[31,36]

A Baron score of 0–1 was defined as endoscopic remission. A Baron score of 2 represents moderate activity, and 3 represents severe activity.

A histological report of 'no significant inflammation' was defined as histological remission. The Truelove and Richards index is categorised into 'no significant inflammation', 'mild to moderate

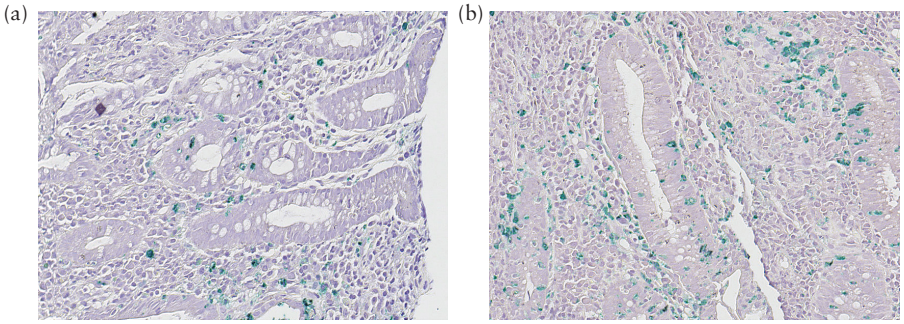

Figure 1. Positive intramucosal calprotectin [iMC] stain with granular cytoplasmic staining associated with clear nuclear counterstain.

inflammation', and 'severe inflammation'. In the original study clinical details, sigmoidoscopy findings, and histological state were all classified into these three groups, potentially to align with each other.

Criteria established for a positive intramucosal calprotectin [iMC] stain were granular cytoplasmic staining associated with clear nuclear counterstain[37] (Figure 1a and b). To avoid scoring artefactual staining, the presence of cytoplasmic staining without a clear associated cell nucleus was discounted. The mean number of calprotectin-positive cells in five high-power fields [HPF] was counted to determine the iMC score [count/HPF].

2.3. Immunohistochemistry for intramucosal calprotectin [iMC]

Formalin-fixed, paraffin-embedded blocks of colonic tissue were retrieved from the department of cellular pathology. Serial sections [4 μm] were cut and mounted on glass slides.

Slides were placed in a 60°C oven for 1 h before de-paraffinisation by incubation in Histoclear solution [National Diagnostics]. The slides were then rehydrated by immersion in graded ethanol solutions.

The sections were stained for the S100A8/9 heterodimer by incubating for 30 min with the primary antibody, monoclonal mouse anti-human myeloid/histiocyte antigen clone MAC387 [DakoCytomation] diluted 1:50 in phosphate buffered saline [PBS] and 10% human serum. These sections were treated with 3% hydrogen peroxide for 15 min to block endogenous peroxidase activity. The slides were then incubated with the secondary antibody (anti-mouse/rabbit horseradish-peroxidase [HRP] Dako Envision™ kit system) for 30 min. The sections were incubated with MenaPath Green Chromagen Kit [A. Menarini Diagnostics] for 10 min for HRP detection. Slides were lightly counterstained with haematoxylin nuclear stain. Sections were washed, dehydrated with graded ethanol solutions, and mounted with non-aqueous mounting media [VectaMount™ Mounting Media].

Appropriate negative [mouse control immunoglobulin IgG1, 1:50, Dako™] and positive controls [inflamed tonsil or inflamed terminal ileal biopsies] for calprotectin were performed with each immunostaining.

2.4. Staining for neutrophils

Identical slides of the biopsies stained for iMC were stained with a Naphthol AS-D Chloroactetate-specific esterase [SPE] kit [Sigma Aldrich] according to the product information.

2.5. Quantification of iMC

Sections were scanned and coded before analysis and investigators blinded to clinical information. Five hig-power fields [HPF] of the biopsy from the most affected segment with the highest iMC score were examined independently by two authors [MG, LMW], to determine the mean number of calprotectin-positive cells per high-power field, according to criteria described above.

2.6. Statistical analysis

Continuous variables were summarised by mean and standard deviation if normally distributed, or the median and interquartile range if not normally distributed. The Mann-Whitney U-test for discontinuous, normally distributed variables was used to compare iMC expression between UC patients and controls. The Kruskal-Wallis test was used to compare iMC between the four variants of remission [clinical, endoscopic, histological, and mucosal biomarker]. Correlations between iMC and clinical, endoscopic, and histological disease activity indices were evaluated using Pearson correlation. SCCAI and iMC were analysed on a logarithmic scale, due to the skewed distribution. Cox regression with Bonferroni correction for multiple comparisons was used to assess the relationship between iMC and steroid use, hospitalisation, and colectomy. Probability [p] values of less than 0.05 were regarded as significant. To accommodate the skewed distribution of iMC score, median iMC values were categorised into three categories to give approximately three equal-sized groups [≤ 5, 6–50, > 50/HPF]. Similarly, specific esterase counts were categorised into three evenly distributed groups: ≤ 10/HPF, 11–50/HPF, and > 50/HPF

3. Results

3.1. Patient demographics

Of the original 100 patients recruited for the activity indices study, outcomes with regard to steroid use, hospitalization, and colectomy of 91 patients were available in 2014.[5] Sufficient tissue from mucosal biopsies taken at the index flexible sigmoidoscopy in 2007 was available for immunohistochemistry to quantify iMC in conjunction with clinical outcomes in 83 patients. Immunohistochemistry to quantify iMC was performed on 372 colorectal biopsies from patients with UC and 20 controls with histologically normal mucosa from surgically resected, distal colorectal tumours. Patient demographics are reported in Table 1.

Table 1. Patient demographics.

Characteristic	Category	Result, *n* [%]
Age at diagnosis[a]	-	37.5 [15.0]
Age at initial assessment[a]		49.3 [15.9]
F/u time from baseline [months][b]	-	72 [53, 75]
F/u time from diagnosis [years][b]	-	13 [9, 22]
Extent of disease	Proctitis	26 [32%]
	Distal	43 [52%]
	Extensive	13 [16%]
Clinical disease activity	0–2	33 [40%]
	3–5	22 [27%]
	6–10	19 [23%]
	> 10	9 [11%]
Endoscopic disease activity	Remission	10 [12%]
	Mild	41 [49%]
	Moderate	22 [27%]
	Sever	10 [12%]
Histological activity	Normal	15 [18%]
	Not significant	27 [33%]
	Mild/moderate	25 [30%]
	Severe	16 [19%]
Medication at baseline	None	8 [10%]
	5-ASA	43 [52%]
	Immunomodulators	10 [12%]
	Anti-TNF-α	3 [4%]
	ASA + immunomodulator	18 [22%]
	Mycophenolate mofetil	1 [1%]

F/u, follow-up; 5-ASA, 5-aminosalicylic acid; TNF, tumour necrosis factor.
[a]Mean [standard deviation] reported.
[b]Median [interquartile range] reported.

3.2. iMC expression and disease activity

Median iMC positively correlated with all three activity scores: clinical, endoscopic, and histological activity indices [$p < 0.001$]. Higher counts of iMC were associated with higher disease activity scores [Figure 2, Table 2]. Overall, there was no statistical difference between a set of biopsies from healthy controls and the entire cohort of patients with UC [$p = 0.29$], of whom 18% had entirely normal histology, 33% had 'no significant inflammation', and 30% had only 'mild to moderate inflammation'.

3.3. iMC and remission

iMC expression in patients with UC was evaluated, based on whether they were in remission by the three conventional definitions of remission [clinical, endoscopic, histological, Table 3]. For each conventional definition of remission, the iMC was significantly lower compared with patients not in remission [i.e. disease activity]. Of note, iMC values significantly decreased with increasing concordance between definitions of remission. Median iMC was lowest in patients with concordance for clinical, endoscopic, and histological remission. There were 42 patients in histological remission, of whom 15 [36%] had a median iMC > 5/HPF. There were 51 patients in endoscopic remission, of whom 23 [45%] had a median iMC > 5/HPF.

3.4. Total cohort: iMC and disease outcomes

Of the total cohort, 65% [53/83] required one or more courses of corticosteroids, 20% were hospitalised [17/83], and 11% underwent surgery [9 of 83]; 56% were free of adverse outcome [treatment with steroids, hospitalisation, or surgery] at 1 year. This figure dropped to 38 % by 6 years.

iMC and clinical, endoscopic, and histological criteria for remission correlated with any adverse outcome [Table 4]. Maintenance therapy, all types of remission, and iMC were significantly and inversely associated with the time to an adverse outcome. In multivariable analysis, disease duration, maintenance therapy, and iMC remained significantly inversely associated with time to an adverse event. The risk of an adverse outcome was almost four times higher in the iMC 6–50/HPF and > 50/HPF group compared with the lowest iMC group [≤ 5/HPF] [Figure 3]. The Kaplan-Meier curve illustrates that 36%, 25%, and 14% of the total cohort with a median iMC > 5 were free of an adverse outcome at 12, 36, and 72 months, respectively, compared with 79%, 70%, 66% in those with an iMC < 5/HPF [Figure 3].

3.5. Patients in histological remission: iMC and disease outcomes

In the histological remission group, 74% of patients had no adverse outcome after 1 year and this fell to 55% at 6 years. The Kaplan-Meier plot illustrates the effect of iMC on time to adverse outcome in patients with histological remission [Figure 4]; 53%, 33%, and 25% of those with a median iMC > 5/HPF were free of an adverse outcome at 12, 36, and 72 months, respectively, compared with 85%, 77%, and 72% of those with an iMC < 5/HPF [Figure 4]. The patients in histological remission with an iMC < 5/HPF had the best outcome, with 72% free from an adverse event at 6 years.

In the histological remission group, men had a lower risk than women for an adverse outcome, although this may reflect small numbers. On the other hand, a higher iMC was associated with a higher

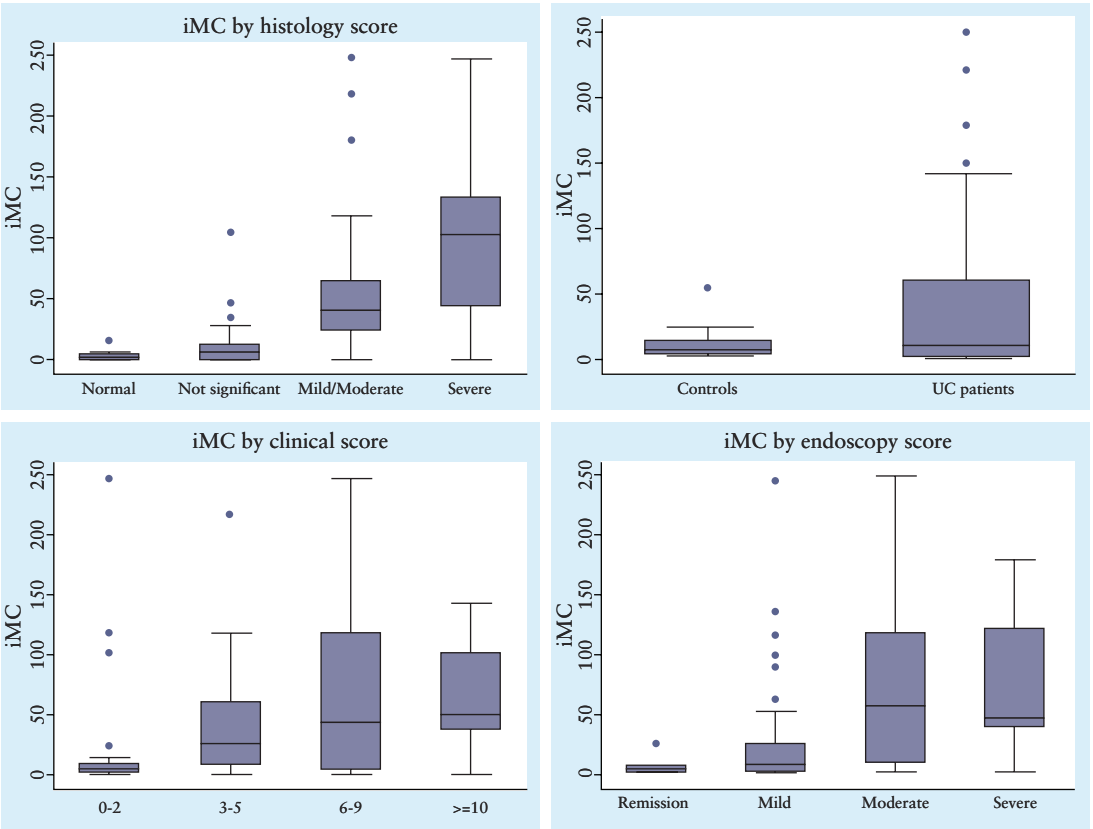

Figure 2. Quantification of intramucosal calprotectin [iMC] in controls and by disease activity index.

risk of adverse outcome [Table 5], which is biologically plausible. In a multivariable analysis, iMC remained statistically significant [HR 5.98, CI 2.15, 16.6, $p < 0.001$] for a risk of needing steroids, hospitalisation, or colectomy during the 6-year follow-up.

3.6. Patients with deep remission [all three markers of remission]: iMC and disease outcomes

A total of 25 patients had all three types of remission. Patients with higher iMC values [> 5/HPF] were at an increased risk of an adverse event [HR 3.04, [0.81, 11.4], p = 0.1] but did not quite reach statistical significance [Figure 5]. A multivariable analysis was performed to examine jointly the factors affecting time to an adverse outcome. Maintenance therapy with 5-aminosalicylic acid [5-ASA] [HR 1.70, 0.17, 17.2, $p = 0.008$], other maintenance therapy [HR 18.5, 1.67, 205, $p = 0.008$], and iMC > 5/HPF [HR 4.85, 1.12, 20.9, $p = 0.03$] were significantly associated with time to an adverse outcome.

3.7. Specific esterase staining for neutrophils and patient outcomes

Higher specific esterase [SPE] values were associated with an increased risk of adverse outcome. Patients with SPE values > 50/HPF had an increased risk of an adverse outcome compared with those with values of 10 or less [HR 5.65, 2.75, 11.6, $p < 0.001$]. In a multivariable analysis, which included SPE counts, only iMC and disease duration remained statistically significant. There was a trend to an association of increased risk of adverse outcome with SPE, which did not quite reach statistical significance [$p = 0.08$].

Table 2. Pearson correlation of intramucosal calprotectin with ulcerative colitis disease activity indices.

Activity index	Correlation coefficient	*p*-Value
Clinical [SCCAI*] activity	0.51	< 0.001
Endoscopic [Baron] activity	0.65	< 0.001
Histological [T and R*] activity	0.53	< 0.001

4. Discussion

A principal aim of this study was to determine the prognostic value of iMC in patients with UC. Our results show that a median iMC > 5/HPF was independently associated with worse outcome [corticosteroid use, hospitalisation, or colectomy during 6 years of follow-up] both in the total cohort [HR 3.36, [1.58, 7.15], $p = 0.001$], and

Table 3. Intramucosal calprotectin [iMC] and remission.

Type of remission	Yes/no	Number of patients	Median iMC and interquartile range [IQR]	*p*-Value
Histological remission	No	41	50.6 [26.4–118.1]	< 0.001
	Yes	42	3.5 [0.4–9.8]	
Endoscopic remission	No	32	49.5 [35.4–117.9]	< 0.001
	Yes	51	4.2 [0.7–22.4]	
Clinical remission	No	50	40.3 [7.7–100.8]	< 0.001
	Yes	33	3.2 [0.7–8.8]	
Number of remissions	-			< 0.001
0		23	61.6 [37.4–118.8]	
1		19	48.5 [4.4–118.1]	
2		16	5.9 [0.2–19.9]	
3		25	2.4 [0.4–5]	

Table 4. Total cohort: univariable and multivariable analysis for predictors of adverse outcomes.

Variable	Category	Hazard ratio [95% CI]	*p*-Value	Hazard ratio [95% CI]	*p*-Value
Age at diagnosis[a]	-	1.08 [0.89, 1.30]	0.44		
Sex	Female	1	0.16		
	Male	0.66 [0.37, 1.18]			
Disease duration [years]	-	0.88 [0.74, 1.04]	0.12	0.79 [0.67, 0.94]	0.008
Maintenance therapy	None	1	0.01	1	0.030
	5-ASA	1.44 [0.43, 4.82]		1.75 [0.50, 6.09]	
	Other	3.08 [0.93, 10.2]		3.39 [0.96, 11.9]	
Histological remission	No	1	< 0.001		
	Yes	0.32 [0.18, 0.57]			
Endoscopic remission	No	1	0.001		
	Yes	0.41 [0.24, 0.70]			
Clinical remission	No	1	0.001		
	Yes	0.36 [0.19, 0.67]			
Concordance of three remissions	No	1	0.003		
	Yes	0.34 [0.16, 0.69]			
iMC	≤ 5	1	< 0.001	1	< 0.001
	6–50	3.98 [1.91, 8.23]		3.36 [1.58, 7.15]	
	> 50	4.74 [2.25, 9.99]		3.60 [1.64, 7.88]	

CI, confidence interval; iMC, intramucosal calprotectin; 5-ASA, 5-aminosalicylic acid.
[a]Mean [standard deviation] reported.

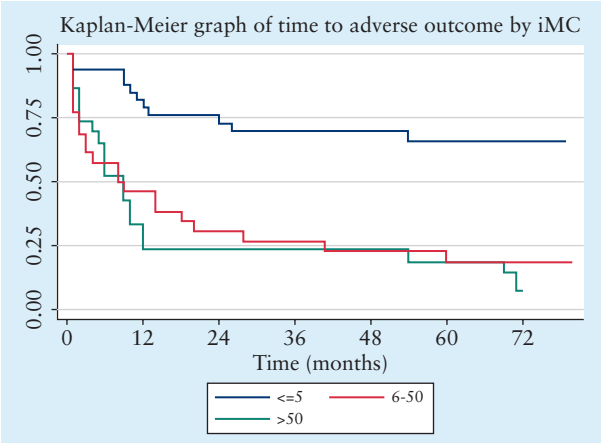

Figure 3. Kaplan–Meier graph for total cohort: time to any adverse outcome stratified by intramucosal calprotectin [iMC]. The subgroup with the lowest iMC [≤ 5/HPF] had the best outcome with 79% (confidence interval [CI] 0.61, 0.90), 70% [CI 0.51, 0.82], and 66% [CI 0.47, 0.80] free from adverse outcome at 12, 36, and 72 months, respectively, compared with 36% [CI 0.23, 0.49], 25% [CI 0.14, 0.38], and 14% [CI 0.06, 0.25] in those with iMC > 5/HPF [$p < 0.001$]. HPF, high-power field.

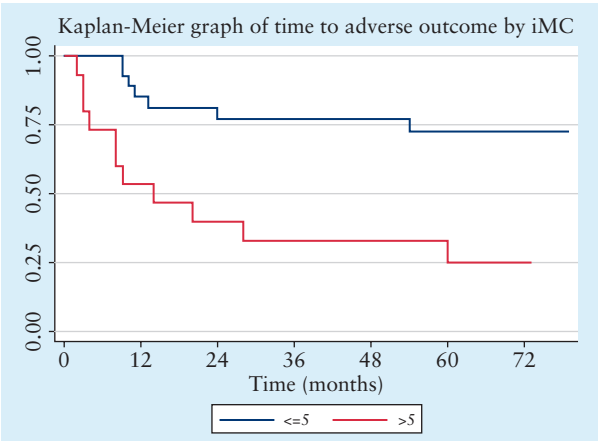

Figure 4. Kaplan–Meier graph for patients in histological remission: time to any adverse outcome stratified by intramucosal calprotectin [iMC]. The subgroup with the lowest iMC [≤ 5/HPF] had the best outcome with 85% (confidence interval [CI] 0.65, 0.94), 77% [CI 0.56, 0.89], and 72% [CI 0.50, 0.86] free from adverse outcome at 12, 36, and 72 months, respectively, compared with 53% [CI 0.26, 0.74], 33% [CI 0.12, 0.56], and 25% [CI 0.07, 0.49] in those with iMC > 5/HPF [$p = 0.003$]. HPF, high-power field.

unexpectedly, in those with apparent histological remission [HR 5.98, 2.15, 16.6, $p = 0.001$]. In patients with histological remission and a median iMC > 5/HPF, 53%, 33%, and 25%, respectively, were free of an adverse outcome at 12, 36 and 72 months compared with 85%, 77%, 72%, with an iMC < 5/HPF. The subgroup of patients with histological remission and an iMC ≤ 5/HPF had the best outcome, with 72% free from an adverse outcome at 5 years. Similarly in patients with all three remissions, iMC > 5/HPF was associated with a trend to adverse outcome, and in a multivariable analysis iMC was significantly associated with adverse outcome iMC > 5/HPF [HR 4.85, 1.12, 20.9, $p = 0.03$].

These data support a potential role for mucosal calprotectin in defining remission and stratifying those in histological remission who are at risk of disease progression. Histological remission has been proposed as a critical therapeutic endpoint of UC treatment, in the light of growing evidence that it is associated with improved clinical outcomes.[2,5,8,9,11,36,38–42] However, until recently, histological remission was not typically used as an endpoint partly because of a lack of a well-validated scoring system and a lack of consensus about definitions. Recent work however, may alter this situation.[35] Even with the use of a quantitative score for histological activity, there is much inter- and intra-observer variation.[5] A recent study comparing the predictive value of faecal calprotectin measurement and histological scoring in UC found that calprotectin measurement performed better.[43] Therefore a simple immunohistochemical score for calprotectin may prove more reliable and robust, either by itself or as an adjunct to histological scoring. Although iMC expression has previously been described in a small number of studies, there are few data in UC.[14,16–18,44–47] No previous studies have examined its role in defining remission or as a predictor of the clinical course of UC.

A study from Chicago of 310 patients with UC in clinical remission reported that patients with complete histological normalisation [CHN] had better clinical outcomes than those with histological activity or histological quiescence. Only CHN was associated with an improved clinical relapse free survival.[48] iMC may therefore have a potential role in defining histological remission [i.e. lack of microscopic disease activity] and possibly CHN, provided that iMC scoring can be validated by further studies in IBD and non-IBD samples.

iMC correlated positively with all of the three commonly assessed activity scores: clinical, endoscopic, and histological. It also correlated with each definition of remission, which is the first time this has been demonstrated in UC. For each type of remission, patients in remission had significantly lower iMC values compared with patients not in remission.

Furthermore, median iMC correlated with the depth of remission, being lowest in patients with concordance for clinical, endoscopic, and histological remission. The median iMC decreased stepwise as patients were classified as being in remission according to each of the three categories: clinical, endoscopic, or histological. The median iMC was 61.6/HPF for those without remission, compared with a median iMC of 2.4/HPF for patients in remission by all three measures [$p < 0.001$]. This suggests a potential role for iMC as a marker of the 'depth' of remission. We have previously shown that histologically defined remission, especially when concordant with endoscopic and clinical measures, predicts a better outcome in the group of patients described here, and was associated with a 5-fold reduction in oral steroid use over a median of 6 years 29 months.[5] This study suggests that iMC could add particular value in defining remission for those patients where there is discordance on other measures, such as endoscopic, clinical, and histological remission. In this cohort, 45% of those meeting endoscopic remission criteria had abnormal histology and an iMC > 5 /HPF. Similarly, 36% of those in histological remission had an iMC > 5/HPF. This discordance underscores the need for a more reliable and reproducible measure of microscopic disease activity.

The results demonstrate that mucosal calprotectin is readily detected using commercially available reagents on paraffin-embedded, formalin-fixed specimens, and that the pattern of staining, which delineates individual reactive cells, can be quantified. The counting is amenable to automated counting using image analysis software. In the context of clinical trials, where iMC staining may provide particular insight into mucosal disease activity, the stained sections, which could be scanned and stored electronically, provide an archivable record subject to independent audit and validation.

Examples of clinical scenarios where iMC may be useful include when a patient undergoes a colonoscopy with biopsies during

Table 5. Histological remission: univariable and multivariable analysis for predictors of adverse outcomes.

Variable	Category	Hazard ratio [95% CI]	*p*-Value	Hazard ratio [95% CI]	*p*-Value
Age at diagnosis[a]	-	0.95 [0.68, 1.34]	0.79		
Gender	Female	1	0.04		
	Male	0.32 [0.11, 0.97]			
Disease duration [years]	-	1.12 [0.71, 1.76]	0.63		
Maintenance therapy	None	1	0.05	1	0.007
	5-ASA	0.71 [0.19, 2.70]		0.87 [0.23, 3.29]	
	Other	2.72 [0.70, 10.6]		5.04 [1.18, 21.5]	
Endoscopic remission	No	1	0.32		
	Yes	0.53 [0.15, 1.85]			
Clinical remission	No	1	0.08		
	Yes	0.44 [0.17, 1.10]			
Concordance three remissions	No	1	0.26		
	Yes	0.59 [0.23, 1.48]			
iMC	≤ 5	1	0.003	1	0.001
	> 5	4.29 [1.65, 11.1]		5.98 [2.15, 16.6]	

iMC, intramucosal calprotectin; CI, confidence interval; 5-ASA, 5-aminosalicylic acid.
[a]Mean [standard deviation] reported.

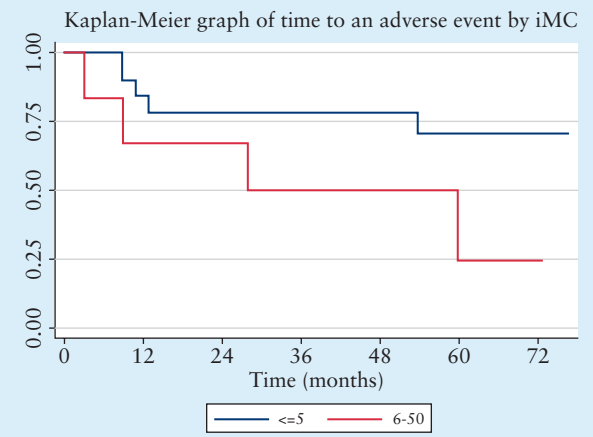

Figure 5. Kaplan–Meier graph for patients with all three definitions of remission: time to any adverse outcome stratified by intramucosal calprotectin [iMC]. The risk of an adverse outcome at any time was 3.04 times greater in those with iMC values > 5/HPF compared with < 5/HPF [$p = 0.10$]. HPF, high-power field.

assessment before change of therapy. Patients with an IMC < 5/HPF may have better outcomes than those with > 5/HPF even if in histological remission. This might aid a decision to stop biological or immunomodulatory therapy. Patients may be reluctant to provide stool samples for disease assessment and iMC may be useful in this scenario. Similarly, in patients undergoing surveillance colonoscopy, iMC may be a candidate prognostic marker for future neoplasia, since neoplasia is closely linked to inflammation in UC.[49]

The limitations of this study are that it is retrospective, with a small sample size of controls and patients with UC. There may be both selection and survival bias, as this study is based on patients from a tertiary referral centre. Faecal calprotectin was also not routinely incorporated into clinical practice at the time of this study, and therefore iMC may not be correlated with this measure. This study merits further validation by prospective studies on iMC, comparing iMC with faecal calprotectin levels, and with a larger sample size to evaluate of inter- and intra-observer agreement, as well as to establish appropriate thresholds for iMC in patients with and without UC.

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Conflict of Interest

To the best of our knowledge, no conflict of interest, financial or other, exists.

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Author Contributions

All authors have made substantial and equal contributions to all of the following: the concept and design of the study, literature search, or acquisition of data, or analysis and interpretation of data, drafting the article, or revising it critically for important intellectual content, final approval of the version to be submitted.

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