

Faecal Calprotectin and 7- α Cholestenone Levels in Microscopic Colitis: Experience from Edinburgh

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Introduction: Microscopic colitis (MC) is an important cause of chronic, watery diarrhoea. Currently, there is no specific biomarker available to guide diagnosis. The use of faecal calprotectin (FCP) as a potential marker has been addressed in only a few studies. Further, bile acid malabsorption (BAM) often accompanies MC. Current practice recommends the selenium-labelled homocholic acid-taurine (SeHCAT) test, but at our centre, 7 α -hydroxy-4-cholesten-3-one (7 α C) is used as a simpler and less expensive alternative to SeHCAT, with values over 22ng/mL indicating BAM. This study aims to evaluate the use of FCP as a biomarker in the diagnosis of MC and the role of 7 α C in detecting concomitant BAM with MC.

Methods: Pathology records were retrospectively reviewed for patients diagnosed with collagenous colitis (CC) between 2000 and 2018 and lymphocytic colitis (LC) between 1995 and 2011. FCP and 7 α C results, if measured within 6 months of pathological diagnosis, were extracted for analysis.

Results: Between 2000 and 2018, 646 CC cases were confirmed on histology. Of 646 patients, 147 had FCP measured; in 111 (75.5%) FCP was elevated with mean levels 238.1 μ g/g (SD \pm 273.0); 140/646 had 7 α C measured; 16 (11.4%) indicated BAM. Mean levels were 10.2ng/mL (SD \pm 9.4). During a 21-year period (1995–2011), 204 LC diagnoses were made: 14/204 had FCP measured; 8 (57.1%) were elevated. Mean levels were 128.4 μ g/g (SD \pm 107.7). Of 204 LC patients, 20 had 7 α C measured, 5 (25%) indicating BAM. Mean levels were 13.95ng/mL (SD \pm 9.4).

Discussion: Both CC and LC were associated with raised FCP during the diagnostic phase, supporting the potential role of its use in clinical practice. Furthermore, we present results of using 7 α C in identifying BAM amongst patients with MC. In our cohort, low levels of 7 α C suggest relatively low concordance of BAM with MC.

Keywords: microscopic colitis, bile salt malabsorption, biomarkers, 7- α cholestenone, faecal calprotectin

Introduction

Microscopic colitis (MC) comprises the clinical disorders collagenous colitis (CC) and lymphocytic colitis (LC).¹ Over the last decade the prevalence of MC has risen, with numbers now comparable to that of Inflammatory Bowel Disease (IBD).^{2,3} A significant contributor to this increase in diagnosis has been raised awareness and recognition of the disease.⁴ Despite this MC is often misdiagnosed as clinical symptoms may mimic a large number of other conditions,⁵ some examples are included in Table 1. Typically, patients with MC present with chronic, painless, non-bloody and watery diarrhoea.⁶ An issue remains that there is no specific biomarker available to guide towards a diagnosis of MC.⁷ Therefore, multiple colonic biopsies, taken from an often macroscopically normal

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Table 1 List of Differential Diagnoses of Microscopic Colitis

Common Differential Diagnoses of Chronic Non-Bloody Watery Diarrhoea
<ul style="list-style-type: none"> • Microscopic colitis (LC or CC) • Irritable bowel syndrome (IBS) • Bile acid malabsorption • Crohn's disease (CD) • Gastroenteritis • Coeliac disease • Medications, eg, metformin • Colonic neoplasia • Post-radiation diarrhoea • Small bowel bacterial overgrowth • Hyperthyroidism • HIV

colon, are required for diagnosis.⁸ With ever increasing pressures on healthcare delivery, patients may not undergo a colonoscopy for many months or years from the onset of symptoms.⁹ Therefore, the use of biomarkers for screening and diagnosis in the setting of MC is desirable.

This study presents data from a large patient cohort evaluating two possible biomarkers, faecal calprotectin (FCP) and 7- α -hydroxy-4-cholesten-3-one (7 α C) amongst patients with histologically confirmed MC. FCP is already well established as a biomarker for detecting inflammation, most typically in IBD.¹⁰ Its role in detecting MC, however, is limited and conflicting and requires further evaluation. The European Microscopic Colitis Group (EMCG) advise that bile acid malabsorption (BAM) should be considered and investigated.¹¹ This association is poorly understood, yet treating BAM early in MC can aid with symptomatic control. Currently, the reference standard for investigating BAM is the selenium-labelled homocholeic acid-taurine (SeHCAT) scan.¹² This is a complex and expensive procedure which also exposes patients to small volumes of radiation.¹³

In this retrospective study, we investigate the use of 7 α C, a bile acid precursor, as a simpler alternative for detecting BAM amongst patients with MC. Furthermore, we evaluate the role of FCP in identifying new cases of MC.

Methods

Study Design

Data were collected from patients diagnosed with MC within the Lothian health board. Pathology records were retrospectively reviewed for all patients with a histologically confirmed diagnosis of CC or LC based on results of colonic biopsies.

Records for confirmed CC diagnoses were available over an 18-year period between 2000 and 2018 and reviewed. Relevant LC data were available over a 16-year period extending from 1995 to 2011. Within this period, all patients who were diagnosed with MC had their biochemical and clinical records reviewed. Data values for FCP and 7 α C were extracted through the electronic patient record system (TrakCare, ©Intersystems, Cambridge, MA), and then exported into Microsoft Excel (©Microsoft, Redmond, WA) for further analysis.

Diagnostic Protocol

Most patients undergoing investigations for MC followed a similar pathway to diagnosis, with the majority presenting with non-bloody watery diarrhoea as the major component of their initial complaint. If other gastrointestinal conditions, aside MC, were also diagnosed during the diagnostic workup, these patients were excluded for concerns over interference with the biomarker values.

Biomarker Testing and Interpretation

FCP is a commonly used marker in determining intestinal inflammation.¹⁰ This is particularly relevant in active IBD, where large volumes can be found in stool samples. Values recorded $>50\mu\text{g/g}$ were noted as elevated above the normal range, with those $<50\mu\text{g/g}$ within the reference range and documented as non-elevated/normal. Laboratory values for FCP reported as $<20\mu\text{g/mL}$ were counted as $20\mu\text{g/mL}$ for the purpose of analysis, as very low values locally are grouped and reported as " $<20\mu\text{g/mL}$ ". 7 α C is used in our centre in determining whether patients with MC have contemporaneous BAM. This biochemical is an intermediate within the pathway of bile acid synthesis, and elevated serum levels indicate an impairment concerning the absorption of bile acids.^{12,24} Values measured $>22\text{ng/mL}$ indicate concomitant BAM, and those $<22\text{ng/mL}$ are within the normal reference range. Importantly only values for FCP and 7 α C measured within the 6 months prior to or following the diagnosis of MC were included. Where a patient had repeat measurements for either biomarker within this timescale a mean value was calculated, and this was used as the single value for analysis.

Results

Collagenous Colitis

Between the years of 2000–2018, a total of 646 patients had pathology records confirming a diagnosis of CC. Of these patients 147 (22.8%) had FCP values recorded and

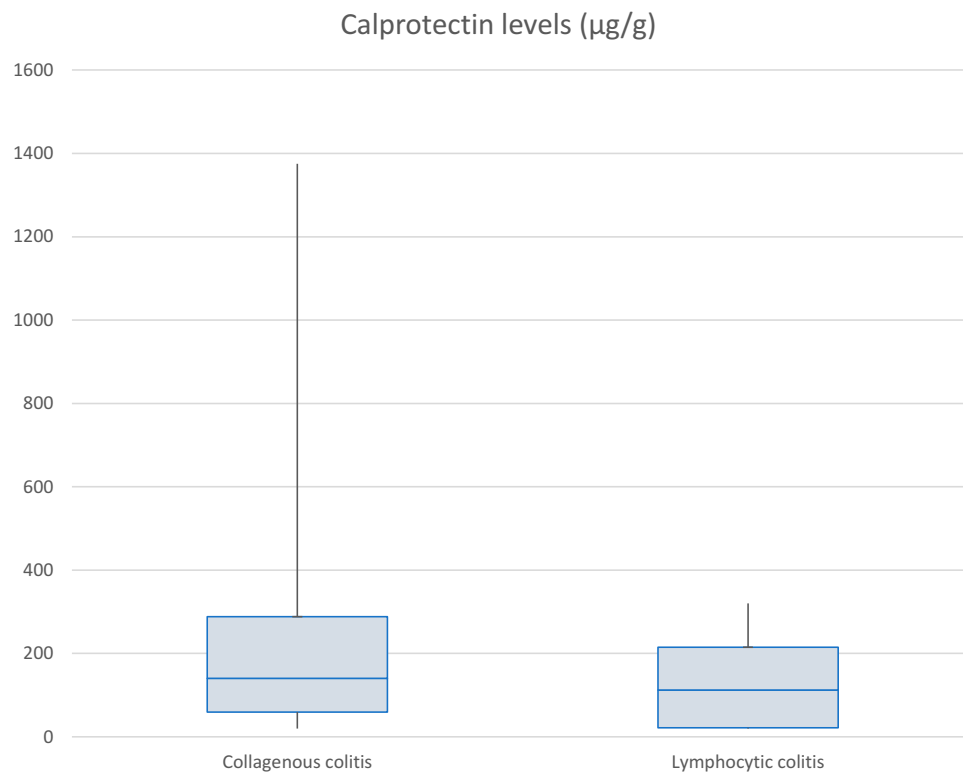


Figure 1 Calprotectin levels measured in collagenous and lymphocytic colitis.



Figure 2 7-a. Cholestenone levels measured in collagenous and lymphocytic colitis.

140 (21.7%) were investigated for BAM using 7 α C. 111/147 (75.5%) patients who had FCP measured had elevated levels >50 μ g/g. Furthermore, 92/147 (62.6%) of these were recorded >100 μ g/g, and 53/147 (36.1%) were >200 μ g/g. The total range of FCP values was between 20–1375 μ g/g, and the mean value recorded was 238.1 μ g/g (SD \pm 273.0), **Figure 1**. Of the 140 patients who had 7 α C measured, 16 (11.4%) had values elevated above the normal reference value of 22ng/mL, indicating clinically evident BAM. The mean value of the 7 α C measurements was 10.2ng/mL (SD \pm 9.4), **Figure 2**. Specifically, there were 73/646 (11.3%) patients who had both FCP and 7 α C values measured during their diagnostic work-up. Amongst this group, 60 had a raised FCP, with just 5 of these patients also having a coexisting elevated 7 α C. The 13 patients with normal FCP levels also had normal 7 α C levels.

Lymphocytic Colitis

204 patients were diagnosed with LC in the time period of 1995–2011. 14 (6.9%) patients had FCP recorded and 20 (9.8%) patients had 7 α C investigated. 8/14 (57.1%) patients who had FCP measured had elevated levels >50 μ g/g. Additionally, all 8 values were >100 μ g/g with 4 being >200 μ g/g, **Figure 1**. The mean FCP value was 128.4 μ g/g (SD \pm 107.7). Separately 20 (9.8%) patients had 7 α C investigated, with 5/20 (25.0%) of the values being elevated above the normal threshold of 22ng/mL. The mean value was 13.95ng/mL (SD \pm 9.4), **Figure 2**. As with the CC cohort, we also identified the population of LC patients who had both FCP and 7 α C measurements undertaken. 9/204 (4.4%) fulfilled this criterion, 6 of these patients had an elevated FCP, with just 2 of this group having a concomitantly elevated 7 α C.

Discussion

Over recent years the prevalence of MC has increased.² Despite raised awareness of the condition, there is a paucity of available literature relating to the use of biomarkers in the diagnostic workup of MC. Previous research evaluating the use of FCP to detect active MC provide generally conflicting results with examples given in **Table 2**. Additionally, there is no literature specifically evaluating the use of 7 α C in investigating BAM in MC, see **Table 3**.

Clinical research looking into FCP in patients with MC has suggested that MC is associated with elevated FCP. Limburg et al showed that patients with chronic colonic

Table 2 Summary of Research Evaluating the Clinical Effectiveness of Faecal Calprotectin in MC

Limburg et al ¹⁴	FCP was higher in those with colorectal inflammation and chronic colitis than in those with no colonic inflammation.
Von Arnim et al ¹⁵	17 patients with active MC and 20 with IBS had FCP levels prospectively reviewed. Higher FCP levels were found within the MC group.
Wildt et al ¹⁶	Higher FCP in active CC, compared with quiescent CC and a control group, who did not have MC.
Larsson et al ¹⁷	No significant differences between the levels of FCP amongst 15 patients with active MC compared to a control group.
Batista et al ¹⁸	34 MC patients were compared with a control group of 60 whom had functional watery diarrhoea. Mean FCP values were; 175 in MC group, and 28 in the control.

Table 3 Sample of Previous Research Evaluating the Association of BAM in MC and/Or the Role of 7- α Cholestenone in Detecting BAM

Ung et al ²¹	Using SeHCAT, BAM was common in a CC cohort (n=12/27, 44%)
Ung et al ²²	BAM less common in LC compared with CC.
Rasmussen ²³	Bile acid diarrhoea accompanied 41% of CC and 29% of LC cases.
Brydon et al ²⁴	Identified a similar effectiveness of 7 α C to that of SeHCAT.
Fernández-Bañares ²⁵	BAM common in both MC (43.1%, n=22/51) and unexplained functional chronic diarrhoea (75%, n=24/32).

inflammation had higher average FCP compared with a control group.¹⁴ Von Arnim et al concluded that FCP levels were greater in a MC cohort compared with an IBS population,¹⁵ while Wildt et al also documented higher FCP in active MC patients, compared with quiescent CC and a control group.¹⁶ However, in another study no differences in FCP values were seen between MC patients and a control group.¹⁷ Nevertheless, all of these studies presented small patient cohorts. Perhaps the best data available are from Batista et al.¹⁸ The authors retrospectively compared 34 patients with MC and 60 patients with functional diarrhoea, identifying mean FCP levels in MC and functional groups of 175 μ g/g and 28 μ g/g, respectively.

Our results suggest that MC is commonly associated with elevated FCP values, with 75.5% (n=111/147) of CC patients, and 57.1% (n=8/14) of LC patients having abnormal FCP values (>50µg/g), and often markedly higher, Table 4. However, as 24.5% (n=36/147) of those with CC and 42.9% (n=6/14) of LC had a FCP within the normal range, the use of FCP cannot exclude the diagnosis of active MC. It is an entirely non-specific biomarker, rising in many causes of diarrhoea, but despite its low specificity and sensitivity for MC, the use of FCP does have value as a supplementary investigation in the diagnostic work-up of both CC and LC.

Bile acid malabsorption (BAM), which also causes non-bloody diarrhoea, mimics many conditions including IBS and MC.¹⁹ Past literature has frequently reported patients to have both BAM and MC, but the true association is poorly understood.¹¹ One theory is that chronic BAM may be a risk factor for developing MC.²⁰ Current practice is to investigate for BAM using the SeHCAT test. Previous research, looking at 27 patients with CC, suggested that the SeHCAT test identified 44% with coexistent BAM.²¹ The association of BAM has been reported as slightly more common with CC than LC.^{22,23}

Our study is the first of its kind evaluating the use of 7αC, a bile acid precursor, as an alternative to SeHCAT for detecting BAM within MC patients. The main benefit of 7αC in practice is that it is considered a cheaper and simpler alternative, with a similar rate of detection to that of SeHCAT.²⁴ In our cohort, a relatively low levels of BAM were identified in MC patients (11.4% of CC, 25% of LC) when compared to the results of past research. An optimal design for this study would have included both 7αC and SeHCAT results being included for analysis. LC was more strongly associated with BAM than CC, conflicting with previously published findings. There were relatively few patients in the LC category included as few had had the tests performed, possibly due to being

a slightly earlier cohort, which could introduce bias. Based on the low levels of BAM reported, it is therefore possible that the association of BAM and MC is weaker than previously recorded. Whilst it is useful in detecting BAM, this study would suggest it has a limited role in the detection of MC prior to diagnostic colonoscopy. Fernández-Bañares et al identified that whilst BAM was common amongst patients with MC, there were also higher rates in the cohort who had functional diarrhoea.²⁵ 7αC, therefore, may arguably be more useful following a histopathological confirmation of MC, as on its own it has limited value in differentiating MC from other conditions. Importantly, it remains of worth for investigating patients with functional diarrhoea, as it can rule out BAM as an alternative cause of their presentation.

Our study also identified little association between FCP and 7αC in patients who had both parameters measured.

As with any study, there are important limitations to reflect upon. The retrospective design involved the reliance upon previously documented clinical information.

This study presents real-world experience and data. This unfortunately means the collection of biomarkers in those ultimately diagnosed with MC was sporadic and inconsistent. We used a period of 6 months as the inclusion criteria for the measurement of the biomarkers of interest, chosen to represent truer levels in active MC. The study design including FCP and 7αC results returning after the diagnosis of MC being made risks including those who have commenced therapy, which could again introduce unaccounted for biases. Many of the initial referrals, as altered bowel habit or new diarrhoea, came with the clinical concern of malignancy. This resulted in many patients undergoing early colonoscopy and so biomarkers went unchecked, excluding them from our study. Despite initial large numbers included in the MC cohort, this resulted in small numbers for final analysis and so the results are hard to generalise. There is also a risk of selection bias as those with a high FCP recorded are more likely to proceed to invasive colonoscopy, leading to their MC diagnosis, than those with a low FCP reading who may not undergo further investigation.

Serial biomarker values would ideally have been used to illustrate response to treatment and changing levels with the degree of MC activity but unfortunately, these data were not available as once the diagnosis was reached further biomarkers were rarely checked.

Conclusion

In summary, both CC and LC were associated with raised FCP. We suggest FCP should be used more frequently as a biomarker in clinical practice to help raise suspicion of

Table 4 Summary Comparing the Findings Between Collagenous and Lymphocytic Colitis

	Collagenous Colitis	Lymphocytic Colitis
Faecal Calprotectin <ul style="list-style-type: none"> • Number of patients tested • Elevated (>50µg/g) 	147 111 (75.5%, n=111/147)	14 8 (57.1%, n=8/14)
7-α Cholestenone <ul style="list-style-type: none"> • Number of patients tested • Elevated (>22ng/mL) 	140 16 (11.4%, n=16/140)	20 5 (25%, n=5/20)

MC, as a raised FCP can lower the threshold for colonoscopy referral, leading to fewer misdiagnoses. Importantly though, FCP is not specific to MC and although it was raised in many cases, not all with MC have a positively elevated level. Therefore, it should not be considered as an alternative to the current approach of a thorough clinical assessment followed by histological confirmation during colonoscopy, but instead considered as a supplementary test to focus investigations.

Our study indicated low levels of coexisting BAM in MC. 7 α C, nevertheless, has a potential role in identifying this particular subgroup of patients. This bile acid precursor is a quick, and cost-effective alternative to SeHCAT. We recommend that further research is carried out investigating its role as an alternative to SeHCAT in clinical practice. Future research should continue to focus upon the development of biomarkers which may have a higher specificity for MC.

Ethics

This study was performed as a service evaluation using routinely collected anonymised data, and as such no ethics committee review was requested. Throughout, the methodology acted in compliance with the Declaration of Helsinki to ensure that all data were protected in order to appropriately safeguard the patients included.

Disclosure

Dr Anastasios Koulaouzidis reportsspeaker/advisory board honoraria and travel support from Dr FalkPharma UK. The authors report no other conflicts of interest in this work.

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