

Relevance of fecal calprotectin and lactoferrin in the post-operative management of inflammatory bowel diseases

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bowel disease (IBD) patients' management. The post-operative setting in both Crohn's disease (CD) and ulcerative colitis (UC) patients has been less investigated although few promising results come from small, cross-sectional studies. Therefore, the current post-operative management still requires endoscopy 6-12 mo after intestinal resection for CD in order to exclude endoscopic recurrence and plan the therapeutic strategy. In patients who underwent restorative proctocolectomy, endoscopy is required whenever symptoms includes the possibility of pouchitis. There is emerging evidence that fecal calprotectin and lactoferrin are useful surrogate markers of inflammation in the post-operative setting, they correlate with the presence and severity of endoscopic recurrence according to Rutgeerts' score and possibly predict the subsequent clinical recurrence and response to therapy in CD patients. Similarly, fecal markers show a good correlation with the presence of pouchitis, as confirmed by endoscopy in operated UC patients. Fecal calprotectin seems to be able to predict the short-term development of pouchitis in asymptomatic patients and to vary according to response to medical treatment. The possibility of both fecal markers to be used in the routine clinical practice for monitoring IBD patients in the post-operative setting should be confirmed in multicentric clinical trial with large sample set. An algorithm that can predict the optimal use and timing of fecal markers testing, the effective need and timing of endoscopy and the cost-effectiveness of these as a strategy of care would be of great interest.

Key words: Calprotectin; Lactoferrin; Fecal markers; Inflammatory bowel disease; Post-operative; Surgery; Crohn's disease; Ulcerative colitis

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Core tip: Inflammatory bowel diseases (IBDs) are chronic conditions, requiring life-long therapy and monitoring. Surgery is not curative and the disease

Abstract

The role of fecal lactoferrin and calprotectin has been extensively studied in many areas of inflammatory

might recur after operation as post-operative recurrence in Crohn's disease patients and pouchitis in ulcerative colitis patients. In both cases, endoscopy with histology is the gold standard procedure to assess disease activity. Non-invasive markers of intestinal inflammation, such as fecal calprotectin and lactoferrin, might be useful in the post-operative management of IBD patients, in order to identify individuals requiring endoscopy, so that they can avoid unnecessary invasive investigations. This paper reviews the current knowledge on the use of fecal markers in this specific setting.

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INTRODUCTION

Inflammatory bowel disease (IBD) is chronic, relapsing-remitting inflammatory conditions of the gastrointestinal tract. Both Crohn's disease (CD) and ulcerative colitis (UC) might require surgical intervention for different indications. Operated patients, both CD and UC patients, need to be followed-up regularly, because the risk of post-operative recurrence (CD patients) and of pouchitis (UC patients) is very common. However, the post-operative management is not clearly defined, in terms of needs of medications and timing of clinical, biochemical and endoscopic follow-up. Therefore, patients often undergo several invasive procedures to re-assess disease activity and exclude complications.

The role of non-invasive markers has been extensively studied in the diagnosis, management and monitoring of IBD patients. In particular, fecal markers, calprotectin (FC) and lactoferrin (FL), represent intestinal infiltration by leukocytes and correlate with the severity of endoscopic and histological intestinal inflammation^[1,2].

Calprotectin is a calcium- and zinc-binding protein of neutrophils, that is released in case of activation or apoptosis/necrosis^[3]. It displays several physiological roles in inflammatory and infectious processes and has anti-proliferative capability. The long stability of FC at room temperature (up to 7 d) is an advantage for its use in clinical practice. Lactoferrin is an iron-binding glycoprotein of neutrophils that, after degranulation, regulates their margination and diapedesis through the intestinal wall in case of inflammation^[4]. Unlike calprotectin, its stability at room temperature is guaranteed only for 2 d.

More recently, a meta-analysis confirmed the usefulness of C-reactive protein (CRP) and FC in excluding IBD in patients with symptoms of irritable bowel syndrome^[5]. Another meta-analysis showed that CRP, FC, and FL might aid in the triage of IBD patients for endoscopic evaluation when they are symptomatic^[6].

Currently, FC and FL do not replace endoscopy with histology, however this might become the future approach.

Still now, due to limited studies on the efficacies of FC and FL as non-invasive biomarkers, no consistent conclusion was made about their use in post-operative management of IBD patients. In this context, we aimed to review the efficacy of FC and FL from the available studies for use of FC and FL as non-invasive diagnostic markers of inflammation in post-operative CD and UC.

CD

CD is a chronic progressive destructive, disabling disease, clinically characterized by relapsing-remitting behavior. Even during periods of clinical remission the disease progresses, leading to structural and irreversible bowel damage in the majority of patients^[7]. In the natural history of CD, intestinal resection is often required to treat strictures, fistula, or abscesses. Historical population-based studies reported that, overall, the cumulative risk for surgery 10 years after diagnosis is around 40%-55%^[8]. There are emerging data suggesting that the early use of immune-modulators and biologics might delay disease progression and thus the timing of first surgical intervention. In an Australian population-based registry, authors observed indeed a fall in surgery rates (the one and 5 year resection rates were 13% and 23%, respectively)^[9]. Similarly, a Hungarian population based study showed that reduction of surgery rates was independently associated with early introduction of immune-modulators^[10].

Although, surgery is not curative and the disease often recurs in many cases (in the neoterminal ileum or in the ileo-colonic anastomosis), that leads to progressive loss of intestinal function and disability. Post-operative recurrence can be clinical, endoscopic, radiological or surgical. The reported incidence rates of post-operative recurrence depend on the definition used, the time of observation and the study design. Unfortunately, the available epidemiological data are heterogeneous and difficult to interpret. Buisson *et al*^[11] summarized the data coming from randomized controlled trials; referral centers studies and population-based studies. Clinical recurrence was higher in population-based studies and referral center studies, reaching 61% at 10 years. Data about endoscopic recurrence at one year derived mainly from referral center studies (rates ranging from 48% to 93%) and randomized controlled trials (rates ranging from 35% to 85%). However, the definition of endoscopic post-operative recurrence was heterogeneous.

The established risk factors for post-operative recurrence are smoking, prior intestinal resection, fistulizing behavior, perianal disease and extensive disease (> 50 cm)^[11]. It is fundamental to identify high-risk patients and offer efficient treatment in order to maintain remission. However, the most appropriate therapeutic approach has still to be established. At present, the only universally accepted preventive measure for post-

operative recurrence is to quit smoking^[12,13].

Landmark studies by Rutgeerts *et al*^[14,15] demonstrated that the post-operative clinical course of CD is predicted by the severity of endoscopic lesions during the first year after surgery. The presence of severe endoscopic lesions (Rutgeerts' score $\geq i2$) gives a high risk of early clinical relapse and complications^[14], but this can be observed already 6 mo after curative resection^[16].

As mentioned, detection of post-operative recurrence is mainly based on endoscopic appearance. Therefore, patients soon after surgery are expected to undergo endoscopy and repeat several evaluations, which timing is still need to be determined.

Fecal markers might be the most realistic alternative to ileocolonoscopy; their reliability as markers of intestinal inflammation has been proved in different settings and they are entering routine management. However, data about their use in the post-operative setting are poor.

Clinical and biochemical recurrence

The correlation between fecal markers and clinical and serological activity is controversial also in the post-operative setting.

We observed in 63 operated CD patients that levels of both FL and FC remained high after a median follow-up of 40.5 mo even in case of clinical remission, suggesting the persistence of subclinical inflammation^[17]. However, episodes of clinical flares predicted higher levels of FL. Only FL significantly correlated with CRP, showing a potential also as a marker of systemic inflammation. We investigated the correlation between FL levels and systemic inflammation in other 36 CD patients in clinical remission after ileo-colonic resection^[18], and demonstrated a significant correlation with IL-6 and CRP and an inverse correlation with albumin and serum iron. A major limitation of both studies was the absence of endoscopic evaluation to confirm endoscopic recurrence and its correlation with fecal markers.

Lamb *et al*^[19] followed-up a small cohort of 13 CD patients for one year after surgery with regular FC and FL measurements. In case of early normalization of these biomarkers (within two months), a subsequent two-fold increase in the upper limit of FC and FL correlated with a relapse. Both markers demonstrated better performance than CRP. The authors studied also a second post-operative cohort of 104 patients in a cross-sectional study. In this study, both FC and FL correlated significantly with the Harvey Bradshaw Index (HBI) of clinical activity; in particular, severely active patients ($HBI \geq 6$) had higher levels of fecal markers (more than twice the upper normal limit). However, surprisingly there was no significant difference between the FC and FL values in those with endoscopic post-operative recurrence (25 patients out of 43 patients who underwent endoscopic assessment) and those without.

Yamamoto *et al*^[20] prospectively investigated the relationship between the severity of endoscopic inflammation and fecal markers in 20 CD patients in remission during 6-12 mo after ileocolic resection. All patients underwent ileocolonoscopy at study entry and were then followed for 12 mo. Both fecal markers were significantly higher in patients (30%) who developed clinical recurrence. A cutoff value of 170 $\mu\text{g/g}$ for FC had 83% sensitivity and 93% specificity to predict a risk of clinical recurrence within 12 mo from the baseline endoscopy, while a cutoff value of 140 $\mu\text{g/g}$ for FL had a sensitivity of 67% and a specificity of 71%.

Endoscopic recurrence

The correlation of fecal markers with the presence of endoscopic recurrence should be the major endpoint in studies evaluating their role in post-operative recurrence. Orlando *et al*^[21] observed that amongst 50 CD patients who underwent intestinal resection a FC level $> 200 \text{ mg/L}$ had 63% sensitivity and 75% specificity to diagnose endoscopic post-operative recurrence one year after the operation.

In the study performed by Yamamoto *et al*^[20] both FC and FL correlated with the presence of endoscopic post-operative recurrence according to Rutgeerts' score. On the contrary, laboratory measurements (white blood cell count, platelet count and CRP level) did not significantly correlate with the endoscopic score.

A recent study performed in Sweden did not confirm the promising results of fecal markers in the post-operative setting proposed by the earlier studies^[22]. Authors evaluated the correlation between FC and the endoscopic findings one year after ileo-caecal resection in 30 CD patients; they observed that the median FC values did not significantly differ between patients in endoscopic remission or recurrence. However, most patients with low values were in remission and all patients with $FC > 600 \mu\text{g/g}$ had recurrence. The collection of the stool sample for FC measurement after colonoscopy might influence FC levels. This happened for only six patients, who collected the sample 1-4 wk after the endoscopy, and might not be sufficient to explain the absence of statistically significant difference between the groups of patients. Furthermore, the longitudinal part of the study, in which stool samples were delivered monthly until ileocolonoscopy, showed an important variability in FC concentrations. According to these findings, a single measurement of calprotectin might not be significant in the decision making process, and this was already demonstrated in the follow-up of patients undergoing anti-TNF treatment^[23].

Recently, results from the Post-Operative Crohn's Endoscopic Recurrence (POCER) Trial became available^[24] and data about the role of FC in monitoring and detecting post-operative recurrence were extracted^[25]. It is a prospective, randomized, controlled, multicenter trial, which evaluated a therapeutic strategy based on risk stratification of patients, with treatment step-up in case of recurrence detected at ileocolonoscopy, performed at

6 and 18 mo after surgery^[26]. This trial clarified that an active strategy based on the postoperative endoscopic monitoring, together with treatment intensification for early recurrence, is more effective (at least in the short term) than standard drug therapy alone and waiting for clinical recurrence. The surgically induced and verified remission (after resection of the macroscopically involved intestine) is an ideal starting point for the use of a noninvasive marker to monitor for recurrent inflammation. In the POCER trial FC concentration was increased markedly before surgery and decreased substantially after resection of all macroscopic diseased segments at 6 mo. Combined 6- and 18-mo FC levels correlated significantly with endoscopic recurrence, whereas CRP and CDAI did not. A cutoff of FC > 100 mg/g detected patients with endoscopic recurrence with 89% sensitivity and 58% specificity; the negative predictive value (NPV) was 91%. In this cohort, colonoscopy could be avoided in 47% of cases without endoscopic recurrence, but at the cost of missing 11% of patients with endoscopic recurrence. FC could be useful also in treatment monitoring, since it decreased in patients who underwent treatment intensification. A FC level of < 51 µg/g in patients in remission at 6 mo after surgery predicted maintenance of remission at 18 mo, with NPV 79%; sensitivity, specificity and PPV in this particular situation were less satisfying (50%, 68% and 36%, respectively), therefore the FC measurement remains of modest value in predicting long term future endoscopic recurrences.

Rapid test

Usually fecal markers are determined through the conventional ELISA method, that is effective, but time-consuming. A new rapid test for FC (FC-QPOCT) has been evaluated for the prediction of endoscopic remission in 115 CD patients^[27]. Twenty nine out of these patients were previously resected and endoscopic activity was scored according to the Rutgeerts' score. Median FC-QPOCT levels were able to discriminate between patients with and without endoscopic post-operative recurrence (98 µg/g vs 234.5 µg/g, respectively; $P = 0.012$). There was no significant difference in FC levels between the different degrees of the Rutgeerts' score. The accuracy of FC-QPOCT in predicting post-operative recurrence presented an AUC of 71.53. A 283 µg/g cut-off value had 67% sensitivity and 72% specificity (similar results were obtained with the ELISA method). However, accuracy was lower than that obtained in non-resected patients (AUC 0.933). Neither clinical activity nor serological biomarkers had a significant correlation with post-operative recurrence.

The validation of rapid fecal tests could be of further utility in the out-patient management of operated patients, avoiding the waiting time of laboratory reports.

Taken these results together, serial measurement of FC at regular intervals in the postoperative period might be the best way to predict future endoscopic behavior^[25,28].

It has been proposed that an algorithm combining FC and colonoscopy, based on the stratification of patients according to the risk of permanent bowel dysfunction, could be a cost-effective strategy to detect asymptomatic recurrence^[28]. This approach need further validation in larger, prospective trials, but might be a cost-effective strategy for the management of operated CD patients.

Results of the major studies in CD patients are reported in Table 1.

UC

The clinical course of UC may range from prolonged periods of remission to acute severe colitis requiring intensive medical treatment. Emergency colectomies are required in case of life-threatening complications of colitis in hospitalized patients unresponsive to medical treatment. Elective colectomy is indicated for refractory disease, intolerance to medical treatment and colonic neoplasia.

Surgery rates at 10 years from diagnosis are approximately 10%^[29,30], showing a decline over the years for elective colectomies (probably due to immunomodulators)^[31]; in contrast, emergent colectomy rates remain stable. Extensive colitis at diagnosis is proposed as a risk factor for colectomy in several studies across different cohorts of patients^[29,30].

Proctocolectomy with ileal pouch-anal anastomosis (IPAA) is the procedure of choice for most patients with UC requiring colectomy. Pouchitis is a non-specific inflammation of the ileal reservoir and the most common complication of IPAA in patients with UC^[32,33]. The incidence of a first episode of pouchitis depends on the duration of follow up, occurring in up to 45% of patients 10 years after surgery^[34,35]. Pouchitis recurs in more than 50% patients and up to 10% of patients develop chronic pouchitis; refractory pouchitis is rare^[33]. Increased bowel movements, urgency, and abdominal pain in patients with IPAA may be caused by different inflammatory conditions (pouchitis, cuffitis, or CD) or non-inflammatory conditions (irritable pouch syndrome). The diagnosis of pouchitis requires therefore endoscopic confirmation with mucosal biopsies^[36,37]. The Pouchitis Disease Activity Index (PDAI) was developed to standardize diagnostic criteria and assess the severity of pouchitis, combining symptoms, endoscopy and histology^[36]; a total PDAI score ≥ 7 is diagnostic for pouchitis. Patients with suspected pouchitis need endoscopy for a proper diagnosis. The use of fecal markers for the detection of pouch inflammation might avoid the repetition of such invasive investigation^[38].

The literature on fecal markers in the post-operative setting in UC patients is quite scarce. Furthermore, the majority of studies was conducted on small samples of patients and have a cross-sectional design, which do not permit to clarify the evolution over time of the disease and the consensual behavior of the fecal markers. However, these studies form the basis of evidence that FC and FL are useful as inflammatory markers also in

Table 1 Studies evaluating fecal calprotectin and lactoferrin in operated Crohn's disease patients

Ref.	FC/FL	Method	No. of patients	Aim	Best cut-off	Sens %	Spec %	Main findings
Scarpa <i>et al</i> ^[17]	FC and FL	ELISA	63 (22 endoscopy)	Role as marker of intestinal inflammation after ileocolonic resection	/	/	/	High FC and FL levels at long-term follow-up after resection even in case of clinical remission Correlation between FL and CRP Higher levels of FL in case of clinical recurrence
Ruffolo <i>et al</i> ^[18]	FL	ELISA (IBD-scan)	36	Correlation with systemic inflammation and prognostic value in terms of need of surgery for recurrence	/	/	/	FL as expression of subclinical intestinal inflammation (through IL6-CRP cascade)
Lamb <i>et al</i> ^[19]	FC and FL	ELISA (PhiCal) ELISA (IBD-Scan)	13 (prospective cohort) 104 (cross-sectional cohort; 43 endoscopy)	Evaluation of the course of FL and FC after ileocaecal resection. Identification of postoperative recurrence; Correlation between FC and FL	/	/	/	Prospective cohort: Normalization of fecal markers by 2 mo after surgery in uncomplicated patients Cross-sectional cohort: Significant correlation between FC and FL Significant correlation of fecal markers with HBI No significant difference between the FC and FL values in those with endoscopic recurrence and those without
Yamamoto <i>et al</i> ^[20]	FC and FL	ELISA (Cell Science) and Colloidal Gold Agglutination reagent (Auto Lf-Plus, respectively)	20	Evaluation of the relationship between endoscopic activity and FC/FL Assessment of FC and FL predictive value for future clinical recurrence	FC 170 mg/g FL 140 mg/g (for prediction of clinical relapse)	83 67	93 71	Significant correlation between FC and FL Correlation with endoscopic activity Ability to predict clinical post-operative recurrence
Orlando <i>et al</i> ^[21]	FC	ELISA	50 (39 endoscopy)	Evaluation of the one year postsurgical endoscopic recurrence	200 mg/L	63	75	FC > 200 mg can be an indication to colonoscopy in patients with negative ultrasound in order to detect early recurrence
Lasson <i>et al</i> ^[22]	FC	ELISA (Buhlmann)	30	Correlation of FC with the endoscopic findings one year after ileocaecal resection Evaluation of the variation of FC in individual patients during 6 mo prior to the ileocolonoscopy	/	/	/	No difference in the concentrations of FC between patients in endoscopic remission and patients with recurrence one year after ileocaecal resection Significant variability of FC concentrations over time
Wright <i>et al</i> ^[25]	FC	ELISA (fCAL, Buhlmann)	135 (319 fecal samples)	To assess whether monitoring FC can substitute endoscopy and be used as surrogate marker of recurrent post-operative disease	100 µg/g	89	58	FC correlated with the presence of recurrent disease at endoscopy and with endoscopic severity FC has sufficient sensitivity and negative predictive values to monitor for recurrence FC can be used to monitor response to treatment after detection of recurrence FC has better diagnostic performance than CRP and clinical index of activity
Lobaton <i>et al</i> ^[27]	FC	ELISA (Buhlmann) FC-QPOCT (Quantum Blue)	115 (29 resected)	To evaluate the performance of a new rapid test for FC in predicting endoscopic remission (in both operated and non-operated CD patients)	283 µg/g	67	72	Significant correlation between ELISA and rapid test FC was able to discriminate between the presence or absence of endoscopic recurrence, but not distinguish different levels of severity

CD: Crohn's disease; CRP: C-reactive protein; ELISA: Enzyme-linked immunoassay; FC: Fecal calprotectin; FL: Fecal lactoferrin; HBI: Harvey Bradshaw Index; Sens: Sensitivity; Spec: Specificity.

the post-operative setting.

In 24 patients with ileo-anal pouch (both UC patients and with familial adenomatous polyposis) FC showed a strong association with pouchitis ($P = 0.0002$), and correlated with the severity of inflammation detected at endoscopy and histology in the 9 patients having pouchitis^[39]. A cut-off of 92.5 µg/g feces in 54 patients who underwent restorative proctocolectomy (46 UC patients and 8 with familial adenomatous polyposis coli) reached 90% sensitivity and 76.5% specificity in diagnosing pouchitis^[40]. No difference was found in symptom scores of patients with FC concentrations above or below 50 µg/g ($P = 0.155$), confirming that the clinical presentation is aspecific.

Thirty-two patients with pediatric-onset of UC who underwent proctocolectomy with IPAA were enrolled in a cross-sectional study to assess whether FC was related to pouchitis^[41]. Patients with recurrent pouchitis had significantly higher FC levels (832 ± 422 µg/g) followed by those with a single episode (290 ± 131 µg/g) and those with no history of pouchitis (71 ± 50 µg/g) ($P = 0.019$). FC levels correlated also with the amount of neutrophilic infiltration of the distal ileum at histology. The cross-sectional design of the study and the small sample size are of course important limits of the study.

Also FL showed satisfactory results in detecting pouch inflammation (due to either pouchitis, cuffitis or CD). In 60 patients with IPAA, a cut-off of 13 µg/mL could distinguish irritable pouch syndrome from pouchitis, cuffitis, or CD, with 97% sensitivity and 92% specificity^[42]. FL levels correlated with the PDAI score (correlation coefficient 0.73; $P < 0.001$), especially with the endoscopic subscore. Although the cut-off level of 13 µg/mL showed the best combination of specificity and sensitivity, authors recommended a cutoff level of 7 µg/mL to decrease the possibility of false negative results. In case of higher levels, pouch endoscopy with biopsy is necessary to distinguish among different causes of inflammation. Lim *et al*^[43] achieved similar results in 2008, evaluating the levels of FL in 32 patients with IPAA, showing 100% sensitivity and 86% specificity in diagnosing pouchitis, according to PDAI.

We evaluated the interplay between the ileal-pouch microbiota and several inflammatory parameters in the pathogenesis of pouchitis in 32 consecutive patients^[44]. Although it was not the primary aim of the study, we observed that FL correlated with the presence of mucosal ulcers, neutrophils and monocytes infiltration and the histologic diagnosis of pouchitis, confirming the ability of the fecal marker in detecting mucosal inflammation.

Recently, Yamamoto *et al*^[45] conducted a longitudinal study to assess the utility of sequential dosage of FC and FL for the early diagnosis and prediction of pouchitis after restorative proctocolectomy for UC. Sixty patients were followed up (with clinical and biochemical assessments) every 2 mo for one year after the ileostomy closure. In case of symptoms suggestive of pouchitis, endoscopic examination was

immediately undertaken; otherwise, asymptomatic patients performed endoscopy at one year. Between 4 and 10 mo before the diagnosis of pouchitis (10 patients, 17%), the median FC and FL levels remained low and stable. However, these levels significantly increased 2 mo before the diagnosis of pouchitis, although patients were asymptomatic. In contrast, in 50 patients without pouchitis fecal levels did not change. In particular, a cut-off value of 56 µg/g for FC had a NPV of 100% and a diagnostic accuracy of 87% to predict pouchitis; a cut-off value of 50 µg/g for FL had a NPV of 98% and a diagnostic accuracy of 88% to predict pouchitis. Again, there was no significant correlation between the clinical subscore of PDAI and fecal biomarkers (FC: $r = 0.230$, $P = 0.08$ and FL: $r = 0.163$, $P = 0.21$); on the contrary, both fecal markers correlated with the endoscopic and histological subscores. In patients with pouchitis who responded to antibiotics (8/10) median FC levels dropped from 106 to 34 µg/g and FL levels from 89 to 31 µg/g; in non-responders the levels of these fecal biomarkers increased, suggesting their usefulness for evaluating the efficacy of medical treatment and possibly for the early detection of pouch inflammation without repeating endoscopy.

In summary, fecal proteins demonstrated the potential to monitor intestinal inflammation in UC patients after proctocolectomy with IPAA. The early detection of subclinical inflammation with serial measurements of fecal markers might facilitate pre-emptive treatments in asymptomatic patients. Prospective studies need to confirm the cost-effectiveness of such strategy, especially evaluating the reduction of rates of chronic pouchitis and pouch failures^[46].

Results of the major studies in UC patients are reported in Table 2.

CONCLUSION

The role of fecal markers in the post-operative management of IBD patients seems promising. Preliminary data in CD patients came from small studies, sometimes relying only on clinical activity, without endoscopic confirmation of recurrence, and produced inconsistent data. More recently, studies have revealed the potential use of fecal markers, especially FC, in the post-operative management of CD, for the diagnosis of post-operative recurrence and possibly for monitoring the response to therapy. In UC patients, studies, although heterogeneous, have more consistently showed the correlation between fecal markers and the presence of inflammation of the pouch. Furthermore, there are no data showing that the early diagnosis of post-operative recurrence in CD patients and of pouchitis in UC patients might alter the long term outcome. The evidence of the reliability of FC and FL as markers of inflammation in the post-operative setting in both CD and UC should be strengthened in larger, longitudinal, multicentric studies, addressing the aim to refine an algorithm that stratifies the use and the optimal timing of fecal markers testing

Table 2 Studies evaluating fecal calprotectin and lactoferrin in operated ulcerative colitis patients

Ref.	FC/FL	Method	No. of patients (No. of patients with inflammation of the pouch) and type of disease	Aim	Best cut-off	Sens %	Spec %	Main findings
Thomas <i>et al</i> ^[39]	FC	ELISA	24 (9) UC and familial polyposis coli	Comparison between single and 24-h stool collections in patients with and without pouchitis (endoscopic, histologic and immunohistochemical indexes)	/	/	/	Mean first morning stool concentration correlated with 24-h collection Levels of FC were significantly higher in patients with pouchitis Correlation with % of mature granulocytes and activated macrophages
Johnson <i>et al</i> ^[40]	FC	ELISA (PhiCal)	54 (20) UC and familial polyposis coli	Differentiation between inflamed and noninflamed pouches Correlation with inflammation severity	92.5 µg/g	90%	76.50%	FC levels significantly higher in pouchitis ($> 50 \mu\text{g/g}$ had higher endoscopic and histological scores) Correlation with endoscopic score ($r = 0.605$) and histological score ($r = 0.708$)
Pakarinen <i>et al</i> ^[41]	FC	ELISA (PhiCal)	32 (22) UC	Cross-sectional assessment of FC after proctocolectomy for pediatric onset UC	300 µg/g (for detection of recurrent pouchitis)	57%	92%	Higher levels of FC in patients with recurrent pouchitis, followed by those with a single episode and those without (832, 290, 71 µg/g respectively, $P =$ 0.019) Correlation with neutrophilic infiltration and overall inflammatory activity in the distal ileum
Parsi <i>et al</i> ^[42]	FL	In-house test	60 (30) UC	Evaluate the usefulness of FL in symptomatic patients with IPAA	13 µg/mL	97%	92%	Higher levels in patients with inflammation of the pouch Not able to distinguish between pouchitis, cuffitis and CD Not able to distinguish between asymptomatic patients and those with irritable pouch syndrome Correlation with PDAI (better for endoscopic subscore)
Lim <i>et al</i> ^[43]	FL	Rapid immuno- chromatographic test	32 (11) Healthy controls and pouchitis patients	Diagnostic yield for pouchitis	/	100%	86%	Sensitive method for the non- invasive diagnosis of pouchitis
Scarpa <i>et al</i> ^[44]	FL	ELISA (IBD-scan)	32 UC	Evaluate the relationship between ileal-pouch microbiota and inflammatory parameters	/	/	/	Correlation with histological inflammation Correlation with mucosal ulcers, mucosal immune infiltration Inverse correlation with <i>Eubacteriaceae</i> spp., <i>Burkholderiaceae</i> spp and <i>Moraxellaceae</i> spp. counts
Yamamoto <i>et al</i> ^[45]	FC FL	ELISA (Cell Science) and Colloidal Gold Agglutination reagent (Auto Lf-Plus, respectively)	60 (10) UC	Evaluate the significance of consecutive monitoring of fecal markers for early diagnosis and prediction of pouchitis	56 µg/g 50 µg/g	100% 90%	84% 86%	Elevation of FC and FL already 2 mo before the diagnosis of pouchitis Correlation with PDAI score (correlation with endoscopic and histological subscores, but not with the clinical subscore) Correlation with response to therapy

CD: Crohn's disease; CRP: C-reactive protein; ELISA: Enzyme-linked immunoassay; FC: Fecal calprotectin; FL: Fecal lactoferrin; PDAI: Pouch disease activity index; Sens: Sensitivity; Spec: Specificity; UC: Ulcerative colitis.

and the effective need of colonoscopy. This should

be based on patients-tailored approach, in order to

improve the cost-effectiveness of several postoperative fecal testing and examine the ability of such a strategy to prevent both clinical relapse and subsequent surgical resections in CD patients and the early identification with prompt treatment of pouchitis in UC patients.

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