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

Roberta Caccaro, Imerio Angriman, Renata D’Incà

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

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.
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.

Caccaro R, Angriman I, D’Incà R. Relevance of fecal calprotectin and lactoferrin in the post-operative management of inflammatory bowel diseases. World J Gastrointest Surg 2016; 8(3): 193-201 Available from: URL: http://www.wjgnet.com/1948-9366/full/v8/i3/193.htm DOI: http://dx.doi.org/10.4240/wjgs.v8.i3.193

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)[10]. 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 ≥ 12) 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 maker 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 ≥ 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 μg/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 μg/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 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 μg/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
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. This approach needs 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.[24,25] 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;[35] a total PDAI score $\geq 7$ 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 investigations.[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

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’s score. Median FC-QPOCT levels were able to discriminate between patients with and without endoscopic post-operative remission (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’s 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]
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 et al[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 et al[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 et al[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 et al[20] | FC and FL 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      | 93      | Significant correlation between FC and FL. Correlation with endoscopic activity. Ability to predict clinical post-operative recurrence. |
| Orlando et al[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 et al[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 et al[23] | FC ELISA (ICAL, Buhlmann) FC-QPOCT (Quantum Blue)  | 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. 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. |
| Lobaton et al[24] | 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 \( \mu g/g \) 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 \( \mu g/g \) \( (P = 0.155) \), 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 \( (832 \pm 422 \mu g/g) \) followed by those with a single episode \( (290 \pm 131 \mu g/g) \) and those with no history of pouchitis \( (71 \pm 50 \mu g/g) \) \( (P = 0.019) \). FC levels correlated also with the amount of neutrophil 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 \( \mu g/mL \) could distinguish irritable pouch syndrome from pouchitis, cuffitis, or CD, with 97% sensitivity and 92% specificity\(^{[42]}\). FL levels correlated with the PDAI score \( (\text{correlation coefficient} 0.73; P < 0.001) \), especially with the endoscopic subscore. Although the cut-off level of 13 \( \mu g/mL \) showed the best combination of specificity and sensitivity, authors recommended a cutoff level of 7 \( \mu g/mL \) 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 \( \mu g/g \) for FC had a NPV of 100% and a diagnostic accuracy of 87% to predict pouchitis; a cut-off value of 50 \( \mu g/g \) 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 \( (FC: r = 0.230, P = 0.08 \text{ and } FL: r = 0.163, P = 0.21) \); 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 \( \mu g/g \) and FL levels from 89 to 31 \( \mu g/g \); 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
be based on patients-tailored approach, in order to

| 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 et al[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 et al[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 μg/g had higher endoscopic and histological scores). Correlation with endoscopic score (r = 0.605) and histological score (r = 0.708) |
| Pakarinen et al[41] | FC     | ELISA (PhiCal)       | 32 (22) UC                                                                        | Cross-sectional assessment of FC after proctocolectomy for pediatric onset UC | 300 μg/g     | 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 et al[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). Sensitive method for the non-invasive diagnosis of pouchitis. |
| Lim et al[43]      | FL      | Rapid immunochromatographic test | 32 (11) Healthy controls and pouchitis patients | Diagnostic yield for pouchitis                                           | /            | 100%   | 86%    | Correlation with histological inflammation. Correlation with mucosal ulcers, mucosal immune infiltration. Inverse correlation with Eubacteriaceae spp., Burkholderiaceae spp and Moraxellaceae spp. counts. |
| Scarpa et al[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 Eubacteriaceae spp., Burkholderiaceae spp and Moraxellaceae spp. counts. |
| Yamamoto et al[45] | FC      | 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     | 100%   | 84%    | 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.
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.

ACKNOWLEDGMENTS

We thank Dr. Surajit Pathak for his careful English language revision of the manuscript.

REFERENCES

1 Caccaro R, D’Incà R, Sturmiolo GC. Clinical utility of calprotectin and lactoferrin as markers of inflammation in patients with inflammatory bowel disease. Expert Rev Clin Immunol 2010; 6: 551-558 [PMID: 20594128 DOI: 10.1586/eci.10.26]
2 Caccaro R, D’Incà R, Pathak S, Sturmiolo GC. Clinical utility of calprotectin and lactoferrin in patients with inflammatory bowel disease: is there something new from the literature? Expert Rev Clin Immunol 2012; 8: 579-585 [PMID: 22992152]
3 Tlibble JA, Bjarmason I. Non-invasive investigation of inflammatory bowel disease. World J Gastroenterol 2001; 7: 460-465 [PMID: 11819911 DOI: 10.3748/wjg.v7.i4.460]
4 Oesas R, Yang JH, Bathum RL, Boxer LA. Lactoferrin: a promoter of polymorphonuclear leukocyte adhesiveness. Blood 1981; 57: 939-945 [PMID: 7214024]
5 Menees SB, Powell C, Kurlander J, Goel A, Chey WD. A meta-analysis of the utility of C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and fecal lactoferrin to exclude inflammatory bowel disease in adults with IBS. Am J Gastroenterol 2015; 110: 444-454 [PMID: 25732419 DOI: 10.1038/ajg.2015.6]
6 Mosli MH, Zoua G, Garg SG, Feagan BG, MacDonald JK, Chands N, Sandborn WJ, Feagan BG. C-Reactive Protein, Fecal Calprotectin, and Stool Lactoferrin for Detection of Endoscopic Activity in Symptomatic Inflammatory Bowel Disease Patients: A Systematic Review and Meta-Analysis. Am J Gastroenterol 2015; 110: 802-819; quiz 820 [PMID: 25964225 DOI: 10.1038/ajg.2015.120]
7 Pariente B, Cosnes J, Danese S, Sandborn WJ, Lewin M, Fletcher JG, Chowers Y, D’Haens G, Feagan BG, Biber T, Hommes DW, Irvine EJ, Kamm MA, Loftus EV, Louis E, Michetti P, Mullin XM, Oresland T, Pandol JP, Peyrin-Biroulet L, Reinish W, Sands BE, Scolmerich J, Schreiber S, Tilg H, Travis S, van Assche G, Vecchi M, Mary JY, Colombel JF, Lennm M. Development of the Crohn’s disease digestive damage score, the Lennm score. Inflamm Bowel Dis 2011; 17: 1415-1422 [PMID: 21560202 DOI: 10.1002/ibd.21506]
8 Peyrin-Biroulet L, Loftus EV, Colombel JF, Sandborn WJ. The natural history of adult Crohn’s disease in population-based cohorts. Am J Gastroenterol 2010; 105: 289-297 [PMID: 19861953 DOI: 10.1038/ajg.2009.579]
9 Niwiadomski O, Studd C, Hair C, Wilson J, Ding NS, Heerasing N, Ting A, McNeill J, Knight R, Santamaria J, Prewett E, Dabkowski N, Plebani M, Sturniolo GC, D’Amico DF, Angirn J. Fecal lactoferrin and lactoferrin as markers for monitoring disease activity and predicting clinical recurrence in Crohn’s disease. Br J Surg 2009; 96: 663-674 [PMID: 19384912 DOI: 10.1002/bjs.6593]
10 Yamamoto T, Shiraki M, Bauma T, Umegae S, Matsumoto K. Fecal calprotectin and lactoferrin as markers for monitoring disease activity and predicting clinical recurrence in patients with Crohn’s disease after ileocolonic resection: a smokers fire. J Gastrointest Surg 2010; 14: 24-31 [PMID: 19962313 DOI: 10.1007/s11605-009-1070-9]
11 Lamb CA, Moolhuddin MK, Gicquel J, Neely D, Bergin FG, Hanson JM, Mansfield JC. Fecal calprotectin or lactoferrin can identify postoperative recurrence in Crohn’s disease. Br J Surg 2009; 96: 663-674 [PMID: 19384912 DOI: 10.1002/bjs.6593]
12 Ruffolo C, Scarpa M, Faggian D, Basso D, D’Incà R, Plebani M, Sturmiolo GC, Bassi N, Angirn A. Subclinical intestinal inflammation in patients with Crohn’s disease following bowel resection: a smoldering fire. J Gastrointest Surg 2010; 14: 24-31 [PMID: 19962313 DOI: 10.1007/s11605-009-1070-9]
13 Lamb CA, Moolhuddin MK, Gicquel J, Neely D, Bergin FG, Hanson JM, Mansfield JC. Fecal calprotectin or lactoferrin can identify postoperative recurrence in Crohn’s disease. Br J Surg 2009; 96: 663-674 [PMID: 19384912 DOI: 10.1002/bjs.6593]
14 Yamamoto T, Shiraki M, Bauma T, Umegae S, Matsumoto K. Fecal calprotectin and lactoferrin as markers for monitoring disease activity and predicting clinical recurrence in patients with Crohn’s disease after ileocolonic resection: a prospective pilot study. United Eur Fnd Gastroenterol J 2013; 1: 366-374 [PMID: 24917985 DOI: 10.1586/2050640613501818]
15 Orlando A, Modesto I, Castiglione F, Scala L, Scimmea D, Rispo A, Teresi S, Mocciaro F, Criscuoli V, Marrone C, Platania P, De Falco T, Maisano S, Nicoli N, Cottone M. The role of calprotectin in predicting endoscopic post-surgical recurrence in asymptomatic Crohn’s disease: a comparison with ultrasound. Eur Rev Med Pharmacol Sci 2006; 10: 17-22 [PMID: 16944106]
16 Lessan A, Strid H, Ohman L, Isaksson O, Olsson M, Rydström B, Ung KA, Stotzer PO. Fecal calprotectin one year after ileocecal resection for Crohn’s disease—a comparison with findings at ileocolonoscopy. J Crohns Colitis 2018; 4: 789-795 [PMID: 24418661 DOI: 10.1016/j.crohns.2013.12.015]
17 De Vos M, Louis EJ, Janshen J, Vanzendoort J, Noman M, Dewit D, d’Haens GR, Franchimont D, Baert F, Torp RA, Henrikssen M, Potvin PM, Van Houtegem PP, Hendryckx PM, Moreels TG, Collard A, Karlsen LN, Kattig E, Lambrecht G, Grimstad T, Koch J, Lygren I, Coche JC, Mana F, Van Gossuin A, Belaiche J, Cool MR, Fontaine F, Maisin JM, Muls V, Neuville B, Staesens DA, Van Assche GA, de Lange T, Solberg JC, Vander Cruysen BJ, Vermeire SA. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. Inflamm Bowel Dis 2013; 19: 2111-2117 [PMID: 23883959 DOI: 10.1097/MIB.0b013e318292ca37]
18 De Cruz P, Kamm MA, Hamilton AL, Ritchie KJ, Krejany EO, Gorelik L, Liew D, Prideaux L, Lawrance IC, Andrews JM,
Bampton PA, Gibson PR, Sparrow M, Leong RW, Florin TH, Geary RB, Radford-Smith G, Macrae FA, Debinski H, Selby W, Kronborg I, Johnston MJ, Woods R, Elliott PR, Bell SJ, Brown SJ, Connell WR, Desmond PV. Crohn’s disease management after intestinal resection: a randomised trial. *Lancet* 2015; 385: 1406-1417 [PMID: 25542620 DOI: 10.1016/S0140-6736(14)61908-5]

Wright EK, Kamm MA, De Cruz P, Hamilton AL, Ritchie KJ, Krejany EO, Leach S, Gorelik A, Liew D, Prideaux L, Lawrance IC, Andrews JM, Bampton PA, Jakobovits SL, Florin TH, Gibson PR, Debinski H, Macrae FA, Samuel D, Bampton I, Radford-Smith G, Selby W, Johnston MJ, Woods R, Elliott PR, Bell SJ, Brown SJ, Connell WR, Day AS, Desmond PV, Geary RB. Measurement of fecal calprotectin improves monitoring and detection of recurrence of Crohn’s disease after surgery. *Gastroenterology* 2015; 148: 938-947.e1 [PMID: 25620670 DOI: 10.1053/j.gastro.2015.01.026]

Vuitton L, Peyrin-Biroulet L. The POCER Trial: Bet on Active Care. *Gastroenterology* 2015; 148: 1474-1475 [PMID: 25935253 DOI: 10.1053/j.gastro.2015.04.034]

Lobatón T, López-Garcia A, Rodriguez-Moranta F, Ruiz A, Rodríguez L, Guadiola J. A new rapid test for fecal calprotectin predicts endoscopic remission and postoperative recurrence in Crohn’s disease. *J Crohns Colitis* 2013; 7: e641-e651 [PMID: 23810085 DOI: 10.1002/jc.20315.05.005]

Schoepfer AM, Lewis JD. Serial fecal calprotectin measurements to detect endoscopic recurrence in postoperative Crohn’s disease: is colonoscopic surveillance no longer needed? *Gastroenterology* 2015; 148: 889-892 [PMID: 25805423 DOI: 10.1053/j.gastro.2015.03.022]

Monstad I, Hovde O, Solberg IC, A Moum B. Clinical course and prognosis in ulcerative colitis: results from population-based and observational studies. *Ann Gastroenterol* 2014; 27: 95-104 [PMID: 24733670]

Bernstein CN, Ng SC, Lakatos PL, Moum B, Loftus EV. A review of mortality and surgery in ulcerative colitis: milestones of the seriousness of the disease. *Inflamm Bowel Dis* 2013; 19: 2001-2010 [PMID: 23624887 DOI: 10.1097/MIB.0b013e318281f3b6]

Kaplan GG, Seow CH, Ghosh S, Molodecky N, Rezaie A, Moran GW, Proulx MC, Hubbard J, MacLean A, Buie D, Panaccione R. Decreasing colectomy rates for ulcerative colitis: a population-based time trend study. *Am J Gastroenterol* 2012; 107: 1879-1887 [PMID: 23165448 DOI: 10.1038/ajg.2012.333]

Cvancarova M, Solberg IC, Vatn M, Moum D. Risk matrix model for prediction of colectomy in a population based study of ulcerative colitis patients. The IBSEN study. *Gut* 2010; 59: A36

Van Assche G, Dignass A, Bokemeyer B, Danese S, Gionchetti P, Moser G, Beaugerie L, Gomollon F, Häuser W, Herrlinger K, Oldenburg B, Panes J, Portela F, Rogler G, Stein J, Tilg H, Travis S, Lindsay JO. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis part 3: special situations. *J Crohns Colitis* 2013; 7: 1-33 [PMID: 23040453 DOI: 10.1016/j.jcjo.2012.09.005]

Mahadevan U, Sandborn WJ. Diagnosis and management of pouchitis. *Gastroenterology* 2003; 124: 1636-1650 [PMID: 12761722]

Penna C, Dozios R, Tremaine W, Sandborn W, LaRusso N, Schleck C, Ilstrup D. Pouchitis after ileal pouch-anal anastomosis for ulcerative colitis occurs with increased frequency in patients with associated primary sclerosing cholangitis. *Gut* 1996; 38: 234-239 [PMID: 8801203]

Shen B, Achkar JP, Lashner BA, Ormsby AH, Remzi FH, Bevins CL, Brzezinski A, Petras RE, Fazio VW. Endoscopic and histologic evaluation together with symptom assessment are required to diagnose pouchitis. *Gastroenterology* 2001; 121: 261-267 [PMID: 11487535]

Pardi DS, Shen B. Endoscopy in the management of patients after ileal pouch surgery for ulcerative colitis. *Endoscopy* 2008; 40: 529-533 [PMID: 18464195 DOI: 10.1055/s-2007-995784]

Navaneethan U, Shen B. Laboratory tests for patients with ileal pouch-anal anastomosis: clinical utility in predicting, diagnosing, and monitoring pouch disorders. *Am J Gastroenterol* 2009; 104: 2606-2615 [PMID: 19603012 DOI: 10.1038/ajg.2009.392]

Thomas P, Rihani H, Roseth A, Sigthorson G, Price A, Nicholls RJ, Bjarnason I. Assessment of ileal pouch inflammation by single-stool calprotectin assay. *Dis Colon Rectum* 2000; 43: 214-220 [PMID: 10696896]

Johnson MW, Maestranzi S, Duffy AM, Dewart DH, Forbes A, Bjarnason I, Sherwood RA, Cichlira P, Nicholls JR. Fecal calprotectin: a noninvasive diagnostic tool and marker of severity in pouchitis. *Eur J Gastroenterol Hepatol* 2008; 20: 174-179 [PMID: 18301296 DOI: 10.1097/MEG.0b013e3282f1c9a7]

Pakarinen MP, Koivusalo A, Natunen J, Ashorn M, Karikoski R, Aitola P, Rintala RJ, Kolho KL. Fecal calprotectin monitors inflammation of the distal ileum and bowel function after restorative proctocolectomy for pediatric-onset ulcerative colitis. *Inflamm Bowel Dis* 2010; 16: 482-486 [PMID: 19685453 DOI: 10.1002/ibd.21069]

 Parsi MA, Shen B, Achkar JP, Remzi FF, Goldblum JR, Boone J, Lin D, Connor JT, Fazio VW, Lashner BA. Fecal lactoferrin for diagnosis of symptomatic patients with ileal pouch-anal anastomosis. *Gastroenterology* 2004; 128: 1280-1286 [PMID: 15131788]

Lim M, Gonsalves S, Thekkinkattil D, Seedat S, Finan P, Sagar P, Burke D. The assessment of a rapid noninvasive immunochromatographic assay test for fecal lactoferrin in patients with suspected inflammation of the ileal pouch. *Dis Colon Rectum* 2008; 51: 96-99 [PMID: 18085334]

Scarpa M, Grillo A, Faggian D, Ruffolo C, Bonello E, D’Inca R, Scarpa M, Castagliuolo I, Angriman I. Relationship between mucosa-associated microbiota and inflammatory parameters in the ileal pouch after restorative proctocolectomy for ulcerative colitis. Surgery 2011; 150: 56-67 [PMID: 21549404 DOI: 10.1016/j.surg.2011.02.009]

Yamamoto T, Shiomaya T, Bamba T, Matsamoto K. Consecutive Monitoring of Fecal Calprotectin and Lactoferrin for the Early Diagnosis and Prediction of Pouchitis after Restorative Proctocolectomy for Ulcerative Colitis. *Am J Gastroenterol* 2015; 110: 881-887 [PMID: 25916224 DOI: 10.1038/ajg.2015.129]

Schoepfer A, Reinsch W. Serial Fecal Calprotectin and Lactoferrin Measurements for Early Diagnosis of Pouchitis After Proctocolectomy for Ulcerative Colitis: Is Pouchoscopy No Longer Needed? *Am J Gastroenterol* 2015; 110: 888-890 [PMID: 26052770 DOI: 10.1038/ajg.2015.170]
