VIEWPOINT

Concept for a rapid point-of-care calprotectin diagnostic test for diagnosis and disease activity monitoring in patients with inflammatory bowel disease: Expert clinical opinion

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1. Introduction

Inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn’s disease (CD) are organic inflammatory diseases, caused by chronic mucosal inflammation of the intestine. They are typically characterized by variable disease activity, often with repeated periods of intermittent disease activity and remission. Irritable bowel syndrome (IBS) is a non-inflammatory functional disorder and, by definition, presumes the absence of organic disease. As the presenting manifestations of IBD and IBS are similar and can include diarrhea, abdominal pain and bloating, obtaining a clinical diagnosis can be difficult, and further invasive diagnostic procedures may be required in order to obtain a confirmed diagnosis.

Treatment strategies based on presenting clinical manifestations have failed to modify the course of IBD. Indeed clinical disease activity indices are non-specific and do not correlate with endoscopic activity.

Mucosal healing has emerged as a new therapeutic goal in clinical practice and is regarded as a major endpoint definition for remission of IBD in clinical trials, due to its association with improved outcomes in IBD, including reduced relapse and hospitalization rates, and reduced need for surgery. C-reactive protein (CRP) is a non-specific marker that reflects systemic inflammation and is useful for the detection of complications (such as abscesses) or extraintestinal manifestations. However, it is not an accurate marker of mucosal healing.

There is currently an unmet medical need for a suitable non-invasive biomarker of mucosal inflammation, arising primarily because of the inherent problems associated with endoscopy, which is an invasive and expensive procedure.

Although cross-sectional imaging methods such as MRI and ultrasound are used for assessing disease activity in patients with UC and CD, there is still room for the use of fecal markers to evaluate mucosal lesions in clinical practice. In addition, a non-invasive test might be used to anticipate flares in IBD activity, which would allow for pre-emptive escalation of treatment. The ideal marker should be specific and sensitive, inexpensive, yield rapid results, which could be available during the patient visit, and have a high negative predictive value.

This article reports the outcomes of a recent meeting of a panel of gastroenterologists with expertise in IBS/IBD and fecal biomarkers to discuss the development of a rapid point-of-care semi-quantitative test to enable discrimination of IBD from IBS, and subsequent disease activity monitoring in patients with IBD.

2. Materials and methods

In April 2012, 11 gastroenterologists with expertise in IBS/IBD and special interest in fecal biomarkers met in Zürich, Switzerland to discuss the potential for development of a rapid point-of-care, semi-quantitative test. The meeting focused on the following main questions:

1. What is the rationale for choosing fecal calprotectin as the most suitable IBD biomarker for the test, compared with other fecal markers such as lactoferrin or M2-pyruvate kinase (M2-PK)?

2. What would be the agreed number and cut-off calprotectin level values for various clinical settings?

3. Would it be useful to have different cut-off levels for UC and CD?

4. Could patients with IBD tailor their treatment to allow earlier escalation of their treatments to prevent a relapse of their disease or, alternatively, reduce their treatment if their disease was known to be in remission as indicated by the results of their fecal marker rapid point-of-care test?

Finally, the expert panel discussed the next steps in terms of a clinical study concept to validate agreed consensus calprotectin cut-off levels.

3. Results

A non-invasive, point-of-care diagnostic test would be of interest in order to reduce the number of invasive and expensive endoscopies, which are undertaken to diagnose suspected IBD, differentiate UC from CD, and determine the extent and severity of mucosal inflammation.

3.1. Candidate markers for a rapid point-of-care diagnostic test

Inflammation of the intestine during the acute-phase IBD is associated with migration of leukocytes to the gut, resulting in the production of a large number of inflammatory proteins which can be measured in the feces. As a result, several leucocyte products and serum proteins have been evaluated for use as fecal markers, which can be used to indicate the presence and severity of intestinal inflammation.

Possible fecal markers for IBD include lactoferrin, M2-PK, S100A12, calprotectin and polymorphonuclear neutrophil elastase (PMN-elastase). The characteristics of these IBD markers are summarized in Table 1.

Lactoferrin is an iron-binding glycoprotein found in neutrophil granulocytes; its levels in feces have been shown to increase quickly following inflammation of the intestine. Studies have reported that lactoferrin is a sensitive marker of IBD activity, however, it is not as widely studied as calprotectin. Walker et al. reported promising results from a study of 148 children and young adults with IBD (141 with IBD and 7 with IBS, plus 22 healthy controls) in which elevated lactoferrin levels were used to identify patients at greater risk of disease relapse.

M2-PK is an enzyme involved in glycolysis that is present in rapidly dividing cells and has been previously used as a marker of gastrointestinal cancers. However, its role in gastrointestinal inflammation is unknown. A study by Chung-Faye et al. reported elevated concentrations of M2-PK in patients with IBD compared with those patients with IBS. However, it has yet to be shown if M2-PK can be used as a potential marker for predicting relapses in asymptomatic patients with IBD.

PMN-elastase is a serine proteinase that is secreted by neutrophils and macrophages during inflammation. The concentrations of PMN-elastase in feces correlate with intestinal inflammation. A study by Schröder et al. identified the use of PMN-elastase as a possible tool for the differential diagnosis of IBS; however, its overall diagnostic accuracy (sensitivity for identifying inflammation and specificity for excluding...
S100A12 (calgranulin C), a member of the human calcium-binding S100 protein family, is a cytoplasmic protein found in neutrophils that acts as a marker of inflammation. Previous studies carried out in both children and adults reported positive results regarding the ability of S100A12 to distinguish between IBD and IBS. However, it has not yet been determined whether S100A12 could be useful as a marker of disease relapse in both the pediatric and adult settings. Calprotectin is a heterocomplex of S100A8 (migration inhibitory factor-related protein [MRP] 8, calgranulin A) and S100A9 (MRP14, calgranulin B), two other members of the calcium-binding S100 protein family. Fecal calprotectin represents up to 60% of cytosolic protein in neutrophils and levels correlate with the influx of neutrophils into the intestine. Calprotectin is stable in feces for up to 7 days at room temperature and this property offers advantages in both clinical practice and in terms of a suitable marker for a diagnostic test. The predictive value of biomarkers in the diagnosis and treatment of patients with IBD has been more widely explored for calprotectin than the other biomarkers mentioned here. Of particular interest is the potential for calprotectin levels to be used to diagnose IBD, differentiate IBD from IBS, assess IBD activity, and predict relapse of active disease. Several clinical studies have demonstrated that not only does fecal calprotectin significantly correlate with endoscopic disease activity in active IBD, but also that a normalization of fecal calprotectin equates to mucosal healing in IBD, which is an important treatment target goal. Studies have shown that fecal calprotectin levels more closely correlate with endoscopic disease findings than CRP, blood leucocytes, Crohn’s Disease Endoscopic Index of Severity, and Crohn’s Disease Activity Index. A report commissioned by the UK Department of Health in 2009 found fecal calprotectin to be more cost effective and accurate in discriminating between IBS and IBD in primary care in comparison to other inflammatory markers of IBD, such as CRP. In addition, the UK National Institute for Health and Clinical Excellence is currently developing guidance on the application of fecal calprotectin as a diagnostic test for the differentiation of IBD from IBS. Use of fecal calprotectin for diagnosing IBD could lead to a reduction in the number of specialist referrals for further invasive endoscopic investigations. A meta-analysis of 13 prospective diagnostic accuracy studies (6 studies involving 670 adults with fecal calprotectin cut-off levels ranging from 24 µg/g to 150 µg/g; and 7 studies involving 371 children with fecal calprotectin cut-off levels ranging from 32 µg/g to 100 µg/g) predicted that screening with fecal calprotectin to exclude IBD could hypothetically reduce the number of endoscopy referrals by 67% in adults and by 35% in children.

On the basis of the current available evidence, the expert panel agreed that fecal calprotectin would be the best potential biomarker for a rapid point-of-care diagnostic test. Currently, most assays for fecal calprotectin are based on the enzyme-linked immunosorbent assay (ELISA) method, which can be associated with a delay of up to 3 weeks before obtaining the results. However, the ELISA method provides a quantitative result, which might be of additional

<table>
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<th>Table 1: Characteristics of existing IBD markers.</th>
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Abbreviations: PMN, polymorphonuclear neutrophils. Information derived from: Judd et al. 23 and Schröder et al. 25
clinical value. Further quantitative tests for calprotectin are available including the Quantum Blue® Calprotectin Rapid Test (Bühllmann Laboratories AG, Schönenbuch, Switzerland), which has been shown to be a suitable alternative to ELISA in a clinical setting. Previous studies have shown that there is some variability in calprotectin values between patients, samples, and between test kits from different suppliers; therefore, the clinical value of quantitative data remains to be determined.29,35,40,51

Thus, the development of a new rapid, semi-quantitative, point-of-care test would dramatically reduce the turnaround time for test results to be available to physicians, thereby allowing a faster decision to be made regarding the current IBD status of a patient and the subsequent suitable treatment options. An ideal rapid, semi-quantitative, point-of-care test needs to be easy to perform, accurate, specific and sensitive, and yield rapid results at the point-of-care, to allow the test results to be available during the patient visit.

3.2. Use of a semi-quantitative rapid point-of-care calprotectin test in clinical settings

The expert panel reached a consensus that a semi-quantitative fecal calprotectin point-of-care test would be useful in several IBD clinical settings: at initial diagnosis (discrimination between organic [IBD] and non-organic diseases [IBS]); monitoring of patients with chronic IBD currently in remission; and monitoring of therapy efficacy in patients with active and severe active diseases. The expert panel also agreed on the number and range of cut-off levels for each setting depending on the intended use (Table 2).

3.3. Diagnosis of IBD and discrimination from IBS in primary and/or secondary care settings

An important goal of a semi-quantitative point-of-care test would be the non-invasive discrimination of IBD from IBS. This point-of-care test would be ideally placed for use during the early diagnosis stage in primary care but it could also be used in the secondary care setting, as well as for differentiation of symptomatic patients with coexisting IBD and IBS. Discriminating possible IBD from IBS at an early stage would avoid unnecessary specialist referral of patients and subsequent endoscopic investigations. A single cut-off level of 100 μg/g was agreed by the expert panel as appropriate for this purpose based on results from previous studies, which reported increased diagnostic precision for discrimination of colorectal inflammation in patients with CD and UC at 100 μg/g.52,53 This agreed cut-off level of 100 μg/g is higher than the levels suggested in recent studies, in which cut-off values of 30 to 50 μg/g were recommended.28,29,37,40,46 It was agreed at the expert meeting that a higher cut-off level would be desirable to maximize the negative predictive value of the test to reduce incorrect diagnoses of IBD. As indicated in Table 2, a negative test result at the lowest cut-off level suggests a diagnosis of a non-inflammatory condition, such as IBS. A positive test result at the cut-off level of 100 μg/g may indicate IBS, with a recommendation to repeat the test in 6 weeks to confirm the initial result. However, a normal fecal calprotectin level alone would not be sufficient to confirm a diagnosis of IBS, without diagnostic criteria, such as Rome III being met.1 Positive results at both the 100 μg/g and 250 μg/g cut-off levels may indicate IBD rather than IBS.

3.4. Detection and prediction of IBD flares while in remission

Another important clinical setting and goal for development of a semi-quantitative test was management of patients with established chronic IBD currently in remission. For patients exhibiting mild or moderate IBD symptoms, a corresponding increase in fecal calprotectin levels (as indicated by a positive point-of-care test result, Table 2) would be used to confirm/predict an active flare of IBD, and suggests to physicians that a

| Table 2 | Proposed interpretation of results of a rapid point-of-care test. |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Fecal calprotectin cut-off levels in semi-quantitative test | Interpretation |
| 100 μg/g | 250 μg/g | 400 μg/g | 1000 μg/g |
| **Patients in primary care with suspected IBS** | |
| Negative | Negative | Negative | Negative | Result confirms IBS 

| Positive | Negative | Negative | Negative | Result suggests IBS, repeat in 6 weeks to confirm initial result |
| Positive | Positive | Negative | Negative | Result suggests possible IBD, repeat in 4 weeks |
| Positive | Positive | Positive | Negative | Result consistent with IBD, refer for specialist opinion |
| Positive | Positive | Positive | Positive | Result indicates IBD |

| **Patients in primary care with established UC** | |
| Positive | Positive | Positive | Positive | Result confirms active disease, consider changing/escalating treatment, consider admitting patient to hospital |
| Positive | Positive | Positive | Negative | Result suggests flare of IBD, consider changing/escalating treatment |
| Positive | Positive | Negative | Negative | Result suggests IBD in remission |
| Positive | Negative | Negative | Negative | Result suggests IBD in remission |
| Negative | Negative | Negative | Negative | Result confirms IBD in remission |

IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; UC, ulcerative colitis.29,46

a In conjunction with Rome III criteria for IBS being met.
change of treatment or dose adjustment is required, without the need for further endoscopic investigations. For patients who are well, with minimal or no IBD symptoms, a point-of-care test with one or two cut-off values would be used to indicate and confirm that the disease is in a period of remission. It was recommended by the expert panel that both positive and negative test results should ideally be repeated to confirm the test result.

A fecal calprotectin value of 250 μg/g was deemed appropriate as a cut-off value for monitoring IBD remission based on results from previous studies. D’Haens et al. reported the results of a study which included 126 patients with IBD (87 patients with CD and 39 patients with UC) and proposed a fecal calprotectin cut-off value of 250 μg/g for indicating IBD remission.37 An earlier study by Sipponen et al., which included 77 patients with CD (106 endoscopies) proposed a cut-off value of 200 μg/g for identification of endoscopically inactive disease.36 In another study of 115 patients with CD, fecal calprotectin levels less than 300 μg/g were associated with a reduced risk of disease relapse.34

3.5. Monitoring of active IBD to optimize treatment

Finally, the use of a point-of-care fecal calprotectin test would help assess therapy efficacy and optimize treatment in patients with active and severe active IBD by monitoring a reduction in fecal calprotectin levels. Fecal calprotectin levels are a sensitive measure of therapeutic efficacy, and test results would aid physicians with treatment modification decisions. A cut-off calprotectin value of 400 μg/g was agreed by the expert panel with a further cut-off level of 1000 μg/g proposed for monitoring therapy efficacy in patients with severe active IBD. These two values were selected by the panel based on data from previously published studies, which reported a range of mean and median fecal calprotectin values of 175 μg/g,37 465 μg/g,17 718 μg/g,15 810 μg/g34 and 1260 μg/g35 in patients with active and severe active UC and CD.

4. Discussion

Experts agreed that a semi-quantitative fecal calprotectin test will be useful in clinical practice settings. The suggested cut-off values agreed by the expert panel are intended for a point-of-care test for use initially in patients with UC. It remains to be established if these proposed cut-off values can be used for patients with CD as the evidence base for interpreting fecal calprotectin results in patients with CD is less robust, which is partly due to the greater variety of disease manifestations in patients with CD.

There are some limitations to using fecal calprotectin as a proposed biomarker for monitoring disease activity in patients with IBD. Fecal calprotectin is a non-specific disease biomarker and, while it is useful for discriminating IBD (organic disease) from IBS (non-organic disease), it is not useful for discriminating UC from CD or to discriminate active IBD from infectious gastrointestinal disease. Other presenting conditions such as non-steroidal anti-inflammatory drugs, tumors, and diverticulitis, which cause an increase in intestinal neutrophils, result in increased concentrations of fecal calprotectin. Fecal calprotectin is more sensitive for detection of mild mucosal inflammation than CRP, although in severely active cases CRP better reflects systemic inflammation.12,14,29

Currently available tests can also yield results that vary substantially between and within patient samples, particularly in patients with CD.34,40,51,56 Results can also vary depending on the mixing, consistency and quantity of the stool sample, necessitating a standardized sample collection and preparation method to ensure that test results are as accurate as possible.35 Due to this variability in test results, individual values can sometimes be difficult to interpret, and it may be more appropriate to look at gross changes in fecal calprotectin levels i.e., a semi-quantitative rather than a quantitative test.

Semi-quantitative tests are fast, cheap, and may have the potential to be performed at home by the patients themselves or in an outpatient setting during patient visits. Home tests are currently being used by patients with UC in Norway who are concerned that they may be having a flare.51 An easy-to-use semi-quantitative test would offer practical advantages in this setting, enabling home based testing to become more widely used for the monitoring of IBD. This would be of particular benefit to patients who are located some distance from medical centers or hospitals. It would also provide patients with a higher degree of ownership and sense of control over their condition. However, the expert panel agreed that both patient education and a good physician–patient relationship would be key support requirements needed to ensure correct usage and good compliance of a home-based semi-quantitative test, to ensure a benefit for patients. As the involvement of General Practitioners (GPs) in the diagnosis and treatment of IBD varies greatly between European countries, GPs do not always have the opportunity to provide their patients with this necessary support.

The expert panel agreed that a benchmarking study should be undertaken comparing the rapid point-of-care test against the standard ELISA test. This proof-of-concept study should evaluate the impact of the point-of-care test on the doctor’s decision-making process and any potential improvement in patient’s health-related quality of life, measured, for example, by reduced hospitalization of patients and a reduction in the number of invasive endoscopies. The prognostic value of the point-of-care test should be included as a study endpoint measure.

In addition, larger prospective studies were suggested to be carried out to validate the cut-off values in clinical practice in patients with UC, using endoscopy as a reference for monitoring intestinal inflammation. Once cut-off values have been established for patients with UC, cut-off values for patients with CD could then be developed and validated, although cut-off values in CD will be harder to establish due to lack of consistent evidence. In addition, small intestinal disease is only reflected to a variable extent by fecal calprotectin values in contrast to colon involvement.28,58 At the time of preparing this paper, the UK National Institute for Health Research Health Technology Assessment Programme is welcoming applications from researchers to develop and validate a fecal biomarker tool, which can be used in primary care and secondary care, for early detection of relapse in patients with CD.59
5. Conclusions

As a sensitive biomarker of mucosal inflammation, fecal calprotectin could be used in a rapid point-of-care test to monitor disease activity in patients with IBD, and help reduce unnecessary specialist referrals and subsequent endoscopic investigations, which can be both uncomfortable and painful for the patient, and time-consuming and costly for health services. As one of the main limitations of current calprotectin tests is the slow turnaround of results, a rapid semi-quantitative test would be an important development. This article reports the consensus opinion of an expert panel of gastroenterologists with expertise in IBD and highlights four clinical situations whereby the use of a rapid semi-quantitative test could be helpful, with recommended fecal calprotectin cut-off values proposed for each situation: early diagnosis and discrimination of IBD from other diseases such as IBS in primary and/or secondary care settings (cut-off level of 100 μg/g); detection and prediction of IBD flares in patients currently in remission (cut-off level of 250 μg/g); and monitoring of active IBD (cut-off level of 400 μg/g); and severe active IBD (cut-off level of 1000 μg/g) to optimize treatment efficacy. Large prospective studies will be required to validate the recommended cut-off values in clinical practice.

Conflicts of interest statement

All authors of this paper have served as a speaker, a consultant and/or an advisory board member for Tillotts Pharma.

In addition:

• Dr Rogler has served as a consultant for Abbott Switzerland and Abbott International, FALK Germany, Essex/MSD Switzerland, Novartis, Roche, and Vifor Switzerland. He has received speaker honoraria from Abbott, FALK, MSD, UCB, and Vifor. He has received educational grants and research grants from Abbott, Ardeypharm, Essex/MSD, FALK, Flamentera, Novartis, Tillotts Pharma, UCB and Zeller
• Dr Aldeguer has received educational and/or research grants from Marato, TV3, Abbott and Shire
• Dr Kruis has served as a speaker, a consultant and an advisory board member for Dr Falk Pharma, Ferring Deutschland, Abbott, Recordati, Novartis and Ardeypharm. He has received educational and/or research grants from Dr Falk Pharma, Ferring, Abbott, Novartis and Ardeypharm
• Dr Lassen has served as a speaker or consultant for Abbott Scandinavia AB, AstraZeneca, Ferring Pharmaceuticals and MSD
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• Dr Sipponen has served as a speaker for Abbott, MSD and Roche
• Dr Vatn has served as a speaker or consultant for Falk Foundation, Abbott, UCB and Schering Plough and has received research funding from Centocor, IPSEN, Ferring, Tillotts, Novartis and MEDA
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References

27. Joishy

23. Judd TA, Day AS, Lemberg DA, Turner D, Leach ST. Update of
20. Kane SV, Sandborn WJ, Rufo PA, Zholudev A, Boone J, Lyerly D,
19. Dai J, Liu WZ, Zhao YP, Hu YB, Ge ZZ. Relationship between fecal
lactoferrin and inflammatory bowel disease. Scand J Gastroenterol

24. Kane SV, Sandborn WJ, Rufo PA, Zholudev A, Boone J, Lyerly D,
et al. Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. Am J Gastroenterol 2003;98(6):
1309–14.


