Rational Use of Fecal Calprotectin in Irritable Bowel Syndrome and Inflammatory Bowel Disease

Roberto Catanzaro1, Alfio Maugeri1, Morena Sciuto1, Fang He2, Baskar Balakrishnan3, Francesco Marotta4

1 Department of Clinical and Experimental Medicine, Gastroenterology Section, Gaspare Rodolico Hospital, University of Catania, Catania, Italy
2 Department of Nutrition, West China School of Public Health, Sichuan University, Sichuan, China
3 Department of Immunology, Mayo Clinic, Rochester, MN, USA
4 ReGenera R and D International for Aging Intervention and San Babila Clinic, Milano, Italy

Received: 03 Sep. 2020; Accepted: 27 Feb. 2021

Abstract: The gastrointestinal pathologies have increased over the last years. The clinical pictures of inflammatory and irritable bowel disease might overlap, leading to expensive and invasive tests. Our study aims to investigate fecal calprotectin as an effective tool for differential diagnosis of gastrointestinal disorders. Two hundred fifty-six patients with the diagnosis of the gastrointestinal disorder and subjected to colonoscopy were collected for the statistical analysis of fecal calprotectin. The differential diagnosis of intestinal inflammation or non-inflammation was performed according to the Receiver Operating Characteristic (ROC) curve that outlines the Area Under Curve (AUC), Sensitivity (Se), Specificity (Sp). Fecal calprotectin was significantly elevated in patients with inflammatory bowel disease compared with patients with irritable bowel syndrome. Especially, the mean values of fecal calprotectin were 522 µg/g (IQR=215-975) and 21 µg/g (IQR=14-34.5) in patients with and without inflammation, respectively (P<0.0001). AUC value of fecal calprotectin was 0.958 (Se=88.9%, Sp=91.1%, with a cut-off value of 50 µg/g) for differentiating between inflammatory bowel disease and irritable bowel syndrome. Fecal calprotectin seems to be a non-invasive and inexpensive biomarker useful for the purpose of a differential diagnosis between inflammatory bowel disease and irritable bowel syndrome.

Keywords: Fecal calprotectin; Irritable bowel syndrome; Inflammatory bowel diseases; Ulcerative colitis; Crohn's disease

Introduction

Irritable bowel syndrome (IBS) is a widespread pathology among the adult world population and significantly affects the quality of life of those affected and the social costs (1). It is characterized by recurrent discomfort or abdominal pain and changes in bowel habits. The causes are not fully known, although several hypotheses have been put forward over the years (2).

Whereas inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is a relapsing and remitting condition. This disease can manifest itself with abdominal pain, changes in bowel habits, weight loss, asthena, etc. (3).

The number of new cases diagnosed with IBS is growing worldwide, and this condition affects 9%-23% of the world population despite the fact that a pathological picture with well-defined contours is not shown (4). The IBD increases in the world, present with a wide geographical variability of the prevalence, which is approximately 40 per 100,000 people and whose incidence is around the age of 20-40-year-old (5).

The two pathologies can therefore present very similar clinical pictures. According to Rome IV Criteria, diagnosis of IBS can be made when the symptoms have been present at least one day a week in the last three months, together with 2 of the following characteristics: reduction/disappearance of pain with defecation, change
Fecal calprotectin in IBS and IBD

in the frequency of defecation, change in the shape of the stool (6). Moreover, for a correct diagnosis of IBS must be excluded other pathological processes, such as lactose intolerance, drug-induced diarrhea, laxative abuse, parasitosis, gastritis or enteritis, colitis, celiac disease (7). Diagnostic testing should be executed when the "red flags" are present (Table 1) (6).

Table 1. Red flags

| Red flags       | 
|-----------------|
| Older age       | 
| Fever           | 
| Weight loss     | 
| Rectal bleeding | 
| Vomiting        |

Instead, the diagnosis of IBD requires performing a colonoscopy with retrograde ileoscopy and the definition of anatomo-pathological picture of intestinal biopsies by histological examination (8,9).

Therefore, an endoscopic examination would be required to rule out an IBD diagnosis.

Therefore, since the two pathologies are very similar to each other from a clinical point of view, it is often necessary to carry out laboratory or instrumental investigations for a correct differential diagnosis. The gold standard would be to carry out an endoscopic examination (10). However, due to the high costs of a colonoscopy, several biomarkers have been considered which can allow to exclude or confirm IBD. Fecal calprotectin is one of these markers (11). In fact, only in the case of an intestinal inflammation, it can be detected in the faeces. Its fecal dosage is a useful method that provides direct indications on the location of inflammation, while the dosage in serum or plasma shows a state of inflammation that it is possible to be found anywhere (12).

This study aims to verify the usefulness of the fecal calprotectin dosage as a non-invasive test to assess its possible role in the differential diagnosis between IBS and IBD pathologies. The fecal calprotectin dosages of patients were performed through an observational retrospective study.

Materials and Methods

This is an observational retrospective study. It has been examined a total number of 854 medical records of patients suffering from IBD (that includes Crohn's disease and ulcerative colitis) or IBS, who were treated at the Internal Medicine Unit-Section of Gastroenterology of "G. Rodolico" Policlinico Hospital of Catania (Italy) in the period between January 2017 and February 2020.

The inclusion criteria for the study were: diagnosis of IBD or IBS-D (diarrhea, abdominal pain), execution of colonoscopy in the period 2017-2020, execution of the fecal calprotectin test. Among the 854 medical records analyzed, 256 belong to eligible patients for the study; respectively, 138 patients were diagnosed with IBD (76 with the diagnosis of Crohn's disease, 62 with the diagnosis of ulcerative colitis), 118 were diagnosed with IBS.

The collected data were organized in a Microsoft Office Excel workbook. The document consists of one sheet, and it reports surname and name, gender, age, type of diagnosis IBD or IBS, endoscopy alterations, values of fecal calprotectin.

This is a retrospective study; therefore, its management was notified to the institutional ethics committee. Our research was conducted in accordance with the principles of the Helsinki Declaration 2008 (6th edition) and the Tokyo Declaration. Since the study we conducted is a retrospective observational study, it was not necessary to gather informed consent.

Statistical analysis

Numerical data were expressed as mean and range or median and interquartile range (IQR), whereas categorical data were expressed as number and percentage. Logistic regression analysis was used to construct Receiver Operating Characteristic (ROC) curves for calprotectin Sensitivity (Se) and Specificity (Sp) in this study, and it was calculated the cut-off values. Statistical analyses were performed with IBM SPSS software (version 20). P<0.05 were considered statistically significant.

Results

The study includes 138 patients with inflammatory bowel disease (78 males/60 females, mean age 44, range age 16 to 75) and 118 patients with irritable bowel syndrome (66 males/52 females, mean age 40, range age 19 to 82). The inflammatory bowel disease included 76 patients with Crohn's disease and 62 patients with ulcerative colitis. Concentrations of fecal calprotectin are shown in Table 2 and Figure 1.

The mean values of fecal calprotectin concentration in patients were 72 µg/g (IQR=22.75-552.5). In particular, the mean values of fecal calprotectin in patients with IBD were 522 µg/g (IQR=215-975), while in patients with IBS, they were 21 µg/g (IQR=14-34.5) (P<0.0001). Fecal calprotectin was significantly
elevated in patients with IBD compared with patients with IBS.

Figure 2 shows the ROC curve for the quantification of fecal calprotectin in the group of patients analyzed. The fecal calprotectin concentration significantly differentiated between IBD and IBS ($P<0.0001$; Area Under Curve 0.958, 95% Confidence Interval 0.908, 0.986). A cut-off value of 50 $\mu$g/g resulted in a Sensitivity (Se) of 88.9% and Specificity (Sp) of 91.1%. Area Under Curve (AUC)=0.958, Sensitivity (Se)=88.9%, Specificity (Sp)=91.1%, cut-off=50 $\mu$g/g.

### Table 2. baseline characteristics of the study subjects

| Variable               | Overall     | IBD         | IBS         |
|------------------------|-------------|-------------|-------------|
| No. of patients        | 256 (100%)  | 138 (54%)   | 118 (46%)   |
| Gender, M/F            | 144/112     | 78/60       | 66/52       |
| Age, mean (range)      | 41 (16-82)  | 44 (16-75)  | 40 (19-82)  |
| FC, median (IQR) - $\mu$g/g | 72 (22,75-552,5) | 522 (215-975) | 21 (14-34,5) |

[IBD = inflammatory bowel disease; IBS = irritable bowel syndrome; M = male; F = female; FC = fecal calprotectin; IQR: interquartile range]

**Figure 1.** Levels of fecal calprotectin in patients of the study (IBD = inflammatory bowel disease, IBS = irritable bowel syndrome)

**Figure 2.** Receiver Operating Characteristic analysis of the fecal calprotectin concentration in the differentiation between patients with inflammatory and non-inflammatory bowel diseases

### Discussion

IBD are relatively frequent pathologies worldwide, especially in Western countries. Their incidence is constantly increasing. In this category of diseases, several subtypes of intestinal inflammation are included, but the two main forms are Crohn's disease and ulcerative colitis (13). IBD are diseases that can affect males and females equally. Although they can occur at any age, there is a peak incidence between 15 and 25-
year-old for Crohn’s disease and between 25 and 35-year-old for ulcerative colitis (3). The exact etiology is currently unknown, but evidence suggests that normal intestinal flora triggers an abnormal immune reaction in patients with a multifactorial genetic predisposition (14). Other risk factors could be stress, dietary changes, smoking, and drugs. These can cause an alteration of the permeability of the intestinal mucosa, which represents a determining element in the pathogenesis of these diseases. From this derives a cell-mediated immune response that causes inflammation of intestinal mucosa resulting in appearance of symptoms (15,16).

Instead IBS is a very common functional disorder in daily clinical practice. In recent years, several events have been illustrated that can lead to the onset of the symptoms that characterize IBS (food intolerance, enteric infections, etc.) (17). According to the Rome IV criteria there are 4 different subtypes of IBS based on the characteristics of the bowel habits. They are distinguished: IBS with predominant constipation (IBS-C), IBS with predominant diarrhea (IBS-D), IBS with mixed bowel habits (IBS-M), and IBS unsubtyped (IBS-U) (6).

A fundamental difference between the 2 pathologies is that in IBD there is a chronic inflammatory state of the intestinal mucosa that is missing in the IBS. In fact in IBS there are no organic pathologies or morpho-structural alterations of the gastrointestinal tract that can be considered the cause of the disturbances present in these patients (18). However, despite this important difference, IBS and IBD are characterized by a very similar clinical features. In particular, the symptoms of IBD are very similar to those of IBS-D: diarrhea and abdominal pain. In IBD there are also weight loss and blood in the stools, which are missing in the IBS (19). In the presence of the symptoms mentioned above it is important to define whether they are caused by organic lesions attributable to an IBD or if they are the result of a functional disorder. This aspect is fundamental for the correct management of the patient (20).

In recent years, in order to limit the execution of invasive diagnostic methods, various serological and fecal markers have been considered. These are inflammatory indices which, by definition, tend to increase in inflammatory diseases (such as IBD) and not in functional disorders (such as IBS). One of these markers is fecal calprotectin (21). Calprotectin is a protein that binds calcium and zinc. It belongs to the S100 family proteins and it is present in plentiful quantities in neutrophil granulocytes, where it represents 60% of cytoplasmic proteins and 5% of total proteins. In lesser amounts, calprotectin has also been found in activated macrophages and monocytes (22,23). Its dosage in the stool offers the greatest advantages in the evaluation of the degree of gastrointestinal inflammation. Hence calprotectin is an extremely stable protein in the faeces where it remains unaltered even for more than seven days (24). In the presence of inflammatory processes, calprotectin is released as a result of the degranulation of neutrophil granulocytes (25).

Thus, as calprotectin increases in the course of inflammation, it can be exploited in the differential diagnosis between IBS and IBD. The cut-off that is commonly used is 50 μg/g. A result below this value is suggestive of a non-inflammatory pathology, such as IBS. Instead, a result above 50 μg/g and, even more, above 100 μg/g is highly suggestive of IBD (12).

In our study, we obtained a mean fecal calprotectin value of 522 μg/g in patients with IBD. Instead, the mean value of this marker in patients with IBS was 21 μg/g. This difference was highly significant. With this result, we can state that this protein, dosed in the faeces, allows us to better define whether a patient with symptoms attributable to both IBS and IBD should undergo invasive and expensive examinations. In fact, there has been an increase in the number of endoscopic examinations required and performed in recent decades. In particular, these are endoscopies of the lower gastrointestinal tract, which in Italian patients undergoing colonoscopies under a conventional health care system, affects for the individual patient in the order of approximately € 51,22 (about USD 57,45), increased by approximately € 47,18 (about USD 52,92) when taking multiple biopsies of the colon mucosa, while the dosage of the fecal calprotectin, totally charged to the patient, has a medical cost of about € 15,00 (approximately USD 16,82). In some cases, these endoscopic investigations are totally inadequate, and the result is to lengthen the waiting time, as well as to increase the health costs, subjecting patients, very often, to the risks of an invasive diagnostic method, with long execution times and, in some cases, from the need to also make use of additional tools (26). The decision to carry out such an investigation should be based on a real diagnostic doubt. Before reaching the endoscopic examination, laboratory methods should be used to define which of these patients, in the absence of the aforementioned “red flags,” are really candidates for a colonoscopy. Therefore, according to our study, fecal calprotectin can be considered as a good marker for implementing this choice (6).

Fecal calprotectin in IBS and IBD
Our results were in line with what has already been reported in the literature. In fact, Otten et al., showed that the dosage of fecal calprotectin is a good test able to exclude the presence of intestinal inflammation (27). In one of their studies, Banerjee et al., found higher calprotectin values than cut-off (50 μg/g) in patients with IBD. While, in patients without macroscopic (endoscopic) and microscopic (on histological examination) organic alteration, the level of this fecal marker were much lower (28). Lozoya Angulo et al., found higher calprotectin values both in patients with IBD and in patients with other organic lesions than in patients with simple functional disorders (29). Finally, Carroccio et al., confirmed that the fecal values of this protein have high diagnostic value in the case of IBD. However, they also pointed out that false positives can be obtained in the case of extra-intestinal diseases or taking drugs (30).

The dosage of calprotectin in faeces is a simple, non-invasive, inexpensive test and appears to be a direct marker of bowel inflammation. It has a significant role in the differential diagnosis between IBD and IBS. Gastrointestinal pathologies affect the quality of patients’ life and they often require additional tests that can be invasive and expensive. All of this fully justifies the effective interest on fecal calprotectin as a non-invasive and inexpensive biomarker useful for the purpose of a differential diagnosis between IBD and IBS, especially in the patient population under the age of 50 yrs and without the presence of signs of alarm (rectal bleeding, weight loss unjustified, etc.) (31-33).

References

1. Catanzaro R, Occhipinti S, Calabrese F, Anzalone MG, Milazzo M, Italia A, et al. Irritable Bowel Syndrome: New Findings in Pathophysiological and Therapeutic Field. Minerva Gastroenterol Dietol 2014;60:151-63.
2. El-Salhy M, Hatlebakk JG, Hausken T. Diet in Irritable Bowel Syndrome (IBS): Interaction With Gut Microbiota and Gut Hormones, Nutrients 2019;11:1824.
3. Flynn S, Eisenstein S. Inflammatory Bowel Disease Presentation and Diagnosis. Surg Clin North Am 2019;99:1051-62.
4. Saha L. Irritable bowel syndrome: Pathogenesis, diagnosis, treatment, and evidence-based medicine. World J Gastroenterol 2014;20:6759-73.
5. Lewis JD. A Review of the Epidemiology of Inflammatory Bowel Disease with a Focus on Diet, Infections and Antibiotic Exposure. Nestle Nutr Inst Workshop Ser 2014;79:1-18.
6. Lacy BE, Patel NK. Rome Criteria and a Diagnostic Approach to Irritable Bowel Syndrome. J Clin Med 2017;6:99.
7. Mearin F, Lacy BE. Diagnostic criteria in IBS: useful or not? Neurogastroenterol Motil 2012;24:791-801.
8. Conrad K, Roggenbuck D, Laass MW. Diagnosis and classification of ulcerative colitis. Autoimmun Rev 2014;13:463-6.
9. Laass MW, Roggenbuck D, Conrad K. Diagnosis and classification of Crohn's disease. Autoimmun Rev 2014;13:467-71.
10. Burri E, Beglinger C. The use of fecal calprotectin as a biomarker in gastrointestinal disease. Expert Rev Gastroenterol Hepatol 2013;8:197-210.
11. Mumolo MG, Bertani L, Cecarelli L, Laino G, Di Fluri G, Albano E, et al. From Bench to Bedside: Fecal Calprotectin in Inflammatory Bowel Diseases Clinical Setting. World J Gastroenterol 2018;24:3681-94.
12. Zhang YZ, Li YY. Inflammatory bowel disease: pathogenesis. World J Gastroenterol 2014;20:91-9.
13. Cho JH, Weaver CT. The genetics of inflammatory bowel disease. Gastroenterology 2007;133:1327-39.
14. Sun Y, Li L, Xie R, Wang B, Jiang K, Cao H. Stress Triggers Flare of Inflammatory Bowel Disease in Children and Adults. Front Pediatr 2019;7:432.
15. De Lange KM, Barrett JC. Understanding inflammatory bowel disease via immunogenetics. J Autoimmun 2015;64:91-100.
16. Hadjivasilis A, Tsioitis C, Michalinos A, Ntourakis D, Christodoulou DK, Agouridis AP. New insights into irritable bowel syndrome: from pathophysiology to treatment. Ann Gastroenterol 2019;32:554-64.
17. Drossman DA. Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features, and Rome IV. Gastroenterology 2016;S0016-5085(16)00223-7.
18. Berens S, Schaefer RT, Baumeister D, Gauss A, Eich W, Tesarz J. Does symptom activity explain psychological differences in patients with irritable bowel syndrome and inflammatory bowel disease? Results from a multi-center cross-sectional study. J Psychosom Res 2019;126:109836.
19. Costa F, Mumolo MG, Marchi S, Bellini M. Differential diagnosis between functional and organic intestinal disorders: is there a role for non-invasive tests? World J Gastroenterol 2007;13:219-23.
20. Kamal A, Padival R, Lashner B. Inflammatory Bowel Disease and Irritable Bowel Syndrome: What to Do When There Is an Overlap. Inflamm Bowel Dis 2018:24:2479-82.
21. Abraham BP, Kane S. Fecal Markers: Calprotectin and Lactoferrin. Gastroenterol Clin North Am 2012;41:483-
Fecal calprotectin in IBS and IBD

22. Ometto F, Friso L, Astorri D, Botsios C, Raffeiner B, Punzi L, et al. Calprotectin in rheumatic diseases. Exp Biol Med (Maywood) 2017;242:859-73.

23. Lamb CA, Mansfield JC. Measurement of faecal calprotectin and lactoferrin in inflammatory bowel disease. Frontline Gastroenterol 2011;2:13-8.

24. Sipponen T. Diagnostics and Prognostics of Inflammatory Bowel Disease with Fecal Neutrophil-Derived Biomarkers Calprotectin and Lactoferrin. Dig Dis 2013;31:336-44.

25. Gimeno-García AZ, Quintero E. Colonoscopy appropriateness: Really needed or a waste of time? World J Gastrointest Endosc 2015;7:94-101.

26. Catalano F, Catanzaro R, Branciforte G, Bentivegna CF, Cipolla R, Brogna A, et al. Colonoscopy Technique With an External Straightener. Gastrointest Endosc 2000;51:600-4.

27. Otten CMT, Kok L, Witteman BJM, Baumgarten R, Kampman E, Moons KGM, et al. Diagnostic Performance of Rapid Tests for Detection of Fecal Calprotectin and Lactoferrin and Their Ability to Discriminate Inflammatory From Irritable Bowel Syndrome: A Prospective Study in Adults and Children. Clin Chem Lab Med 2008;46:1275-80.

28. Banerjee A, Srinivas M, Eyre R, Ellis R, Waugh N, Bardhan KD, et al. Faecal Calprotectin for Differentiating Between Irritable Bowl Syndrome and Inflammatory Bowel Disease: A Useful Screen in Daily Gastroenterology Practice. Frontline Gastroenterol 2015;6:20-6.

29. Lozoya Angulo ME, de Las Heras Gómez I, Martinez Villanueva M, Noguera Velasco JA, Avilés Plaza F. Faecal Calprotectin, an Useful Marker in Discriminating Between Inflammatory Bowl Disease and Functional Gastrointestinal Disorders. Gastroenterol Hepatol 2017;40:125-31.

30. Carroccio A, Iacono G, Cottone M, Di Prima L, Cartabellotta F, Cavataio F, et al. Diagnostic Accuracy of Fecal Calprotectin Assay in Distinguishing Organic Causes of Chronic Diarrhea From Irritable Bowel Syndrome: A Prospective Study in Adults and Children. Clin Chem 2003;49:861-7.

31. Adriani A, Ribaldone DG, Astegiano M, Durazzo M, Saracco GM, Pellicano R. Irritable bowel syndrome: the clinical approach. Panminerva Med 2018;60:213-22.

32. Canavan C, West J, Card T. Review article: the economic impact of the irritable bowel syndrome. Aliment Pharmacol Ther 2014;40:1023-34.

33. Hillilä MT, Färkkilä NJ, Färkkilä MA. Societal costs for irritable bowel syndrome - a population based study. Scand J Gastroenterol 2010;45:582-91.