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Role of fecal calprotectin in gastrointestinal disorders

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Abstract. – BACKGROUND: Fecal calprotectin (FC) has been proposed as a useful and non-invasive marker of acute intestinal inflammation.

AIM: We summarize recent evidences on FC, providing practical perspectives on its diagnostic and prognostic role in different gastrointestinal conditions.

MATERIALS AND METHODS: We performed a MEDLINE search for all articles published on FC in human gastroenterology field up to December 2011. We chose evidences from well-designed and controlled studies when available. A meta-analysis was not performed because of the heterogeneity of these studies.

RESULTS: Most of relevant data derived from studies on inflammatory bowel disease (IBD). FC concentrations (FCCs) showed a good diagnostic precision for separating organic and functional intestinal diseases and well correlated with IBD activity. FCCs were higher in subjects with NSAID enteropathy, but the actual correlation between FC and endoscopy is under investigation. FCCs can not be recommended for colorectal neoplasia population screening purpose. Few and heterogeneous studies have been performed in order to evaluate role of FC in other gastrointestinal conditions.

CONCLUSIONS: FC has been widely proposed as a filter to avoid unnecessary endoscopies. Nevertheless, it should not be considered as a marker of organic intestinal disease at all; rather it represents a marker of "neutrophilic intestinal inflammation". In IBD, more and larger studies are needed to confirm FC's capacity to correlate with IBD extent, to predict response to therapy and relapse, and the presence of a subclinical intestinal inflammation in asymptomatic first-degree relatives of patients. For NSAID enteropathy, the actual correlation between FC and endoscopic results needs further confirmation. Finally, as regarding other gastrointestinal conditions, available data are still insufficient to draw any final conclusion and further studies should be encouraged.

Key Words:
Fecal calprotectin, IBD, Intestinal inflammation.

Fecal Calprotectin: a General Overview

Calprotectin is a 36 kDa calcium and zinc binding protein, mainly derived from neutrophils, and to a lesser extent, from monocytes and reactive macrophages. It belongs to S100 proteins, a family of more than 20 calcium-binding proteins characterised by a tissue-specific expression pattern; in particular calprotectin represents a non-covalently associated complex of S100A8/S100A9 proteins[1]. Calprotectin accounts for approximately 60% of the total protein of the cytosol and comprises two heavy chains of 14 kDa and one light chain of 8 kDa[2]. It has a well-known antimicrobial activity, by competing for zinc and by inhibiting zinc dependent enzymes[3]. Elevated concentrations of calprotectin can be measured in plasma, synovial fluid, urine, liquor, saliva and feces when an inflammation process with recruitment of neutrophils is ongoing[4,5]. In particular, the presence of calprotectin in feces quantitatively relates to neutrophil migration towards the gastrointestinal tract[6] and fecal calprotectin concentrations (FFCs) closely correlated with the fecal excretion of ^{111}In-labelled leukocytes[7]. Therefore, it is considered a useful marker of intestinal inflammation[8], also because, unlikely other systemic inflammatory markers, like erythrocyte sedimentation rate (ESR) and C reactive protein (CRP), its levels seem to be unaffected by other causes of inflammation other than intestinal ones[1].

Calprotectin can be assayed in simple buffer extracts of small (5 g) fecal samples[2] and it shows an excellent stability in feces at room temperature for as long as a week[9,10]. Its quantification is inexpensive and easy and commonly performed by an a commercially available ELISA immunoassay. The original assay was first described by Roseth et al[8]; then, an improved assay was developed in 2000, and the units were

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changed from mg/l to μg/g[11]. In the last years, rapid, qualitative or semi-quantitative tests are suitable, showing still questionable results[12].

Up to now, an optimal cut-off has not been identified yet. The cut-off value differs according to the specific assay used and different cut-off values are suggested for varied patient categories, i.e., higher for patients with known inflammatory conditions while lower for screening purposes[13]. Nowadays, is commonly thought that concentrations of < 50 μg/g are normal; 50-100 μg/g represent a weakly positive test; concentrations of > 100 μg/g indicate a positive result[1,14].

In this review we tried to summarize the most recent evidences on faecal calprotectin, providing practical perspectives on its diagnostic and prognostic role in different gastroenterological conditions. For the present article we performed a MEDLINE search for all articles published on fecal calprotectin in human gastroenterology field up to December 2011. The search strategy for Medline was "fecal calprotectin". We restricted our search to studies published in English only. We chose evidences from well-designed and controlled studies when available. A meta-analysis was not performed because of the heterogeneity of the studies cited in this review, in terms of sample number, age, reference standard test, cut-off values, etc.

Fecal Calprotectin in Inflammatory Bowel Diseases (IBDs)

Physicians commonly apply a combination of symptoms, clinical examination, laboratory indices, radiology, and endoscopy with histology to make the diagnosis, to assess severity, and to predict the outcome of IBDs[6]. Endoscopy (and histology) remains the gold standard method for detecting and assessing bowel inflammation. Nevertheless, it has the disadvantage of being invasive, time consuming and not well tolerated by patients.

Within the last years various laboratory markers have been investigated in search to provide non invasive, cheap and rapid methods able to help in diagnosis and assessment of IBD activity. The most widely used laboratory parameters of inflammation, such ESR and CRP resulted not sufficiently specific or sensitive and poorly correlated with symptoms and disease activity index[1,15,16]. On the other hand, a series of studies indicate fecal calprotectin as the most useful marker able to quantify bowel acute inflammation[3,6,10,11,16].

Diagnosis

Fecal excretion of indium-labeled autologous granulocytes has been suggested for a long time as the gold standard test in assessing bowel inflammation in IBD. However, this technique is expensive, it involves exposure to radiation and a prolonged fecal collection[17]. Since FCCs are directly proportional to neutrophil migration towards the intestinal tract, a strong correlation between leukocyte excretion measured by ^{111}Indium and fecal calprotectin has been showed[6,18].

In the last years different studies have been carried out to evaluate diagnostic accuracy of fecal calprotectin in IBD patients in respect to IBS people or healthy controls[1,19-22]. Discriminating irritable bowel syndrome (IBS) from IBD, especially with mild disease activity, represents a common clinical challenge, since both conditions share a symptom complex with abdominal pain and altered bowel habits, and IBS-like symptoms are frequently reported in patients before the diagnosis of IBD[22].

Cut-off levels of fecal calprotectin able to discriminate between IBD and IBS were quite different among different works (generally 50 or 100 μg/g). Despite this heterogenicity, von Roon et al[23] performed a meta-analysis to evaluate the diagnostic accuracy of FC for IBD. Data from 9 studies comparing FCCs in IBD subjects in respect to IBS subjects or healthy controls, were analyzed, showing an overall sensitivity and specificity of fecal calprotectin of 95% and 91% respectively for the identification of patients with IBD, compared with those without. In particular FCCs were higher by 219.2 μg/g in patients with IBD compared with healthy controls. The analysis also demonstrated that at a cutoff of 100 μg/g, the diagnostic precision of FC for IBD appeared to be better than at a cutoff of 50 μg/g. In a similar more recent analysis including 670 adults with suspected IBD who performed endoscopy, van Rheenen et al[24] found that in adults the sensitivity and specificity of calprotectin was 0.93 and 0.96.

In general, the precision of FC for the diagnosis of IBD appears to be superior to serological markers such as CRP, ESR, anti-*Saccharomyces cerevisiae* antibody (ASCA), perinuclear antineutrophil cytoplasmic antibody (ANCA). In the meta-analysis by von Roon et al[23], four studies showed comparative values for CRP and ESR, showing a sensitivity of CRP ranging from 35% to 40%, and specificity ranging from 78-100%. For ESR, sensitivity ranged from 18% to 52%, and specificity was 78-100%. Another meta-analysis[25] including sixty

studies comprising 3.841 ulcerative colitis (UC) and 4.019 Crohn's disease (CD) patients found the combination of a positive ASCA test and a negative ANCA test to be 55% sensitive and 93% specific for diagnosing of CD, whereas a positive ANCA test alone was found to be 55% sensitive and 89% specific for diagnosing UC.

As for the adults, diagnostic accuracy of FC was also evaluated in in children. Diamanti et al[26] prospectively evaluated FCCs in a cohort of children with recurrent abdominal pain and altered bowel habits associated or not with other symptoms suggestive of IBD, showing that, compared to histology, the cut-off of 100 μg/g reached a sensitivity and specificity of 100% and 68% respectively, in IBD diagnosis. However, the cut-off value of 160 μg/g, produced the best joint estimate of sensitivity and specificity (100% and 80%, respectively). In von Roon's meta-analysis[23], diagnostic precision of FCCs for IBD diagnosis was even higher in children than adults using a cut-off of 50 μg/g, with a sensitivity of 83% and specificity of 85% (vs 71% and 80% in adults), while in Rheenen's meta-analysis[24] on 271 children and teenager, the sensitivity and specificity of calprotectin was 0.92 and 0.76, respectively (vs 0.93 and 0.96 in adults).

Therefore, it is possible that testing for fecal calprotectin could represents a useful screening tool for identifying patients who are most likely to need endoscopy for suspected IBD, substantially reducing the number of invasive procedures, as well as their associated cost. Just at this regards, a recent Swedish work by Mindemark et al[27] showed that FC pre-evaluation reduced demand for colonoscopies by 50% with the 50 μg/g and 67% with the 100 μg/g cut-off, corresponding to a cost-avoidance of approximately € 1.57 million and € 2.13 million, respectively.

However, due to false-negative test results, this approach would also delay diagnosis in about 6% of adults and 8% of children[24]. Moreover, as previously described, fecal calprotectin is not a specific test for IBD and levels can be increased in subjects with other conditions, such as acute gastrointestinal infection, and nonsteroidal anti-inflammatory drug (NSAID)-induced enteropathy. More recently, a new neutrophil specific protein S100/A12 has been reported to be an even more accurate fecal marker of inflammation than fecal calprotectin[28] As an example, in a study by Sidler et al[29] evaluating 61 presenting with gastrointestinal symptoms requiring endoscopy examination, faecal S100A12 was highly specific than fecal calprotectin (97% vs 67%) in distinguishing paediatric patients with IBD from children without IBD. It is probable that the different diagnostic accuracy of these two proteins could result from their different expression patterns, since S100/A12 is very specifically expressed in neutrophils, while calprotectin is also inducible in epithelial cells[28]. These results have to be confirmed by further studies.

Correlation with IBD activity

The assessment of IBD activity is based on a combination of symptoms, clinical findings, and endoscopy. However, there is often an insufficient correlation between these diagnostic elements. Considering that active inflammation in IBD patients is associated with an acute-phase reaction and migration of leukocytes to the gut, FCCs have been proposed as valid marker for monitoring IBD activity[30].

Different studies showed a close correlation between fecal calprotectin levels and the degree of IBD activity[1,3,11,20,30-33]. As an example, Sipponen et al[33] evaluated the correlation of fecal calprotectin with the Crohn's disease index of severity (CDEIS) in 77 CD patients, showing that fecal calprotectin could discriminate inactive from all the other activity groups (mild to moderate and highly active endoscopic disease). In particular, with a cut-off level of 200 μg/g for a raised fecal calprotectin concentration, sensitivity and specificity in predicting endoscopically active disease (CDEIS ≥ 3) were 70% and 92% respectively[33].

Calprotectin determination appears to better reflect disease activity than other serum biomarkers, such as CRP and blood leukocytes, and than clinical activity score. In a recent work by Schoepfer et al[30], 134 UC patients undergoing colonoscopy were prospectively enrolled and scored according the endoscopic and clinical section of the Rachmilewitz Index for activity monitoring. The overall accuracy for the detection of endoscopically active disease (score ≥ 4) was 89% for calprotectin (at a cut-off of 50 μg/g), 73% for Clinical Disease Activity Index (CDAI), 62% for elevated CRP, and 60% for leukocytosis[30]. As the same, when correlated to the Simple Endoscopic Score for Crohn's disease (SES-CD), the overall accuracy for detection of endoscopically active disease was 87% for calprotectin (at a cut-off 70 μg/g), 66% for elevated CRP, 54% for blood leukocytosis, and only 40% for the CDAI ≥ 150[34].

Also in pediatric population, fecal calprotectin correlated well with the histologic and endoscop-

ic grade of colonic inflammation[31,35-37]. Canani et al[35] found in 58 paediatric patients (26 CD, 32 UC) that fecal calprotectin showed a high correlation (r=0.655) with the histological grade of mucosal inflammation, and it resulted the most accurate tool (sensitivity 94%, specificity 64%) to detect the presence of active mucosal inflammation when compared to clinical scores and ESR (sensitivity 42%, specificity 68%) and CRP (sensitivity 44%, specificity 82%).

In conclusion, fecal calprotectin has been described as the more specific biomarker that could reliably discriminate inactive from mild, moderate, and high endoscopic activity and normalization of its levels represents an accurate indicator of endoscopic healing. Therefore, the role of fecal calprotectin in supporting endoscopic and histological evaluation in the assessment of IBD activity has been widely accepted, while clinical score presented the worst accuracy for detection of endoscopically active disease, mainly because it may improbably detect subclinical activity, frequently occurring in Crohn's disease[34].

Further studies should be encouraged to provide the more appropriate cut-offs.

Correlation with IBD Localization

Data of eventual differences of FCCs based on the localization of IBD are still few and controversial.

Sipponen et al[32] reported higher FCCs in colonic than in ileal Crohn's disease patient, but showing a lack of correlation among ileal, both endoscopic and histological findings, and fecal markers. It is likely that in this study the number of patients with purely ileal disease was limited (16 in respect to 66 with colonic or ileocolonic disease). However, it seems that the limited extent of ileal disease, although endoscopic and histological inflammation was present, resulted in lower fecal marker concentrations than predictable by endoscopic activity[32]. More recently, Jensen et al[38] evaluated FCCs in 40 CD patients undergoing endoscopic evaluation (13 with small bowel involvement, 15 with colonic and 11 with ileo-colonic one), showing similar FCCs in small bowel or colonic CD (median 890 mg/kg and 830 mg/kg, respectively).

Also in pediatric population, there are still controversial results on this topic. Fagerberg et al[37] showed in 39 IBD children undergoing colonscopy that FCCs correlated significantly both to the microscopic (Spearman ρ 0.71) and macroscopic extent (Spearman ρ 0.6) of colonic inflammation, defined as the number of colonic segments with a regional (histological) score ≥ 1 (possible range, 0-8). On the contrary, Bremner et al[39] showed no significantly different fecal calprotectin levels in ileal CD from those with colonic or ileocolonic disease.

Therefore, so far FCCs can not be used as a marker of localization of disease.

Response to Therapy

Recent studies suggest "mucosal healing", that is the absence of mucosal ulceration, as becoming the therapeutic target for treatment of IBDs[1,32-34]. Therefore, biological markers able to recognize the achievement of a mucosal healing should be necessary to avoid multiple and uncomfortable endoscopic examinations. In consideration of its close correlation with disease activity, FC has been suggested as a reliable marker able to assess response to treatment.

In a prospective study, Kolho et al[40] measured FCCs in 15 pediatric patients with IBDs receiving glucocorticoid therapy, showing that FCCs declined in line with clinical improvement, but seldom fell in normal ranges. This result suggested that although the achievement of a clinical remission, complete remission was more difficult, perhaps because of the persistence of a suclinical inflammation, as previously reported. Nevertheless, histological follow-up was not performed in this study and FCCs were only compared to clinical evaluation.

A following study by Sipponen et al[41] showed in 15 CD patients on TNF-α therapy that FCCs significantly declined in treatment responders and normalized in almost all those who reached endoscopic remission (scored by using the CDEIS). In particular, median FCCs fell from 1173 μg/g to 130 μg/g, and in the 5 patients who achieved endoscopic remission (CDEIS < 3), median FCCs declined from 1891 μg/g to 27 μg/g. Nevertheless, this study was designed to explore fecal markers only during the induction (0 and 8 weeks) therapy, leaving behaviour of these markers during maintenance treatment unanswered[41]. A few years later, the same Authors showed that in 19 adult CD patients needing enhancement therapy, FCCs significantly correlated with endoscopic score, measured at baseline and at 2-3 and 4-6 months, with endoscopic responders achieving normalization of fecal biomarkers, whereas remaining abnormal in the majority of endoscopic non-responders[42]. Moreover, in 90 patients with ASUC (acute severe UC), FCCs were significantly higher in patients who have failed medical therapy

and required emergency colectomy (1,200 *vs* 887,0 μg/g)[43].

More recently, Turner et al[44] prospectively evaluated 128 children suffering from severe UC who underwent intravenous steroid therapy and were followed up for 1 year after discharge. In this study, the Pediatric UC Activity Index (PUCAI) clinical score resulted as the best parameter able to predicte response when compared with other clinical scores (Travis, Seo and Lindgren indices), and both serum (C-reactive protein level) and fecal (FCCs) markers[43]. Moreover, Hämäläinen et al[45] showed that FCCs were more reliable than clinical activity indices or blood markers of inflammation in identifying response to TNF-α therapy in 36 pediatric IBD subjects. Nevertheless, a comparative endoscopic and histological evaluation was not performed in both the studies.

Up to now, available data appear still quite weak to support the role of fecal calprotectin as a promising surrogate marker of mucosal healing, reducing the need for endoscopic evaluation, since only few and on small smaple size studies have been carried out on this topic. Future larger studies are warranted to confirm these findings before widely adapting this approach to clinical practice.

Prediction of IBD Relapse

Inflammatory bowel diseases (IBD) are chronic intestinal disorders with a typically relapsing course. Disease flares occur in a random way and are often unpredictable. However, if a relapse could be reliably predicted, it would be possible treating with early treatment regimes[6].

It is thought that, even in cases of successful treatment, subclinical inflammation of the intestinal wall may persist, contributing significantly to the risk of relapses[15]. It is likely that most quiescent IBD patients have, to some extent, residual inflammation in intestinal mucosa, and symptomatic relapse probably occurs only when the inflammatory process reaches a critical threshold intensity[25]. The most widely used laboratory parameters of inflammation, such as the erythrocyte sedimentation rate (ESR) and C reactive protein (CRP), as well the clinical indices of disease activity, such as the CDAI, are not specific, reflecting a systemic host response rather than being specific for intestinal inflammation.

From different recent studies, fecal calprotectin appears as a promising marker able to predict IBD relapse[7,15,46-47]. Tibble et al[7] reported that in 80 IBD patients in clinical remission, basal calprotectin levels of 50 mg/g (≡ 250 μg/g) or more, predicted a 13-fold increased risk for relapse within a year. On the contrary, serum markers CRP and ESR resulted unable to predict IBD relapse[7]. In a following study, Costa et al[15] included 38 CD and 41 UC patients in remission, showing that a baseline level of calprotectin of 150 μg/g or more was predictive for a relapse in the next year. They found an high sensitivity for both CD (87%) and UC (89%), while specificity was much lower in the case of CD (43%) compared with UC (82%). Also in this study, ESR or CRP was not predictive of relapse[15]. It is probable that the different specificity of fecal calprotectin in predicting relapse in UC and CD could reflect differences in the inflammatory pattern of these two diseases, since UC clinical remission is more frequently accompanied by endoscopic and histological normalization than in CD[15]. D'Incà et al[46] showed in 97 patients with UC and 65 with CD in clinical remission, that a significant correlation emerged between a positive calprotectin test (at a cut of level of 130 mg/kg) and the probability of relapse within a year in UC patients, while in CD patients, this significant correlation was reported only for cases of colonic CD. In a more recent study, 66 patients with CD and 69 UC in clinical remission were prospectively evaluated for at least 3 months, showing values of FC significanly higher among the patients with relapse than in those that remained in remission (444 μg/g versus 112 μg/g)[47]. Conversely, Sipponen et al[48] reported in 72 IBD children in clinical remission that the predictive value of fecal calprotectin for an overt clinical relapse was low ranging from 0.396 to 0.429 for fecal calprotectin values > 100 μg/g or > 1000 μg/g, respectively, with a negative predictive value was 0.75 for values < 100 μg/g.

It should be considered that the cut-off value differs accordingly to the specific assay used and the results of these few studies are not directly comparable, also in consideration of the differences in patient selection, in the extent of disease and remission time. Therefore, although fecal calprotectin appears to have a good diagnostic precision in predicting IBD relapse, possibly more so in UC than in CD[15,23], further studies are necessary to confirm these results and to define the period of time between increase of fecal calprotectin and the occurrence of clinical relapse, as well as to establish the frequency of FCC determinations.

FCCs in IBD Patients Undergoing Bowel Resection

Crohn's patients undergoing bowel resection have a high risk of recurrence, with occurrence

of fever, diarrhea, abdominal pain and rectal bleeding. Extensive ileocolonic resections may result in diarrhea, bloating, and pain without recurrence of active disease. Therefore, a correct differential diagnosis between these two conditions is often difficult. To avoid multiple endoscopic examinations, the ideal assessment of the postoperative would be a reliable, non-invasive marker able to correlate both with symptom relapse and mucosal ulceration[49].

Scarpa et al[50] found that 63 CD patients undergoing ileocecal resection maintained high levels of FCCs (247 ± 22.7 ng/ml) during the follow-up even in case of clinical remission. This finding suggested that surgical resection does not remove all of the active disease and that, despite a clinical remission, there is an ongoing subclinical intestinal inflammation. In this study no significant difference in calprotectin levels was evident between patients who experienced a clinical recurrence compared to patients without recurrence as well as in patients diagnosed to have an anastomotic recurrence compared to those with a negative endoscopy. Moreover, serial measurements of fecal calprotectin from the time of surgery were not performed.

In a more recent work, Lamb et al[49] investigated FCCs in two CD patient cohorts having undergone ileocecal resection (13 patients followed prospectively for 1 year with FCCs and a second postoperative cohort of 104 patients provided a single stool sample within a period of median 24 months after resection). The Authors found a normalization of the fecal marker within 2 months postoperatively in cases with uncomplicated disease course. FCCs were elevated in a high proportion of CD patients with severe clinical activity, whereas low levels were measured in patients with clinically inactive disease. This fecal marker was more accurate at predicting clinical disease activity than CRP, platelet count, or endoscopic appearance. Nevertheless, an accurate follow-up was reserved only to a very small number of patients, and further studies involving larger sample size are not still available to confirm these results.

Ileal Pouch-anal Anastomosis Patients

Patients undergoing restorative proctocolectomy for UC have a high risk (about 40%) of developing pouchitis during the following years. The diagnosis requires both endoscopic and histological evidence of inflammation with neutrophilic infiltration of the pouch mucosa[14]. Very few data are available about role of FCCs in monitoring these patients. In a study by Thomas et al[51], all 9 patients with endoscopic and histologic evidence of pouch inflammation had elevated FCCs compared with only 2 of 15 patients with noninflamed pouches. More recently Johson et al[14] found in 54 patients who had undergone restorative proctocolectomy (46 patients with ulcerative colitis and 8 with familial adenomatous polyposis coli) that patients with pouchitis had significantly higher FFCs compared with those with uninflamed pouches. Moreover, FCCs closely correlated with the Pouch Disease Activity Index and endoscopic and histological inflammatory scores.

Therefore, available data are still insufficient to draw final conclusions and these preliminary data should be confirmed in larger and well designed studies.

FCCs in Asymptomatic Relatives of IBD Patients

The pathogenesis of IBD involves a complex interaction of genetic, environmental, and immunoregulatory factors. In particular, the importance of genetic susceptibility in IBD is well known. At present, a number of alterations, such as changes of intestinal permeability or immunological abnormalities (i.e., altered immunoglobulin secretion, autoantibody production, etc.), have been found in relatives of IBD-affected patients[52,53].

From this consideration, it has been suggested[54-56] that direct measurement of intestinal inflammation may be a more sensitive way of assessing the prevalence of subclinical intestinal abnormalities in relatives of patients with IBD. Significantly higher FCCs have been found in first-degree relatives of IBD patients in respect to healthy controls. In particular, a study by Thjodleifsson et al[54] showed increased FCCs in 49% of the first-degree relatives of patients with Crohn's disease, suggesting a high prevalence of subclinical inflammation in these subjects. More recently, our group[55] showed significantly greater FCCs in first-degree relatives of UC patients as compared with controls. Moreover, FCCs were significantly higher in a group of spouses than in controls and lower in respect to relatives, suggesting that both genetic and environmental factor may be responsible for fecal calprotectin changes. In a more recent study, Pham et al[56] measured fecal S100 A12 levels and FCCs in 13 children with CD and 36 siblings and 41 parents. They showed that fecal S100A12 levels in siblings and patients differed significantly from pediatric controls. Conversely, FCCs in siblings were lower than that of pediatric controls.

Further studies on a larger series of patients are necessary to clarify whether subclinical intestinal inflammation is the consequence of a genetic predisposition, of environmental factors, or the interaction of both. Follow-up trials could evaluate if FCC may identify relatives at risk of developing IBD.

Fecal Calprotectin in NSAID enteropathy

NSAIDs cause small bowel injuries in 20-65% of patients receiving these drugs[57]. Different techniques have been used to detect NSAID enteropathy: the recently introduced enteroscopy and capsule endoscopy, and other indirect methods, such as the assessment of intestinal inflammation by the ^{111}Indium labeled leukocyte technique or intestinal permeability tests, as well as fecal calprotectin measurement[58].

^{111}In white cell studies and/or enteroscopy are time consuming, expensive, cumbersome, and not widely available; on the other hand, permeability test does not represent a direct measure of intestinal inflammation[59]. Since the essential feature of NSAID enteropathy is the increased influx of neutrophils to the intestinal mucosa and subsequent excretion into the bowel lumen, measurement of fecal calprotectin has been suggested as a simple method for the diagnosis of NSAID induced enteropathy[60].

In the first study on this topic, Meling et al[61] reported a significant increase of FCCs with respect to baseline values in healthy volunteers taking indomethacin or naproxen for 14 days, while a 7 days treatment of lornoxicam failed to increase FCCs. In this work, shedding of fecal calprotectin significantly correlates to endoscopic assessment of the NSAID-induced gastroduodenal lesions. Interestingly, FCCs significantly increased only during the first week of treatment with indomethacin and naproxen and not during the following 7 days. Therefore, the possibility of an adaptation to NSAID-intestinal damage was suggested. Nevertheless, the sample was to small to draw any final conclusion in this respect and , when FCCs were evaluated in a consistent group of chronic NSAID-users, this last hypothesis was not longer confirmed.

In a more extensive work, Tibble et al[60] compared FCCs with the four day faecal excretion of ^{111}In labelled white cells in 47 patients taking NSAIDs showing a strong correlation between these two techniques. Moreover they also assessed the prevalence and severity of NSAID enteropathy by fecal calprotectin measurement in other 312 patients (192 with rheumatoid arthritis, 65 with osteoarthritis, 55 with other conditions) taking 18 different NSAIDs. FCCs were significantly higher in these patients than in 48 healthy controls[60], but, unlike Meling et al, the prevalence and severity of NSAID enteropathy was independent of the particular type of NSAID being taken. Nevertheless, a direct visualization of intestinal injuries by NSAID was not performed in any subject.

In the last few years, diagnosis of NSAID enteropathy has been improved by introduction of new endoscopic techniques, such as video capsule endoscopy (VCE) and double balloon enteroscopy (BE). Whether the detection ability is higher for fecal biomarkers or for endoscopy, is still an open matter. In a study by Maiden et al[59], after treatment with diclofenac show-release for 14 days, 75% of subjects had increased FCCs above the upper limit of normal, while capsule enteroscopy showed new pathology in 68% (mainly mucosal breaks, reddened folds, petechiae or red spots, denuded mucosa and blood in the lumen without a visualized source). However, no significant correlation was found between FCCs and capsule enteroscopy results, since those with mucosal breaks had the same increase in intestinal inflammation as those with no small bowel abnormality detected by the capsule. At this regard, the Authors suggested that some small bowel damage could be missed with the capsule method, but this remains questionable.

In a more recent study, Hawkey et al[62] assessed NSAID-small-bowel injury by VCE, fecal calprotectin and small intestinal permeability test in 139 subjects taking enaproxen plus omeprazole (n= 45), or lumiracoxib (n=47) or placebo (n=47) for 16 days, showing that, as assessed by 3 different measures, acute small-bowel injury on lumiracoxib treatment was significantly lower than with naproxen plus omeprazole, and similar to placebo.

Therefore, FC could represent a valid marker to detect NSAID enteropathy; nevertheless further studies are needed to clarify the actual correlation between fecal biomarkers and endoscopy in detection of NSAID enteropathy, and the possibility of real and clinically significant differences among NSAIDs.

Data on acetylsalicylic acid (ASA) induced intestinal injury are still controversial. An our previous study[63] demonstrated no significant changes in calprotectin excretion after administration of 100 mg of aspirin for cardiovascular prophylaxis in 22 patients. In a recent study by

Smecuol et al[64], FCCs significantly increase in 20 healthy volunteers after exposure to 100 mg of enteric-coated ASA, together with worsening of intestinal permeability and detection of new lesions at VCE examination. However, only 3 of 20 subjects exhibited post-ASA fecal calprotectin exceeding the upper normal limit. Further studies should clarify whether a more prolonged use of low-dose ASA will induce a greater increase of calprotectin levels and will have clinical implications in some patients, or whether chronic ASA use can induce an adaptation, limiting intestinal inflammatory reactions.

Fecal Calprotectin in Colorectal Cancer

Colorectal neoplasia (CRC) is one of the most common malignancies in the Western World. Survival rates are closely related to the stage of cancer at the time of diagnosis, so detection at an early stage could result in reducing mortality[65]. Colonoscopy remains the gold standard to detect benign or malignant tumors, but it does not represent a reliable primary screening tool, because of its invasiveness, the risk for procedure-related complications, the substantial expense and the low compliance rate, mainly due to unpleasant preparation. In contrast, stool testing has been suggested a non-invasive and relatively inexpensive screening approach[66].

The most widely used screening method for CRC is represented by fecal occult blood (FOB) testing, with a reported 15-30% reduction of mortality from CRC by testing asymptomatic persons for FOB[67]. Nevertheless, sensitivity of this test for detecting CRC is quite low (less than 30% for the most commonly used guaiac based FOB tests when testing strictly asymptomatic CRC)[66], probably because of the intermittence of blood loss from the tumour or fecal haemoglobin levels below the detection threshold (2-4 ml of blood/100 g stool)[68].

Fecal calprotectin has been suggested as another candidate fecal biomarker for CRC screening. Increased FCCs have been reported in patients with known CRC[66,68-72] showing increased FCCs in patients with known CRC and a lower intrasubject variability compared with fecal Hb levels[73]. Immunohistochemical examination of CRC specimens has shown reactivity confined to neutrophilic granulocytes with no reactivity seen in neoplastic cells[68]; therefore, it has been suggested that the high FCCs are probably due to polymorphonuclear cell recruitment and infiltration of the tumour in response to intraluminal antigens or chemotactic factors, with subsequent shedding into the intestinal lumen[66,68].

Different studies[68-72] evaluated the accuracy of fecal calprotectin as a CRC screening biomarker, mainly in comparison with FOB testing reporting not concordant results. Kronborg et al[69] found higher median FCCs among patients with adenoma and adenocarcinomas in respect to subjects with negative colonscopy. With a cut off limit of 10 mg/l (50 μg/g), the sensitivity of fecal calprotectin for cancer was 74% and for adenoma 43%. Tibble et al[68] compared fecal calprotectin levels among 96 healthy volunteers, 62 colorecal cancer patients, and 233 subjects undergoing colonoscopy with an increased risk for CRC. They found significantly higher FCCs in subjects with CRC and adenomatous polyps than in healthy volunteers. Sensitivity for detection of adenomatous polyps was 55% using the calprotectin method and 10% using fecal occult blood testing. The overall sensitivity and specificity of calprotectin for colorectal cancer and adenomatous polyps as a combined group was 79% and 72%, respectively, compared with a sensitivity and specificity of fecal occult blood of 43% and 92%. Nevertheless, increased FCCs were also found among subjects with non-neoplastic conditions. According to these results, both the Authors concluded that faecal calprotectin method represents a useful adjuvant to the investigation of patients at high risk for colorectal neoplasia. The same conclusion was sustained by Kristinsson et al[70] who evaluated FCCs in 237 first degree relatives from 148 patients with CRC, showing that FC test was more sensitive than FOB in detecting colorectal neoplasia but the specificity was lower.

It has to been considered that these studies have been performed in high risk individuals undergoing colonoscopy, rather than in asymptomatic subjects on a screening evaluation[68], and in the following years, these promising results were not confirmed by further studies involving larger e more heterogeneous sample size. Limburg et al[66] evaluated the diagnostic accuracy of fecal calprotectin in detecting colorectal neoplasia within a cohort of 412 patients at above average risk. They found not significant differences in FCCs between subjects with *versus* subjects without colorectal neoplasm, with a sensitivity of 37% and a specificity of 63% for any colorectal neoplasm. Subgroup analyses based on tumor size, number, anatomic site and histopathological features did not show significant differences, leading

to the conclusion that fecal calprotecin seems to have little, if any, potential utility in the context of CRC screening. In a Norvegian screening trial reporting analysis of fecal calprotectin in 2321 individuals[74], fecal calprotectin showed lower specificity and sensivity in respect to immunochemical FOB test, and no differences of FCCs have been reported among subjects with no adenoma, low risk adenoma, and high risk adenoma, whereas only the group with CRC showed significantly higher FCCs than each of the other groups. Using meta-analytical techniques, von Roon et al[23] reported that patients with CRC had not significantly higher FCCs compared with noncancer controls, with an estimated sensitivity and specificity of FC for the diagnosis of CRC of about 0.36 and 0.71, respectively.

For a screening test to be used appropriately it needs to be sensitive, specific, easy and acceptable for the target patients to comply with. At present, fecal calprotectin does not fulfill all of these criteria, therefore, it can not be considered as a specific marker of CRC and is not be recommended for population screening purpose. Moreover, an high proportion of false positive tests would create an unacceptably high workload for endoscopic laboratories[70].

Other Gastrointestinal Conditions

The presence of an inflammatory infiltrate has been described in the colonic mucosa of patients affected by diverticular disease (DD) rather than in healthy controls; moreover inflammatory grading seems to be related to the clinical severity of the disease[75]. Tursi et al[75] showed that FCCs were significantly increased in 16 subjects with acute uncomplicated diverticulitis and in 16 symptomatic uncomplicated DD, than in 16 healthy controls, 16 IBS patients and 16 with asymptomatic diverticulosis. Moreover, FCCs significantly correlated with inflammatory infiltrate (assessed by histological evaluation) and decreased after treatment to normal values both in acute uncomplicated diverticulitis and in symptomatic uncomplicated DD. Nevertheless, it is little probable that FC may be useful in differentiating symptomatic DD from IBD. Most patients affected by symptomatic DD or IBD may show the same symptoms (diarrhoea, abdominal pain, rectal bleeding, etc), but, at the same time, they both may show increased FCCs.

Celiac disease is an intestinal autoimmune disorder caused by intolerance to gluten-derived peptides of wheat, barley and rye. The typical histological lesion in patients with active celiac disease is characterized by striking changes in mucosal architectural, with absent villi and hyperplastic crypts, increased numbers of intraepithelial lymphocytes, plasma cells and lymphocytes in the lamina propria. Nevertheless, some studies have suggested a neutrophil-prevalent infiltration in the biopsies of celiac children and adults. Hallgren et al[76] demonstrated that neutrophil activity, as reflected by the measurement of myeloperoxidase of the small bowel, was increased in patients with celiac disease, reflecting an unspecific, enhanced turnover of granulocytes in the intestine without a defined pathophysiologic role. A few years ago, we evaluated FCCs in 28 untreated celiac patients[77], showing no significant differences from those in 30 controls. FCCs also did not significantly differ in relation to the level of clinical score, lesion severity or neutrophil infiltration; in particular, neutrophil granulocyte infiltration was present in only 4 out of 28 patients (14.3%), and was considered as "mild". Therefore, these results may reflect a low presence of neutrophils in duodenal biopsy samples, as commonly found in celiac disease. Conversely, a more recently study by Ertekin et al[78] performed in children, showed that mean FCCs of 29 newly diagnosed children with CD were significantly higher (13.40 mg/dl) than in healthy children (4.3 mg/L). Moreover, there was a significant statistical difference between untreated patients and those under GFD for 1 year, while there was no statistical difference between FCCs of those under GFD and healthy children. Nevertheless, no correlation between FCCs and neutrophilc infiltrate was found[78]. Therefore, additional studies on larger series have to been performed to confirm these findings, and to clarify the eventual differences between adults and children.

Up to now, no specific studies have been designed to evaluated FCCs in upper gastrointestinal tract diseases. The few available data on this topic can be only gathered from studies evaluating FCCs in different conditions throughout the gastro-intestinal tract. In this regard, only Summerton et al[79], in 2002, performed a study evaluating FCCs in different gastro-intestinal inflammatory and cancer conditions. In particular, in 26 patients showing upper-gastrointestinal inflammation due to gastritis and duodenitis, FCCs were in normal range. Nevertheless, a correlation between FCCs and histological severity of inflammation was not performed in this study. In a recent work[80] we showed that FCCs in 35 pa-

tients with chronic active gastritis were not significantly different in respect to FCCs both in 26 subjects with not active gastritis and in 74 healthy controls. Moreover, among patients with chronic active gastritis, FCCs did not significantly differ according to activity score. Therefore, it was recommend that in subject with high FCCs, causes of gut inflammation other than chronic gastritis should be checked.

Small intestinal bacterial overgrowth (SIBO) is defined by any condition in which the proximal part of the small bowel harbors for a long time > 10^5 bacteria/ml of the intestinal juice. Whether the presence of SIBO leads to small intestinal mucosal changes is not well-known. In an experimental blind loop syndrome in rats, changes of villus and crypt architecture and an increase in chronic inflammatory cells have been reported[81]. FCCs were evaluated in 40 consecutive patients with SIBO resulting positive to hydrogen glucose breath test, and 40 adult healthy volunteers[82]. FCCs in patients with SIBO were not significantly different compared to controls, suggesting that no subclinical intestinal inflammatory changes involving principally the neutrophils occur in SIBO. Therefore, it is likely that the presence of high FCC in patients with SIBO cannot be justified by the bacterial overgrowth itself and a search for other associated intestinal diseases might be appropriate.

The acute exposure of the intestinal mucosa to ethanol may result in structural and functional intestinal injuries, such as infiltration of inflammatory cells in the lamina propria[83]. In chronic alcohol consumers a reduced both villous height and a mucosal surface area of villi, an increased number of intra-epithelial mononuclear cells, globet cells hyperplasia and gastric metaplasia have been reported[83]. Increased levels of FCCs in alcoholics and their normalization after a period of alcohol abstinence have been described. Nevertheless, these data were reported only in an abstract[84]. Our recent study[85] showed that FCCs are not significantly increased in chronic alcoholics with respect to healthy controls, concordantly with literature data not showing the presence of a conspicuous neutrophilc intestinal infiltrate in these subjects.

Conclusions

Many and heterogeneous studies have been performed in order to evaluate role of fecal calprotectin in different gastrointestinal conditions. A good diagnostic precision for separating organic and functional intestinal diseases has been widely reported, thus leading to propose fecal calprotectin as a filter to avoid unnecessary endoscopies. Nevertheless, it should not be considered as a marker of organic intestinal disease at all; rather it represents a marker of "neutrophilic intestinal inflammation". On the other side, high FCCs could represent a strong motivation to carry out a colonoscopy in order to rule out presence of IBD or other organic pathologies.

So far, the most relevant data derived from IBD subjects, since the considerable number of large sample size and well conducted studies. FCCs better correlate with IBD activity (more in UC than in CD patients) than the other classically recommended inflammatory parameters (ESR, CRP) and normalization of FCCs represents an accurate indicators of endoscopic healing. Conversely, more and larger studies are needed to confirm fecal calprotectin's capacity to correlate with IBD extent, or to predict response to therapy and IBD relapse. High FCCs found in asymptomatic first-degree relatives of patients with IBD could suggest the presence of a subclinical intestinal inflammation in these subjects, but data are not still consistent.

Fecal calprotectin has been suggested a valid marker to detect NSAID enteropathy; nevertheless, the actual correlation between fecal biomarkers and endoscopy in detection of NSAID enteropathy need further confirmation, since the recently introduction of new specific and more accurate diagnostic techniques, like enteroscopy and capsule endoscopy.

Studies aimed to evaluated the role of FCCs in CRC screening, mainly in respect to FOB, show conflicting results, and, nowadays, this fecal marker can not be recommended for population screening purpose.

Finally, as regarding the role of fecal calprotectin in other gastrointestinal conditions (celiac disease, gastritis, SIBO, alcohol enteropathy and diverticulitis), only few and small sample studies have been still performed, and available data are still insufficient to draw any final conclusion.

Conflict of Interest

None to declare

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