Fecal calprotectin

Overview

Calprotectin (CP) is an abundant, widespread protein in the human body. It binds calcium and belongs in the S100 family. It is mainly found in neutrophilic polymorphonuclear (PMN) leukocytes (neutrophils), and to a lesser extent in monocytes and reactive macrophages (1) CP represents approximately 5% of total protein contents in neutrophils, and 60% of total protein contents in their cytoplasm (2).

It has obvious bacteriostatic and fungicide properties, and its plasma levels develop 5- to 40-fold increases in the presence of infection and/or inflammation (3).

It is also present in the feces, and fecal calprotectin (FCP) concentration is much higher than plasma calprotectin levels (approximately by 6-fold) (4).

Increased CP levels are found in the feces of patients with various bowel inflammatory conditions, both localized in the small bowel and anywhere in the colon (5-7).

FCP measurement method and reference values

In 1992 Roseth et al. (2) developed the first FCP measurement method using ELISA. Since then it has been improved and extensively validated, and very small fecal samples are used (0.1 g), which are mixed with a buffer solution in a 5-mL tube (8). Results show a very good correlation to 3-day fecal excretion of granulocytes labeled with a radionuclide such as indium⁹⁹ (9).

New methods express FCP concentration as micrograms per gram, whereas these were previously expressed as milligrams per liter.

CP is a highly stable protein resistant to proteolytic fecal degradation. Samples may be stored for up to 5 days at room temperature with no apparent concentration loss, and may be shipped by mail without refrigeration.

The upper normal limit is established at 50 µg/g. Reference values for children are similar to those found in adults; however, interestingly, healthy children in their first year of life exhibit values up to 10-fold higher than normal for older children, and no clear explanation thereof has been reported (10).
Usefulness of biological markers in inflammatory bowel disease

So-called “biological activity markers” or “acute phase reactants” have been used for decades in the study and follow-up of patients with inflammatory bowel disease (IBD) with a number of goals, including diagnosis, clinical outcome monitoring, differential diagnosis with other bowel conditions, and therapy effectiveness monitoring.

The advent of biological therapies in IBD has renewed interest in marker use, particularly in the use C-reactive protein (CRP), which may help select patients who will respond to therapy.

CRP is the most widely studied marker among acute phase reactants and has offered excellent results, as is an objective inflammation marker and correlates to activity extent both in Crohn’s disease (CD) and ulcerative colitis (UC). It also has a short half-life, and thus increases early in the course of inflammation and then decreases with it. Its measurement is cheap, straightforward and fast using a commercial ELISA test, which may be performed even at the emergency room. The presence of increased CRP levels is associated with a better response to biological therapies; in contrast, normal levels predict a better response with placebo.

However, despite potential advantages, routine CRP and other marker testing has also disadvantages. For instance, CRP has been seen to better relate to inflammatory bowel disease activity in patients with CD versus UC (11).

Other laboratory markers, including erythrocyte sedimentation rate (ESR), white blood cell and platelet counts, and serum albumin and alpha-1 acid glycoprotein (orosomucoid) levels, have been studied in IBD and are usually less useful than CRP. Having a very long half-life, ESR has a number of shortcomings including the fact that it is influenced by many factors such as age, presence of anemia or polycythemia, serum levels of selected proteins, and even smoking and various drugs; hence its measurement cannot compare to CRP in terms of sensitivity or specificity for inflammatory conditions (12,13).

Fecal markers seem more promising and specific for the identification of bowel inflammation in patients with established IBD. Promising results have been reported with the use of fecal calprotectin (FCP) both in patients with CD and UC. However, recent data suggest that, in contrast to CRP, the effectiveness of FCP has a greater diagnostic sensitivity in patients with UC versus CD.

Taken together, biological markers for inflammatory activity are all useful tests that should be considered in the study and management of patients with IBD. However, in the absence of further information, their use should be considered an additional tool for patients with IBD, and added to clinical observation, physical examination, and other lab test findings, as well as imaging, endoscopic and biopsy findings, when possible and needed; their isolated use will never suffice or ultimately replace a full, thorough exploration with most of the diagnostic aids that are currently available.

Some of these new serologic methods for IBD seem to have a higher association with complicated disease, as has been recently reported in a large study (14).
Organic versus functional intestinal conditions

A common diagnostic difficulty is telling mild-to-moderate patients with various inflammatory intestinal conditions from those with no organic illness.

While data obtained through history-taking, as well as from physical examination and laboratory findings, are often revealing, many patients need digestive endoscopy to confirm or exclude this diagnosis.

In this respect fecal calprotectin, a marker reflecting the presence and extent of bowel inflammation, is a measurement that has proven useful for differential diagnosis and patient follow-up, albeit its discriminating diagnostic power is not enough to differentiate all cases.

At least 2 prospective studies in adult patients have looked at these aspects. In two of them patients were referred to tell IBD apart from functional disease, mainly irritable bowel syndrome (IBS) (15,16). We also reviewed two additional such studies in children (17,18).

Tibble et al. studied 220 consecutive adult patients to distinguish between IBD and IBS. Using FCP with a 10-mg/L discrimination level, sensitivity was 82% and specificity was 83% (15).

Another study by these same authors measured fecal calprotectin concentrations in 602 adults: 263 (44%) were categorized as with organic disease, and 339 (56%) as with functional disorders. FCP sensitivity and specificity for a threshold of 10 mg/L were 89 and 79%, respectively (16).

Studies in children have found better positive and negative predictive values when compared to those of adults, maybe related to smaller sample sizes; such results should be corroborated by other authors including in their series a greater number of patients (17,18).

In the present issue of our journal, Bonnín-Tomáš et al. (19) measured FCP values in a series of 42 children, and compared their usefulness for diagnosis and follow-up regarding the distinction between organic and functional disease; they concluded that this marker is sensitive but nonspecific, and allows a selection of patients with IBD who require colonoscopy for a definite diagnosis, thus decreasing or preventing colonoscopy use in patients with functional gastrointestinal disease in view of the inconveniences and difficulties entailed by this technique in pediatric subjects.

In this same respect other studies were previously performed with this aim, which had closely similar results (20).

A higher discriminating value to separate inflammatory from functional conditions is obtained when FCP threshold values are increased to 100 µg/g, as some authors recently confirmed (21).

Other intestinal and extraintestinal disorders

In patients with microscopic colitis several studies have shown FCP values that were usually not as much increased as in IBD, both in children and adults (22).
In patients with celiac disease diagnosed at any age increased FCP levels are seen in around 50% of patients, more commonly in the presence of active disease, which gradually decline after 1 month on a gluten-free diet (23).

However, a study recently reported by Montalvo et al. (24) in 22 untreated celiac adult patients found no significant differences in FCP levels versus control subjects.

FCP is also elevated in extraintestinal conditions such as liver cirrhosis. Thus, in a study performed in 53 patients the authors found that FCP levels were increased in all patients, with values paralleling Child-Pugh functional stage \( p < 0.001 \) and a trend towards higher concentrations in patients with alcohol-related cirrhosis \( p = 0.1 \). All this may be related to some degree of associated intestinal inflammation, probably as a consequence of accompanying bacterial translocation (25).

High FCP levels are found in patients with colorectal carcinoma, and also in subjects who recently received NSAIDs even in the presence of normal colonoscopy, as recently described by García-Sánchez et al. at Hospital Reina Sofía, Córdoba, Spain (26).

It should be borne in mind that in children on corticoids for IBD FCP levels gradually decrease with clinical improvement, and remain high for up to several weeks after treatment completion, which suggests prolonged silent inflammation after said clinical improvement (27).

Following complete colonic and rectal resection with ileo-anal anastomosis, 10-40% of patients develop chronic pouch inflammation, both in adults and children (28,29).

Regular FCP measurements have proven useful to monitor pouchitis, as have other similar markers such as fecal lactoferrin (30,31).

Final comments and conclusions

FCP measurement was not intended as a marker of organic intestinal disease but rather as a parameter to measure neutrophilic intestinal inflammation.

Many organic bowel conditions, including celiac disease, colonic diverticulosis, and colon carcinoma, have shown highly variable results regarding this marker (32,33).

Therefore, a negative FCP measurement should not be interpreted as absence of organic bowel disease (at least in the adult), but rather would suggest the absence of intestinal inflammation by polynuclear neutrophils.

This is useful to differentiate IBD from IBS. It is also useful when symptoms in a patient with a known inflammatory bowel condition result from disease reactivation or other disorders, and also helps assess treatment response for the various drugs used in these diseases.

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References


Editorial