Fecal calprotectin as a biomarker to distinguish between organic and functional gastrointestinal disease

A. Bonnín Tomàs, M. Vila Vidal and A. Rosell Camps

Biochemistry Section. Clinical Analysis Service. Gastroenterology Unit. Pediatric Service. Son Dureta University Hospital. Palma de Mallorca, Spain

RESUMEN

Introducción: la determinación de calprotectina en heces se está afianzando en los últimos años como un marcador no invasivo para el diagnóstico diferencial entre patología gastrointestinal orgánica y funcional. Su uso es útil sobre todo en niños que requieren anestesia general para una colonoscopia. El objetivo de este estudio es evaluar la sensibilidad y utilidad de la calprotectina fecal (CPF) en pacientes pediátricos con signos y síntomas sugestivos de enfermedad inflamatoria intestinal (EI1) con el fin de evitar técnicas invasivas innecesarias y poder discriminar entre patología gastrointestinal orgánica y funcional.

Material y métodos: se determinó la concentración de calprotectina mediante enzimoinmunoanálisis en una única muestra de heces de 47 niños (edad media: 10.1 años) con algún síntoma de patología gastrointestinal sugestivo de organicidad. Trece niños fueron diagnosticados de patología funcional y 34 de patología orgánica. Entre estos, 15 con EI1 y el resto con patologías orgánicas de distinto origen (no-EI1). Se incluyeron 13 niños sanos como controles.

Resultados: el grupo de niños con EI1 presentó valores de CPF (mediana [rango intercuartil]: 1.219 µg/g [322.2-967]) significativamente más altos que el grupo con patología gastrointestinal funcional [20 µg/g (16-25); p < 0.0001], el grupo con patología orgánica no-EI1 [113 µg/g (36-193); p = 0.002] y el control [25 µg/g (19-32); p < 0.0001]. Las concentraciones también fueron más altas en el grupo de niños con patología orgánica no-EI1 respecto al grupo con patología funcional (p = 0.002) y al control (p = 0.004). No hubo diferencias entre el grupo control y los niños con patología funcional (p = 0.264).

Discusión: la CPF es un marcador sensible, pero no específico, que permite seleccionar pacientes con EI1, que requieren colonoscopia para el diagnóstico definitivo y evitar así pruebas invasivas a pacientes con patología gastrointestinal funcional.


ABSTRACT

Introduction: there is growing evidence showing the importance of the fecal calprotectin assay in differentiating organic from functional gastrointestinal disease. It is a simple, non-invasive biomarker that is especially useful in children, who may require general anesthesia for colonoscopy. The aim of this study was to assess the use and sensitivity of fecal calprotectin (FCP) in pediatric patients with signs and symptoms of IBD to avoid unnecessary invasive techniques and to distinguish between organic and functional gastrointestinal pathology.

Material and methods: a single stool sample was collected from 47 children (mean age: 10.1 years) referred for non-specific gastrointestinal symptoms suggestive of organicity. On the basis of clinical criteria 13 children had functional bowel disorders and 34 had organic gastrointestinal disease, 15 with IBD and 19 with other organic (non-IBD) gastrointestinal conditions. Thirty healthy children were included as controls. Calprotectin concentrations were measured by enzyme immunoassay.

Results: children with IBD had FCP levels [median (interquartile range); 1.219 µg/g (322.2-967 µg/g)] higher than children with functional gastrointestinal disease [20 µg/g (16-25 µg/g); p < 0.0001], those with organic non-IBD disease [113 µg/g (36-193 µg/g); p = 0.002], and healthy children [25 µg/g (19-32.5 µg/g); p < 0.0001]. Fecal calprotectin concentration also was significantly higher in children with organic (non-IBD) disease as compared to controls (p = 0.004) and children with functional pathology (p = 0.002). FCP levels were similar in controls and children with functional gastrointestinal disease (p = 0.264).

Discussion: FCP is a sensitive, but not disease-specific, marker to identify patients with IBD who should undergo diagnostic colonoscopy, and to avoid unnecessary invasive procedures in patients with functional gastrointestinal disorders.

Key words: Fecal calprotectin. Inflammatory bowel disease. Functional gastrointestinal diseases. Pediatric.
INTRODUCTION

Functional abdominal pain is a frequent cause of primary care visits and gastroenterology consultations that on some occasions even requires hospitalization. The main challenge is to differentiate it from an organic pathology, which may mean multiple diagnostic tests with the risks that these may entail from their side effects and with loss of school hours for children and work hours for their parents.

Many clinical symptoms present in functional abdominal pain are also common in other organic disorders, in particular inflammatory bowel disease (IBD) (1). When there is suspicion of IBD, it is important to carry out a scintigraphy with labeled leukocytes and a colonoscopy and biopsy in order to confirm the diagnosis and to establish the inflammation extent. This is an invasive technique with an inherent risk of complications and radiation, and general anesthesia is needed when used on children. The classic biochemical parameters of inflammation, ESR (erythrocyte sedimentation rate) and C-reactive protein, lack diagnostic specificity and sensitivity (2). In recent years calprotectin in feces is becoming a new useful marker for intestinal inflammatory pathology (3-7). Various studies have also shown that there is an association between calprotectin levels and inflammation degree; as a consequence, this could be used to monitor treatment response and to predict risk of relapse (8-12).

Calprotectin is a 36.5-kD calcium and zinc binding protein in the S100 family. Its molecular structure consists of a heterotrimer made up with 2 heavy chains and 1 non-glycosylated light chain. This protein constitutes approximately 60% of cytosol soluble proteins in neutrophils, and it is also abundant in monocytes and macrophages (13).

Although its biological function is not completely known, it may play an important role in the regulation of inflammation and in neoplastic processes. It appears to exert a protective function in inflammatory and infectious conditions, and also has anti-proliferative activity. However, an excessive increment may induce cell damage (14). Its concentration has been measured in plasma and other biological fluids (saliva, CSF, sinovial fluid), as well as in the feces, where it is most concentrated. High levels may be seen in multiple pathological conditions, both inflammatory (cystic fibrosis, rheumatoid arthritis, Crohn’s disease, ulcerative colitis, bacterial infections) and neoplastic (colorectal cancer) (15).

In the gastrointestinal tract inflammatory conditions of varying etiology increase mucosal permeability, thus inducing the migration of granulocytes and monocytes towards the intestinal lumen (15). The subsequent activation and death of these cells result in the release of large amounts of calprotectin (16), which is excreted in the feces. Calprotectin measurement in the feces exhibits a good correlation with the fecal excretion of In-111-labeled leukocytes, considered the gold standard for measuring inflammatory bowel activity (9).

When bound to calcium calprotectin has a high resistance to heat and metabolic degradation bacterial enzymes and intestinal proteases (8,17). These properties allow it to be eliminated intact in the feces, and give it an advantage as a non-invasive biochemical marker for the screening of intestinal inflammation over other markers used (lactoferrin, neutrophil elastase, or leukocyte esterase).

Nowadays an improved assay is available to determine calprotectin using ELISA (18). When compared to the original it requires smaller samples, and increases the performance of the extraction solution. It is a simple, sensitive, accurate, reproducible, and cheaper technique.

Various studies have been carried out in adults and in children with gastrointestinal diseases, but results are frequently not comparable due to the use of distinct methodologies with distinct units and sensitivities. With the original ELISA method a higher reference limit of 10 mg/L was considered, but with the improved method the limit was established at 50 µg/g of feces.

The objective of this study was to evaluate the use and sensitivity of FCP in pediatric patients with signs and symptoms suggestive of IBD with the aim of avoiding unnecessary invasive techniques, and to be able to discriminate between organic and functional GI pathology.

MATERIAL AND METHODS

A retrospective study (from 2003-2005) in 47 patients with gastrointestinal symptoms who had been referred to the Pediatric Gastroenterology Unit of Son Dureta University Hospital (Palma de Mallorca), and in those who had had fecal calprotectin ordered on presenting with signs and symptoms suggestive of organic pathology (intense abdominal pain, chronic diarrhea, weight loss, rectal bleeding). Mean age of children was 10.1 years, with ages ranging from 3 months to 15.3 years. Following clinical criteria, laboratory, image and endoscopic test results, in relation with their evolution, and complying with the functional pathology criteria established in the Rome II meeting (19), thirteen children were diagnosed with functional pathology and thirty-four with organic pathology. Among the latter group 15 had inflammatory bowel disease (IBD): 3 had ulcerative colitis (UC) and 12 Crohn’s disease (CD); the remaining subjects had organic conditions of varying origin (infectious colitis, alimentary allergies, etc.). Thirteen healthy children were included as controls, relatives of laboratory staff who consented to the study.

A single stool sample was collected from each patient in a plastic container, which was sent to the laboratory in less than 48 hours; samples were then frozen at -70 ºC until analyzed. Calprotectin is stable in the feces at room temperature for at least 72 hours, and for more than 6
months when frozen. Samples need not be homogenized, as they show no variability between different takes (18). Samples were prepared and analyzed in accordance following manufacturer instructions (Calprest, Eurospital, Trieste, Italy).

Calprotectin concentrations were calculated from a 4th-order polynomial regression curve obtained with standard kits (6.25, 12.5, 25, 50, 100 ng/mL). Results were expressed in µg/g. Samples exceeding the technique’s linearity, with absorbance readings greater than those of the maximum concentration calibrator, were diluted as indicated by the manufacturer as follows: 1:100, 1:200, and up to 1:500. Calprotectin can be frozen and unfrozen up to 4 times without changes. The lower limit of detection is 15.6 µg/g.

We used the SPSS v.11 for Windows for the statistical analysis of data. A Kolmogorov-Smirnov test showed that calprotectin concentration values do not follow a normal distribution. Therefore, results were expressed in median values and interquartile range. Comparisons between groups were performed using the Mann-Whitney U statistical test. We assumed a statistically significant difference when *p* < 0.05.

**RESULTS**

The results obtained for FCP concentrations in the various groups are shown in table I. Not included in table I are 2 patients with Crohn’s disease (CD), a patient with ulcerative colitis, and another child with multiple allergies, whose calprotectin values exceeded the technique’s linearity with concentrations > 250 µg/g with us being unable to carry out greater dilutions.

The group of children with IBD had FCP values significantly higher than those of children with functional gastrointestinal pathology (*p* < 0.0001) and non-IBD organic pathology (*p* = 0.002), or than healthy control children (*p* < 0.0001). Concentrations were also significantly higher in the group of children with other types of organic pathology (non IBD) with respect to the group with functional pathology (*p* = 0.002) and with healthy children (*p* = 0.004). On the other hand, there were no differences in calprotectin concentrations between healthy children and children with gastrointestinal pathology of functional origin (*p* = 0.264).

Figure 1 represents the distribution of calprotectin values in each of the groups. No child diagnosed with IBD had PCF values lower than 50 µg/g; the lowest value was 91.9 µg/g in a child with CD. In the functional pathology group only one child with recurring abdominal pain had a calprotectin level of 137.5 µg/g, and in the rest it was < 50 µg/g. Calprotectin levels were > 50 µg/g in 13 out of 19 children (68.4%) with organic pathology of etiology other than IBD. Within this group we found levels > 200 µg/g in 4 children (21.0%): One with allergies to multiple foods (> 250 µg/g), another one with celiac disease and chronic diarrhea from dietetic transgressions (354.7 µg/g), one with rectal bleeding and normal colonoscopy with probable anal fissure (1,264.2 µg/g), and in one child with IPEX syndrome (4,011.1 µg/g) who, of all patients, had the highest calprotectin value. With values between 50-200 µg/g two children were included with gastritis from *Helicobacter pylori*, two with allergy to cow milk proteins and other foods, one with intolerance to lactose, one with biliary lithiasis, one child with Hirschprung’s disease, one with Wiscott-Aldrich syndrome and chronic diarrhea, and one with idiopathic gastritis. In the IBD group calprotectin levels were > 200 µg/g in 12 of the 15 children studied (80%).

**Table I. Median, range, and 95th percentile for fecal calprotectin (µg/g) in patients with inflammatory bowel disease (IBD), organic gastrointestinal pathology (non IBD), functional gastrointestinal pathology, and the control group**

<table>
<thead>
<tr>
<th></th>
<th>IBD</th>
<th>Organic (non IBD)</th>
<th>Functional</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>12</td>
<td>18</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Median</td>
<td>1,218.9</td>
<td>112.6</td>
<td>20.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>321.8-2,966.8</td>
<td>36.5-193.1</td>
<td>15.6-25.4</td>
<td>19.2-32.5</td>
</tr>
<tr>
<td>95th percentile</td>
<td>3,797.0</td>
<td>4,011.1</td>
<td>137.5</td>
<td>61.3</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In general medical practice there is a need for a simple test that helps select patients with chronic diarrhea or chronic abdominal pain in need of more complex diag-
nostic tests. Colonoscopy is the reference method for the diagnosis of inflammatory bowel disease, but it is an expensive, invasive technique that cannot be frequently repeated. If we suspect IBD with normal colonoscopy, then there is video-enteroscopy available. However, it is an invasive method with little experience in children and unavailable in a majority of centers (20). The use of a non-invasive, reliable, simple and repeatable marker is especially important in children (21), as general anesthesia is required to carry out a colonoscopy (9).

Our study supports the results of previous studies in children and adults (5,18,22) that show how a sole measurement of FCP can help gastroenterologists in differentiating between IBD and other gastrointestinal symptoms of functional etiology. Taking 50 μg/g as the upper reference limit, our data confer a sensitivity of 100% and a specificity of 92% in discriminating between IBD and other functional gastrointestinal pathology. The only child with functional abdominal pain and 137 μg/g of FCP returned to normal concentrations in a second determination (15.6 μg/g) not included in the study after 4 months. On the other hand, patients with confirmed IBD maintained elevated levels. However, to optimize the test’s diagnostic efficiency some studies postulate the upper limit of reference calprotectin concentrations is closer to 100 μg/g (6). A recent study shows an optimum cut off point of 217 μg/g with the aim of discriminating adult patients with organic colon disease (4,23). The manufacturer establishes the margin values between 50 and 100 μg/g. If we consider that the main utility of this test is to select patients requiring invasive diagnostic tests, the use of a lower cut off point confers greater diagnostic sensitivity for IBD, but reduces specificity.

Reference values in healthy children between 4 and 17 years of age (24) have been established at below 50 μg/g in accordance with the limit indicated in the assay. However, other studies show calprotectin values higher than this limit in healthy control children (6,25). In our control group a seven-year-old child had a calprotectin concentration of 61.3 μg/g. On the other hand, distinct reference values for fecal calprotectin have been proposed in the newly born. During the first days of life, with increased intestinal permeability, calprotectin values are higher (25,26) than in older children or adults. Higher intestinal permeability is related to higher transepithelial neutrophil migration, as observed in adults with IBD.

Even though the study was carried out with a reduced number of patients, results confer FCP measurement utility as a candidate test for selecting patients with IBD, although from the beginning of its application it has proven nonspecific for this type of organic intestinal pathology. Apart from IBD, pathologies of varying etiology such as infections, food allergies, cystic fibrosis, neoplasms, etc., or treatment with nonsteroidal anti-inflammatory, can give elevated calprotectin results (4,14). In our data series, calprotectin concentrations were significantly higher in the group of children with IBD when compared to the group with other types of organic pathology. Even so, our results do not allow us to establish calprotectin values that discriminate between both groups of diseases. FCP may serve as a first link when clinical manifestations do not allow differentiating patients with possible organic gastrointestinal pathology, passing on to a second level with more expensive, complex, and invasive explorations.

Scintigraphy with leukocytes labeled with radionuclides is the reference technique for measuring intestinal inflammatory activity, but the method is expensive, emits radiation, and is not available in all centers (27). Fecal calprotectin levels have a good correlation with this reference technique, and therefore with intestinal inflammation degree. For this reason it may be used to monitor response to treatment (8,9). However, calprotectin levels often exceed the technique’s linearity. This means that we must dilute samples further, meaning that it becomes more expensive and delays results and diagnosis. The manufacturer proposes the use of calprotectin only as a screening test for the diagnosis of IBD. Recently, a new immunofluorometric assay has been published that solves this technique’s limitation, as it quadruplicates the range of ELISA, and thus reduces the number of samples with concentrations higher that the average range from 30 to 4% (28).

In conclusion, we consider that FCP is a rapid, reliable, and reproducible technique that allows the selection of patients with nonspecific gastrointestinal symptoms who should be submitted to invasive and more costly techniques, and thus it may be used as a routine, non-invasive biochemical marker for the diagnosis and follow-up of patients with IBD, as well as the technique of choice in the differential diagnosis between functional and organic gastrointestinal conditions.

Despite all this, more comprehensive studies are needed with a higher number of patients, above all to reach consensus regarding a cut-off point that would be most useful in discriminating patients.

REFERENCES


