Diagnostic utility of fecal calprotectin in chronic diarrhea of bacterial etiology in pediatric patients

Utilidad diagnóstica de la calprotectina fecal en la diarrea crónica de etiología bacteriana en pacientes pediátricos

Dear Editor:

Chronic diarrhea is defined as a diarrheal process that lasts more than 14 days and that may be associated, although not exclusively, with an infectious cause (persistent diarrhea). It is a relatively common problem in pediatric age, originating approximately 2–5% of pediatric consultations, and it is generally of functional origin.[1,2]

In this context, it is important to assess the inflammatory status and the quantification of fecal calprotectin is a useful, simple, and non-invasive test that can rule out intestinal inflammation in children with gastrointestinal symptoms. However, diagnostic accuracy of fecal calprotectin has not yet been adequately evaluated in pediatric persistent diarrhea.[3]

We report the diagnostic utility of fecal calprotectin in chronic diarrhea of bacterial etiology in pediatric patients. Seven hundred and seventy-seven patients (52.3% males, 47.7% females) with a diarrheal process lasting more than 14 days and aged between 1 and 14 years (median: 6 years) were included. This study met ethical recommendations of the Declaration of Helsinki[4] and it was approved by the Sevilla Sur Research Ethics Committee (No.1569-M1-17).

Calprotectin was quantified by fluorescence enzyme immunoassay in a Phadia®100 analyzer (Thermo Fisher Scientific, Germany). A laboratory study of infectious etiology was carried out by molecular techniques (LightMix® Modular-Gastro-Bacteria-Parasite-Virus Multiplex Testing. Roche, USA). Data were processed using R-software (R-Core Team, 2014) and MedCalc 13.0 software (Ostend, Belgium).

The results showed that in 11.3% of patients an infectious cause was detected and in 12.2% an inflammatory process was detected, although, in many cases, these processes were not isolated findings, but they were interrelated. Non-infectious causes were detected in 693 patients with a median calprotectin of 12 μg/g (median absolute deviation (MAD): 2; confidence interval (CI) 95%: 10–14.2) (Fig. 1a). Regarding cases in which an infectious origin of diarrhea was found, bacteria of clinical interest were detected in 31 patients (4%) with a median calprotectin of 123 μg/g (MAD: 44; CI95%: 95–218) (Fig. 1): the most prevalent bacteria were *Campylobacter* spp. with 20 cases (64.5%), followed by *Salmonella* spp. with 11 cases (35.5%). The presence of parasites was detected in 55 patients (7.1%) with a median calprotectin of 10 μg/g (MAD: 0; CI95%: 10–19) (Fig. 1a); in 22 cases (40%) the parasites had a proven clinical value (*Giardia* spp., *Cryptosporidium* spp.) while in 33 cases (60%) the parasites were of uncertain clinical significance (*Blastocystis* spp., *Dientamoeba* spp.) (Fig. 1a). Viral etiology was not very prevalent, being detected in 2 patients (0.3%), and both corresponding to an infection by adenovirus. Furthermore, in 4 patients, there was a concomitant bacterial and parasitic infection: 2 cases of *Giardia* spp. and 1 case of *Blastocystis* spp. associated with diarrhea caused by *Salmonella* spp., and 1 case of *Blastocystis* spp. associated with diarrhea caused by *Campylobacter* spp.

Calprotectin results greater than 50 μg/g have been observed in some patients with a parasitic infection and in some patients without an infection. These patients presented a concomitant bacterial infection, inflammatory bowel disease, hematochezia, processes associated with immunoglobulin A deficiency, or autoimmune disease. Specifically, in the hematochezia cases studied, the median calprotectin was 118.5 μg/g; other recent studies show that calprotectin represents approximately 30% of neutrophil cytosol and, therefore, calprotectin levels increase as the extent of inflammation and bleeding increases.[5]

According to the results, the optimal cut-off point to differentiate bacterial diarrhea in the pediatric community was calculated in the ROC (receiver operating characteristic) curve. A calprotectin value of 74 μg/g (Fig. 1b) presented an adequate discriminatory value with a sensitivity of 96.77%, specificity of 97.45%, positive predictive value of 80.6% and negative predictive value of 99.6%.

These results agree with other studies which indicate that fecal calprotectin may be a useful biomarker in children with infectious diarrhea, since calprotectin values increase in an acute bacterial infection and they are related to the severity of symptoms. In the case of chronic diarrhea, different studies have reported its utility in the evaluation and discrimination of infectious diarrhea of bacterial etiology in children.[6] Usefulness of calprotectin in parasitic diarrhea in children has not been studied much, but the results of the only available study agree with the findings of our study: in absence of other associated underlying causes, the values of calprotectin in a parasitic infection are very low, except in an *Entamoeba histolytica* infection.[7]

In conclusion, our findings suggest the use of calprotectin as a biomarker of chronic diarrhea of bacterial etiology in pediatric patients. However, the discriminatory value of calprotectin can be affected in other situations such as hematochezia, inflammatory bowel disease, celiac disease, or the inclusion of children under 1 year of age. Therefore, their values should be interpreted in conjunction with clinical symptoms and other laboratory tests.

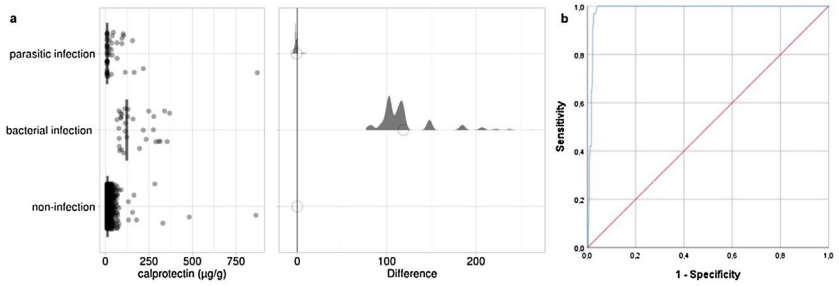

Fig. 1. (a) Fecal calprotectin in symptomatic patients without an infection, in patients with bacterial infection and in patients with parasitic infection. (b) ROC curve to differentiate patients with and without bacterial infection.

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Colonic anisakiasis, an infrequent case molecularly characterized by PCR-RFLP☆

Anisakiosis colónica, un caso infrecuente caracterizado molecularmente mediante PCR-RFLP

Anisakiasis is an emerging and underestimated zoonosis in Spain, where the incidence is the highest in Europe.[1] The main aetiological agents belong to the *Anisakis simplex* sensu lato (s.l.) complex, which is made up of three sibling species: *Anisakis simplex* sensu stricto (s.s.), *Anisakis pegreffii and Anisakis berlandi.* There are different clinical forms of anisakiasis: gastric, gastroallergic, intestinal and ectopic. In the intestinal form, the terminal ileum is usually the most affected segment[2] and, conversely, the colon and jejunum, the least involved.[3]

In this study, we present a case of colonic anisakiasis in a 69-year-old Spanish male with a history of colon polyps and endoscopic polypectomy of an adenomatous lesion 30 mm in size two years earlier, who was having a follow-up colonoscopy. The patient reported no gastrointestinal, or any other, symptoms.

In addition to 3 sessile polyps, which were removed, the colonoscopy detected a transparent whitish worm 6–8 mm in length penetrating into the mucosa in the descending colon (Fig. 1A). The worm was sent to the laboratory for molecular identification.

Initially, a morphological identification of the extracted larva was made under the microscope using the Petter and Maillard keys (1988).[4] The partially destroyed whitish, 19-mm-long larva was identified morphologically as compatible with *A. simplex* s.l. Automated extraction of genomic DNA was then performed using the commercial QIAamp® DNA FFPE kit (QIAGEN, Hilden, Germany) with prior overnight incubation at 56 °C with proteinase K and ATL buffer.

For molecular characterisation, the PCR products corresponding to the ITS1-5.8S-ITS2 region of ribosomal DNA were amplified and subsequently digested with HhaI and HinfI restriction enzymes (New England Biolabs, Massachusetts, USA) as per the protocol described by D'Amelio et al. in 2000.[5] The PCR results (Fig. 1B) established that the amplified DNA of the larva had a molecular weight (960 pb) compatible with that expected for the *A. simplex* s.l. complex. The larval transport fluid also showed an amplification at 960 pb, although with less intensity (amplification of free DNA).

In the analysis of the restriction fragment length polymorphisms (RFLP) of the amplified DNA, the larva had the characteristic profiles described for the species *A. simplex* s.s.[5] (Fig. 1C). Sequencing confirmed the identification of the *A. simplex* s.s. genotype and the sequence has been annotated in GenBank (NA: MT516319). This shows high homology with other isolates of the same genotype (99.9% NA: MN871437.1, NA: KY973681.1).

Anisakiasis is unusual in the more distal regions of the gastrointestinal tract; although cases have been reported in the ascending segment, it is rare to find it in the colon.[6,7] Until now Joo et al.[8] were the only authors to have described cases in the descending segment, and none have been reported in Spain.

The clinical manifestations of intestinal anisakiasis are quite diverse, from an asymptomatic condition, to diffuse abdominal pain with nausea, vomiting, etc., to acute abdomen due to intestinal perforation, obstruction, intussusception, etc.[8] An association between colonisation by *Anisakis* spp. and cancer has been suggested, due to the possible tumorigenic effect resulting from persistent local inflammation, as well as that induced by certain secretions of the parasite.[9]

The case we present here was an asymptomatic patient, in whom the anisakiasis was found incidentally. If it had been left to develop freely, there is no saying what the outcome might have been. The location of the larva in the descending colon makes our case more unique. One of the reasons for these larvae being more commonly found in the ascending colon is that intestinal peristalsis is slowed down in that area, and a larva therefore has the ideal conditions for penetration and is not forced to migrate to more distal regions of the colon.[8]

Molecular techniques are very useful in the diagnosis of intestinal anisakiasis, as they work even when the larva is in a poor state of preservation, which is common due to manipulation during the extraction process or the patient's immune response when attempting removal. These techniques also provide genotyping, with unequivocal characterisation at the species level, unlike the morphological study, in which not only must the integrity of the larva be taken into account, but also that it is impossible to distinguish sibling species. Also important is the high sensitivity of molecular techniques, enabling amplification and identification even with the DNA in the sample transport fluid, as demonstrated here.

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