

## Fecal Calprotectin in Children with the Enthesitis-related Arthritis Subtype of Juvenile Idiopathic Arthritis

To the Editor:

Subclinical gut inflammation is present in two-thirds of adult and pediatric patients with spondyloarthritis (SpA)<sup>1,2</sup> and predicts a chronic course of arthritis<sup>2,3</sup>. Thus, there may be value in evaluating the gut in patients with SpA. However, commonly used tests, such as colonoscopy, barium studies, and computed tomography, are limited by expense, invasiveness, or radiation exposure<sup>4</sup>, prompting a need for noninvasive surrogate markers. Serologic markers revealed a large number of false-positive tests<sup>5</sup>.

Fecal calprotectin is a sensitive and specific marker for the presence of inflammatory bowel disease or other intestinal illnesses<sup>6</sup>. This test has not heretofore been used to assess for subclinical gut inflammation in patients with arthritis. In this study, we measured fecal calprotectin levels in children with enthesitis-related arthritis (ERA), comparing them to children with non-SpA subtypes of juvenile idiopathic arthritis (JIA), as well as children with unrelated connective tissue diseases (CTD) and noninflammatory control subjects.

We enrolled 4 groups of children: (1) 9 with the ERA subtype of JIA; (2) 17 with other subtypes of JIA [persistent oligoarticular, n = 6; extended oligoarticular, n = 1; rheumatoid factor-negative (RF-) polyarticular, n = 8; RF+ polyarticular, n = 2]; (3) 9 with unrelated CTD (dermatomyositis, n = 3; localized scleroderma, n = 5; microscopic polyangiitis, n = 1); and (4) 6 noninflammatory control subjects (e.g., hypermobility syndrome). All children with JIA met the International League of Associations for Rheumatology criteria<sup>7</sup>. All children with ERA met the criteria on the basis of a history of arthritis in an HLA-B27+ boy with onset after the 6th birthday; additional SpA features included clinically diagnosed enthesitis in 2, sacroiliitis in 3, family history in 2, and acute anterior uveitis in one. We excluded children with acute onset (< 30 days) of gastrointestinal (GI) symptoms as well as those with prior abnormal studies of the GI tract. We defined GI symptoms as being one or more of a history of chronic abdominal pain that interfered with activity, hematochezia, persistent diarrhea, or poor growth<sup>8</sup>. Active arthritis was defined by having at least one joint with swelling or limitation accompanied by joint pain or tenderness<sup>7</sup>. For chil-

dren with unrelated CTD, disease activity was assessed clinically by the attending physician. Each enrolled child submitted a stool specimen for measurement of calprotectin; the specimen was provided on the day of the visit, hand-delivered to the hospital, or shipped overnight to the hospital by a commercial carrier. This study was approved by the Institutional Review Board at University of Texas Southwestern Medical Center. Informed consent was obtained from each subject's legal guardian, and assent was obtained in children age 10 years or older.

At the clinical laboratory, stool specimens were aliquoted into transport media provided by a commercial laboratory (ARUP, Salt Lake City, UT, USA) and were stored at -25°C and shipped frozen overnight to ARUP for measurement of calprotectin, performed using ELISA. Calprotectin is stable at room temperature for up to 7 days<sup>9</sup>.

Calprotectin was measured on a continuous scale. Comparisons between the groups were made with the Kruskal-Wallis test. Values were also dichotomized to < 121 µg/g or ≥ 121 µg/g, the cutoff suggested by ARUP. Comparisons of the dichotomized values were performed by chi-squared test.

The patient population is described in Table 1. GI symptoms were reported in 2 patients with ERA (abdominal pain and loose stools in one; abdominal pain and weight loss in another) and in one patient with polyarticular JIA (chronic poor weight gain).

Fecal calprotectin levels are shown in Table 1 and Figure 1. Median levels were highest in the children with ERA, and lowest in the CTD controls (p = 0.034). Among patients with ERA, nonsteroidal antiinflammatory drug (NSAID) use was not associated with an elevated fecal calprotectin level (125 µg/g in NSAID users vs 201 µg/g in NSAID non-users; p = 1.000). Of the 3 patients who reported GI symptoms, 2 (1 ERA, 1 polyarticular JIA) had normal calprotectin levels, while 1 ERA patient had a calprotectin level of 310 µg/g. The majority of ERA patients, but a minority of patients in each of the other groups, had abnormal levels (p = 0.024; Table 1).

The primary implication of this study is that a safe and noninvasive test can be used to evaluate for subclinical gut inflammation in arthritis patients. In addition, we confirm prior studies showing evidence of gut inflammation in children with SpA<sup>2</sup>.

Table 1. Study subjects.

Characteristic	Noninflammatory Controls	CTD Controls	JIA Controls	ERA	p
N	6	9	17	9	—
Age, yrs <sup>†</sup>	3.8 (3.0–9.6)	11 (8.0–14)	6.2 (2.8–11)	12 (9.5–14)	0.014
Female, %	67	56	82	0	0.001
Ethnicity, %					0.070
Latino	17	56	12	0	
Non-Latino White	67	33	82	67	
African American	17	0	6	22	
Other	0	11	0	11	
Treatment, %					
NSAID	33	0	65	56	0.013
Systemic corticosteroid	0	33	0	11	0.042
Methotrexate	0	78	65	56	0.019
Etanercept	0	0	5.9	33	0.055
Other DMARD*	0	33	0	0	0.009
Active disease	0	67	100	78	< 0.001
GI symptoms, %	0	0	5.9	22	0.242
Fecal calprotectin, µg/g (range) <sup>†</sup>	80 (28–120)	26 (0–38)	51 (36–98)	171 (34–280)	0.034
Fecal calprotectin, > 120 µg/g, %	17	11	18	67	0.024

<sup>†</sup> Continuous data are presented as medians (intraquartile range). \* One patient was taking mycophenolate, another was taking both mycophenolate and hydroxychloroquine, and a third was taking 6-mercaptopurine. DMARD: disease-modifying antirheumatic drug; CTD: connective tissue disease; ERA: enthesitis-related arthritis.

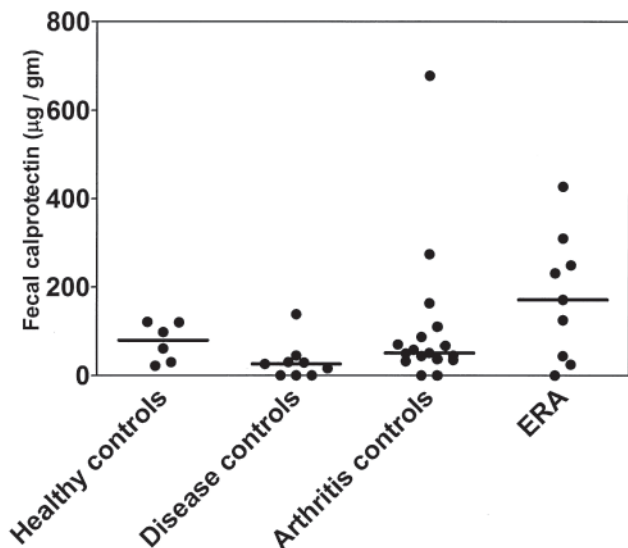


Figure 1. Calprotectin levels in children with enthesitis-related arthritis (ERA), arthritis controls (oligoarticular and polyarticular juvenile idiopathic arthritis), noninflammatory controls, and disease controls (unrelated CTD).

Our study has several limitations. Our sample size was relatively small, and reflected patients who were heterogeneous with respect to treatment. Several patients with JIA were receiving NSAID, which could potentially have influenced the fecal calprotectin levels<sup>10</sup>; however, no clear association was observed in this study. Additionally, although fecal calprotectin is stable for up to 7 days at room temperature<sup>9</sup>, several specimens were shipped to us over the summer, when temperatures in Texas exceeded room temperature. The possibility for selection bias exists, in that patients with GI symptoms may have been more likely to participate in the study. However, no obvious relationship was observed between GI symptoms and calprotectin levels. All subjects with ERA were male, introducing a potential confounding factor. However, levels were similar between male and female patients in each of the other groups (data not shown), suggesting that gender is not likely to be an independent predictive factor. Additionally, all of the ERA patients were HLA-B27+, potentially indicating a higher risk of developing ankylosing spondylitis in the future, and thus may not be reflective of all patients with ERA. Finally, fecal calprotectin levels were obtained at a single timepoint only.

We show for the first time that stool biomarkers may be used to evaluate for intestinal inflammation in children with JIA. Future studies should be performed using validated “gold standard” assessments to confirm the significance of elevated fecal calprotectin levels in this population, and also to define the role of routine assessments of fecal inflammation in children and adults with SpA.

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