

## Association between defective spleen function and primary eosinophilic gastrointestinal disorders



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### Clinical Implications

- Splenic hypofunction may complicate the course of eosinophilic gastrointestinal disorders, and is reverted by steroid therapy. Further studies are needed to ascertain whether hyposplenism may predispose patients with eosinophilic gastrointestinal disorders to autoimmunity or thromboembolism.

### TO THE EDITOR:

Primary eosinophilic gastrointestinal disorders (EGID) are inflammatory diseases of unknown etiology characterized by eosinophilic mucosal infiltration of one or more segments of the gastrointestinal tract in the absence of known causes of secondary eosinophilia, such as inflammatory, infectious, malignant, or hypersensitivity disorders.<sup>1</sup> The precise incidence of EGID is unknown, due to its rarity, but it is rising in the latest years, probably because of a better recognition and an increasing interest. Although both pathogenic mechanisms and natural history are mostly unrevealed, there seems to be a common association of EGID with atopy, such as IgE-mediated food allergy, asthma, atopic dermatitis, and allergic rhinitis or, to a lesser extent, autoimmunity, such as Hashimoto's thyroiditis, rheumatoid arthritis, and systemic lupus erythematosus.

Recently, we described the first case of primary EGID complicated by spleen hypofunction,<sup>2</sup> an acquired impairment of both the reticuloendothelial and immune functions of the spleen, accompanied or not by a reduction in spleen size, which potentially predisposes to encapsulated bacterial infections, thromboembolism, and autoimmunity.<sup>3</sup> Splenic hypofunction has been described in a number of gastrointestinal disorders, including celiac disease<sup>4,5</sup> and inflammatory bowel disease,<sup>6</sup> although the mechanisms implicated in its pathogenesis are still unknown. On this basis, we aimed to assess spleen function in a series of consecutively enrolled patients with EGID.

Peripheral blood samples were obtained from 21 patients affected by primary EGID at diagnosis (mean age 43.7 years, range 15-79 years). Twenty-two healthy volunteers (mean age 42.5 years, range 26-61 years) and 18 splenectomized patients (mean age 43.1 years, range 24-68 years) were used as negative and positive controls, respectively. Diagnosis of EGID was based on gastrointestinal symptoms associated with a pathologic eosinophilic infiltration of the gastrointestinal wall, without evidence of parasitic infection, other causes of secondary eosinophilic infiltration, and after ruling out hypereosinophilic

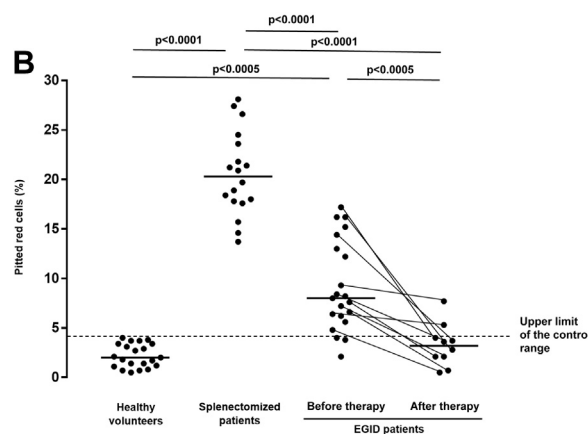
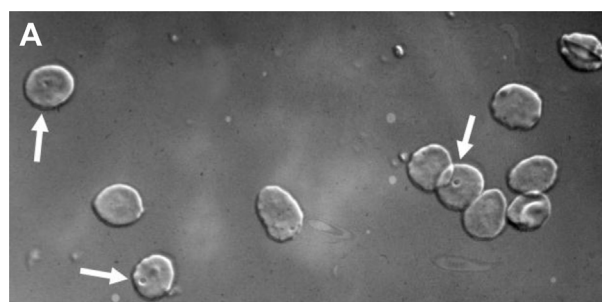


**FIGURE 1.** A, Pitted red cells (PRC) were recognized by “pit” on the erythrocyte membrane (arrows). B, PRC in 21 patients with eosinophilic gastrointestinal disorders (EGID) at diagnosis, in 10 patients with EGID after therapy, 22 healthy volunteers, and 18 splenectomized patients.

syndrome.<sup>1</sup> All patients with EGID (3 with esophagitis, 8 with gastroenteritis, and 10 with colitis) underwent upper and/or lower endoscopy with collection of biopsies. Patients with esophagitis were on high-dose proton pump inhibitors at the time of the biopsy. The number of eosinophils in the lamina propria in each high power field (HPF) was counted as the peak of eosinophil count (the highest number of eosinophils per HPF; 40× objective). In most cases of gastroenteritis and colitis, clusters of eosinophils within the lamina propria and occasional intraepithelial eosinophils were also present, and no cases of mucosal erosions or ulcerations were seen. One of 21 patients with EGID has been already described by us.<sup>2</sup> Seven patients had concomitant autoimmune disorder(s), that is, Hashimoto's thyroiditis (n = 5), autoimmune atrophic gastritis (n = 2), autoimmune hemolytic anemia (n = 1), and rheumatoid arthritis (n = 1). To assess the response to therapy, in 10 patients with EGID, a further peripheral blood sample was collected after an 8-week course of therapy, that is, swallowed fluticasone in 2 esophagitis cases, oral beclomethasone dipropionate in 5 colitis cases, and oral prednisone in 3 gastroenteritis cases.

Splenic function was assessed by counting pitted red cells (PRC), that is, red cells with membrane abnormalities visible under phase-interference microscopy, the so-called pits.<sup>3</sup> One

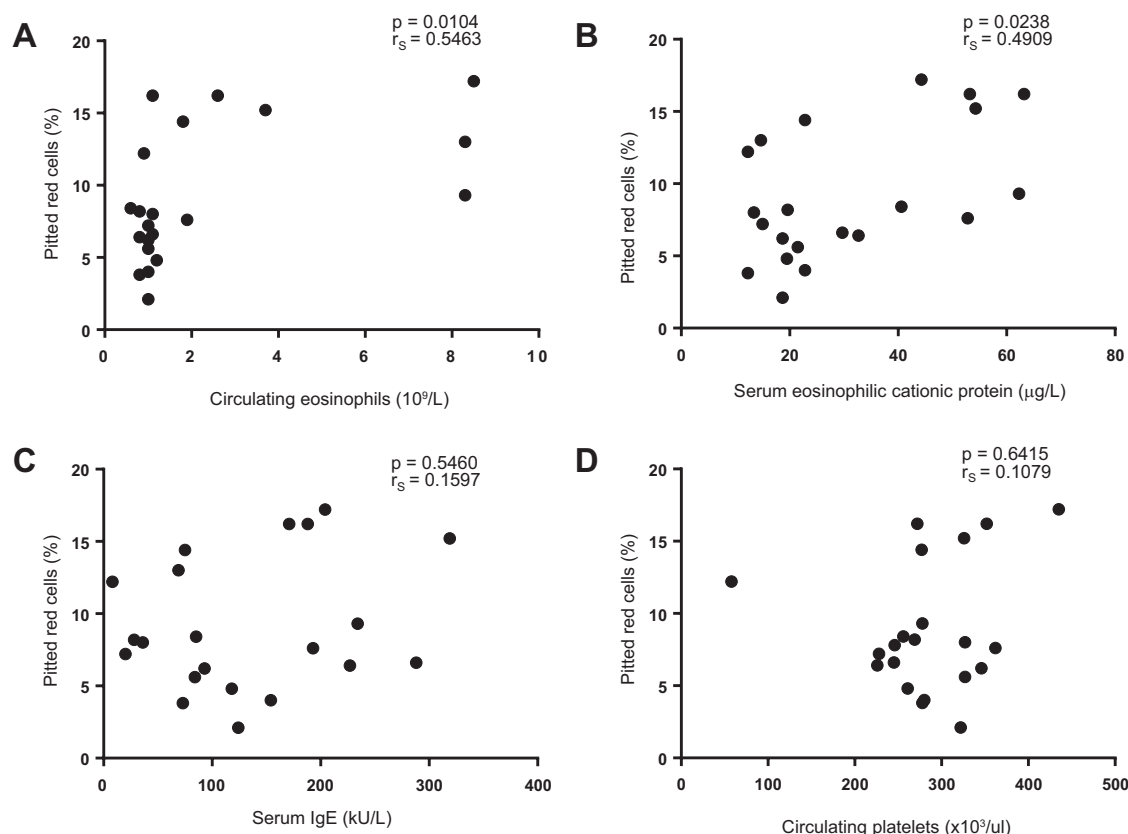


**FIGURE 2.** Correlation between pitted red cells and (A) circulating eosinophils, (B) eosinophilic cationic protein, (C) IgE, and (D) platelets.

thousand red cells were examined in a wet preparation (magnification  $\times 1000$ ) with a direct-interference contrast microscope (Leitz Dialux 20, Cape Coral, Fla) equipped with Nomarsky optics. The percentage of PRC was calculated and taken as a measure of splenic function (upper normal limit of 4%).<sup>3</sup> Data were analyzed by means of the nonparametric Mann-Whitney *U*-test. Correlations were studied by Spearman's rank correlation test. Differences in frequencies were tested using Fisher's exact test. For all statistical analyses, a 2-tailed *P* value of  $<.05$  was considered significant.

Eighteen of 21 patients with EGID at diagnosis (85%) had PRC values higher than 4% and thus were considered hyposplenic (see Table E1 in this article's Online Repository at [www.jaci-inpractice.org](http://www.jaci-inpractice.org)). As a further method of splenic function evaluation, Howell-Jolly bodies were assessed through May-Grünwald-Giemsa staining in the peripheral blood smear of all patients with EGID, but they were detected only in the 2 patients with the highest PRC values (16.2% and 17.2%, respectively). Howell-Jolly bodies were 5 and 6 per 10,000 peripheral red cells, respectively. As expected, none of the healthy volunteers and all the splenectomized patients had PRC values higher than 4%. Numerous PRC were evident in the peripheral blood smear of a patient with EGID (Figure 1, A). As shown in Figure 1, (B), patients with EGID at diagnosis showed a median PRC value (8.0%, range 2.1% to 17.2%) significantly ( $P < .0001$ ) higher than that of healthy volunteers (1.9%, range 0.4% to 4.0%). In the 10 patients with EGID followed-up after diagnosis, the 8-week steroid treatment significantly ( $P < .0005$ ) decreased the median PRC value (3.2%, range 0.7% to 7.7%).

The 7 patients with EGID with concomitant autoimmune disorders had higher median PRC values (9.3%, range 6.6% to 17.2%) than those without autoimmune comorbidities (7.0%, range 2.1% to 16.2%), although this difference was not statistically significant ( $P = .21$ ). Notably, none of the patients with esophagitis had a concomitant autoimmune disease, whereas 3 of 8 patients with gastroenteritis and 4 of 10 patients with colitis had at least 1 autoimmune disease. No significant difference was found in the percentage of PRC between patients with esophagitis (median 8.4%, range 4.0% to 14.4%), those with gastroenteritis (median 8.1%, range 2.1% to 17.2%), and those with colitis (median 6.9%, range 3.8% to 16.2%). As expected, splenectomized patients showed median PRC values (20.3%, range 13.7% to 28.1%) significantly ( $P < .0001$ ) higher than that of both patients with EGID and healthy volunteers.

In all the 21 patients with EGID, the median circulating eosinophil count was  $1.0 \times 10^9/L$  (range 0.6-8.5/L), the median serum eosinophilic cationic protein (ECP) level was 21.5  $\mu g/L$  (range 12.3-63.2  $\mu g/L$ ), the median serum IgE concentration level was 124 kU/L (range 8-319 kU/L), the median circulating platelet count was  $278 \times 10^3/\mu L$  (range 58-435/ $\mu L$ ), and the median spleen longitudinal diameter measured at abdominal ultrasound was 9.9 cm (range 6.5-11.4 cm) (normal lower limit 7.5 cm for females and 8.0 cm for males).<sup>7</sup> As shown in Figure 2, in patients with EGID at diagnosis, a significant positive correlation was found between PRC and either circulating eosinophil count or serum ECP. In contrast, no significant correlation was identified between PRC and serum IgE, circulating platelet count, spleen diameter, hemoglobin levels, albumin levels, or

patients' age. The median peak eosinophil count per HPF was 77 (range 28-187) in esophagitis, 88 (range 30-252) in gastroenteritis, and 88 (range 60-110) in colitis. Only in the subgroup of eosinophilic colitis, a significant positive correlation between PRC and mucosal peak eosinophil count ( $r_s = 0.71$ ,  $P = .02$ ) was found. All the 10 patients with EGID followed up for 8 weeks achieved a clinical response; however, the spleen function reverted to normal only in 8 of them. The median pretreatment PRC value was 8.0% (range 4.8% to 17.2%), and significantly decreased ( $P = .0003$ ) to 3.2% (range 0.5% to 7.7%) after therapy (Figure 1, B).

We here show that 85% of this small cohort of patients with EGID had splenic hypofunction as evidence by PRC. The prevalence of hyposplenism in this cohort of patients appears to be higher than that found in celiac disease (33%)<sup>4</sup> and inflammatory bowel disease (40%).<sup>6</sup> A concomitant autoimmune disease was found in 40% of patients with colitis, 37% of patients with gastroenteritis, and in none of the patients with esophagitis. Overall, the degree of functional hyposplenism seems to correlate with peripheral eosinophilia and could potentially be reverted by therapy, but larger studies are needed to confirm these findings in patients with EGID. The demonstration that the spleen is the major site for eosinophil disposal,<sup>8</sup> together with the massive eosinophil accumulation observed in the spleen of experimental models of eosinophilia,<sup>9</sup> suggests that the spleen filtering function might be impaired by the eosinophil engorgement of the red pulp. Further studies are needed to ascertain whether splenic hypofunction may predispose patients with EGID to autoimmunity and thromboembolism.<sup>10</sup>

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## ONLINE REPOSITORY

**TABLE E1.** Clinical, laboratory, and abdominal ultrasound features of the 21 patients affected by eosinophilic gastrointestinal disorders

| Pt | Sex | Age (y) | Disorder type   | EOS (10 <sup>9</sup> /L) | ECP (μg/L) | IgE (kU/L) | PLT (×10 <sup>3</sup> /μL) | PRC (%) | Spleen longitudinal diameter (cm) | Concomitant autoimmune disorder |
|----|-----|---------|-----------------|--------------------------|------------|------------|----------------------------|---------|-----------------------------------|---------------------------------|
| 1  | M   | 21      | Gastroenteritis | 2.6                      | 63.2       | 188        | 352                        | 16.2    | 7.8                               | None                            |
| 2  | M   | 29      | Gastroenteritis | 8.5                      | 44.3       | 204        | 435                        | 17.2    | 6.5                               | AAG, Hashimoto's thyroiditis    |
| 3  | M   | 61      | Gastroenteritis | 1.0                      | 21.5       | 84         | 327                        | 5.6     | 7.1                               | None                            |
| 4  | F   | 49      | Esophagitis     | 1.0                      | 22.8       | 154        | 280                        | 4.0     | 9.5                               | None                            |
| 5  | F   | 53      | Colitis         | 1.0                      | 18.7       | 93         | 346                        | 6.2     | 10.3                              | None                            |
| 6  | F   | 60      | Gastroenteritis | 0.8                      | 19.6       | 28         | 269                        | 8.2     | 11.4                              | None                            |
| 7  | M   | 71      | Esophagitis     | 1.8                      | 12.8       | 75         | 277                        | 14.4    | 9.3                               | None                            |
| 8  | F   | 35      | Colitis         | 1.1                      | 53.2       | 171        | 272                        | 16.2    | 10.0                              | None                            |
| 9  | M   | 79      | Colitis         | 8.3                      | 14.7       | 319        | 246                        | 13.0    | 9.6                               | Rheumatoid arthritis            |
| 10 | M   | 41      | Colitis         | 3.7                      | 54.3       | 289        | 326                        | 15.2    | 10.4                              | None                            |
| 11 | F   | 15      | Colitis         | 0.8                      | 32.7       | 227        | 226                        | 6.4     | 9.9                               | None                            |
| 12 | F   | 57      | Gastroenteritis | 1.1                      | 13.4       | 36         | 327                        | 8.0     | 9.0                               | Hashimoto's thyroiditis         |
| 13 | F   | 38      | Colitis         | 1.0                      | 15.0       | 20         | 228                        | 7.2     | 11.2                              | Hashimoto's thyroiditis         |
| 14 | F   | 49      | Esophagitis     | 0.6                      | 40.6       | 85.3       | 256                        | 8.4     | 8.6                               | None                            |
| 15 | M   | 43      | Colitis         | 8.3                      | 62.3       | 234        | 278                        | 3.8     | 10.5                              | None                            |
| 16 | M   | 41      | Colitis         | 1.9                      | 52.8       | 193        | 362                        | 7.6     | 10.4                              | Hashimoto's thyroiditis         |
| 17 | F   | 19      | Colitis         | 1.1                      | 29.7       | 288        | 245                        | 6.6     | 9.23                              | AAG, Hashimoto's thyroiditis    |
| 18 | M   | 34      | Gastroenteritis | 1.2                      | 19.5       | 118        | 261                        | 4.8     | 11.1                              | None                            |
| 19 | M   | 27      | Colitis         | 0.8                      | 12.3       | 73         | 278                        | 3.8     | 10.5                              | None                            |
| 20 | F   | 53      | Gastroenteritis | 1.0                      | 18.7       | 124        | 322                        | 2.1     | 8.7                               | None                            |
| 21 | M   | 43      | Gastroenteritis | 0.9                      | 12.3       | 8.13       | 58                         | 12.2    | 10.9                              | AHA                             |

AAG, Autoimmune atrophic gastritis; AHA, autoimmune hemolytic anemia; ECP, eosinophilic cationic protein; EOS, eosinophil; F, female; Ig, immunoglobulin; M, male; PLT, platelet; PRC, pitted red cell; Pt, patient.

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