Platelets

Effects of a *Helicobacter pylori* eradication regimen on anti-platelet autoantibody response in infected and uninfected patients with idiopathic thrombocytopenic purpura

Thirty-seven patients with idiopathic thrombocytopenic purpura (ITP) were treated with a standard *Helicobacter pylori* eradication regimen irrespective of *H. pylori* infection. Our results indicate that platelet recovery results from the disappearance of *H. pylori* itself, and is mediated, in part, through suppression of anti-platelet autoantibody production.

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It has been proposed that *Helicobacter pylori* (*H. pylori*) infection is associated with idiopathic thrombocytopenic purpura (ITP), based on increased platelet counts after successful eradication of *H. pylori*.[1,2] However, the prevalence of *H. pylori* infection is similar in ITP patients and the general population,[3] suggesting that ITP is not necessarily linked to *H. pylori* infection. A critical question is how the *H. pylori* eradication regimen increases platelet counts. The most plausible explanation is that the disappearance of *H. pylori* has a therapeutic effect. However, a 1-week regimen of a combination of antibiotics should eradicate bacteria other than *H. pylori*, which may include a bacterium truly associated with the pathogenesis of ITP, or induce prominent changes in the normal flora. Moreover, immunomodulatory effects are reported for drugs used for *H. pylori* eradication. To evaluate these possibilities, we conducted an open-label, prospective study involving 37 consecutive patients with ITP (aged 24 to 73, 14 male) who satisfied the following criteria: thrombocytopenia persisting >6 months, normal or increased bone marrow megakaryocytes without morphologic evidence of dysplasia, no secondary immune or non-immune diseases that could account for thrombocytopenia, and a platelet count $<50\times10^9$/L at ≥3 measurements during the preceding 3 months. None of the patients satisfied the classification criteria for systemic lupus erythematosus,[4] but 13 (35%) had a low titer (≤ 80) of anti-nuclear antibodies. All patients were assessed for *H. pylori* infection and given amoxicillin (1.5g daily), clarithromycin (800 mg daily), and lansoprazole (60 mg daily) for 7 days, irrespective whether they did or did not have *H. pylori* infection.

All patients visited our hospital at 0, 1, 4, 8, 12, and 24 weeks, and all responders were followed for ≥56 weeks. The anti-GPIIb/IIIa autoantibody response was evaluated by detecting circulating B cells producing anti-GPIIb/IIIa antibodies.[5] The patients were allowed to continue other therapy (danazol, n=4), provided their dosages were maintained at a constant level until 24 weeks, except for prednisolone (≥10mg daily), which was allowed to be decreased or discontinued after platelet counts reached $>100\times10^9$/L.

The study protocol conformed to the ethical principles of the World Medical Association Declaration of Helsinki as reflected in a priori approval from the Institutional Review Board. Twenty-six patients (70%) who had positive results in a 13°C urea breath test plus serum anti-*H. pylori* antibodies or stool *H. pylori* antigen were regarded as *H. pylori*-positive, whereas 11 patients negative for all three tests were *H. pylori*-negative. Eradication was successful in all *H. pylori*-positive patients according to a negative urea breath test at 12 weeks. When a therapeutic response was defined as a platelet count $>100\times10^9$/L at 24 weeks, 16 *H. pylori*-positive patients (62%) were responders, while none of the *H. pylori*-

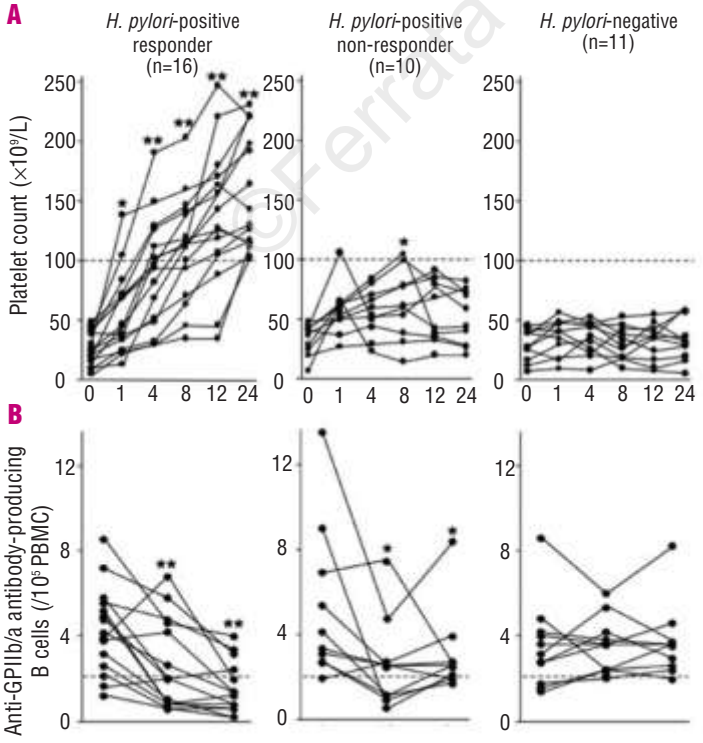

Figure 1. Serial platelet counts (A) and number of circulating anti-GPIIb/IIIa antibody-producing B cells (B) before and after *H. pylori* eradication regimen in *H. pylori*-positive ITP responders (n=16) and non-responders (n=10), and *H. pylori*-negative ITP patients (n=11). Changes in the absolute values at different time points from the baseline value taken at week 0 were compared by repeated measures analysis of variance. When the *p* value for this overall comparison was statistically significant, post-hoc pairwise comparisons were performed using Dunnett's test. *<0.05 and **<0.01 compared with week 0. Broken lines indicate the cut-offs for the platelet response (100×10^9/L; A) and the number of circulating anti-GPIIb/IIIa antibody-producing B cells ($2/10^5$ peripheral blood mononuclear cells; B. At week 0, a positive anti-GPIIb/IIIa antibody response was detected in 14 H. pylori-positive responders (88%), nine *H. pylori*-positive non-responders (90%), and eight *H. pylori*-negative patients (73%).

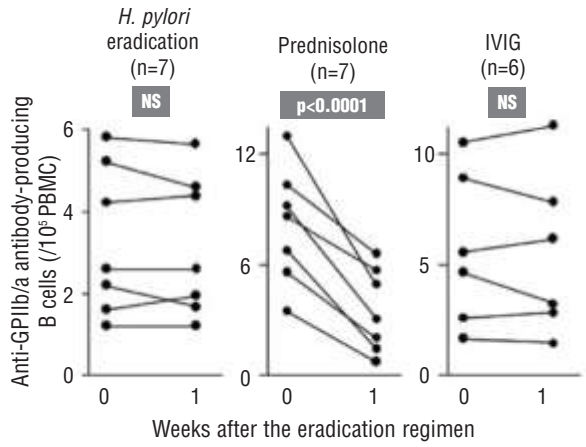

Figure 2. The number of circulating anti-GPIIb/IIIa antibody-producing B cells in ITP patients who responded to *H. pylori* eradication regimen (n=7), high-dose prednisolone (n=7), or IVIG (n=6) before and 1 week after the therapeutic regimen. The prednisolone group received 1 mg/kg prednisolone for at least 1 week while the IVIG group received 400 mg/kg human immunoglobulins for 3 days. Absolute values at weeks 0 and 1 were compared by a paired t-test. NS: not significant.

negative patients was a responder (p=0.0006). The platelet response lasted for ≥56 weeks in all responders. Serial platelet counts in *H. pylori*-positive responders and non-responders, and *H. pylori*-negative patients are shown in Figure 1A. Almost no fluctuation was observed in the *H. pylori*-negative patients. In 13 *H. pylori*-positive patients (50%), platelet counts increased two-fold or more at 1 week irrespective of the platelet response at 24 weeks. Anti-GPIIb/IIIa antibody-producing B cells were significantly decreased at 12 and 24 weeks in *H. pylori*-positive responders (p<0.0001) and, to a lesser extent, in non-responders (p=0.02), but not in *H. pylori*-negative patients (Figure 1B). There was no difference in frequencies of responders between anti-nuclear antibody-positive and negative *H. pylori*-positive ITP patients (50% versus 65%).

Despite a rapid platelet increase by a 1 week, there was no reduction in anti-GPIIb/IIIa antibody-producing B cells at this time point in seven *H. pylori*-positive responders (Figure 2). For reference, we additionally measured anti-GPIIb/IIIa antibody-producing B cells in a separate cohort of ITP patients who responded to high-dose prednisolone (n=7) or intravenous immunoglobulin (IVIG) (n=6). The antibody-producing B cells were significantly reduced at 1 week in patients treated with prednisolone (p<0.0001), but not in patients treated with IVIG.

In this prospective study, the efficacy of the *H. pylori* eradication regimen in *H. pylori*-infected ITP patients was in agreement with previous studies,[1-3] but platelet recovery was observed in none of *H. pylori*-negative patients. Taken together with previous case series of *H. pylori*-negative patients who failed to respond to the *H. pylori* eradication regimen,[6-8] it is likely that platelet recovery results from the eradication of *H. pylori* itself, rather than from other *H. pylori*-independent mechanisms. Whether the *H. pylori* eradication regimen influences the anti-platelet autoantibody response is still under debate.[9,10] Our study clearly showed that anti-GPIIb/IIIa autoantibody response was suppressed at 12 and 24 weeks after successful *H. pylori* eradication, suggesting involvement of *H. pylori* infection in the process of anti-platelet autoantibody production. However, a rapid platelet increase at 1 week might be mediated by other mechanisms, potentially similar to the actions of IVIG. These different actions of *H. pylori* eradication suggest that multiple processes are responsible for platelet recovery in ITP patients.

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Key words: idiopathic thrombocytopenic purpura, autoantibody, Helicobacter pylori, platelet.

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