

Original article

Platelet count response to *Helicobacter pylori* eradication for idiopathic thrombocytopenic purpura in northeastern Brazil



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ABSTRACT

Background: Several studies have demonstrated that platelet counts in *Helicobacter pylori*-positive patients with chronic idiopathic thrombocytopenic purpura improved significantly after successful eradication of the infection. However, depending of the geographical region of the study the results have been highly divergent.

Objective: The purpose of this study was to evaluate the effect of *H. pylori* eradication therapy on platelet count in a cohort of chronic idiopathic thrombocytopenic purpura patients from northeastern Brazil.

Method: *H. pylori* status was determined in 28 chronic idiopathic thrombocytopenic purpura patients using the rapid urease test and histology. *H. pylori*-positive patients received standard triple therapy for one week. The effect of the eradication therapy was evaluated using the 13C-urea breath test two to three months after treatment.

Results: The prevalence of *H. pylori* infection was similar to that found in the general population. Twenty-two patients (78.5%) were *H. pylori*-positive. Fifteen were treated, 13 (86%) of whom successfully. At six months, 4/13 (30%) displayed increased platelet counts, which remained throughout follow-up (12 months). Platelet response was not associated to mean baseline platelet count, duration of chronic idiopathic thrombocytopenic purpura, gender, age, previous use of medication, or splenectomy.

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Conclusions: *H. pylori* eradication therapy showed relatively low platelet recovery rates, comparable with previous studies from southeastern Brazil. The effect of *H. pylori* eradication on platelet counts remained after one year of follow-up suggesting that treating *H. pylori* infection might be worthwhile in a subset of chronic idiopathic thrombocytopenic purpura patients.

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Introduction

Helicobacter pylori, a gram-negative microorganism first isolated by Warren & Marshall in 1984, colonizes the human stomach and may cause type B gastritis and peptic ulcers. Colonization of the stomach by *H. pylori* is associated with increased risk of gastric cancer,¹ and a number of other non-gut-related disorders, such as coronary disease² and autoimmune diseases including autoimmune thyroiditis³ and chronic idiopathic thrombocytopenic purpura (cITP).⁴

cITP is a poorly understood acquired hemorrhagic disease which involves the destruction of platelets in the reticuloendothelial system induced by anti-platelet antibodies.⁵ To date an effective and safe treatment for cITP has not been established. cITP treatment has been restricted to therapies with the potential of causing significant toxicity and risks including immunosuppressive agents, such as corticosteroids, intravenous immunoglobulin therapy (IVIg), anti-D immunoglobulin (anti-D), rituximab and salvage splenectomy. Furthermore, 20–30% of cITP patients are resistant to these therapies.⁶

After the discovery by Gasbarrini that platelet counts in *H. pylori*-positive cITP patients improved significantly after successful eradication of the infection,⁴ several authors from different geographical regions have evaluated the effect of *H. pylori* eradication therapy on platelet counts in this patient population. However, results have been highly variable (0–100%).⁷

The highest response rates (>50%) are of cohorts in Italy,⁸ Japan,⁹ Korea¹⁰ and Colombia.¹¹ On the other hand, in a study from Spain, only 13% experienced a significant increase in platelet counts as a result of *H. pylori* eradication,¹² whereas a study from the United States found no difference between groups.¹³

Differences in the genetic background of the host and in the virulence of *H. pylori* strains may explain the discrepancies observed in studies on *H. pylori* eradication therapy in cITP patients. This bacterium has several virulence genes, showing a high variability of distribution with the most important being the vacuolating cytotoxin A gene (*VacA*) and cytotoxin-associated gene A (*cagA*). The *cagA* gene is part of a 40 kb cluster of genes (*cag* pathogenicity island) that codes a type IV secretion system that injects the CagA protein into gastric epithelial cells and is also associated with increased secretion of interleukin-8, a strong proinflammatory chemokine.¹⁴ It has been postulated that CagA evokes host systemic immune responses, producing autoantibodies that cross-react with

host platelet surface antigens promoting platelet aggregation via immune complex formation with augmented platelet clearance rates resulting in thrombocytopenia.¹⁵

The few Brazilian studies that have evaluated the role of *H. pylori* infection in adult cITP patients were based on cohorts from southeastern Brazil.¹⁶ Although the prevalence of *H. pylori* infection is high (80%) in northeastern Brazil¹⁷ and infection is often intrafamilial with onset in early childhood,¹⁸ no study to our knowledge has evaluated the association between *H. pylori* and cITP in cohorts from this region.

Objective

The purpose of the present study was therefore to evaluate the effect of *H. pylori* eradication therapy on platelet counts in a cohort of cITP patients from northeastern Brazil.

Methods

This prospective, observational, study evaluated 28 patients with cITP selected from those who attended the Centro de Hematologia e Hemoterapia do Ceará, a referral center for cITP in Fortaleza, Ceara, Brazil. Patients, recruited through convenience sampling from August 2013 to August 2014, were followed up for one year.

cITP was diagnosed according to the guidelines of the American Society of Hematology.¹⁹ Inclusion criteria were i) platelet count $<100 \times 10^9/L$ for over six months, ii) normal or increased bone marrow megakaryocytes, and iii) no other thrombocytopenia-related conditions or factors. The exclusion criteria were i) platelet counts $\geq 100 \times 10^9/L$ and $\leq 25 \times 10^9/L$, ii) treatment with corticosteroids, immunosuppressants or other specific drugs for cITP as well as splenectomy during the study period, iii) treatment with antimicrobial drugs, steroidal or non-steroidal anti-inflammatory agents or proton pump inhibitors in the 30 days preceding the study, iv) active gastrointestinal bleeding, v) pregnancy, vi) history of treatment for *H. pylori* infection, and vii) history of gastric surgery. cITP patients with platelet counts $\leq 25 \times 10^9/L$ were excluded to reduce the confounding effect of concomitant cITP therapies during the study period.

Gastroendoscopy was performed at the university hospital Walter Cantideo, Fortaleza, Ceara, Brazil. All cITP patients were submitted to upper gastrointestinal endoscopy including biopsy of the gastric mucosa from the five sites recommended

by the Houston-updated Sydney System for the Classification of Gastritis²⁰ and to evaluate *H. pylori* status. Antral fragments from biopsies were submitted to the urease test.¹⁷ The biopsies were stained with hematoxylin and eosin and with Giemsa stains to evaluate the presence of *H. pylori*. Patients were considered *H. pylori*-negative when their results were negative in both histology and the rapid urease test.

Only *H. pylori*-positive patients were given eradication therapy (standard triple therapy: 20 mg omeprazole and 500 mg clarithromycin once a day and 1000 mg amoxicillin twice a day, for one week). Patients with unsuccessful eradication were retreated with 20 mg omeprazole and 500 mg levofloxacin once a day and 1000 mg amoxicillin twice a day, for one week.²¹

Eradication status was assessed using the ¹³C-urea breath test at least one month after the end of therapy.²¹ The sensitivity and specificity of the *H. pylori* detection test are 90% and 95%, respectively. Briefly, after a fasting period of six hours, the patient provided an initial breath sample, followed by the ingestion of 100 mL orange juice containing 75 mg ¹³C-urea. Thirty minutes later a second breath sample was collected. The samples were submitted to infrared spectrometry (IRIS, Wagner Analysen Technik GmbH, Bremen, Germany). The breath samples were considered positive if the delta-over-baseline value (DOB) was greater than 4%.²²

To determine the platelet count, blood samples were collected at baseline and at 3, 6 and 12 months. Six months after therapy, the effect of the eradication therapy on platelet recovery was evaluated. Platelets were counted using an automated electronic counter (XT-1800i Automated Hematology Analyzer, Sysmex Corporation, Kobe, Japan).

Complete response to *H. pylori* eradication was defined as a final platelet count of $>100 \times 10^9/L$ with an increase of at least $30 \times 10^9/L$ in comparison to baseline. Partial response was defined as an increase of at least $30 \times 10^9/L$ compared to baseline.^{11,12,16} Patients displaying complete or partial response were considered 'responders'. The remainder were considered 'non-responders'.

Statistical analysis

Group differences were analyzed with the Statistical Package for the Social Sciences (SPSS) software for Windows, v. 17.0 (SPSS Inc., Chicago, Illinois). Pearson's chi-square test and Fisher's exact test were used for categorical variables, while Student's t-test was used for continuous variables. The results are expressed as means $\pm$ standard deviation (SD). The level of statistical significance was set at 5% (p -value <0.05).

Ethics

The study protocol was approved on 10 July 2013 by the Research Ethics Committee of the Universidade Federal do Ceará (UFC), Brazil and filed under number 055.05.11. All participants gave their informed written consent. The study was conducted in accordance with the Helsinki declaration as revised in 2008.

Results

The cohort consisted of 28 cITP patients, of whom 22 (78.5%) were *H. pylori*-positive. Five dropped out, one became pregnant, and one had a platelet count $<25 \times 10^9/L$ during the study period. The seven *H. pylori*-positive patients who were excluded did not differ significantly from the remaining 15 with regard to gender (male=2 and female=5 vs. male=1 and female=14; p -value=0.38 – chi-square), mean age (40.2 ± 19.2 years vs. 47.4 ± 12.6 years; p -value=0.70 – Student's t-test), baseline platelet count ($73.45 \pm 25.00 \times 10^9/L$ vs. $72.93 \pm 21.62 \times 10^9/L$; p -value=0.977 – Student's t-test) or cITP duration (10.8 ± 3.6 years vs. 7.36 ± 7.07 years; p -value=0.524 – Student's t-test). Figure 1 is a flowchart of the study from recruitment to outcomes.

The *H. pylori*-negative patients ($n=6$) were similar to infected patients ($n=15$) with regard to gender (female=5; p -value=0.115 – chi-square), mean age (25.6 ± 15.6 years; p -value=0.411 – Student's t test), cITP duration (5.16 ± 3.92 years; p -value=0.52 – Student's t-test), and baseline platelet count ($70.40 \pm 20.08 \times 10^9/L$; p -value=0.96 – Student's t-test).

Six (40%) of the 15 *H. pylori*-positive patients had never been treated for cITP, five (33%) had a history of treatment with prednisone only and four (26%) had undergone splenectomy at least two years before the beginning of this study (two also received prednisone and two received prednisone plus immunosuppressants). Three (50%) of the *H. pylori*-negative patients had never been treated for cITP and three (50%) had a history of treatment with prednisone only. Note that no cITP patient in this study used immunosuppressants or corticosteroids for at least two months before the study. At endoscopy, antral gastritis was observed in five patients, corporal gastritis in three, pangastritis in two, and a duodenal ulcer in one. The remaining four patients displayed no histological abnormalities. *H. pylori*-negative patients had antral gastritis ($n=3$) or no abnormalities ($n=3$).

Eradication was successful in 13 (86.6%) of the 15 patients treated for *H. pylori*. Eradication failed in the remainder ($n=2$; 13.4%) even after retreatment. Six months after treatment, four patients (30.7%) displayed increased platelet counts. Half of these ($n=2$) were classified as complete responders and half as partial responders. Platelet counts were $112.8 \pm 24 \times 10^9/L$ for responders and $57.7 \pm 24.0 \times 10^9/L$ for non-responders at six months, and $119.8 \pm 20.7 \times 10^9/L$ for responders and $70.9 \pm 49.9 \times 10^9/L$ for non-responders at 12 months (Figure 2).

One of the four responders with increased platelet counts after six months had been submitted to splenectomy seven years prior to follow-up, two had a history of corticosteroid treatment, and one had never used medications. Age was <50 years for one patient (25%) and >50 years for three (75%).

Responders and non-responders did not differ significantly with regard to gender, age at diagnosis, mean baseline platelet count, mean disease duration, history of immunosuppressive and corticosteroid therapy, or history of splenectomy (Table 1).

Fourteen (93%) of the 15 patients submitted to eradication therapy were followed up for 12 months. Persistent increases in platelet counts were observed in the four patients with initial responses (including one case in which splenectomy had been ineffective). The platelet count of non-responders

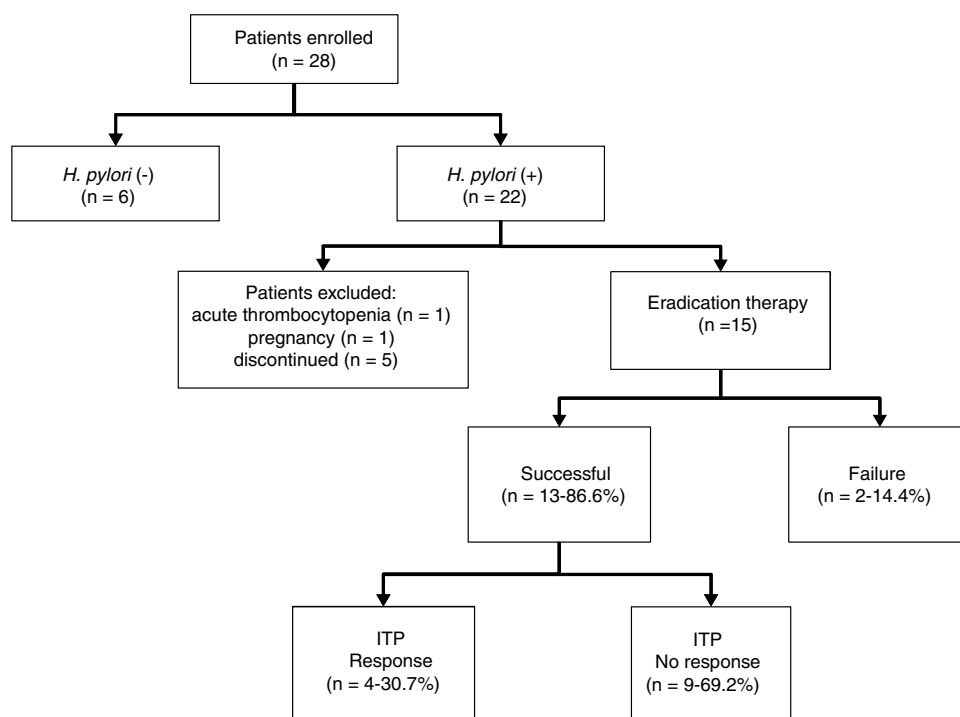


Figure 1 – Flowchart of a cohort of 28 patients with chronic idiopathic thrombocytopenic purpura (cITP) from Northeastern Brazil, 2016.

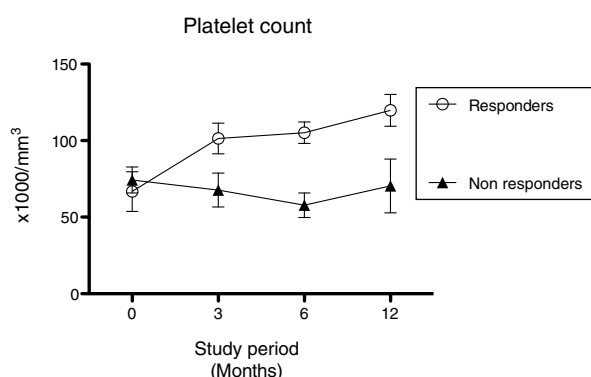


Figure 2 – The mean platelet count $\pm$ standard deviation in responsive and non-responsive patients with chronic idiopathic thrombocytopenic purpura (cITP) at baseline and 3, 6 and 12 months after *Helicobacter pylori* eradication therapy.

Table 1 – Clinical characteristics of patients with chronic idiopathic thrombocytopenic purpura (cITP) that successfully eradicated *H. pylori*.

	Responder (n = 4)	Non- responder (n = 9)	p-value
Male/female ratio	0/4	1/8	0.49
Age – years	53.8 $\pm$ 9.2	44.0 $\pm$ 15.2	0.19
Age at diagnosis – years	50.0 $\pm$ 6.1	41.9 $\pm$ 15.3	0.21
Previous treatment – n (%)			0.31
Yes	3 (75)	4 (44.4)	
No	1 (25)	5 (55.6)	
Splenectomy – n (%)	1 (25)	3 (75)	0.76
Platelet count at baseline – $\times 10^9/L$	66.9 $\pm$ 25.8	79.3 $\pm$ 24.3	0.43

Data are presented as means $\pm$ (standard deviation) or number and %. The p-values were calculated using the Statistical Package for the Social Sciences (SPSS) for Windows (SPSS Inc., Chicago, Illinois). Pearson's chi-square test and Fisher's exact test were used for gender, previous treatment and splenectomy variables, while Student's t-test was used for age, age at diagnosis and platelet count at baseline.

remained low, and two received corticotherapy. No remission of cITP was observed at six months in two patients who were refractory to eradication; one presented hemorrhagic symptoms that were subsequently treated with prednisone. The platelet count of the six *H. pylori*-negative patients (baseline $72.1 \pm 19.6 \times 10^9/L$ vs. $85.0 \pm 17.0 \times 10^9/L$ at six months follow-up; p -value=0.134) remained practically unchanged during the 12-month follow-up, except for one patient who displayed a response at six months ($103.0 \times 10^9/L$), which reverted to $89.3 \times 10^9/L$ at twelve months. *H. pylori*-negative patients

reported no bleeding or need for treatment, splenectomy or specific medications during the follow-up.

Discussion

The association between *H. pylori* infection and cITP is based on the observation of improvements in platelet count following the eradication of *H. pylori*.⁸⁻¹¹ However, the prevalence of

H. pylori infection among cITP patients and the frequency of response in platelet count following eradication therapy vary greatly between geographical regions.²³

Some reports indicate an increased prevalence of *H. pylori* infection among patients with cITP compared to the general population.^{9,11} In this study, the prevalence of *H. pylori* in cITP patients was 75.6%, similar to the findings of an earlier study by our group evaluating the prevalence of *H. pylori* among dyspeptic adults (80%) in Fortaleza, northeastern Brazil¹⁷ and the findings of studies from the southeast of Brazil,¹⁶ Italy⁷ and Japan.²⁴

In the present study, the *H. pylori* eradication rate was 86.6% (13/15) using standard triple therapy. This is compatible with several other studies reporting eradication rates above 70%, with the exception of a single study from Japan (42.9%).²³

Clinical response was positive in 30.7% of *H. pylori*-positive patients after eradication therapy. Similar post-eradication remission rates were reported from southeastern Brazil (17/59; 28.8%),¹⁶ a multicenter study conducted in Italy and England (17/52; 33.0%)²⁵ and a study from Japan (6/22; 28%).²⁶ Lower rates were reported in studies from Iran (3/41; 7.3%),²⁷ Spain (3/23; 13%)¹² and the United States (1/14; 7.1%),¹¹ and higher rates were observed in studies in Colombia (21/26; 80.1%),¹¹ Japan (10/15; 67%)⁹ and Korea (39/42; 92.9%).¹⁰ It is important to mention that higher rates were also observed in a study conducted in children and adolescent patients from the southeast of Brazil (6/10; 60%).²⁸

Differences in clinical findings between responders and non-responders submitted to *H. pylori* eradication therapy have been the object of much study, but results have been variable. It has been suggested that platelet recovery in response to *H. pylori* eradication may be influenced by the duration of cITP,²⁴ age at onset, baseline platelet count and history of prednisone therapy.²⁵ In the present study, no association was found between background characteristics and platelet recovery rates, similar to other reports.^{8,26,29}

Nearly all (93%) of the patients submitted to *H. pylori* eradication therapy were followed up for 12 months. At this time, platelet recovery was sustained in all four patients with initial response, even in a case of unsuccessful splenectomy. In contrast, platelet counts did not improve in patients with successful eradication but poor initial response, nor in patients with unsuccessful eradication. Generally, *H. pylori*-negative patients displayed no change in platelet count between baseline and six months, except for one patient with a slight improvement, who reverted at 12 months. Studies following cITP patients for longer periods found that in most cITP patients responding to *H. pylori* eradication therapy, improvement was sustained without idiopathic thrombocytopenic purpura treatment for more than 12 months.^{8,30} Thus, our results are in agreement with other studies and raise the possibility that treatment for cITP may be discontinued without any negative impact in this subgroup of patients.

The mechanism linking *H. pylori* infection to cITP is not fully understood, but some attribute it to the virulence factor of CagA. Infection with CagA-positive *H. pylori* strains conceivably induces the formation of platelet autoantibodies by chronic immune stimulation or the molecular mimicry of CagA to platelet antigens.¹⁵ Furthermore, clinical studies reinforce a correlation of *H. pylori*-specific CagA with clinical

idiopathic thrombocytopenic purpura. It has been demonstrated that successful *H. pylori* eradication therapy in cITP patients improves the total circulating platelet count and decreases anti-CagA antibodies.²⁴

This is the first study evaluating the effect of *H. pylori* eradication therapy on platelet count in patients with cITP from northeastern Brazil. The study was limited by the small sample size; however it is important to mention that cITP is not a common disease and it is difficult for a single center study to enroll a large number of patients. Another limitation is the short follow-up period. Further investigations are necessary to clarify the role of *H. pylori* infection in cITP.

Conclusion

In conclusion, cITP patients were found to have relatively low platelet recovery rates (31.4%) in response to *H. pylori* eradication therapy in this study, but the effect remained during one year of follow-up. These results suggest that treating *H. pylori* infection might be worthwhile in a subset of cITP patients. Further studies are required to understand the mechanism underlying the response to eradication therapy.

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Conflicts of interest

The authors declare no conflicts of interest.

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