Proton-pump inhibitors for the treatment of functional dyspepsia

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Abstract: In the Rome III classification, functional dyspepsia (FD) has been further subcategorized into two different syndromes, namely, epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS). Acid-related pathophysiology seems to be mainly responsible for EPS, and antisecretory agents such as proton-pump inhibitors (PPIs) seem to be effective mainly against EPS. However, recent information as to the relationship between initial duodenal acid sensitization in the early postprandial phase and delayed gastric emptying in the later postprandial phase would suggest the amelioration of PDS by antisecretory agents. In the present review, we summarized the recent literature on the direct and indirect effect of PPIs in FD (including not only Rome III, but also Rome II criteria). The effects of PPIs against FD are heterogeneous, depending on the protocol of the clinical studies, and the inclusion criteria of each randomized controlled trial (primary care or tertiary care population). As the placebo effects cannot be ignored in this disease, a placebo-controlled study would be necessary, at least for the evaluation of the effect of each agent on symptom relief in patients with FD. Further studies directly comparing PPIs with suitable placebos in terms of their effects in reducing the symptoms of endoscopically confirmed, Rome III-based FD are awaited.

Keywords: antisecretory agents, functional gastrointestinal disorders, *Helicobacter pylori*, randomized controlled study, uninvestigated dyspepsia

Introduction

Many patients with functional gastrointestinal disorders (FGIDs) have chronic symptoms pertaining to the gastroduodenal region. The Rome III consensus, which is based on the consensus opinion of an international panel of clinical investigators who reviewed the available evidence, proposed a classification of functional gastroduodenal disorders [Suzuki *et al.* 2006; Tack *et al.* 2006]. Among four categories of functional gastroduodenal disorders, the first category is functional dyspepsia (FD), associated with symptoms thought to originate from the gastroduodenal region, specifically manifesting as one or more of the four main symptoms of epigastric pain or burning, postprandial fullness, and early satiety. Furthermore, FD has been further subclassified into postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS). This Rome III classification requires further research and careful validation; however, the criteria appear to be of value in clinical practice, for epidemiological, pathophysiological, and clinical management studies, and for drug development.

Gastric acid secretion and the perceived sensation associated with it are among the potentially important players in the genesis of dyspeptic symptoms. In patients with FD, especially those with EPS, suppression of gastric acid secretion by antisecretory agents such as proton-pump inhibitors (PPIs) or histamine type 2-receptor antagonists (H_2 blocker) seems to ameliorate the epigastric pain or burning. Furthermore, even in PDS, as the initial gastric acid emptying may play a pathogenetic role on symptom generation through the early onset of duodenal brake, acid suppression might be effective, at least in part, against the bothersome postprandial fullness [Grudell *et al.* 2006].

PPIs are among the strongest of drugs available for gastric acid suppression. In the CADET-HN study [Veldhuyzen van Zanten *et al.* 2005], the effect of omeprazole to dyspepsia symptoms

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was evaluated. *Helicobacter pylori*-negative patients recruited with dyspepsia symptoms of at least moderate severity were randomized to a 4-week treatment protocol of omeprazole 20 mg o.d. or placebo, followed by on-demand therapy for an additional 5 months. Those who participated in this study were not previously investigated FD patients, but the success rate (no complete disappearance or minimal residual symptoms) of the omeprazole group at 4 weeks was 51% ($N=69/135$, 95% CI 43–60%) and were significantly superior to the placebo group (23%, $N=31/133$, 95% CI 16–31%, $p<0.05$). Furthermore, the proportion of patients who were responders at 4 weeks and at 6 months was significantly greater among those receiving omeprazole (31%, $N=42/135$, 95% CI 23–39%) than placebo (14%, $N=18/133$, 95% CI 8–20%, $p=0.001$). These results may suggest that treatment with omeprazole provides superior symptomatic relief as compared with placebo in the treatment of *H. pylori*-negative primary care uninvestigated dyspepsia patients.

Review methodology of articles concerning PPIs on functional dyspepsia

A literature search of PubMed was performed using the keywords "functional dyspepsia", "nonulcer dyspepsia", and "proton-pump inhibitor", and was limited to studies published in the English language over the past 14 years (1997–2010) and to human studies (meta-analyses, randomized-controlled trials (RCTs), clinical trials, comparative studies, and systemic reviews). After compiling a list of the 79 studies identified, 46 publications not directly linked to FD and PPIs (but concerning mainly *H. pylori* eradication therapy [Suzuki *et al.* 2007]) were excluded. Furthermore, three articles which have only simple abstracts without details were also excluded.

Of the remaining 30 studies, 1 study dealing with only acid-related dyspepsia which seems to overlap with gastroesophageal reflux disease (GERD), 4 descriptive reviews of other studies, and 11 manuscripts not concerned with PPI therapy on FD were further excluded. Finally, 14 articles including 4 systematic reviews, 7 RCTs, and 3 non-RCTs were reviewed in detail (Figure 1), and the results are presented here.

Results of the literature review

Randomized controlled trials. The results of five RCTs [Grudell *et al.* 2006; Van Zanten *et al.* 2006; Peura *et al.* 2004; Wong *et al.* 2002; Talley *et al.* 1998] on the efficiency of PPIs in functional dyspepsia are shown in Table 1.

First, Talley and colleagues conducted an RCT to evaluate the efficacy of omeprazole in patients with FD [Talley *et al.* 1998]. Patients ($n=1262$, mean age 41–43) with a clinical diagnosis of FD were enrolled in their study. The participant patients had a history of persistent or recurrent

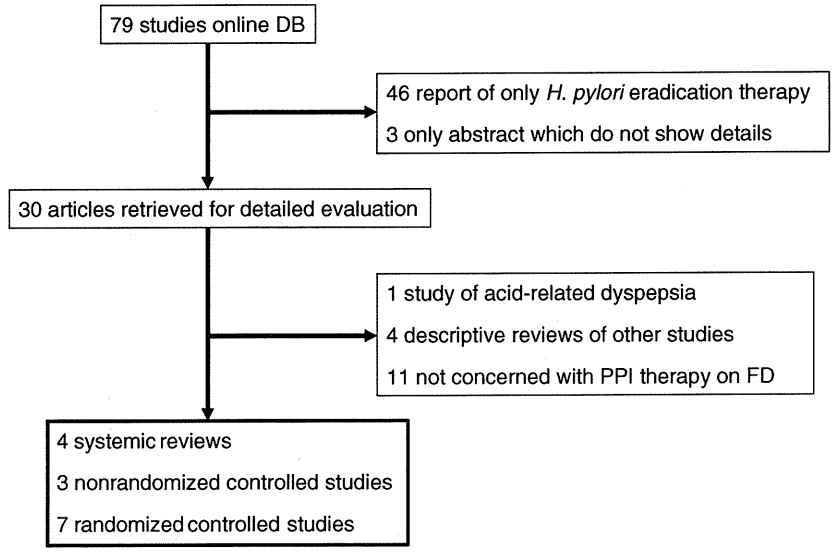

Figure 1. Literature selection flow. DB, database; FD, functional dyspepsia; PPI, proton-pump inhibitor.

epigastric pain or discomfort for at least 1 month, and the symptoms were perceived on at least 25% of the days of the month, with negative findings on esophagogastroduodenoscopy (EGD). Patients were randomized to receive omeprazole 20 or 10 mg, or identical placebo, for 4 weeks. On an intention-to-treat (ITT) analysis ($n=1248$), complete symptom relief was observed in 38% of the patients on omeprazole 20 mg, as compared with 36% of patients on omeprazole 10 mg and 28% of the patients on placebo ($p=0.002$ and 0.02, respectively). In addition, the patients were classified into three subgroups based on the type of the symptoms: ulcer-like dyspepsia, reflux-like dyspepsia, and dysmotility-like dyspepsia. The symptom relief was compared among these patient groups. Complete relief was obtained in 40% and 54% of patients with ulcer-like dyspepsia and reflux-like dyspepsia, respectively, on omeprazole 20 mg, and in 35% and 45% of patients with ulcer-like dyspepsia and reflux-like dyspepsia, respectively, on omeprazole 10 mg, as compared with in 27% and 23% of the above two respective patient groups on placebo treatment (all $p<0.05$, except omeprazole 10 mg in ulcer-like dyspepsia, $p=0.08$). On the other hand, there was no significant benefit of omeprazole over placebo in the group with dysmotility-like dyspepsia. The results suggest that symptom subgrouping based on symptom predominance has clinical utility because it predicts treatment response.

Wong and colleagues examined the effect of different doses of lansoprazole for the treatment of FD in Chinese patients [Wong *et al.* 2002]. A total of 453 patients with a clinical diagnosis of FD according to the Rome II criteria and normal EGD were randomized to receive lansoprazole 30 mg, lansoprazole 15 mg, or placebo, for 4 weeks. Dyspepsia symptom scores and quality of life (QOL; using SF-36 score) were evaluated before and after 4 weeks treatment. There was no difference in the proportion of patients with complete symptom relief in the lansoprazole 30 mg (23%, $N=35/149$, 95% CI 17–30%) and lansoprazole 15 mg (23%, $N=35/152$, 95% CI 17–30%) groups as compared with that in the placebo group (30%, $N=45/152$, 95% CI 23–37%). Furthermore, the mean dyspepsia scores of the three groups were further improved after 4 weeks ($p<0.001$). This result means that there were no significant differences among these groups in terms of the QOL.

In contrast to the above findings, according to the study conducted by Peura and colleagues, in which 921 patients were assigned randomly to receive lansoprazole 30 mg, lansoprazole 15 mg, or placebo for 8 weeks, a significant reduction in the percentage of days with upper abdominal discomfort was observed for patients treated with lansoprazole 30 mg (34%) or 15 mg (35%), but not for those treated with placebo (19%) ($p<0.001$) [Peura *et al.* 2004]. Similarly, a larger percentage of patients treated with lansoprazole 30 mg (44%) or 15 mg (44%) reported complete symptom resolution (defined as no episodes of upper abdominal discomfort during the 3 days prior to the study visit) than in the group treated with placebo (29%) ($p<0.001$). In their

Table 1. Randomized controlled trials.

Study	*n*	Drug	Dosage	Subjects	Outcome	Period	Effect
Talley *et al.* [1998]	1248	Omeprazole	20 mg/10 mg/ placebo	With normal EGD; epigastric pain or discomfort	Symptom relief at interview	4 weeks	Effective
Wong *et al.* [2002]	453	Lansoprazole	30 mg/15 mg/ placebo	With normal EGD; clinical diagnosis of FD	Dyspepsia symptom score Quality of life (SF-36 score)	4 weeks	Ineffective
Peura *et al.* [2004]	921	lansoprazole	30 mg/15 mg/ placebo	With normal EGD; predominant upper abdominal discomfort	Symptom relief	8 weeks	Effective
Van Zanten *et al.* [2006]	224	Esomeprazole	40 mg/ placebo	With normal EGD; moderate severity of symptom (GOS scale)	Symptom relief (GOS ≤ 2)	4 weeks 8 weeks	Effective Ineffective

n, number of patients; EGD, esophagogastroduodenoscopy; SPECT, single photon emission computed tomography; GOS scale, Global Overall Symptom scale

study, however, improvement of upper abdominal discomfort was seen only in patients who had at least some symptoms of heartburn at enrollment. They concluded that lansoprazole was significantly better than placebo in reducing the symptom of persistent or recurrent upper abdominal discomfort associated at least with some heartburn. The fact that about 75% of the enrolled patients had some symptom of heartburn in spite of excluding patients with predominant symptoms of GERD indicated that it was not only a characteristic symptom of gastroesophageal reflux. The presence of some degree of heartburn may be a marker of acid sensitivity and a useful predictor of response to PPIs, indicating that there was no difference in the effects of the groups receiving the 30 mg dose and 15 mg dose. The effect of PPI on FD may not be dependent on the dose and this may be related to the mechanism of FD.

Concerning esomeprazole, Van Zanten and colleagues designed an RCT in which 224 adult patients who had dyspepsia symptoms of at least moderate severity and no findings on EGD were enrolled [Van Zanten *et al.* 2006]. The subjects were randomized to receive esomeprazole 40 mg or placebo once daily for 8 weeks. With regards to the primary outcome measure of symptom relief at 8 weeks, there was no statistically significant difference between the esomeprazole 40 mg (55.1%, $N=60/109$, 95% CI 45.2–64.6) and placebo (46.1%, $N=53/115$, 95% CI 36.8–55.6) once daily groups ($p=0.16$), although at 4 weeks esomeprazole provided significantly greater symptom relief (50.5%, $N=55/109$, 95% CI 40.7–60.2) than placebo (32.2%, $N=37/115$, 95% CI 23.8–41.5) ($p=0.009$). The difference in therapeutic gain between 4 and 8 weeks was considered to be largely attributable to the higher placebo response rate at 8 weeks.

Randomized-controlled trials not concerned directly with the efficacy of PPIs against functional dyspepsia. FD patients have been demonstrated to have enhanced visceroperception and a decreased duodenal motor response to intraduodenal acid infusion. Schwartz and colleagues conducted antropyloroduodenal manometry before and after the treatment in patients randomly assigned to receive treatment with pantoprazole ($n=10$) or placebo ($n=9$) for 2 weeks, and showed that pantoprazole decreased the duodenal acid hypersensitivity to some extent ($p=0.07$), but did not improve the impaired duodenal motor response [Schwartz *et al.* 2001].

On the other hand, Bolling-Sternevald and colleagues investigated the factors predictive of a good response to PPIs [Bolling-Sternevald *et al.* 2003]. Patients ($n=826$) with FD who were treated with omeprazole 10 or 20 mg or placebo for 4 weeks were collected from another RCT [Talley *et al.* 1998]. Fewer days with symptoms during the first week was associated with a higher response rate at 4 weeks ($p<0.0001$), suggesting that the initial response to PPIs could predict the subsequent response. The most discriminating predictor of treatment success was the number of days with epigastric pain/discomfort during the first week of treatment ($p<0.0001$). In addition, age > 40 years ($p=0.03$), bothersome heartburn ($p=0.003$), low scores for bloating ($p=0.006$), epigastric pain ($p=0.02$), and diarrhea ($p=0.03$), history of symptoms for <3 months ($p=0.009$) and low impairment of vitality ($p=0.03$) at baseline were identified as positive predictors of the outcome.

Symptom relapse after PPI treatment in patients with FD is another important issue that needs to be discussed. Reimer and colleagues [Reimer and Bytzer, 2010] investigated the effects of a PPI against symptom relapse in the absence of abnormal endoscopic findings. A total of 31 patients were randomized to 7 days of esomeprazole 40 mg or placebo. Successful effect of therapy after 7 days was observed in 12 of 15 patients (80%) in the esomeprazole group *versus* 2 of 16 (13%) in the placebo group ($p<0.001$), suggesting that short-term esomeprazole therapy was superior to placebo in patients with recurrence of symptoms not associated with abnormalities on endoscopy. They showed on the other RCT that PPI therapy for 8 weeks induced acid-related symptoms in healthy persons after withdrawal [Reimer *et al.* 2009]. The 44% ($N=26/59$) of those randomized to 8 weeks of esomeprazole 40 mg/day followed by 4 weeks with placebo reported acid-related symptom in weeks 9–12 compared with 15% ($N=9/59$; $p<0.001$) in the placebo group, indicating that this phenomenon was caused by rebound acid hypersecretion (RAHS).

Nonrandomized studies. There were three non-RCTs (Table 2), as described in the following; for

Table 2. Nonrandomized controlled studies.

Author	Year	*n*	Drug	Dosage	Subjects	Outcome	Period	Effect
Mundo-Gallardo *et al.*	[2000]	189	Rabeprazole	20 mg	With normal EGD; functional dyspepsia	Symptom relief	4 weeks	Effective
Ghosh *et al.*	[2008]	46	Rabeto plus (rabeprazole + itopride)	1 capsule	Functional dyspepsia overlapped with NERD	Symptom relief global assessment of efficacy and tolerability	4 weeks	Effective
Miyamoto *et al.*	[2010]	467	Rabeprazole lansoprazole omeprazole + mosapride citrate (prokinetics)	10 mg 30 mg/ 15 mg 20 mg/ 10 mg 5 mg	GERD (NERD + RE)	Total score/ reflux score/ dyspeptic score in FSSG	2 weeks + 4 weeks	Effective

n, number of patients; EGD, esophagogastroduodenoscopy; NERD, nonerosive reflux disease; GERD, gastroesophageal reflux disease; RE, reflux esophagitis

two of these, only the abstract could be referred [Ghosh *et al.* 2008; Mundo-Gallardo *et al.* 2000].

Ghosh and colleagues indicated the efficiency of a fixed-dose combination (FDC) of rabeprazole and itopride [a prokinetic (PK) agent] in the management of FD [Ghosh *et al.* 2008]. They designed an open, prospective, noncomparative, multidose study; a total of 46 adult patients with FD [overlapped with non-erosive reflux disease (NERD)] were given one capsule of the rabeto plus formulation (FDC) for 4 weeks, and completed the study. Most patients showed near total symptom relief by the end of the study period and response to the drug was reported as excellent or good by 93% of the patients and their treating physicians.

Mundo-Gallardo and colleagues investigated the effect of rabeprazole on FD in a multicenter, open-label study [Mundo-Gallardo *et al.* 2000]. They assessed the clinical efficacy and tolerability of rabeprazole 20 mg in 189 patients with FD. The clinical efficacy rate was 86% after only 4 weeks of treatment, and the symptom control was remained until the end of 4 weeks without treatment.

In the Rome III criteria, overlap between FD and NERD is recognized. Miyamoto and colleagues designed a study to investigate the effects of treatment in patients with NERD and RE: factors predictive of a poor response to PPIs and also the effects of PKs were evaluated [Miyamoto *et al.* 2010]. The subjects were 467 GERD patients (NERD 349, RE 118). PPI treatment (rabeprazole 10 mg: $n = 214$; lansoprazole 30 mg: $n = 97$; lansoprazole 15 mg: $n = 63$; omeprazole 20 mg: $n = 54$; omeprazole 10 mg: $n = 39$) was administered for 2 weeks. The total score (TS) for symptoms, the reflux score (RS), and the dyspeptic score (DS) were assessed using the frequency scale for the symptoms of GERD (FSSG). There was higher tendency towards nonresponse to PPI treatment among the patients with NERD (52.7%, 184/349) than among those with RE (42.4%, 50/118) ($p = 0.0516$). In NERD, younger age ($p = 0.0066$), presence of constipation ($p = 0.0014$), higher TS ($p = 0.0177$), DS ($p = 0.0012$) and scores for the four items of FSSG (bloated stomach: $p = 0.01547$; heavy stomach: $p = 0.0009$; sick feeling after meal $p = 0.0297$; and early satiety during a meal: $p = 0.0004$) were associated with a poor response to PPIs. In addition, PKs were added to PPI for another 4 weeks in NERD patients nonresponsive to PPIs. Significant improvement of the TS (pretreatment: 17.4 ± 7.7 *versus* PPI only after 2 weeks 14.6 ± 6.0 *versus* PPI + PK after 6 weeks 7.7 ± 5.2, $p < 0.0001$) was observed in the nonresponders to PPIs among the NERD patients after the addition of PKs (mosapride citrate 5 mg t.i.d., a selective 5-HT_4 receptor agonist, stimulates upper gastrointestinal motor activity, and is free of dopamine D_2 receptor antagonist properties).

Systematic reviews. There were five systematic reviews [Sanaka *et al.* 2010; Kleibeuker and

Theijs, 2004; Moayyedi *et al.* 2004; Talley and Lauritsen, 2002; Chiba *et al.* 2000].

Moayyedi and colleagues conducted a systematic review of eight RCTs conducted to evaluate the effect of PPI therapy in patients with nonulcer dyspepsia ($n = 3293$, including five trials of omeprazole and three of lansoprazole), identified through a search of articles published in MEDLINE, EMBASE/Excerpta, Medica, Cochrane-Controlled Trials Register, CINAHL, and SIGLE until September 2002 [Moayyedi *et al.* 2004]. The relative risk of residual dyspepsia symptoms following PPI therapy *versus* placebo was 0.86 (95% CI, 0.78–0.95; $p = 0.003$), with a number needed to treat of 9 (95% CI 5–25). On the other hand, from an economic standpoint, the PPI strategy (PPI price, US$90/month, evaluated over a 1-year period) would cost an additional US$278/month for freedom from dyspepsia than over-the-counter (OTC) antacid therapy at US$57/month (OTC price US$ 19.99). However, since the monthly cost of PPI would fall each year, the updated calculation for FD management should be performed.

Talley and Lauritsen summarized the data of BOND and OPERA [Talley *et al.* 1998], PILOT, and a 3-month follow up study, ENCORE, and indicated that omeprazole 20 or 10 mg may be superior to placebo for obtaining relief from FD symptoms [Talley and Lauritsen, 2002]. Pooling the BOND and OPERA trials, complete relief of symptoms was achieved in 38.2% of the 20 mg omeprazole group ($p = 0.002$) and in 36.0% of the 10 mg omeprazole group ($p = 0.02$) compared with 28.2% in the placebo group. In patients with ulcer-like and reflux-like dyspepsia, complete relief of symptoms was achieved in 40% and 54% of the 20 mg omeprazole group ($p = 0.05$) and in 35% and 45% of the 10 mg omeprazole group ($p = 0.08$ and $p = 0.05$, respectively) compared with 27% and 23% in the placebo group in pooled analysis. In addition, these trials showed that symptom relief may be unrelated to the *H. pylori* status, and that successful treatment may have a positive impact on the patient QOL assessed over a 3-month period after treatment cessation. Compared with patients in whom symptoms still persisted at the end of trial, patients with complete absence of symptoms at the end of active or placebo treatment had fewer days on medication (9.2 *versus* 22.7, $p < 0.001$), fewer clinical visits (1.5 *versus* 2.0, $p < 0.001$) and the psychological general well being index ($p < 0.001$).

Another review [Kleibeuker and Theijs, 2004], based on the examination of several trials, indicated the efficacy of PPIs in the treatment of FD (RR reduction of 14%), and that the best predictor of a beneficial effect was the response during the first week of treatment. Additional predictors included a history of symptoms of less than 3 months duration and a history of bothersome heartburn [Peura *et al.* 2004; Bolling-Sternevald *et al.* 2003]. They pointed out that PPIs provide only a small beneficial effect in FD patients, and that this effect is largely attributable to a reduction in gastroesophageal reflux-induced complaints. They emphasized that the response during the first week of PPI therapy can probably be used as an indicator to decide on whether or not to continue using the drug for treatment.

On the other hand, Sanaka and colleagues reviewed several studies (1979–2009) that investigated the rate of gastric emptying [Sanaka *et al.* 2010]. They suggested that PPIs (omeprazole and lansoprazole, but not rabeprazole) delayed the gastric emptying of solid meals, while having variable effects on gastric liquid emptying. They hypothesized that PPIs impair hydrolytic digestion by inhibiting acid-dependent peptic activity, thereby delaying the gastric emptying rate of solids, while the gastric emptying rate of liquids largely depends on the volume and energy density of the intragastric contents. PPIs variably modify the volume and energy density by reducing gastric fluid secretion, thereby modifying the rate of gastric emptying of liquids in an unpredictable manner.

Summary

Taken together with the abovementioned information in relation to PPI efficacy against the symptoms of FD, the placebo effect, which is approximately 30–40% among patients in RCTs, cannot be ignored in patients with FD [Moayyedi *et al.* 2004]. The placebo response may, in part, reflect the natural fluctuations of upper gastrointestinal tract-originated symptoms, although this is not the only likely factor [Thompson, 2000]. According to a recent report by Talley and colleagues, independent predictors of a weak placebo response were a lower BMI and a more consistent predominant symptom pattern (both $p < 0.05$), while no association was seen with the age, gender, center type,

baseline symptom score, baseline rate or change in rate of gastric emptying, or the baseline QOL [Talley *et al.* 2006]. Their data indicated that aside from BMI and a consistent predominant symptom pattern, the aforementioned factors (symptom severity, age, gender, center type and rate of gastric emptying) were not useful predictors of a placebo response [Talley *et al.* 2006]. As shown in the previous studies, the placebo effects cannot be ignored in the field of FGIDs; a placebo-controlled study would be necessary, at least for the efficacy evaluation of each treatment agent on the symptoms of FD. Further studies directly comparing PPIs with suitable placebos in terms of their effects in reducing the symptoms of endoscopically confirmed, Rome III-based FD are awaited.

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Conflict of interest statement

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Helicobacter

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Extragastric Manifestations of *Helicobacter pylori* Infection

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Diabetes mellitus, idiopathic thrombocytopenic purpura, glaucoma, liver fibrosis, prurigo, chronica multiformis, parkinsonism.

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Abstract

In the previous year, some extragastric diseases, possibly linked to *Helicobacter pylori* infection, have been largely investigated. There are, in fact, several studies concerning cardiovascular diseases, lung diseases, hematologic diseases, eye and skin diseases, hepatobiliary diseases, diabetes mellitus, and neurological disorders. Among them, the relationship between bacterial CagA positivity and coronary heart disease is reportedly emphasized. Concerning normal tension glaucoma, new interesting data are playing in favor of the association with *H. pylori* infection. For other diseases, there are many interesting results, although more studies are needed to clarify the reality of the proposed association.

The topic of the extragastric manifestations of *Helicobacter pylori* infection continues to capture the attention of many researchers all over the world, as demonstrated by the number of studies published. Here, we review the results of the studies published last year.

Cardiovascular Diseases

Several studies have been published in the last year on the possible role of *H. pylori* infection in cardiovascular diseases. Jafarzadeh et al. [1] focused on the prevalence of CagA-positive strains in patients with acute myocardial infarction (MI), unstable angina (UA), and healthy controls. They clearly showed that the seroprevalence of CagA-positive strains was significantly higher in patients with acute MI and UA than controls (86.7, 91.7, and 58.3%, respectively). Another study conducted by our group in Rome on the role of CagA positivity in patients undergoing coronary angiography showed that the titer of anti-CagA antibodies was significantly higher in patients with coronary atherosclerosis than in subjects with normal coronary arteries [2]. Interestingly, a positive correlation between CagA antibody titer and the extent score of the atherosclerotic disease was also found. Moreover, patients infected with CagA-positive strains had a more extensive coronary artery disease (CAD) compared with those infected with CagA-negative strains and, at multivariate analysis, anti-CagA antibody titer was the only predictor of the extent of coronary atherosclerosis [2].

Another study by Agrawal et al. [3] conducted on diabetic patients with or without *H. pylori* infection reported a higher prevalence of *H. pylori* infection in patients with diabetes mellitus (DM). Moreover, *H. pylori*-positive diabetic patients showed a higher prevalence of CAD than *H. pylori*-negative diabetic subjects. Nevertheless, this is still a debated topic. In fact, these data were not confirmed by the study of Schimke et al. [4], in which CagA positivity was not shown to be a risk factor for chronic vascular complications in patients with type 2 diabetes.

Concerning the pathogenic mechanisms by which *H. pylori* may eventually concur to the pathogenesis of ischemic heart disease (IHD), two studies were published last year. The first one aimed at investigating whether CagA-positive *H. pylori* strains may influence serological levels of high sensitivity C-reactive protein, total cholesterol, low-density protein (LDL), oxidized LDL (oxLDL), and apolipoprotein B. Interestingly, the levels of all those markers were significantly increased in CagA-positive patients compared with negative; moreover, CagA-positive patients showed a more severe coronary atherosclerosis [5].

The second study presents a meta-analysis of all studies published in the field of *H. pylori* infection, platelet aggregation, and thrombosis [6]. Results showed that some *H. pylori* strains are able to bind to the von Willebrand factor, to interact with glycoprotein Ib, and to induce platelet aggregation in humans. The final hypothesis is that *H. pylori* may eventually affect IHD by eliciting thrombosis [6].

Diabetes Mellitus

The consistency of a role of *H. pylori* infection in the pathogenesis of DM as well as in the gastric abnormalities of patients with diabetes has been analyzed and critically discussed. Several controversies emerge from the epidemiological data. The clinical consequence of *H. pylori* infection in terms of metabolic control seems to be low. Regarding interventional studies, the bacterial eradication rate is significantly lower in patients with DM than in controls [7]. The difference in the *H. pylori* eradication rate observed between adults and children affected by diabetes could be due to the fact that the latter have no history of repeated infectious diseases and antibiotic treatments, leading to less antibiotic-resistant *H. pylori* strains. Ojetti et al. showed that a higher *H. pylori* reinfection rate occurs in patients with DM than in the general population [8].

Lung Diseases

In the last few years, a positive correlation between seroprevalence of *H. pylori* and lung cancer has been described. A study by Behroozian et al. seems to confirm these findings [9]. In particular, they looked for the prevalence of anti-*H. pylori* antibodies among 66 patients with lung cancer and 66 controls. Interestingly, they found a higher prevalence of *H. pylori* in patients with lung cancer compared with controls (73 vs 51%; odds ratio (OR): 2.51; [95% CI: 1.14–5.54]; $p < .05$). Nevertheless, whether the higher prevalence of *H. pylori* in patients with lung cancer is casual or causative still remains undetermined. Smoking habits might be confounding in both events.

Interestingly, a case report was published by Riviere et al. showing the disappearance of pulmonary sarcoidosis in a patient after *H. pylori* eradication [10]. Also in this case, whether *H. pylori* is the cause or a coincidence is still unknown.

Hematologic Diseases

Helicobacter pylori is a well-recognized cause of idiopathic thrombocytopenic purpura (ITP) [11,12]. Studies published in the last year are in favor of this association. A study by Kikuchi et al., who re-evaluated 11 patients with ITP 8 years after *H. pylori* eradication, clearly showed the presence of a complete remission in all patients [13]. Fan et al. tested the efficacy of amifostine, a cytoprotective agent reducing reactive oxygen species, in treating patients with refractory ITP. Interestingly, all patients treated with this drug experienced a long-lasting remission, except for two, and one of these two patients relapsed following an *H. pylori* infection [14]. Matsukawa et al. focused on a peculiar interaction between *H. pylori* infection and peripheral platelet count in patients without ITP. In particular, the authors reported a significant decrease in peripheral platelet counts in patients with *H. pylori* infection, after its successful eradication [15]; the clinical significance of such a phenomenon is still unclear. A study conducted by Gursel et al. showed that *H. pylori* infection may cause dysfunction of platelets in children and can be reversed by *H. pylori* eradication [16]. Those studies clearly demonstrate the existence of a close interaction between *H. pylori* and platelets, which surely merits further investigation.

Diamantidis et al. reported a high prevalence of *H. pylori* infection in Greek patients with myelodysplastic syndromes; nevertheless, there is no evidence for a causal relationship between those conditions so far [17]. Finally, Matsukawa et al. described the case of a patient with *H. pylori*-positive atrophic gastritis, who showed a significant increase in IgE and eosinophils after successful eradication of the infection [18].

Ophthalmology, Skin, and Mucosal Diseases

Rahbani-Nobar et al. evaluated the effect of *H. pylori* treatment on remission of idiopathic central serous chorioretinopathy [19]. Twenty-five patients with idiopathic central serous chorioretinopathy who were infected with *H. pylori* were treated with an anti-*H. pylori* treatment; another 25 patients with the same clinical symptoms served as the control. The difference between the mean visual acuity at the end of 16 weeks and the time of subretinal fluid reabsorption was compared between the two groups. Subretinal fluid reabsorption time was 9.28 ± 3.20 weeks in the *H. pylori* eradication group and 11.63 ± 3.18 weeks in the control group, which was statistically significant ($p = .015$). On the other hand, visual acuity improvement did not represent a statistically significant difference. *Helicobacter pylori* eradication regimen can be considered as effective in the treatment of patients with idiopathic central serous chorioretinopathy given that it leads to a faster reabsorption of subretinal fluid.

Kim et al. investigated whether *H. pylori* infection is associated with normal tension glaucoma (NTG) [20]. One hundred consecutive patients with NTG (group 1) from an outpatient glaucoma clinic were enrolled. Medical records of the 88 control participants (control 1) of the outpatient clinic as well as 104 patients with NTG (group 2) and 1116 healthy controls (control 2) (1220 subjects in total) from a primary health care center

were reviewed retrospectively to compare the results. The distribution of the results of *H. pylori* serology of the patients with NTG and controls was compared. Patients with NTG had significantly more positive *H. pylori* serology than did the healthy controls. There were significant differences between group 1 and control 1 patients ($p = .020$; OR: 2.05; [95%CI: 1.12–3.75]), group 1 and control 2 patients ($p = .016$; OR: 1.73; [95%CI: 1.10–2.72]), and group 2 and control 2 patients ($p = .008$; OR: 1.83; [95%CI: 1.17–2.86]). This study suggests that *H. pylori* infection may be associated with an increased risk of NTG and that *H. pylori* may play a role in the development or progression of NTG.

Akashi et al. studied the relationship between *H. pylori* and chronic urticaria and prurigo chronica multiformis [21]. Eighty-two patients with chronic urticaria and 17 patients with prurigo chronica multiformis were tested with a polyclonal *H. pylori* stool antigen test. *H. pylori* antigen was detected in 25 (30.5%) of the 82 patients with chronic urticaria and in 10 (58.8%) of the 17 patients with prurigo chronica multiformis. This *H. pylori* positivity was not significantly higher than the positivity observed in healthy age-matched controls. The therapeutic efficacy of antibacterial treatment for the chronic urticaria and the prurigo chronica multiformis was examined. The effectiveness of treatment was evaluated by scoring the skin conditions and by using the Skindex-16, a measure of quality of life. Although *H. pylori* eradication therapy was more effective in treating prurigo chronica multiformis and the skin symptoms started to improve within 3–14 days after the start of treatment, such eradication therapy was not always effective in treating chronic urticaria. *Helicobacter pylori* may be an important pathogenetic factor, especially for prurigo chronica multiformis, and eradication therapy would be considered to treat intractable cases.

Helicobacter pylori has been found in the oral cavity and stomach. Zou et al. studied whether there might be any associations between isolates of *H. pylori* in the oral cavity and those in the stomach by meta-analysis [22]. Studies reporting raw data on the prevalence of *H. pylori* infection in the oral cavity in gastric *H. pylori*-positive and *H. pylori*-negative patients, in patients with gastroesophageal diseases (GERD), and in healthy individuals and studies reporting data on the eradication rate in the oral cavity or stomach were identified. The prevalence of *H. pylori* infection in the oral cavity in gastric *H. pylori*-positive patients was significantly higher (45.0%) than that in gastric *H. pylori*-negative patients (23.9%) (OR: 3.61, [95% CI: 1.91–6.82], $p < .0001$). The 44.8% (91/203) prevalence of *H. pylori* infection in the oral cavity of patients with clinical and/or histologic GERD was significantly higher than the 13.2% (21/159) prevalence in patients with nonulcer dyspepsia or healthy controls (OR: 5.15, [95% CI: 2.97–8.92], $p < .00001$). The eradication rate in the stomach was 85.8% (187/218), while it was only 5.7% (9/158) in the oral cavity (OR: 55.59, 95%CI: 8.69–497.46, $p < .00001$), indicating that the oral cavity may be a source or reservoir of reinfection by *H. pylori*.

Hepatobiliary Diseases

Ki et al. reported that *H. pylori* promotes hepatic fibrosis in a murine model [23]. To elucidate the mechanism by which *H. pylori* accelerates liver fibrosis, they investigated the changes in expression levels of mitogen-activated protein kinases (MAPKs), p53-related proteins, antioxidants, and pro-inflammatory cytokines in liver samples. *Helicobacter pylori* and/or CCl_4-induced MAP kinase activation was investigated. *Helicobacter pylori* infection enhanced CCl_4-induced MAP kinase activation and the p53 signaling pathway as well as Bax- and proliferating-cell nuclear antigen expressions, whereas *H. pylori* alone induced neither of these expressions nor hepatic fibrosis. Moreover, mRNA expression of inflammatory cytokines, glutathione peroxidase expression, and the proliferative index were strongly augmented in livers with *H. pylori* in the CCl_4-treated group compared with those without *H. pylori* in the CCl_4-treated group, whereas there was no difference in apoptotic index between these two groups. Interestingly, *H. pylori* treatment increased the number of alpha-fetoprotein-expressing hepatocytes, independent of CCl_4 intoxication. In vitro analyses, using an immortalized rat hepatic stellate (Ito) cell line, revealed that *H. pylori* lysates increased the proliferation of hepatic stellate (Ito) cells, which was boosted by the addition of transforming growth factor-beta1 (TGF-β1). Furthermore, the treatment of *H. pylori* lysates promoted the translocation of the nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) into the nucleus based on an increase in the degradation of NF-κB inhibitor alpha, in the presence of TGF-β1, as did H_2O_2 treatment. They concluded that *H. pylori* infection along with an elevated TGF-β1 might accelerate hepatic fibrosis through increased TGF-β1-induced pro-inflammatory signaling pathways in hepatic stellate cells. Moreover, they suggest that *H. pylori* infection would increase the risk of TGF-β1-mediated tumorigenesis by disturbing the balance between apoptosis and proliferation of hepatocytes.

Bacterial infection is accepted as a precipitating factor in cholesterol gallstone formation, and recent studies have revealed the presence of *Helicobacter* species in the hepatobiliary system. Lee et al. utilized PCR to establish

the presence of bacterial DNA, including from *Helicobacter* species, in gallstones, bile juice, and gallbladder mucosa from patients with gallstones [24]. At cholecystectomy, 58 gallstones, 48 bile samples, and 46 gallbladder mucosal specimens were obtained and subjected to nested PCR using specific 16S rRNA primers of *H. pylori* and other bacteria. Bacterial 16S rRNA was detected in 25 of 36 (69.4%) mixed cholesterol gallstones, one of 10 (10%) pure cholesterol gallstones, and 9 of 12 (75%) pigmented stones, and 16S rDNA sequencing identified *Escherichia coli, Pseudomonas, Citrobacter, Klebsiella,* and *Helicobacter* species. Helicobacter DNA was detected in 4 of 58 (6.9%) gallstones, 6 of 48 (12.5%) bile samples, and 5 of 46 (10.9%) gallbladder specimens. Direct sequencing of Helicobacter amplicons confirmed *H. pylori* strains in all four gallstones, in five of 6 (83.3%) bile samples, and in three of 5 (60%) gallbladder specimens. Although almost all mixed cholesterol gallstones appear to harbor bacterial DNA, predominantly *E. coli, H. pylori* was also found in the biliary system, suggesting that these bacteria play a role in the gallstone formation.

Helicobacter pylori has been suggested to be involved in pancreatic diseases, namely autoimmune pancreatitis and pancreas cancer. Jesnowski et al. investigated the presence of conserved sequences of Helicobacter in pancreatic tissue and pancreatic juice from patients with chronic nonautoimmune and autoimmune pancreatitis as well as pancreatic ductal adenocarcinoma [25]. They collected 35 pancreatic juice samples during routine endoscopic retrograde cholangiopancreatography and 30 pancreatic tissue samples and performed a nested PCR to detect *H. pylori* in the isolated DNA samples. However, they could detect no *H. pylori* DNA, suggesting that a direct infection of the microbial agent in the pancreas seems unlikely.

Neurological Disorders

Dobbs et al. examined the effect of eradicating *H. pylori* in idiopathic parkinsonism by a randomized, placebo-controlled study [26]. Thirty idiopathic parkinsonism patients infected with *H. pylori* and taking no anti-parkinsonian medication were enrolled. Stride length improved (73 mm/year; [95% CI: 14–131]; $p = .01$) in favor of successful blinded active over placebo, irrespective of anti-parkinsonian medication. Gait did not deteriorate in years 2 and 3 post-eradication. This study suggested that *H. pylori* plays a role in the progression of idiopathic parkinsonism.

Kountouras et al. tested the hypothesis that eradication of *H. pylori* infection could improve survival in a Greek cohort of patients with Alzheimer's disease, in a 5-year follow-up [27]. Forty-six patients diagnosed with probable Alzheimer's disease were enrolled in their analysis. The study population was classified into three groups: 1, patients for whom *H. pylori* eradication therapy was successful; 2, patients for whom eradication therapy of *H. pylori* had failed, those who refused the treatment, and those who were noncompliant with eradication therapy; and 3, patients who were *H. pylori* negative at baseline. During the 5-year follow-up, 21 patients died and 25 patients remained alive. Patients who died were older and exhibited lower mean Mini-Mental State Examination scores compared with the patients still alive. Successful eradication of *H. pylori* infection was associated with a significantly lower mortality risk (HR: 0.287; [95% CI: 0.114–0.725]; $p = .008$). The results were similar in adjusted and unadjusted models, for age and Mini-Mental State Examination at baseline (HR: 0.29; [95% CI: 0.11–0.765]; $p = .012$). *Helicobacter pylori* eradication regimen in patients with Alzheimer's disease is associated with a higher 5-year survival rate. A limitation of this series was the small number of patients studied. Therefore, these findings were considered rather as preliminary, thereby requiring future confirmation.

In conclusion, in the last year, several diseases have been investigated to possibly be associated with *H. pylori* infection. For some of those, such as ITP, there is consistent evidence of a causative role, while for the others, further studies are needed to verify the association.

Conflicts of Interest

The authors have declared no conflicts of interest.

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Letter to the Editor

Digestion

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Trainee Participation during Colonoscopy Adversely Affects Polyp and Adenoma Detection Rates

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Dear Sir,

Training of future endoscopists is essential for meeting the increasing demands for colonoscopy. It is still unknown whether the polyp detection rates might be compromised when trainees perform colonoscopy under the supervision of experienced examiners. We therefore attempted to assess whether there might be any differences in the polyp detection rates between procedures that are performed by experienced staff physicians alone (EP) and those that are performed with trainee participation (TP).

Procedural data from all screening colonoscopies performed at the Tokyo Medical Center between January 1, 2006 and December 31, 2010 were reviewed. All patients had a positive result on the fecal occult blood test. The exclusion criteria included suboptimal bowel preparation and incomplete examination (inability to reach the cecum). Demographic information (age, gender) and the colonic lesion detection profile (size and number) were recorded. The polyps were classified as 1–9, 10–19 or ≥20 mm in size. Polyps that were ≤7 mm in diameter were measured by placing the open biopsy forceps, which have a span of 7 mm, against the lesion. Larger lesions were measured with calipers after they were removed. Advanced adenomas were defined as follows: size ≥10 mm, presence of a villous component and presence of high-grade dysplasia. In the presence of multiple polyps, only the size of the largest polyp was considered for the purpose of the analysis in this study. Seven staff physicians (T.N., M.T., H.K., Y.F., H.K., H.N. and S.T.) with extensive experience in endoscopy performed or supervised all the procedures. Ten trainees in their third, fourth or fifth year participated in a proportion of the procedures. The average number of colonoscopies staff physicians and trainees took part in was 2,528 ± 1,409 and 115 ± 38, respectively. According to previous reports, the minimum length of time during colonoscopic withdrawal was 6 min in both groups [1, 2]. Differences between groups were compared by Student's t test for continuous variables and Fisher's exact test or a χ^2 test for categorical variables.

A total of 853 screening colonoscopy procedures that fulfilled the inclusion criteria for this study were recorded in the Tokyo Medical Center endoscopic database. Of the 853 procedures, 342 were conducted with TP, and the remaining 511 were performed by EP. The distribution of the baseline characteristics of the patients in the two groups was similar (table 1).

The polyp size distributions and detection rates in the TP and EP groups are outlined in table 1. The overall polyp detection rate was significantly higher in the EP group (67.5%) than in the TP group (56.4%). The detection rate of polyps that were 1–9 mm in diameter was significantly higher in the EP group than in the TP group. The histological subtype of the polyps and the detection rates are outlined in table 1. Polyps could not be retrieved in 4.6% of the procedures in the TP group and in 5.7% of the procedures in the EP group. The adenoma detection rate in the EP group was significantly higher than that in the TP group. There were no differences in the detection rates of cancer or advanced adenoma between the EP and TP groups.

This is the first report to show that TP during screening colonoscopy adversely affects polyp and adenoma detection rates. To the best of our knowledge, only one prospective study comparing adenoma detection rates for colonoscopies with and

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Table 1. Patient characteristics, polyp size distribution and polyp detection rates, and histological subtype and detection rates in the two study groups

	TP	EP	p
Patient characteristics			
Age	64.8 ± 13.9	64.6 ± 13.1	0.8836
Male/female	188/152	248/263	0.0652
	Detection rate, % (n)	**Detection rate, % (n)**	
Polyp size			
None	43.6 (149/342)	32.5 (166/511)	0.0010**
1–9 mm	36.8 (126/342)	46.2 (236/511)	0.0068**
10–19 mm	12.0 (41/342)	12.9 (66/511)	0.6885
≥20 mm	7.6 (26/342)	8.4 (43/511)	0.6697
Histological subtype			
Advanced cancer	2.9 (10/342)	3.1 (19/511)	0.5304
Early cancer	6.7 (23/342)	5.1 (26/511)	0.3139
Advanced adenoma	2.3 (8/342)	4.3 (22/511)	0.1266
Adenoma	36.3 (124/342)	44.0 (225/511)	0.0236*
Hyperplastic polyp	5.3 (18/342)	8.2 (42/511)	0.0980
Others	0.6 (2/342)	0.7 (4/511)	0.7345
Not retrieved	4.6 (16/342)	5.7 (29/511)	0.5233

* $p < 0.05$; ** $p < 0.01$.

without TP has been published to date [3]; a total of 368 consecutive patients were entered into this study, and adenomas were detected in 19.3% of the procedures performed by EP and in 14.9% of the procedures performed with TP. The study reported no significant difference in the adenoma detection rate between the two groups, although the adenoma detection rate was 4.4% lower in the TP group. Thus, the authors were not able to exclude a lack of statistical significance as a result of a type II statistical error.

The detection of small adenomas may have little effect on the risk of colon cancer since the majority of these lesions do not progress to cancer. On the other hand, enhanced detection of adenomas could provide long-term benefits for patients. By definition, tubular adenomas are neoplastic lesions with the potential to progress to cancer, and even small polyps can occasionally contain cancer [4]. Patients who have adenomatous polyps that were overlooked during a colonoscopy may be at risk for progression to cancer, either because of a longer interval between colonic examinations than is appropriate or because of the patient's own decision to forgo colorectal cancer screening in the future.

In conclusion, TP during colonoscopy appears to have a significant adverse effect on the detection rates of polyp and adenoma. Our results indicate the necessity for careful supervision of colonoscopies conducted with TP.

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Bovine antibody-based oral immunotherapy for reduction of intragastric *Helicobacter pylori* colonization: A randomized clinical trial

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CM den Hoed, AC de Vries, PBF Mensink, et al. Bovine antibody-based oral immunotherapy for reduction of intragastric *Helicobacter pylori* colonization: A randomized clinical trial. Can J Gastroenterol 2011;25(4):207-213.

BACKGROUND: Antibiotic-based regimens are frequently used for the treatment of *Helicobacter pylori* infection. These regimens fail to eradicate *H pylori* in 15% to 40% of patients, primarily due to antimicrobial resistance and insufficient patient compliance. Effective prevention and eradication of *H pylori* by passive immunization with orally administered bovine antibodies has been demonstrated in animal studies, and may serve as an alternative therapy in humans.
OBJECTIVE: To study the efficacy and safety of orally administered bovine anti-*H pylori* antibodies for the reduction of intragastric bacterial load and eradication of *H pylori* in humans.
METHODS: Dairy cows were immunized against *H pylori*. After confirmation of the presence of anti-*H pylori* antibodies in the milk, the milk was subsequently processed into a whey protein concentrate (WPC). In a prospective, double-blind, placebo-controlled randomized clinical trial, *H pylori*-infected subjects were randomly assigned to treatment with the WPC preparation or placebo. Study medication was continued for 28 days; subjects were followed-up for 56 days.
RESULTS: Of the 30 subjects included, 27 completed the protocol. Of these 27 evaluable subjects, 14 were treated with WPC and 13 with placebo. There was no significant difference in urea breath test decrease between the WPC- and placebo-treated group (P=0.75). *H pylori*-associated gastritis and density were not significantly reduced in either group after treatment (P>0.05 for all).
CONCLUSION: Bovine antibody-based oral immunotherapy appears to be safe, but does not significantly reduce intragastric *H pylori* density in humans. Further studies are needed to determine whether WPC treatment has additional value to conventional antibiotic treatment for *H pylori*.

Key Words: H pylori; *Eradication treatment; Immunotherapy; Gastritis*

Une immunothérapie orale à base d'anticorps bovin pour réduire la colonisation intragastrique de *Helicobacter pylori* : un essai clinique aléatoire

HISTORIQUE : On utilise souvent des antibiotiques pour traiter les infections à *Helicobacter pylori*. Chez 15 % à 40 % des patients, ces antibiotiques n'éradiquent pas le *H pylori*, surtout en raison d'une antibiorésistance et d'une observance insuffisante de la part des patients. Des études auprès d'animaux ont démontré une prévention et une éradication efficaces du *H pylori* par immunisation passive au moyen d'anticorps bovins administrés par voie orale, ce qui pourrait constituer une autre thérapie chez les humains.
OBJECTIF : Étudier l'efficacité et l'innocuité de l'administration d'anticorps anti-*H pylori* pour réduire la charge bactérienne intragastrique et l'éradication du *H pylori* chez les humains.
MÉTHODOLOGIE : Des vaches laitières ont été immunisées contre le *H pylori*. Après confirmation de la présence d'anticorps anti-*H pylori* dans leur lait, ce lait a été transformé en concentré de protéine de lactosérum (CPL). Dans un essai clinique à double insu aléatoire et contrôlé contre placebo, des personnes infectées par le *H pylori* ont été réparties au hasard entre le traitement à l'aide de la préparation de CPL et un placebo. L'étude du médicament s'est poursuivie pendant 28 jours, et les sujets ont été suivis pendant 56 jours.
RÉSULTATS : Des 30 sujets participants, 27 ont terminé le protocole. De ces 27 sujets évalués, 14 ont été traités par CPL, et 13, par placebo. On n'a constaté aucune différence significative de diminution du test respiratoire à l'urée au sein du groupe traité par CPL et de celui traité par placebo (P=0,75). La gastrite et la densité associées au *H pylori* n'avaient pas diminué de manière significative dans les deux groupes après le traitement (P>0,05 dans tous les cas).
CONCLUSION : Une immunothérapie orale à l'anticorps bovin semble être sécuritaire, mais elle ne réduit pas la densité intagastrique de *H pylori* de manière significative chez les humains. D'autres études s'imposent pour déterminer si un traitement au CPL a une valeur supplémentaire par rapport à l'antibiothérapie classique du *H pylori*.

Helicobacter pylori infection causes chronic active gastritis in virtually all infected patients and is associated with an increased risk of peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma and gastric cancer (1-3). Therefore, *H pylori* eradication therapy is frequently prescribed in patients in whom the presence of *H pylori* colonization has been confirmed. The current European guidelines state that a positive *H pylori* test is an indication for eradication treatment (4). Such treatment regularly consists of the combination of two to three antimicrobial drugs in combination with a proton pump inhibitor (PPI). However, treatment failure occurs in up to 40% of patients, primarily due to bacterial resistance and insufficient patient compliance, among others, and as a result of gastrointestinal side effects (Table 1) (5,6). Consequently, new therapeutic strategies with broader approaches to treating, suppressing or possibly preventing *H pylori* infections to circumvent problems with drug resistance and side effects are required.

Passive immunization with orally administered antibodies against *H pylori* may constitute one of these alternatives. This approach has been shown to be effective in the prevention and treatment of a variety of pathogens such as *Candida albicans*, rotavirus, *Clostridium difficile* and *Campylobacter jejuni* (7-11). Animal studies have shown that bovine antibodies against *H pylori* reduce bacterial load and that *H pylori* infection can thereby be prevented and even eradicated (12,13). In humans, breastfeeding seems to protect infants from early acquisition of *H pylori*, also suggesting that passive delivery of immunoglobulin (Ig) A antibodies affect *H pylori* colonization (14).

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TABLE 1
Eradication rates, and first-, second-, third- and fourth-line treatments for *Helicobacter pylori* infection

Author (reference), year	Country	Study type	Treatment	Eradication rate, %
Rokkas et al (41), 2009	Greece	Prospective (n=540)	First line: Omeprazole + amoxicillin + clarithromycin	76
			Second line: Omeprazole + bismuth + metronidazole + tetracycline	73
			Third line: Omeprazole + amoxicillin + levofloxacin	70
Hojo et al (42), 2001	Japan	Meta-analysis	Second-line treatment:	
			Proton pump inhibitor + 1 antimicrobial agent	45.8
			Proton pump inhibitor + 2 antimicrobial agents	69.8
			Ranitidine-bismuth + 2 antimicrobial agents	80.2
			Proton pump inhibitor + bismuth + 2 antimicrobial agents	75.8
Seppälä et al (43), 2000	Finland	Prospective (n=644)	First-line treatment of choice of treating physician:	81
			Second line: Bismuth + metronidazole + amoxicillin/tetracycline or omeprazole + bismuth + metronidazole + amoxicillin/tetracycline or triple therapy* based on susceptibility test	58
			Third line: One of the treatments mentioned under second line	76
			Fourth line: Another one of the treatments mentioned under second line	100
Pontone et al (44), 2010	Italy	RCT (n=84)	Sequential therapy: Lansoprazolen + amoxicillin for 5 days and lansoprazole + clarithromycin + metronidazole for an additional 5 days	83
			Rescue therapy: Lansoprazole + levofloxacin + amoxicillin	100
Kearny and Brousal (45), 2000	United States	RCT (n=224)	Bismuth + metronidazole + tetracycline	81
			Lansoprazole + metronidazole + clarithromycin	90
			Proton pump inhibitor + bismuth + metronidazole + tetracycline	87
Vaira et al (46), 2007	Italy	RCT (n=300)	Proton pump inhibitor + amoxicillin (5 days) and proton pump inhibitor + clarithromycin + tinidazole (5 days)	89
			OR	
			Proton pump inhibitor + clarithromycin + amoxicillin (10 days)	77

**Triple therapy: Proton pump inhibitor plus two different antibiotics. RCT Randomized controlled trial*

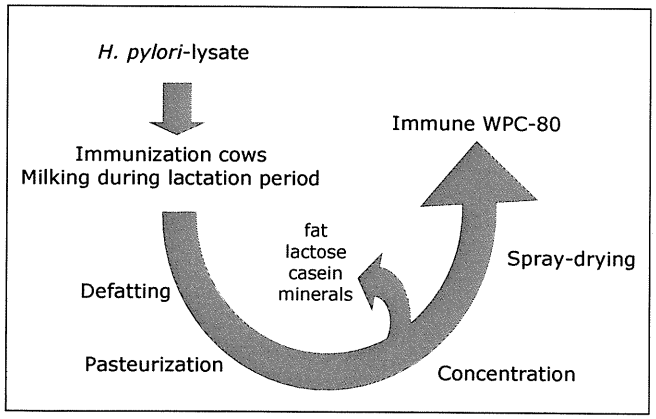

Figure 1) *Whey protein concentrate (WPC) preparation. H. pylori* Helicobacter pylori

The primary underlying mechanism is probably based on inhibition of adherence of *H pylori* to the gastric mucosa by specific antibodies to the main *H pylori* surface-binding antigens (15). However, results of clinical studies on the effect of specific anti-*H pylori* antibodies are scarce. The studies that are available have reported conflicting results (16-20). While two studies did not demonstrate any effect of treatment with bovine antibodies (16,18), three others reported that treatment with bovine antibodies could eradicate *H pylori* infection in all patients (19,20) or decrease *H pylori* colonization density and the extent of gastritis (17). None of these studies were, however, placebo controlled. Therefore, we performed a randomized, placebo-controlled clinical study to evaluate the efficacy and safety of specific anti-*H pylori* polyclonal bovine IgA antibodies for the reduction of intragastric bacterial load and gastritis activity in humans.

METHODS

Study medication: Immune whey protein concentrate preparation

A polyclonal antibody-enriched immune whey protein concentrate (WPC-80 [80% protein]) was prepared from milk collected from cows immunized with whole antigen lysates of eight clinical *H pylori* isolates.

The immunization of six dairy cows comprised repeated nasal (mucosal every two weeks) and supramammary lymph node administration (percutaneous once every month). After immunization, the presence of polyclonal secretory component (sIgA) anti-*H pylori* antibodies in the milk was confirmed by ELISA. Immune WPC-80 was prepared using standard milk industry techniques (Figure 1). The whey fraction was pasteurized, concentrated by ultra filtration and spray-dried to yield the final whey powder. One gram of the final enriched WPC preparation contained approximately 80% protein, of which approximately 20% consisted of Ig, and was completely free of lactose. Inhibition of the adherence of *H pylori* to gastric tissue by specific antibodies in the WPC-80 preparation was demonstrated in vitro using fluorescently labelled *H pylori*. The adherence of *H pylori* in the presence of phosphate-buffered saline/bovine serum albumin and nonspecific antibodies was used as the control.

Study design

The present study was designed to be a double-blind, placebo-controlled trial (Figure 2). Adult *H pylori*-positive subjects (18 years of age and older), as demonstrated by both a positive ^{13}C-urea breath test and histopathology confirming *H pylori* gastritis were eligible for inclusion (Figure 3). Thirty *H pylori*-positive subjects were randomly assigned to either immune WPC treatment (15 subjects) or placebo (15 subjects). Randomization occurred within blocks containing four subjects each, according to a randomization table designed by Department of Statistics at the University of California, Los Angeles (USA). Consecutive eligible patients were included after informed consent, and a coded study number was assigned to each patient.

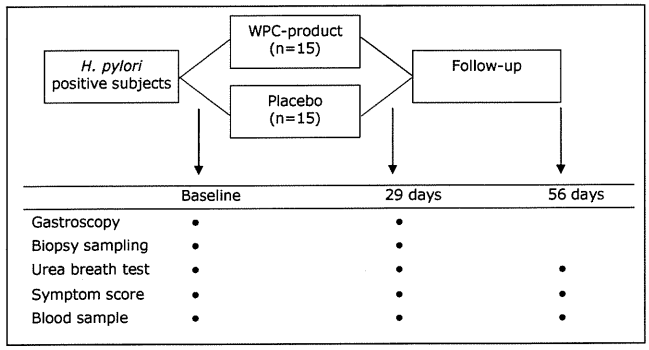

Figure 2) *Study design. H. pylori* Helicobacter pylori; *WPC Whey protein concentrate*

Treatment with H_2-blockers or PPIs was allowed, provided that the doses were stable at least two weeks before the start of the study medication, including the time of the ^{13}C-urea breath test and sampling of gastric biopsies, and remained stable during the study period. Subjects who underwent previous treatment for *H pylori* infection with standard therapy were also included. Exclusion criteria were as follows: lactose intolerance, pregnancy or lactation, active peptic ulcer disease, malignancy, significant systemic comorbidity and use of antibiotics within four weeks before the start of the study.

All subjects received 2.5 g of immune WPC or placebo, to be taken three times daily during meals for 28 days. The medications had identical appearances. Adherence to study medication was monitored by recording the number of empty medication sachets and conducting interviews. The follow-up period was 56 days.

Gastroscopy with biopsy sampling was performed at baseline and at the end of treatment (at 29 days). During gastroscopy, two antral and two corpus biopsies were obtained for histological assessment. In addition, one antral and one corpus biopsy were obtained for *H pylori* culture. ^{13}C-urea breath tests and serological tests were performed at the start of the study, after completion of study medication and at the end of follow-up. Serological evaluation comprised hematological and biochemical markers, and serum levels of gastrin, pepsinogen I, pepsinogen II and *H pylori*-specific IgG. In addition, serum anticytotoxin gene A (CagA) protein IgG antibodies were evaluated at the start of the study.

Dyspeptic symptoms including heartburn, acid regurgitation and epigastric discomfort were evaluated using a validated questionnaire. All items were rated according to seven criteria of the Gastrointestinal Symptom Rating Scale (21,22). The questionnaires were completed before treatment, each week during treatment, just after completion of treatment and at 56 days. In addition, all adverse events during follow-up were recorded.

The Institutional Review Board of the Erasmus University Medical Centre (Rotterdam, The Netherlands) approved the study protocol. All subjects provided informed written consent before enrollment.

Histological and culture methods

Gastric biopsy specimens were fixed in buffered formalin and embedded in paraffin. Hematoxylin-eosin stained sections were used for standard histological evaluation. A single, expert gastrointestinal pathologist performed all histological assessments. The pathologist was blinded to the timing of biopsies, treatment assignment, and clinical and endoscopic data. The samples were assessed according to the updated Sydney classification. The sections were graded for *H pylori* density, polymorphonuclear neutrophil activity, mononuclear cells, gastric glandular atrophy and intestinal metaplasia. All parameters were scored using the following four-point scale: absent = 0, mild = 1, moderate = 2 and severe = 3. Culture of *H pylori* was performed under microaerophilic conditions (5% oxygen, 10% carbon dioxide and 85% nitrogen) using blood agar plates (Dent-plates, Biotrading, The Netherlands).

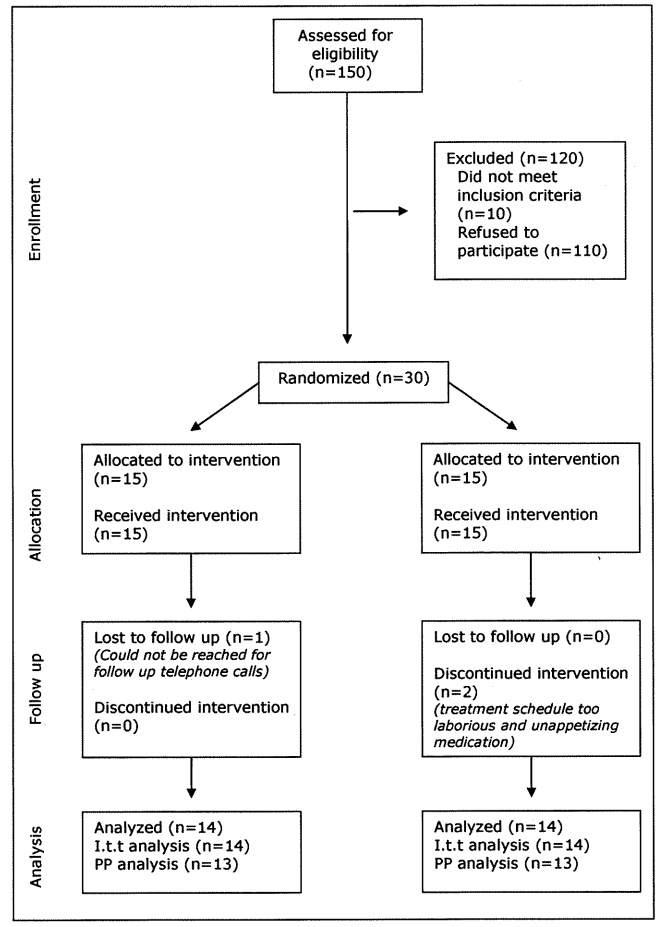

Figure 3) *Consort diagram. I.t.t Intention to treat; PP Per protocol*

Data analysis

The efficacy of the WPC preparation to reduce intragastric *H pylori* bacterial load was evaluated according to the reduction in intragastric urease activity measured by ^{13}C-urea breath tests and reduction of bacterial colonization, as well as acute gastritis quantified by histological assessments according to the updated Sydney system. The number of patients was sufficient to demonstrate a 30% difference in the above mentioned parameters, with a predictive power of 90%. An intention to treat and per-protocol analysis were performed comparing the immune WPC-80 treatment group and placebo group. Statistical analysis was performed using Wilcoxon's rank sum test for changes from baseline to end of treatment for histological severity grades of gastritis. A subgroup analysis of patients receiving acid-suppressive drugs was performed. A two-sided $P<0.05$ was considered to be statistically significant. Power was not calculated due to the explorative nature of the study.

RESULTS

Participants

In total, 30 subjects were included and randomly assigned to the placebo- or WPC-treated group. One patient in the WPC group was lost to follow-up immediately after randomization before the start of the study medication. Two patients in the placebo group discontinued the study medication within two weeks – one due to the intensity of the treatment regimen, the other due to the unappetizing flavour of the placebo; therefore, follow-up data on these patients were not available.

TABLE 2
Baseline epidemiological, histological, serological and ^{13}C urea breath test results of both treatment groups

	Treatment group		
	WPC (n=14)	Placebo (n=15)	P
Sex, n (male/female)	5/9	9/6	0.27
Age, years (mean ± SD)	44.7±14.0	51.3±14.4	0.22
Range	25–67	25–75	
Acid-suppressant use, n			
Proton pump inhibitor	4	5	1.0
H_2-blocker	3	2	
Previous eradication treatment, n	6	6	1.0
Country of birth, n			
The Netherlands	8	6	0.81
Turkey	3	5	
Morocco	1	1	
Other	2	3	
Histological findings			
Antrum			
H. pylori colonization	1.86 (1.03)	1.67 (1.05)	0.63
Activity	1.14 (0.77)	1.47 (0.92)	0.31
Inflammation	2.28 (0.47)	2.07 (0.80)	0.37
Corpus			
H. pylori colonization	1.0 (0.82)	1.33 (0.82)	0.29
Activity	0.92 (0.76)	0.80 (0.68)	0.66
Inflammation	1.54 (0.66)	1.73 (0.59)	0.42
Premalignant gastric lesions, n (%)	1 (7.1)	4 (26.7)	0.33
Urea breath test, mean delta value ± SD	24.2±17.4	23.8±16.3	0.95
H. pylori IgG, mg/mL (mean ± SD)	202±178	152±127	0.40
CagA positive, n (%)	9 (64)	11 (73)	0.43
Gastrin, ng/L (mean ± SD)	20.7±24	24±25	0.73
Pepsinogen I, ng/mL (mean ± SD)	163±90.0	147±61	0.58
Pepsinogen II	14.2±3.7	15.9±4.3	0.85
Pepsinogen ratio I/II, mean ± SD	16.5±4.9	17.0±8.8	0.86

CagA Cytotoxin-associated gene A; H. pylori Helicobacter pylori; *Ig Immunoglobulin; WPC Whey protein concentrate*

Baseline characteristics

Following randomization, 14 patients were treated with the WPC-80 preparation and 15 patients with placebo. The groups were similar with respect to several baseline characteristics (Table 2). At baseline, 14 (48%) patients used acid-suppressive drugs; these patients were equally distributed between both treatment arms (P=1.00). Infection with CagA-positive *H pylori* strains was present in nine (64%) patients included in the WPC-80 group, compared with 11 (73%) patients in the placebo group (P=0.43). Premalignant gastric lesions, atrophic gastritis and intestinal metaplasia were found in one patient in the WPC group and in four patients in the placebo group (P=0.33).

H pylori bacterial load

In the intention to treat analysis, no significant differences between groups were found in urea breath test results at baseline or follow-up and, similarly, in the effect of treatment on urea breath test results (P>0.05 for all) (Table 3). Moreover, no significant differences were observed between subgroups within treatment arms (P=0.57), or in patients with or without simultaneous use of acid-suppressive drugs (P=0.35).

Histological assessment did not demonstrate an effect of active or placebo treatment on acute gastritis and the *H pylori* colonization scores in the antrum (Figures 4 and 5). The *H pylori* colonization scores in the corpus even seemed to increase after treatment with WPC-80 (P=0.03) (Figure 5). In addition, no significant differences in outcome were observed between treatment groups. Subgroup analysis of patients with or without simultaneous use of acid-suppressive drugs showed no significant differences (P>0.05 for all). In one patient, *H pylori* eradication was achieved after concomitant use of the WPC preparation and a one-day course of metronidazole; this patient was not included in the per-protocol analysis. Twenty-six patients were considered in the per-protocol analysis, which did not demonstrate an effect of the WPC-80 or placebo treatment. No differences were obtained using the per-protocol analysis versus the intention to treat analysis (P>0.05 for all).

Serology

Comparison of anti-*H pylori* IgG antibody levels did not demonstrate any differences between the WPC-80 group and the placebo group at day 29 (P=0.63) nor at day 56 (P=0.33) (Table 2). No significant differences with respect to the mean reduction in serum *H pylori* antigen concentration at day 0 (P=0.33) and day 56 (P=0.54) in the WPC-80 group and the placebo group, respectively, were found.

No differences were demonstrated between the WPC-80 group and the placebo group when the mean values of gastrin, pepsinogen I and pepsinogen II levels, and the pepsinogen ratio at baseline at day 29 or day 56 were compared. The levels of these parameters did not change significantly within either group during follow-up, nor were there significant changes between groups in this respect (Table 3).

Safety

Twenty-seven subjects completed the study – 14 in the WPC group and 13 in the placebo group. None of the patients experienced adverse effects due to WPC-80 treatment, and the preparation was well tolerated. No significant differences were identified in the quality of life scores of the WPC-80 group compared with the placebo group on days 7, 14, 21, 28 and 56 (P>0.05 for all). Quality of life scores remained stable during the course of the study in both groups.

TABLE 3
Urea breath test and serology results in both treatment groups at day 29 and day 56

	Day 29			Day 56		
	WPC treatment group (n=14)	Placebo group (n=13)	P	WPC treatment group (n=14)	Placebo group (n=14)	P
Urea breath test						
Mean delta value	24.3±24.2	22.9±14.4	0.85	25.5±25.5	24.0±15.8	0.85
Mean reduction	−0.15±13.4	2.3±14.4	0.66	−1.2±14.0	0.15±11.8	0.78
Serology						
Gastrin, ng/L	4.5±3.6	6.4±4.8	0.25	7.9±5.2	8.4±6.3	0.84
Pepsinogen I, ng/mL	1524±727	1825±829	0.33	1716±923	1829±753	0.72
Pepsinogen II, ng/mL	102±74	121±82	0.55	109±74.5	125±78	0.57
Pepsinogen ratio I/II	17.4±5.5	18.1±8.0	0.79	18.0±6.7	17.0±6.6	0.66

Data presented as mean ± SD unless otherwise indicated. WPC Whey protein concentrate

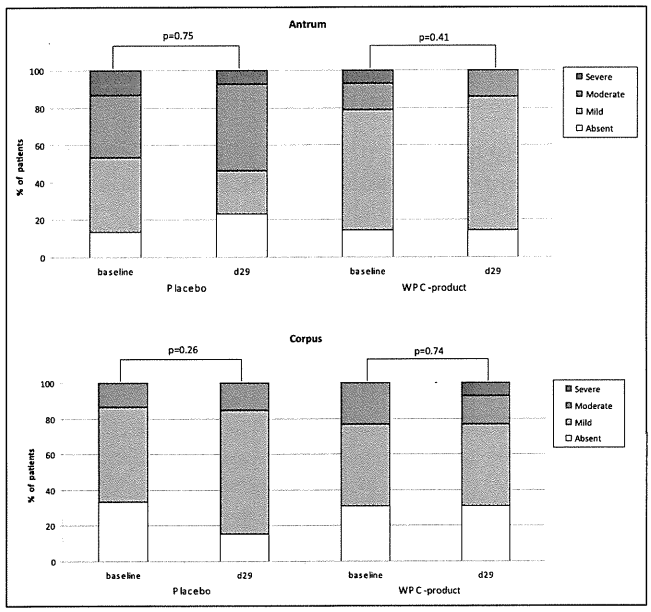

Figure 4) *Histological grade of active gastritis in antrum and corpus in patients randomly assigned to placebo or whey protein concentrate (WPC) preparation. d Day*

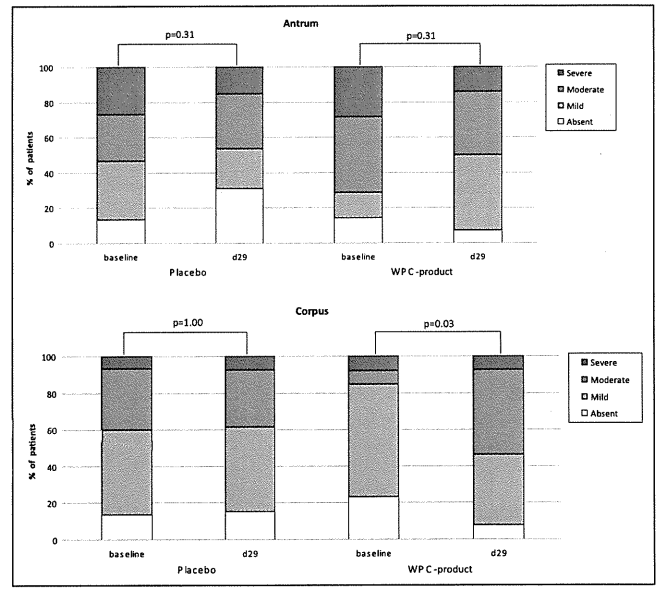

Figure 5) *Histological grade of* Helicobacter pylori *colonization in antrum and corpus in patients randomly assigned to placebo or whey protein concentrate (WPC) preparation. d Day*

Analysis of general blood test parameters revealed no significant changes in either treatment group, nor were there changes in serum liver enzymes or additional serological parameters (eg, hemoglobin, leukocytes, etc) (P>0.05 for all).

DISCUSSION

The present clinical study did not demonstrate a positive effect on *H pylori*-associated gastritis or colonization density following administration of a polyclonal antibody-enriched (sIgA) WPC-80 preparation in humans. No significant reduction was demonstrated in urea breath test levels determined on days 0, 29 and 56. Treatment in either arm had no effects on serum *H pylori* IgG antibody titres, gastrin, pepsinogen I and II levels, or on the pepsinogen I/II ratio. Correcting for the use of PPIs did not influence differences in the outcomes. However, we demonstrated that WPC-80 was well tolerated and did not cause any adverse effects or a decrease in quality of life scores.

H pylori infection is widespread in humans. Although it can be cured by antimicrobial therapy, large-scale use of antibiotics has led to the increasing emergence of antibiotic-resistant strains. Furthermore, side effects of current eradication treatments, although partially preventable by the coadministration of probiotics, limit their efficacy by induction of early treatment withdrawal (23). This has prompted investigators to focus on several alternatives. These alternatives must be effective; however, considerations such as costs, side effects and ease of administration should also be taken into account.

Because previous studies have shown protection against early acquisition of *H pylori* through breastfeeding in breastfed infants (14), the concept of passive immunization (ie, mimicking mechanisms of natural protection) as a logical approach has emerged. An in vitro study conducted in 2001 confirmed the possible effectiveness of antibodies by showing a complement-dependent bactericidal effect of WPC with *H pylori*-specific antibodies. The WPC prevented adherence of *H pylori* to the gastric mucosa (24,25). In addition, an early clinical study in 1991 (19) demonstrated *H pylori* eradication in 20 patients receiving an *H pylori*-specific bovine Ig. The outcomes of more recent studies with *H pylori*-infected patients (16-18,20) have shown modest, albeit encouraging, results. However, one of these studies was open labelled (18) and used a treatment period of only two days, while two others included children only (16,17). Furthermore, the methodology of the other studies were unclear (16-18,20).

A possible limitation of the present analysis was the size of the study population, which may have been too small to demonstrate significant reductions in intragastric bacterial load or gastritis activity by treatment with bovine antibody-based oral immunotherapy. Furthermore, because the optimal length of treatment remains unknown, our study may have been limited by the relatively short duration of WPC-80 treatment. Previously conducted studies (15) used a duration of between two days and four weeks. However, given the total absence of any effect after four weeks in the population studied, we consider it unlikely that an increase in the study population or prolongation of treatment with the same dose would have led to measurable changes.

We cannot exclude the possibility that an increase in dose and/or dosing frequency would have led to an effect on *H pylori* colonization. The dose and administration frequency used in the present study were chosen on the basis of previous experience with similar products, particularly those against C *difficile* (11). Finally, our study may have been limited by the fact that intragastric antibody availability has yet to be clarified (25). Therefore, future research in human subjects is necessary to obtain additional information regarding the optimal antibody dose, frequency of administration and ratio.

The strengths of the present study were the strictly defined outcome parameters, the double-blinded design with repeated follow-up assessments and the exclusion of a possible concomitant antibiotic effect. Except for one subject, all patients completed the four-week treatment course and no adverse effects linked to treatment were identified.

Current research has focused on several alternatives to replace antibiotic eradication therapy. In contrast to our study of bovine-derived IgA antibodies, Japanese researchers have described positive effects of egg yolk-derived IgY, demonstrating a decrease in urea breath test parameters in patients treated with IgY (26,27). Similar studies examining the natural antimicrobial properties of colostrum and egg yolk have focused on the possible use of lactoferrin and lysozyme, which are also components of the humoral immune reaction. Positive effects of bovine antibodies were demonstrated in vitro and in animal models, but conflicting results were obtained in human studies (28). Some studies described a suppressive effect of lactoferrin and lysozyme on *H pylori* colonization (28,29), while others even described an increase in *H pylori* growth and gastric inflammation (30). However, supplementing current eradication therapy with lactoferrin appears to increase eradication rates and could be helpful in patients who fail